INTERNATIONAL CONFERENCE ON
Pharmaceutical and Pharmacological Sciences

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Innovation through diversity
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Letter of Welcome from the School of Pharmacy, North-West University (Potchefstroom campus)

Dear International and National Congress Delegates,

Three years have passed since we last met during the 4th International Congress on Pharmaceutical and Pharmacological Sciences and it is this exciting time again on the calendar of the Academy of Pharmaceutical Sciences and the South Africa Society for Basic and Clinical Pharmacology, as well as the Southern African Neuroscience Society. Yes, the 5th International Congress on Pharmaceutical and Pharmacological Sciences (5th ICPPS) for 2009, hosted by the School of Pharmacy at the North-West University, Potchefstroom Campus in the City of Potchefstroom, North-West Province, South Africa, look forward to meeting with previous participants as well as the new delegates participating for the first time at ICPPS. A hearty welcome to all the international and national participants, invited guest speakers, presenters in the podium and poster sessions, participants at the various pre-ICPPS workshops, accompanying persons, young scientists and honoured guests. Indeed a prestigious event on calendar of the health sciences occurring every 3 years that started in 1996 when the 1st ICCPS was held in Midrand, Halfway House, Johannesburg. The exciting scientific program of the 5th ICPPS, with the theme “Science to Wellness”, indeed addresses health sciences and care delivery issues that are not only facing South Africa and Africa but the world at large. This Congress seeks to find solutions to these challenges. The social functions are structured in such a way to allow you to experience the North-West University and the relaxing City of Potchefstroom, which is situated in a unique part of South Africa, with the “big five” game reserves, “Sun City, the “Cradle of Humankind” and many other attractions close at hand. To all the sponsors and exhibitors that have so generously supported the ICPPS meetings your contributions are sincerely appreciated. Your involvement enables us to serve and contribute to a better world.

We wish you a scientifically fruitful and socially exciting 5th ICPPS and that you not only gain scientific and clinical knowledge but also strengthen and build friendships during your brief visit to the City of Potchefstroom.

Douglas Oliver
Director: School of Pharmacy, North-West University
Dear Colleagues
Welcome to the 5th International Conference on Pharmaceutical and Pharmacological Sciences. This joint conference between the Academy of Pharmaceutical Sciences and the South African Society for Basic and Clinical Pharmacology promises to be the highlight on the academic calendar of 2009. It has attracted both top international and local scientists who are working at the cutting edge of their fields. You will, thus, be exposed to the most recent developments in the pharmaceutical and pharmacological sciences.

The value of a congress is not limited only to listening to presentations, but extends also to interaction amongst delegates of various institutions. In fact, it is common for new collaborations to be established during breaks between conference proceedings. I am appealing especially to our young scientists to engage with senior scientists and overseas delegates during intervals. It often happens that new opportunities, such as an offer to spend a summer in an overseas or different local laboratory, open up for those who are deemed enthusiastic and keen to do research.

Finally, I wish all of you a productive conference. Be assured that your participation contributes significantly to strengthening the local pharmaceutical and pharmacological sciences and helps to maintain their world-class standard.

Henry M. J. Leng
Chairman: Academy of Pharmaceutical Sciences
Letter of Welcome from the South African Society for Basic and Clinical Pharmacology

Dear Delegates, Sponsors and Friends,
Welcome to the 5th ICPPS hosted in Potchefstroom. A special word of welcome to the intrepid young scientists who take part in the competitions, welcome to new members and members of the three Societies.
We hope that our hospitality ensures that our International guests return to our shores again. To the members who attend these meetings regularly, there is always more to learn and contribute. That is what makes the world of science gratifying.
Therefore dear delegate use the opportunity to learn, share and contribute. Not only should this be on an academic level but also on a social level, called networking.
I would like to appeal to everybody to work diligently to get academia back to where it belongs – the academics and run by academics. For too long have Universities and Technikons been driven by administrative expediency and not by academic need.
A word of appreciation to the organisers Sandra van Dyk and Brian Harvey. We look forward to a successful congress.
Last but not least welcome and thanks to our sponsors, without whom we would not be able to have successful conferences.
Wim du Plooy
President: SA Soc. for Basic and Clinical Pharmacology
Letter of Welcome from the Southern African Society for Neurosciences

Dear Friends
On behalf of the Southern African Neuroscientists Society, we would like to warmly welcome you to our meeting here in Potchefstroom. In view of present fiscal restraints, I would like to thank you sincerely for making the effort of joining us at this meeting. Your support of the development and growth of research and its associated activities are inspiring and encouraging.

We eagerly look forward to your participation and sincerely hope that you too will have an enriching experience, make new friends and establish fruitful collaborations.

We are greatly indebted to the organizers and sponsors for their hard work and generosity, and making this event a reality.

Best wishes,
Willie Daniels
Chair: SA Society for Neurosciences
BIOSKETCHES

BS 01  Prof David B Bylund

David Bylund is a Professor in the Department of Pharmacology and Experimental Neuroscience at the University of Nebraska Medical Center in Omaha, Nebraska, USA. He received his Ph.D. in Biochemistry in 1974 from the University of California, Davis in the laboratory of Nobel Laureate Edwin G. Krebs. Following postdoctoral work in the laboratory of Solomon H. Snyder at Johns Hopkins University in Baltimore, MD, he became a faculty member in the Department Pharmacology, University of Missouri-Columbia. In 1988, he assumed the Chair of the Department of Pharmacology at University of Nebraska Medical Center in Omaha. Dr. Bylund has served as the President (2002-2005) of the American Society for Pharmacology and Experimental Therapeutics, and as a member of the Board of Directors of the Federation of American Societies for Experimental Biology (2003-2007).

Prof Bylund has been the Editor of Pharmacological Reviews (1995-2001) and an Associate Editor of the Journal of Pharmacology and Experimental Therapeutics (1987-1995 and 2001-2007), and is a co-editor of xPharm, a comprehensive, web-based database. He received the University of Missouri Teaching Award for Medical Pharmacology in 1987 and is included in the ISI database of highly cited researchers in Pharmacology (http://isihighlycited.com).

Prof Bylund has had a long term interest in the classification and regulation of adrenergic receptors with a current emphasis on adolescent depression. His research activities have been well-funded by NIH and he is the author of over 200 papers. He is actively involved in the development of subtype-selective alpha-2 adrenergic agents for the treatment of a variety of disorders. He is also the Director of a NIH-sponsored Integrative and Organ Systems Pharmacology (IOSP) Short Course.
William (Willie) Daniels graduated in 1983 with a BSc degree in Biochemistry and Botany at the University of the Western Cape. He later obtained his BSc-Hons (1985) and MSc (1988) in Medical Biochemistry, a PhD in Chemical Pathology (1993), and MBA (2003) in Business Administration at the University of Stellenbosch. He completed his postgraduate studies as a postdoctoral fellow on melatonin (1994) under Prof Russ Reiter (University of Texas Health Science Center, San Antonio, Texas, USA), he continued his education on Survival Skills and Ethics (2003) under Michael Zigmond and Beth Fischer (Snowmass, Aspen, Colorado, USA), and he obtained a fellowship to study proteomics (2005) under Prof Seth Grant (Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, UK).

He has held numerous appointments and positions. He was a senior scientist in the Department of Chemical Pathology at Tygerberg Hospital (Feb. 1986 – Jan. 1996), a lecturer (Feb. 1996 – Dec. 1999), a senior lecturer (Jan. 2000 – Dec. 2002), and Associate Professor (Jan. 2003 – Dec. 2005) in the Department of Medical Physiology, University of Stellenbosch. He became Director of the Animal Research Program MRC Unit for Anxiety and Stress Disorders (Jun. 2004 –), Director of the Cape Universities Brain and Behaviour Initiative (Oct. 2005 –) and Head of the Division of Medical Physiology at the University of Stellenbosch (Jan. 2006 – Nov. 2007). Currently, Prof Daniels is a Professor in the Department of Human Physiology, and heads the School of Medical Sciences at the University of KwaZulu-Natal (Westville Campus). His research fields of interests include Neuroproteomics and Bioinformatics, Basic Behavioural Neuroscience, Neurodegenerative Diseases, and Stress and Anxiety Disorders.

He has been a member of numerous professional and scientific societies including the Current Chair of the South African National Committee for IBRO (2002 –), the Current Chairperson of the South African Neuroscience Society (2002 –), the Treasurer and President of the Society for Neuroscientists in Africa (2004 – 2005). He has received numerous honours. In 1991 he received the Beckman Postgraduate Award, in 1994 the Boehringer Young Research Award, the Bristol-Myers Squibb Most Promising Young Scientist Award, the Harry Crossley Travel Award and FRD Travel Award, in 1995 the Mushet Education Trust Postgraduate Travelling Scholarship, in 1996 the Lundbeck Travel Bursary, in 2000 the International Society for Neurochemistry Travel Bursary, in 2003 the International Brain Research Organisation Travel Award, in 2004 the British Commonwealth Fellowship, and in 2006 the Italian Education Exchange Program.

He has received research funding from the NRF, MRC, Harry Crossley Foundation, Lundbeck Pharmaceuticals, Pfizer Pharmaceuticals, a Fogarty Grant from the NIH, and an NIH RO1 Grant. He is a prolific researcher and has thus far published more than 50 articles in international journals.
Professor Elly Grossman is currently the acting Head, Division of Experimental Odontology in the School of Oral Health Sciences, University of the Witwatersrand. She is a basic scientist who graduated with an Honours degree in the Department of Botany, specialising in Electron Microscopy. Her return to academia occurred when she was appointed to the Electron Microscope Unit of the then Medical Research Council run National Research Institute for Occupational Diseases. There she was involved with research projects dealing with aspects of asbestosis, mesothelioma and fibrosing alveolitis. Two years later she was transferred to the Dental Research Institute (DRI) where she remained until 2005. Initially she worked on oral mucosa and tooth ultrastructure before finding her metier in dental materials and the tooth restoration interface which has remained her research interest ever since. During her 26 years of MRC service she has risen to the post of Senior Specialist Scientist and Honorary Lecturer at the University of the Witwatersrand, obtained an MSc and PhD, both in the Faculty of Dentistry, has published over 70 papers in peer reviewed journals, 30 abstracts and has presented close to 70 papers at conferences in South Africa and abroad. She has served as Acting Director of the DRI, on the EXCO of the Microscopy Society of Southern Africa, the South African Chapter of the Dental Materials Group and the South African Division of the International Association of Dental Research as well as on the Faculty Boards of Dentistry and Health Sciences. She has been a referee for numerous journals and has supervised and acted as examiner for many higher degrees. She has just completed a longitudinal study, extending over 55 years, on the research activities and subsequent careers of 132 DRI postgraduates.

Her abiding interest has been in the fusion of research techniques and supervision skills within the Faculty of Health Sciences. She has been integral to the only formally recognised research techniques course in the Faculty of Health Sciences. This course has run on an annual basis since 1978 and has had over 1 700 attendees, from both inside and outside the University. She has been instrumental in initiating a course for Novice Supervisors and started a Supervision Support Group which serves to transfer skills from seasoned to inexperienced supervisors at the Faculty of Health Sciences. Her experience in postgraduate and supervisor activities has led to her secondment to the Wits Postgraduate Project Office in 2009.
Lois Harden has been employed by the University of the Witwatersrand in the School of Physiology as a lecturer since 2004. She obtained her PhD on “Cytokines as mediators of fever and sickness behaviour” in 2008, has published 8 peer-reviewed articles and has recently been invited to give a plenary lecture entitled “Immune-to-brain signaling by interleukin-6: a pathway for lipopolysaccharide-induced fever and sickness behavior”, at the 3rd International Symposium on Physiology and Pharmacology of Temperature Regulation held in Matsue, Japan, from July 23 - 26, 2009. She is currently the chairperson of the Fever research section of the Brain Function Research Group, a research entity recognized by the University of the Witwatersrand. Her research interests lie in the fields of fever, sickness behaviour, and interactions between the immune system and the brain during acute and chronic infection. Lois has recently received a Young Investigators Award at the XXXVIth International Congress of Physiological Sciences, Kyoto, Japan, 2009 for her work on cytokines as mediators of fever and sickness behaviour during infection.
Richard Haynes is in the Department of Chemistry, The Hong Kong University of Science and Technology, Hong Kong, China. He obtained his Ph.D. in 1970 from the University of Western Australia, having been a Commonwealth Scholar (1965-67) and General Motors Holden Fellow (1967-69), as well as receiving the CSR Chemicals Prize in 1967. Before taking up his present position in 1993, he undertook post-doctoral training at the Institut für Organische Chemie, University of Karlsruhe, Germany (1970-72), followed by appointments to the Departments of Chemistry, Imperial College, London (1972-1974), and Monash University, Melbourne, Australia (1975-78), Department of Organic Chemistry, University of Sydney, Australia (1978-92), Institut für Organische Chemie, Zürich (1985), Département de Chimie Organique, Université de Genève (1991-1992), and Exchange Fellow, Australian Academy of Science - Chinese Academy of Science Exchange Programme (1988, 1991). Richard is a fellow or council member for various chemical institutes and societies, including the Royal Society of Tropical Medicine and Hygiene, as well as being on various committees, including the World Health Organization (WHO). He also consultants for a number of international pharmaceutical companies and government agencies. His research has focussed on organic and medicinal chemistry, specifically drug design, synthesis and development of antimalarial agents, antiproliferative agents, antivirals and drugs for other parasitic targets. Prof Haynes has 135 publications in scientific journals, 9 patents, two world patents for antimalarial drug development, 2 book chapters, 51 technical and consulting reports, as well as being invited to talk at many symposia and seminars around the world. He has mentored 24 Ph.D., 13 M.Phil., 3 M.Sc. and 39 honours students, as well as 13 postdoctoral fellows.
BS 06  Dr John A Joska

Dr John Joska is senior lecturer, Department of Psychiatry and Mental Health, University of Cape Town (UCT), senior specialist and director of the HIV/Neuropsychiatry Program, Groote Schuur Hospital, Cape Town, and Honorary senior lecturer, Infectious Diseases Unit, Department of Medicine, University of Cape Town. He obtained his training in medicine and psychiatry at the University of Cape Town, and has received a number of awards in his career, including the Entrance Merit Scholarship of the University of Cape Town (1989), the Lynn Gillis Medal of the Fellowship of College of Psychiatrists of South Africa (2000), the MMed (Psychiatry) degree awarded with distinction (2004), and Best presentation for a young psychiatrist at the Biological Psychiatry congress, Somerset West, Cape Town (2007). He has 10 publications in the peer reviewed literature, as well as 2 book chapters. His current research activities include the study of neurocognitive disorders in young adults commencing anti-retroviral treatment, and studying the assessment of needs and services at a psychiatric hospital.
Peter Kamerman obtained his BSc, BSc (Honours) and PhD at the University of the Witwatersrand, Johannesburg, where he currently is senior lecturer in the School of Physiology. Peter is a member of the International Association for the Study of Pain, a member of Pain SA, the Royal Society of South Africa and also of the Physiology Society of Southern Africa. He has received a number of awards and accolades, including a Y2 rating as a young researcher with the National Research Foundation, the Friedel Sellschop Award for Outstanding Young Researchers (2005), a Young Investigators Award from the International Union of Physiological Sciences (2001) and a Best Poster award from the Zoological Society of South Africa (2001). He has supervised 2 MSc and 2 PhD students and is an ad hoc referee for a number of international pain and behaviour journals, as well as a reviewer for the National Research Foundation. He has chaired workshops and sessions at various local and international congresses, and has published nine papers in international peer review journals, as well as contributed one book chapter.
Gary Maartens is an infectious disease physician and clinical pharmacologist. He has a joint appointment as chief specialist physician and professor at Groote Schuur hospital and University of Cape Town, South Africa, where he is head of the Division of Clinical Pharmacology, Department of Medicine. He is a consultant to the largest managed care HIV programme in Africa. He has acted as a consultant to the World Health Organisation. He is currently the principal investigator on 6 studies, including two randomised controlled trials of interventions for HIV-associated tuberculosis. He has published over 80 peer reviewed articles (including an invited review on tuberculosis in the Lancet), is co-editor of the Handbook of HIV Medicine (Oxford University Press Southern Africa, now in its second edition) and has written several book chapters. He is on the editorial boards of Lancet Infectious Diseases, Antiviral Therapy, Cochrane Collaboration (HIV/AIDS Review Group) and PLoS ONE.
Prof Sarel F Malan

Sarel Malan is currently Professor and Director of the School of Pharmacy, University of the Western Cape and Extraordinary Professor of Pharmaceutical Chemistry, School of Pharmacy, North-West University. He obtained the degrees B.Pharm, M.Sc., DTE and Ph.D. from the then Potchefstroom University for Christian Higher Education and is registered as a pharmacist with the South African Pharmacy Council.

He has supervised or co-supervised 43 M.Sc. and 6 Ph.D. studies in Pharmaceutical/Medicinal Chemistry and Pharmaceutics and is author and co-author of 43 peer reviewed articles in subsidised journals, 5 papers in professional journals, 4 papers in conference proceedings, 2 research reports and various national and international conference contributions; has acted as external examiner and moderator for undergraduate courses, M.Sc. dissertations and Ph.D. thesis for University of Port Elizabeth, University of the Western Cape, South African Pharmacy Council, Rhodes University, North-West University (M.Sc. and Ph.D), University of Pretoria, University of the Witwatersrand and National University of Lesotho and is an Editorial Board Member of The Open Medicinal Chemistry Journal (TOMCJ) and Open Medicinal Chemistry Letters (OMCL) and reviewer/evaluator for the National Research Foundation (Rating, funding and Innovation fund), and various national and international scientific journals.

He has served in various capacities on executive committees of the Academy of Pharmaceutical Sciences and Pharmaceutical Society of SA and as member of Expert Committees of Medicines Control Council (MCC) and World Health Organisation (WHO), Advisory Panels and Steering Committees of the NRF, Standards Generating Body for Pharmacy and Pharmacy Council Inspection Panels.

He is a National Research Foundation (NRF) C2 rated scientist and Fellow of the Pharmaceutical Society of South Africa.

His current research interests are Drug design and molecular modelling of novel entities with ion channel or neuroprotective activity, and pro-drugs and the influence of chemical structure and conformation on physicochemical properties and membrane permeability of drugs.
Andrew Marston is Professor of Organic Chemistry, University of the Free State, Bloemfontein, South Africa. He obtained his BSc. in chemistry at University College, London University, England in 1975, and thereafter worked at the Institut de Chimie, Université de Neuchâtel, Switzerland (1975 – 1976). He obtained his PhD at Liverpool University, England in 1979, thereafter taking up a research fellow position at the Deutsches Krebsforschungszentrum, Heidelberg, Germany (1979 – 1983). Between 1983 and 2009, he worked at various institutes, including the Institute of Pharmacognosy and Phytochemistry, School of Pharmacy, Lausanne University, Switzerland, and the Laboratory of Pharmacognosy and Phytochemistry, School of Pharmaceutical Sciences, Geneva University, Switzerland. His research interests include the isolation and structure determination of biologically-active plant constituents, as well as the applications of new separation techniques, with emphasis on countercurrent chromatography methods. He is the co-author of 12 books, 33 book chapters and 131 research articles in scientific journals. Prof Marston has received a number of honours, including the Rhône-Poulenc Rorer Award of the Phytochemical Society of Europe, and is a Central European Committee representative of the Phytochemical Society of Europe.
Andreas Reif trained in medicine and psychiatry at the University of Wurtzburg, Germany. Since the completion of his medical studies, he has been intensely active in research into the neurobiology of attention deficit hyperactivity disorder (ADHD) and schizophrenia, most notably as principal investigator in studies concerned with the behavioral phenotyping of transgenic animals for ADHD, and in investigating glutamatergic candidate genes in schizophrenic disorders. Dr Reif is the recipient of a number of postgraduate awards and honors, including the PHOENIX-Pharmazie Science Award (2000), the German Society of Psychiatry, Psychotherapy, and Neurology Poster Award (2001), Promotion Award of the Lower Franconia Remembrance Foundation (2002), the CINP Young Investigators Award (2004), the Lundbeck Institute Neuroscience Foundation Sponsorship Award (2004), the WFBPS Young Scientist’s Award (2005), the ECNP Fellowship Award (2006), the AEP Young Psychiatrists Fellowship Award (2007) and the Essex Research Award for Biological Psychiatry (2008). Dr Reif is currently resident and senior scientist (group leader) in the Dept. of Psychiatry, University of Würzburg in Germany. Current and past projects include investigating the involvement of nitrergic signalling in schizophrenia, genotyping of glutamatergic candidate genes in schizophrenia, studying ADHD with special emphasis on its molecular pathogenesis and endophenotypes during the course of therapy, behavioral phenotyping of transgenic animals with respect to ADHD, and investigating the functional genomics and gene-environment interactions in dimensional endophenotypes of fear and anxiety. He has authored or co-authored more than 70 manuscripts published in international peer-reviewed journals.
Vivienne Russell is a neuroscientist in the Department of Human Biology at the University of Cape Town (UCT). She graduated from UCT with her BSc and BSc(Hons) degrees in Chemistry, in 1968 and 1969, respectively. She subsequently obtained her MSc in chemistry at UCT in 1971 and later joined Professor Brian Shanley’s Neurochemistry Research group at the University of Stellenbosch, where she graduated with her PhD in 1978. Based on this research, Dr Russell produced a series of publications that provided insight into the neurochemistry of acute porphyria. Professor Russell joined UCT in 1997. During the past three decades she has supervised several MSc and PhD students and published extensively on topics of interest in the neurosciences. Professor Russell's international reputation rests on her pioneering work in developing an animal model for Attention-Deficit/Hyperactivity Disorder (ADHD). The first of a series of publications on ADHD, in 1995, has garnered 93 citations. In a recent paper published in Biohavioral and Brain Function in 2006, she introduced a novel hypothesis – based on neuronal and glial energetics – for understanding ADHD. This paper has attracted a great deal of attention, is the second most highly accessed paper from this journal (with > 14,000 downloads from the BioMed Central website), and was rated by Faculty 1000 Biology as a major advance in ADHD and a “must read”. Professor Russell has presented her research at more than 150 scientific conferences, and has 97 publications in international journals and 7 book chapters. She was recently honoured by an invitation from the Norwegian Academy of Science and Letters to take an active role in an international project on “ADHD: from Genes to Therapy”.

Professor Russell is the recipient of competitive grants from the National Institutes of Health in the USA, as well as the MRC and NRF in South Africa, to study the neural basis of ADHD as well as the effects of developmental stress on survival of neurons in the brain. Stress experienced during the early stages of development can have long-lasting effects, and Professor Russell's research group was the first to show that voluntary exercise has beneficial effects on the brain that are offset by stress experienced either prenatally or postnatally. Professor Russell has done innovative research on animal models of prenatal and postnatal maternal separation stress in collaboration with Professor Willie Daniels at the University of KwaZulu-Natal and Professor Michael Zigmond of the University of Pittsburgh, USA. This work falls under the UCT signature theme, the Brain-Behaviour Initiative (BBI), a cross-faculty, multidisciplinary, collaborative framework designed to promote research in the cognitive and affective neurosciences. As Director of BBI, Professor Dan Stein is also involved in this project as well as other projects within BBI which focus on trauma and resilience with the aim of understanding why some people develop mental dysfunctions such as post-traumatic stress disorder in response to stressors, while others do not. Professor Russell has also played a major role in promoting neuroscience training in Africa, by organizing international neuroscience schools for junior faculty and postgraduate students. She serves on the Schools board of the International Brain Research Organisation (IBRO), IBRO’s Neuroscience Programme Network and the IBRO African Regional Committee whose function it is to distribute funds and organize neuroscience training in Africa. She is also involved in coordinating the new Masters degree in Neuroscience being offered at UCT. Professor Russell has also been honoured by appointment as an Adjunct Professor of Neurology in the School of Medicine at the University of Pittsburgh and a Fellow of UCT.
BS 13  Dr Anil K Saxena

Anil Saxena is Deputy Director and Head of the Medicinal & Process Chemistry Division, Central Drug Research Institute, Lucknow in India. After completing his M.Sc and PhD degrees, Dr Saxena undertook his post-doctoral research as Alex. V. Humboldt Fellow at the Borstel Research Institute in Germany. His primary area of research is in Medicinal Chemistry & Computer Aided Drug Design (CADD) where he has 39 years of experience in drug development research, CADD & CAMM. He has published 134 research papers and 18 reviews/articles in books and/or monographs, and is the owner of 67 patents. Anil has presented 123 papers, 114 invited lectures, chaired 29 sessions at congresses and symposia, and supervised more than 160 research projects. He has received various awards, including the Alexander von Humboldt Fellowship (Alexander von Humboldt foundation Bonn, W. Germany, 1977-78), the INSA Young Scientist Medal (Indian National Science Academy, New Delhi, 1983), the Themis Chemicals UDCT Diamond Jubilee Distinguished Fellowship in Pharmaceutical Sciences (U.D.C.T., Mumbai, 1997-98), the Ranbaxy Research Award in Pharmaceutical Sciences (Ranbaxy Research Foundation, Gurgaon, 2001), an Honorary Medal for outstanding contributions to Medicinal Chemistry and International Scientific collaboration (Scientific Partnership Foundation, Moscow, Russia, 2004) and the Prof. P.K. Bose Memorial Award (Indian Chemical Society, 2006). Anil has also received Social Recognition awards, including Kayastha Ratna (Akhil Bhartiya Kayastha Maha Sabha, India, 2006) and Kayastha Shiromani (Akhil Bhartiya Kayastha Maha Sabha, India, 2009).

Anil Saxena’s major research achievements include the invention of Centbutidole, released for marketing in 1986, development of the antihypertensive candidate molecule CENTHAQUIN up to phase I, inventor of CDRI 93/478, co-inventor of Pervosine (Phase II) developed by Ranbaxy Laboratories Ltd and many more new lead compounds (>10) under preclinical/clinical drug development. He also created and established a center of excellence in emerging areas of Drug Discovery Research at CDRI, now a premier centre on QSAR and computer aided drug design in India. Dr Saxena is an editorial board member of Medicinal Chemistry Research, SAR and QSAR in Environmental Research, Online International journal ARKIVOC, ARKAT USA Inc and Patent Evaluator: Current Drugs, U.K. He is a member of the American Chemical Society, USA, member of the ‘Board of Directors’ in American Bibliography Inc. USA, secretary for the QSAR society of India, life member of the Indian Chemical Society, member of the Indian Association of Medicinal Chemists and life member of the UP Association for Science and Technology Advancement.
Daniel Stein qualified in medicine and psychiatry at the University of Cape Town, thereafter obtaining his FRCPC at Columbia University, New York, and later his PhD (Clinical Neuroscience) and D.Phil (philosophy) at the University of Stellenbosch. He is currently chair of the Department of Psychiatry at the University of Cape Town and Visiting Professor of Psychiatry at the Mount Sinai Medical School, New York, USA. Professional memberships include Associate Member of the American College of Neuropsychopharmacology; Executive Committee of the Collegium Internationale Neuro-Psychopharmacologicum (CINP); Chairperson of the Society of Biological Psychiatry of South Africa and member of the European College of Neuropsychopharmacology (ECNP). Prof Stein has received various accolades for his research, including the Max Hamilton Award (CINP, 2002), OCDi Award (International Council on OCD, 1999), Young Investigator Award (American Association of Anxiety Disorders, 1997), Lebensohn Award (American Association of General Hospital Psychiatrists, 1993), Distal Fellowship Award (Society of Biological Psychiatry, 1993), APA Resident Research Award (American Psychiatric Association, New York County, 1992) and the NARSAD Louis Pardes Investigator award (NARSAD, 1992). He has published over 350 peer-reviewed papers, and 100 book chapters. His current research interests include: Investigating the psychobiology of the anxiety disorders; A multimedia social support intervention to promote adherence to HIV care in South Africa; The effects of heavy alcohol abuse on adolescent brain structure and function; Brain changes that occur with cannabis and metamphetamine abuse; Psychiatric sequelae of torture in South Africa; The epidemiology of psychiatric disorders in South Africa; Psychopathology in survivors of gross human rights violations in South Africa, and investigating candidate genes (e.g. COMT) involved in mediating obsessive-compulsive disorder in selected populations in South Africa.
Trevor Stone obtained his BPharm at the University of London, his PhD at the University of Aberdeen and his DSc at the University of London. He has received numerous awards, including the MRC Scholarship for Training in Research Methods, the William Ramsay Henderson Travelling Fellowship, Research Fellowships at the National Institutes for Mental Health in Washington DC, the Wellcome Trust Travel Fellowship and a Pfizer Travelling Fellowship. He is currently Professor of Pharmacology at the University of Glasgow, Scotland, UK, where his research focus is on the role of purines, kynurenines and serine proteases in synaptic plasticity, neurodegeneration and neuroprotection. He is Editor-in-Chief of the Journal of Receptor, Ligand & Channel Research, and a past or present member of the editorial boards of the British Journal of Pharmacology, Journal of Tryptophan Research, General Pharmacology, Pharmaceutical Sciences, Journal of Alzheimer's Disease and PharmacologyOnline, while he is a regular referee for approximately 20 other journals. He has published over 375 full research papers in refereed international journals, including three papers in Nature and one in Science, over 200 proceedings abstracts and 13 book contributions (3 authored, 10 edited). To date he has successfully supervised 26 research students for the PhD degree. Prof Stone is a research consultant to Beecham's, Pfizer, Fisons, Wellcome Foundation, Fujisawa, Citrox UK Ltd., Mitsubishi Pharma, and Consultant Research Pharmacologist to the Royal Hospital for Neuro-disability. He is also on the panel of various funding committees, including the MRC Fellowships and Career Training Panel, European Community-Neuroscience Framework 5 and 6 funding panels, while his society memberships include Fellow of the Royal Society of Medicine, Member of the New York Academy of Sciences (1987), Member of the British Pharmacological Society (1973), Member of the Physiological Society (1972), Member of the Brain Research Association (UK), Member of the European Neuroscience Association and a Member of the Society for Neuroscience (USA). Prof Stone has delivered over 50 invited lectures at international meetings, and organised and/or chaired 30 international symposia.
Prof Alvaro M Viljoen

Prof Alvaro Viljoen completed a BSc, BSc Hons. (*cum laude*) and MSc (*cum laude*) in Botany at Stellenbosch University. The topic of his research was the “Essential oil chemistry of indigenous *Pelargonium* species”. In 1994 he commenced with a PhD at the Rand Afrikaans University (now the University of Johannesburg) on the chemotaxonomy of the genus *Aloe*. A total of 16 research papers (all ISI recognised journals) emanated from this PhD study on *Aloe*, challenging various aspects of the infrageneric taxonomy of this commercially important genus. The study also led to the isolation of novel phytochemicals of which some have significant chemotaxonomic value. He completed his doctorate in 1999. Due to the commercial interest in *Aloe*, he visited the International Aloe Science Council (IASC) in Texas in 1999 and was invited back to Dallas in 2000 to present his research results on *Aloe* to an international meeting of the IASC. In 1999 he was appointed lecturer in Pharmaceutical Chemistry in the Department of Pharmacy, University of the Witwatersrand. In 2000 he established a Pharmacognosy research group in the Department of Pharmacy and Pharmacology and supervised 25 postgraduate students in the group. Twenty-six post-graduate students have graduated (14 with distinction) under his supervision since 2002.

His research interest is the phytochemistry and biological activity of medicinal and aromatic plants which have led to extensive international collaboration with scientists in the USA, Italy, Portugal, France, Turkey, England and many countries in Africa. In 2002 he was promoted to senior lecturer at the University of the Witwatersrand and in 2005 to Associate Professor. In July 2005 he was appointed as a research fellow in the Department of Pharmaceutical Sciences, Tshwane University of Technology (TUT, Pretoria).

He has been actively involved in research administration at various institutions serving on the Faculty Research and Faculty Postgraduate Committees. Presently he is the focus area leader for “Intervention Technologies in Health Sciences” and the NRF-IRDP niche area leader for the program “Innovate Naturopharmaceutics”. In addition to these responsibilities he is often requested to review several project proposals for The National Research Foundation and the Medical Research Council in South Africa and he is frequently requested to review papers for high impact journals (Phytochemistry, Journal of Ethnopharmacology, Natural Product Communications and South African Journal of Botany). He has authored / co-authored over 100 peer reviewed papers mostly on the phytochemical exploration and biological activity of indigenous medicinal and aromatic plants. A total of 200 papers / posters have been presented at various scientific conferences including five invited plenary lectures at international meetings.

In 2006 he co-ordinated the first special issue of the Journal of Essential Oil Research dedicated to the chemistry and biological activity of South African aromatic plants. Based on research contributions in the field of medicinal and aromatic plants he has been elected to the editorial board of the Journal of Essential Oil Research (Allured, USA), the International Journal of Essential Oil Therapeutics (France), Journal of Ethnopharmacology (Elsevier) and I am reviewing-editor for South African Journal of Botany (Elsevier).

He is also the recipient of various prestigious awards.
PL 01  Tryptophan metabolism and neuro-psychiatric disorders

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Although the kynurenine pathway of tryptophan metabolism was recognised for many years as the primary route for the synthesis of NAD, a major cellular co-factor, none of the intermediates along the pathway were thought to have any specific biological role or activity. In 1981 we demonstrated that quinolinic acid was an agonist at a subpopulation of receptors for the neurotransmitter glutamate, namely those responding to the analogue N-methyl-D-aspartate (NMDA). Subsequent work revealed that another tryptophan metabolite, kynurenic acid, was an antagonist at glutamate receptors, raising the possibility that the activation of glutamate receptors might be under the control of tryptophan metabolism via the balance between quinolinic acid and kynurenic acid formation. This view has been borne out by several thousand reports published over the past 30 years, with the additional realisation that other tryptophan metabolites in the pathway have marked redox activity and can therefore modulate cellular levels of oxidative stress.

In parallel with these developments, it has been recognised that the kynurenine compounds have potent actions on cells of the immune system, contributing to immunomodulation and influencing the types and relative amounts of immune-active cells. As a result, the kynurenine pathway is increasingly being viewed as playing a key role at the neuro-immune interface, with physiological and pathological relevance to CNS disorders such as depression, neurodegenerative disease and stroke. There is also developing interest in the role of this pathway in the control of infection and tumorigenesis. The possibility that changes in neural function which affects the kynurenine pathway would have secondary effects on aspects of immune function, and vice versa, is opening the prospect to understanding the ways in which psychiatric health and immune activity are intimately interwoven.

The development of pharmacological tools with which to explore the kynurenine pathway experimentally or clinically has lagged behind the basic science, but the use of the available tools to reduce cerebral damage and inflammation is increasing interest in their use for therapeutics. Two specific examples are in the treatment of malaria and trypanosomiasis, in both of which conditions inhibitors of the kynurenine pathway have marked therapeutic and life-saving activity in animal models. Our recent results demonstrate that kynurenine pathway activation is involved in generation of the severe inflammation associated with late-CNS disease, and raise further the hope that future generations of inhibitors may be of value in human trials.
A number of different approaches to obsessive-compulsive disorder and to the obsessive-compulsive spectrum of disorders have been taken over the years, with different authors emphasizing the psychodynamic, cognitive-behavioural, and neurobiological mechanisms thought to underlie various OCD subtypes and spectrums. There is growing evidence that the recognition of certain subtypes of OCD may be useful in clinical settings; these include early-onset OCD, OCD with tics, and OCD with predominant hoarding. Advances in the cognitive-affective neuroscience of obsessive-compulsive disorder (OCD), including its neuropsychopharmacology, may be useful in validating such subtypes. Furthermore, such advances may also suggest novel ways of delineating the obsessive-compulsive spectrum of disorders in terms of cortico-striatally mediated control and reward mechanisms. The space defined by the obsessive-compulsive spectrum of disorders is likely best conceptualized as multidimensional in nature.
PL 03  Understanding attention-deficit/hyperactivity disorder (ADHD): from bench to bedside

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Attention-deficit/hyperactivity disorder (ADHD) is a heterogeneous developmental disorder of childhood resulting from complex gene-gene and gene-environment interactions which give rise to variable expression of the defining symptoms of impaired sustained attention, impulsivity and hyperactivity. ADHD is highly heritable. A genome-wide association scan of quantitative traits for ADHD identified a wide range of genes implicating dopaminergic, noradrenergic, serotonergic, GABA and nicotinic transporters and/or receptors in the pathogenesis of ADHD. Neuroimaging studies suggested increased dopamine transporter (DAT) density, decreased dopamine synthesis (decreased DOPA uptake) and decreased dopamine release (decreased methylphenidate-induced decrease of D2 receptor availability) in striatum of adult patients with ADHD, in line with the notion of dopaminergic hypofunction in ADHD.

The Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) delineates three subtypes of ADHD based on the relative predominance of the two symptom clusters of inattention and hyperactivity/impulsivity, predominantly inattentive (ADHD-PI), predominantly hyperactive/impulsive (ADHD-HI), and combined type (ADHD-C) ADHD. Besides motor activity and impulsivity, there are qualitative differences between the subtypes. Children with PI are more likely to appear sluggish and display slower processing speed. They are less likely to display disruptive behaviours, they have distinct patterns of social deficits, characterized by greater passivity, lower aggression, and less assertiveness than ADHD-C. Although controversy exists as to whether PI is a separate subtype of ADHD or a variant of the combined subtype, there is consensus that the two are similar in their response to stimulant medication.

Psychostimulant treatment and animal models have provided insight into the underlying disturbances of ADHD. Methylphenidate-induced increases in extracellular striatal dopamine were negatively correlated with scores of impulsivity, impaired/inconsistent attention and memory problems, supporting the hypothesis that in ADHD psychostimulants act by enhancing dopaminergic neurotransmission. Many of the results obtained in human studies were previously demonstrated in animal models of ADHD. Although evidence largely supports abnormal dopamine function as a result of a primary defect in dopamine neurons (neuronal firing, dopamine transporter, synthesis, receptor function) or an indirect result of impaired glutamate and/or noradrenergic regulation of dopamine neurons, other neurotransmitters (GABA, serotonin) and second messenger systems (Ca2+) are likely to play an important role in the development of the many different patterns of symptoms displayed by individuals with ADHD.
In a substantial number of patients, ADHD can persist into adulthood and often goes along with substantial comorbidity. Although it is assumed that adult ADHD (aADHD) has an even higher genetic background than childhood ADHD, studies on the genetics of aADHD are scarce. Furthermore, as aADHD most likely features a complex mode of inheritance, large samples are needed to uncover risk genes. Several strategies have therefore to be used in concert to uncover the molecular mechanisms of ADHD. First, genome-wide approaches using SNP chips are able to detect common variants with small effects in large samples in a hypothesis-free approach. Second, high-density linkage analysis provide a means to identify rare variants segregating with disease in large pedigrees. Third, the genome-wide arrayCHG technology can point to rare copy number variants conveying high risk. Finally, case-control association studies are used to test for both rare as well as common variants and provide high power to detect small effects. Converging evidence from all experimental paradigms

However, replication is paramount in complex genetics and any identified risk gene has in the next stage to be confirmed in independent cohorts. To do so, a multicenter project termed IMpACT (International Multicenter persistent ADHD CollaboraTion) was put together in 2007. At present, the consortium consists of five European and one US sites representative of more than 2700 aADHD patients. High throughput genotyping facilities are available at each site enabling rapid replication of candidate genes in the complete sample. In principle, IMpACT carries out two different study designs: meta-analyses of risk gene variants, and replication studies of novel candidate genes. Since the formation of the consortium, several meta-analyses on functional genetic variants in genes like DAT1, 5HTT, NOS1 and BDNF were performed and demonstrated e.g. an association of the 9/6 haplotype in the DAT1 gene with aADHD. Furthermore, candidate genes from hypothesis-free studies, such as CDH13, could be confirmed in large-scale case-control association studies. Since the formation of IMpACT two years ago, it thus became evident that international multicenter efforts are the prerequisite to obtain meaningful genetic data. IMpACT provides a powerful resource for genetic studies on aADHD and already proved to be fruitful.

Taken together, the identification of ADHD risk genes is a daunting task and requires to usage of all pertinent technologies, both hypothesis-driven as well as hypothesis-free. Most likely, both common variants with small effect and rare variants with large effect will play a role in ADHD genetics; in either case, large patient samples are required to unequivocally identify these variants calling for international multi-center efforts.
PL 05  A novel class of antipsychotic agents: Octa/decahydropyrazinopyridoindoles

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Rational drug design implies the identification or creation of novel candidate drug(s) using the knowledge about the information of the structure of a drug receptor or its natural ligands. The substructure approach, based on anneleation or incorporation of the pharmacophores (substructure analysis) with or without the concept of medicinal chemical hybridization (MCH) has been most rewarding in generating new prototype molecules of desired biological potential (Lead generation). The follow up of the lead using state of art lead optimization techniques has been very useful in the discovery and identification of candidate molecule(s) for drug development. The 2-substituted 1,2,3,4,6,7,12,12a-octahydropyrazino[20,10:6,1]pyrido[3,4-b]indoles, which incorporate piperazine and tryptamine in a rigid conformation were designed and synthesized as potential antipsychotic agents using above approaches. One of the compounds 2-[γ-(4-fluorobenzoylpropyl)-1,2,3,4,6,7,12,12a-octahydropyrazino[2',1':6,1]pyrido[3,4-b]indole (centbutindole, biriperone) emerged as a neuroleptic drug from this work. The QSAR studies have led to the identification of 1-(4-fluorophenyl)-butanone substructure for this activity. Its incorporation into the reduced prototype has led to invention of yet another potent antipsychotic compound, (6aR*,11bS*)-1-(4-fluorophenyl)-4-{7-[4-(4-fluoro-phenyl)-4-oxobutyl]-1,2,3,4,6,6a,7,11b,12,12a(RS)-decahydropyrazino-[2',1':6,1]pyrido-[3,4-b]indol-2-yl}-butan-1-one derivative which has shown D1, D2 and 5-HT2A receptor blocking activity where the ratio pKi (5-HT2A) to pKi (D2) is 1.42, which is better than risperidone (1.15). It blocks amphetamine induced hyperactivity/stereotypy and secondary conditioned avoidance responses in rodents at lower doses than those required for the neuroleptic drugs haloperidol and centbutindole (biriperone).
Neurodegenerative conditions, such as Parkinson’s (PD) and Alzheimer’s diseases, are increasingly becoming a burden to society as the world’s population grows older. Neurodegeneration and the development of neuroprotective agents have thus in recent years become an increasingly important focus of research. Currently, symptomatic therapy for many of these diseases exists, but no proven therapies that prevent cell death (neuroprotection), or restores damaged neurons to a normal state (neurorescue) are available.

The lethal triplet of metabolic compromise, oxidative stress and excitotoxicity may act separately or cooperatively to cause neuronal cell. Intracellular calcium homeostasis, or rather the lack thereof, has in many studies been implicated in neuronal degeneration and is currently believed to be one of its main causes. An intracellular calcium overload leads to the activation of various enzyme systems, as well as intracellular accumulation of free radicals. These and other calcium related processes ultimately lead to cell death and neurodegeneration. Earlier studies evaluating the biological activity of the polycyclic cage amines indicate activity that includes amongst others, L-type calcium channel antagonism – leading to possible neuroprotective activity – and high affinity for the sigma-binding site. The pentacyclo-undecylamine derivatives also show structural similarities to the known NMDA antagonist, memantine, currently used in the treatment of Parkinson’s disease. The potential antiparkinsonian and neuroprotective activity of the pentacycloundecylamines and D3-trishomocubanes is thought to reside in the observed antiscataleptic activity, selective sigma receptor antagonist activity and ion channel modulating effects thereof. The MPTP parkinsonian mouse model also indicated neuroprotective activity for compounds in this class. Based on this information and the structural similarities of the polycyclic cage amines and memantine, a series of functionalized cage compounds was synthesised and evaluated for neuroprotection through various, and possibly dual mechanisms. The highly lipophilic polycyclic cage was also utilised to afford blood-brain barrier permeability to drugs with known antioxidant and possible neuroprotective potential.

In these studies, the polycyclic cage has proven to be an excellent scaffold for design of neuroprotective compounds and it is postulated that derivatives thereof could yet be of therapeutic value in the treatment of neurodegenerative disorders like Parkinson’s and Alzheimer’s disease.
From methylene blue to chloroquine to artemisinin: Drug development and questions of mechanism

Diego Monti, Paolo Coghi, Donatella Taramelli, Nicoletta Basilico, Richard K Haynes

The use of methylene blue 1 by Ehrlich in 1891 represents the first example of the use of a totally synthetic compound for treatment of malaria. Although its use subsequently languished, it triggered an analogue search at Bayer Elberfeld in the 1920s, resulting in the development of the quinoline antimalarials santochn, and, with eventual completion in other hands, the des-methyl analogue resochn or chloroquine 2. The latter became the most successful single drug for the treatment of malaria; it was relatively well tolerated, affordable, and was the drug of choice in the World Health Organization (WHO) Global Eradication Program of the 1950s. However, the emergence of resistance by the malarial parasite to chloroquine that first appeared in Asia in the 1960s led to the demise of this drug. Fortunately, in the 1970s, artemisinin 3 was discovered, and with the derivatives dihydroartemisinin (DHA) 4, artesunate 5 and artemether 6 have been notably successful in reatment of malaria in combination therapy with longer half-life drugs.

Although structurally unrelated, an intriguing mechanistic relationship between each of methylene blue, chloroquine and the artemisinins is beginning to emerge. Methylene blue acts synergistically with the artemisinins both in vitro and in vivo against the malaria parasite, and, like the artemisinins, appears to act antagonistically towards chloroquine. Methylene blue acts as a subversive substrate resulting in co-factor destruction, and thereby appears to shut down enzymes associated with maintenance of redox balance within the parasite. However, this is not easily reconciled with the assumed mechanisms of actions of artemisinins and chloroquine. For the artemisinins, it is widely assumed that Fe^{2+} ‘activates’ the artemisinins via Fenton cleavage of the peroxide to generate short-lived alkoxyl, and thence, C-centered radicals, which alkylate ‘vital parasite proteins’. Apposite suggestions have been put forward for the mechanism of action of chloroquine implicating heme formed within the parasite food vacuole, although the precise mechanism of action does indeed remain unclear. We submit an alternative proposal that emerges from our work in this area relating to the ability of artemisinins to dramatically accelerate redox cycling of methylene blue and its effect of chloroquine; the proposal calls for neither the involvement of heme-Fe^{2+} or non-heme-Fe^{2+} for so-called ‘activation’ of artemisinins.
The gaseous messenger nitric oxide (NO) has been implicated in a wide range of behaviors, including aggression, anxiety, depression, and cognitive functioning. Behavioral phenotyping of mice lacking the neuronal isoform of nitric oxide synthase (NOS-I), the major source of NO in the central nervous system, revealed only little differences in activity-related parameters or depression-related tests, yet a subtle anxiolytic phenotype. The most prominent feature however was cognitive impairment in spatial learning and memory. A set of > 120 differentially expressed genes was identified in a gene chip study. Among the most significantly up-regulated genes were CCAAT/enhancer binding protein alpha, 3 genes involved in GABA(B) signalling and the glucocorticoid receptor. Furthermore, male Nos1 knockout mice were shown to display increased aggressive behaviours, while female knockout mice on the other hand lack maternal aggression. However, this was only observed under conditions of adverse environment.

In contrast to rodent behavior, little is known about the function of NO in humans. We could identify a promoter polymorphism in the transcriptional control region of exon 1f of the human NOS1 gene. By a reporter gene assay, we could show that this polymorphism alters the expression of the gene; furthermore, it impacted on the transcriptome of postmortem brain tissue (n=105). Most notably, amongst the top dysregulated genes were candidate molecules for psychiatric disorders such as alpha-synuclein or the NMDA receptor subunit 1. We then aimed to investigate this polymorphism for an association with behavioral traits. Emphasis was placed on outward-bound, disruptive behaviors. Five different samples have been ascertained for this study: a sample of patients with adult ADHD (n=383), a sample of subjects with Cluster B and Cluster C personality disorder (n=403), a sample of prison inmates stratified for violent and non-violent criminality (n=233), a sample of suicide attempters (n=189) and, finally, a control sample of 1914 subjects. Short NOS1 exon 1f alleles were significantly associated with Cluster B personality disorder, especially histrionic personality disorder. Likewise, short alleles were found significantly more in patients suffering from adult ADHD. Finally, short allele carriers were more prone to commit violent crime, when childhood adversity was controlled for, as well as suicide. Short promoter repeat alleles were also associated with personality traits related to impulsivity. Collectively, these data argue for a role of NOS-I in human behaviour. It appears that especially interpersonal, disruptive, impulsive behaviours are influenced by NOS1 genotype. We have tested for environmental effect in an independent cohort, which was followed up longitudinally, and found that NOS1 ex1f-VNTR affects impulsive traits along the developmental trajectory. Finally, short promoter repeats appear to influence prefrontal brain functioning as tested by a Continuous Performance Test and event related EEG potentials. Taken together, these findings correlate to previous animal studies where Nos1 knockout mice were found to be highly aggressive; whether or not cognitive dysfunctioning, as found in our Nos1 knockout colony, is also a function of exon 1f VNTR, has yet to be investigated. Further analyses on the impact of NOS1 genotype on personality dimensions therefore are under way, which will clarify the role of NO in human behaviour.
The discovery that quinolinic acid was an agonist at NMDA receptors, and kynurenic acid was an antagonist, led to immediate interest in their potential pathophysiological roles. There are striking similarities between the effects of quinolinic acid and Huntington’s disease, which have led many to propose a causative role for quinolinic acid in this disorder. Quinolinic acid lesions of the striatum in monkeys produce dystonia and dyskinesia closely resembling those of human Huntington’s disease and those effects can be suppressed by lesions of the pallidum. Quinolinic acid lesions are associated with the induction of the huntingtin gene in rats, which can appear within 6 hours of quinolinic acid treatment. Chronic infusions of quinolinic acid into the rat striatum induced deficits of spatial learning in a radial arm water maze, leading the authors to propose that chronically raised quinolinic acid could induce the related deficits seen in Huntington’s disease. When acute intrastrialal injections were used and the animals studied in a range of behavioural paradigms together with a crude histological asse ssment, authors concluded that quinolinic acid provided a good model of the earlier symptoms of Huntington's disease while 3-nitropropionic acid produced more severe effects which could be a model for the later symptoms. A large volume of evidence has now accrued suggesting that several of the tryptophan metabolites in the kynurenine pathway may be relevance to the aetiology of Huntington's disease.

Although a large volume of evidence suggests a role for glutamate receptors in the aetiology of schizophrenia, there is no abnormality in plasma kynurenine levels in schizophrenic patients. However, a single injection of amphetamine reduced the levels of kynurenic acid in the young rat striatum, as a result of which the sensitivity of the animals to the neurotoxic effects of NMDA is substantially increased. Since repeated administration of amphetamine is known to induce a psychosis closely resembling that of schizophrenia, it is possible that schizophrenia itself could involve a decline of kynurenic acid levels with a secondary hyperactivation of NMDA receptors and a loss of neurones in limbic regions of the brain. Of a series of metabolites measured, 3-hydroxykynurenine gave the best indication of clinical response to neuroleptic treatment in schizophrenia. There may, therefore, be value in examining further the possible role of kynurenines in schizophrenia, especially since quinolinic acid lesions of the ventral striatum of rats have the effect of suppressing prepulse inhibition, an attention disorder characteristic of schizophrenia.
Alzheimer’s disease (AD) is the most frequent cause of dementia in elderly persons, with presently more than 30 million people who suffer from the disease. The principal strategy for treating AD consists of correcting the acetylcholine (Ach) deficit in the brain by the use of inhibitors of acetylcholinesterase (AChE). Galanthamine, first isolated in the 1950s from the snowdrop Galanthus nivalis (Amaryllidaceae) [1], is now one of the few therapeutics used in the management of Alzheimer’s disease, by a mechanism involving maintenance of acetylcholine levels in the brain.

The clinically-interesting alkaloid huperzine A was originally isolated from the Chinese clubmoss Huperzia serrata (Lycopodiaceae) [2]. Huperzine A is a reversible inhibitor of acetylcholinesterase (AChE). Several clinical trials have been reported in China and the efficacy of huperzine A was demonstrated in the treatment of 447 patients suffering from age-related memory dysfunction or dementia. A 5-chloro-vanillin derivative (ZT-1) of huperzine A is currently undergoing clinical trials in Switzerland. This derivative is used in the form of an implant.

Since plants can provide inhibitors of AChE which delay the progression of the disease, a test for the inhibition of the enzyme on TLC plates has been developed [3]. In a screening programme using this assay, an extract of Gentiana campestris (Gentianaceae) showed a high level of inhibition of the enzyme. The xanthones which were responsible for this inhibition were isolated and found to have activity similar to that of galanthamine [4]. Another promising plant from mountainous regions of central Europe is masterwort (Peucedanum ostruthium, Apiaceae). Coumarins isolated from the roots also inhibit AChE. These and other active constituents, such as the flavonoid linarin from the leaves of Buddleja davidii (Buddlejaceae), have been isolated by centrifugal countercurrent chromatography. Linarin inhibited the enzyme acetylcholinesterase to the same extent as galanthamine.

As South Africa is a rich source of plants belonging to the Amaryllidaceae, a programme to study certain genera of this family, notably Nerine, has been instigated.
PL 11 Natural products in the quest for the body beautiful

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Health and beauty are integral components of physical and psychological well-being. The pharmaceutical and cosmetic industries have been placed under immense pressure to produce innovative products for these lucrative industries. Both industries turn to nature for guidance, inspiration and as a source of novel compounds for commercial development.

In the past decades, several ambitious attempts have been made in the search for the definitive panacea to cure disease and produce the elixir of everlasting beauty. An overview of own work in the field of ethnopharmacology and cosmetic science will be presented. The various tried and trusted approaches will be critically assessed. For example, the reductionist approach of ‘one molecule – one target’ is often short-sighted and contradicts the essential philosophy of herbal and traditional medicine. This is somewhat ironic as the traditional use is often presented as a basis and motivation for ethnopharmacological studies.

The major thrust of natural product research in pharmacy has been to discover new molecules with specific pharmacological activity (e.g. anticancer or antimicrobial). Recently, a novel research initiative was established in the School of Pharmacy at TUT. This innovative application of natural products in pharmaceutical science (“Naturopharmaceutics”) for both drug development and drug delivery will be discussed.

South Africa represents a global epicenter of medicinal and aromatic plants. Despite this rich botanical diversity (which is matched by chemical diversity) it is surprising that pharmacognosy has been a neglected discipline in Pharmacy Schools in South Africa over the past 25 years. Many of the indigenous botanical assets remain latent and are not systematically studied, an obvious pre-requisite for the indigenous resources to be transformed into consumer products. These unique South African opportunities will be highlighted.
The pharmacological treatment of major depressive disorder in children and adolescents is difficult due to a lack of effective treatments for this age group. Many of the antidepressants used to treat adults with depression cannot be used for pediatric depression because of a lack of efficacy or side effects. Specifically, the tricyclic antidepressants show good efficacy in the treatment of adult depression, but are no better than placebo in treating pediatric depression. In order to better understand this differential response of children and adolescents, as compared to adults, to antidepressant drugs, it is useful to compare juvenile rats with adult rats. We have found both neurochemical and behavioral differences between adult and juvenile animals after antidepressant treatment. Juvenile animals have differences as compared to adult animals in the maturation of the serotonergic and noradrenergic systems, and in the dose of antidepressant drug needed to achieve similar brain levels. Differences after administration of antidepressant drugs have been found in the regulation of alpha-2 adrenergic receptors and in behavioral responses in two animal models of depression, the forced-swim test and learned helplessness. A better understanding of the effects of antidepressants in juvenile animals should be beneficial for studying and finding new treatments for pediatric depression.
PLENARY LECTURES

PL 13  Cytokines as mediators of sickness behaviours

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Having cared for a sick individual, caregivers recognize that most often it is the dramatic changes in behaviour that provide signs of illness. During a period of illness individuals often are “feverish”; they do not feel like eating food, have an increased sensitivity to pain and lose interest in their physical and social environments. They feel fatigued and are reluctant to engage in their normal daily activities. Their sleep is also often fragmented and they develop a depressed mood. Each of these sickness behaviours negatively impact on the sick individuals’ quality of life and are extremely uncomfortable, which is often what necessitates medical intervention.

The discovery that these non-specific behavioural symptoms are not only particular to patients with a “common cold” or flu, but in fact also appear to occur in patients with some of the most harmful, costly and debilitating diseases currently experienced in the Western World: coronary heart disease, cancer, obesity, type II diabetes and neurodegenerative disorders associated with aging, has enhanced the effort of basic scientists and clinicians to identify the molecular and cellular mechanisms underlying the triggering of sickness behaviours during illness, with the view to developing treatment strategies aimed specifically at managing them.

A major breakthrough in identifying the possible mechanisms involved in mediating sickness behaviours arose serendipitously from clinical studies in which proteins important for immune function, known as pro-inflammatory cytokines, were used in the treatment of the immunosuppression present in cancer patients. Injecting cancer patients with these cytokines produced a suite of sickness responses, including fever, fatigue, malaise, headaches, anorexia and depression, similar to those noted during infection. From these clinical studies showing that administration of pro-inflammatory cytokines induce dramatic changes in behaviour resembling those seen during infection, the question has arisen as to whether these proteins, produced by immune cells of the host in response to a variety of disease-causing pathogens, are likely mediators of the sickness behaviours experienced during infection and inflammation. In this presentation, I shall provide data from human and animal studies investigating the involvement of cytokines as endogenous mediators of sickness behaviours, with a focus on identifying possible targets for the treatment of sickness behaviours, in particular the anorexia and fatigue experienced by patients.
PL 14  Novel developments in the treatment of HIV-tuberculosis co-infection

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Co-administration of antitubercular and antiretroviral therapy is common in high burden countries where tuberculosis is the commonest opportunistic infection. Concomitant use of rifampicin and many antiretroviral drugs is complicated by drug-drug interactions due to the potent induction by rifampicin of genes involved in drug metabolism and transport, which may result in sub-therapeutic antiretroviral drug concentrations. This talk focuses on drug-drug interactions involving antiretrovirals used in resource-limited settings: the non-nucleoside reverse transcriptase inhibitors (NNRTIs) efavirenz or nevirapine, and ritonavir-boosted protease inhibitors. The reduction of nevirapine concentrations with concomitant rifampicin is greater than with efavirenz, particularly during the lead-in dose period when sub-therapeutic concentrations occur in the majority of patients. There is reassuring data on the effectiveness of standard doses of efavirenz with concomitant rifampicin, but the largest cohort study found a higher risk of virological failure with nevirapine. The drug-drug interaction between rifampicin and ritonavir-boosted protease inhibitors is more marked than with the NNRTIs, and therapeutic concentrations have only been achieved with increased doses of some protease inhibitors. The major barrier to using adjusted dose protease inhibitors with rifampicin is the high rates of hepatotoxicity seen in healthy volunteers. The alternative strategy followed in resource rich settings is to replace rifampicin with rifabutin, but even if the price of rifabutin were to be dramatically reduced it would be difficult to implement in high burden countries where standardised antitubercular regimens with fixed dose combinations are used.
Infection with Human Immuno-deficiency Virus (HIV) results in early invasion of the central nervous system, probably by CD4-bearing circulating monocytes. A cascade of neurotoxicity ensues. The events leading up to this have only been partly understood to date. These include neurotransmitter mechanisms, viral protein effects, and inflammatory pathways. Host factors such as Apo E genotype, drug use and age related neuropathology could be considered as vulnerability factors. Among the consequences of this neurotoxic cascade are a range of HIV-associated Neurocognitive Disorders (HANDs), the most severe being HIV-Associated Dementia (HAD). Despite the advent of highly active antiretroviral therapy (HAART), these disorders persist. The limited success of HAART in eradicating HAD suggests that further work to elucidate targets for prophylactic neuroprotective or other CNS-specific drugs is sorely needed.
Publish (or Perish)

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Publishing the results of your investigations is a requirement for success in science. Scientific papers are written in a clear and concise style. Their purpose is to inform other scientists about an important issue and to document the particular approach you used to investigate that issue. A well-written scientific paper explains your motivation for doing the experiments, the experimental design, what you discovered and the meaning of those results. Good English is critical in science writing and a standard format is used for most scientific papers: Abstract; Introduction; Methods; Results; Discussion; References. You will need to decide who will be included as authors and the journal to which you will submit (and likely resubmit) your manuscript. Once the reviews come back you will need to deal with the comments of the reviewers. If you do not publish your work in appropriate journals, you will not gain the recognition you need to advance your scientific career.
In order to meet future knowledge based economic demands, the Department of Science and Technology (DST) has proposed a sixfold increase in PhDs by 2018, better feeders from Masters to PhD programmes and a concomitant increase in Honours candidates to supply the academic production line. This is occurring at a time when South African tertiary educational institutions are coping with the massification of higher education and addressing the needs of a diverse research student population. Additionally, tertiary institutions are vying for top students and scarce skills, maximizing subsidies and upgrading the academic profile of its permanent staff to address DST aims. Skilled, experienced supervisors are pivotal to achieving government and institutional targets but the multiple demands consequent on increased postgraduate throughput is creating tensions within the supervisor cohort. It is timeous to examine these tensions and how they impact on institutional-supervisor-postgraduate relationships.

Tensions are generated in three broad areas:
Supervision tensions
The magnitude and scope of supervisor intervention in postgraduate work
Supervisory arrangements
Postgraduate management tensions
Part-time postgraduates
Postgraduate student-centred development programmes
New knowledge and skills required to complete projects
Thresholds in conceptual thinking
Statement of principles / memorandum of understanding
Institutional tensions
Pressures placed on newly qualified research staff
Changing supervisor ethics within the “knowledge-based economy”
Knowledge transfer within subsidy deadlines
Undergraduate load

The presentation will explore and determine the origins of each area of tension. Suggestions will be made to enable supervisors to effectively tackle each matter, thereby effectively dealing with the complexities of new era postgraduate research supervision. In this way supervisors will be better equipped to meet postgraduate targets set by the DST.
PLENARY LECTURES
IL 01  Research project on the services for which pharmacists may levy a fee

Vincent Tlala, Sue Putter, Johan van Zyl, Martie Lubbe, Andries Gous, Nadine Butler, David Bayever, Desmond Nazer, Michael Naidoo, Panjasaram Naidoo, Ilse Truter, Head of Pharmaceutical Services in Provinces and Metros, South African Pharmacy Council


Purpose:

Sections 35A(b)(iii) and 49(4) of the Pharmacy Act, 1974 (Act 53 of 1974) as amended, entitle the South African Pharmacy Council to make rules as to the services for which a pharmacist may levy a fee as well as guidelines for levying such a fee. Section 49(1)(a) of the Pharmacy Act, entitles the Minister, in consultation with the Council to make regulations relating to the tariff of fees payable to a pharmacist in respect of professional services rendered by him/her. After a pilot project, specifically focusing on dispensing, that was presented to the Pricing Committee, the SAPC identified that a more comprehensive study encompassing all the professional services, which can be provided by a pharmacist, was needed. In 2006 the SAPC embarked upon a further project in collaboration with the pharmacy schools with the specific research objectives to

- assign unit values to the procedures (services) described in the Rules relating to the services for which a pharmacist may levy a fee;
- determine the cost of providing these services as well as a profit component for the provision of services in a pharmacy; and to
- establish norms for the staffing of institutional and community pharmacies based on the volume of services provided per pharmacy.

Research Methodology:

The research project was conducted in two phases. The results obtained in Phase 1 (n=2200) were used to determine the sample for Phase II, through a stratified random sampling 680 pharmacies, consisting of 502 community, 64 private and 114 public institutional pharmacies throughout South Africa. During Phase II time analysis data collection tools were utilised to measure the duration of each of the services and procedures that are listed in the Rules relating to services for which a pharmacist may levy a fee and guidelines for levying such fees. A total of 597 (88%) pharmacies were surveyed by trained fieldworkers. Financial and human resource information was obtained through structured questionnaires from 399 (58.7 %) and 401 (59%) pharmacies respectively. Ethical approval was obtained from the North-West University (No NWU 00058-07-S4).

Results and Conclusion:

The results of the analyses were used to postulate a costing model that was developed in accordance with the guidelines that were provided in the Regulations relating to the obtaining of information and the processes of determination and publication of reference price list of the National Health Act. Based upon the research that was conducted and an application of the principles that have been prescribed through the relevant regulations and the guidelines to those regulations, a coding structure and set of associated reference prices for the professional services for which a pharmacist may levy a fee were presented.
IL 02 Pharmacological and non-pharmacological treatment of ADHD: A pharmacoepidemiological perspective

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Purpose:
Attention Deficit/Hyperactivity Disorder (ADHD) is a common neuropsychiatric disorder that impairs social, academic and occupational functioning in children, adolescents and adults. Claims are often made that methylphenidate, the mainstay in the treatment of ADHD, is overused or even abused in South Africa, especially in children of school-going age. Yet, few studies have investigated the prescribing patterns for ADHD in South Africa. In addition, more adults are being diagnosed with ADHD and limited research has been conducted on their treatment. The primary aim is to provide an overview of the pharmacological and non-pharmacological treatment of ADHD from a pharmacoepidemiological perspective. An overview is provided of international trends in the treatment of ADHD and the results of six recent South African pharmacoepidemiological studies on ADHD are discussed.

Methods:
Four retrospective studies using medical aid claims data and two questionnaire surveys were conducted. The first database study analysed the prescribing patterns of medicine for ADHD of 106 patients in 2002, the second study focussed on prescriptions for ADHD in the nine provinces of South Africa in 2004, the third study investigated medicine prescribing for ADHD in 3 879 patients (1994 to 2006) and the fourth study investigated prescribing for ADHD in 350 patients in 2007. The two questionnaire surveys investigated in detail the treatment and care of 18 patients in 2005, and 51 patients in 2006, who were diagnosed with ADHD in the Eastern Cape.

Results:
In all four database studies, most patients were male (ranging from 75% to 84% of patients). Methylphenidate was the most often prescribed active ingredient, especially the 10 mg tablet formulation. Other products prescribed for ADHD included risperidone, atomoxetine and imipramine. Dosages were investigated, but varied according to age. Methylphenidate was prescribed off-label to a small percentage of patients, especially in children younger than six years. Common co-morbid conditions with ADHD were allergic rhinitis, asthma and epilepsy. From both questionnaire surveys, it was found that approximately two-thirds of patients had tried one or more complementary and alternative medicine (CAM) therapies for ADHD. Only 21.57% of patients in the 2006 study were still using CAM since no noteworthy improvement in behaviour was observed. Natural products (for example, Eye q®, Biostrath® and Evening Primrose Oil) were the most often used, followed by dietary modifications. Behavioural therapy proved to be beneficial in combination with pharmacotherapy. Treatment of ADHD in South Africa is generally in agreement with that in other developed countries. There is a place for CAM in the treatment of ADHD, but in combination with pharmacological treatment. More studies are needed to determine the extent of use and the cost-effectiveness of the different treatments for ADHD in both children and adults. Studies should also measure the quality of life of patients with ADHD.
Attention deficit hyperactivity disorder (ADHD) is a heterogeneous, highly heritable, behavioural disorder. Research in animals and neuroimaging studies of patients with ADHD suggest that the major symptoms (difficulty with tasks that require sustained attention, impulsivity and hyperactivity) may be attributed to disturbances in the prefrontal cortex and its connections to the striatum and cerebellum. The prefrontal cortex receives information from different parts of the brain and regulates behaviour, attention and affect using representational knowledge. It functions to sustain attention over a delay and inhibit distractions, while more posterior parietal cortical areas are involved in perception and the allocation of attentional resources. The right prefrontal cortex is particularly important for behavioral inhibition, an aspect of behaviour that is deficient in ADHD. Evidence for the involvement of the prefrontal cortex is provided by studies on animals and patients with lesions to the prefrontal cortex. They display ADHD-like behaviour, they are easily distracted, forgetful, impulsive, plan poorly, and they display locomotor hyperactivity. There is also evidence obtained from animal models of ADHD to suggest that impairment of dopamine signalling is a major contributor to ADHD-like behaviour. Rats that were inoculated with a lentivirus that allowed localized delivery of a dopamine transporter (DAT) gene enhancer to the nucleus accumbens, became impulsive risk-takers. Neonatal dopamine-lesioned rats and DAT knock-out mice displayed hyperactivity and impaired learning. Several animal models of ADHD have disturbances in dopamine neurotransmission. However, more recent animal studies suggest that the drugs that are used to treat ADHD selectively target the noradrenergic system at the low doses used to improve prefrontal cortex regulation of attention and behaviour. Both dopamine and norepinephrine are required for optimal function of the prefrontal cortex. Norepinephrine has been suggested to enhance "signals" by activating postsynaptic $\alpha_{2A}$-adrenoceptors, while dopamine decreases "noise" through modest levels of D1 receptor stimulation. Both D1 receptor and $\alpha_{2A}$-adrenoceptor stimulation have been suggested to strengthen the functional connectivity of prefrontal cortex networks. Blockade of $\alpha_{2A}$-receptors in the monkey prefrontal cortex recreated the symptoms of ADHD, resulting in impaired working memory, increased impulsivity, and locomotor hyperactivity. $\alpha_{2A}$-Adrenoceptor agonists have been used to reduce ADHD-like symptoms in both patients and animal models of ADHD. Although the evidence points to dopamine and norepinephrine, it is also argued that altered dopaminergic and noradrenergic transmission may reflect an attempt to compensate for a defect in the neural circuits that they modulate.
An animal model of early life stress

**Willie Daniels, Joachim Uys, Jackie Faure, Lelanie Marais, Ilse Pienaar, Dan Stein**

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There is substantial clinical evidence that suggest a clear link between the occurrence of adverse events early in life and the development of psychiatric disorders later in life. We have subsequently performed a series of experiments to investigate the impact of early life stress on behaviour and neuron function. Maternal separation, where rat pups were removed form their dams, was used as the adverse early life event. We have studied the consequences of this separation in the development of (1) depressive behaviour, (2) addictive behaviour, (3) neuron vulnerability to toxins, and (4) the response to subsequent trauma. At a behavioural level we found that maternal separation led to the development of anxiety-like symptoms, proneness for depression, increased neuron susceptibility to toxins, but no effect on additive behaviour. Interestingly maternal separation together with subsequent trauma resulted in a behaviour that suggested resilience rather than exaggerated behavioural disturbance. At the molecular level these behavioural changes were associated with abnormal hypothalamic-adrenal axis function, significant differences in neurotrophin concentrations especially in the hippocampus, down-regulation of serotonergic receptors in the hippocampus, and alterations in proteins concerned with energy metabolism, cell structure, cell signaling, neurotransmission and oxidative stress.
There is a strong association between pain and psychiatric illness, especially depressive and anxiety disorders. This association is bidirectional, such that chronic pain predisposes affected individuals to the development of psychiatric illness, and psychiatric illness increases the risk for developing chronic pain.

Most animal models of acute and chronic pain test changes in nociceptive thresholds to mechanical or thermal stimuli rather than testing high-order pain processing. Consequently, it would be expected that these animal models would be of limited use for investigating the link between pain and psychiatric illness. Yet, animal models of pain that involve testing nociceptive thresholds have provided valuable insights on the functioning of descending pain-modulating pathways, and that inappropriate activity in these pathways, which originate in the brainstem and are regulated by input from higher brain centres, may have a significant role in mediating the increased risk for developing chronic pain in individuals with psychiatric illness.

I will provide examples of commonly used animal models of pain and methods used to test changes in nociceptive thresholds, and describe how these pain models and testing methods have been used to investigate the functioning of descending pain-modulating pathways. The examples I use will show how these pain models and testing methods have provided a theoretical basis for our understanding of the efficacy of tricyclic antidepressants and serotonin and noradrenaline reuptake inhibitors (SNRIs) in treating the painful symptoms of major depression independently of their antidepressant effects, and how changes in activity in higher brain centres, such as may occur in some psychiatric illnesses, may increase pain sensitivity by increasing activity in descending pain facilitatory pathways.
INVITED LECTURES

IL 06  Linking animal behaviour to human depression: Are parallels being drawn responsibly?

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Major depression is an anxiety-related disorder predicted to become the second most debilitating illness after cardiovascular disease by 2020. Current drug treatments of major depression are plagued with bothersome side-effects, a delayed onset of action and a significant percentage of treatment resistance. While the neurobiological basis of depression and the mechanisms of antidepressant action remain elusive, the best described hypotheses include variations of the classical monoaminergic hypothesis of depression. These hypotheses involve dysregulation of adrenergic, serotonergic and dopaminergic neurotransmission in the brain. While cholinergic hypersensitivity, and glutamate and GABA dysfunction have also been postulated to play a role in the neurobiology of depression, newer hypotheses also involve dysregulation of the HPA axis and circadian rhythms, altered neuroplasticity and altered brain reward pathways.

Consequently, there is a need for a better understanding of the neurobiological basis of depression, as well as of the mechanisms underlying the drug treatment of this disorder, especially if we are to discover new targets for drug development. While the human brain and psychological functioning are extremely complex, whether viewed at biological subcellular or systematic level, research into psychiatric disorders remains one of our biggest challenges. Human studies are also complicated by a maze of ethical considerations that often preclude in-depth investigation into the neurobiology of these illnesses. On the other hand, in vitro research becomes too reductionistic to answer complex questions relating to the human mind and psyche, such that the best alternative remains appropriately validated animal simulation models. With these models it becomes possible to investigate behavioural, anatomical, histological, neurobiological and biomolecular changes in depression and drug treatments. However, before this can be attained, the models in use need to demonstrate proven validity for their intended use.

Several animal models of depression or models to evaluate antidepressant activity have been developed and validated. These models focus on symptoms of depression (such as anhedonia – i.e. face validity), and/or that they have been demonstrated to be responsive to antidepressant treatment (i.e. predictive validity), and/or that they show some comparison with deviations in anatomical or neurobiological markers associated with depression (construct validity). Examples of these models include the rodent forced swim test, the tail suspension test, behavioural despair, chronic mild stress and olfactory bulbectomized rats. With the evidence of gene X environment interactions in depression, genetic animal models such as the Flinder’s sensitive line (FSL) rats are also particularly interesting and useful.

The basis for the validity of these animal models, as well as their value and limitations with respect to depression and antidepressant drug discovery, will be discussed with a focus on the rodent forced swim test. We shall also present recent data on a novel interaction between the NMDA/NO/cGMP pathway and the cholinergic system in producing an antidepressant-like response in this model.
Obsessive Compulsive Disorder (OCD) is a psychiatric condition classified as an anxiety disorder by the American Psychiatric Association. OCD is fourth on the list for most occurring psychiatric disorders, affecting between 1-2% of the world’s population. Although several different types of behavior can be classified as “obsessive” and/or “compulsive”, the two dominant clinical features in all OCD patients are inappropriate intrusive thoughts or “obsessions” (currently thought to be the cause of severe anxiety) and seemingly senseless, persistent repetitive behaviors or “compulsions” that are evoked in an attempt at reducing the anxiety caused by the obsession(s).

Due to the nature of all psychiatric conditions, it is practically difficult to investigate the underlying cause of these disorders at the neurobiological level without the aid of animal simulation models. Although it is impossible to mimic the human condition completely, animal models of disease serve as frameworks in which several different aspects of a given disorder can be investigated, and includes symptom presentation, underlying biology and response to drug treatment. Many of the currently applied models can reproduce the stereotypic quality of locomotor behavior that is characteristic of OCD, albeit doing so by different means. Mimicking compulsions are thus less complex as they are observable behaviors. However, what is especially problematic in modeling OCD in animals is the cognitive “obsessational” dimension of the disorder.

Several established and putative models for OCD are available or are being developed, each with different advantages and drawbacks. This presentation will discuss three principle approaches, viz. 1) using animals genetically manipulated to simulate the disorder, 2) inducing abnormal behaviors in normal animals that are akin to behaviors seen in OCD using pharmacologic agents, and 3) using animals naturally exhibiting abnormal behavior, or using animals trained to exhibit abnormal behavior, that closely resemble typical behaviors observed in patients with OCD. The latter group of models will be discussed more extensively.
Because of the inadequacies of the current pharmacotherapies to treat neurodegenerative diseases such as Parkinson’s disease (PD), a variety of molecular drug targets and treatment strategies have been pursued. Emerging evidence suggests that neurodegenerative disorders are multifactorial in nature and involves several different biological processes. Therapies that act at multiple targets, in principle, may therefore be more effective in treating complex neurodegenerative diseases such as PD. The discovery that styrylcaffeines act as antagonists of adenosine A2A receptors and as inhibitors of monoamine oxidase B (MAO-B) has raised the possibility of designing dual-target-directed drugs for the treatment of PD. A2A antagonists have emerged as promising agents for the symptomatic treatment of PD, possibly as adjuvants to dopaminergic drugs. The therapeutic benefits of A2A antagonists are additive to those of dopaminergic therapy and as a result it may be possible to reduce the dose of the dopaminergic drugs and the occurrence of their side effects. Furthermore, A2A antagonists may also protect neurons against the underlying neurodegenerative processes. Inhibitors of MAO-B are also utilized in the treatment of PD. MAO-B inhibitors are combined with levodopa to enhance the elevation of dopamine concentrations following levodopa treatment. This may be particularly useful when used in early stages of the disease when dopamine production is not as severely compromised. Furthermore, MAO-B inhibitors may also act as neuroprotective agents, in part by reducing the levels of hazardous metabolic by-products of MAO-B catalyzed dopamine oxidation in the brain. The inhibition of MAO-B is especially important when considering that MAO-B activity in the brain increases with age. This lecture summarizes recent efforts to develop such dual-acting drugs using caffeine as the lead compound.
The cost of coping and stroke risk in North-West African educators: The SABPA Study

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Dissociation of a physiological active coping (AC) style implying a shift from central β-adrenergic to vascular α-adrenergic responses in urbanized black Africans has been associated with hypertension prevalence (HT). Our aim was to assess if an AC style can contribute to stroke risk. The SABPA Study included 200 fasting urbanized black men and women (mean age: 41.4 and 43.1 yrs). High responders on a validated Coping questionnaire stratified Africans into AC and passive coping (PC) groups. Stroke risk factors included: smoking, HT (24 hour blood pressure, BP), myocardial infarction (MI) and diabetes profile, sympathetic nervous system activity (SNS). AC men consumed more alcohol compared to PC men. Independent of alcohol consumption, data (p ≤ 0.05) in AC men showed higher disturbed sleep patterns, resting diastolic BP, microalbuminuria (p = 0.07), larger Na:K ratio and smaller phenylalanine/tyrosine ratio compared to PC men. They also reported a perception of good health (GHQ) but showed major depression symptoms (PHQ-9). Prevalence of HT in AC men is 59 % whilst 59.3% had MI incidents (ECG). Odds ratios exist for vascular BP, 1.2 (CI, 0.06, 0.26) and disturbed sleep patterns, 2.5 (0.10, 1.73) contributing to HT. AC women revealed increased heart rate variability, phenylalanine, gamma gluthamyl transferase coupled to major depression compared to PC women. In the AC women, 41% are HT and 33% had MI incidents. Odds ratios exist for a high AC score contributing to HT, 2.7 (CI, 0.05, 1.91). To conclude, higher 24 hour SNS, major depression and an AC style in Africans could increase stroke risk.
Generic substitution (GS) plays a major role in the reduction of health care costs and has come to stay. National economies as well as individuals benefit from more affordable medicines produced as substitutes of innovative products.

An important disadvantage of GS is that it may act as a disincentive for innovation. Clinicians are often apprehensive about the "swap ability" of generic substitutes.

GS rests on bioequivalence (BE) which, in turn is based on bioavailability (BA).

The significance and methods of determination of BA will be explained and discussed.

The methods of assessment of BE will be explained.

Various possible outcomes of BE studies will be considered.

The statistical approach in testing BE will be presented.

Generic substitution will be explained in terms of BE.

The concepts of pharmaceutical alternatives, pharmaceutical equivalents and therapeutic equivalents will be explained.

The FDA requirements for therapeutic equivalence will be presented and the concept of individual equivalence discussed.

The approach regarding equivalence of biotechnology products will be discussed.
Glucose clamp studies

F Burger
Director and Head: Feasibility and Consulting, PAREXEL South Africa

The glucose clamp model is widely recognized as the gold standard to demonstrate the time-action profiles of compounds such as insulin and to describe the Pharmacokinetic and Pharmacodynamic properties of glucose lowering drugs.

Clamp studies can be divided into euglycaemic, hyperglycaemic and hyperinsulinaemic glucose clamp methods.

With the euglycaemic glucose clamp model, a feedback control method is used, to keep the subject’s basal blood glucose level constant, during exogenous insulin administration. After the administration of a glucose-lowering drug, such as insulin, the subject’s blood glucose is closely monitored and a glucose infusion is started to maintain an euglycaemic level, by adjusting the glucose infusion rate according to the subject’s fluctuating blood glucose level. Maintenance of euglycaemia ensures patient safety and prevents the endocrine response normally associated with hypoglycaemia - that is, the release of for instance glucagon and adrenaline.

The euglycaemic, hyperinsulinaemic glucose clamp model may be used to determine insulin sensitivity where as the hyperglycaemic glucose clamp model permits the estimation of glucose utilisation at elevated blood glucose levels. With the latter model, insulinotropic compounds such as sulfonylureas can be evaluated.
INVITED LECTURES

IL 12  Focused surveillance of the safety of anti-retroviral treatment, traditional, complementary and alternative medicines – Medunsa National Pharmacovigilance Centre (MNPC)

du Plooy WJ¹, Osuch E,¹ Summers RS², Tsepe W³, Mayamise G³, Meyer A², Mzileni O⁴, Kangawasa ⁵, Mokgatle M⁶

¹Pharmacology and Therapeutics, ²Pharmacy, ³MNPC, ⁴Internal Medicine, ⁵Tsepang Wellness Clinic, ⁶Public Health,

The Medunsa National Pharmacovigilance Center or MNPC was established to monitor patients receiving ARV’s as part of the Comprehensive Care, Management and Treatment Plan (CCMT), taking into account that many patients may also be using complimentary, alternative or traditional medicines (CAT).

The aim is to collect data from a cohort of at least 10 000 patients from specific sentinel sites. Five sites in four provinces have been developed. Data is captured as either retrospective or prospective. Specific parameters include incidence and prevalence of specific events, time to event, CAT use, categories of adverse drug reactions and change in regimens.

Data from over 1 400 patients has been collated. Almost 4 700 ARV related adverse events were recorded. Two thirds of reactions experienced during the first year of treatment occurred within four months of ARV initiation. About half the patients switched regimens at a median of 147 days. Reasons included toxicity (49%) and treatment failure (29.5%). Detailed results will be presented.

Very little information is divulged by the patients about using traditional medicines, complementary or alternative medicines.

Partnerships with other role players were formed which necessitated an upgrade of the database to make it compatible with others and provide automated reports, details will be shown.
IL 13  Use of radiopharmaceuticals in SA research; Status and new opportunities

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R&D on diagnostic radiopharmaceuticals in the developed world mainly focuses on PET (Positron Emission Tomography), predominantly using F-18 and C-11 isotopes while SPECT (Single Photon Emission Computerized Tomography) remains a routine procedure. The first F-18-FDG (Fluoro-Deoxy-Glucose) scan in South Africa only took place in January 2006 mainly due to the cost of infrastructure associated with PET. Although there are 9 PET-CT and 3 PET cameras currently in the country, Tc-99m remains the radionuclide of choice for diagnostic procedures as it is widely available (through the Mo-99/Tc-99m generator) and has a variety of procedural options via kits. In the third world it is anticipated that Tc-99m will maintain its market share predominantly due to the cost of PET scans as well as the distances between major sentra.

As a major producer of Mo-99, Necsa has a small but active research programme aimed at bringing Tc-99m radiopharmaceuticals to light. It also has an active collaborative R&D programme on therapeutic radiopharmaceuticals. The underlining principles of radiochemistry and radiopharmacy and will be briefly illustrated based on the current projects at Necsa. The DST national initiative to promote these activities, NTeMBI, will also be explained.
Optimum therapeutic outcomes require not only proper drug development, but also effective drug delivery. The human skin is a readily accessible surface for drug delivery. Only a small number of drug products are currently available via transdermal delivery. In many cases, a drug's physical properties, including molecular size and polarity, have limited its capacity to be delivered transdermally. Similarly, the biological properties of drug molecules, including dermal irritation and insufficient bioavailability, have been problematic.

Transdermal drug delivery offers several important advantages over more traditional dosage forms. Some of the greatest disadvantages to transdermal drug delivery is the possibility that local irritation will develop at the site of application and the skin's low permeability limits the number of drugs that can be delivered in this manner.

Bearing in mind that the basic functions of the skin are protection and containment, it would seem exceptionally difficult to target the skin for drug delivery. Many of the recent developments in transdermal drug delivery target the more hydrophilic compounds that were previously undeliverable via this method. With a greater understanding of the structure and function of the skin, and how to alter these properties, more and more new drug products are being developed for transdermal delivery.
A successful new product development (NPD) project is one that is in fact launched in the market and one that has reached the targeted consumer. Successful NPD projects rely on cross-functional teamwork, well defined processes, strict timelines, clear roles and responsibilities and self discipline. Project management is therefore of essence in bringing any concept to life in the new product development (NPD) process.

The NPD processes typically need to accommodate the following type of product developments: new technologies, customer needs, line extensions, re-launch of existing products, new products and the so-called 'me too' offerings. It is therefore essential that a flexible and efficient process is designed that can accommodate any or all of the above, instead of a multitude of different processes.

The NPD process typically consists of the following phases: Ideation and Concept, Feasibility, Development, Implementation and Production and delivery. Each phase consists of numerous tasks, well integrated with one another, with specific time lines and clear roles and responsibilities, e.g. cost objectives, sales figures, development of formula and packaging, packaging design, product testing, batch up scaling, regulatory issues etc.

Various stakeholders are involved in these tasks, including management, marketing, sales, creative or design groups, R&D, supply chain, suppliers, legal and third party manufacturers (TPM) – all with the same goal, but with different approaches and ideas – a challenge in itself.

The process therefore needs to define clear stage gates for top level decision making to track and approve the process, e.g. concept, feasibility, implementation and post launch learning gates. Stage gate sign-off prevents wrong products being marketed and/or product and project failures. This disciplined approach ensures new launches within the shortest possible lead-time, improves cost effectiveness and is in alignment with business strategies.

A knowledgeable and professional team can lead the way to a successful new product launch, through efficient project management tools and techniques.
**IL 16** Topical and/or transdermal growth factors in cosmetics

**Minja Gerber, Berenice Campbell, Lize van Niekerk, Anne Grobler, Lissinda du Plessis and Jeanetta du Plessis**  
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**Purpose:**
Peptides undergo degradation when administered orally due to gastrointestinal enzymes and can benefit from controlled release administration technologies, i.e. through the skin. The skin has less enzymatic activity when compared to other epithelial membranes, which prolongs the delivery of the peptide and provides a more stable dosage form. There are two functions of growth factors in skin biology that were considered, i.e. treatment against pigmentation and alopecia.

**Methods:**
Three peptides (IGF-1, VEGF and KGF) against alopecia and two peptides (TNF-α and TGF-β) against hyper-pigmentation were used. In vitro permeation in phosphate buffer solution (PBS) and Pheroid™ was measured through excised female human abdominal skin in Franz diffusion cells and the skin (epidermis and dermis) was also analysed to observe the quantity that penetrated into it through tape stripping.

**Results:**

<table>
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<th>Growth factors</th>
<th>Cone. (pg/ml)</th>
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<tbody>
<tr>
<td>IGF-1</td>
<td>2000</td>
</tr>
<tr>
<td>VEGF</td>
<td>1500</td>
</tr>
<tr>
<td>KGF</td>
<td>1000</td>
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<tr>
<td>TNF-α</td>
<td>500</td>
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<tr>
<td>TGF-β</td>
<td>50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tape stripping</th>
<th>Cone. (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-1</td>
<td>50</td>
</tr>
<tr>
<td>VEGF</td>
<td>100</td>
</tr>
<tr>
<td>KGF</td>
<td>200</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1000</td>
</tr>
<tr>
<td>TGF-β</td>
<td>5000</td>
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</tbody>
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**Conclusion:**
For the growth factors against alopecia, IGF-1 gave the best diffusion through the skin and VEGF gave the best penetration into the skin. TGF-β gave the best diffusion through the skin and the best penetration into the skin of the growth factors used against pigmentation. Pheroid™ improved the diffusion through the skin and into the skin for all the growth factors, except TGF-β.
Inhibitors of HMG-CoA reductase (3-hydroxymethylglutaryl coenzyme A) have revolutionized the treatment of dyslipidemia since their clinical introduction. Several of these inhibitors have reached clinical status in several countries with others currently in clinical trial phase. Treatment with the statin drugs has clearly induced a reduction in the morbidity and mortality associated coronary artery disease in patients. Currently there are three main groups of statins compounds namely those that are derived from *Aspergillus terreus*, e.g. lovastatin, simvastatin and pravastatin; the prodrug group simvastatin, lovastatin and those derived synthetically, e.g. atorvastatin, cerivastatin, fluvastatin, rosuvastatin. These drugs inhibit the HMG-CoA reductase, the enzyme that is involved in the conversion of HMG-CoA to mevalonate. This conversion is the rate-limiting step in the biosynthesis of cholesterol and a significant reduction in the levels of low-density lipoproteins (LDL) and triglycerides with an increase in high-density lipoproteins.

![Examples of drugs inhibiting the HMG-CoA reductase enzyme](image)

The X-ray crystal structures of the HMG-CoA reductase enzyme in the absence and presence of the HMG-CoA substrate has been reported, clearly showing the uniqueness of the catalytic site of this reductase for the conversion to mevalonate. A D2 symmetry for the catalytic HMG-CoA reductase forming a tetramer with monomers being arranged as two dimers each having two active sites. Several of the statin drugs have subsequently been co-crystallized with the HMG-CoA reductase enzyme, clearly illustrating the unique active site interactions. It is also evident that mark conformational differences exist for the HMG-CoA reductase enzyme bound to the substrate when compared to the inhibitor bound enzyme. The molecular similarity between the substrate and the inhibitors are still evident, whereas significant molecular evolution has occurred in the heterocyclic moieties of the inhibitors. The equieffective dose ratios of these statins also differ significantly, with the more recently approved drugs being superior with respect to their efficacies. The safety of the statins has received significant attention in view of a numbers of deaths associated with rhabdomyolysis, which subsequently lead to the withdrawal of cerivastatin by the Food and Drug Administration in the USA.

In conclusion, the statin drugs proved to be the choice for the treatment of dyslipidemia and hypercholesterolemia and the introduction of novel statins in the near future will continue to play an important role in the primary and secondary prevention of coronary heart disease associated events and the integration of current knowledge will indeed facilitate these strategies.
IL 18 Artemisone – A new artemisinin derivative: Cerebral malaria and the fall of the heme model

Ronald Wai Keung Wu, Gigi Wing Chi Chan, Diego Monti, Paolo Coghi, Donatella Taramelli, Nicoletta Basilico, Richard K. Haynes

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The isolation of artemisinin and discovery of antimalarial activity by Chinese scientists is one of the great events in medicine in the latter half of the 20th Century.1 Artemisinin and derivatives dihydroartemisinin (DHA), artemunate and artemether are used for treatment of malaria in combination therapy with longer half-life drugs.1-3 However, artemunate is rapidly hydrolysed in vivo to DHA, and artemether is metabolized to DHA. The artemisinins, especially DHA, elicit neurotoxicity in laboratory screens4 and the sense of the problem is not diminished by a fatality induced by an artemunate overdose.5 Toxicity must be borne in mind, particularly if artemisinins are to be developed against other targets where protracted doses may be required.3

In a program designed to generate new artemisinins for use against malaria and other targets, we have enhanced drug permeability through the attachment of polar, non-metabolizable groups at C-10. One such compound, artemisone, possesses negligible neurotoxicity, and is a designated drug development candidate.6 The lecture commences with a brief overview of current artemisinins, then gives newer details on artemisone that include activity against cerebral malaria in a mouse model, other parasitic targets, and chemical behavior against various heme species including carboxy hemoglobin and carboxyheme. The latter results lead to the demise of the heme model for mode of action of artemisinin antimalarials.
With the advance of modern synthetic peptide chemistry, many more drugs of peptide origin are being used clinically (Frokjaer & Hovgaard, 2000). This can mainly be attributed to the fact that peptides are an important class of molecule in rational drug design, for the following reasons: (i) metabolism and pharmacokinetics of peptides are easily determined; (ii) except for glycine all other amino acids contain multiple chiral carbons that increase receptor specificity and reduce toxicity and (iii) the synthesis of peptides is relatively simple and economically (Vogler et al., 2002).

The discovery of several cyclic dipeptides (also known as 2,5-diketopiperazines [DKPs]; 2,5-dioxopiperazines [DOPs]; cyclo dipeptides or dipeptide anhydrides) from natural sources and the introduction and development of solid – and liquid-phase synthetic techniques have all contributed to the development and design of new drug entities from peptide origin (Prasad, 1995).

DKPs, which are the smallest cyclic peptides, are a common motif in several natural products with therapeutic properties. Included are inhibitors of mammalian cell cycle, of plasminogen activator-1 and of topoisomerase I, as well as competitive antagonists to Substance P at the neurokinin-1 receptor. Furthermore, DKPs have been shown to be useful scaffolds for rational design of several drugs (Fresno et al., 1998).

As a result of the structural similarity of DKPs to peptides, and their appearance in biological active natural products has inspired medicinal chemists to use DKPs to circumvent the limitations of peptides (Dinsmore and Beshore, 2002)
Neuropathy, which frequently is painful, is a common neurological complication of HIV infection and its treatment with nucleoside-analogue reverse transcriptase inhibitors (NRTIs). Our knowledge of HIV-associated neuropathies is based largely on studies conducted in industrialized countries, where the clade of HIV-1, the routes of virus transmission, and the demographics of the infected population differ substantially from those of developing countries, especially countries in sub-Saharan Africa. Also, whereas less neurotoxic antiretroviral drugs are available in industrialised countries, treatment regimens in developing countries almost exclusively rely on neurotoxic NRTIs as first and second-line therapy. With approximately 90% of HIV-infected individuals living in developing countries, and expanding treatment programmes that incorporate neurotoxic NRTIs in these countries, there is potential for antiretroviral toxic neuropathy to become a significant burden on the healthcare systems of developing countries.

In this presentation, I shall provide data on the prevalence of, and risk factors for, antiretroviral toxic neuropathy in Africa, with a focus on South Africa. I also shall present data from animal studies investigating the pathogenesis of antiretroviral toxic neuropathy.
IL 21  Exploitation of the power of nanotechnology to lift neuropharmaceutics to new heights

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Nanotechnology has a long and fairly unexplored history in neuropharmaceutics. Due to the small size of nanostructures (measured in nanometers), they are able to penetrate the BBB and are therefore easily imbibed within cells, allowing for efficient drug accumulation at targeted sites within the brain. The incorporation of biodegradable polymeric material in the formation of drug-loaded nanoparticles or nanotubes allow for sustained drug release at the targeted site over a period of months or even years after implantation and ideally should erode in vivo resulting in the release of a desired quantity of drug. Polymers offer a unique platform for the development nano-enabled neuro-devices for different applications over a wide range of fields ranging from biomedicine, drug delivery, membranous scaffolds, cell growth and wound healing to mention a few. The evolving technology in polymer science coupled with nanotechnology has focused on combining different polymers to produce new materials for a wide range of applications depending on the requirements placed upon a certain material. Our work focuses on the design, biometric simulation and optimization of intracranial Nano-Enabled Scaffold Devices (NESD) for the site-specific delivery of drugs as a strategy to minimize the peripheral side-effects of conventional forms of therapy for neurodegenerative disorders such as Alzheimer’s disease, Parkinson's disease, AIDS Dementia Complex, Motor Neuron Disease and Spinal Cord Injury. The NESD are modulated through biometric simulation and computational prototyping to produce crosslinked polymeric scaffolds embedded with stable drug-loaded polylipo nanoparticles optimized in accordance with statistical designs. Our results have indicated that the strategy of coupling polymeric scaffold science and nanotechnology enhanced the site-specific delivery of drugs from the NESD for various neurodegenerative disorders.
IL 22 The use of the Pheroid™ to enhance the efficacy of anti-infective agents

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Purpose:
The rapidly escalating prevalence of drug-resistant strains of Plasmodium falciparum and Mycobacterium tuberculosis, the high cost of conventional treatment regimes and the small number of available chemoprophylactic and chemotherapeutic agents have contributed to the globally increasing number of malaria and tuberculosis cases. Drug resistance results in treatment failure and rising mortality. The currently prescribed TB treatment regime suffers from potential drug interactions between rifampicin and isoniazid and possibly also between rifampicin and ethambutol. These interactions may contribute to the development of drug resistance. The studies to be discussed will address mainly the formulatory, preclinical and analytical investigation of the significant enhancement of the efficacy of existing anti-infective agents and candidate drugs by their entrapment or formulation in a drug delivery system. The delivery system of choice is a patented, fatty acid based, non-toxic self-emulsifying system, named the Pheroid™. The objective of this study was to determine whether alternative formulation strategies, such as Pheroid™ technology, can be used to alter drug bioavailability and efficacy.

Methods:
The following aspects of the drug development process will be addressed in terms of the role of drug delivery and alternative formulation strategies: in vitro efficacy, stability, in vivo pharmacokinetics and efficacy in animal models and in vitro/in vivo correlations. The antimalarials used in these studies include some of the existing drugs (artesunate, artemether, mefloquine, chloroquine) and the candidate drug artemisone.

Results:
Results from the following studies will be presented: (i) In a phase I study on human volunteers, the entrapment of rifampicin, isoniazid, ethambutol and pyrazinamide in the Pheroid™ drug delivery system led to increased plasma levels of rifampicin and isoniazid but not of ethambutol and pyrazinamide. (ii) Efficacy studies in Balb/C mice infected with Mycobacterium tuberculosis H37Rv (ATCC 27294), mice treated with the same 4 drugs entrapped in Pheroid™. (iii) The pharmacokinetics of the same 4 drugs in a parallel mice bioavailability study where rifampicin, isoniazid and ethambutol, but not pyrazinamide were significantly enhanced by entrapment, as shown by LCMS analysis of plasma (p<0.01). (iv) In an in vitro solubility model, the Pheroid™ system enhanced drug solubility and decreased particle size by an average factor of a 1000 times. (v) Bioavailability studies of chloroquine, amodiaquine, artemisone and artemisone, which show that the effect of entrapment in PheroidTM is differential and not easily predictable. (vi) Stability studies showing a possible relationship between enhanced bioavailability and stability of Pheroid™ formulations.
Sharing teaching approaches in pharmacy

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Teaching Philosophy

Teaching is fulfilling to me because I feel that I am contributing to the healthcare of our communities by playing a significant role in developing future pharmacists of which there is a recognized scarcity in South Africa. My interactions with students are also an opportunity for me to personally grow, learn and be challenged by them. My main goals as a teacher are to: 1) be recognized and appreciated by students for being knowledgeable and competent in teaching in a manner that facilitates their understanding and success; 2) to produce graduates with pharmaceutical knowledge, skills and attitude relevant to the current practice of the profession who are capable of problem solving and critical thinking and 3) to support and nurture students holistically for facilitating their success.

My main areas of teaching are Physical Pharmacy, which provides students with a physico-chemical background to the formulation of dosage forms, and Pharmaceutical Technology, which provides knowledge and skills to manufacture and evaluate dosage forms.

One of the challenges facing pharmacy education within South Africa and globally is ensuring relevance to the curriculum due to radical transformation and advances in the profession. It is therefore important for a teacher to keep abreast with the pharmacy profession and its advances to ensure that students are presented with a curriculum that will enable them to optimally meet their practice needs once qualified. I believe in being thoroughly knowledgeable, prepared and organised for lectures because this instills confidence in me during the lecture to present it in a clear, systematic and understandable manner with the most relevant and recent information to the students. Together with the timeous provision of well-designed and organised teaching material to students, this contributes significantly to facilitating student understanding, learning and success in a module.

A didactic lecture that is well organised, clear, concise and systematic, is still regarded as one of the most effective approaches to communicate a large amount of core information, such as those often encountered in the basic pharmaceutical sciences modules which I teach, and for ensuring that students acquire essential theoretical concepts. I therefore use the didactic approach because it is important to me that students have a thorough and detailed knowledge and understanding of basic scientific principles, as this underpins their ability to correctly and accurately apply their knowledge in the practice of the profession. Within Pharmacy education, the traditional didactic approach has been debated on and criticized for involving more passive learning. While I believe that the didactic approach is appropriate for me, with respect to the modules and large student numbers I teach as well as current resource constraints, it is nevertheless important for a teacher to further enhance the didactic approach with other teaching activities that promote student engagement, active learning, critical thinking and problem solving skills. They must also include opportunities for students to be able to seek and interpret rather than just recall information. These activities are important to ensure that graduates have the confidence and skills to adapt and respond to the rapid changes and information explosion in dynamic professions, such as Pharmacy, which are under constant change and innovations. It is
also important to integrate theory and practice in Pharmacy, since a sound understanding of theory is important and the practical application thereof is a key skill for professional competence. I believe that a teacher should inspire students especially in core science-based modules that may at first appear abstract to them. This is necessary to motivate and instill enthusiasm in the students, which makes learning easier, and also for them developing an appreciation for the importance of the module to their role as pharmacists. Motivated and inspired students help to develop graduates who can use this attitude to seek excellence in their professional activities.

One of the challenges currently facing higher education in South Africa is the significant drop out and exclusion rates of students, which may be attributed to, amongst other factors, students who are under-prepared due to previously disadvantaged schools, have English as a second language and have financial, health and emotional difficulties. A teacher should therefore realize that their role in ensuring academic success does not end at delivering a good lecture and providing academic support only. I therefore believe that a teacher should strive to be a nurturer and to provide an enabling and supportive environment both inside and outside of the lecture room. This is necessary for giving students hope, making them feel secure and cared for, creating a more conducive environment to learning and for personally motivating them; which are all critical for achieving academic success as well. In this way, I believe this also helps them to appreciate the power of a compassionate attitude to make a difference in the lives of future patients under their care as pharmacists.

An urgent need for developing academics for universities in Africa, including South Africa, is being emphasized in higher education due to several reasons including an ageing research and academic workforce. I therefore believe that it is important for a teacher to also be involved in capacity building initiatives for academics as they are required for training the future generation of students to support a knowledge-based economy for South Africa.
OP 01  Time-dependent sensitisation as a behavioural model for investigating the neurobiology of post-traumatic stress disorder (PTSD)

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Posttraumatic stress disorder (PTSD) may follow exposure to severe emotional trauma resulting in changes in monoamine activity as well as diverse changes in hypothalamic-pituitary adrenal (HPA) axis function, including both elevated and reduced cortisol levels. The exact underlying biology of PTSD remains a mystery. Hypocortisolemia specifically, and subsequent reduced control over monoaminergic systems, may underlie core symptoms of anxiety, hyperarousal and in particular increased fear- and aversive memory and reduced explicit memory performance. The latter changes may underlie flash-backs, re-experiencing, deficits in memory relating to the event as well as other cognitive changes. However, increased cortisol is also implicated in PTSD, while brain imaging studies indicate reduced hippocampal volume in PTSD patients that is positively correlated with cognitive deficit. This suggests the presence of neurodegenerative mechanisms, possibly involving cortisol-glutamatergic signaling, although the nature of this involvement is obscure. These drawbacks in our understanding, and that many patients suffer the burden of ineffective and/or non-optimal drug treatment, highlights the urgent need for identifying new neurobiological targets for the treatment of PTSD. To meet this need, well-validated analogous animal models of PTSD are required.

We have validated a time dependent sensitisation (TDS) stress paradigm to model the symptomology (face validity), drug treatment response (predictive validity) and neuro-anatomical and neuro-physiological characteristics (construct validity) of PTSD. By modelling the bio-behavioural response to acute trauma plus re-experience in rats, TDS stress significantly impacts on cortical/hippocampal serotonin 5HT₁A/2A receptor binding and monoamine levels, increases anxiety and aversive memory but impairs spatial memory. Moreover, considering the importance of glucocorticoids, glutamate and nitric oxide (NO) in memory as well as neurotoxicity and plasticity, TDS stress markedly attenuates HPA-axis function, while also increases excitatory hippocampal glutamate NMDA receptor-NO synthase signalling with an associated reduction in GABA. Stress increases acetylcholine release, which in turn modulates glutamate-mediated hippocampal learning and attentional processes. TDS stress significantly increases cortico-limbic muscarinic receptor binding together with a bolstering of sensory associated aversive memory. Many of the above-mentioned TDS-directed changes are sensitive to intervention with serotonergic active drugs, as well as drugs that modify HPA-axis activity. In this presentation, the above-mentioned data demonstrating the important face, predictive and construct validity of TDS stress for PTSD will be discussed. The model therefore offers a valuable platform with which to study the complex neurobiology of PTSD, and as such may provide new insight into novel drug development.
OP 02  Services for which pharmacists may levy a fee: Time it takes to dispense a prescription in different pharmacy sectors

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Purpose:
The primary aim of the study was to determine the time it takes to dispense a prescription according to the different phases and in different pharmacy sectors (community, private and public institutional pharmacies) in South Africa.

Research Methodology:
A national research project was undertaken during 2008 by the South African Pharmacy Council on the services for which a pharmacist may levy a fee. During this research project, the time it takes to dispense 6862 prescription was determined in 578 pharmacies of which 421 were community, 54 were private institutional and 103 were public institutional pharmacies.

Results and discussion:
Dispensing was divided into three phases, namely Phase I (interpretation and evaluation of the prescription), Phase II (preparation and labelling of the prescribed medicine) and Phase III (provision of information and instructions to the patient). In 84.52% of prescriptions it was possible to separate between the different phases. Phase I was performed in 97.15% of cases, Phase II in 99.38% and Phase III in 80.62%.. The weighted mean time it took to dispense a prescription in a pharmacy was 307.45 (Standard error (SE) = 5.34) seconds (slightly over 5 minutes). Phase II took the longest with 163.59 (SE = 3.86) seconds and dispensers spent only 73.41 (SE = 3.17) seconds on Phase III. In 71.60% of the cases the pharmacist was the only person involved in the dispensing of the prescription, in 17.85% of the cases the pharmacist was supported by other personnel (such as post-basic pharmacist assistants). Prescriptions dispensed only by pharmacists (71.60%) took 304.11 (SE = 5.76) seconds compared to 351.71 (SE = 12.71) seconds in cases (17.85%) where the pharmacist was supported by other personnel (such as post-basic pharmacist assistants). The weighted mean time of dispensing prescriptions (10.55%) where pharmacists were not involved was 297.02 (SE = 13.97) seconds.

Another factor that influenced the dispensing time was the pharmacy sector. The majority of these prescriptions were dispensed in community (retail) pharmacies (70.42%), followed by public institutional pharmacies (20.52%) and then private institutional pharmacies (9.06%). The weighted mean time in community pharmacies was slightly longer, 317.76 (SE = 5.91) seconds compared to 290.62 (SE = 26.17) seconds and 269.64 (SE = 13.23) seconds for private and public institutional pharmacies respectively. The weighted mean time spent on Phase III was the longest in the public institutional pharmacies (81.95, SE = 6.49 seconds). Most of the prescriptions (85.51%) were manually dispensed in public institutional pharmacies compared to computerised dispensing in community (97.47%) and private institutional pharmacies (95.66%).

Conclusion and recommendations:
The results clearly indicated that pharmacists in all sectors should be encouraged to focus on the provision of drug information and usage instructions to patients when dispensing prescriptions.
OP 02  Services for which pharmacists may levy a fee: Dispensing time of different types of prescriptions

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Purpose:
The primary aim of the study was to determine whether the type of prescription and the number of items dispensed per prescription would have an influence on the dispensing time of prescriptions in different pharmacy sectors (community, private and public institutional pharmacies) in South Africa.

Research Methodology:
A national research project was undertaken during 2008 by the South African Pharmacy Council on the services for which a pharmacist may levy a fee. During this research project, the time it takes to dispense a prescription (n = 6862) was determined in 578 pharmacies of which 421 were community, 54 were private institutional and 103 were public institutional pharmacies. Prescriptions (n = 6782) were categorised as i) First time or acute (n = 3917); ii) Repeat (n = 2343); iii) Antiretroviral-not indicated repeat or first time (n = 93); iv) First time antiretroviral (n = 83); v) Repeat antiretroviral (n = 224); vi) First time or acute and repeat prescriptions (n = 114); vii) First time and repeat and Antiretroviral prescriptions (n = 8).

Results and discussion:
The weighted mean time it took to dispense a prescription in a pharmacy was 307.45 (SE = 5.34) seconds (slightly over 5 minutes). The results revealed that 75% of the prescriptions evaluated had four and less items per prescription. The time taken depended on the number of items per prescription dispensed. The weighted mean dispensing time was 279.59 (SE = 5.14) seconds if there were ≤4 items per prescription (n = 5882) and 513.10 (Standard Error (SE) = 16.12) seconds for prescriptions with >4 items (n = 980). The weighted mean dispensing time for prescriptions in community pharmacies was slightly longer for prescription with ≤4 items per prescription (293.07, SE = 5.08 seconds) (n = 4298) and for prescriptions >4 items per prescription (562.32, SE = 22.08 seconds) (n = 457). However, the dispensing time of prescriptions in public institutional pharmacies was much shorter with 226.47 (SE = 16.40) seconds for prescriptions with ≤4 items per prescription (n = 868) and 368.58 (SE = 18.30) seconds for prescriptions >4 items per prescription (n = 531). For all three sectors the weighted time spent on Phase III was less than 80 seconds for prescriptions with ≤4 items per prescription and less than 111 seconds for prescriptions with more than 4 items per prescriptions.
The weighted mean dispensing time of first time or acute prescriptions (320.34, SE = 5.82 second) was longer than repeat prescriptions (275.29, SE = 6.20 seconds). The weighted dispensing time of first time (489.19, SE = 35.04 seconds) antiretroviral prescriptions was also longer than repeat (296.90, SE = 22.67 seconds) antiretroviral prescriptions. The weighted time spent on Phase III for first time or acute prescriptions (excluding antiretroviral prescriptions) was only 76.46 (SE = 3.07) seconds and 54.39 (SE = 2.62) seconds for repeat prescriptions

Conclusion and recommendations
Dispensing antiretroviral prescriptions took longer. The dispensing time for repeat prescriptions were less than first time or acute prescriptions independent of the pharmacy sector and number of items per prescription. Pharmacists should be encouraged to counsel patients thoroughly, also during dispensing of repeat prescriptions.
OP 03  Services for which pharmacists may levy a fee: Primary Care Drug Therapy (PCDT)

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Purpose:

Primary Care Drug Therapy (PCDT) is defined as a face-to-face consultation with a patient
where a pharmacist personally takes down a patient’s history, performs an appropriate health
examination including observations, and plans appropriate interventions/treatment, which may include
referral to another health care professional. The pharmacist has to be qualified and registered as a
PCDT pharmacist. Pharmacists who provide PCDT services have undergone an additional training
course and have obtained a permit issued in terms of Section 22A(12/15) of the Medicines and
Related Substances Act 101 of 1965. The primary aim of the study was to determine the extent of
provision of PCDT services in pharmacies in South Africa, as well as to determine the time it takes to
provide such services.

Research Methodology:

A national research project was undertaken during 2008 by the South African Pharmacy Council
on the services for which a pharmacist may levy a fee. The focus of this study is on one component
of the larger study, namely the PCDT service. A service was considered to constitute PCDT when,
firstly, the pharmacist examined a patient in order to determine a health need, and secondly, that the
consultation took place in a private consultation area. The pharmacist had to indicate to the
fieldworker that it was a PCDT service and the fieldworker had to document whether the pharmacist
providing the service had been trained in PCDT and whether he/she was in possession of a permit.

Results and discussion:

Forty-four pharmacies indicated that they provided PCDT services. All the pharmacies were
community (retail) pharmacies. A total of 104 PCDT services (cases) were measured. The PCDT
service was divided into five phases, namely a pre-consultation procedure (performed in 83.65% of
cases), history taking and anamnesis (performed in 90.38% of cases), a health examination
(performed in 82.69% of cases), pharmacological and non-pharmacological treatment (performed in
90.38% of cases) and follow-up (only performed in 43.27% of cases). Approximately 70% of the
service (the five phases) was performed by the pharmacist himself or herself. Where the pharmacist
did not perform the entire service, it was mostly the nursing practitioner who assisted the pharmacist.
The procedures mostly performed as part of the PCDT service were the dispensing of a prescription,
the measurement of blood pressure, and the administration of an intra-muscular or subcutaneous
injection. It took on average (weighted) 638.16 seconds (approximately 10.5 minutes) per PCDT
service (standard error of mean 73.70 seconds).

Conclusion and recommendations:

PCDT is an important service that community pharmacists can deliver where the need exists. It
is recommended that pharmacists be encouraged to obtain this additional qualification.
OP 03  Services for which pharmacists may levy a fee: Pharmacist Initiated Therapy (PIT)

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Background:

Pharmacist Initiated Therapy (PIT) means the supply of medicine to meet the health needs of a patient or group of patients without a prescription of a person authorised to prescribe medicines. It includes any situation where a patient comes into the pharmacy and asks for health advice or assistance and the pharmacist recommends a specific product and/or therapy together with appropriate counselling. For the purposes of this study, PIT also included cases where the patient came into the pharmacy and requested an over-the-counter (OTC) product and no counselling of the patient by the pharmacist or pharmacist’s assistant took place.

Primary aim:

The primary aim of the study was to determine the extent of provision of PIT services in pharmacies in South Africa, as well as to determine the time it takes to provide this service.

Methodology:

A national research project was undertaken during 2008 by the South African Pharmacy Council on the services for which a pharmacist may levy a fee. The focus of this study is on one component of the larger study, namely the PIT service.

Results and discussion:

A total of 369 pharmacies indicated that they provide PIT services. A total of 3 133 PIT services (cases) were measured. The majority of these services were delivered by community (retail) pharmacies (95.79%), followed by private institutional pharmacies (4.21%). The PIT service was divided into three phases, namely Phase I (pre-administration procedure), Phase II (preparation and labelling of the prescribed medicine) and Phase III (provision of information and instructions to the patient to ensure the safe and effective use of medicine). Phase I was performed in 98.21% of cases, Phase II in 97.19% of cases and Phase III in 91.67% of cases. Pharmacists delivered all three phases themselves in over 70% of the cases. They were supported by mostly post-basic pharmacist assistants in delivering this service. The weighted average time it took for a PIT service to be delivered in a pharmacy was 199.02 seconds (just under 3.5 minutes). The standard error of the mean was 5.57 seconds. The weighted average time in community pharmacies was slightly less (192.82 seconds) compared to 312.15 seconds in private institutional pharmacies. The time taken was dependent on the number of items dispensed. The weighted average time taken was 160.76 seconds if there was less or equal to one item dispensed, 220.31 seconds for more than one and equal to two items dispensed, and 327.19 seconds if more than two items were dispensed.

Conclusion and recommendations:

PIT is an important service that pharmacists deliver where the need exists. It is recommended that pharmacists be encouraged to counsel patients thoroughly when delivering a PIT service.
Purpose:
Compounding of an extemporaneous item for a specific patient is one of the services delivered in pharmacies. It refers to the compounding of any non-sterile pharmaceutical product prepared as a single item for a patient (a new product is manufactured) including the necessary documentation. The primary aim of the study was to determine the extent of provision of extemporaneous compounding services in pharmacies in South Africa, as well as to determine the time it takes to provide such service.

Research Methodology:
A national research project was undertaken during 2008 by the South African Pharmacy Council on the services for which a pharmacist may levy a fee. A total of 597 pharmacies were surveyed by fieldworkers. The focus of this study is on one component of the larger study, namely compounding of an extemporaneous item.

Results and discussion:
One hundred and fifty-eight pharmacies provided extemporaneous compounding services during the study period (79.75% were community pharmacies). This service was provided in all nine provinces. A total of 201 extemporaneous items were prepared by these 158 pharmacies (79.10% by community pharmacies). Most prescriptions (80.10%) were first-time acute prescriptions, followed by repeat prescriptions (14.43%). Compounding was divided into three phases, namely interpretation and evaluation of the prescription (Phase I), preparation and labelling of the prescribed medicine (Phase II), and provision of information and instructions to the patient (Phase III). The weighted mean time (all types of pharmacies) for Phase I was 92.84 (SE = 11.00) seconds (approximately 1.5 minutes), for Phase II was 521.85 (SE = 39.49) seconds (just less than 9 minutes), and for Phase III was 82.76 (SE = 12.17) seconds (just more than one minute). In total, it took a weighted mean time of 669.25 (SE = 39.44) seconds to compound an extemporaneous item (all three phases), that means it took approximately 11 minutes for the extemporaneous compounding of an item. The longest weighted mean time was recorded in community pharmacies (698.74 seconds), followed by 527.08 seconds in public institutional pharmacies, and only 332.02 seconds in private institutional pharmacies. The number of prescriptions in private institutional pharmacies was, however, low (only seven prescriptions). Extemporaneous compounding was mostly performed by pharmacists (in 74.62% of cases only the pharmacist was involved). Post-basic pharmacist assistants and interns were also involved to a limited extent. Of the total weighted mean time of 669.25 seconds, the pharmacist was on average responsible for 550.54 seconds of the compounding process.

Conclusion and recommendations:
It took on average 11 minutes to compound an extemporaneous item in a pharmacy. It will be valuable to investigate the type of preparations compounded and for what conditions they are mostly prepared.
OP 04  Services for which pharmacists may levy a fee: Provision of information concerning a patient’s condition / medicine

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Purpose

The provision of information to patients is a professional service which is offered as part of the scope of practice of a pharmacist, whether or not a medicine is dispensed, (GPP). The South African Pharmacy Council (SAPC) embarked on a study in 2008 to determine the time it takes to provide information to patients on the patients’ conditions in order to determine a professional fee for such services. This fee is to be included in the National Health Reference Price List (NHRPL). Provision of patient information comprises a pre-consultation process followed by the consultation. Pharmacists are required by Good Pharmacy Practice (GPP) to also keep patient records for such procedures. As yet no reasonable and reliable time has been determined for the provision of patient information. The primary aims were to determine the:

- extent to which pharmacists provide information to patients with respect to the patient’s condition and
- the time taken by pharmacists to provide such information in community and institutional pharmacies (public and private) in South Africa.

Research Methodology

A national, observational study of 680 pharmacies providing patient information was undertaken. A total of 680 pharmacies were randomly selected from the national pharmacy database (N=2200).

Results and discussion

A total of 1034 cases of information being provided in a total of 356 pharmacies were observed with a response rate of 88%. Of the pharmacies where information provision was observed, 311 (71%) were community pharmacies, 17 (21%) were private institutional pharmacies and 28 (26%) were public institutional pharmacies. The weighted mean time for providing information in pharmacies in the study population of 356 pharmacies was 217 (SE = 9.8) seconds. Among the community pharmacies a total of 933 cases were observed (n=311) with a weighted mean time of 189.99 (SE = 7.5) seconds. Among private institutional pharmacies 45 cases were observed with a weighted mean time of 504.12 (SE = 37.56) seconds; public institutional pharmacies had 56 cases with a weighted mean time of 395.36 (SE = 97.45) seconds. Community pharmacists took half the time for patient information that institutional pharmacists required, community pharmacies however, represented nearly 90 per cent of all cases observed.

Conclusion

Provision of information to patients regarding their condition and/or medication was highest among community pharmacies (71%) whereas both the institutional pharmacy sectors had similar incidences. The weighted mean time for providing information was twice as long among institutional pharmacies when compared to community pharmacies.
OP 05  Services for which pharmacists may levy a fee: Time analysis of primary screening and monitoring services in pharmacies of South Africa

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Purpose:
Pharmacists perform screening services such as blood glucose testing, blood cholesterol and/or triglycerides testing, urine analysis, blood pressure monitoring, HIV and AIDS testing with pre-and post-test counseling, pregnancy screening and peak flow measurement which is within their scope of practice. The Pharmacy Act makes provisions for the SAPC in terms of Section 35A(b)(iii) of the Pharmacy Act, 53 of 1974 as amended to make rules as to the services for which a pharmacist may levy a fee as well as guidelines for levying such a fee/s. In 2007 the SAPC published its final set of Rules relating to services for which a pharmacist may levy a fee as well as guidelines for levying such a fee or fees which linked each procedure to GPP standards. However, what became apparent was the need to identify the time taken by a pharmacist to perform such procedures. A study was therefore undertaken to inform the SAPC as to the time taken to perform such screening services with the object of assigning unit values to the procedures.

Research Methodology:
The study was conducted in 2 phases. The results obtained in Phase 1 (n=2200) were used to determine the sample for Phase 2. Stratified random sampling was used to select pharmacies from each category of pharmacy in each province. Pharmacies were categorised according to community, private institutional and public institutional pharmacies. Time analysis data collection tools were utilised to collect the data. The data collection was coordinated by the Pharmacy schools. The data were analysed using the SAS System for Windows, Release 9.1 TS Level 1M3 (SAS Institute Inc., 2003).

Results:
A total of 438 pharmacies responded. The weighted mean seconds taken to measure blood glucose was 269 (n=147) blood cholesterol and/or triglycerides 439 (n=102), urine analysis 401 (n=25), blood pressure monitoring 238 (n=366), HIV/AIDS pre-test counselling 1417.71 (n=7), HIV/AIDS testing and post-test counselling 1010.16 (n=9), pregnancy screening 427 (n=15), peak flow measurement 214 (n=24). Possible fees structure inclusive of vat (in 2009 terms) for blood glucose was R41.10, blood cholesterol and/or triglycerides R67.20, urine analysis R61.40, blood pressure monitoring R36.50, HIV/AIDS pre test counselling R282.00, HIV/AIDS testing and post-test counselling R200.90, pregnancy screening R65.40, peak flow measurement R32.80. The majority of the services were performed in community pharmacies.

Conclusion:
The fee that pharmacists can levy for procedures and services performed in a pharmacy has been presented, calculated from the time taken to perform such procedures or services.
OP 05 Services for which a pharmacist may levy a fee: Preparation of sterile products and parenteral preparations

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Purpose
Preparation of sterile products e.g. eye drops and parenteral preparations like cancer chemotherapy, total parenteral nutrition (TPN) and admixing of parenteral solutions are routine functions performed by pharmacy staff in institutional practices. To prepare these products can be very time demanding and labour intensive. The time and category of pharmacy staff involved in providing these services were unknown. The purpose of the study was to determine the time taken to prepare sterile products and parenteral preparations, as well as to determine the staff performing these services.

Research Methodology
The South African Pharmacy Council commissioned a national research project on the services for which a pharmacist may levy a fee. The research was carried out collaboratively by the Schools of Pharmacy in South Africa. The time taken to perform the different phases in preparing the sterile products was recorded. Phase 1 was the pre-compounding procedures with the performance of the compounding and the post-compounding and record keeping Phases 2 and 3. The categories of staff involved at the different stages were also noted.

Results and Discussion
The weighted mean time to prepare the six recorded sterile products in five public institutional pharmacies was 811 seconds (13.5 minutes). Sixty seven percent (549 seconds) was spent on the compounding phase. The duration of the pre and post compounding phases were 17% and 15% respectively. One product was prepared by a post-basic pharmacist assistant and the rest by either a pharmacist or pharmacist intern. The weighted mean time to prepare chemotherapy, TPN and parenteral solutions were 1105 (n=57), 805 (n=5) and 381 (n=13) seconds. Fifty four percent of the time spent to prepare chemotherapy was on Phase 2. Phases 1 and 3 took 35% and 11% respectively. Pharmacists prepared 80% of the chemotherapy dosages. TPN was only prepared in two private sector facilities. Most of the time the preparation of TPN was done by the pharmacist assistant. Sixty eight percent of the time the preparation a parenteral solution (all phases) was that of a pharmacist or pharmacist intern.

Conclusion and Recommendations
Not many sterile products and parenteral preparations except for chemotherapy are prepared regularly. Post-basic pharmacist assistants could be used more to perform these services.
OP 05 Services for which a pharmacist may levy a fee: Provision of pharmacokinetic consultation, pharmaceutical care and medicine review


Purpose
The purpose of the study was to determine the time taken to render pharmacokinetic consultations, pharmaceutical care and medicine review as well as to determine the staff performing these services.

Research Methodology
The SAPC commissioned a national research project on the services for which a pharmacist may levy a fee. The research was carried out collaboratively by the Schools of Pharmacy in South Africa. The time taken to perform the different clinical functions was recorded. Categories of staff involved at the different stages were also noted.

Results and Discussion
The weighted mean time taken for rendering pharmacokinetic consultations was 234 seconds. For pharmaceutical care and medicine review the weighted mean times were 201 and 266 seconds respectively. Only eight observations were recorded for pharmacokinetic consultations of which seven were done in private institutions. Pharmaceutical care serves were recorded in 34 cases (85% in private institutions). All 118 cases of medicine reviews were recorded in community pharmacies. All these services were rendered only by a pharmacist.

Conclusion and Recommendations
Not many clinical functions were performed and the recorded times may not be a true reflection of the time needed to perform these patient care functions. These services provide the pharmacist with the opportunity to strengthen their clinical involvement.
OP 06 Services for which a pharmacist may levy a fee: Emergency Post-Coital Contraception (EPC) and provision of a reproductive health service

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Purpose
Emergency post-coital contraception (EPC), often referred to as the “morning-after-pill”, implies the use of high-dose contraceptive pills taken within 72 hours of unprotected sexual intercourse. A community or institutional pharmacist can render a comprehensive reproductive health service only if necessary and only if supplementary training has been obtained and registered with the South African Pharmacy Council (SAPC). The aim of the study was to determine the extent of provision of EPC as a reproductive health service, as well as the time taken to render these services.

Research Methodology
A national research project on the services for which a pharmacist may levy a fee, was commissioned by the SAPC and carried out collaboratively by the Schools of Pharmacy in South Africa. Phase I (baseline study in 2007) provided data on which services are currently provided; phase II (conducted in 2008) measured activity times and cost of providing these services. This study reflects one component of Phase II of this larger study, viz. the provision of EPC and reproductive health service.

Results and Discussion
Out of a total of 438 community pharmacies sampled, 65 EPC services (in 54 pharmacies), predominantly in the Gauteng (15; 23.08%), Western Cape (13, 20%) and North West (10; 15.38%) provinces, and 63 reproductive health services (in 37 pharmacies), predominantly in the Western Cape (27; 42.86%) and Eastern Cape (11, 17.46%) provinces, were measured. One hundred per cent of EPC and 96.92% of reproductive health services were delivered in community pharmacy settings. EPC service is divided into four phases: Pre-administration procedure (performed in 89.23% of cases), provision of EPC (performed in 95.38% of cases), counselling (performed in 87.69% of cases) and documentation and record-keeping (only performed in 63.08% of cases). Reproductive health service is also divided into four phases: Pre-consultation procedure (performed in 95.24% of cases), evaluation of suitable method of contraception (performed in 87.3% of cases), administration of injectable contraceptive (performed in 57.14% of cases) and documentation and record-keeping (performed in 96.83% of cases). Over 80% of EPC was delivered by the pharmacist and approximately 80% of reproductive health service by a nurse. EPC provision took on average 254.51 sec (slightly over 4 min) with a std. error of mean 27.79 sec. Reproductive health service took on average 391.36 sec (about 6.5 min), with a std. error of the mean 53.96 sec.

Conclusion and Recommendations
Provision of EPC and reproductive health services were found to be offered in only a small proportion of community pharmacies; pharmacists should be encouraged to expand the delivery of these services.
OP 07 Services for which pharmacists may levy a fee: Intramuscular and subcutaneous injections and immunisation

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Purpose
Administration of intramuscular or subcutaneous injections and immunization is one area which encompasses the expanded role of the pharmacists. The pharmacist involvement with immunization may vary with each practice setting, with activities incorporating educating the public and other healthcare professionals, advocating paediatric immunisation, providing immunisation for international travel, screening patients at risk of preventable infections by occupation, lifestyle or underlying disease state, administering immunisation agents, recording immunisation data and using data base to generate reminders for booster doses. The purpose of the study was to determine the extent of provision of intramuscular and subcutaneous injections and immunisation services in community pharmacies and private institutional pharmacies in South Africa and the average time taken to complete the service in the sample.

Results
From the study population, 107 (1.96%) community pharmacies and private institutional pharmacies provided both intramuscular(IM)/subcutaneous(SC) injections and immunisation services. 220 IM/SC injections were administered in community pharmacies. 271 immunisation cases were recorded of which 270 were community pharmacies and 1 was a private institutional pharmacy. Western Cape and the North West Province were the leading pharmacies rendering the service at 29.55% and 20.91% respectively. Mpumalanga recorded the lowest percentage at 0.45%. Western Cape rendered 32.84% of the immunisation service was rendered. Limpopo contributed only 0.74% toward the immunisation service. Measuring of both IM/SC injection and the immunisation service was done by dividing it into 3 phases; a pre-administration procedure which was performed in 92.27% of the cases in IM/SC and 97.79% in immunisation), administration of injection (performed 100% in both IM/SC and immunisation) and documentation and record keeping (performed in 83.64% of cases in IM/SC and 95.57% in immunisation). All three phases of the service were provided by the pharmacist at least 49% of the time during IM/SC and 35% of the time in immunisation. On average the pharmacist provided the immunisation service at least 35% of the time. Administering of an IM/SC injection took a weighted mean time of 316.58 seconds (5.28 minutes) (Standard error (SE) = 28.04) seconds. To successfully complete all three phases in immunisation, the weighted mean time taken was 349.87 seconds (5.83 minutes) (SE = 28.47 seconds). Provided the pharmacy has met with the requirements to provide these services, a proposed fee of R39.70 inclusive of VAT for an IM/SC injection and R44.70 inclusive of VAT for immunisation has been estimated from this study.

Conclusion
Injection and immunisation services are underexplored as part of the pharmaceutical services. Such services can be more effectively achieved by employment of nursing staff. A further study of morbidity and mortality versus the availability of these services by pharmacists may highlight the value of this pharmacist role.
The distrust in using multi source generic medicines often stems from the anecdotal reports of clinical inferiority. Most clinicians will be able to relate to an incidence of a drug failing to achieve the clinical anticipated outcome and they therefore intrinsically distrust products with only bioequivalence data for registration purposes. “The product was not tested in the clinic” is then a common comment.

The rules governing the “quality” of the registration process of multi source generic products are globally universal and are often entrenched in legislation e.g. Act 90; Medicines and Related Substances act as well as Act 101: South Africa. This however, has also failed to establish the desired levels of trust and on the contrary, it often is the reason for distrust. The skepticism in clinical practice therefore remains rife.

Evidence from clinical trials as well as from submission documents for registration, is now emerging, supporting the quality or lack thereof of specific branded multi source generic products. Clinicians in future will be able to select their trusted product based on data not only from bioequivalence studies but also from clinical studies supporting the products clinical outcomes.
OP 09  The quantification of the Pharmacist Daily Workload (PDW) in a district hospital in the public health sector of South Africa

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Purpose:
In the community pharmacy setting a figure of 150 prescriptions per day per pharmacist has been used as a suggested maximum dispensing workload consistent with patient safety and minimising potential for error, but this formula is not easily transferred into the hospital pharmacy environment.

Prescription items dispensed by a hospital pharmacy are often more complex than those in a community pharmacy, requiring more extensive or specialised preparation, detailed counselling or additional documentation.

Some means of recognising these variations in complexity is required in determining a safe dispensing workload limit for a Hospital Pharmacist and this study will also help the managers in making a scientific decision on human resource allocation.

Methods:
A system of assigning a Relative Value Unit (RVU) to each of a range of different types of dispensing or medicine supply categories was designed. The RVU reflects the workload involved in the supply, so that a ‘normal’ or simple dispensed item has an RVU of 1, but a more complex item such as an intravenous cytotoxic drug requiring manufacture, has a RVU of 7.

By applying the RVU to the number of items dispensed in each of the supply categories, an ‘RVU adjusted items’ figure can be obtained. This figure reflects the workload involved in supplying the items and provides a more meaningful measure for determining a safe dispensing workload limit.

The equivalent ‘maximum’ of 150 dispensed items per pharmacist per day used in community pharmacy could then be applied to the hospital setting where the measure would be 150 dispensed items ‘adjusted for RVU’. The RVU for each of the types of items dispensed to individual patients were scaled to a hospital pharmacy setting and calculated based on an adjusted item scale value.

Results:
The above formula sought to calculate the number of pharmacists required for provision of these services over a regular eight hour shift in the hospital. The term Pharmacist Daily Workload (PDW) has been used as a means of equating dispensing and clinical workload in terms of what can be reasonably is performed by a pharmacist in a regular eight hour shift. The results seem to indicate a variance in the number of pharmacists/pharmacists assistant needed in the public hospital in South Africa where the study was conducted.
OP 10  Implementation of intravenous to oral antibiotic switch therapy guidelines in the general medical wards of a tertiary level hospital

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Background:
Antibiotic switch therapy describes the conversion of intravenous (IV) to oral antibiotic therapy as soon as patients are judged clinically stable, according to specified criteria, without the loss of antimicrobial potency. Switch therapy has several advantages such as reduced antibiotic expenditure, treatment costs and nursing time, early discharge from hospital with the simultaneous promotion of recovery at home and the elimination of intravenous line complications.

Methodology:
The aim of the survey was to determine whether IV to oral antibiotic switch therapy (IVOST) guidelines were employed in South African government hospitals. An IV Switch Therapy Questionnaire was sent via postal service to 74 hospitals which included all regional, provincial tertiary and national central hospitals as designated by the Hospitals and Health Facilities Management section of the National Department of Health. After a poor response of 21.6% (16; n=74), a follow-up was conducted telephonically and questionnaires were e-mailed to those pharmacists that had not responded.

Results:
After follow-up the response increased to 40.5% (30; n=74). Of all the hospitals from which a questionnaire was received, only 3.3% (1; n=30) had a written IVOST guideline, 13.3% (4; n=30) had a verbal agreement between the Pharmacy and Therapeutics Committee (PTC) and prescribers to initiate switch at the appropriate time and 83.3% (25; n=30) had no IVOST guidelines at all. Only 76.6% (23; n=30) of pharmacists commented on adherence to any antibiotic guidelines. Adherence to available guidelines was poor as 39.1% (9; n=23) of pharmacists who commented on adherence indicated that prescribers did not adhere to guidelines at all and 30.4% (7; n=23) indicated partial adherence even though 83.3% (25; n=30) of hospitals had an active PTC.

Conclusion:
From the survey it can be concluded that IVOST guidelines are not used in South African government hospitals and therefore guidelines need to be implemented.
OP 11  The Metabolic Syndrome: Estimates of prevalence in the south african private health care sector

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Purpose:
It is estimated that the metabolic syndrome affects between 10 and 30 per cent of adult populations worldwide, especially populations in developed countries or urban areas of developing countries. Furthermore, one in eight children might have three or more risk factors for the metabolic syndrome. The prevalence and impact of the metabolic syndrome on the South African population is largely unknown and needs to be determined, as the syndrome has or will soon have a significant impact on South African’s cardiovascular disease morbidity and mortality. The purpose of this study was therefore to determine the prevalence of the metabolic syndrome and its components in patients in the private health care sector of South Africa.

Methods:
A quantitative retrospective drug utilisation review was performed using dispensing records from a medicine claims database. Metabolic syndrome was defined according to the American Heart Association/National Heart, Lung and Blood Institute criteria. The study population consisted the 202 026 patients on the database who had a prescription(s) for one or more drugs, from one (or more) of the following therapeutic drug classes based on the Monthly Index of Medical Specialities or “MIMS” classification: antidiabetics (MIMS classification 19.1.1-2), antihypertensives (7.3.1-7.3.10), and hipolipidaemics (7.7.1-3). Data for a 1-year period (1 Jan. 2007 to 31 Dec. 2007) was used. Data were analysed by using the Statistical Analysis System® SAS 9.1® (SAS institute Inc., 2002-2003).

Results:
Approximately 8% (n = 15 632) of patients in the study population received only antidiabetics, compared to 45.4% (n = 91 674) of patients that received only antihypertensives and 12.6% (n = 25 512) that received only hipolipidaemics. A further 7.7% (n = 15 553) of patients received antidiabetics in combination with antihypertensives, and 1.3% (n = 2 625) of patients received antidiabetics in combination with hipolipidaemics. The majority of patients (18.5%; n = 37 408) received a combination of antihypertensives and hipolipidaemics. The metabolic syndrome (receiving medicine from all three drug classes) was present in 6.2% (n = 12 500) of patients in the study population.

Conclusion:
This study estimated the prevalence of the metabolic syndrome in the private health care sector of South Africa. Further studies are needed to quantify the economic impact of the syndrome on the scarce resources utilised by this health sector.
Prescribing medicines is the primary intervention that most doctors offer to influence their patients’ health; however, concerns have been expressed about the extent to which graduates are prepared by medical schools to assume prescribing responsibility. Both students and clinical teachers have identified a gap between workplace prescribing demands placed on newly qualified doctors and their preparation for this complex activity during undergraduate training.

This study explored the exit-level prescribing performance of final-year students in the Graduate Entry Medical Programme at the University of the Witwatersrand compared with students’ perceptions of their prescribing competence using both quantitative and qualitative research methods.

The results indicated a disparity between students’ competence and confidence. Examination marks showed that 83.6% of students were competent to prescribe according to the graduating standards of the University; however, questionnaire data revealed that 66% of students did not feel that their training had enabled them to prescribe rationally. This inconsistency was explored by analysis of the examination papers according to Bloom’s Revised and the SOLO Taxonomies.

It was concluded that students score well on questions which test recall and application of knowledge, but some do not manage questions involving evaluation. Since prescribing is a complex skill that requires evaluative competence, this may explain why, despite high examination scores, students remain insecure. Exploration of the structure of knowledge through a Bernsteinian lens revealed that curricular components including problem-based learning and horizontal integration constrain epistemic access to the structure of rational prescribing knowledge for some students.

It is recommended that rational prescribing skills should be taught as a synchronous strand within the curriculum, rather than in the current integrated mode. Learning could also be improved by innovative pedagogies associated with active learning and improved feedback.
ORAL PRESENTATIONS

**OP 13** Cardiovascular effects of (13S)-9α, 13α-epoxylabda-6β(19), 15(14)dil diactone from *L. leonurus* leaves in anaesthetized normotensive rats

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**Introduction**

Plants used in traditional medicines have served as sources of some of the drug compounds used in medicines today, and could still serve as leads for the development of new drugs to treat existing conditions. *Leonotis leonurus* is a plant commonly used in traditional medicine in South Africa for the treatment of hypertension and other cardiac problems, and aqueous and organic extracts have been shown to exhibit cardiovascular effects. The study was aimed at isolating a cardioactive compound from the plant and exploring the possible mechanisms for its effect.

**Materials and methods**

Fractionation of the organic extracts of the leaves led to the isolation of a novel diterpene, (13S)-9α, 13α-epoxylabda-6β(19), 15(14) diol diactone (EDD) whose structure was elucidated using infra red (IR), nuclear magnetic resonance (NMR), and mass spectroscopy as well as X-ray diffraction analysis. In anaesthetized normotensive male Wistar rats, EDD (0.5mg/kg – 5.0mg/kg IV) produced slight non-significant decreases in systolic pressure (SP), diastolic pressure (DP), and mean arterial pressure (MAP) with the lower (0.5mg/kg – 2.0mg/kg) doses, while significant increases in SP, DP and MAP occurred with the higher (3.0 mg/kg – 5.0 mg/kg) doses. All doses of EDD administered also produced significant decreases in heart rate (HR). In animals pre-treated with reserpine (0.5mg/kg), EDD produced significant dose-dependent decreases in MAP. EDD also abolished the increases in HR produced by dobutamine (20µg/kg) and prazosin (7mg/kg). With atenolol (2mg/kg) pre-treatment, EDD administration enhanced the decreases in HR and slight increases in MAP.

**Conclusion**

We conclude that EDD has a dual effect on the cardiovascular system; vasoconstriction possibly via release of catecholamines and a negative chronotropic effect via β<sub>1</sub>-adrenoceptor antagonism. Further studies are however required to fully determine mechanism by which EDD produces its cardiovascular effects.
**Purpose:**
The liver plays an important role in drug elimination and detoxification and liver damage may be caused by alcohol consumption, malnutrition, infection, anaemia and certain medications [1]. Despite the fact that hepatic problems are responsible for a significant number of liver transplantation and deaths recorded worldwide, available pharmacotherapeutic options are very limited. *Byrsocarpus coccineus* Schum. and Thonn. (Connaraceae) is a scrambling or climbing shrub widely dispersed across tropical West and Central Africa. Preparations of the plant are employed in Traditional African Medicine (TAM) to treat diverse ailments, including mouth and skin sores, swellings, tumours, earache, muscular and rheumatic pains, venereal diseases, pile, dysentery, and jaundice [2]. In this study, the hepatoprotective effect of the leaf aqueous extract of *Byrsocarpus coccineus* (BCLAE) was investigated based on its use in TAM for the treatment of jaundice and results obtained from previous toxicological evaluation which suggest that the plant extract possibly possess hepatoprotective activity.

**Method:**
The hepatoprotective effect of BCLAE was investigated using the carbon tetrachloride (CCl₄) induced hepatotoxicity model in rats [1]. Rats were divided into 7 groups of 5 rats each. Treatment was then carried out as follows: Group I (control): 20% tween 20 solution v/v 10 ml/kg p.o.; Group II: CCl₄ (1:1 in olive oil) – 0.7 ml/kg i.p.; Group III: BCLAE 1000 mg/kg p.o.; Group IV – VI: BCLAE 200, 400, and 1000 mg/kg + CCl₄; Group VII (standard): livolin 20 mg/kg + CCl₄. Carbon tetrachloride was given on alternate days for a period of 7 days while control, test, and standard agents were administered for 7 successive days. On the seventh day, the animals were sacrificed by cervical dislocation and blood was withdrawn by cardiac puncture. The levels of serum biochemical parameters were determined using Roche and Cobas commercial kits and Roche/Hitachi analyzer.

**Results:**
Carbon tetrachloride increased ALT, AST, ALP, total cholesterol, and total bilirubin levels but decreased albumin and total protein levels. However, effects were only significant ($P < 0.05$) in respect of ALT (liver specific enzyme), AST, and total protein. BCLAE (1000 mg/kg) alone produced effects not significantly different from control for ALT, AST, and total protein but its effect was significantly different for total cholesterol (↑) and albumin (↑). Challenged and compared with CCl₄, BCLAE (200 – 1000 mg/kg) dose dependently and significantly ($P < 0.05$) decreased ALT, AST, and ALP levels with peak effect produced at the highest dose. Conversely, BCLAE (1000 mg/kg) produced significant increases in albumin and total protein levels. The standard drug (livolin 20 mg/kg) produced significant increases in albumin and total protein levels. The standard drug (livolin 20 mg/kg) produced significant ($P < 0.05$) effects, challenged with CCl₄, in respect of ALT (↓), albumin (↑), and total protein (↑). In general, the effects of the standard drug were comparable and not significantly different from those of BCLAE.

**Conclusion:**
The results obtained in this study suggest that the leaf aqueous extract of *Byrsocarpus coccineus* possess hepatoprotective effect. Further work is on going to determine the effect of BCLAE on antioxidant enzymes and to observe the histopathological presentation of liver samples.
OP 15  The antimalarial activity and toxicity profile of ten South African Commiphora species

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Purpose:
The emergence of drug-resistant strains of Plasmodium falciparum has resulted in an urgent need to develop new antimalarial chemotherapeutic agents. Phytochemicals have led the way in drug development, with quinine and artemisinin being used in the treatment of malaria infections in sub-Saharan Africa. Traditionally, Commiphora (Burseraceae) species, commonly known as ‘corkwood or kanniedood’, have been used in the treatment of fever, infections and malaria. In this first report on the antiplasmodial activity of South African Commiphora species, ten species were investigated along with their red blood cell cytotoxic properties. In addition, the concurrent administration of standard antimalarial agents and traditional medicines has been investigated to foresee an interaction that could compromise the treatment regimen of the patient and possible therapeutic failure.

Methods:

Extract preparation: Aerial parts were collected from natural populations in the Limpopo Province (South Africa) and the leaves and stems extracted with chloroform:methanol (1:1). Antimalarial activity: The 20 extracts were screened for in vitro antimalarial activity against the chloroquine-resistant (FCR-3) strain of Plasmodium falciparum (0.5% parasitaemia and 1% haematocrit). The incorporation of tritiated hypoxanthine was used as an indicator of parasite growth and the concentration required to inhibit 50% parasite growth (IC50 value) calculated from log sigmoid dose response curves. Quinine and primaquine were used as the positive controls.

Combination studies: The more active extracts were combined in varying concentration ratios with quinine or primaquine. Isobolograms were constructed to assess whether they interacted in a synergistic, antagonistic or additive manner.

Haemolytic activity: The extracts were incubated with a 1% haematocrit of human erythrocytes for 48 hours at 37°C and the absorbance read at 412nm. The haemolysis was expressed as a percentage of the positive control, 0.2% (v/v) Triton-X100.

Results:

Of the twenty extracts, C. marlothii (leaf and stems), C. pyracanthoides (leaf and stem) and C. africana (leaves) were the most potent in inhibiting parasite growth with IC50 values less than 10μg/ml. At 500μg/ml, C. mollis (stem), C. pyracanthoides (stem and leaf) and C. neglecta (leaf) caused more than 50% haemolysis; but at the concentrations required to inhibit 50% parasite growth, there was no haemolytic activity of the extracts. C. Africana (leaf and stem) combined with quinine or primaquine potentiated each others inhibitory actions as observed by a synergistic interaction. Thus, the haemolytic properties of certain South African Commiphora species did not contribute to their promising antimalarial activity. Caution should be taken when combining Commiphora with primaquine in the treatment of P. vivax. When used concurrently with quinine, the combination possesses therapeutic benefits in treating P. falciparum infections.
Purpose:

Hoodia gordonii (Masson) Sweet ex Decne. contains the perceived active ingredient P57, which is used in popular weightloss products that are highly susceptible to adulteration due to increased public demand and limited availability of the raw material. A rapid and simple method to authenticate raw material and products as well as quantification of P57 is desirable for the quality control of H. gordonii-containing products.

Methods:

High performance thin layer chromatography (HPTLC) analysis was carried out on silica gel plates using toluene:chloroform:ethanol (40:40:12.5, v/v/v) as the mobile phase. Liebermann-Burchard reagent was used as derivatising agent and the plates were viewed under UV-light at 365 nm. Fourier transform near infrared (FT-NIR) spectroscopy combined with chemometric techniques were used to attempt the quantification of P57 in raw plant material. The concentration of P57 (a triterpene glycoside) in 146 raw plant material samples was determined with liquid chromatography coupled to mass spectrometry (LC-MS) and used to develop a calibration model based on the partial least square projections to latent structures (PLS) and orthogonal projections to latent structures (OPLS) regression algorithms. The performance of each calibration model was evaluated according to the root mean square error of prediction (RMSEP) and correlation coefficient.

Results:

The HPTLC system produced good separation of the complex plant constituent mixture including that of P57 which was confirmed by LC-MS after preparative thin layer chromatography (TLC). The OPLS model with 2nd derivative pre-processing predicted P57 content based on the FT-NIR spectra with the best accuracy and a correlation coefficient ($R^2$) value of 0.9064 and the lowest RMSEP of 0.04%.

Conclusions:

The HPTLC method was applied successfully to develop a chemical fingerprint for authentication and for confirmation of the presence of P57 in H. gordonii raw material and products. FT-NIR spectroscopy can be used to rapidly quantify P57 in H. gordonii raw material with high accuracy.
Several psychiatric disorders, including depression, have been associated with increased oxidative stress. In recent years dysfunctional neuroplasticity has also been demonstrated in the neurobiology of major depression, while improved neuroplasticity has been postulated to play a role in antidepressant action. Amongst many other neurodegenerative mechanisms, suppressed neuroplasticity may render the brain more vulnerable to oxidative stress. The latest evidence in support of a role for oxidative stress in depression will be discussed.

Ozone (O₃) is a pro-oxidant and environmental pollutant known to have central effects. Being a highly reactive molecule, ozone is known to form reactive oxygen species (ROS) upon contact with biological membranes, which are ultimately responsible for its systemic oxidative effects. While ozone is used for various industrial applications, as well as for medical treatments in alternative medicine, most pre-clinical studies, particularly at biochemical level, have been done in plants, with very limited data in animals. We set up a protocol in our laboratory to facilitate acute and chronic exposure of rats to ozone by inhalation. Using this technique, we have studied the effect of ozone inhalation in the presence and absence of a known antidepressant, imipramine. The resulting effects on immobility in the forced-swim test (FST) and on markers of neuronal oxidative stress in various regions of the rat brain were then assessed. The protocol for this \textit{in vivo} ozone exposure technique will be discussed.

The proof of concept regarding this newly introduced ozone exposure technique as a model of oxidative stress in rat brain will be presented. Supported by data from our laboratory, we will demonstrate that acute and chronic exposure to ozone increases oxidative stress and evidence for associated oxidative tissue damage in the rat brain, as well as inducing notable changes in the behavioural response to antidepressants. These studies will also demonstrate that the response to ozone exposure in rats per inhalation may differ in a concentration-dependent manner with respect to acute versus chronic administration. Finally, we will argue that controlled ozone inhalation represents a viable model to investigate the mechanisms of oxidative stress in the rodent brain and how these may be countered. Moreover, it provides a means of investigating the action of antidepressants on brain redox status. Since oxidative stress has become increasingly evident in a number of neuropsychiatric disorders, the model may prove useful in similar studies in other animal models validated for these illnesses.
OP 18  Acute, but not repeated, administration of fibroblast-stimulating lipopeptide-1 impairs short-term spatial learning in rats

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Purpose:
Immune stimulation, such as occurs during infection, induces febrile, behavioural and impaired cognitive responses. Since tolerance appears not to develop in either the fever or the anorexia caused by Gram-positive pyrogens, recurrent community-acquired infections, commonly caused by Mycoplasma or other Gram-positive organisms, may have chronic deleterious effects on growth and cognition. We investigated the acute and chronic effects of the novel synthetic lipopeptide fibroblast-stimulating lipopeptide-1 (FSL-1), derived from Mycoplasma salivarium, on fever and sickness responses, especially on learning and memory in rats.

Methods:
Male Sprague-Dawley rats received randomly one acute or three repeated intraperitoneal administrations of either phosphate-buffered saline (PBS; 1ml.kg⁻¹) or FSL-1 (500µg.kg⁻¹ in PBS). Based on pilot experiments, repeated injections were spaced 10d apart, to avoid tolerance. All injections were administered at 16:00. Temperature and activity sensitive radiotransmitters, implanted intra-abdominally, continuously measured core body temperature and cage activity. Body mass and food intake were measured daily. Spatial learning and memory were assessed in the Morris water maze 16h after the acute administration of either FSL-1 or PBS, or 10d after the third injection of either FSL-1 or PBS. Cued tests, where the platform is clearly visible above the water, were conducted before and after FSL-1 or PBS treatment, to assess visual and physical abilities as well as motivation. Learning was assessed across four days of training (four trials per day) with the platform submerged. Memory was tested on day five during a probe trial, with the platform removed from the water. Latency, distance travelled and swim speed were our performance indices in the maze.

Results:
Rats receiving acute or repeated administration of FSL-1 had a significant increase in body temperature and decrease in cage activity, food intake and body mass following each administration, compared with rats receiving PBS. There were no significant differences in the magnitude and duration of the fever, lethargy and anorexia induced by the three repeated administrations of FSL-1. Acute administration of FSL-1 impaired acquisition of the task (longer latencies and distance travelled) on the first day of training in the maze. Despite significantly reduced cage activity following acute FSL-1 administration, there were no significant differences in swim speed between the groups, indicating that FSL-1 did not impair motivation or sensorimotor abilities. Furthermore, there were no significant differences between the groups during the probe trial testing memory. Rats tested 10d after the last of three repeated injections of FSL-1 showed normal performance in the maze. Our results show that both acute and repeated administration of FSL-1 induced fever, lethargy and anorexia, and that acute administration of FSL-1 impaired short-term learning but had no effect on memory. Repeated administration of FSL-1, simulating recurrent infection, did not leave residual impairment of learning or memory after the fever had resolved.
OP 19  Nutritional supplement, trends, perceptions and product use, (including South African participants at the Olympic and Paralympic Games in Beijing 2008)

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Purpose:
Nutritional supplement products are used widely to promote health and physical performance. It is estimated that the dietary supplement industry generates annual sales in excess of US $10 billion, and up to $800 million of this is spent on “sports supplements”. In the context of South Africa, the local turnover for nutritional supplements was estimated at R1.5 billion per year (HPA survey 1998-2000), with the industry continuing to grow. In many cases the efficacy of these supplements are not known. There is concern that some of the supplements exert their effect as a result of contaminants such as steroids, or other banned ergogenic substances which may be present. Athletes, may inadvertently ingest the banned substance(s) because package labels do not indicate that banned substances may be present. Of equal concern should be the potential negative health ramifications of the broader general public, who may consume nutritional supplements, on an ongoing basis. In South Africa the content and purity of supplements is not regulated but rather is left to the manufacturer to take responsibility for the accuracy of the reported contents. This problem is compounded by the lack of data on the consumer trends, particularly at the elite level of sports performers. Therefore the aim of this study was to determine usage patterns of nutritional supplements by participants, including elite athletes competing at the Olympic and Paralympic Games. These consumer trends would assist with the planning of subsequent research aligned to policy development.

Methods:
Subjects: Were gained from various sport training venues, including participants who formed part of South African Team to the Beijing 2008, Olympic and Paralympic Games, in China. The questionnaires were administered in similar classroom setup, and returned, by the personal that were entrusted with the process.

Self-administered questionnaire: Comprised of 44 questions, which included, general information of participants, health/wellness status, supplement use/none use, perceptions, information sources and knowledge, and aspects marketing and labelling related.

Human ethics and informed consent: The research was approved by the Faculty of Health Science Research Ethics Committee of the University of Cape Town, (Reference REC REF 275/2008). Informed consent of the subjects was a requirement to participate in study.

Descriptive statistics- Windows-based Microsoft ® Excel 97 SR-2 (Excel © 1985-1997 Microsoft Corporation), was used to capture completed returned questionnaire data, and for doing the necessary descriptive statistical analysis and graphic representations.

Results
Returned questionnaires - A total of 128 returned questionnaires were received and processed (Olympics and Paralympics = 65, Other = 63).

Health/wellness: 53% of participants indicated that their health (wellness) was “very good”, and 42% indicated the quality of their diet was “good”.

Supplement Use/Non-Use: 41% of the respondents indicated that supplements may contain banned substances, and 31% indicated their non-use of supplements was the potential for negative side effects.

Perceptions, information sources and knowledge: 40% of respondents gained most of their information on supplements from fellow athletes.

Marketing and labelling: 66% of the respondents indicated they looked at the ingredients on the labels/information of the supplement products.

Conclusion
The research has shown the need for continuous education and scientific information provision to the consumer, as well as the ongoing monitoring and evaluation of nutritional supplements.
ORAL PRESENTATIONS

OP 20  A mixture model showing bimodal rifampicin absorption and clearance: Do drug transporter polymorphisms play a role?

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Purpose:
Low antituberculosis drug concentrations may predispose to the development of drug resistance, treatment failure and tuberculosis (TB) relapse. Rifampicin (RIF) is a key component of contemporary antituberculosis regimens. The absorption of RIF is particularly variable and patients with poor RIF absorption achieve low drug plasma concentrations. RIF is a known substrate of p-glycoprotein (p-gp), which affects its absorption and biliary excretion. We describe the pharmacokinetics (PK) of RIF in patients during the intensive phase of TB treatment and investigate possible causes of interindividual variability in drug concentrations.

Methods:
Participants: The study was approved by the University of Cape Town Research Ethics Committee. 79 patients on first line TB drugs were enrolled after voluntary informed consent.
PK sampling: Sparse sampling methods were used. 4-5 blood samples per participant were collected at steady state over a period of 8 hours.
Drug analysis: RIF was assayed using LC-MS/MS methods published in literature which were optimized and validated in our pharmacology laboratory.
Population PK modeling: Non-linear mixed effects modeling with NONMEM VI was used for population PK analysis. A transit absorption compartment model described variable absorption in a one compartment model. Log-normal distributions were assumed for the parameters. Covariates i.e. weight (W), body surface area (BSA), HIV status, age, and sex were investigated in a stepwise fashion to determine if they influenced clearance (Cl/F), volume of distribution (Vd/F), mean transit time (MTT) or the absorption rate constant, Ka.

Results:
A bimodal population distribution for RIF absorption and clearance was observed. A mixture model identified two subpopulations. One group of patients (65%) had an average MTT of 0.86h and a Cl/F of 8L/h, thus having an area-under-the-curve (AUC) of 66mg.h/L. The other subpopulation (35%) had an average MTT of 3.17h and a Cl/F of 12.4L/h, with an AUC equal to 42.5mg.h/L therefore being at higher risk of failing treatment. This difference was statistically significant (p<0.05). No significant bimodality was detected in other model parameters. BSA and W were found to be positively correlated with Cl/F, whilst age was negatively correlated. Females had a 20% lower Vd/F than males. HIV status did not significantly affect RIF PK in our population. Interindividual variability (as CV%) in Cl/F, Vd/F, Ka and MTT was approximately 36%, 30%, 92% and 44% respectively.

Discussion:
Polymorphisms in the gene encoding p-gp (ABCB1; MDR1) lead to protein of variable amounts and activity and can possibly explain the bimodality in Cl/F and absorption seen in our study.
Furthermore, hepatocellular uptake of RIF is mediated by the SLCO1B1 (OATP-C) transporter, which is also polymorphic. Apart from SNPs in ABCB1 having an impact on RIF concentrations, polymorphisms of the pregnane (steroid) X receptor (PXR; NR1I2) and the constitutive androstane receptor (CAR) which regulate the expression of ABCB1 transcription can indirectly impact on drug concentrations. Thus our findings need confirmation by genotyping for polymorphisms that affect p-gp expression and activity.
This information will be useful in early identification of patients who may need higher RIF doses.

Acknowledgements:
We are greatly indebted to the DELFT study team for recruiting participants and data collection.
Background

Contemporary chemotherapy of disseminated cancer is often thwarted by dose related systemic toxicity and multidrug resistance (MDR). Owing to a unique multi-mechanistic action, Riminophenazines are capable of circumventing both classical (transporter mediated) and non-classical drug resistance mechanisms. Considering that combination chemotherapy is standard practise, the vision directing R&D efforts is that Riminophenazines could speculatively be included within any and all standard chemotherapeutic regimes.

The strategic intent is to attain gains in both pharmacodynamic and pharmacokinetic specificity for cancer cells. Tactically, this is to be achieved through the use of in vitro optimised, synergistic fixed-ratio drug combinations (FRDC) encapsulated within tumour targeting, nanoparticulate drug delivery systems (NDDS). The intent behind this project is more than merely academic - efforts are focused on progressing towards an approved, marketable product. As such, the development strategy has been devised with due consideration to appropriate international regulatory guidelines.

Methods

The drug interactions of a range of FRDC comprising either of the two lead Riminophenazines (B663 and B4125) in combination with either Etoposide, Paclitaxel or Vinblastine has been evaluated in vitro at various dose levels using a Pgp expressing neoplastic cell line (COLO320DM). Median effect analysis (conforming to the law of mass action) was performed where the measure of synergy is defined by combination indexes (CI) at particular fraction affect ($f_a$) levels. CI=1, CI<1, CI>1 indicate additive, synergistic and antagonistic drug interactions respectively. Dose reduction indexes (DRI) were also generated.

Results and Discussion

The results obtained are very promising and demonstrate that either of the Riminophenazines could provide clinical benefit if used in optimized combinations with any of the tested standard chemotherapeutics. B4125-Vinblastine combinations were particularly impressive as all of the tested fixed-ratio’s (50:1, 25:1, 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:10, 1:25, 1:50) demonstrated synergistic activity (CI<1) at all $f_a$ levels. Owing to inhibition of Pgp as well as their direct antiproliferative activity, Riminophenazines afford large reductions in the dose of standard chemotherapeutics required to incur a particular $f_a$ level.

Progress

NDDS represent the enabling technology that allows synergistic FRDC to be translated into in vivo applications through maintaining an optimized ratio (irrespective of the inherent pharmacokinetic differences between drugs) and through cancer specific delivery. Currently, a novel NDDS that encapsulates a synergistic FRDC is under development.
Background
The concept of informed consent is central to present-day medical ethics. It indicates the right of individuals over their health and health care. This right is enshrined in legal and ethical documents throughout the world. It means that the doctor-centered approach has given way to the patient-centered approach. It signifies patient autonomy and self-determination. The right is accompanied by responsibility.
A mentally competent adult has the right to give or withhold consent to any diagnostic procedure or therapy. Consent must be legally valid and informed. A patient has the right to the information necessary to make his/her decision. The practitioner has the duty to inform the patient in words that he/she will understand. He is obliged to warn the patient about any material risk inherent in the proposed procedure. The yardstick is that of the reasonable man.
A necessary requirement for informed consent is good communication between practitioner and patient. Explanations must be given in simple language. The patient must understand the significance of treatment options including the advantages and disadvantages of each. A major obstacle may be differences in language and culture. Competent and informed patients have the right to refuse a certain treatment even if this means disability or death.
Research involving human participants
This type of research requires review and approval by an Ethics Committee. The Declaration of Helsinki of 1964, itself based on the Nuremberg Code of 1947, is a summary of research ethics. According to this document research in humans must comply with the following general requirements:
Scientific merit
Social value
Risks and benefits
Informed consent
Confidentiality
Individuals must be free to decide whether to become research participants. Coercion or manipulation is a violation of the right to self-determination. Information must be given in a comprehensible fashion. This involves a careful explanation of the project and all that participation in it will mean to the person involved including aim, method, anticipated benefits and potential discomfort and harm. Informed consent should be demonstrated by the participants signing a consent form. In it subjects are informed that they are free to withdraw their consent at any time, even after the project has begun.
Effect of bulk and fat content meals on the bioavailability of a single 900mg dose rifapentine in healthy male volunteers

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Purpose:
Rifapentine (RPT) and its primary metabolite, 25-desacetyl rifapentine (25-DRIF), are active against mycobacterium tuberculosis. The objectives of this study was to describe the population pharmacokinetics (PK) of RPT and 25-DRIF in the fasting and fed states (4 meals with different fat and bulk content).

Methods:
35 male healthy volunteers were enrolled in an open-label, randomized, sequential, five-way crossover-design study. Participants were randomised to meal sequences and received a single 900mg dose of RPT with either of the five breakfasts: high fat (meal A); bulky and low fat (meal B); bulky and high fat (meal C); Soup and low fat (meal D); 200mls of water (meal E). Serial blood samples, collected during 72h PK profiles (separated by 14 days), were analysed for RPT and 25-DRIF using a validated high-performance liquid chromatography method. Concentration-time data were analysed by nonlinear mixed-effect modeling using NONMEM. Steady-state concentrations following multiple dosing regimens were simulated.

Results:
One-compartment disposition models adequately described both the parent and the metabolite data. Drug absorption and metabolite formation were described using transit compartment models. Compared with the fasting state (meal E), the RPT mean absorption transit time increased 14%, 22%, 39%, and 20% for meals A, B, C, D, respectively. Likewise, the RPT absorption rate constant (ka) increased 21%, 26% and 81% for meals B, C, D, respectively. Treatment A lowered RPT ka by 24%. Meals A, B, C, D resulted in 90%, 32%, 46%, and 48% increase in RPT bioavailability, respectively. Similarly, these effects followed the same trend for 25-DRIF formation and subsequent exposure.

Conclusion:
We developed a model to describe the population PK of RPT and 25-DRIF simultaneously. All meals affected the rate and extent of RPT absorption. The high fat meal had the greatest effect on RPT bioavailability. Simulations of multiple-dose regimens confirmed the meal effects at steady-state. In patients with suspected sub- or supratherapeutic RPT concentrations, meal behavior should be investigated and optimized.
OP 24  The effect of food extracts on P-glycoprotein mediated cimetidine efflux

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Background:
P-glycoprotein (P-gp) is a 170-kDa membrane-bound transporter protein, which has been implicated as a primary cause of multidrug-resistance in tumours. It is also localized in the apical domain of normal epithelial cells and actively pumps substrates from within the cell back into the intestinal lumen, which significantly reduces the bioavailability of some drugs. Many drugs, herbs and food substances inhibit or stimulate P-gp, which may influence the absorption of co-administered drugs. For example, grapefruit juice increases the bioavailability of cyclosporine through inhibition of the function of P-gp and CYP3A4 enzyme. This study aims to determine whether selected materials from natural origin influence drug absorption via inhibition of P-gp.

Methods:
Extraction of plant materials: Extracts of different materials of natural origin, including fruits and aerial parts of plants were obtained by various extraction techniques such as blending of the fruit pulp and preparation of infusions, which were then freeze-dried and packaged in opaque and moisture-free containers.

In vitro transport experiments: Transport of cimetidine across Caco-2 cell monolayers was investigated both in the apical-to-basolateral and basolateral-to-apical directions in the absence (negative control) and presence of the extracts or verapamil (positive control). The apparent permeability coefficient ($P_{app}$) values were calculated for cimetidine and statistically analysed (ANOVA, single factor) with Microsoft Excel® (Redmond, USA). Extracts that significantly inhibited the P-gp efflux of cimetidine were fractionated by column chromatographic techniques and the effect of each fraction on transport of cimetidine in both directions across Caco-2 cell monolayers was determined.

Results:
Sclerocarya birrea (marula) and Psidium guajava (guava) extracts inhibited P-gp efflux of cimetidine across Caco-2 cell monolayers, while Beta vulgaris (beetroot) enhanced cimetidine transport but seemingly not by inhibiting P-gp efflux. Some of the fractional components of S. birrea and P. guajava increased the apparent permeability coefficient values of cimetidine statistically significantly ($p < 0.5$), indicating these plants have specific phytoconstituents that act as P-gp inhibitors. No statistically significant change in the apical-to-basolateral transport of cimetidine was observed for the crude extracts of Fragaria ananassa (strawberry), Prunus persica (peach), Dovyalis caffra (kei-apple), Daucus carota (carrot), Aspalathus linearis (rooibos tea) and Prunus domestica (plum). However, A. linearis and D. carota showed induction of P-gp with increased cimetidine transport in the basolateral-to-apical direction.

Conclusion:
Certain constituents of S. birrea and P. guajava act as P-gp inhibitors and thereby show potential to increase the transport of certain drugs across the intestinal epithelium, while B. vulgaris enhance drug transport via another mechanism.
Anxiolytic and Muscle Relaxant Activity of Aqueous Extract of *Ipomoea batatas*

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**Purpose:**

*Ipomoea batatas* (Linn.) Lam. belongs to the family Convolvulaceae. It originated from Central and South America where it was cultivated by 2500 B.C and later introduced to the subtropical regions and became an important food crop commonly called sweet potato. It is useful as food and medicinally in the management of diarrhoea, fever, nausea, stomach distress among others. This study aims at determining the anxiolytic and muscle relaxant potential of the whole plant based on the effects observed in routine pharmacological studies carried out in our laboratory.

**Methods:**

The anxiolytic effect of aqueous extract of *Ipomoea batatas* (AIB) whole plant was investigated using the elevated plus maze and Y maze tests (Adeyemi et al., 2006; Dhara et al., 2002). Its effect on the exploratory activity of mice was also carried out with the head dip test using the whole board method. The muscle relaxant activity of the extract was studied using the inclined screen, chain climbing, traction and the chimney tests (Adeyemi et al., 2006). Acute toxicity study for the oral route of administration was carried out using the method of Miller and Tainter (1944). Preliminary phytochemical screening was also carried out.

**Results:**

AIB (100 - 400 mg/kg) produced a dose dependent and significant ($P < 0.05$) anxiolytic effect in the elevated plus maze, Y maze, and head dip tests. This effect was comparable to that of diazepam (1mg/kg). A significant ($P < 0.05$) and dose dependent muscle relaxant activity was also exhibited in the inclined screen and climbing tests by AIB (100 – 400 mg/kg). Preliminary phytochemical screening showed the presence of saponins, phlobatanins and tannins in the extract. The extract showed no lethal effect or morbidity at oral dose of up to 10g/kg for acute toxicity test. The results obtained in this study showed that AIB has sedative and muscle relaxant effects which can be further explored for clinical use.

**Acknowledgements:**

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OP 26  Pharmacokinetic evaluation of antimalarial compounds in mice

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Introduction
Malaria is one of the most serious infectious diseases of the tropics, and has a significant impact on health and economic systems worldwide. The malaria situation is deteriorating faster at present than at any time in the past, due to drug and insecticide resistance and environmental and social changes. Malaria control relies strongly on drug treatment, but many of the first-line treatments are failing due to the parasites ability to develop resistance against drug action over time. There is an urgent need to discover new classes of antimalarial compounds, which use different mechanisms of action against the *Plasmodium* parasites, and to develop some of these compounds into antimalarial drugs.

Pharmacokinetic evaluation of active *in vitro* compounds is one of the most important secondary screening procedures in drug development and should also be included on a larger scale in preliminary antimalarial screening programmes. An understanding of the absorption, distribution, metabolism and excretion of a test compound is essential in order to be able to design effective treatment regimens. The *P. berghei* mouse model is the most frequently used animal model for efficacy testing, but is rather a challenging model for pharmacokinetic and metabolic studies, because of the animal's small blood volume.

Methodology
Sensitive and selective LC/MS/MS assays were developed to study clinically used antimalarial drugs and pre-clinical hit compounds in mouse plasma or whole blood. Pharmacokinetic models were constructed with WinNonlin® software.

Results
Pharmacokinetic models in mice were developed for the following antimalarials:

Clinically used antimalarial drugs (formulation and comparison application): chloroquine, amodiaquine, mefloquine, artemether/DHA and artesunate/DHA

Drugs in clinical development (formulation application): artemisone and artemiside

Pre-clinical hit compounds (drug resistance application): phenylequine and a chalcone-quinoline hybrid.
YSP 01 Effect of isolation rearing on schizophrenia-like behaviors and cortico-striatal parameters of oxidative stress in rats, and response to clozapine

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Purpose:
Psychotic (positive) symptoms are the most distinctive feature of schizophrenia, although negative symptoms such as emotional flattening, social withdrawal and cognitive disturbances are the most treatment resistant manifestation of the illness. Schizophrenia is a progressive degenerative illness that has been causally linked to environmental and neurodevelopmental factors, as well as dysfunctional redox balance. Animal models are useful in studying the molecular underpinnings for a neuropsychiatric illness. Post weaning social isolation rearing (SIR) in rats has been proposed to model the neurodevelopmental aspects of schizophrenia. We validated the SIR model with respect to effects on sensorimotor gating and social interaction, deficits of which are core negative symptoms of schizophrenia. Furthermore, cortico-striatal levels of oxidative stress were determined, as well as overall response to treatment with the atypical antipsychotic, clozapine.

Methods:
Male Sprague-Dawley (SD) rats (10 rats/group) were used. In a non-treatment arm, two groups of rats were randomly separated at weaning and exposed to either 8 weeks SIR or 8 weeks social housing. In the treatment arm, SIR rats received saline or clozapine (5mg/kg i.p.) for the last 11 days of SIR. The remaining two social-housed groups received either saline of clozapine. At 8 weeks, mean startle amplitude (at 120dB) and percentage prepulse inhibition (%PPI) of the acoustic startle (AS) reflex (at 72, 76, 80 and 86dB prepulse) were accessed and analysed using mixed statistical models and post hoc tests. Social interactive and self-directed behaviors were accessed using the open field test (OFT) and analysed using 2-way ANOVA plus post hoc tests. In a parallel neurochemical study, animals were sacrificed and superoxide dismutase activity, reduced/oxidised glutathione and lipid peroxidation were analysed in the frontal cortex and striatum. A mixed linear models analysis with repeated measures and Bonferonni’s post hoc tests was applied.

Results:
%PPI was significantly reduced in SIR versus social housed rats, together with deficits in various social interactive behaviours and increased locomotor activity and self-grooming. Superoxide dismutase activity and reduced/oxidized glutathione were significantly decreased and lipid peroxidation significantly increased in isolates versus social housed rats. Following treatment, %PPI in isolates was significantly elevated by clozapine versus saline treatment. %PPI was unaltered in group-housed animals receiving either treatment. Social interactive behaviors were significantly impaired in isolates receiving saline, while locomotor activity and self-grooming were increased. SIR rats receiving only saline showed altered redox state compared to all the other treatment groups. Clozapine reversed deficits in %PPI, aberrant social behaviours and altered redox state in SIR rats, with limited effects in group housed controls. SIR thus significantly disrupts sensorimotor gating and social behaviours in male SD rats, while there is significant evidence for altered redox state in both the frontal cortex and striatum of these animals. Importantly, both altered behaviour and redox state were reversed by sub-chronic clozapine treatment. SIR is therefore a useful, non-lesion and non-pharmacological neurodevelopmental animal model of schizophrenia that presents with robust face, predictive and possibly construct validity for schizophrenia.
YSP 02 The effect of selected South African plant extracts on haemolysis and coagulation

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The use of herbal preparations for staunching blood flow and reducing the risk of formation of vascular thrombosis is common worldwide. In this study, aqueous and methanolic extracts of 12 plants known to be used in the treatment of blood associated complaints were investigated to determine their effects on red cell haemolysis and coagulation. The extent of haemolysis was determined spectrophotometrically. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) as indicators of coagulation were determined using a coagulometer. All of the plant extracts tested had a significant effect on coagulation time, prolonging the aPTT. Only *Cassia petersiana*, prolonged the PT compared to the control. As all of the herbal extracts tested had a delaying effect on coagulation, patients using herbal therapies should be cautioned to stop their use before surgery.
YSP 03 Novel nucleoside analogues in the induction of colonic cell differentiation and apoptosis

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Introduction:
Colon cancer is the third most common cancer worldwide and the second most common in the western world. More than 40% of colon cancer sufferers develop metastases and chemotherapy is often used alone or in combination with radiotherapy as adjunctive therapy for the progressed disease.

Over the past few decades, a major effort has been made to develop anti-cancer agents through both empiric screening and rational design of new compounds. These attempts are made to reduce the severe adverse effects associated with existing cancer chemotherapeutic agents, as well as to reduce the development of drug resistance.

Cytotoxic nucleoside analogues were among the first cancer chemotherapeutic agents introduced. The aim of the study was thus to screen novel nucleoside analogue derivatives for cytotoxic activity, as well as to determine the level of differentiation and the induction of apoptosis in the colonic carcinoma cell lines, viz. HT-29 and Caco-2.

Materials and methods:
All compounds were synthesised using standard organic chemistry techniques. In vitro cytotoxicity of the synthetic compounds were tested against the HT-29 and Caco-2 cell lines. In order to determine the cytotoxic effects of these compounds, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was used. The measurement of cell differentiation was determined by the alkaline phosphatase assay, and a colorimetric assay was used for the determination of caspase activity. The percentages of apoptosis were determined using the Annexin V-FITC apoptosis detection kit and cytochrome c levels were determined by Elisa.

Results:
Compounds JLP 26.5, JLP 27.2 and JLP 38.2 showed significant cytotoxic activity (p<0.05) against both cell lines, with IC₅₀ values ranging from 25.59-37.20 µM. The JLP compounds did not produce a significant reduction in white blood cell viability (p>0.05) in comparison to camptothecan or the JLP derivatives. Compound JLP 26.5 induced cell differentiation in the HT-29 cells, whereas JLP 38.2 induced cell differentiation in the Caco-2 cells. JLP 38.2 induced the highest amount of apoptosis. Maximal caspase 3 expression was observed between 4-12 hours after exposure to the JLP compounds.
A pilot study to investigate the predictability of efavirenz plasma levels in HIV-1 infected South African children

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Purpose:
Efavirenz, a non-nucleoside reverse transcriptase inhibitor, used in the treatment of HIV-1-infected children, is principally metabolized via oxidative metabolism by the liver enzyme, Cytochrome 2B6 (CYP2B6), and to a lesser extent by Cytochrome 3A4 (CYP3A4). Altered efavirenz metabolism in HIV patients is associated with CYP2B6 polymorphism. The CYP2B6 allelic variant is associated with significantly greater efavirenz exposure due to lower clearance of the drug in African-Americans compared to European-Americans. It is important to be able to predict plasma levels in a population to prevent sub-therapeutic levels which can lead to resistance developing against the drug, or toxic drug levels that may occur and lead to side-effects. The main aim of this study was to investigate the accuracy of certain literature clearance values to predict efavirenz levels for South African children. (Wintergerst: 0.3 l/h/kg; Saitoh: 3/l/h/m² and Goodman and Gilman 0.186 l/h/kg)

Methods:
Plasma was collected from 21 black HIV-1 infected South African children, who have been on efavirenz combination antiretroviral treatment for at least 6 months. The study participants visited Harriet Shezi Children’s Clinic, Soweto, on two separate occasions, where 2ml whole blood was drawn from each participant twice on each visit and at least 2 hours apart. Plasma levels were analyzed by a validated liquid chromatography tandem mass spectrometer coupled with electrospray positive ionization mode, method. The projected steady state plasma levels were calculated with the following formula: \( Cp^{ss} = \frac{F*D}{Cl * 24} \)

Results:
Median (range) age, body weight, dose, dose/kg, Body Surface Area (BSA), and steady state plasma level were 7.2 (4 -13.6) years, 20.24 (12.45 – 43.05) kg, 271 (200 – 400) mg, 14.01 (9.2 – 16.4) mg/kg, 0.79 (0.578 – 1.3) kg/m², 1.8 (0.14 – 7.29) mg/L respectively, for the first visit.

No correlation was found by using any of the published clearance values.

Conclusion:
Our population differs substantially from the literature and it is important to expand the study to establish representative clearance values for this specific population.
Purpose:
Dysfunctional monoaminergic neurotransmission, especially decreased levels of noradrenalin, serotonin and dopamine, play an important role in the neurobiology of depression. Available antidepressants attempt to correct these deficiencies, although are at best 65% effective. Consequently, new antidepressant treatments, as well as new targets for antidepressant action, are urgently needed. The nitric oxide (NO)-cGMP cascade is implicated in the pathophysiology of depression. Methylene blue (MB), which is structurally dissimilar to any known antidepressant, inhibits NO synthase (NOS) and guanylate cyclase and demonstrates significant anti-depressive-like activity in rodents. The structural-activity relationship of MB and its analogues will be studied as potential new lead antidepressant compounds using the forced swim test (FST). In addition, their potential monoamine oxidase A (MAO-A) inhibitory activity will be studied.

Methods:
MB analogues (selected from the Merck Index according to structural similarities) included methylene green (MG), methylene violet (MV), thionin acetate (THI), phenothiazine (PHE), tacrine (TAC) and acriflavine (ACR). Male Sprague-Dawley rats (200-250g) were exposed to acute MB or MB-analogues over a dosage range of 0.5-60 mg/kg, depending on the analogue tested. Saline and imipramine (IMI) were used as negative and positive controls, respectively. Rats were introduced to a 15 min pre-swim in the FST apparatus before the first intra-peritoneal injection 24 hours prior to the final swim test. One and six hours prior to the final swim test; rats received their second and final dose of the drug. Locomotor activity was then assessed in the open field arena. One hour after the final dose, immobility, climbing and swimming were recorded in the FST over 5 min. In a separate study, the inhibitory potential on human recombinant MAO-A were determined for the above compounds, including IMI, by spectrofluorometric assay using kynuramine as substrate.

Results:
IMI significantly reduced immobility in the FST, as did MB (15, 30, 60 mg/kg) and MG (7.5, 25, 40 mg/kg), with limited effects on locomotor activity, thus indicating substantial antidepressant-like activity. ACR, TAC, MV, THI and PHE failed in this regard, with ACR and TAC significantly reducing locomotor activity at all doses tested. Higher doses of MB and MG were more effective than IMI in reducing immobility. MB and MG increased climbing behaviours, suggestive of more pronounced noradrenergic activity, with less marked increases in swimming, a serotonergic-driven behaviour. IMI markedly increased climbing behaviour, arguing for a more dominant noradrenergic response. MB was found to be a potent MAO-A inhibitor (IC_{50}=0.073 \mu M) as well as MG (IC_{50}=0.169 \mu M), while the other analogues showed no inhibition. These data would support the above noted effects on noradrenergic-serotonergic behaviours. Concluding, only MG presents with noteworthy antidepressant-like activity that is similar to both MB and IMI. Considering the potent MAO-A inhibitory actions of MB and MG, actions on the NO-cGMP pathway may not be the only mechanism whereby these compounds evoke an antidepressant-like response. Further characterisation of their NOS inhibitory activity may reveal MB and MG to be novel dual action antidepressants.
YSP 06  Comparison of Methods to determine the oxidative properties of medicinal plant extracts (T. camphoratus and T. parvicapitulatus)

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Introduction:

Tarchonanthus camphoratus L. is a well-known medicinal plant in Southern Africa. Various parts of the plant are used for asthma, bronchitis, whooping cough and abdominal pain among others. Morphologically this species can be divided into five different species. In this study two species were investigated: T. camphoratus and T. parvicapitulatus.

Aim:

The aim of this study is to compare the oxidative properties of T. camphoratus and T. parvicapitulatus using thin layer chromatography method and chemiluminiscence assay.

Method:

1. Thin layer chromatography: A solution of 10mg/ml was prepared from each extract. The TLC plates were developed in two different solvent systems and thereafter visualized under UV light. For further detection of chemical compounds, the plates were sprayed with 0, 2% diphenyl picrylhydralazine (DPPH) in methanol.

2. Chemiluminiscence assay: 30ml of blood was withdrawn from six healthy volunteers (n=6) into heparinized tubes. Neutrophils were isolated immediately after the blood was drawn and the cells were kept on wet ice in order to prolong the lifespan of the cells. The cells were incubated with 100 µl of the different plant extract for 20 minutes at room temperature and bioluminometer was used to run the assay. PMA was used as the stimulant and paired t-test was used for statistical analysis (n=6)

Result:

The Thin layer chromatography (TLC) method of qualitative antioxidant detection showed that the acetone extracts of T. camphoratus and T. parvicapitulatus displayed antioxidant compounds due to their DPPH free radical scavenging activity. The chemiluminiscence assay also showed that T. camphoratus and T. parvicapitulatus has significant antioxidant properties.

Discussion and conclusion:

Of the two methods, both indicated that the two plants extracts could inhibit superoxide production; however, the TLC method was more accurate and could distinguish between the two plants extract revealing that T. parvicapitulatus has more antioxidant properties than T.camphoratus. The strongest concentration (10mg/ml) of the plants extracts significantly suppressed superoxide production.
YSP 07  Stereotypy in the deer mouse, correlation with cortico-striatal oxidative status and dopamine turnover, and response to combined fluoxetine/risperidone treatment

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Purpose:
Obsessive compulsive disorder (OCD) is a debilitating disorder characterised by obsessions and compulsions, involving dysfunctional cortico-striatal-thalamic-cortical circuits in the brain. OCD responds preferentially to serotonin selective agents, such as fluoxetine, although a clinical response is only observed in 40 – 60 % of patients. In non-responding patients, augmentation with dopamine-blocking agents, especially atypical antipsychotics, may be effective. The deer mouse (Peromyscus maniculatus bairdii) presents with repetitive jumping, pattern running, and backward somersaults, that are variable within a population, and which closely resemble repetitive behaviours seen in OCD. Recently, OCD has been linked to altered redox status. Since dopamine signalling can promote oxidative stress and also increase stereotypies, we postulated that deer mouse stereotypy may be correlated with increased cortico-striatal redox status and dopamine turnover. Moreover, deer mouse stereotypies may show an improved response to low dose fluoxetine plus risperidone as compared to either drug alone, thus supportive of predictive validity for the model.

Methods:
Deer mice were separated into high stereotypic behaviour (HSB), low stereotypic behaviour (LSB), or non-stereotypic behaviour (NS) groups. Frontal cortico-striatal dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), as well as oxidized (GSSG) and reduced (GSH) glutathione were determined by tandem liquid chromatography-mass spectrometry (LC-MS). Superoxide dismutase (SOD) activity was determined using a commercially available SOD kit. Saline, low dose fluoxetine (5mg/kg/day), standard dose risperidone (2mg/kg/day), and a combination of these, were administered to HSB mice for 3 weeks. Baseline, weekly and endpoint behavioural analyses were performed. Animals were sacrificed, and the above mentioned neurochemical parameters determined. Dopamine turnover and oxidative stress changes versus stereotypy were analysed by one way ANOVA and post hoc Bonferroni, plus Pearson correlation tests. Drug treatment data were analysed using two way ANOVA and Bonferroni post-tests.

Results:
While chronic treatment with fluoxetine only separated from control in the 3rd week, with risperidone being completely ineffective, the fluoxetine/risperidone combination was remarkably effective in attenuating stereotypy, also demonstrating an earlier onset of action compared to fluoxetine. This provides further evidence for the predictive validation of the deer mouse model. Cortical GSH and GSSG levels were significantly lower in HSB mice, compared to NS mice, and showed a significant correlation with the severity of stereotypy, suggesting an involvement for a deficient glutathione system in deer mouse stereotypy. A similar but non-significant trend was evident in the striatum. SOD activity and levels of dopamine and its metabolites did not differ with respect to stereotypy. At the time of writing, the neurochemical parameters for the drug treatment groups were still undergoing analysis.
**YSP 08  Genotype vs. phenotype and the allelic variation of CYP2D6 and CYP2C19 in a demographic sample of the South African population**

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**Purpose:**

Adverse drug reactions are a growing concern in medical practice. Pharmacogenetics identifies interpersonal variation particularly with regard to the cytochrome P450 isoenzymes and delineates a critical element in so-called personalised medicine. The “gold standard” regarding pharmacogenetic testing has been genotyping which is used to predict poor, intermediate, extensive and ultra-rapid metabolisers (PM, IM, EM and UM respectively). It is becoming increasingly apparent that numerous environmental factors play an equally important role in drug metabolism. Therefore the aim of this study was to investigate the relationship between genotype and phenotype for CYP2D6 and CYP2C19 in a demographically representative sample of the South African population.

**Method:**

**Sample collection and preparation:** Venous blood samples were collected from probe drug (dextromethorphan for CYP2D6 and omeprazole for CYP2C19) treated subjects immediately before and at 2, 3 and 4 hours after administration. Plasma was used for phenotyping and DNA was extracted from the leukocyte layer for genotyping.

**Genotyping:** Two genotyping approaches were utilised. First, the FDA-approved Roche AmpliChip CYP450 test allowing accurate identification of 33 CYP2D6 and 3 CYP2C19 alleles based microarray technology. Second, each sample was amplified using specific primers for CYP2D6 and the full gene was sequenced. This combined approach allowed for identification of existing and novel polymorphisms.

**Phenotyping:** An efficient semi-automated on-line solid phase extraction (SPE) liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) method was developed in-house for the simultaneous detection of both probe drugs, their metabolites and an internal standard. Phenotype was expressed as the ratio of metabolite to parent drug concentration.

**Results:**

**Genotypic information:** The CYP2D6 wild type *1 (EM) allele was predominant at 27% followed by *17 (IM) at 24%, *41 (IM) at 19% and *4 (PM) at 6%. It was found that 47% of the population were IM, 47% EM, 2% were PM and 4% could not be classified using the AmpliChip. Sequencing data revealed that some of the *10 alleles (IM) as classified by the AmpliChip are in fact *56B alleles (PM). CYP2C19 allele frequencies were identified as follows: 79.7% *1 (EM) and 20.3% *2 (PM). Ninety-four percent of the population were EM and 6% PM for CYP2C19. No UMs were found.

**Phenotypic information:** Phenotypic distributions for both CYP2D6 and CYP2C19 lacked distinct phenotypic limits demonstrating variation in metabolism.

**Genotype vs. phenotype:** Evidence shows discordance between genotype and phenotype.

**Conclusion:**

Discordance between genotype and phenotype illustrates the need for more accurate diagnostic tests and a careful scrutiny of the validity of existing tests.
YSP 09  Pilot study of wound healing parameters of Absorbatox™-containing dressings in the porcine model

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Introduction:
Wound healing is the restoration of traumatised tissue to re-establish integrity. A malfunction in the healing cascade can lead to chronic wounds that are difficult to treat. Although wound dressings remain the mainstay of treatment, they are often not effective in maintaining a moist environment without fluid leakage and subsequent maceration of peri-wound margins. Absorbatox™ is a potentiated aluminosilicate that can aid in creating an ideal wound healing environment due to its adsorbent nature and cation exchange capacity. The aim of this study was to determine whether Absorbatox™ improved healing in a porcine wound healing model.

Methods:
In an in vivo study, twenty full skin thickness wounds were created via punch biopsy on each of six female large white pigs. Four randomly assigned treatments included wound dressings containing a sachet filled with Cerdak™ particles (positive control), micronised or granular Absorbatox™ particles (treatment groups) or no particles (negative control). Wound dressings were changed at regular intervals and multidimensional wound size was measured to determine rate of wound closure. Seventeen days after wounds were made, biopsies of the wound regions were collected and histological evaluation following Haematoxylin and Eosin, Alcian Blue and Giemsa staining of the wound areas was performed.

Results:
Treatment with Absorbatox™ resulted in a rapid decrease of wound depth when compared to both Cerdak™ and the negative control. This was apparent from day 7 with the largest difference on day 10 where micronised and granular Absorbatox™ resulted in 96 percent and 92 percent wound closure respectively vs. 88 percent for both Cerdak™ and the negative control. Absorbatox™ and Cerdak™ treatments limited wound surface contraction. Histological evaluation found that wound closure in the Absorbatox™ and Cerdak™ groups was associated with the establishment of new epithelium with a well-defined basal layer, normal collagen arrangement and neovascularisation. These healing characteristics were absent in the negative control groups.

Conclusion:
The wound healing properties of both micronised and granular Absorbatox™ treatments are comparable to that of the positive control and are superior to that of the negative control. Absorbatox™, therefore, is a promising wound healing agent that warrants further investigation in a human clinical trial.
YSP 10  Basal versus KCl-evoked frontal cortical glutamate release in Flinders Sensitive Line (FSL) and Flinders Resistant Line (FRL) rats: An in vivo microdialysis study.

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Purpose:

In vivo intracerebral microdialysis is a valuable method for obtaining multiple extracellular fluid samples from discrete brain areas in live, freely moving animals, allowing good temporal real-time resolution of brain transmitters and metabolites. Multiple sampling using this technique is devoid of stressful handling of the animals, or any other unintended external stimuli. This is particularly valuable when sampling neurotransmitters that are sensitive to internal and external stimuli, be it chemical, mechanical or physiological. The objective of this study was to set up the apparatus and surgical procedures needed for in vivo microdialysis in our laboratory, as well as the validation of procedures for analysing the dialysate. In a subsequent application study, we studied basal and KCl-evoked release of glutamate in extracellular fluid in the frontal cortex of the Flinders Sensitive Line (FSL) rat, a genetic model of depression, and its healthy control, the Flinders Resistant Line (FRL) rat.

Methods:

Eight adult male FSL and FRL rats (250 - 350g) were anesthetized with pentothal and halothane. A guide cannula was surgically implanted in the frontal cortex according to stereotaxic coordinates. Following a 5 day recovery period, the microdialysis probes were positioned in the frontal cortex via the guide cannula under light halothane anesthesia, and perfused with artificial CSF at a flow rate of 1.5μl/min. Following a 2 hr stabilization period, 30μl dialysate was collected every 20min over 5 hrs in glass inserts located in a refrigerated microfraction collector. Basal glutamate release was determined in FSL and FRL rats (n=2 each), while KCl-evoked glutamate release was assessed after perfusing the probe with 100mM KCl (n=2 each). Two microdialysis stations enabled the head-to-head comparison between FSL and FRL rats under the above conditions. Animals were thereafter euthanized and probe placement verified histologically. Microdialysate glutamate levels were analyzed with HPLC coupled with fluorescence detection.

Results:

Correct placement of the probe in the frontal cortex was confirmed histologically. Frontal cortical glutamate levels in naive FSL rats were approximately 10-fold lower than that in FRL rats (0.142±0.067μM versus 1.10±0.742μM), while FRL glutamate levels were in accordance with values for rats described in the literature. FSL rats showed a much greater KCl-evoked glutamate release in the frontal cortex than did FRL rats (±246% versus ±116%). While these data are preliminary, the depressogenic phenotype of the FSL rat may have its origins in a hyper-responsive glutamatergic system in the frontal cortex. Further studies are needed and are currently underway.
SERVIER SASBCP YOUNG SCIENTIST PRESENTATIONS

YSP 11 Development of a new platelet-rich plasma kit for the extraction and partial purification of platelet-derived growth factor and transforming growth factor beta 1 for treatment of photo-aging damage

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Introduction:
Skin wrinkling may be considered a chronic wound that cannot heal completely due to ongoing injury from sun exposure. Normal wound healing is orchestrated via an ordered sequence of specific cytokine and growth factor release. These factors have the potential to reverse the effects of sun damage and to rejuvenate the skin by decreasing the appearance of photo-aging. Dermal fibroblasts normally produce extracellular matrix (ECM) proteins responsible for maintaining the skin’s youthful appearance. A number of growth factors, but in particular, transforming growth factor beta (TGF-beta) and platelet-derived growth factor (PDGF) have demonstrated an increased synthesis of ECM protein in the dermis. Aesthetic practitioners are using autologous Platelet-Rich Plasma (PRP) for skin rejuvenation in patients with severe photo-aging. The autologous PRP is produced by the practitioner using a commercially available “easy-to-use centrifugal system” kit.

Purpose:
This study assessed and compared the concentrations of PDGF-AB and TGF-β1 that can be extracted and partially purified from blood from a small sample population using a centrifugal system.

Methods:
A variety of growth factor extraction procedures were compared and the least complicated method with the ability to deliver the highest concentration of growth factors optimized further. The concentration of partially purified growth factors from the developed kit was compared to the available literature of the commercial kits. Furthermore this study determined the optimal concentration of PDGF-AB and TGF-β-1 required to increase ECM protein production using in vitro primary fibroblast cultures. A range of recombinant growth factor concentrations were used to establish the optimum conditions under which primary fibroblasts produce ECM proteins using the crystal violet assay after different incubation periods.

Results:
The concentration of growth factors extracted by the developed kit is comparable to the commercial kits. From the crystal violet assay it is apparent that rTGFβ-1 is more potent stimulant than PDGF of fibroblasts in vitro. A concentration of 10ng/ml rTGFβ-1 was sufficient to increase ECM proteins production by 50% after 1 day of primary fibroblast incubation. The developed kit proved to extract sufficient TGFβ-1 to achieve treatment. The developed kit is comparable to the commercially available kits and warrants further clinical investigation.
YSP 12 The effects of phosphodiesterase type 5 inhibitors on cellular plasticity in a neuronal and non-neuronal cell line

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Background:
Depression is the most debilitating psychiatric disorder of our time, whilst clinically used drug treatments are plagued with troublesome side-effects, delayed onset of action and treatment resistance. In addition, a comprehensive understanding of the biological basis of depression and its treatment remains illusive, prompting extensive ongoing research. While the neuroplasticity hypothesis of depression has gained recent support from various lines of experimental and clinical evidence, the nitric-oxide (NO)/cGMP pathway is believed to play an important role in dysregulated neuroplasticity in depression and in its reversal and enhanced synaptogenesis by antidepressants. Recent studies in our laboratory demonstrated antidepressant-like effects of the phosphodiesterase type 5 (PDE5) inhibitors sildenafil and tadalafil when combined with the antimuscarinic drug atropine in rats, while unpublished data also suggest that sildenafil may upregulate genes encoding for the expression of anti-apoptotic proteins in vitro. These data warranted the current study to investigate the effects of PDE5 inhibitors and other modulators of the NO/cGMP pathway on neuroplasticity.

Methods:
Human neuroblastoma (SH-SY5Y) and Chinese hamster ovary (CHO-K1) cell lines were maintained in culture medium at 37°C, 10% CO₂ and saturated humidity. Cells were subjected to stress (modelling neuronal stress associated with major depression) by inducing excitotoxicity (glutamate) or oxidative stress (serum deprivation or H₂O₂), and the condition (concentration and duration of exposure) that induces a 50% reduction in mitochondrial activity, as measured by the standard MTT cell viability assay, was determined for each of these stressors. Thereafter, implementing these optimal stress conditions, the effects of the PDE5 inhibitors sildenafil, tadalafil and zaprinast, rolipram (PDE4 inhibitor), 3-isobutyl-1-methylxanthine (nonselective PDE inhibitor), imipramine (tricyclic antidepressant), fluoxetine (selective serotonin reuptake inhibitor) and tianeptine (neuroprotective antidepressant), ODQ (selective inhibitor of soluble guanylyl cyclase), db-cGMP (cGMP analogue), sildenafil + atropine, as well as sildenafil + ODQ were determined on neuroplasticity, measuring mitochondrial activity (MTT).

Results:
Optimal stress conditions (50% reduction in mitochondrial activity) in both neuroblastoma SH-SY5Y and CHO-K1 cells were demonstrated to be either 24-hour serum-deprivation, 15 minutes incubation with 1.75 μM H₂O₂, or a 24-hour incubation with 12.5 mM glutamate (for SH-SY5Y) and 17.5 mM glutamate (for CHO-K1), respectively. From a concentration series, only 0.16 nM sildenafil significantly improved mitochondrial activity (MTT) after 24-hour serum deprivation, while all other concentrations, drugs and in all other stress conditions there was no neuroprotective effect.

Conclusions:
Sildenafil protects against neurodegenerative effects of serum deprivation in neuronal cells at relatively low concentrations (about 10-fold below its Kᵦᵦ value for PDE5), while this effect was not observed in non-neuronal cells. Neuroprotection may play a role in the antidepressant-like activity by sildenafil, as observed in rats in earlier studies.
Antiradical and antioxidant activity of theanine

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Introduction

Free radicals that are generated in vivo result in damage of DNA, small molecules and lipids (Halliwell et al., 1995). Lipid peroxidation results from free radicals attacking membrane lipids which leads to the destruction of the cell membrane, cell integrity and the proper functioning of membrane bound enzymes and receptors. Free radicals have also been shown to play a role in cardiovascular diseases (Dorman et al., 2003). Importance of an antioxidant in vivo cannot be emphasized enough as these agents protect the human body against damage by ROS. Thus in the search for agents that have antioxidant activity, Theanine which is a natural component of green tea was studied as it has been shown to prevent lipid peroxidation (Tsunge et al., 2003).

The free radical scavenging activity of a potential antioxidant is evaluated in vitro by its ability to scavenge the stable 2, 2-diphenyl-1-picryl-hydrazyl (DPPH*) radical which acts as both an oxidizable substrate and as a reaction indicator molecule (Dorman et al., 2003).

Method

A modified method of free radical scavenging of Brand-Williams et al., (1995) was used to measure the free radical scavenging ability of theanine and melatonin. Vitamin C was used as a positive control (Brand-Williams et al., 1995; Toit et al., 2001).

Results

Theanine scavenges 32% of the radicals on average over the entire concentration range tested which is significantly lower than the free radical scavenging ability of vitamin C which is 95%. Flavonoids in tea account for most of the antioxidant activity in tea and thus the presence of Theanine in green tea also increases the antioxidant capacity of tea.

Conclusion

Theanine can be an antioxidant in vitro due to it antiradical activity.
YSA 01  Formulation and evaluation of a stimuli-responsive colon-targeted drug delivery system for the treatment of local colonic pathologies

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Purpose:
Ulcerative Colitis, a relapsing and remitting disease characterized by chronic non-infectious inflammation of the colorectal mucosa, is most effectively treated by the topical application of an anti-inflammatory at the mucosal level. Despite the numerous technologies evaluated for this purpose an effective and physiologically triggered drug delivery system is yet to be developed. For the purposes of this study Mesalamine (5-ASA) was incorporated into a novel in situ crosslinked drug delivery system to achieve targeted colonic delivery in response to colonic bacterial populations.

Methods:
Preparation of crosslinked drug-loaded tablets: Crosslinked chitosan (CHT) drug-loaded granules were prepared using various concentrations of sodium tripolyphosphate (TPP) as a solvent, and the wet mass was passed through a 1mm mesh sieve. The granules were allowed to dry for 24 hours. The dried granules were then incorporated within matrices of pectin, xanthan gum, BaCl₂ and CMC. These were subsequently compressed into concave tablets.
Friability of uncoated tablets: Friability studies were conducted according to USP test parameters for tablets of weight ≥1g.
Coating of compressed tablets: All tablets were coated using a Fluidised Bed Dryer. A novel coating solution was prepared by blending Surelease® with an aqueous pectin solution to achieve a total mass gain (TMG%) of between 9-10%. In vitro release studies: Performed using USP 32 apparatus 3. Each formulation was subjected to a standard oscillating rate of 10dpm. The tablets were subjected to pH 1.2 (2 hours), pH 6.8 (4 hours) and pH 5.92 (18 hours) with and without pectinase and β-glucosidase. Drug release was evaluated by UV spectrophotometry at the applicable wavelengths.
In vivo studies: Conducted on female pigs using conventional systems of 5-ASA, placebos and the stimuli-responsive drug-loaded system. Blood samples were taken over a 24 hour period and analyzed by UPLC.

Results and Discussion
Results from friability studies showed a loss in mass ranging from 0.07-0.1%. In vitro release studies showed that for the first 6 hours no drug was released. After 12 hours in the presence of colonic enzymes, 100% of drug was released, however, when no enzymes were present <50% of drug was released. This basic phenomenon was observed for all formulations. After analysis of all plasma samples it was established that the polymers had no physical or metabolic adverse effects on the pigs and that the drug-loaded system was superior to that of the conventional as 5-ASA release was not dependent on the highly variable pH profile of the GIT.

Conclusions
An enzyme-responsive drug delivery system was therefore successfully developed for the site-specific delivery of 5-ASA to the colon.
YSA 02  Enzyme Inhibition in the prevention of neuronal apoptosis: a molecular modelling study

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Purpose:
Many enzymes are involved in the apoptotic processes that contribute to neurodegenerative diseases like Alzheimer's and Parkinson's disease. These enzymes include CDK5/p25 (Cyclin dependent kinase 5 in complex with activator protein p25), calpain I, caspase 3 and GSK3\textsubscript{β} (Glycogen synthase kinase 3\textsubscript{β}). Inhibition of these enzymes may have the potential to counteract the neurodegeneration caused by various apoptotic processes.

Methods:
In this study, computational pharmacophore hypotheses for CDK5/p25, calpain I, caspase 3 and GSK3\textsubscript{β} were formulated to predict the geometry of the chemical features necessary to exhibit inhibitory activity against these enzymes. The in-house developed library was screened to determine which compounds comply with the hypotheses for the respective enzymes. Docking studies were subsequently performed using the compounds in the in-house library to determine which compounds have the ability to fit into the respective enzyme cavities thus acting as potential inhibitors. Using a combination of docking results and hypothesis compliance, the compounds with the most promising combined results were selected for biological screening against some of the investigated enzymes.

Figure 1: Calpain I with co-crystallised ligand

The selected compounds for calpain I were tested using a Calpain-Glo\textsuperscript{™} Protease Assay kit (purchased from Promega) to determine the inhibitory activity of the compounds. This is a luminescence based biochemical assay, measuring the luminescence emitted by the luciferase enzyme after cleavage of its substrate.

Results:
The compounds exhibited activities as calpain I inhibitors with IC\textsubscript{50} values in the micro molar range. In conclusion, this study demonstrated that the application of hypothesis generation and docking studies show promise to predict compounds with the potential to inhibit specific enzymes to counteract apoptosis and neurodegeneration.
YSA 03  The synthesis and evaluation of 3-phenylazetidine and 3-hydroxy-3-phenylpyrrolidine as substrates of monoamine oxidase B (MAO-B)

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Purpose:
Parkinson’s disease is a neurodegenerative disorder that is prevalent in elderly patients. This condition affects the dopaminergic neurons of the substantia nigra, and manifests itself with motor problems in the patient. One compound, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 1) has been shown to induce parkinsonism in humans. This compound proved to be a good substrate for MAO-B that catalyzes the enzymatic activation of MPTP to the toxic metabolite MPP⁺. This spurred the investigation for other possible substrates of MAO-B. For example, the five membered ring analogues of MPTP, 1-methyl-3-phenyl-3-pyroline (2) and 3-phenyl-3-pyrroline (3) also are good MAO-B substrates. In the present study, we have also synthesized a four membered ring analogue of MPTP, 3-phenylazetidine (4) and evaluated it as a substrate of MAO-B. In an attempt to evaluate the requirement of the 3,4-π-bond for substrate oxidation and the possibility that an electron donating substituent may stabilize reaction intermediates in the catalytic cycle, we have also prepared and evaluated 3-hydroxy-3-phenylpyrrolidine (5). As positive controls (known MAO-B substrates), 2, 3, the pyrrolidine (6) and azabicycloclohexane (7) were included while piperidine (8) served as negative control.

Methods:
All compounds were synthesized according to literature methods and evaluated as MAO-B substrates by measuring the stoichiometric production of hydrogen peroxide by baboon liver mitochondrial MAO-B. In the catalytic cycle of MAO-B, one mole of hydrogen peroxide is generated for each mole of substrate oxidized.

Results:
The assay procedure was validated by the finding that all of the positive controls included in the study acted as substrates of MAO-B, with steady-state kinetic parameters (Kₘ and Vₘₐₓ values) similar to those in literature. As expected, the negative control failed to exhibit any MAO-B substrate activity. 3-Phenylazetidine (4) also was not a MAO-B substrate. 3-Hydroxy-3-phenylpyrrolidine (5) however proved to be a very good MAO-B substrate with a Kₘ value of 139.4±40.0 µM. These data show that four membered ring analogues of MPTP are not MAO-B substrates and also demonstrate that a wide variety of five membered ring analogues act as MAO-B substrates. The 3-hydroxy group of 5 does not appear to play a role in the catalytic cycle since pyrroline 3 is approximately as good a substrate as 5.
Purpose:
Like other chronic medical conditions, HIV infection requires exceptionally high adherence to highly active antiretroviral therapy for successful treatment. The primary aim of this study was to investigate the refill adherence of antiretroviral medication in a section of the private health care sector of South Africa.

Research methodology:
Data from a medical claims database were analysed by means of a quantitative, retrospective drug utilisation review (DUR) for the years 2005, 2006, and 2007. An overall adherence rate (AR) was calculated by using the following equation: \( \text{AR} = \frac{\text{total days of antiretroviral items supplied} - \text{days supplied at the last refill}}{\text{date last claimed} - \text{date first claimed}} \).

Results:
Antiretroviral prescriptions (n = 220 095) represented 0.86% of the total number of prescriptions claimed during the study period (N = 25 586 877). ARVs (antiretroviral medicine items) represented 0.91% of all medicine items (N = 59 971 226) claimed during the three years at a total cost of 1.92% (N = R 5 758 783 544).

A total number of 51 235 ARVs were prescribed once or repeatedly to 27 890 HIV patients. Of these, 9268 ARVs (0.01% of the total ARV cost) were claimed only once and were excluded. ARs were calculated for 41 967 ARVs which accounted for 90.46% (N = R110 728 214.00) of the total ARV cost. ARVs incorporated in the calculations were prescribed for 384 ± 305 days (13 months) during the three periods. Based on the number of days supplied, as indicated in the database, approximately 61% of all ARVs were prescribed for shorter than a year, 23.24% between one and two years, 12.72% between two and three years and 3.23% for more than three years.

Less than 50% of all ARVs had acceptable adherence rates (ARs between 90% and 110%). Stavudine had the lowest acceptable AR of all active ingredients at only 20.07%, while lamivudine had the highest acceptable AR at 44.32%.

AR analyses based on age and gender showed that only 34.99% of ARVs prescribed to children younger than 9 years were acceptable compared to 36.03% in young adolescents (10-19 years). Acceptable ARs for older adults (patients older than 29 years) were all below 44%. Among males and females alike the total percentage of ARVs that revealed acceptable ARs (between 90% and 110%), amounted to almost 42%.

ARVs with adherence rates below 90% (possibly under-supplied), accounted for 14.30% (n = R15 829 876.53) of the total cost of all ARVs (N = R110 728 214.00) while ARVs that were possibly over-supplied accounted for 25.60% (R28 347 266.48).

The results revealed that the acceptable adherence rate for ARVs dispensed is likely to be higher the longer the period that patients receive their medication (number of days supplied).

Conclusion:
The calculated refill adherence rates indicate that most ARVs were either possibly over- or under-supplied for the specific treatment period. This has a significant impact on the success of treatment, as well as costs incurred.
YSA 05  A discoidal multi-layered compressed matrix for use in chronotherapeutic disorders

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Purpose
The purpose of this study was to develop a multi-layered multi-disc tablet for use in chronotherapeutic disorders such as hypertension, asthma, cardiac disease and stomach ulcers.

Methods
Preparation of the device: To produce a base disk, various ratios of the polymers HEC and EC were granulated with model drug theophylline (THP) using an aqueous dispersion of EC as granulating fluid. The granules were then compressed using a Beckman Hydraulic Press into drug disks. The crown disk comprised THP with lactose used as a bulking agent. The disks were suspended between layers of HPMC and pectin using a flat faced punch and die set. Drug release studies: Performed using a USP dissolution apparatus II comprising 900mL PBS (pH 6.8; ±37°C). Matrices were subjected to dissolution for 24 hours at 50rpm. FTIR: FTIR analyses was undertaken to assess any possible structural variations in the polymeric backbone as a result of drugs/excipients/polymer interactions. Analysis was performed on the native polymers, the granulation blend and the compressed formulations. Thermal analysis: Differential scanning calorimetry was undertaken to analyse changes, such as the glass transition temperature ($T_g$) of the polymer, melting point and any interaction between the polymers, excipients or drug during formulation. Samples were run from 0-300°C at a heating rate of 10°C/min. Analyses were performed on the native polymers, the granulation blend and the compressed formulations.

Results and Discussion
Drug release for all formulations displayed biphasic release with an initial lag phase of approximately 3 hours, followed by a burst of drug release between $t=3$ hours and $t=5$ hours. Thereafter drug release was sustained for the remainder of the 24 hours ranging from 70-90%. Formulations with a 2:1 ratio of EC to HEC however, display a drug-free period of approximately 5 hours after the initial burst. As a result, this formulation was able to provide two phases of drug release and may have applications in chronotherapeutic drug delivery. FTIR analyses showed no significant changes in both the polymeric and drug structure as a result of granulation and compression. This is further motivated by DSC analyses that displayed no change in the $T_g$ and melting point values of the various polymers and model drug THP.

Conclusions
The device produced biphasic drug release achieved through the use of two drug loaded disks, with the crown disk providing the first phase of drug release and the base disk providing the second phase of drug release with an intermittent drug-free period.
YSA 06  Synthesis and evaluation of novel quinolones for antioxidant properties in neuroprotection

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Introduction:
Energy production by mitochondria ultimately leads to production of harmful free radicals, which is responsible for oxidative stress, lipid peroxidation, and damage to cells, proteins and DNA. This results in age related neurodegenerative disorders (Alzheimer’s and Parkinson’s disease), which may be prevented by antioxidant mechanisms such as scavenging of free radicals, iron chelation and degradation of hydrogen peroxide. Flavonoids exhibit potent antioxidant activity when substituted with OH-groups on R2, R3, R4 and the R1 aromatic substitution’s C-3’ and C-4’. 1 4-Quinolones are structurally similar to the flavonoids (Scheme) and might possess antioxidant activity when the above-mentioned structure activity relationships are applied. The aim of the study is to prove that 4-quinolones have relevant antioxidant activity when compared to flavonoids and may therefore assist in neuroprotection.

Methods:
A series of 4-quinolones were synthesised according to the Conrad-Limpach synthesis and characterised by NMR, IR and MS, to establish the structure activity relationships of hydroxyl substituted quinolone structures.

The 4-quinolone series was compared to the correlating flavonoid structures in a selection of biological assays, to establish the relative antioxidant properties of the 4-quinolones. The biological assays employed were the superoxide anion Nitro Blue Tetrazolium (NBT) assay and lipid peroxidation Thiobarbituric Acid (TBA) assay.

Results:
The NBT assay evaluated the ability of the 4-quinolones to scavenge free radicals and consequently inhibit the reduction of NBT to nitro blue diformazan, the free radical indicator. Results obtained compared favourably to that of the flavonoids, which are known radical scavengers.1 The ability of 4-quinolones and the correlating flavonoids to inhibit toxin induced lipid peroxidation, was assessed in the TBA assay and showed inhibition when compared to the known antioxidant Trolox.

Conclusion:
Data obtained confirmed the antioxidant activity of the synthesised 4-quinolone series and correlating flavonoids. The results demonstrated the potential for employing these novel structure activity relationships in the development of antioxidant approaches to neurodegenerative disease.
YSA 07  Pharmaceutical evaluation of Phela capsules used as traditional medicine

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Introduction:

Phela is a traditional herbal medicine comprising of four medicinal plants which together are claimed to have immune boosting properties. The IKS Lead Programme of the MRC focuses on investigating such claims. The aim of this study was to assess the pharmaceutical quality of Phela, in capsule form, and its suitability for use in clinical trials. Thus the organoleptic features and physical and chemical properties of the individual plant powders and the content uniformity, release characteristics and shelf-life were determined.

Methods:

Freshly prepared powders of the 4 individual plant materials and Phela were assessed for their organoleptic features, ash value, flowability, moisture content, extractable matter and microbial contamination using standard pharmacopoeia methods. The capsules of Phela were manufactured at the MRC’s IKS Lead Programme under GMP conditions. The in vitro dissolution of the capsules was determined using the USP basket method and release of the plant drug monitored by UV spectroscopy. The capsules were subjected to accelerated stability testing for estimation of a shelf-life. An HPLC analysis and fingerprint method was employed for this purpose.

Results:

The 4 crude plant powders (RM, PT, CG, S and the mixture) were moderately fine powders with irregular particle shapes, had similar flow properties (angles of repose of 39.23±3.85°, 39.41±1.85°, 35.91±3.24°, 38.16±4.59° and 37.92±1.28°, respectively) and acceptable moisture content level (9.28±0.31%, 8.58±0.43%, 9.31±0.06%, 10.29±0.53%, respectively) which was constant in the final mixture. The solubility studies showed for plant PT contained high level of aqueous extractable matter (77.98±5.82%) and low level for plant S (32.79±2.87%). The content uniformity of manufactured capsules (446.5 ±0.01 mg) was highly acceptable and 50.39 ± 6.76% of the capsule content was released in 45 min. The chromatogram of Phela fingerprints of the capsule content showed minor indications of chemical instability of Phela compounds.

Conclusion:

The 4 constituent plant powders and the final Phela mixture had acceptable pharmaceutical properties that did not complicate capsule manufacturing. The manufactured capsules complied with general pharmaceutical specifications standards, was not rapid releasing but immanently suitable for use in clinical trial.
Evaluation of antihistamines for antimalarial activity against *Plasmodium falciparum in vitro*

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**Purpose:**

The declining efficacy of antimalarial drugs against resistant *Plasmodium falciparum* strains in several endemic regions of the world has highlighted the need for alternate strategies for chemotherapy and chemoprophylaxis. Since malaria is prevalent primarily in third world countries, it is critical for novel therapies to be affordable. Previous research has found that some antihistamines possess inherent antimalarial activity and to cause a marked reversal of chloroquine resistance *in vitro* and *in vivo*. The purpose of this study is to investigate mainly off-patent (generic) antihistamines for antimalarial activity. In this study, the comparative efficacy of a total of 24 antihistamines, representing Histamine₁ (H₁), Histamine₂ (H₂), and Histamine₃ (H₃) receptor antagonists, has been evaluated against chloroquine-sensitive (CQ⁰) and chloroquine-resistant (CQ⁰) strains of *Plasmodium falciparum*. In addition, the study investigated if synergistic interactions could occur between antihistamines and chloroquine that may manifest either as potentiation of the effect of chloroquine on D10, a CQ⁰ strain, or reversal of chloroquine resistance of DD2, a CQ⁰ strain. A preliminary quantitative structure-activity relationship (QSAR) study was also done in which several physicochemical parameters (Pka, molecular mass, LogP, and Log D) were regressed against IC₅₀ values obtained for each drug.

**Methods:**

The parasites were maintained in continuous culture by a method modified from that of Trager and Jensen. The in vitro susceptibility tests for the parasites in the presence and absence of tested compounds were performed using a modified method by Makler et al. Using a non-linear regression analysis in GraphPad Prism, fractional inhibitory concentrations (IC₅₀ = 50% inhibitory concentration) of the synthesized compounds were determined graphically from semi-logarithmic plots.

**Results:**

Amongst the first generation of H₁ receptor antagonists, cyproheptadine and ketotifen (IC₅₀ 1.5-2.5 µg/ml), were the most potent in both CQ⁰ and CQ⁰ strains. The 2nd generation H₁ receptor antagonist’s loratadine and its active metabolite, desloratadine, had the highest activity (IC₅₀ 1.5-2.5 µg/ml). None of the H₂ blockers had any antimalarial effects. Significant potentiation of the antimalarial effect of chloroquine on *P. falciparum* was not observed with any of the antihistaminic drugs. Cyproheptadine and ketotifen exerted a marked synergistic action with chloroquine against CQ⁰ parasites Results obtained from the SAR study showed that antimalarial activity correlated strongly with the Log D values of the test drugs.
YSA 09  A randomised controlled trial of Absorbatox™ C35 in irritable bowel syndrome: A pilot study

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Background:
Irritable Bowel Syndrome (IBS) is a common gastrointestinal disorder with some uncommon aetiology. Various healthcare professionals encounter the syndrome, especially general practitioners and gastroenterologists. IBS sufferers are likely to complain of abdominal discomfort, bloating and altered defecation patterns. Many sufferers have turned to complementary and alternative medicine (CAM) mainly because of ineffective syndrome management through conventional western approaches.

Aim:
To assess the efficacy of Absorbatox™ C35, a natural, non-toxic zeolite, with enhanced ion exchange capacity, as well as water and gas adsorbing properties, in the treatment of IBS in a 6-week randomised, double-blind, placebo-controlled trial with parallel group assignment.

Methods:
Sixty-seven IBS candidates were recruited of which thirty-three patients met the trial entry criteria. A 2-week run-in phase evaluated baseline symptoms. Patients were randomised to receive either 750 mg Absorbatox™ three times daily or placebo for 4 consecutive weeks. Validated questionnaires were used to assess treatment outcomes. Patients were characterised as overall responders if they reported “adequate relief” in 50% of treatment weeks. The severity of patients’ symptoms was evaluated using the IBS Severity Scoring System (IBS-SSS).

Results:
Seventeen patients received Absorbatox™ C35 and sixteen received placebo. A total of twenty-nine (29) patients completed the entire study. At the end of treatment 80% and 50% of patients were classified as overall responders in the Absorbatox™ C35 and placebo groups respectively (p = 0.085). Interestingly, both Absorbatox™ C35 and the placebo groups were associated with significant decreases in the total severity score (p < 0.001 and p = 0.005, respectively). Likewise, both groups were associated with significant decreases in clinical parameters like pain, distension, bowel habit satisfaction and disease interference with life in general. However, no significant differences were observed between the Absorbatox™ C35 and placebo groups in terms of total severity score and separate symptoms ratings. Adverse events were of similar nature in both groups during the entire study (p = 0.259).

Conclusion:
Although the placebo effect was largely present during the trial, Absorbatox™ C35 showed a trend towards better improvement in several endpoint measurements. A larger trial, conducted over an extended period of time, is recommended to elucidate the potential role of Absorbatox™ in IBS management.
YSA 10  Effect of a combination of absorption enhancers on the transport of insulin across Caco-2 cell monolayers

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Purpose:
Insulin is poorly absorbed from the gastrointestinal tract. Oral administration of insulin thus requires formulation intervention to improve absorption for this to be a viable route of administration. \textit{N}-trimethyl chitosan chloride (TMC) and monocaprin (MC) are reported to be effective absorption enhancers for hydrophilic macromolecules. In this study the ability of TMC and MC to enhance insulin permeability across Caco-2 cell monolayers, singly or in combination, was determined at pH 5.0, 6.2 and 7.4. Insulin concentration was determined using HPLC.

The chemical interaction between insulin and TMC as well as between insulin and MC was investigated using differential scanning colorimetry (DSC). As an indication of the potential cytotoxicity of TMC and MC, alone or in combination, Caco-2 cells in suspension were subjected to a tetrazolium salt colorimetric assay.

Results:
TMC at all the studied pH values and MC at pH 5.0 inhibited insulin transport. MC at pH 6.2 and 7.4 significantly ($p \leq 0.05$) enhanced insulin permeability. The combination of TMC and MC enhanced insulin permeation at pH 6.2 and inhibited permeation at pH 5.0 and 7.4. DSC revealed that insulin interacts with TMC at pH 5.0 and 7.4. MC interacts with insulin at pH 5.0, however there appears to be no interaction at pH 7.4. TMC and insulin singly are not toxic to Caco-2 cells, while MC and all the combinations with TMC and/or insulin are cytotoxic.
YSA 11  Synthesis of artemisinin esters as more effective prodrugs against resistant P. falciparum strains

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Background and purpose
The silent killer of Africa, malaria, causes the death of someone every 30 seconds, in mostly women and children. This translates to 300-500 million people infected every year, fatally infecting more than a million people. Artemisinin and its derivatives are developing into a very important new class of antimalarials and their usage is becoming more common in the fight against malaria. The most commonly used and applied of these derivatives are artesunate, artemether and dihydroartemisinin.

One of the problems of dihydroartemisinin is its short half-life, although this is likely to be the reason that resistance hasn’t occurred as of yet.

Aim
The aim of this study is to evaluate the effects of certain compounds on the potential half-life of dihydroartemisinin, thus trying to lower recrudences in malaria patients and improving efficacy. In conjunction, it would lower the total absolute cost of treatment, making it more accessible to the population who needs it most.

Method
Attempting to synthesize a range of ester prodrugs (3-8) of dihydroartemisinin (1) and to determine their efficacy against P. falciparum. Using dihydroartemisinin and a selection of the acyl chlorides (2) enables the synthesis of a wide variety of artemisinin-based ester prodrugs (3) – (8).
Eflornithine derivatives for enhanced oral bioavailability in the treatment of human African trypanosomiasis

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Introduction

Human African trypanosomiasis (HAT), or sleeping sickness, is caused by the parasite protozoa *Trypanosoma brucei gambiense* and *T. b. rhodesiense* and is transmitted to humans by the tsetse fly (*Glossina*). The gradual breakdown of countermeasures, war and civil instability have caused a re-emergence of sleeping sickness with an estimated 30 000 new cases per year. In the first or haematolymphatic stage of the disease the parasite is restricted to the bloodstream and the extracellular tissue; if this stage is left untreated it will progress to the second or meningoencephalitic stage where the parasite penetrates the central nervous system. The first stage is treated with either suramin or pentamidine and has a high success rate with only minor side effects. Treatment for the second stage consists of melarsoprol which is highly toxic. Eflornithine which has fewer side effects is an alternative but is too hydrophilic for oral administration and has to be given intravenously each day for 14 days making this drug too expensive and difficult to administer under African healthcare conditions. Oral eflornithine would greatly decrease the cost of administering the drug and would make the drug available to more people.

Aim

The aim of this study was to synthesize and evaluate a series of eflornithine derivatives for increased oral bioavailability with the ultimate goal to obtain a more affordable alternative to melarsoprol.

Methodology

Computer modelling

Discovery studio, a computer modelling program, was used to predict the fitting of the potential derivatives to the target enzyme. The results of the computer modelling, calculated pharmacokinetic parameters (pKₐ values, log P values and molecular weight) and pharmacodynamic parameters (passive diffusion, facilitated diffusion and carrier mediated transport) were used as criteria to select the most promising prodrugs as target compounds for synthesis.

Experimental

All the derivatives were synthesized in three steps or less by α- and/or δ-amidation and/or esterification.

In the first step eflornithine was dissolved in sodium hydroxide (5N) and reacted with an acyl chloride to form the monosubstituted δ-amides [1-3].

In the second step the monosubstituted δ-amide was dissolved in DMF and bromoethane was added to form the ethyl esters [4-6].

In the third step the δ-amide ethyl ester was dissolved in MeCN with TEA as a base, and the same acyl chloride as in Step 1 was then reacted on the α-amine to give the target compounds [7-9].

All compounds were purified by silica gel column chromatography and the structures confirmed by nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS).

Results

Seven derivatives have been synthesized successfully and their oral bioavailability determined *in vitro* (using an Ussing chamber) and *in vivo* (using rats with a venous catheter). An HPLC method using o-phthalaldehyde (OPA) and N-acetyl-L-cysteine (NAC) was used to identify and qualify the two enantiomers of eflornithine.
YSA 13 The Inhibitory Effect of Erucic acid on Fatty Acids in the Sprague Dawley rat

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Introduction:
X-linked adrenoleukodystrophy (ALD) is a severe neurodegenerative disease causing demyelination of the central nervous system. Accumulation of very-long chain fatty acids (VLCFA), particularly hexacosanoic acid (C 26:0) and tetracosanoic acid (C24:0), in tissues and biological fluids is the hallmark of this disease. To date, there is no effective therapy, except for Lorenzo’s oil, consisting of oleic acid (C18:1) and erucic acid (C22:1). Treatment with Lorenzo’s oil gives a good biochemical response leading to decreased blood VLCFA levels in patients, however, improvement of neurological symptoms has not been reported. Erucic acid competes for the microsomal elongation system, inhibiting saturated VLCFA and could also lead to the inhibition of polyunsaturated fatty acids that are synthesised by the same elongation system.

In this study we determined the optimum dosage at which erucic acid lowers levels of VLCFA and its effect on the polyunsaturated fatty acids.

Methods:
Sprague Dawley rats were divided into 5 groups (no=10) including a control group. Dosages of 400, 575, 600, 625 and 800 mg/kg of erucic acid were given for 7 days by gavage. On day 7 the rats were decapitated and blood collected. For the determination of fatty acids in plasma, a standardised method employing gas chromatography-mass spectrometry (GC/MS) was used.

Results and discussion:
Sprague Dawley rats treated with several dosages of erucic acid showed a decrease in their C22:0, C24:0 and C26:0 concentrations compared to the control group. A dosage of 600 mg/kg erucic acid gave the best inhibition of VLCFA, with a decreased C24:0/C22:0 ratio.
YSA 14  Synthesis of amide derivatives of eflornithine to increase lipid solubility to improve oral bioavailability

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Introduction
Human African trypanosomiasis (HAT) is an endemic disease of sub-Saharan Africa caused by two species of parasitic protozoa, *Trypanosoma brucei gambiense* and *T.b. rhodesiense*, which are transmitted to humans by the bite of various species of *Glossina* (tsetse fly). Eflornithine, given intravenously, is the drug of choice for late stage trypanosomiasis but has been used with little result due to difficult dosage regimes and high cost. This can be attributed to low cerebrospinal fluid (CSF) concentration. Increasing the lipophilicity of eflornithine would enhance its oral bioavailability and thus administer the drug orally with greater efficiency. This could have several advantages such as easier dosage regimes and fewer side effects.

Aim of study
The aim of this study is to synthesise lipophilic derivatives of eflornithine, determine their physicochemical properties namely log D, aqueous solubility and evaluate their oral bioavailability.

Method
Molecular modelling was used to determine ODC cavity binding of several derivatives, given in scheme 1. To decrease the polarity of eflornithine, heterocyclic rings or hydrocarbon chains were attached to the δ amino groups (1-8) by acylation. The target compounds were obtained after purification by silica gel column chromatography.

Scheme 1 – Reaction pathways in the synthesis of eflornithine derivatives

Results
δ-Substituted derivatives of eflornithine have been synthesized and their structures confirmed by nuclear magnetic resonance (NMR). Physicochemical properties, log D and aqueous solubility will be determined. Bioassays are being conducted by an outside institution (Goteborg University, Sweden) to determine the oral bioavailability.
Purpose:
Parkinson’s disease is the second most common neurodegenerative disease after Alzheimer’s dementia. The principal pathological feature of Parkinson’s disease is a loss of dopaminergic neurons in the substantia nigra of the brain. Monoamine oxidase B (MAO-B) is a flavin-adenine dinucleotide (FAD)-containing enzyme that catalyzes the deamination of biogenic and xenobiotic enzymes, such as dopamine, in the periphery as well as in the central nervous system. Inhibition of MAO-B in the brain reduces the catabolism of dopamine and it is used in the symptomatic treatment of Parkinson’s disease. The purpose of this study is to synthesize a series of C-2 substituted benzimidazole analogues and evaluate them as possible MAO-B inhibitors.

Methods:
We have previously shown that (E)-2-styryl-1-methylbenzimidazole analogues (1) are potent inhibitors of MAO-B. In the present study we explored the possibility of designing benzimidazole analogues with enhanced MAO-B inhibition potencies. For this purpose benzimidazole was substituted with a variety of substituents at C-2 to yield a series of 8 compounds. We have also prepared a series of 12 acrylamide analogues (2) in an attempt to determine the importance of the benzimidazole moiety for binding to the active site of MAO-B. All the analogues were evaluated as inhibitors of recombinant human MAO-B. In order to determine the selectivity of inhibition by the compounds, they were also evaluated as inhibitors of recombinant human MAO-A. To gain additional insight into the binding modes and affinity, the compounds were also docked into the active site of human MAO-A and –B using the molecular docking software GOLD®.

Results:
All of the compounds synthesized were found to be inhibitors of MAO-B. For example, (E)-N,3-diphenylacrylamide inhibited MAO-B with an IC$_{50}$ value of 5.65 ± 0.014 μM. This is approximately 16 fold more potent than the lead compound (E)-2-styrylbenzimidazole (IC$_{50}$ = 91.8 μM). This difference in potencies between the acrylamide and benzimidazole analogues may be explained by the possibility that the carbonyl oxygen of the phenylacrylamide analogue can act as an effective hydrogen-bond acceptor in the active site of MAO-B.
YSA 16  The availability and distribution of registered community pharmacies among the South African population, 2003 and 2008

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Purpose:
The South African (SA) health care system is committed by the constitution and National Drug Policy to ensure that every citizen has access to health care including safe, good, quality and essential drugs. Since 2003, various regulatory measures were introduced in an effort to regulate the distribution of community pharmacies (CPs) within SA. This study aims to investigate the changes in the distribution of the CPs among the SA population; which occurred in the presence of the regulations introduced from the year 2003 vs. 2008.

Research Methodology:
The South African Pharmacy Council’s registers of pharmacies of August 2003 and August 2008 were analyzed using the SAS® programme and the CPs in SA of those years were located for the different geographical regions. The total population of each of the geographical regions, for the years 2003 and 2008, was estimated using the Census 2001 data and estimations published in the mid year population estimates of 2008 by Statistics South Africa. The pharmacy per population ratio of the CPS for the geographical regions was calculated for the year 2003 and 2008. The pharmacy per population ratio calculated were then used as benchmark ratio to calculate the number of CPs expected in a particular local municipality, should the CPSs be evenly distributed among the country’s population. The expected number of CPs, as calculated, was then compared to the existing number of CPs in the particular geographical region, to determine which regions was possible “oversupply” and which in “undersupply” of CPs. The results of the 2003 and 2008, were compared to determine any changes that occurred between the two years.

Results:
The total number of registered CPS in SA was 2530 for the year 2003 and 2811 for the year 2008. The pharmacy per population ratio of the CPs improved from one community pharmacy (CP) per 18,216 people in 2003, to one CP per 17,330 people in 2008. The interprovincial variation of the CPs per population ratio ranges from 1:10,531 (Gauteng) to 1:55,393 (Limpopo) in 2003. The range is 1:10,785 people (Gauteng) to 1:36,403 people (Limpopo) for the year 2008. The CPs were distributed as such that, in 2003, 30% of the country’s municipalities were without registered CPs; this decreases to 26% in the year 2008. The total estimated population of the municipalities without a CP was 5.2 million in 2003 and 4.1 million in 2008. Despite the overall improvement in the distribution and availability of CPs in SA, the results demonstrate that, should the CPs in the country be more evenly distributed among the country’s population, the percentage of municipalities in the country that are in “undersupply” of CPs in relation to the population within them, increased from 48% in 2003 to 52% in 2008.

Conclusion:
The availability of registered CPs to the SA population has improved with the increase in the total number of the CPs between 2003 and 2008. Despite this however, the possible misdistribution of CPS among the population has worsened. This aspect needs further investigation.
YSA 17 Dietary treatment of Adrenomyeloneuropathy with Lorenzo’s oil and docosahexaenoic acid. A case study

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Introduction:
Adrenomyeloneuropathy (AMN), one of the variants of X-linked adrenoleukodystrophy (X-ALD), is an inherited genetic disorder classified as a single peroxisomal enzyme disorder that affects the peroxisomal β-oxidation pathway. Biochemically this disease is associated with the accumulation of very long chain fatty acids (VLCFA) in plasma, the central and peripheral nervous system, adrenal glands and testes, primarily causing adrenal insufficiency and neurological deterioration. Lorenzo’s oil (LO), which contains erucic acid (C22:1) and oleic acid (C18:1), is currently used as the treatment for X-ALD patients. Although VLCFA plasma levels of ALD patients were lowered within 4 weeks after treatment with LO, the clinical efficacy is disputed widely as neurological symptoms seem to persist and sometimes even progress. Clinical symptoms like demyelination, visual impairment and psychomotor impairment in peroxisomal disorders may be owed partly to a docosahexaenoic acid (DHA) deficiency, because approximately 30-40% of neural membranes consist of DHA.

This study aims to provide evidence that additional supplementation with DHA to LO may increase plasma and red blood cell DHA levels and therefore improve the neurological prognosis of ALD patients.

Methods:
The fatty acid levels of an AMN patient as well as one female carrier were determined. Both were taking LO for at least 16 months, which included 10 months of additional DHA supplementation. An MRI scan was done before and after 6 months of DHA treatment.

Results and discussion:
The MRI scan illustrated that our AMN patient already showed white matter lesions typical of ALD. DHA treatment of the AMN patient increased red blood cell DHA. Although the MRI showed no neurological improvement after 6 months of DHA treatment, no selective progression of demyelination was detected.
YSA 18 Novel polycyclic analogues of non-steroidal anti-inflammatory drugs for increased blood-brain barrier permeability

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Background:
Various drug candidates to treat Alzheimer’s and Parkinson’s diseases are under investigation and studies are being done to increase drug permeability across the blood-brain barrier (BBB) for drug delivery into the central nervous system (CNS). Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the groups of drugs receiving much attention in the search for neuroprotective compounds. Most of these compounds however, show unfavourable BBB permeability and various strategies to overcome this shortcoming are being investigated. The chemistry and pharmacological properties of polycyclic cage compounds have intrigued many researchers over the past few decades. Many of these compounds have important pharmaceutical applications, ranging from the symptomatic and proposed curative treatment of neurodegenerative diseases such as Parkinson’s disease and Alzheimer’s disease. Studies done in our laboratory indicated favourable distribution of the polycyclic structure, pentacyclo[5.4.0.0²,6.0³,10.0⁵,9]undecane into the brain. It is thus hypothesised that this structure can be used as carrier molecule to deliver drugs into the CNS.

Objective:
In this study our main objective was to enhance CNS delivery of the NSAIDs, ibuprofen and acetylsalicylic acid. This was done by conjugating the drugs by means of a chemical linker to the polycyclic moiety, pentacyclo[5.4.0.0²,6.0³,10.0⁵,9]undecane by amination, amidation and esterification. These novel synthesised compounds have the potential to be used in the prevention and treatment of neuronal inflammation and oxidative stress as observed in Parkinson’s disease and Alzheimer’s disease. Previous compounds synthesised in our laboratory, where both ibuprofen and acetylsalicylic acid were directly conjugated to the polycyclic moiety to yield a rearranged cage structure, were used to compare BBB permeability as well as antioxidant activity to the newly synthesised prodrugs.

Methods:
The test model developed in our laboratory was applied to determine the BBB permeability of the synthesised compounds. A highly sensitive ESI-MS/MS analytical procedure, which was developed for the detection of test compounds in biological preparations, was applied. Adult male C57BL/6 laboratory mice (25-30g) were used to compare the CNS delivery of the prodrugs with that of the free drugs. A lipid peroxidation assay was performed to determine the antioxidant activity of the synthesised compounds.

Results:
This procedure gave a clear indication that the synthesised prodrugs cross the BBB and enter the CNS in more favourable quantities than the free drugs. The compounds synthesised in this study thus presented good BBB permeability as well as increased antioxidant activity when compared to the free drugs and previously described cage structures.
The synthesis and evaluation of 8-benzyloxycaffeine analogues as inhibitors of MAO

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Purpose:
Monoamine oxidase (MAO), specifically MAO-B plays a key role in the therapy of Parkinson’s Disease (PD) since it is the major enzyme responsible for the catabolism of dopamine in the substantia nigra of the brain. Inhibition of MAO-B may thus conserve dopamine in the brain and provide symptomatic relief to PD patients.

Selegiline is a MAO-B inhibitor currently being used for the treatment of PD. Selegiline has psychotoxic and cardiovascular side effects along with the additional disadvantage that it is an irreversible inhibitor. This justifies the need to develop new, safe and reversible inhibitors of MAO-B for the treatment of PD.

Rationale:
Recently discovered reversible MAO-B inhibitors include safinamide and (E)-8-(3-chlorostyryl)caffeine (CSC). Safinamide has a benzyloxy side chain which is thought to be crucial for inhibition of MAO-B. CSC, on the other hand, consists of a caffeine ring with a styryl substituent at C-8 which is also a critical feature for its inhibitory activity. In this study the caffeine ring and the benzyloxy side chain will be combined to produce a series of 8-benzyloxycaffeine analogues as potent new MAO-B inhibitors.

Methods:
All the target compounds were synthesized by condensing 8-chlorocaffeine with the appropriate benzylalcohol under high temperatures in the presence of metallic sodium. Inhibition activities were determined using baboon liver MAO-B, recombinant human MAO-B and MAO-A. The inhibitor potencies were expressed as IC\textsubscript{50} values.

Results:
The results showed that the target compounds potently inhibit human as well as baboon MAO-B. The most potent inhibitor was 8-(3-bromobenzyloxy)caffeine with an IC\textsubscript{50} of 0.068 ± 0.003 µM toward human MAO-B. The values obtained for human MAO-B compared very well to that of baboon MAO-B, which indicates that the active site and inhibitor specificities of these two enzymes are similar. Additionally, the inhibitors showed inhibition activity towards human MAO-A with the most potent inhibitor having an IC\textsubscript{50} of 0.397 ± 0.013 µM.

It was determined that the target compounds bind reversible to all three enzymes and that the mode of inhibition is competitive. A Hansch-type structure-activity relationship (SAR) study showed that MAO-B inhibition activities correlate with the Hansch lipophilicity constant and the Swain-Lupton electronic parameter.

The observation that 8-benzyloxycaffeine analogues inhibit both MAO-B and MAO-A makes these compounds ideal drug candidates since both enzymes are targets for the treatment of PD.
YSA 20  Artemisinin based amine analogues for increased anti-malarial efficacy

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Background
Drug-resistance to classical and existing anti-malarial drugs is a challenging problem in malaria control in most parts of the world, contributing to the emergence of developing new compounds for malaria treatment. Artemisinin and its derivates are of special biological interest because of their outstanding anti-malarial activity against chloroquine-resistant \textit{P. falciparum} and cerebral malaria. The reason for this is because of their unusual chemical structures and the difference in their mechanism of action compared to other anti-malarials. However, the use of such endoperoxides is restricted by their poor oral bioavailability, a short plasma half-life (30 minutes in plasma) and the high rate of recrudescent infections when used as mono-therapy in short-course treatments, even if these drugs have a rapid onset of action and low reported toxicity.

Purpose
The aim of this study was to synthesise artemisinin derivatives, attempting to overcome the pharmacokinetic deficiencies of the artemisinin endoperoxides, so that it can be possible to produce a new one-day treatment alternative in the future, as well as a more cost effective treatment.

Methods
The compounds were synthesised using standard organic chemical procedures to produce artemisinin based amine analogues (2 – 7). The following amines were selected, based on Lipinski’s rule of 5, to employ in this research project as substituents on C-9 of DHA (1): 4(2-aminoethyl)morpholine, N,N-dimethylethylenediamine, 1-amino-4-methylpiperazine, 4-aminomorpholine, 1(3-aminopropyl)imidazole and 4-amino-4H-1,2,4-triazole. Dihydroartemisinin (1) is linked to the various amine compounds through a one step reaction that is catalyzed by boron trifluoride diethyl etherate, to form artemisinin based amine analogues.

\begin{equation}
\text{R-NH}_2 + \text{Dihydroartemisinin (DHA) (1)} \rightarrow \text{Amine analogues}
\end{equation}

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Results
The structures of the products were confirmed by physical methods such as nuclear magnetic resonance spectroscopy (NMR), mass spectroscopy (MS) and the \textit{in vitro} anti-plasmodial activity determined against cultures of \textit{P. falciparum} (chloroquine sensitive and chloroquine resistant).
YSA 21  Formulation and development of a gastroretentive drug delivery system for the delivery of narrow absorption window drugs

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Purpose

A gastroretentive drug delivery system has been developed in order to deliver metformin over a 12 hour period. The system comprised ionically crosslinked mucoadhesive gastrospheres that are also buoyant within the stomach.

Methods

A polymeric emulsion was prepared by blending an aqueous solution of alginate, pectin, PAA and metformin with an organic solution of PLGA. The emulsion was then added drop-wise through an 18G needle into a crosslinking solution of 2% Ca(OH)$_2$. The gastrospheres were allowed to cure for 30 minutes, after which they were collected, washed and frozen at -72°C for 24 hours. Lyophilisation was conducted on the gastrospheres at -60°C and 25mtorr for 24 hours. A randomized Box-Behnken statistical experimental design was constructed in order to model the number of formulations required for optimization. The design consisted of 15 statistically derived formulations of various polymer combinations. Drug entrapment was determined by immersing gastrosphere samples in 100mL PBS (pH 7.4, 37°C) and allowing complete drug release. Drug release studies were performed using a USP 32 type 2 dissolution apparatus. Samples were immersed under a wire mesh in 900mL SGF (pH 1.2, 37°C) at 50rpm and dissolution media was removed at predetermined intervals and analysed by UV. Buoyancy studies were conducted by immersing the gastrospheres in simulated gastric fluid (SGF) and observed. Bioadhesion was determined using a TA.XT plus Texture Analyser (Stable Microsystems, UK) with a simulated gastric membrane covering both the probe and platform stage. In vivo drug release studies have been conducted on pigs which have surgically implanted chronic jugular catheters. The drug delivery system was administered via an intragastric tube while under sedation. Blood samples were extracted via the jugular catheter Metformin concentrations were determined by UPLC.

Results and Discussion

Drug release profiles were classified into three distinct types comprising zero-order release, burst release and first-order release. Drug entrapment ranged between 25-53%. It was observed that the gastrospheres displayed immediate buoyancy. The average buoyancy after 8 hours was 98% and 96% after the full 12 hour period in SGF. Bioadhesion was primarily due to PAA, while the inclusion of PLGA lowered bioadhesion. The Box-Behnken design resulted in the optimisation of the gastrosphere formulation, achieving maximum bioadhesion as well as zero-order drug release. In vivo studies revealed that the gastrospheres resulted in drug release over a 12 hour period, the profile of which depicted a much more controlled release than the gold standard (Glucophage® 500).

Conclusions

The gastrosphere drug delivery system has proven to be retained within the stomach, resulting in a more desirable drug release profile, which will reduce dosing frequencies and side-effects and improve the bioavailability of NAW drugs such as metformin, thus improving patient compliance.
Drug-resistant malaria poses a major crisis to public health in large parts of the world and the urgent need for safe and effective new therapies are recognised globally. Artemisinin and its derivates are currently the benchmark of antimalarial drugs, but they have the drawback of a very short half-life.

The purpose of this study was to synthesise novel mutual prodrugs of artemisinin with potent antimalarial activity and a longer half-life. These mutual artemisinin prodrugs are built from dihydroartemisinin (DHA) and are linked to the different antimalarial drugs currently available. This was a chemical combination which distinguishes it from what is the WHO’s treatment of choice - artemisinin combination therapy (ACT), which is a physical combination. When a drug with a long half life is combined with another drug that has a short half life, the latter drug’s half life will be prolonged. This is based on a mechanism called formation rate limited (FRL) metabolite kinetics. The selection of the current available antimalarial drugs was based on chemical structure and pharmacological activity. In order of priority these drugs are: primaquine; pyronaridine; isoquine and mefloquine. Para-nitrophenylchloroformate (p-NPC) was used to activate DHA in order to form the linkage between the two drugs through nucleophilic substitution. This activated DHA (structure confirmed with NMR) could form a carbamate or a carbonate prodrug with the existing antimalarial drugs through the attack of either an R-OH or an R-NH on the carbonate carbon.

These drugs will be evaluated by means of various biological tests, which include: in vitro antiplasmodial activity; cytotoxicity assay; in vivo test for antimalarial activity in mice infected with Plasmodium berghei; pharmacokinetic evaluation of artemisinin prodrugs and formation rate limited (FRL) metabolite kinetics. Ultimately these tests will be important determinants of whether artemisinin-based antimalarial mutual prodrugs had significant pharmacological improvements to those most in need of antimalarial treatment.
**Purpose:**
The primary aim of this study was to determine prescribing patterns of antibiotics for in- and outpatients at hospitals in Lesotho and to determine the possible impact of appropriate prescribing of antibiotics on treatment outcomes, days of patient hospitalisation and costs of antibiotic treatment.

**Research methodology**
Relevant data from case notes of all patients on antibiotic treatment in inpatient departments and all antibiotic prescriptions from outpatient departments were collected from June 15th to July 15th 2006 at five (5) study site hospitals. All prescriptions were assessed and classified into categories of appropriateness based on their conformities to criteria developed from principles of antibiotic prescribing. Analyses were further conducted to determine the possible impact that appropriate prescribing may have on treatment outcomes, days of patient hospitalisation and costs of antibiotic treatments.

**Results:**
A total of 307 inpatients and 867 outpatient prescriptions were assessed. Total frequencies of prescribed antibiotics were 584 and 1073 for inpatients and outpatients respectively. Antibiotics most frequently prescribed for inpatients were ampicillin (29.0%) metronidazole (16.1%), cloxacillin (12.8%), cotrimoxazole (10.8%), gentamicin (8.9%) and penicillin (7.9%). Most frequently prescribed antibiotics for outpatients included ampicillin (23.9%), cotrimoxazole (17.0%) erythromycin (15.6%), cloxacillin (10.6%) penicillin (10.2%), metronidazole (7.7%) and ciprofloxacin (5.3%). For inpatients antibiotics were most often prescribed for skin and soft tissue (31.7%) and respiratory tract (28.9%) infections. Outpatients received the most antibiotics for respiratory tract (42.0%) and skin and soft tissue infection (21.9%) respectively. Of the 307 inpatient prescriptions 41.6% were appropriately written for either empiric treatment or prophylaxis of infections while 57.1% did not conform to antibiotic prescribing principles. The majority (81.0%) of the 867 outpatient prescriptions were in line with the prescribing principles. Prescriptions written appropriately were also composed in part of 34.8% and 43.6% of total prescription that were written for established and suspected bacterial infections. Inpatient antibiotic prescribing patterns based on principles of antibiotic prescribing have had a positive impact on treatment outcomes, days of hospitalisation and costs of antibiotic treatment. In outpatients prescribing of antibiotics based on principles of antibiotic prescribing failed to show any significant impact on costs of antibiotic. Due to geographical challenges the outcome for treatment could not be determined.

**Conclusion:**
Ampicillin was the most frequently prescribed antibiotic for both in- and outpatients. More outpatients received antibiotics according to the principles than inpatients. Rational prescribing had a positive impact on treatment outcomes. Promoting rational antibiotic prescribing remains a challenge.
Primary aim:
The aims of this project were to i) determine bacterial pathogens commonly associated with infections in patients at hospitals in Lesotho; ii) determine their sensitivity patterns to formulary antibiotics; and iii) develop procedures for appropriate selection of antibiotics in empiric treatment of infections.

Research Methodology
Data on bacterial pathogen sensitivities to formulary antibiotics were collected from records of culture sensitivity test results from January 2000 to June 2005 from microbiology laboratories of selected hospitals in Lesotho. The data were analysed to determine pathogen associations with various infections and their sensitivity patterns to formulary antibiotics. A formula was developed from first principles applied in selecting preferable antibiotics in the empiric treatment of given infections using data on pathogens’ sensitivities to antibiotics.

Results:
Analysis of data revealed the following pathogens as predominantly associated with infections in the study sites. They include *Staphylococcus aureus* - lower respiratory tract, eye, ear, skin and soft tissue infections and penile and vaginal discharges; *Streptococcus pneumoniae* - meningitis; *Escherichia coli* - ascites and urinary tract infections; non haemolytic streptococci - throat infections and *Proteus* - septicaemia. Increases in yearly resistance rates of bacterial pathogens against given antibiotics between January 2000 to December 2005 were seen in the following organisms and antibiotics: *Staphylococcus aureus* - ampicillin (47.0% to 71.0%), penicillin (68.9% to 87.1%), cloxacillin (17.1% and 36.4%), cotrimoxazole (54.0% to 84.0%), cefotaxime (11.0% to 53.0%) and gentamicin (25.0% to 42.0%); *Streptococcus pneumoniae* - tetracycline (14.0% to 39.0%); non haemolytic streptococci - gentamicin (14.0% - 66.7%); *Escherichia coli* - erythromycin (51.0% to 86.0%) and cotrimoxazole (43.0% to 79.0%) cefotaxime (7.9% to 33.3%); *Klebsiella* spp - cotrimoxazole (48.0% to 79.0%); Based on rates of isolation and sensitivities to various antibiotics of bacterial pathogens associated with indicated infections as applied in the use of the formula developed for appropriate selections of antibiotics, the following antibiotics were identified from the group of agents for which data were available as most appropriate in the empiric treatment of infections indicated against them. They are in order of 1st, 2nd and 3rd choices: Chloramphenicol, ciprofloxacin and ampicillin for meningitis; Ciprofloxacin, tetracycline chloramphenicol for ascites; Ciprofloxacin, tetracycline chloramphenicol for lower respiratory tract infections with pleural effusions; Ciprofloxacin, chloramphenicol and ampicillin lower respiratory tract infections without pleural effusions; Ciprofloxacin, chloramphenicol and ampicillin for throat infections; Ciprofloxacin, tetracycline chloramphenicol for ear infections; Ciprofloxacin, chloramphenicol, tetracycline for urinary tract infections; Ciprofloxacin, tetracycline, chloramphenicol for urinary tract infections complicated with penile and vaginal discharges.

Conclusion:
Knowledge of local bacterial pathogen sensitivity patterns to formulary antibiotics and patterns of bacterial pathogen associations with infections enabled appropriate selection of antibiotics for empiric treatment of infections. Periodic updates of lists of pathogens associated with infections and also of bacterial pathogen antibiotic sensitivity data are required to make antibiotic selections always current and effective.
Factors influencing prescribing patterns of antibiotics in a section of health service areas in Lesotho

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Purpose:
The primary aim of this study was to determine factors that influenced the prescribing patterns of antibiotics by prescribers at a section of hospitals in Lesotho.

Research Methodology:
A structured questionnaire survey aimed at investigating factors influencing antibiotic prescribing patterns in Lesotho and which targeted all prescribers both doctors and nurses within Health Service Areas abounding five (5) hospitals selected for this study was carried out. Out of 74 prescribers targeted, 69 questionnaires were reached with questionnaires. Out of the total 69 questionnaires distributed 51 were returned. Data were analysed by means of the SAS 9.1® programme to determine factors attributing to or influencing manners of antibiotic prescribing by prescribers.

Results:
Of a total of 51 respondents, 70.6% (n=36) composed only of doctor respondents who have laboratory facilities at their hospitals. Of the 36 respondents, 43.2% reported their laboratories do not provide information on gram stain properties of microbial isolates. Twenty-seven per cent of respondents reported not considering patients’ clinical conditions when they decide to prescribe antibiotics. Similarly, 25.5% of respondents also in the minority said they were influenced by patients’ request for antibiotics as they prescribe antibiotics. Majority 90.2% of respondents would always prescribe antibiotics if they were not sure of their diagnosis. On the question if diagnostic means used in establishing presence of bacterial infections before prescribing antibiotics, 58.8% and 70.6% indicated using respectively laboratory investigations and physical examination to establish the presence of bacterial infections. In a test of prescribers’ knowledge in bacteriology and principles of antibiotic prescribing as many as 58.8% of respondents showed performance levels classifying them as having poor to very poor knowledge in these areas. Test of respondents’ practical demonstration of knowledge in recognition of signs and symptoms and of bacterial pathogens associated with upper and lower respiratory tract infections (URTI & LRTI) and non-sexually transmitted urinary tract infection (NSTUTI) showed 78.4%, 39.2% and 33.3% of respondents respectively incorrectly indicated signs and symptoms of bacterial infections of URTI, LRTI and NSTUTI. Similarly, 51.0%, 49.1% and 58.8% incorrectly indicated bacterial pathogens associated with these infections. Test of use of knowledge in morphological characteristics of organisms in antibiotic selections also showed as many as 98.0% and 56.9% of respondents not being able to select the correct antibiotic from a group of three antibiotics in treating surgical wound infections of gram positive cocci and gram negative bacilli.

Conclusion:
Conclusions drawn from results indicate the following as factors influencing antibiotic prescribing patterns in hospitals in Lesotho: deficiencies in systems of laboratory results reporting inabilitys on the part of some prescribers to establish presence of bacterial infections; prescribers’ lack of adequate knowledge of the characteristics of bacterial pathogens and activity patterns of antibiotics.
Isolation of antiplasmodial metabolites from the marine alga *Sargassum heterophyllum*

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**Purpose:**

Malaria remains one of the most deadly diseases in sub-Saharan Africa with reported clinical cases and mortality rate of 300–500 million and 1–3 million respectively each year. Some form of resistance has been reported for each available anti-malarial agent. For example, compounds such as chloroquine are no longer useful in many parts of the world where malaria is endemic. This emphasizes the urgent need for development of new anti-malarial agents. This presentation describes the isolation, characterization and antiplasmodial activity of natural product from *Sargassum heterophyllum*.

**Method:**

*Extraction and Isolation:* The alga was sequentially extracted with MeOH and CH₂Cl₂:MeOH and the combined organic crude extracts were fractionated by solvent-solvent partitioning. Silica gel chromatography followed by normal phase HPLC afforded four pure compounds. Structures of the pure compounds were elucidated by analysis of one- and two-dimensional NMR data.

*Antiplasmodial Assay:* All samples were tested in duplicate against a chloroquine sensitive (CQS) *Plasmodium falciparum* (D10 strain) using the lactate dehydrogenase assay.

**Result:**

Four compounds, sargaquinoic acid (1), sargahydroquinoic acid (2), sargaquinal (3) and fucoxanthine (4), were isolated from *S. heterophyllum*. Compounds 1 and 2 showed moderate antiplasmodial properties against CQS *P. falciparum* D10 strain (IC₅₀ 12.0 and 15.2 µM, respectively) while 3 and 4 showed the most promising activity with IC₅₀ values of 2.0 and 1.5, µM respectively.
Antimycobacterial activity of South African medicinal plants

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The recent increase in the incidence of tuberculosis with the emergence of multidrug-resistant (MDR) cases has lead to the search for new drugs that are effective against MDR strains of \textit{Mycobacterium tuberculosis} and can augment the potential of existing drugs against tuberculosis. In the present study, we used a Microplate Alamar Blue Assay (MABA), an \textit{in vitro} high throughput screening method, to investigate the activities of 20 medicinal plants against \textit{M. tuberculosis}. Six of the tested plant extracts exhibited the Minimum Inhibitory Concentration (MIC) ranging from 0.15 - 5 mg/ml. The ability of these six plant extracts to inhibit mycobacterial growth is promising and they may become lead compounds for future drug development for the treatment of \textit{M. tuberculosis} infections.
Introduction:
Medicine-taking behaviour is influenced by patient knowledge, beliefs, attitudes and expectations. Self-efficacy, which is a key factor influencing adherence, refers to patient confidence in the ability to self-manage medicine-taking. The HIV Medication Taking Self Efficacy Scale (HIV-ASES) was developed for use in an English-speaking, literate, North American population. In contrast, many South African patients on antiretrovirals (ARVs) are low-literate and are unfamiliar with the concept of rating scales. Instruments measuring behaviours therefore require modification and testing in the target population prior to their use.

Objectives:
The objectives of this research were to modify an existing self-efficacy instrument for use in a Xhosa low-literate population to ensure cultural acceptability and understanding of questions, to develop a visual analog scale as an alternative to the existing numerical rating scale, and to evaluate these tools.

Method:
The study was conducted in Grahamstown. In a group discussion, the 11 individual HIV-ASES questions were scrutinized for clarity and simplicity and cultural acceptability. The resulting questions were translated into isiXhosa using a multi-stage translation - back translation process. Four modified versions of the numerical rating scale (0-10) were developed, with three of these including visual images. The questions and the rating scale underwent preliminary qualitative evaluation in focus group discussions with two groups of varying degrees of low literacy. The discussions were recorded and responses subsequently analysed. A pilot study was conducted in 20 isiXhosa AIDS patients on ARVs prior to the instrument being used in a clinical trial. Ethical approval for the study was obtained from all academic and health care institutions involved.

Results:
The self efficacy scale appeared to be well understood. Interestingly, 100% of the patients felt confident about taking their medication correctly, which could be a result of the intensive counselling they receive. Confidence in persisting with ARVs decreased to 84% when side effects occurred. Confidence was also negatively influenced (90%) by being in the presence of others who were unaware of the patient’s HIV status. Patients in this population did not appear to fully appreciate or utilise the full range of the scale. The visual analog scale was preferred over the numerical scale.

Conclusions:
Instruments used to measure medicine-taking behaviour must be adapted to specific patient populations and be evaluated in that population before use. Further research is needed to develop and validate alternatives to the 11-point rating scale used in the original test.
Antimicrobial and antifouling metabolites from the South African marine alga, Laurencia flexuosa

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Purpose

The emergence of drug resistant microorganisms is a major concern for human health and new drugs to combat this problem are urgently required. In our search for new antimicrobial agents we have focused our attention on marine algae. Marine algae protect themselves from infection and fouling organisms by producing secondary metabolites. In this study we have investigated the antimicrobial extract of an endemic South African marine alga, Laurencia flexuosa Kützing.

Methods

Isolation of metabolites: The alga was collected near Port Elizabeth and extracted with MeOH followed by MeOH-dichloromethane (1:2). Isolation of the metabolites followed our standard purification procedure which incorporates solvent-solvent partitioning, silica gel column chromatography and semi-preparative normal phase HPLC.

Antimicrobial and antifouling activity: The pure compounds were tested for antimicrobial and antifouling activity against Pseudomonas aeruginosa using a simple microtitre plate method and staining using crystal violet.

Structure elucidation: The structures of the pure compounds were determined by analysis UV, IR one- and two-dimensional NMR (1H, 13C NMR, COSY, HSQC, HMBC and NOESY) spectroscopy and mass spectrometry.

Results

A new cuparene sesquiterpene 4-bromo-2-(5-hydroxy-1,2,2-trimethylcyclopent-3-enyl)-5-methylphenol was isolated along with two geometric isomers of the vinyl acetylene bromofucin. An halogenated monoterpene 3S*,4R*-1-bromo-3,4,8-trichloro-9-dichloromethyl-1-E,5-E,7-Z-octatriene was also isolated but was suspected to be a contaminant and an investigation into its biological source revealed that it originated from Plocamium suhrii. This compound showed excellent antibacterial activity but could not prevent biofilm formation when employed as a film on the surface of microtitre plate wells.
Introduction:

Gunnera perpensa, also known as river-pumpkin, ughobo or uklenza (isiZulu), ipuzi lomlambo (isiXhosa) and qobo (Sesotho), is a common traditional medicine for various maternal ailments, as well as for inflammation and cancerous sores. Drewes et al. (2005) reported that the main active ingredient of G. perpensa, z-venusol, inhibits the growth of various human pathogens, such as S. epidermidis, E. faecalis, S. aureas and B. cereus, in vitro. To date, no mechanism of action has been elucidated, nor has the anti-cancer potential of venusol been screened.

Purpose:

To investigate the in vitro regulation of cell proliferation by venusol using the human cervical cancer cell line HeLa.

Methods:

Commercial HeLa cells were cultured to approximately 50% confluence before incubation for 24 hours with freshly-prepared venusol, at serial dilutions ranging from 2400ug/ml to 2.2ug/ml. The conventional MTT assay was used to determine cell proliferation, measured against non-exposed controls. All dilutions were performed in quadruplicate and the experiments repeated three times.

Results:

There was a significant decrease in cell proliferation at concentrations of 2400ug/ml (23%, p < 0.0001) and 1200ug/ml (9%, p = 0.023), while, interestingly, at a concentration of 9.4ug/ml venusol, there was an increase in proliferation of 15.5% (p = 0.015). No gross cytotoxic damage, including cell necrosis, was observed at any venusol concentration for the duration of incubation period, even when extended to 48 hours.

Conclusion:

These preliminary findings suggest that venusol may have the potential to influence cervical carcinoma cell proliferation. Further investigations, including apoptosis and cell cycle assays, are planned to confirm these results and elucidate a mechanism of the action of venusol.
Purpose:
The aim of this study is to investigate the presence of antimicrobial compounds in the leaves of *Syzygium cordatum* and further to isolate and identify the antimicrobial compounds.

Methods:
Phytochemical analysis: Chemical constituents of the extracts were analysed by thin layer chromatography (TLC) using aluminium-packed TLC plates. The TLC plates were developed under saturated conditions with one of the three eluent systems that separate components of Syzygium extracts well i.e. Ethyl acetate/methanol/water (40:5.4:5): (EMW) Chloroform/ethyl acetate/formic acid (5:4:1): (CEF);Benzene/ethanol/ammonia hydroxide (90:10:1): (BEA). The dried extracts of the solvents were reconstituted to a concentration of 10 mg/ml in acetone. Approximately 100 µg aliquots (10 µl of a 10 mg/ml solution) of each of the extracts were loaded in 1 cm bands on three TLC plates and each of these was developed with EMW, CEF or BEA. Once developed, the separated compounds were observed under UV light at 360 nm and 254 nm and the fluorescing (360 nm) or quenching (254 nm) compounds marked. To detect the separated compounds, vanillin-sulphuric acid (0.1 g vanillin: 28 methanol: 1 ml sulphuric acid) was sprayed on the chromatograms and heated at 110 °C to optimal colour development.

TLC-DPPH antioxidant screening: Ten microlitres of each extract was loaded as a 1cm band, 1cm on the origin of the TLC plates. Plates were developed using BEA, CEF and EMW. To detect antioxidant activity, chromatograms were sprayed with 0.2 % 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) in methanol, as an indicator until just wet, and dried in the fume hood. The presence of antioxidant compounds was detected by yellow spots against a purple background.

Minimum inhibitory concentration: A serial microdilution assay was used to determine the minimum inhibitory concentration (MIC) values of the extracts using p-iodonitrotetrazolium violet (INT) reduction as an indicator of growth. Residues of the different extracts were dissolved in acetone to a concentration of 10 mg/ml. 100µl of the extracts were serially diluted 50% with water in 96 well microtitre plates Antimicrobial cultures were transferred into fresh Sabouraud dextrose broth, and 100µl of this was added to each well. Acetone blanks were included as negative control. As an indicator of growth, 40 ml of 0.2 mg/ml of INT dissolved in water was added to each of the microplate wells. The covered microplates were incubated for two days at 35°C and 100% relative humidity. The MIC was recorded as the lowest concentration of the extract that inhibited antifungal growth after 24 hours.

Results:
The TLC plates showed separation of components of the *S. cordatum*. For the TLC-DDPH antioxidant screening the acetone extract in CEF shows the presence of antioxidant compounds followed by the methanol extract. For the MIC the acetone extract and methanol extract were very active against all bacteria species tested with MIC varying between 0.16 and 2.5 mg/ml.
PG1 10 Services for which pharmacists may levy a fee: Intramuscular and subcutaneous injections and immunisation

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Purpose
Administration of intramuscular or subcutaneous injections and immunization is one area which encompasses the expanded role of the pharmacists. The pharmacist involvement with immunization may vary with each practice setting, with activities incorporating educating the public and other healthcare professionals, advocating paediatric immunisation, providing immunisation for international travel, screening patients at risk of preventable infections by occupation, lifestyle or underlying disease state, administering immunisation agents, recording immunisation data and using data base to generate reminders for booster doses. The purpose of the study was to determine the extent of provision of intramuscular and subcutaneous injections and immunisation services in community pharmacies and private institutional pharmacies in South Africa and the average time taken to complete the service in the sample.

Results
From the study population, 107 (1.96%) community pharmacies and private institutional pharmacies provided both intramuscular(IM)/subcutaneous(SC) injections and immunisation services. 220 IM/SC injections were administered in community pharmacies. 271 immunisation cases were recorded of which 270 were community pharmacies and 1 was a private institutional pharmacy. Western Cape and the North West Province were the leading pharmacies rendering the service at 29.55% and 20.91% respectively. Mpumalanga recorded the lowest percentage at 0.45%. Western Cape rendered 32.84% of the immunisation service was rendered. Limpopo contributed only 0.74% toward the immunisation service. Measuring of both IM/SC injection and the immunisation service was done by dividing it into 3 phases; a pre-administration procedure which was performed in 92.27% of the cases in IM/SC and 97.79% in immunisation), administration of injection (performed 100% in both IM/SC and immunisation) and documentation and record keeping (performed in 83.64% of cases in IM/SC and 95.57% in immunisation). All three phases of the service were provided by the pharmacist at least 49% of the time during IM/SC and 35% of the time in immunisation. On average the pharmacist provided the immunisation service at least 35% of the time. Administering of an IM/SC injection took a weighted mean time of 316.58 seconds (5.28minutes) (Standard error (SE) = 28.04) seconds. To successfully complete all three phases in immunisation, the weighted mean time taken was 349.87 seconds (5.83 minutes) (SE = 28.47 seconds). Provided the pharmacy has met with the requirements to provide these services, a proposed fee of R39.70 inclusive of VAT for an IM/SC injection and R44.70 inclusive of VAT for immunisation has been estimated from this study.

Conclusion
Injection and immunisation services are underexplored as part of the pharmaceutical services. Such services can be more effectively achieved by employment of nursing staff. A further study of morbidity and mortality versus the availability of these services by pharmacists may highlight the value of this pharmacist role.
PG1 11 An analysis of the usage and cost of hipolipidaemics in the private health care sector

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Purpose:
More than 5.5 million South Africans aged 30 years and older are at risk of chronic disease by virtue of their triglyceride levels (Maritz, 2006). Lowering of cholesterol levels can result in a 12% reduction in all-cause mortality; a 19% reduction in cardiovascular heart disease mortality and 21% reduction in major vascular events (Baigent et al., 2005:366). However, the second highest therapeutic group, based on expenditure, is the hipolipidaemic drugs (Bester et al., 2005:8) in the already financially strained private health care sector of South Africa. The purpose of this study was therefore to characterise the usage and cost of hipolipidaemic drugs in the private health care environment in South Africa.

Methods:
A quantitative retrospective drug utilisation review was performed using dispensing records from a medicine claims database. Data for a two-year period (1 Jan. 2005 to 31 Dec. 2006) was used to determine the utilisation patterns and costs of drugs associated with the management of dyslipidaemia.

Results:
The database consisted of 19,860,593 and 21,473,062 medicine item claims for 2005 and 2006 respectively, at a total cost of R 1,893,376,921.00 (for 2005) and R 2,046,944,383.00 (for 2006). Hipolipidaemics represented between 3.2% and 3.3% of the total number of items claimed during the study period. The total cost of hipolipidaemics accounted for 5.6% and 5.8% respectively of the total cost of all medications claimed during the study period. The average cost per item of hipolipidaemics (i.e., R170.63 ± 70.19 and R167.08 ± 71.93) was relatively higher compared to the average cost of all items on the database (R95.33 ± 192.21 and R95.33 ± 227.99 respectively).

Statins represented the highest percentage (96.03% and 95.23% respectively) of hipolipidaemics claimed on the database [N =626,759 (2005); N =705,424 (2006)]. Of the items claimed for both study periods, simvastatin was the most commonly claimed, accounting for 45.35% and 46.21% respectively of the number of hipolipidaemic items claimed, at a total cost of 30.97% and 31.38% for 2005 and 2006 respectively. Atorvastatin followed second at 41.50% and 37.79% respectively; at a cost of 53.10% (N = R106,943,348.53) and 50.13% (N = R117,862,631.87) of the total cost of hipolipidaemic active ingredients in 2005 and 2006 respectively. Of all the hipolipidaemic drugs utilised on the database, only three active ingredients (bezafibrate, simvastatin and pravastatin) had generic equivalents available at the time of the study. With total substitution (100%) of these three drugs with the average price of the available generic hipolipidaemic equivalents on the database, a cost saving of R1 744 462.27 or 1.63% (N = R106 943 348.53) was possible in 2005. In 2006, a total cost saving of R1 526 985.79 or 1.30% (N = R117 862 631.87) was calculated.

Conclusion:
The high average cost per prescription of hipolipidaemic drugs indicates that they are relatively expensive in comparison to other medications. Generic (and therapeutic) substitution should be investigated as potential cost-saving mechanisms in the private health care sector of South Africa.
Objective:
The evaluation of the number of vancomycin therapeutic drug levels monitored by the Tygerberg Toxicology Laboratory over a five year period (2003 – 2007).

Method:
Vancomycin levels were determined by FPIA technique. Data was extracted from laboratory records and analyzed for the number of requests per year, as well as trough and peak levels.

Results:
A total of 10 379 vancomycin levels were determined (9967 trough and 412 peak levels). Yearly totals were 2003 (1547), 2004 (1966), 2005 (2249), 2006 (2270) and 2007 (2347). Of the levels requested, 32% trough levels and 45% peak levels were in the therapeutic range.

Discussion:
Vancomycin is indicated for treatment of septicaemia and endocarditis due to methicillin-resistant Staphylococcus aureus strains (MRSA). Internationally, the trend is to monitor trough levels. However, in high-risk patients both trough and peak levels should be measured. The Toxicology Laboratory references for therapeutic levels are: trough levels 5 – 10ug/ml and peak levels 20 – 40ug/ml. A considerable increase in requests for therapeutic monitoring was observed for the period 2003 – 2005. The reason for this might be an increased incidence of MRSA. According to our laboratory guidelines, only 32% of trough levels were therapeutic, whereas 50% were potentially toxic. The high incidence of toxic levels reported could be due to incorrect information on whether samples represented trough or peak levels.

Recommendations:
Closer co-operation between medical personnel and laboratory staff is required to ensure that correct information is recorded on the request form and that levels are interpreted correctly. Development and implementation of standardized guidelines are important.
Purpose:
Complete suppression of viral replication in HIV-positive patients prevents the development of resistance but is only achieved if more than 95% of doses of Anti Retroviral drugs (ARV) are taken according to prescribed schedule. Concurrent DUR has the potential to identify a problem after the prescription was dispensed but while the patient is still using that medication prescribed, which means that the patient will still benefit from a corrective change in therapy. The purpose of this study is to identify potential non-adherent patients early and prevent resistance to HIV medication regimes.

Methods:
From February 2009 up till now, 1,030 patients were registered in the program for HIV treatment at the hospital; however only around 444 prescriptions (43.11%) were dispensed every month. The ARV prescriptions are dispensed by using a computer program where each patient’s profile, demographical details as well as prescription were recorded. The dispensed ARV items were then send with patient specific instructions to the two 24 hour clinics in the surrounding informal settlement, from where patients can go and collect their medication.

On the prescription the date on which the patient must return to the clinic for the next repeat is indicated. In the back of the prescription a space is indicated where the clinic staff indicates the date on which the patient came to collect his/her treatment as well as a table for a tablet count of the remaining tablets and comments.

By comparing the date on which the patient came to collect his/her medication, the pharmacist can determine if the patient came on the correct date (within 28-30 days) for a refill before the current medication were finished. The tablet count also indicates the number of tablets left in the containers currently used by the patient. By comparing these numbers and additional comments from the clinic staff about the patients, the pharmacist can determine if the patient used his/her medication in the intended way.

Results:
Concurrent DUR was performed during the course of drug therapy where interventions based on. Formal and informal counselling at clinics were used to correct problems encountered in patient adherence issues. The results seem to indicate a higher compliance rate after initiating interventional strategies.
PG1 14  Identifying perceptions of UKZN graduates on the relevance and adequacy of the pharmacy curriculum to current pharmacy practice


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Purpose:
Pharmacy, like every other profession, is undergoing radical changes. The knowledge and skills base required by the profession are affected by external changes including patient demographics and expectations, emerging disease state priorities, technological developments, regulatory requirements and development in other professions. Pharmacy education therefore needs to timely and effectively respond to professional and social change to ensure optimal education and training of pharmacists. The aim of this study was therefore to determine the relevance and adequacy of the B.Pharm curriculum at University of KwaZulu-Natal amongst its own graduates to identify areas to enhance the curriculum.

Methods:
A cross sectional descriptive study was conducted. A structured questionnaire was developed and administered to UKZN graduates. The sample population of 323 included graduates that qualified between 2003-2007 to determine their perceptions towards the pharmacy curriculum in relation to current practice. The questionnaires were hand delivered, emailed and faxed as per the participant’s request after obtaining their consent. Data was collected and captured electronically and analyzed using the Statistical Package of Social Sciences (SPSS) for Windows version 15. Ethical clearance was obtained from UKZN Faculty of Health Science Ethics Committee

Results:
The response rate was 57% with 69% of respondents being females. The majority of respondents indicated that all modules in the current programme were relevant to their practice (72.7%-100%). However, while relevant, the respondents also reported that the various modules were inadequate in meeting their practice needs (5.7%-44.6%). It was also established that there were several areas in the curriculum that were lacking. Only a minority of respondents felt adequately equipped in categories such as complementary and alternative medicines (26.5 %), traditional healing (10.6%), drug utilization review (31.8%), overall pharmacy management (14.5%-41.2%) and screening (54.9%), whilst over 90% of the graduates felt they were particularly adequately equipped in areas such as communication, reconstitution of medicines and pharmacy laws. Except for computer skills (86.2%), Research (74.7%), Pharmacy Laws (82.8%), First Aid (77.0%), the study also showed that skills acquisition in all the other indicated subject areas from the University during their training were low (1.1%-54.0%). Overall, 58.1% of graduates felt that their training at university equipped them with knowledge and skills required to perform their tasks confidently.

Conclusions:
The findings of the study confirmed a need for the School to make specific amendments to the curriculum of the B.Pharm programme at UKZN in terms of content, to consider introducing new modules and also to explore alternative teaching methodologies to enhance skills acquisition in students during their training.
PG1 15 Prevalence of drug-drug interactions between antiretroviral regimens as prescribed according to the recommended antiretroviral dosing

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Purpose:
The aim of this investigation was to determine the prevalence of drug-drug interactions (DDIs) between antiretrovirals (ARVs) and if prescribed according to or/not according to the recommended dosages for antiretroviral agents and according to patients’ age.

Methods:
A quantitative, retrospective drug utilisation study was performed on 49 995, 81 096 and 88 988 ARV prescriptions prescribed to 7 664, 10 162, and 10 061 human immunodeficiency (HIV) patients for 2005, 2006 and 2007 and claimed through a pharmacy benefit management company. Potential DDIs between ARVs were identified and dosages evaluated according to guidelines indicated in the literature and treatment guidelines.

Results:
The results revealed that 4.49%, 4.07% and 2.99% of the ARV prescriptions for 2005, 2006 and 2007 respectively had one ARV item, 43.77%, 43.52%, and 55.70% had two items; and 51.76%, 51.93% and 43.38% had three or more ARV items. The total number of DDIs according to age group for the three years was 779 (2005), 1 155 (2006) and 1 177 (2007) respectively, of which 4.37%, 2.94% and 2.55% were prescribed to patients 0<=12 years, 1.03%, 0.00% and 0.42% to patients 12<=19 years and 69.28%, 67.97% and 69.24% to patients 19<=45 years and 25.32%, 29.09% and 27.79 to patients 45<=59 years and older.

The most prevalent antiretroviral drug regimens that were prescribed not according to the recommended ARVs dosing and where DDIs were identified according to patients’ age were: Lopinavir/ritonavir 800mg/200mg and Efavirenz 200mg prescribed to patients 0<=12 years (n = 20), Lopinavir/ritonavir 320mg/80mg and Nevirapine 2600mg (n = 8). Then Lopinavir/ritonavir 1066.4mg/264mg and Efavirenz 600mg to patients 19<=45 years (n = 347), Lopinavir/ritonavir 1066.4mg/264mg and Nevirapine 400mg (n = 41), Indinavir 1600mg and Ritonavir 800mg (n = 16). Then Lopinavir/ritonavir 1066.4mg/264mg and Efavirenz 600mg to patients 45<=59 years (n = 49 ), Lopinavir/ritonavir 1066.4mg/264mg and Efavirenz 600mg (n = 22), Lopinavir/ritonavir 1066.4mg/264mg and Efavirenz 400mg (n = 7), Lopinavir/ritonavir 1066.4mg/264mg and Nevirapine 500mg (n = 10), Saquinavir 800mg and Ritonavir 800mg (n = 18); Then Lopinavir/ritonavir 1066.4mg and Nevirapine 400mg to patients >59 years (n = 3), Lopinavir/ritonavir 1066.4mg/264mg and Efavirenz 600mg (n = 9), Lopinavir/ritonavir 1066.4mg/264mg and Efavirenz 500mg (n = 2).

Conclusion and Recommendation:
Drug-drug interactions were identified between ARVs prescribed in different age groups, age group 3(19<=45), furthermore there were identified in ARV regimens with Prescribed Daily Doses that were not according to the recommended ARV dosing. There is need for more education to prescribers to adhere to the recommended standard dosing for ARVs for HIV/AIDS patients to achieve maximum virological suppression and lower costs in terms of possible adverse effects.
Introduction:
Global prescriptions for antidepressants are increasing. The introduction of Selective Serotonin Reuptake Inhibitors (SSRIs) has lead to a considerable increase in pharmacotherapeutic treatment rates. But this increase in treatment has also created a considerable degree of controversy. Questions have been raised on the efficacy of treatment of depression and anxiety, suicidality rates and tolerability of drugs. This research is a product of that controversy and aims to shed light on antidepressant prescriptions. Evaluation of prescriptions to determine prevalence of use and to data mine for any possible problems or commendations is of value. A Drug Utilization Review (DUR) combines all these aims and furthers our understanding of the environment surrounding prescriptions in order to optimize treatment plans.

Aim:
Aim of this study was to serve as a pilot research topic for a larger and more comprehensive drug utilisation review on current antidepressant prescriptions.

Method:
Records of antidepressant use were obtained from a private hospital in the Eastern Johannesburg region. Computerised prescriptions were collated over an 8 month period and reviewed according to prescriber and patient information, type and dosage of antidepressant. Simple statistical analysis was completed using Microsoft Excel.

Results:
Data for 321 patients and 583 prescriptions were obtained. 62.93% of patients were female. The average age of patients was 44.2 years and 25.86% had a co-morbid condition. 2 prescribers (3.5%) were psychiatrists but accounted for 46.1% of prescriptions, the reminder prescribers were non-specialist. The most frequently prescribed antidepressant was fluoxetine (25.21%), followed by amitryptiline (19.73%), citalopram (14.07%), escitalopram (10.29%), sertraline (6.69%) and mirtazepine (5.15%). Only 19.18% of prescriptions were on a chronic basis with an average duration of treatment of 4.57 months. SSRIs were prescribed in doses which very closely matched their Defined Daily Doses (DDD), in contrast TCAs were not. The PDD for TCAs were less than 50% of their DDD. 45.17% patients were on concurrent benzodiazepine treatment.

Discussion and conclusion:
Following international trends, antidepressant prescriptions have shifted to favour SSRIs ahead of TCAs. Duration of treatment differs greatly from treatment guidelines which suggest that antidepressant treatment should continue for a minimum of 6 months to achieve remission of symptoms. Reasons for this are considered.
Being a pilot study and the basis for a much wider study, the limitations encountered are important. These include: indication of use and diagnosis of patients were unknown as the data was not sufficiently comprehensive; information on follow-up prescriptions filled in other dispensaries was not available; the time frame of data was short and the sample size small. A future study will ideally address these limitations and aim to acquire a dataset that is larger, more comprehensive and spans a longer time frame.
PG1 17  Evaluating the potential of DNA Model-building activities in a problem-based learning pharmacy programme

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Purpose:
Watson and Crick, who unraveled the structure of DNA in 1953, attributed their success to the use of two kinds of physical models: rough cardboard cutouts and then finally a model crafted by Cambridge technicians. This raises two questions. Firstly, will students benefit from interaction with physical models, when learning about DNA? Secondly, which of these two kinds of models have the greater pedagogical content knowledge potential?

Methods:
This paper focuses on collecting empirical evidence about student activities and learning when they are exposed to model-building in a Problem-based Learning environment. Thirty-nine third year students enrolled in a Problem-based Learning Pharmacy program were given a pretest (to determine their prior knowledge), followed by two model building activities and a post-test. These students then completed an anonymous survey using Lickert scales that probed their sense of engagement, their perception of the impact of the models on their learning; as well as an evaluation of the fitness for purpose of each of the models.

Results:
These students scored an average of 26% on the pre-test as compared to 73% in the post-test. Furthermore, in the survey the students strongly agreed by overwhelming majorities that model building activities were: challenging and interesting and that it helped them to learn more effectively.
Services for which pharmacists may levy a fee: Dispensing time of different types of prescriptions

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Purpose:
The primary aim of the study was to determine whether the type of prescription and the number of items dispensed per prescription would have an influence on the dispensing time of prescriptions in different pharmacy sectors (community, private and public institutional pharmacies) in South Africa.

Research Methodology:
A national research project was undertaken during 2008 by the South African Pharmacy Council on the services for which a pharmacist may levy a fee. During this research project, the time it takes to dispense a prescription (n = 6862) was determined in 578 pharmacies of which 421 were community, 54 were private institutional and 103 were public institutional pharmacies. Prescriptions (n = 6782) were categorised as i) First time or acute (n = 3917); ii) Repeat (n = 2343); iii) Antiretroviral-not indicated repeat or first time (n = 93); iv) First time antiretroviral (n = 83); v) Repeat antiretroviral (n = 224); vi) First time or acute and repeat prescriptions (n = 114); vii) First time and repeat and Antiretroviral prescriptions (n = 8).

Results and discussion:
The weighted mean time it took to dispense a prescription in a pharmacy was 307.45 (SE = 5.34) seconds (slightly over 5 minutes). The results revealed that 75% of the prescriptions evaluated had four and less items per prescription. The time taken depended on the number of items per prescription dispensed. The weighted mean dispensing time was 279.59 (SE = 5.14) seconds if there were ≤ 4 items per prescription (n = 5882) and 513.10 (Standard Error (SE) = 16.12) seconds for prescriptions with > 4 items (n = 980). The weighted mean dispensing time for prescriptions in community pharmacies was slightly longer for prescription with ≤ 4 items per prescription (293.07, SE = 5.08 seconds) (n = 4298) and for prescriptions > 4 items per prescription (562.32, SE = 22.08 seconds) (n = 457). However, the dispensing time of prescriptions in public institutional pharmacies was much shorter with 226.47 (SE = 16.40) seconds for prescriptions with ≤ 4 items per prescription (n = 868) and 368.58 (SE = 18.30) seconds for prescriptions > 4 items per prescription (n = 531). For all three sectors the weighted time spent on Phase III was less than 80 seconds for prescriptions with ≤ 4 items per prescription and less than 111 seconds for prescriptions with more than 4 items per prescriptions.

The weighted mean dispensing time of first time or acute prescriptions (320.34, SE = 5.82 second) was longer than repeat prescriptions (275.29, SE = 6.20 seconds). The weighted dispensing time of first time (489.19, SE = 35.04 seconds) antiretroviral prescriptions was also longer than repeat (296.90, SE = 22.67 seconds) antiretroviral prescriptions. The weighted time spent on Phase III for first time or acute prescriptions (excluding antiretroviral prescriptions) was only 76.46 (SE = 3.07) seconds and 54.39 (SE = 2.62) seconds for repeat prescriptions.

Conclusion and recommendations
Dispensing antiretroviral prescriptions took longer. The dispensing time for repeat prescriptions were less than first time or acute prescriptions independent of the pharmacy sector and number of items per prescription. Pharmacists should be encouraged to counsel patients thoroughly, also during dispensing of repeat prescriptions.
Purpose:

The primary aim of the study was to determine the time it takes to dispense a prescription according to the different phases and in different pharmacy sectors (community, private and public institutional pharmacies) in South Africa.

Research Methodology:

A national research project was undertaken during 2008 by the South African Pharmacy Council on the services for which a pharmacist may levy a fee. During this research project, the time it takes to dispense 6862 prescription was determined in 578 pharmacies of which 421 were community, 54 were private institutional and 103 were public institutional pharmacies.

Results and discussion:

Dispensing was divided into three phases, namely Phase I (interpretation and evaluation of the prescription), Phase II (preparation and labelling of the prescribed medicine) and Phase III (provision of information and instructions to the patient). In 84.52% of prescriptions it was possible to separate between the different phases. Phase I was performed in 97.15% of cases, Phase II in 99.38% and Phase III in 80.62%. The weighted mean time it took to dispense a prescription in a pharmacy was 307.45 (Standard error (SE) = 5.34) seconds (slightly over 5 minutes). Phase II took the longest with 163.59 (SE = 3.86) seconds and dispensers spent only 73.41 (SE = 3.17) seconds on Phase III. In 71.60% of the cases the pharmacist was the only person involved in the dispensing of the prescription, in 17.85% of the cases the pharmacist was supported by other personnel (such as post-basic pharmacist assistants). Prescriptions dispensed only by pharmacists (71.60%) took 304.11 (SE = 5.76) seconds compared to 351.71 (SE = 12.71) seconds in cases (17.85%) where the pharmacist was supported by other personnel (such as post-basic pharmacist assistants). The weighted mean time of dispensing prescriptions (10.55%) where pharmacists were not involved was 297.02 (SE = 13.97) seconds.

Another factor that influenced the dispensing time was the pharmacy sector. The majority of these prescriptions were dispensed in community (retail) pharmacies (70.42%), followed by public institutional pharmacies (20.52%) and then private institutional pharmacies (9.06%). The weighted mean time in community pharmacies was slightly longer, 317.76 (SE = 5.91) seconds compared to 290.62 (SE = 26.17) seconds and 269.64 (SE = 13.23) seconds for private and public institutional pharmacies respectively. The weighted mean time spent on Phase III was the longest in the public institutional pharmacies (81.95, SE = 6.49 seconds). Most of the prescriptions (85.51%) were manually dispensed in public institutional pharmacies compared to computerised dispensing in community (97.47%) and private institutional pharmacies (95.66%).

Conclusion and recommendations:

The results clearly indicated that pharmacists in all sectors should be encouraged to focus on the provision of drug information and usage instructions to patients when dispensing prescriptions.
Background:
In 2006 the South African Pharmacy Council (SAPC) and the Research and Development Task Team of the SAPC embarked upon a national research project with the specific research objective to assign unit values to the procedures (services) for which a pharmacist may levy a fee and to determine the cost of providing these services in both community and private institutional pharmacies. The purpose of this poster is to focus on the unique research methodology that was followed to achieve above-mentioned aims.

Research Methodology:
The research project was conducted in two phases. The purpose of Phase I was to determine which of the services for which a pharmacist may levy a fee are provided currently in community and institutional (public and private) pharmacies. Three structured questionnaires for community and private and public institutional pharmacies were developed and applied by means of a telephonic interview conducted by fieldworkers from various pharmacy schools. The results obtained in Phase 1 (n=2200) were used to determine the sample for Phase II.

A stratified random sampling method was used to select a sample (n = 680) of pharmacies from each category of pharmacy in each province (community [n =502], public [n = 114] and private [n = 64] institutional pharmacies). The sampling was done on a provincial level to ensure that it would geographically representative and would include under-serviced areas. Schools of Pharmacy were responsible for implementation of the research project in different geographical areas. A pilot study was conducted to test the different research instruments. All fieldworkers were trained and complied with certain criteria. During Phase II time analysis data collection tools were utilised to measure the duration of each of the services and procedures that are listed in the Rules relating to services for which a pharmacist may levy a fee and guidelines for levying such fees. A total of 597 (88%) pharmacies were surveyed by trained fieldworkers. Financial and human resource information was obtained through two different structured questionnaires from 399 (58.7 %) and 401 (59%) pharmacies respectively. Data were analysed by using the Statistical Analysis System. All analyses for the cost and human resource questionnaires and the time-analysis for the different procedures were conducted by using the SURVEYMEANS procedure of SAS (SAS Institute Inc. 2004. SAS OnlineDoc® 9.1.3). This statistical tool makes it possible to correctly calculate significance tests and 95% confidence intervals, taking into account the weighting of the data. Ethical approval was obtained from the North-West University (No NWU 00058-07-S4).

Conclusion
The results of the analyses were used to arrive at possible fees for services for which a pharmacist may levy a fee in the different pharmacy sectors in South Africa.
In developing resource-constrained countries challenges to successful HIV/AIDS care have been linked to poor access to health care facilities and although in some instances progress is recognised, much work remains to achieve the goal of universal access (Ojikutu, 2008). In these countries, scaling up of ART has been challenging, especially in sub-Saharan Africa. Rapid scale up of HIV/AIDS services in these settings is most ambitious and complex while the most challenge has been that of efficiency and cost minimisation. Criticism of the South African ART Programme has been centered around lack of political will and the slow pace of treatment provision within the public sector. The health system infrastructure deficits have slowed the pace of the ART roll-out. This is of great concern especially in remote, rural areas where there is a poor health care capacity. According to Pillay (2008), poor service delivery in health care can be attributed to a number of management factors. There is urgent need to develop simple and sustainable models of delivering ART in less-developed health-care delivery systems (Miles, 2007). This is important because inefficient Programmes can be costly and time wasting and this is clearly not desirable in struggling health care systems or in developing countries where resource allocation should be well planned to manage scarce resources.

Most health care problems have been linked to a lack of management capacity within the public sector in South Africa and there is a significant gap between private and public sectors (Pillay et al., 2008) in this regard. It is against this background of challenges that the main objective of the study is to assess technical efficiencies of ART roll-out Programmes by measuring the performances of ART roll-out facilities as a function of structures and processes of governance of the Programmes. It is sensible to provide evidence on the performance of current models of ART roll-out given the increasing demand for HIV/AIDS care and the management systems put in place. The term ‘efficiency’ usually refers to the ability of a firm to produce maximum output given its Inputs (Badunenko et al., 2006).

Because of a number of factors that play a role in the performance of ART roll-out, a phased multi-method approach is proposed for this study to assess and describe factors of governance/management and the roles they play on technical efficiencies of ART roll-out Programmes. This study will employ Data envelopment analysis (DEA) technique to measure technical efficiencies of antiretroviral (ARV) delivery facilities. DEA is a relatively new “data oriented” approach for evaluating the performance of a set of peer entities called Decision Making Units (DMUs) which convert multiple inputs into multiple outputs (Copper et al., 2004). DMUs in this study will mean the ART roll-out facilities. Data Envelopment Analysis (DEA) constructs an efficiency frontier which reveals the least input requirement for obtaining a given output level, or, from an output-oriented framework, the highest output obtainable from a given input set (Masiye, 2007). Otherwise other qualitative and quantitative methods will be used in this study. Thematic content analysis will be used to analyse qualitative data, while quantitative and semi-quantitative data (descriptive statistics) will be analysed using appropriate data management software packages such SPSS or excel spreadsheet. This study will inform public–private mix debate, and hence, guide the identification of ARV delivery models that are effective given certain management practices. An ideal scenario at all levels of care should be recommended as a best ‘management-delivery-output’ model.
POSTER SESSION 1

PG1 22 Services for which pharmacists may levy a fee: Provision of information concerning a patient’s condition / medicine

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Purpose
The provision of information to patients is a professional service which is offered as part of the scope of practice of a pharmacist, whether or not a medicine is dispensed, (GPP). The South African Pharmacy Council (SAPC) embarked on a study in 2008 to determine the time it takes to provide information to patients on the patients’ conditions in order to determine a professional fee for such services. This fee is to be included in the National Health Reference Price List (NHRPL). Provision of patient information comprises a pre-consultation process followed by the consultation. Pharmacists are required by Good Pharmacy Practice (GPP) to also keep patient records for such procedures. As yet no reasonable and reliable time has been determined for the provision of patient information.

The primary aims were to determine the:

- extent to which pharmacists provide information to patients with respect to the patient’s condition and
- the time taken by pharmacists to provide such information in community and institutional pharmacies (public and private) in South Africa.

Research Methodology
A national, observational study of 680 pharmacies providing patient information was undertaken. A total of 680 pharmacies were randomly selected from the national pharmacy database (N=2200).

Results and discussion
A total of 1034 cases of information being provided in a total of 356 pharmacies were observed with a response rate of 88%. Of the pharmacies where information provision was observed, 311(71%) were community pharmacies, 17 (21%) were private institutional pharmacies and 28 (26%) were public institutional pharmacies. The weighted mean time for providing information in pharmacies in the study population of 356 pharmacies was 217 (SE = 9.8) seconds. Among the community pharmacies a total of 933 cases were observed (n=311) with a weighted mean time of 189.99 (SE = 7.5) seconds. Among private institutional pharmacies 45 cases were observed with a weighted mean time of 504.12 (SE = 37.56) seconds; public institutional pharmacies had 56 cases with a weighted mean time of 395.36 (SE = 97.45) seconds. Community pharmacists took half the time for patient information that institutional pharmacists required, community pharmacies however, represented nearly 90 per cent of all cases observed.

Conclusion
Provision of information to patients regarding their condition and/or medication was highest among community pharmacies (71%) whereas both the institutional pharmacy sectors had similar incidences. The weighted mean time for providing information was twice as long among institutional pharmacies when compared to community pharmacies.
PG1 23  Services for which pharmacists may levy a fee: Time analysis of primary screening and monitoring services in pharmacies of South Africa

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Purpose:
Pharmacists perform screening services such as blood glucose testing, blood cholesterol and/or triglycerides testing, urine analysis, blood pressure monitoring, HIV and AIDS testing with pre-and post-test counseling, pregnancy screening and peak flow measurement which is within their scope of practice. The Pharmacy Act makes provisions for the SAPC in terms of Section 35A(b)(iii) of the Pharmacy Act, 53 of 1974 as amended to make rules as to the services for which a pharmacist may levy a fee as well as guidelines for levying such a fee/s. In 2007 the SAPC published its final set of Rules relating to services for which a pharmacist may levy a fee as well as guidelines for levying such a fee or fees which linked each procedure to GPP standards. However, what became apparent was the need to identify the time taken by a pharmacist to perform such procedures. A study was therefore undertaken to inform the SAPC as to the time taken to perform such screening services with the object of assigning unit values to the procedures.

Research Methodology:
The study was conducted in 2 phases. The results obtained in Phase 1 (n=2200) were used to determine the sample for Phase 2. Stratified random sampling was used to select pharmacies from each category of pharmacy in each province. Pharmacies were categorised according to community, private institutional and public institutional pharmacies. Time analysis data collection tools were utilised to collect the data. The data collection was coordinated by the Pharmacy schools. The data were analysed using the SAS System for Windows, Release 9.1 TS Level 1M3 (SAS Institute Inc., 2003).

Results:
A total of 438 pharmacies responded. The weighted mean seconds taken to measure blood glucose was 269 (n=147) blood cholesterol and/or triglycerides 439 (n=102), urine analysis 401 (n=25), blood pressure monitoring 238 (n=366), HIV/AIDS pre-test counselling 1417.71 (n=7), HIV/AIDS testing and post-test counselling 1010.16 (n=9), pregnancy screening 427 (n=15), peak flow measurement 214 (n=24). Possible fees structure inclusive of vat (in 2009 terms) for blood glucose was R41.10, blood cholesterol and/or triglycerides R67.20, urine analysis R61.40, blood pressure monitoring R36.50, HIV/AIDS pre test counselling R282.00, HIV/AIDS testing and post-test counselling R200.90, pregnancy screening R65.40, peak flow measurement R32.80. The majority of the services were performed in community pharmacies.

Conclusion:
The fee that pharmacists can levy for procedures and services performed in a pharmacy has been presented, calculated, and the procedures necessary to perform such procedures or services.
PG1 24  Services for which a pharmacist may levy a fee: Emergency Post-Coital Contraception (EPC) and provision of a reproductive health service

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Purpose

Emergency post-coital contraception (EPC), often referred to as the “morning-after-pill”, implies the use of high-dose contraceptive pills taken within 72 hours of unprotected sexual intercourse. A community or institutional pharmacist can render a comprehensive reproductive health service only if necessary and only if supplementary training has been obtained and registered with the South African Pharmacy Council (SAPC). The aim of the study was to determine the extent of provision of EPC as a reproductive health service, as well as the time taken to render these services.

Research Methodology

A national research project on the services for which a pharmacist may levy a fee, was commissioned by the SAPC and carried out collaboratively by the Schools of Pharmacy in South Africa. Phase I (baseline study in 2007) provided data on which services are currently provided; phase II (conducted in 2008) measured activity times and cost of providing these services. This study reflects one component of Phase II of this larger study, viz. the provision of EPC and reproductive health service.

Results and Discussion

Out of a total of 438 community pharmacies sampled, 65 EPC services (in 54 pharmacies), predominantly in the Gauteng (15; 23.08%), Western Cape (13, 20%) and North West (10; 15.38%) provinces, and 63 reproductive health services (in 37 pharmacies), predominantly in the Western Cape (27; 42.86%) and Eastern Cape (11, 17.46%) provinces, were measured. One hundred per cent of EPC and 96.92% of reproductive health services were delivered in community pharmacy settings. EPC service is divided into four phases: Pre-administration procedure (performed in 89.23% of cases), provision of EPC (performed in 95.38% of cases), counselling (performed in 87.69% of cases) and documentation and record-keeping (only performed in 63.08% of cases). Reproductive health service is also divided into four phases: Pre-consultation procedure (performed in 95.24% of cases), evaluation of suitable method of contraception (performed in 87.3% of cases), administration of injectable contraceptive (performed in 57.14% of cases) and documentation and record-keeping (performed in 96.83% of cases). Over 80% of EPC was delivered by the pharmacist and approximately 80% of reproductive health service by a nurse. EPC provision took on average 254.51 sec (slightly over 4 min) with a std. error of mean 27.79 sec. Reproductive health service took on average 391.36 sec (about 6.5 min), with a std. error of the mean 53.96 sec.

Conclusion and Recommendations

Provision of EPC and reproductive health services were found to be offered in only a small proportion of community pharmacies; pharmacists should be encouraged to expand the delivery of these services.
PG1 25  Institutionalising Service-learning in Pharmacy (SLIP) at the University of the Western Cape

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Purpose:
Pharmacy education is responsible for preparing students to enter into professional practice settings with the skills and values necessary to serve society. The South African Pharmacy Council (SAPC) requires that pharmacy education and training equips pharmacists for the roles they will take on in practice. The SAPC framework however, is technically orientated, with very little emphasis on a values-driven profession. Service-learning is defined as experiential learning in which students engage in structured activities that address community needs. The purpose of this prospective study is to design, implement and evaluate an undergraduate service-learning program that will enable interaction at various facets across public sector health facilities. It is envisioned that graduates would become technically and culturally competent as well as industry able and socially responsive. Furthermore, this study aims to establish a triad partnership between the School of Pharmacy (students), the health facility and the community (patients) in the Western Cape.

Methods:
The SLIP course is directed to UWC undergraduate pharmacy students who are in their 2nd, 3rd and 4th years of study. The 4th year SLIP program comprises an already established year of block sessions at tertiary and secondary hospitals and community health centres (CHCs). Pre- and post- intervention qualitative and quantitative methods were used in evaluating the impact of the 4th year SLIP program (Parker, 2007). Students and pharmacists who were involved in SLIP comprised the study cohort. Focus group discussions with students were conducted to assess knowledge and expectations of SLIP. Covert observation was used to assess pharmacists’ views and receptivity toward student activities during the SLIP course. Student competency assessments in hospital pharmacy practice (compounding, dispensing and clinic/ward pharmacy), and self-administered questionnaires to pharmacists comprised the quantitative study.

Results:
The 4th year SLIP evaluation resulted in improved perceptions and receptivity of pharmacists to service-learning initiatives. Students’ competency increased in pharmacy practice skills and they contributed to service delivery at public sector pharmacies. Students were both enthusiastic about this style of learning (“saw the pharmacy profession with new eyes”) and realized the need for more skills development in clinic-based pharmacy practice. Gaps identified in the 4th year SLIP program included a lack of patient-centeredness and values-based learning. Findings from an independent reviewer (Pollack, 2008) further endorsed the need for more exposure to patient-centered practices based on the tenets of pharmaceutical care. The 2nd and 3rd year SLIP programs, which are currently being implemented as pilot programmes in the fields of Drug Supply Management and Primary Health Care respectively, aim to address the gaps identified in the 4th year SLIP program.
PG1 26 Prescribing patterns of products containing methylphenidate and atomoxetine in a section of the private health care sector of South Africa

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Purpose:
The general objective of this study was to investigate the prescribing patterns of products containing methylphenidate as well as atomoxetine in Attention Deficit Hyperactivity Disorder (ADHD) in a section of the private health care of South Africa.

Research Methodology:
A quantitative, retrospective drug utilisation review was performed on medicine claims data of a Pharmacy Benefit Management company for the study years 2005, 2006 and 2007.

Results:
The total number of patients who receiving ADHD medication in 2005 were 7,990 in 2006 8,575 and in 2007 it decreased to 7,828. The female gender increased from 27.75% in 2005 to 29.06% in 2007 and males decreased from 72.03% of ADHD patients in 2005 (N=7,990) to 70.89% in 2007. (N=7,828). The ratio of gender-related prescribing of ADHD medicine items in this section of the private health care sector of South Africa for male to female was ±2.5 to 1 compared to the international standard of 3 to 1.

The percentage of young children (age 0 to 5 years) receiving ADHD medicine items increased from 0.91% in 2005 (N=7,990) to 1.11% in 2007 (N=7,828). The number of children (age 5 to 12 years) decreased from 53.62% in 2005 to 49.23% in 2007. The number of adolescents increased from 26.32% in 2005 to 27.38% in 2007. Adults increased from 19.15% in 2005 to 22.28% in 2007. The 5 top ranking prescribed items in 2005 were: Ritalin LA 20mg®, Ritalin 10mg®, Ritalin LA 30mg® (methylphenidate), Concerta 36mg® (atomoxetine) and Methylphenidate HCl-Douglas®. In 2006 the top 5 prescribed items included Ritalin LA 20mg®, Ritalin 10mg®, Concerta 36mg®, Ritalin LA 30mg® and Concerta 18mg®, while this ranking order merely stayed the same for 2007 with only the last 2 items changing places in the order. ADHD prescriptions represented 0.32% of all prescriptions claimed during 2005 (N=8,522,798), 0.35% during 2006 (N=9,048,541) and further increased to 0.41% in 2007 (N=8,015,538). Atomoxetine medicine items represented 4.69% while methylphenidate medicine items represented 95.31% of all ADHD medicine items claimed in 2005 (N=29,424). Atomoxetine items increased to 12.91% in 2006 (N=33,576), but slightly decreased to 12.34% in 2007 (N=34,850).

The average cost per ADHD prescription was R318.29 ± 162.09 in 2005 and increased to R358.91 ± 208.10 in 2007.

Conclusion:
The number of ADHD prescriptions has increased throughout the study period of 2005 to 2007, with prescriptions with methylphenidate as medication still in the majority. The average cost for ADHD drug therapy increased from 2005 to 2007.
Purpose:

Primary Care Drug Therapy (PCDT) is defined as a face-to-face consultation with a patient where a pharmacist personally takes down a patient’s history, performs an appropriate health examination including observations, and plans appropriate interventions/treatment, which may include referral to another health care professional. The pharmacist has to be qualified and registered as a PCDT pharmacist. Pharmacists who provide PCDT services have undertaken an additional training course and have obtained a permit issued in terms of Section 22A(12/15) of the Medicines and Related Substances Act 101 of 1965. The primary aim of the study was to determine the extent of provision of PCDT services in pharmacies in South Africa, as well as to determine the time it takes to provide such services.

Research Methodology:

A national research project was undertaken during 2008 by the South African Pharmacy Council on the services for which a pharmacist may levy a fee. The focus of this study is on one component of the larger study, namely the PCDT service. A service was considered to constitute PCDT when, firstly, the pharmacist examined a patient in order to determine a health need, and secondly, that the consultation took place in a private consultation area. The pharmacist had to indicate to the fieldworker that it was a PCDT service and the fieldworker had to document whether the pharmacist providing the service had been trained in PCDT and whether he/she was in possession of a permit.

Results and discussion:

Forty-four pharmacies indicated that they provided PCDT services. All the pharmacies were community (retail) pharmacies. A total of 104 PCDT services (cases) were measured. The PCDT service was divided into five phases, namely a pre-consultation procedure (performed in 83.65% of cases), history taking and anamnesis (performed in 90.38% of cases), a health examination (performed in 82.69% of cases), pharmacological and non-pharmacological treatment (performed in 90.38% of cases) and follow-up (only performed in 43.27% of cases). Approximately 70% of the service (the five phases) was performed by the pharmacist himself or herself. Where the pharmacist did not perform the entire service, it was mostly the nursing practitioner who assisted the pharmacist. The procedures mostly performed as part of the PCDT service were the dispensing of a prescription, the measurement of blood pressure, and the administration of an intra-muscular or subcutaneous injection. It took on average (weighted) 638.16 seconds (approximately 10.5 minutes) per PCDT service (standard error of mean 73.70 seconds).

Conclusion and recommendations:

PCDT is an important service that community pharmacists can deliver where the need exists. It is recommended that pharmacists be encouraged to obtain this additional qualification.
Background:
Pharmacist Initiated Therapy (PIT) means the supply of medicine to meet the health needs of a patient or group of patients without a prescription of a person authorised to prescribe medicines. It includes any situation where a patient comes into the pharmacy and asks for health advice or assistance and the pharmacist recommends a specific product and/or therapy together with appropriate counselling. For the purposes of this study, PIT also included cases where the patient came into the pharmacy and requested an over-the-counter (OTC) product and no counselling of the patient by the pharmacist or pharmacist’s assistant took place.

Primary aim:
The primary aim of the study was to determine the extent of provision of PIT services in pharmacies in South Africa, as well as to determine the time it takes to provide this service.

Methodology:
A national research project was undertaken during 2008 by the South African Pharmacy Council on the services for which a pharmacist may levy a fee. The focus of this study is on one component of the larger study, namely the PIT service.

Results and discussion:
A total of 369 pharmacies indicated that they provide PIT services. A total of 3 133 PIT services (cases) were measured. The majority of these services were delivered by community (retail) pharmacies (95.79%), followed by private institutional pharmacies (4.21%). The PIT service was divided into three phases, namely Phase I (pre-administration procedure), Phase II (preparation and labelling of the prescribed medicine) and Phase III (provision of information and instructions to the patient to ensure the safe and effective use of medicine). Phase I was performed in 98.21% of cases, Phase II in 97.19% of cases and Phase III in 91.67% of cases. Pharmacists delivered all three phases themselves in over 70% of the cases. They were supported by mostly post-basic pharmacist assistants in delivering this service. The weighted average time it took for a PIT service to be delivered in a pharmacy was 199.02 seconds (just under 3.5 minutes). The standard error of the mean was 5.57 seconds. The weighted average time in community pharmacies was slightly less (192.82 seconds) compared to 312.15 seconds in private institutional pharmacies. The time taken was dependent on the number of items dispensed. The weighted average time taken was 160.76 seconds if there was less or equal to one item dispensed, 220.31 seconds for more than one and equal to two items dispensed, and 327.19 seconds if more than two items were dispensed.

Conclusion and recommendations:
PIT is an important service that pharmacists deliver where the need exists. It is recommended that pharmacists be encouraged to counsel patients thoroughly when delivering a PIT service.
Services for which pharmacists may levy a fee: Compounding of an extemporaneous item

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Purpose:

Compounding of an extemporaneous item for a specific patient is one of the services delivered in pharmacies. It refers to the compounding of any non-sterile pharmaceutical product prepared as a single item for a patient (a new product is manufactured) including the necessary documentation. The primary aim of the study was to determine the extent of provision of extemporaneous compounding services in pharmacies in South Africa, as well as to determine the time it takes to provide such service.

Research Methodology:

A national research project was undertaken during 2008 by the South African Pharmacy Council on the services for which a pharmacist may levy a fee. A total of 597 pharmacies were surveyed by fieldworkers. The focus of this study is on one component of the larger study, namely compounding of an extemporaneous item.

Results and discussion:

One hundred and fifty-eight pharmacies provided extemporaneous compounding services during the study period (79.75% were community pharmacies). This service was provided in all nine provinces. A total of 201 extemporaneous items were prepared by these 158 pharmacies (79.10% by community pharmacies). Most prescriptions (80.10%) were first-time acute prescriptions, followed by repeat prescriptions (14.43%). Compounding was divided into three phases, namely interpretation and evaluation of the prescription (Phase I), preparation and labelling of the prescribed medicine (Phase II), and provision of information and instructions to the patient (Phase III). The weighted mean time (all types of pharmacies) for Phase I was 92.84 (SE = 11.00) seconds (approximately 1.5 minutes), for Phase II was 521.85 (SE = 39.49) seconds (just less than 9 minutes), and for Phase III was 82.76 (SE = 12.17) seconds (just more than one minute). In total, it took a weighted mean time of 669.25 (SE = 39.44) seconds to compound an extemporaneous item (all three phases), that means it took approximately 11 minutes for the extemporaneous compounding of an item. The longest weighted mean time was recorded in community pharmacies (698.74 seconds), followed by 527.08 seconds in public institutional pharmacies, and only 332.02 seconds in private institutional pharmacies. The number of prescriptions in private institutional pharmacies was, however, low (only seven prescriptions). Extemporaneous compounding was mostly performed by pharmacists (in 74.62% of cases only the pharmacist was involved). Post-basic pharmacist assistants and interns were also involved to a limited extent. Of the total weighted mean time of 669.25 seconds, the pharmacist was on average responsible for 550.54 seconds of the compounding process.

Conclusion and recommendations:

It took on average 11 minutes to compound an extemporaneous item in a pharmacy. It will be valuable to investigate the type of preparations compounded and for what conditions they are mostly prepared.
PG1 30  Prescribing of antimigraine preparations in a primary care setting in South Africa

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Background:
Migraine is a prevalent condition in Western countries, affecting 8% to 14% of the population. In the United Kingdom, it occurs in 15% of the adult population. Migraine affects primarily the economically active sector of the community. Females around 40 years of age have the highest prevalence of migraine. Not many studies have been published in South Africa on the prescribing patterns of antimigraine drugs.

Primary aim:
The primary aim of the study was to determine the prescribing patterns of antimigraine preparations in a private sector patient population and to establish similarities and differences with previous studies in other patient settings.

Methodology:
A retrospective drug utilisation consumption study was conducted. Data for six months during 2008 were obtained from a private pharmacy group serving patients from different regions in South Africa. All prescriptions for antimigraine preparations were identified and analysed. Main outcome measures were frequency and cost of prescribing as well as whether it constituted rational prescribing.

Results and discussion:
A total of 11 613 patients were included in the study, of which 71.38% were females. The average age of patients was 44.98 (SD = 13.80) years. A total of 20 820 antimigraine products were prescribed at a cost of R1 776 481. Females received on average slightly more antimigraine preparations. Fourteen different trade name products were prescribed. Tablets were the preferred dosage form (87.28%). Most drugs (49.29%) were for the prophylaxis of migraine, of which clonidine was the most frequently prescribed. Of the drugs prescribed specifically for the management of migraine, the ergot derivatives (32.27%) were the most frequently prescribed, followed by the selective 5HT1-receptor agonists (triptans) (18.44%). Rizatriptan constituted 61.58% of all triptan prescriptions. The triptans were relatively expensive compared to the other two classes of antimigraine drugs (accounting for 18.44% of prescribing frequency and for 53.95% of prescribing cost).

Conclusion and recommendations:
Differences were observed between the results of this study and previous South African studies. A lower prescribing rate for triptans has been observed. Since migraine affects primarily the economically active sector of the population, studies on the pharmacoepidemiology of migraine can greatly enhance the understanding of this disease state in South Africa to ensure that the condition is optimally managed.
Potential abuse of medicine: A South African database analysis

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Background:

Medicine abuse is defined as the recurrent use of a medicine in a non-medical manner for non-medicinal purposes. The abuse of prescription drugs is increasing worldwide. Little has been published about the detection and prevalence of medicine abuse using prescription databases.

Primary aim:

The primary aim of the study was to detect the potential abuse of medicine using a medicine claims database and to make recommendations to control this form of abuse.

Methodology:

A retrospective exposure-cohort drug utilisation study was conducted on a medicine claims database of a medical aid administrator. The medicine file contained 1 357 717 records of 53 147 medical aid beneficiaries for 2007.

Results and discussion:

Analgesics and respiratory products were the most commonly prescribed over-the-counter (OTC) medicines. Of the 20 345 products that were classified as pharmacist-initiated therapy, 42.0% were analgesics and 33.1% musculoskeletal system agents. Just over 18% of these products were claimed illegally. Two patients received more than 100 Schedule 2 products during 2007. One of these patients claimed a syrup containing promethazine, codeine phosphate and ephedrine 47 times during 2007. Although it was rejected 15 times, it was still dispensed by the same pharmacy 32 times. Another patient received 1 129 capsules of a cold and flu preparation, together with three different trade name cough syrups with clear therapeutic duplication. Combination analgesic tablets and capsules were furthermore dispensed to children in large quantities. For example, 38 prescriptions of a specific Schedule 2 combination analgesic product were dispensed to children younger than 12 years (10 of these prescriptions were for 100 tablets). There was also concern about the quantities prescribed to specific families. It was clear that if the medical aid benefit of one family member was exhausted, the product was claimed under another family member’s name.

Conclusion and recommendation:

It was concluded that a medical aid database can be used to detect and control medicine abuse. It was possible to develop a set of guidelines for the medical aid administrator for implementation that can assist in controlling abuse. It was recommended that prescribing patterns in the different geographic areas be investigated.
POSTER SESSION 1

PG1 32   Prescribing for Alzheimer’s Disease: A database analysis of a South African pharmacy group

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Background:

Alzheimer's disease is an irreversible progressive disease that affects cognitive, behavioural and functional abilities. The prevalence of Alzheimer's disease is increasing as the world's population is aging. Memantine, the first product in a new category of drugs effective against dementia, was launched in March 2006 in South Africa.

Primary aim:

The primary aim of the study was to determine the prescribing patterns of drugs for Alzheimer’s disease in South Africa.

Methodology:

A retrospective, exposure-cohort drug utilisation study was conducted. Data were obtained from a South African private pharmacy group for six months in 2008. The database consisted of 789 737 medicine records.

Results and discussion:

A total of 560 patients (286 females and 274 males) received 1 827 medicine items for Alzheimer’s disease at a total cost of R890 024.70. The average cost per item was therefore R487.15. The average age of patients was 75.19 (SD = 10.37) years. Donepezil was the most frequently prescribed active ingredient (37.88%), followed by galantamine (35.08%), memantine (26.05%) and rivastigmine (0.99%). Memantine (Ebixa®) 10 mg tablets was the single most frequently prescribed trade name product, accounting for 25.95% of all trade name products prescribed for Alzheimer's disease. Average Prescribed Daily Doses (PDDs) of all active ingredients were generally lower than their respective Defined Daily Doses (DDDs). For example, the DDD for memantine is 20 mg. The average PDD was 14.80 mg for females and 14.42 mg for males. Average PDDs generally increased with increasing age.

Conclusion and recommendations:

Donepezil and galantamine accounted for over 70% of the prescriptions for Alzheimer's disease. It will be important to investigate how prescribing patterns will change in the next few years as memantine become more established on the South African market. Treatment outcomes could not be measured and it is recommended that qualitative studies be undertaken to determine the effectiveness of the different treatment options according to family members and caretakers.
Background:

Pharmaceutical advertising involves the advertising of medicines, medical devices and health care services. A literature review indicated the belief that pharmaceutical advertisements negatively affect health care decisions made by consumers. Little research has been conducted to determine how consumers in South Africa are affected by these advertisements.

Aim and objectives:

This study aimed to determine how consumers in the Nelson Mandela Metropole (NMM) perceived pharmaceutical advertisements. More specific objectives included the investigation of legislation in South Africa applicable to pharmaceutical advertisements, the interpretation and misinterpretation of the advertisements, and the identification of problematic areas in this form of advertising.

Methodology:

A qualitative study was conducted consisting of a focus group, a survey and the semiotic decoding of pharmaceutical advertisements.

Results and discussion:

South African legislation applied to pharmaceutical advertisements was investigated by means of a comprehensive literature review. A qualitative research design was used by conducting a focus group consisting of six randomly selected participants in the NMM. Thereafter, a consumer survey of 100 consumers from 10 randomly selected community pharmacies within the NMM was conducted. The themes identified in the focus group were incorporated into the questionnaire for the consumer survey. The results of the survey supported the findings of the qualitative study. Three randomly selected pharmaceutical advertisements were also decoded to interpret the components employed in each. The results indicated that pharmaceutical advertising is a marketing tool that incorporates various emotional and psychological techniques to persuade consumers. It was evident that consumers can misinterpret pharmaceutical advertisements. Various legal and ethical problems were identified.

Conclusion and recommendations:

The results showed that pharmaceutical advertisements have the possibility of negatively affecting consumers’ health care decisions and therefore warrants further investigation.
POSTER SESSION 1

PG1 34 Services for which a pharmacist may levy a fee: Preparation of sterile products and parenteral preparations


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Purpose
Preparation of sterile products e.g. eye drops and parenteral preparations like cancer chemotherapy, total parenteral nutrition (TPN) and admixing of parenteral solutions are routine functions performed by pharmacy staff in institutional practices. To prepare these products can be very time demanding and labour intensive. The time and category of pharmacy staff involved in providing these services were unknown. The purpose of the study was to determine the time taken to prepare sterile products and parenteral preparations, as well as to determine the staff performing these services.

Research Methodology
The South African Pharmacy Council commissioned a national research project on the services for which a pharmacist may levy a fee. The research was carried out collaboratively by the Schools of Pharmacy in South Africa. The time taken to perform the different phases in preparing the sterile products was recorded. Phase 1 was the pre-compounding procedures with the performance of the compounding and the post-compounding and record keeping Phases 2 and 3. The categories of staff involved at the different stages were also noted.

Results and Discussion
The weighted mean time to prepare the six recorded sterile products in five public institutional pharmacies was 811 seconds (13.5 minutes). Sixty seven percent (549 seconds) was spent on the compounding phase. The duration of the pre and post compounding phases were 17% and 15% respectively. One product was prepared by a post-basic pharmacist assistant and the rest by either a pharmacist or pharmacist intern. The weighted mean time to prepare chemotherapy, TPN and parenteral solutions were 1105 (n=57), 805 (n=5) and 381 (n=13) seconds. Fifty four percent of the time spent to prepare chemotherapy was on Phase 2. Phases 1 and 3 took 35% and 11% respectively. Pharmacists prepared 80% of the chemotherapy dosages. TPN was only prepared in two private sector facilities. Most of the time the preparation of TPN was done by the pharmacist assistant. Sixty eight percent of the time the preparation a parenteral solution (all phases) was that of a pharmacist or pharmacist intern.

Conclusion and Recommendations
Not many sterile products and parenteral preparations except for chemotherapy are prepared regularly. Post-basic pharmacist assistants could be used more to perform these services.
PG1 35 Services for which a pharmacist may levy a fee: Provision of pharmacokinetic consultation, pharmaceutical care and medicine review

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Purpose

The purpose of the study was to determine the time taken to render pharmacokinetic consultations, pharmaceutical care and medicine review as well as to determine the staff performing these services.

Research Methodology

The SAPC commissioned a national research project on the services for which a pharmacist may levy a fee. The research was carried out collaboratively by the Schools of Pharmacy in South Africa. The time taken to perform the different clinical functions was recorded. Categories of staff involved at the different stages were also noted.

Results and Discussion

The weighted mean time taken for rendering pharmacokinetic consultations was 234 seconds. For pharmaceutical care and medicine review the weighted mean times were 201 and 266 seconds respectively. Only eight observations were recorded for pharmacokinetic consultations of which seven were done in private institutions. Pharmaceutical care serves were recorded in 34 cases (85% in private institutions). All 118 cases of medicine reviews were recorded in community pharmacies. All these serves were rendered only by a pharmacist.

Conclusion and Recommendations

Not many clinical functions were performed and the recorded times may not be a true reflection of the time needed to perform these patient care functions. These services provide the pharmacist with the opportunity to strengthen their clinical involvement.
PG1 36  Antioxidant Properties of a Methanolic Extract of Garcinia Kola "Agbilu" Seeds

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Purpose:

Garcinia kola is used in treatment of various diseases such as diarrhea, hepatitis, asthma, dysmenorrhea and dysentery in traditional practice in Nigeria. Spasmolytic interaction between G. kola and oxytoxin, in the estrogen primed virgin rat uterus, markedly diminished toxic spasms. G. kola extract as a potential natural antioxidant and carcinogen could be used as replacement therapy for chemoprevention of cancer and other health threatening diseases. The effect of a methanolic extract of G. kola on ascorbic acid oxidation was evaluated.

Methods:

The method of Harris and Ray (1935) was used with modification. A 2,6-dichlorophenol-indophenol solution was prepared by dissolving 40 mg of the dye in 100 ml of distilled water. Each ml of the solution is equivalent to 0.02 mg ascorbate. The solution was freshly prepared at appropriate intervals and standardization of dye was checked against an ascorbic acid solution with a concentration of 0.04 g/ml. The stock solution of ascorbic acid was placed in an organ bath stabilized at a temperature of 37°C. The stock solution of ascorbic acid was titrated from a burette at 5 minutes intervals in triplicate. The point at which ascorbic acid was reduced to a Colonels lencobase in the indicator solution was recorded. The effect of various substances on vitamin C oxidation was evaluated by adding each substances (50 mg NaCl, 5 mg cysteine, 50 mg CuSO₄ and 1 g G. kola extract) to the stock solution.

Results:

The titration values at 20 minutes were 6.12 ml for ascorbic acid alone, 15.00 ml with NaCl, 21.53 ml with cysteine, 2.63 ml with CuSO₄ and 34.92 ml with the G. kola extract in the presence of ascorbic acid. The G. kola extract therefore illustrates antioxidant properties.
Histomorphological evaluation of a mucoadhesive, porous matrix for controlled transbuccal drug delivery

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Purpose
This study focused on designing a mucoadhesive porous matrix, assessing its ex vivo drug permeation initiating and sustaining kinetics as well as evaluating the buccal mucosal histology post administration using the pig model.

Methods
The porous matrix was prepared by homogenizing both polymeric and non-polymeric release modifying, permeation enhancing, and mucoadhesive compounds dispersed in a co-solvent system to produce a homogenous blend that was lyophilized. Ex vivo drug permeation was investigated using Franz Diffusion Cells with simulated saliva (pH 6.8) and plasma (pH 7.4) at 37°C over 8 hours. A Texture Analyzer fitted with a 10mm cylindrical probe was employed for measuring the ex vivo bioadhesivity of the formulation. Fresh porcine buccal mucosa (0.9±0.1mm thick) was employed for the bioadhesivity and permeation tests. Drug loading capacity was computed and the surface morphology was evaluated using SEM and porosity was measured by porositometric analysis. In vivo histomorphological studies were conducted using 3 healthy Large White Pigs (36±3kg) and a drug-loaded and placebo formulation was applied to the non-keratinized region of the buccal mucosa. Experimental biopsy samples and their controls, about 3mm deep (1mm² area), were taken from the administration site and control sites. The specimens were stored in 10% formalin for analysis. They were processed with routine histological methodology in an automated tissue processor after which tissue blocks were sectioned at 5µm and the slides that were produced stained using haematoxylin and eosin in an automated stainer before evaluation under the electron microscope.

Results and Discussion
The formulation bio-adhered to the buccal mucosa (1.07±0.02N). 90.05±1.15% of carbamazepine was loaded into the matrix and 83.56±3.62% permeated through the porcine tissue in a controlled manner over 8 hours following pseudo-zero kinetics. The formulation was mesoporous in nature (80.12±3.42Å) and presented with a widespread internal spherical pore structure with asymmetrical channels. The pig model employed tolerated the formulation well indicating its non-toxicity. SEM images revealed that the formulation had minimal, non significant pathological effects on the buccal epithelium and lamina propria of the porcine buccal mucosal epithelium. This implied that the polymeric, non-polymeric and active drug components of the porous matrix formulation had no irritable or toxic effects on the porcine buccal mucosa thereby making it potentially suitable for its intended transbuccal delivery application.

Conclusions
The porous matrix displayed the potential to initiate and sustain tissue permeation of drug molecules in a controlled manner for possible transbuccal drug delivery applications.
**Aim:**
Nevirapine (NVP) is an antiretroviral agent that is used for prophylaxis and treatment of HIV. Unfortunately, its use is associated with occurrence of hypersensitivity reactions and hepatotoxicity, the mechanism of which remains unclear. Furthermore, NVP is an enzyme inducer that is metabolised by cytochrome P450, as such, it is liable to P450-drug interactions during combination therapy with other drugs, particularly the protease inhibitors, leading to toxic or sub therapeutic concentrations. Therefore, the use of NVP requires plasma concentration monitoring for which a method for analysis is required. Unfortunately, the existing methods could not be adopted owing to the solvents and instrumentation used, while the rapid methods posed a problem regarding the column-life. Therefore, a high performance liquid chromatography (HPLC) assay for determination of NVP concentrations in plasma was developed.

**Methods:**

**Sample preparation:** To 100 µl of plasma spiked with NVP, chlorzoxazone (IS) was added and proteins were precipitated with perchloric acid, centrifuged and the supernatant was purified by solid phase extraction on C18 cartridges with acetonitrile:water (80:20, v/v), and 40 µl of the eluent was injected into the HPLC.

**HPLC conditions:** The sample was analyzed on HP 1100 series with an isocratic pump and a UV detector set at 210nm. The mobile phase was TEAP buffer and acetonitrile (60:40; v/v) at a flow rate of 1 ml/min. Separation of the compounds was performed on a C18, 5 micron (150 x 4.6 mm) analytical column with a run time for of 8 minutes.

**Method Validation:** Calibration curves (0 µg/ml – 10 µg/ml) were run over 5 days and the linear regression and correlation coefficient (r) were calculated using the GraphPad® statistical program. Accuracy was tested at 1, 5 and 10 µg/ml, while stability was tested at room temperature, 4°C and -20°C at 8 hours, 24 hours, and 1 week.

**Results:**
Nevirapine eluted at 2.6 min. while the IS at 5.2 min., and both peaks were sharp and symmetrical. The average 5 days calibration curve was linear (y = 0.012x + 0.051;  r = 0.9985) with a CV% of ±5.25%, while accuracy at 1, 5 and 10 µg/ml was 89.9%, 100.6% and 96.3%, respectively. Stability at -20°C was 91.7% and 86.1% at 24 hours and 1 week, respectively. The method was used to monitor NVP in rat plasma.

**Conclusion:**
An accurate and effective HPLC method for measurement of NVP in a small plasma volume (100 µl) was developed. This method will be useful for analysis of NVP in patients, particularly in the newborns and in small research animals (mice and rats) where a small volume of blood is often available.
Introduction:
In 2007, about 2 million people died of AIDS, 33 million were living with HIV and 2.5 million were newly infected with the virus. HIV / AIDS is one of the greatest challenges in developing countries with a huge impact on social-economics and resources. The impact on children is a severe and growing problem. In 2007, 420 000 children under age 15 were infected with HIV and 290 000 died of AIDS. Combination antiretroviral therapy has proven to be the most effective approach in treating HIV positive patients. This combination therapy leaves us with a major patient compliance problem for children and babies. It would be difficult for children and babies to swallow large and many tablets. Therefore, an alternative dosage form to the conventional fixed dose combination tablets is desired and would be of importance for paediatric HIV patients. It may also proof to be advantageous for those like the elderly, who cannot swallow oral dosage forms such as capsules and tablets.

Background:
The Pheroid delivery system consists of the interspersion of two liquid phases and a gas phase. The two liquid phases consist of an aqueous base and an oily base. Pro-Pheroid formation thus involves the process of gassing the oily phase with nitrous oxide, followed by mixing the drug into this oily phase.

Objectives:
The aim of this study was to evaluate the stability of nevirapine and butylparaben when formulated into single API dosage forms containing the pro-Pheroid delivery system.

Method:
The formulation was subjected to an accelerated stability study over a period of three months. The ARV drug was constituted in the pro-Pheroid and stored at 25°C+60%RH, 30°C+65%RH and 40°C+75%RH for three months. HPLC analysis was done at 215nm and 245nm after each month according to the assay method. The method was developed by Kühn, A.

Results:
The content of nevirapine and butylparaben remained stable throughout the first two months and decreased after month 3.

Conclusion:
Preliminary stability studies showed that nevirapine and butylparaben could be successfully formulated in the pro-Pheroid delivery system.
PG2 04  Optimisation of salbutamol sulphate sustained release matrix tablets using a central composite design

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Purpose:
Salbutamol sulphate (SS) is a short-acting β₂ agonist that is used for the treatment of bronchoconstriction and bronchospasm in patients with reversible obstructive airway disease and chronic obstructive pulmonary disease. Sustained release formulations are designed to release an active pharmaceutical ingredient (API) at a specific rate, resulting in sustained drug plasma levels. Response surface methodology (RSM) involves the use of systematic experimental design to characterise the relationship between input and response variables. Polynomial equations and response surfaces may be used to optimise a formulation to achieve specific target goals. The objective of this study was to use RSM methodology, specifically a central composite design for the optimisation of a sustained release matrix formulation of SS.

Methods:
A central composite design with four (4) input formulation variables viz., Methocel® K100M, xanthan gum, Carbopol® 974P and Surelease® E-7-10910 at predetermined low, medium and high levels were manufactured using a wet granulation procedure. The API, Methocel® K100M, xanthan gum, Carbopol® 974P and Avicel® PH101 were dry blended in a rapid mixer for 15 min at 100 rpm. Thereafter, 120 g of Surelease® E-7-10910 was sprayed onto the powder blend while mixing at 120 rpm for the main impeller and 1000 rpm for the chopper. Granules were dried at room temperature for 24 hr, after which they were mixed with 0.5% w/w colloidal silica and 1% w/w magnesium stearate. The tablets were compressed to a uniform weight of 220 mg using a Manesty® B3B rotary press. Dissolution testing was conducted using USP Apparatus 3 (reciprocating cylinder) with phosphate buffer (0.1 M) of varying pH over a 12 hr period. The release of SS was monitored using an isocratic HPLC method that had been previously validated.

The percent SS released in the early (1 hr), middle (6 hr) and late (12 hr) stages of the dissolution test were fitted to linear and quadratic models using the Model-Based Calibration toolbox on Matlab® R2008a.

Results:
The drug release pattern after 1 hr and 6 h were best described using quadratic models, whereas the release after 12 hr was best fitted to a linear model. The results indicated that Methocel® K100M and xanthan gum had the greatest impact on the release of API from the matrix formulations. Increasing the proportion of these hydrophilic matrix formers resulted in significant reduction in the rate of drug release, whereas Surelease® had the greatest impact on drug release in the latter stages of the dissolution test. Increasing the Carbopol® 974P content resulted in a minor increase in the rate of drug release. A minimisation procedure was used for the optimisation of a formulation with the desired release characteristics. The formulation that was predicted from the optimisation procedure produced the desired rate of release, when tested using the conditions described above.

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PG2 05 Crosslinking of polymethacrylates for controlled drug release in the small intestine

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Purpose

This study explored various polymeric crosslinking techniques for site-specific drug release in the small intestine employing polyspheres. Furthermore, the effect on drug entrapment has been assessed due to the varying crosslinking approaches employed.

Methods

A 50mL formulation of a INH-loaded latex containing 30mL deionised water, 20% polymethacrylate (PMA) and 5% 1M NaOH was prepared. PMA powder was weighed and added in specific quantities to 30mL deionised water. NaOH (1M) was then added to the latex in a drop-wise manner to allow neutralisation. Triethyl citrate (TEC) was added as a plasticizer followed by a 20%w/v ethyl cellulose solution with the entire latex agitated under a Heidolph® propeller stirrer for 30min. INH (6%w/v) was then added with further agitation. The first electrolyte solution used for crosslinking was a 25%w/v AlCl₃ followed by crosslinking in either a 30%w/v BaCl₂ or a 30%w/v MgCl₂ solution to provide Formulations A and B respectively. After a predetermined washing in deionised water, the spheres were then dried under ambient conditions overnight. Samples were subsequently tested routinely for drug release, surface area, porosity and drug entrapment efficacy (DEE).

Results and Discussion

Polysphere formulations A and B revealed a drug entrapment of 42 % and 50%, respectively. This can be attributed to the specific molecular structure (and mass) of the individual cationic crosslinkers and the volume that each constituted. The cationic crosslinkers similarly had a direct effect on the drug release and pore size of the spheres. Formulation A shows a BJH adsorption of 315.31Å and BJH desorption of 292.00Å, which is much larger than Formulation B with a BJH adsorption of 234.60Å and a BJH desorption of 210.55Å. This pore size difference is one explanation for the more controlled in INH release observed with Formulation B as compared to A. The Ba²⁺ ions only penetrated a limited area of the matrix relative to the Mg²⁺ ions, due to the much larger ionic radius. Hence crosslinking was not as saturated as with the Mg²⁺ ions resulting in a burst phase of drug release.

Conclusions

Results from this study revealed that Formulation B displayed a superior control of INH release in the small intestine, higher DEE values and a more regulated (limited in size) pore distribution. Mg²⁺ served as the optimal divalent cation with regards to crosslinking for producing more controlled drug release kinetics. Further investigation into optimizing the drug release is warranted for future investigations.
Purpose
This study focused on the design of an electro-spun fibrous zidovudine (AZT)-loaded membrane for rapid drug delivery.

Methods
Preparation of fibrous membranes: PVA was dissolved in a 2:1 mixture of water and isopropanol at 15% w/v. A drug-loaded solution was produced by dissolving PVA and AZT in a 2:1 mixture of water and isopropanol at 15% w/v and 3% w/v, respectively. Electro-spinning of the solutions was performed at 20kV with a tip-to-collector distance of 5cm, instituting a custom-built electro-spinning device equipped with a voltmeter. Fibres were collected on aluminium foil. Disintegration: The disintegration time was tested using a modified USP Basket Rack Assembly. Fibrous membranes were placed on a wire mesh and the basket rack assembly was lowered into and raised out of a beaker containing 150mL of simulated saliva (pH 6.75; 37°C) using a water bath. Drug encapsulation efficiency: The DEE was determined at pH 6.75 using UV spectrophotometry. Mucoadhesion: The mucoadhesive profile of the fibrous membranes was evaluated using a TA.XTplus Texture Analyser (Stable Microsystems, UK), fitted with a cylindrical probe affixed with a dialysis membrane. All tests were performed in triplicate (N=3).

Results
The resultant fibrous membranes were thin, flexible and porous. The thickness of the membranes was dependent on the electro-spinning time. The average disintegration time was recorded as 6 seconds for plain fibrous membranes and 5 seconds for AZT-loaded membranes. The average AZT entrapment ranged from 96-101%. The average work of adhesion was 0.719mJ for plain fibres and 0.924mJ for AZT-loaded fibres. The average force of adhesion was 1.3577N and 0.5187N for plain and AZT-loaded fibres, respectively.

Conclusions
The fibrous membranes exhibited porous morphologies, desirable AZT entrapment efficiency, bioadhesiveness and a rapid disintegration rate and thus demonstrate the propensity for rapid drug delivery.
PG2 07  An in vitro evaluation of triclosan in combination with Pheroid™ technology for antimalarial treatment

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Introduction
Malaria is an escalating world-wide problem and consequently, new and more efficacious treatments to combat the disease are urgently needed. Triclosan 5-chloro-2-(2,4-dichlorophenoxy)phenol), widely used as an antibacterial, has recently demonstrated antimalarial activity. The very low solubility of triclosan (~10 mg.L⁻¹) can give rise to problems in formulation and variable bioavailability. For effective treatment of malaria, systemic absorption of the drug at a therapeutic dose is necessary. To overcome these problems drug delivery systems can be used. The Pheroid™ system is a patented drug delivery system which entraps drugs with high efficiency and delivers it to target sites in the body. It consists of a unique submicron emulsion type formulation. The aim of this study was to evaluate if triclosan, in combination with Pheroid™ technology, can be an effective option for malaria treatment.

Method
A chloroquine resistant strain of *P. falciparum* at 5% hematocrit and 0.5% parasitemia was used. Parasite red blood cells were incubated with different concentrations of triclosan alone and Pheroid™-triclosan combinations at 37°C in an atmosphere of 5% O₂ 5% CO₂ and 90% NO₂ for 48 hours. Inhibition of parasite growth was measured in relation to the control without drugs, by counting the parasites in Giemsa-stained thin blood smears and by flow cytometry.

Results
Both triclosan and triclosan in Pheroid™ formulations showed antimalarial activity against chloroquine resistant parasites. Triclosan in Pheroid™ (10 µM) showed higher *in vitro* activity, with 90% reduction in parasitemia, compared to 70% reduction by triclosan alone. The efficacy of triclosan in Pheroid™ increased between 1 and 2 fold with the different concentrations used (0.075 – 10 µM). The IC₅₀ value for triclosan in Pheroids were 0.68 µM compared to 1.05 µM of triclosan alone. This resulted in an increased therapeutic efficacy of triclosan.

Conclusion
Triclosan in combination with Pheroid™ vesicle is more efficacious than triclosan alone. Pheroid™ technology can thus be considered in the development of antimalarials aiming at chloroquine resistant parasites.
MeHg is a toxin that is released into the environment by both natural and artificial sources. In humans, MeHg exposure is predominantly through the consumption of contaminated fish and the anthropogenic release into waterways resulting in contamination of freshwater sources. MeHg is readily absorbed in the gastrointestinal tract and crosses the blood brain/placental barrier. Poisoning by MeHg is associated with neurotoxic effects which are seen in both human and animals. The immature central nervous system is extremely vulnerable to the harmful effects of MeHg and pregnant women showing little or no signs of poisoning have given birth to affected offspring. The neurotoxic effect of metals such as mercury is through the increased production of reactive oxygen species which have been implicated in Parkinson’s disease, a neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta. In the present study, pregnant rats were prenatally stressed by the administration of 5 mg/kg s.c MeHg in the last week of pregnancy. Adult offspring were lesioned with the neurotoxin 6-hydroxypamine (6-OHDA) to create a mild parkinsonian rat model. Melatonin, a potent antioxidant was administered for two weeks immediately following 6-OHDA lesion. We hypothesize that exposure to MeHg during pregnancy will exacerbate the neurotoxic effects of 6-OHDA and injection of melatonin immediately following the lesion will attenuate dopamine neuron destruction in the substantia nigra pars compacta.
PG2 09 Cytochrome P1A2-mediated o-deethylation of phenacetin to produce paracetamol: in vitro optimization and determination of enzymatic kinetic parameters

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Purpose
The investigation the in vitro metabolism of N-(4-ethoxyphenyl)ethanamide (phenacetin) to produce N-(4-hydroxyphenyl)ethanamide (paracetamol) using liver microsomal cytochrome P1A2 (CYP1A2), and the establishment of an optimal laboratory assay technique for the determination and quantification of the metabolite and estimated enzyme kinetic parameters was undertaken.

Methods
A method of chromatographical separation and quantitative determination of paracetamol and phenacetin was developed using a highly sensitive Ultra Performance Liquid Chromatography (UPLC) system. Phenacetin solutions (0.5-50.0mmol/L) were incubated with 0.25mg/mL human liver microsome (HLM) in 24-well plates for 25 minutes in a shaking incubator (100rpm; 37°C). An NADPH-regenerating system prepared with glucose-6-phosphate dehydrogenase and reduced NADP+ in a cofactor solution was used to initiate the metabolic reaction. The reaction was halted by the addition of ice-cold acetonitrile (-20°C). The resulting mixture was centrifuged at 4°C at 4000g to precipitate the microsomal proteins prior to quantifying the substrate content. Supernatants were analysed by a UPLC method injecting 1.7µL of sample containing equal volume of 0.05M loperamide (internal standard) through a BEH phenyl column (1.7µm; 2.1x100mm) with a binary mobile phase comprising 0.025M KH2PO4 (pH 2.5) and acetonitrile (gradient=95:5-5:95, 5 minutes) at a flow rate of 0.2mL/min absorbing at 200nm (7000psi, delta<20) in order to quantify the rate of paracetamol formation that was profiled against phenacetin concentrations to determine the enzyme kinetic parameters.

Results and Discussion
The UPLC assay yielded a linear calibration curve through the origin with the relationships y=0.0031x (R²=0.99) and y=0.002x (R²=0.99) for phenacetin and paracetamol, respectively. The ‘x’ and ‘y’ variables represented the substrate concentrations and the ratio of the AUCs. Typical Michaelis-Menten curves were obtained upon profiling phenacetin concentrations against the ratio of paracetamol formation to enzyme activity. The maximum rate of paracetamol formation (Vmax) was 122.6µmole/min/mg HLM. Phenacetin concentration corresponding to 50% of the Vmax (Vmax/2) also referred to as the Michaelis-Menten constant (Km) was 0.5mM. Although literature values vary widely, kinetic parameters obtained through this method were reproducible, thereby validating the method for further analytical enzymatic determinations.

Conclusions
A validated method for the in vitro metabolism of phenacetin by HLM was developed and optimized. At the determined Km value, paracetamol formation was optimal. Studies have shown that there is a close similarity between in vitro and in vivo HLM metabolic behaviour. The Km value obtained in this determination is applicable in the therapeutic use of phenacetin as a prodrug, its metabolism to yield active paracetamol, metabolic induction in cases of phenacetin toxicity and metabolic inhibition in case of paracetamol toxicity.
PG2 10  *In vitro* investigation of the effects of quercetin, naringin and naringenin on the metabolism of felodipine by human liver microsomes

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**Purpose**

To investigate the effects of flavonoid content of grapefruit juice on the metabolism of felodipine by human liver microsomes (HLM) following the reported observation of an increase in the oral bioavailability of felodipine due to concomitant grapefruit juice consumption, and comparing such effects to that of verapamil, a known CYP 3A4 inhibitor.

**Methods**

A highly sensitive assay method for felodipine was developed using Ultra Performance Liquid Chromatography (UPLC) which yielded a linear calibration curve with the relationship $y=0.0158x$. The ‘x’ and ‘y’ variables represented the substrate concentrations and the ratio of the AUC’s. Laboratory conditions for *in vitro* felodipine metabolism using pooled HLM were optimized by incubating felodipine over a range of 0.03-40.0mmol/L with 0.25mg/mL HLM in 24-well plates for 10 minutes in a shaking incubator (100rpm; 37°C). An NADPH-regenerating system prepared with glucose-6-phosphate dehydrogenase and reduced NADP⁺ in a cofactor solution was used to initiate the metabolic reaction. The reaction was halted by the addition of ice-cold acetonitrile (-20°C). The resulting mixture was centrifuged at 4°C at 4000g to precipitate the microsomal proteins. Supernatants were analysed with UPLC by injecting 1.7µL samples containing equal volume of 0.05M loperamide (internal standard) through a BEH phenyl column (1.7μm; 2.1x100mm) in a binary mobile phase comprising 0.025M KH₂PO₄⁻ (pH 2.5) and acetonitrile (50:50) at a flow rate of 0.2mL/min absorbing at 200nm in order to quantify the extent of substrate metabolism in solution. The rate of metabolism was profiled against felodipine concentrations to determine the enzyme kinetic parameters. A typical Michaelis-Menten curve was obtained upon profiling the felodipine concentrations against the rate of its metabolism due to enzyme activity. The maximum rate of metabolism ($V_{max}$) was 500µmol/min/mg HLM. The substrate concentration corresponding to 50% of the $V_{max}$ ($V_{max}/2$) also referred to as the Michaelis-Menten constant ($K_m$) was 5mM. Further incubation of felodipine was performed at its determined $K_m$ value with verapamil, quercetin, naringin and naringenin over a range of 10-1000µM. The inhibitory potencies of the probe substances were measured as their IC₅₀ values determined through a non-linear regression of the inhibition (%) of felodipine metabolism against the inhibitor concentration.

**Results and Discussion**

All three flavonoids showed significant inhibition of felodipine metabolism compared to verapamil. The IC₅₀ value for verapamil was 107.88M while for naringin, naringenin and quercetin it was 177.81M, 121.97M and 208.65M, respectively. The low IC₅₀ value of naringenin further suggested that it is the aglycone portion of the glycoside (naringin) that was responsible for the inhibitory effect.

**Conclusions**

The flavonoid contents of grapefruit juice responsible for the observed increased oral bioavailability of felodipine following concomitant consumption of grapefruit juice may be due to first-pass inhibition mediated by intestinal and hepatic microsomal CYP 3A4.
PG2 11 Development and validation of an HPLC method for the determination of clobetasol 17-propionate in cream formulations  

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Introduction:  
Potent corticosteroid molecules marketed by innovator companies are expensive and as 33% of the South-African population reportedly suffers from common dermatological conditions, there is a need for the development of generic versions of topical corticosteroid formulations. Clobetasol 17-propionate (CP) is a Class 1 corticosteroid that is highly potent drug and is formulated as a 0.05% w/w cream for the treatment of significant skin inflammation in disease states such as psoriasis, atopic dermatitis, eczema and lichen planus. The analysis of Clobetasol 17-propionate in semi-solids has been performed using normal-phase HPLC, ultraviolet spectroscopy, liquid chromatography-mass spectrometry (LCMS) and LCMS®. The development of a simple reverse phase high performance liquid chromatography (HPLC) was attempted due to the simplicity, relatively cheap cost, and selectivity of the technique. The objective of this study was to develop a simple, stability indicating RP-HPLC method using UV detection for the quantitative analysis of CP in cream formulations.

Methods  
CP stock solutions (100μg/ml) were prepared daily by dissolving CP in 100ml methanol. Separation was achieved using a Nova-Pak® 60 Å C18 4 μm (3.9 i.d. x 150mm) column, maintained at ambient temperature and a mobile-phase comprised of a mixture of degassed methanol and water (68:32) at a flow rate of 0.9 ml/min. The modular HPLC system consisted of a Spectra-Physics® solvent delivery module, a Spectra system® Model 710B Wisp auto sampler, a Linear® UV-200 detector and a dual-pen Hitachi® strip chart recorder. Betamethasone 17-valerate was used as the internal standard and the eluant was monitored at a wavelength of 239nm. The method was validated in terms of linearity, precision, accuracy, limits of quantitation (LOQ) and detection (LOD). Stability studies of CP and BV alone and in combination were initiated with a CP concentration of 12μg/ml on the workbench (22 °C), in the autosampler (22 °C) and in the refrigerator (4 °C) for a period of 7 days.

Results  
Sharp well resolved peaks were obtained for CP and BV with fully defined baseline resolution. The retention times for CP and BV were 6.0 min and 8.0 min respectively. The calibration curve over the range of 0.15μg/ml to 15μg/ml was found to be linear with an equation for the line y=0.1183x-0.0245 (R²= 0.9998). Initial precision (n=3) studies reveal that the method is precise with resultant %RSD values between 0.01 and 1.21% for the concentrations studied. Initial accuracy (n=3) studies revealed % bias values for all the concentrations of < ±5%. The LOQ and LOD were found to be 0.15μg/ml and 0.045μg/ml respectively. The preliminary results of stability studies indicate that the solutions of CP are stable for the 7 day period whereas BV appears to be stable for only 3 days.

Conclusion:  
A rapid, reliable analytical RP-HPLC method with appropriate precision and accuracy to analyse Clobetasol 17-propionate in topical formulations has been developed and validated. The method appears to be stability indicating and will be applied to the analysis of CP in formulation development and assessment studies.

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Characterisation of Chitosan-poly(ε-Caprolactone) interpenetrating polymeric complexes

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Purpose

This study investigated the effect of varying the composition of chitosan (CHT), a biodegradable and biocompatible polymer, and poly(ε-caprolactone) (PCL), a pH-responsive polymer, on the resultant formulation and characterisation of lipid-based formulations.

Methods

Solutions of CHT (1-2% w/v) in acetic acid and (PCL) (2-5% w/v) in chloroform were prepared. Phosphatidylcholine (10mg) was incorporated into the PCL solution. All solutions were agitated overnight. The CHT solution was subsequently added to the PCL solution in the presence of sonication. A surfactant, Tween 80 (2mL), was added drop-wise to the emulsion during homogenisation. The resultant emulsion was subjected to rotary evaporation under vacuum (30rpm; 90°C) for 12 hours. The final solution was allowed to evaporate under a fume hood for 48 hours. The polymer composition of two exemplary formulations were as follows: Formulation 1: CHT (2% w/v; 100mL), PCL (5% w/v; 25mL); Formulation 2: CHT (1% w/v; 50mL), PCL (2% w/v; 100mL). Microscopy, size and zeta analysis, FTIR spectroscopy, and differential scanning calorimetry were undertaken for in-depth characterisation of the complexes.

Results

Macroscopic analysis exhibited morphological distinctions between the final products of each formulation. Formulation 1 produced porous macroparticles, whilst a semi-membranous structure with a microbead-like surface morphology resulted from Formulation 2. Microscopy studies revealed a more consistent size distribution in Formulation 2 (~100-200µm), as compared to Formulation 1. Vibrational transitions demonstrated minimal deviation from that of the spectra of the native polymers and lipids, signifying a minor degree of inter-polymeric/ lipid interaction, with the spectra for each of the formulations displaying greatest similarity to the spectrum of the dominant polymer in the formulation. This was further confirmed following evaluation of the thermal transitions of the complexes, in which individual glass transition and melting point peaks, representative of each native polymer employed was elucidated for the formulations. Significant variation in the stability of these formulations was also highlighted by their zeta potential, with Formulation 1 demonstrating greater stability.

Conclusions

The relative proportions of the polymeric components had a substantial influence on the resultant complexes. This principal can be manipulated to develop multi-functional polymeric systems with precise properties for specific drug delivery functions.
PG2 13  A selective, sensitive and rapid Liquid Chromatography- Tandem Mass Spectrometry method for the determination of artemether and dha in human plasma

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Purpose:
Approximately one million deaths occur each year of malaria. Further, the treatment of uncomplicated malaria occurring in pregnant women in Africa, is also a challenge, still to be met. The consequences of prolonged malaria attacks can have grave effect for the mother, and the developing foetus. Whilst the shift for the general population to artemisinin combination therapies (ACT’s) in first line therapy are coming into place, throughout the African continent, considered clinical trials have not been performed in pregnant women. This is particular acutely necessary as ACT’s become more widely available. As part of a efficacy study in pregnant women, attending the ante-natal clinic of Mbarara, National Referral Hospital, in Uganda, Coartem®, a fixed combination of Artemether/Lumefantrine, has been used (since 2006), to treat uncomplicated malaria.

In the context as sketched, a validated liquid chromatography tandem mass spectroscopy bioanalytical method was developed in the laboratory for the quantification of artemether and dihydroartemisinin (DHA) in human plasma, using deuterated internal standards.

Methods:
Instrumentation, and Sample Storage: An Agilent 1200 series HPLC system and Applied Biosystems API 4000 triple quadrupole mass spectrometer was used, to develop and validate the method. The reference standard, calibration standards and quality control standards were stored at -70 °C.

Chromatography and Detection: Chromatography was performed on a Phenomenex Luna, PFP (50 x 2.0 mm, 5 μm) analytical column. The mobile phase consisted of methanol and ammonium acetate (10 mM) with 0.1 % acetic acid (65:35, v/v) and was delivered with a gradient at a flow rate of 0.5 ml/min. Detection was performed on the mass spectrometer with ESI in the positive ion mode.

Extraction procedure: The liquid-liquid extraction procedure, was performed on ice in polypropylene test tubes, followed the aliquotting of samples (100 µl). 100ul of internal standards in blank plasma (100 ng/ml), and Universal Buffer (200 µl) was added, followed by Ethyl acetate (2ml), in consequential steps. Samples were then vortexed (1 minute), and spun at high speed (5 minutes) after which the organic phase was subjected to evaporation (30°C for 1 hour). Mobile phase (100 µl) was added to the dry samples, which was vortexed (30 seconds), and then subjected to analysis. 20 µl was injected onto the HPLC column.

Results and Conclusion:
Alternative cost effective method: A rapid, sensitive and selective method has been established for the simultaneous determination of Artemether and DHA in plasma, using high-performance liquid chromatography separation with tandem mass spectrometric detection.

Clinical studies: This method has been used for the analysis of Artemether and DHA in a Ugandan clinical study recently, and will further be used in a South East African Combination Anti-malarial Treatment (Seacat) study.

Validation: The method performed well during intra- and inter-batch validations for peak areas with the use of the internal standard and quadratic regressions for Artemether and DHA calibration curves, respectively.

Calibration range: The method was validated between the range 2 and 500 ng/ml for both the analyte and metabolite, with the percentage coefficient of variation (% CV) being at most 14.3% for Artemether, and 10.2% for DHA at 2ng/ml, respectively.
**Purpose:**

Each year 350-500 million cases of malaria occur worldwide and over 1 million people die; most of them young children in sub-Saharan Africa. Lumefantrine (LF) is an antimalarial that is widely available as a co-formulated product Coartem® (lumefantrine/artemether). This combination has proved to be well tolerated and highly efficacious in children and adults. Lumefantrine is a lipophilic compound that is more than 99.9% bound to plasma proteins and has a longer elimination half-life. Previous published methods for the determination of LF in plasma using liquid-liquid extraction techniques used 1ml plasma samples to achieve lower limits of quantification of 25 and 13 ng/ml. The aim of this work was to develop and validate a sensitive and selective high throughput analytical method for the determination of LF using only 25 µl of plasma sample.

**Methods:**

*Instrumentation:* An Agilent 1200 series HPLC system and Applied Biosystems API 3200 triple quadruple mass spectrometer was used to analyse samples and validate the method. Chromatography was performed on a Phenomenex Luna, PFP (50 x 2.0mm, 5µm) analytical column. The mobile phase consisted of acetonitrile and 0.1% formic acid (70:30, v/v) and was delivered at a constant flow rate of 0.5 ml/min for 3 minutes.

*Protein Precipitation Extraction Procedure:* 25 µl of the thawed and vortexed plasma sample was pipetted into 1.5 ml eppendorf tubes. The protein precipitation solvent mixture was prepared and spiked with the deuterated internal standard (ISTD); 2µl of a 1mg/ml ISTD stock solution was added to 22.5 ml acetonitrile and 7.5 ml 0.1% formic acid. 250 µl of this precipitation solvent was added to the 25 µl plasma sample. The samples were vortexed for 1 minute, ultra sonicated for 5 minutes then vortexed again for 30 seconds. They were then centrifuged for 10 minutes at 13000 rpm. 150 µl of the supernatant was then transferred to a 96 well plate and 2 µl was injected onto the HPLC column.

**Results and Conclusions:**

*Alternative Cost Effective Method:* A rapid, sensitive and selective method has been developed for the determination of LF but only uses 25 µl of plasma; this is significant as the majority of malaria infected patients are small children. This high throughput assay using protein precipitation should prove to be more cost effective than established solid phase extraction methods.

*Calibration Range:* The method was validated for LF with 20 µg/ml as the highest level of quantitation and 0.02 µg/ml as the lower limit of quantitation.
PG2 15 Evaluation of chlorpromazine release from a polyamide-ethylcellulose implantable mini-disc

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Purpose
The purpose of this study was to investigate the possibility of developing a biodegradable intracranial implantable device capable of chlorpromazine hydrochloride (CPZ) release over a prolonged period of time.

Methods
Preparation of mini-discs: Polyamide 6,10 (PA 6,10) membranes were prepared by an immersion precipitation reaction. The PA 6,10 was firstly dissolved in formic acid (3mg/mL), cast in appropriate molds and immersed in de-ionized water (F1), a 5% w/v CaCl₂ solution (F2), a 1.15% w/v NaCl solution (F3) and a 5% w/v NaOH solution (F4). Resultant membranes were dried, ground, sieved and granulated with ethylcellulose (EC) and CPZ. Mini-discs with dimensions of 8×2mm were compressed using a Carver Hydraulic Press. In addition, a fifth disc (F5), comprising the PA 6,10 powder, EC and CPZ, was compressed. FTIR spectrophotometric Analysis: FTIR was performed on resultant membranes and the PA 6,10 powder to assess major structural variations that may have occurred during the immersion precipitation reaction. In vitro drug release and matrix erosion studies: Drug release studies were performed in 100mL PBS (pH 7.4, 37°C) at 25rpm using an orbital shaker bath. Samples were taken at predetermined intervals and analyzed at 254nm using UV spectroscopy. Matrix erosion was determined at 168 hours.

Results
All formulations barring F4 exhibited negligible structural changes thereby confirming the integrity of major functional groups on the polyamide backbone. F4 displayed a lack of minor peaks characteristic of the polyamide backbone indicating the formation of a structurally distinct compound with purportedly novel properties. F2 and F4 exhibited total drug release within a period of 27 and 15 hours, respectively. In addition, these two formulations demonstrated an average mass loss of ~91% after 168 hours. F1, F3 and F5 demonstrated desirable drug release behavior with drug release ranging from 5.8-19.8% over 168 hours. Furthermore, these longer-acting formulations revealed an average mass loss of ~17%.

Conclusions
In vitro assays highlighted the potential of the fabricated systems for controlling drug release over extended periods of time. This could prove beneficial for the management of psychotic patients over prolonged periods, overcoming patient non-compliance and relapse.
PG2 16 Development and validation of an HPLC method for the determination of ketoconazole in pharmaceutical dosage forms

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Purpose:
During the development and assessment of pharmaceutical formulations rapid, simple, sensitive and selective analytical methods for the determination and quantitation of API are required. The analysis of ketoconazole, an imidazole antifungal, has been accomplished in biological fluids using high performance liquid chromatography (HPLC). However HPLC has rarely been used to analyze ketoconazole in pharmaceutical dosage forms. HPLC methods that have been reported are complicated, and require mobile phase compositions that include the use of hydrophobic ion pair reagents or amine modifiers. The aim of this study was to develop a simple reversed-phase HPLC assay for the determination of ketoconazole in pharmaceutical dosage forms viz., oral paediatric formulations.

Method:
The method was developed using a modular isocratic reversed-phase HPLC-UV system. The analysis was performed at room temperature (22°C) and separation achieved using a Beckman Coulter® ODS column (5µm, 150 × 4.6 mm I.D.). Clotrimazole was used as an internal standard and the eluant was monitored at 206 nm. Optimal chromatographic conditions were determined by manipulation of the mobile phase composition, buffer pH and molarity and flow rate. A binary mobile phase mixture of acetonitrile and 80 mM potassium dihydrogen phosphate buffer (60:40) adjusted to pH 6.0 using 1 M sodium hydroxide was used for the separation. A 10 µL sample was injected using a Waters® Wisp 710B autosampler and the analytical run time was approximately 12 min at a flow rate of 1 ml/min. The method was validated as outlined in the ICH guidelines and included an assessment of linearity, precision, accuracy, limits of detection (LOD) and quantitation (LOQ).

Results:
The resultant retention times for ketoconazole and clotrimazole were 3.7 min and 8.6 min, respectively. Validation results reveal that the assay was linear over the range of 2-120 µg/ml and the calibration curve yielded a linear regression coefficient ($r^2$ value) of 0.998 with the equation of the line y = 0.0154x − 0.0021. The precision determined at 5, 45 and 115 µg/ml and expressed as percentage relative standard deviation (% RSD) and was found to be between 0.35-3.91% RSD (n=6) for intra-day precision and 1.11-2.52% RSD (n=6) for inter-day precision (1-3 days). Accuracy, reported as percentage bias was found to be 0.56-4.07%. The LOQ and LOD were found to be 2 µg/ml and 0.6 µg/ml using the standard deviation method.

Conclusion:
A reversed-phase HPLC method has been developed and validated and is simple, linear, accurate, precise, sensitive and selective for ketoconazole. The method has application during the development and assessment of ketoconazole formulations.

Acknowledgements:
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PG2 17  Antibacterial activity of selected liposome encapsulated cyclic dipeptides

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Purpose:
Liposomes are small vesicles that are currently under investigation as drug carriers for the delivery of therapeutic agents. A number of liposome formulations are currently under clinical trial review, whilst some of them have already been approved for clinical use. Cyclic dipeptides (CDPs) are amino-acid-based compounds, some of which possess antimicrobial activity. The encapsulation of certain drugs into liposomes has been found to improve their activity in terms of bioavailability and duration of action. The aim of this study was to formulate and assess the antimicrobial activity of liposome-encapsulated CDP, as compared to that of free CDP.

Methods:
The liposomes were manufactured, using dimyristoylphosphatidylcholine (DMPC) and cholesterol, by the dehydration-rehydration method. The antimicrobial activity was assessed as the Minimum Inhibitory Concentration (MIC) of the CDPs was tested against S. aureus and B. subtillis, E. coli, K. pneumoniae and C. albicans using a serial dilution method and MTT as an indicator of bacterial viability. CDPs tested were cyclo(Tyr-Pro) and cyclo(Trp-Pro) which have already been shown to exhibit mild antibacterial activity. Concentrations tested ranged from 0.0781 – 10 mg/ml and chloramphenicol and liposomal chloramphenicol were used as positive controls.

Results:
Both CDPs showed a significant increase in antibacterial activity against Gram positive bacteria when encapsulated into the liposome formulation with the greatest activity being that of encapsulated cyclo(Tyr-Pro) against S. aureus and B. subtillis with both having an MIC of 0.3125 mg/ml which was comparable to the positive control of chloramphenicol. The MIC value for free cyclo(Tyr-Pro) was 10 mg/ml, indicating a 32-fold increase in activity while encapsulation of chloramphenicol only increased its activity by 2-fold.

These results therefore indicate that the antimicrobial activity of cyclo(Tyr-Pro) shows a large increase when encapsulated in liposomes which may be as a result of poor permeation of the free drug into bacterial cells.
PG2 18 Simultaneous analysis of ranitidine and metronidazole in pharmaceutical dosage forms

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Purpose:
Ranitidine and metronidazole have been used in combination therapy with clarithromycin for the treatment of peptic ulcers. The current HPLC methods available for analysis of ranitidine and metronidazole in solid oral dosage forms, are specific for assessment of individual active ingredients. The development of an HPLC method that can be used for simultaneous determination both compounds, will be useful for analysis of either fixed dose combination or single dosage forms of ranitidine and/or metronidazole.

Methods:
A modular reversed-phase HPLC system with UV detection was used for development of suitable analytical method. Ranitidine and metronidazole standards were procured from SIGMA-ALDRICH™. Separation was achieved in a Beckman® 5μm octyl 4.6mm i.d. x 150mm analytical column with mobile phase comprised of acetonitrile 20% v/v and potassium dihydrogen orthophosphate 80% v/v (pH 7; 50mM) and a flow rate of 1ml/min. The injection volume was 20μl and the eluant was monitored at a wave-length of 317nm. Ranitidine was used as an internal standard for analysis of metronidazole and their role reversed when analyzing ranitidine.

Results:
The resultant retention time for ranitidine and metronidazole were 5.8 and 3.5 minutes respectively. The method was found to be linear over the concentration range studied for both drugs (ranitidine 65-110μg/ml, metronidazole 175-300μg/ml). The resultant equation of the line was, y=3566.7x-58050 ($R^2=0.9585$) for ranitidine and y=5519.8x-74066 ($R^2=0.9920$) for metronidazole. The precision of the method in terms of repeatability expressed as percent Relative Standard Deviation (%RSD) was found to be between, 0.26%-0.56% (n=3) for ranitidine and 0.29%-0.5% (n=3) for metronidazole.

Conclusion:
The resultant method has a necessary precision for the analysis of ranitidine and metronidazole in fixed dose or single entity dosage forms, and has application in formulation development and quality control studies.

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PG2 19 **Enhancement of the in vitro efficacy of chloroquine with Pheroid™ technology on multi-drug resistant malaria parasites**

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**Introduction:**
Global malaria morbidity and mortality figures are escalating due to the emergence and spread of antimalarial drug resistance. There is an urgent need for improved antimalarial compounds. One approach may be the use of drug delivery systems to improve the efficacy of existing compounds such as chloroquine. Pheroid™ technology, a novel drug delivery system, entraps drugs with high efficiency and delivers it to targets in the body. The aim of this study was to evaluate the in vitro antimalarial efficacy of chloroquine in combination with a Pheroid™ formulation on multi-drug resistant parasite strains.

**Methods:**
The in vitro efficacy of chloroquine (0 nM - 500 nM) alone and in combination with Pheroid™ vesicles was investigated on two *P. falciparum* chloroquine resistant strains (RSA11 and W2). The percentage reduction in parasitemia was determined and used to derive an effective concentration of the test and reference formulations at fifty percent (EC₅₀) using GraphPad Prism™.

**Results:**
At the highest concentration (500nM) chloroquine in combination with Pheroid™ vesicles decreased parasite levels by 74.15 % and 76.81% respectively for W2 and RSA11. In comparison the control reference formulation of low hypoxanthine culture medium only rendered parasite growth reduction of 41.38% and 64% for W2 and RSA11 correspondingly. The measured EC₅₀ values were 7.19e-010 ± 0.27 and 4,66e-011 ± 0.46 for the Pheroid™ vesicles on W2 and RSA11 and 2.72e-009 ± 0.13 and 1.55e-009 ± 0.23 for the control formulation for the respective strains.

**Conclusion:**
Pheroid™ technology shows promising results in vitro. These effects should be evaluated in vivo to determine if chloroquine can be an effective treatment option when used in combination with a drug delivery system.
The purpose of this study was to synthesize and determine the *in vitro* transdermal penetration of cytarabine and its 5’-alkyl esters and to establish a correlation, if any with selected physicochemical properties. The *n*-alkyl esters were synthesized by acylation of cytarabine (1) at its pharmacophoric 5’-OH. The transdermal flux values of (1) and its esters were determined *in vitro* using Franz diffusion cell methodology. Aqueous solubility and log D (pH 7.4) values were determined and assessed for correlation to transdermal flux. An inverse relation was observed between the water solubility ($S_w$) and log D values. Of all esters, (4) exhibited the highest flux value of 22.2 nmol.cm$^{-2}$.h$^{-1}$, which is significantly different to that of the parent drug cytarabine (3.70 nmol.cm$^{-2}$.h$^{-1}$). No trend was found between water solubility and flux values.
PG2 21  Fingerprinting of Phela, a traditional medicine, in plasma by High Performance Liquid Chromatography (HPLC)

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Purpose:

Phela is a traditional medicine, prepared using well defined parts of four African medicinal plants. In a phase 1 clinical study [PLWA] of Phela as an immune booster in HIV positive and AIDS patients, Phela led to an increase in appetite, weight gain and 200 % increase in CD 4 cell count with an overall increase in quality of life from as low as 30 % to 100 %. Currently Phela is being studied in rat model to understand its mechanism of action. For the purpose of this study a chromatographic fingerprinting method was developed to monitor Phela in plasma.

Methods:

Preliminary extractions and HPLC runs of standard solutions of Phela led to identification a marker peak by which to analyze the product.

Sample Preparation: To 1ml of plasma, IS was added after which it was acidified with 0.5M HCL and extracted with 5ml of hexane. The organic phase was evaporated to dryness under nitrogen and the residue was reconstituted with 100µl of mobile phase of which 50µl were injected in the HPLC.

HPLC Analysis: The sample was analyzed on HP 1100 series with an isocratic pump and a UV detector set at 245nm. Separation was done on C18 column (250mm x 4.6 mm x 5microns) coupled to a C18 guard column. The mobile phase was Acetonitrile: water (70/30: v/v), and the rate was 0.5ml/min with a runtime of 30 minutes.

Method Validation: Calibration curves (2 mg/ml – 10 mg/ml) were run over 5 days and the linear regression and correlation coefficient (r) were calculated using GraphPad® statistical program. Accuracy was tested at 2, 6 and 10 mg/ml, while stability was tested at room temperature, 4°C and -20°C at 24 hours, 48 hours and 1 week.

Results:

A marker peak for Phela was identified at a retention time of 25 minutes at which there was no interference in plasma. The average 5 days calibration curve was linear (y = 0.02x – 0.59; r = 0.9983) with a CV% of ±20%, while accuracy at 2, 6, 10 mg/ml was 103%, 85% and 102%, respectively. Stability was at -20°C was 97%, 77% and 124% at 24 hrs, 48 hrs and 1 week, respectively. The method was used to monitor Phela in rat plasma. In conclusion, an HPLC method for fingerprinting of Phela and monitoring its time profile in plasma was successfully developed.
Introduction
We have recently demonstrated that N-methyl-2-phenylmaleimide analogues (1) are moderately potent competitive inhibitors of the enzyme monoamine oxidase B (MAO-B). In this study a series of structurally related N-substituted phthalimide analogues (2 and 3) were prepared and evaluated as inhibitors of MAO-B. MAO-B is of therapeutic importance since this enzyme catalyzes the oxidation of dopamine in the brain. Inhibitors of MAO-B are being used for the treatment of Parkinson’s disease (PD) since they may conserve the depleted dopamine in the parkinsonian brain as well as enhance dopamine levels resulting from exogenously administered levodopa. In addition, MAO-B inhibitors may have a neuroprotective effect by reducing the formation of potentially hazardous by-products such as hydrogen peroxide in the MAO catalytic cycle.

Purpose
Various N-substituted phthalimide analogues (2 and 3) were investigated to establish their potential as reversible competitive inhibitors of monoamine oxidase B (MAO-B).

Method
A series of eleven N-substituted phthalimide (2) and homophthalimide (3) analogues were synthesized from the appropriately substituted amine and pthalic anhydride or homophthalic anhydride, and the enzyme-inhibitor dissociation constants (K_i values) for competitive reversible inhibition of MAO-B for each compound was determined. In addition a series of 4-phenoxyphthalimides (4) were synthesized by the following reaction, 4-nitrophthalonitrile was reacted with the appropriate phenol to yield the 4-phenoxyphthalonitrile. This intermediate was treated with sodium ethoxide resulting in the target 4-phenoxyphthalimide derivative.

Results
In general, all the N-substituted phthalimide analogues evaluated were found to be relatively weak reversible competitive inhibitors of MAO-B compared to the corresponding N-methyl-2-phenylmaleimides. The 4-phenoxyphthalimide was found to be more potent than the N-substituted phthalimide analogues. A possible reason for this is that the N-substitution prevents hydrogen-bond interactions between the phthalimide carbonyl oxygen and the enzyme’s amino acid residues and the integral water molecules. Inspection of the active site of MAO-B by molecular docking studies reveals that N-substitution on the phthalimidyl ring would prevent the interaction of the carbonyl oxygens with the polar substrate binding site of the enzyme.
Background:
Ozone (O$_3$), a major industrial tropospheric pollutant is associated with excessive oxidative stress resulting in central nervous system effects while oxidative stress is suggested to play a role in major depression. We therefore set out to investigate the effects of acute and chronic ozone inhalation in the presence and absence of imipramine, on immobility in the forced-swim test (FST) and on markers of neuronal oxidative stress in various regions of the rat brain.

Methods:
Male Sprague Dawley rats were exposed to 0, 0.25 or 0.7 ppm ozone by inhalation 4 hours daily for either 30 days (chronic) or once (acute). 24 Hours before the final FST scoring, rats were exposed to 15 min forced swimming, then received a 1st intraperitoneal (IP) injection of 0 or 10 mg/kg imipramine, followed by the last (or only) 4 hour exposure to ozone. Thereafter, the rats received corresponding imipramine administrations 5 and 1 hour before the FST scoring, whereafter locomotor activity was measured and the final 5 minute FST recorded. Rats were decapitated and the frontal cortex, hippocampus, striatum and hypothalamus dissected for determination of superoxide accumulation and lipid peroxidation.

Results:
Imipramine evoked a significant antidepressant-like effect that was independent of acute or chronic ozone exposure in the FST, although alone imipramine failed to evoke any noteworthy effects on superoxide and lipid peroxidation on rats exposed to acute ozone inhalation. Acute ozone (0.7 ppm) and chronic ozone (0.25 ppm) significantly elevated superoxide accumulation and the resultant lipid peroxidation in all the brain areas. Although imipramine failed to reverse superoxide accumulation in the brain regions tested, it reversed chronic ozone-induced lipid peroxidation in the hippocampus.

Conclusion:
Imipramine prevents cellular damage induced by chronically induced oxidative stress in the rat hippocampus, although does not have a direct anti-oxidant action on released reactive oxygen species.
POSTER SESSION 2

PG2 24 Development of a novel polymeric triple-layered solid matrix for flexible zero-order drug release

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Purpose
The study assessed the drug release kinetics from two polymeric triple-layered matrix formulations. The matrices were loaded separately with two model drugs namely diphenhydramine HCl (DPH) (high solubility; 100mg/mL) and theophylline (THP) (low solubility; ~1.80mg/mL).

Methods
Preparation of formulations: Triple-layered matrices were prepared by direct compression using a Carver Hydraulic Press. Formulations containing various polymer combinations were prepared. The optimal formulations were evaluated further. In formulation 1 the first layer comprised 300mg polyamide 6,10 (PA 6,10) and 50mg sodium sulphate (SS); the middle layer comprised 350mg PEO and the third layer comprised 300mg of salted-out PLGA and 50mg PEO. In formulation 2 layer one contained 200mg PA 6,10 and 150mg SS; layer 2 had 350mg PEO and layer 3 had 50mg salted-out PLGA and 300mg PEO. Either DPH or THP was loaded separately into the different layers of both formulations. In vitro release studies: Formulations were subjected to dissolution testing in simulated gastric fluid (SGF) (pH 1.2; 37°C; 900mL) and PBS (pH 6.8; 37°C; 900mL) using a USP 25 rotating paddle method set at 50rpm over 24 hours. Samples were taken at predetermined time intervals and analysed via UV spectroscopy. Assessment of BHN: A TA.XTplus Texture Analyzer (Stable Microsystems, UK) was used to determine matrix hardness of the formulations. DSC Analysis: Thermograms of native and compressed polymer samples were generated using a Mettler Toledo differential scanning calorimeter.

Results
In vitro results for the first layer showed that the formulations containing SS did not have an undesirable burst release that occurred in its absence. Formulation 1 with a higher quantity of PA 6,10 provided linear release profiles for DPH and THP from the first layer, drug release was also extended over a longer period than formulation 2, indicating a greater ability to retard drug release. The release from the middle PEO layer was linear for DPH and THP with both formulations. The most linear release profiles from the third layer was achieved with a higher PEO:salted-out PLGA layer (Formulation 2) for DPH and a lower ratio for THP. Formulation 1 exhibited an average BHN of 30.80N/mm² while the average BHN of formulation 2 was 28.15 N/mm² indicating the enhanced rigidity of formulation 1 compared to formulation 2 due to the higher quantities of PA 6,10 and salted-out PLGA. DSC analysis showed no significant changes in thermal events after compression of the polymers.

Conclusions
The triple-layered matrices proved to have significant potential for the controlled, linear release of drugs of both high and low water solubility. The matrices may be able to deliver various drug combinations for the treatment of diverse disease states.
PG2 25 The chemotherapeutic action of synthetic dyes against *Plasmodium falciparum*

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**Purpose:**
Malaria remains a major health threat to more than 50% of the global population with sub-Saharan Africa carrying 90% of the disease burden. *Plasmodium falciparum* is responsible for over 80% of the clinical malaria cases that result in high mortality rates especially amongst children below 5 years old and pregnant women. With the increasing resistance of *P. falciparum* to classic antimalarial drugs and the absence of a vaccine, there is a critical need to identify new chemotherapeutic agents to control the disease. In previous studies thiazine dyes such as methylene blue have demonstrated antimalarial activity. For the purpose of this study, the effect of selected synthetic monoazo and heterocyclic dye compounds on the *in vitro* growth of erythrocytic stages of *P. falciparum* was investigated.

**Methods:**
- **Parasite culture:** A synchronized, chloroquine-sensitive strain of *P. falciparum* (3D7) was maintained in culture.
- **Tritiated ([3H])-hypoxanthine incorporation assay:** The parasites were exposed to the drugs for 48 hrs and the uptake of [3H]-hypoxanthine into the DNA measured with a betascintillation counter. Lead compounds that inhibited *in vitro* growth of parasites by 50% at concentrations (IC50 values) <100 μM were identified for further tests.
- **Red blood cell (RBC) toxicity assay:** A 1% haematocrit suspension was prepared from washed human blood. The haemoglobin from the lysed RBCs was measured spectrophotometrically at 412nm subsequent to 48 hrs exposure to the test compounds.
- **_haematin formation inhibition assay:** The ability of the test compounds to inhibit haemozoin formation was tested by measuring _haematin crystal formation (induced under acidic conditions) in the presence of the drugs.

**Data analysis:** The IC50 values were obtained from the log sigmoid dose-response curves generated using Enzfitter® software. The experiments were done in triplicate (*n* = 3) and mean±S.D. calculated. The Student-T test was used to analyse the data and statistical significance reported at *p* < 0.05.

**Results:**
Of the 32 compounds tested, all but three were inactive at less than 45 μM. The three compounds that demonstrated antimalarial activity at IC50 values <20 μM, methylene blue, safranine O and mercury orange were the most active with IC50 values of 2.09±0.70, 4.12±2.21 and 16.13±2.28 μM, respectively. These compounds were significantly less active compared to quinine (*p*<0.0001). The compounds did not inhibit _haematin formation at equimolar ratios (1:1) of haem to drug. However, at a ratio of 1:4, eriochrome black (T, B and R), palatine chrome black 6BN and ponceau 4R inhibited approximately 90% crystal formation. Mercury orange was toxic to RBCs at an IC50 of 4.11±1.03. The high antimalarial activity of several of these colourants warrants further investigation into their mechanism of action and interaction with clinically used antimalarial drugs such as quinine.
Determination of plasma concentrations using LC/MS and pharmacokinetics of ofloxacin in patients with multi-drug resistant tuberculosis and in patients with multi-drug resistant tuberculosis co-infected with HIV

Pierre Mugabo

Background:
Many studies have investigated the pharmacokinetics (PK) of anti-tuberculosis (anti-TB) drugs in patients infected with TB. However, little is known about the PK of the drugs that are used in the treatment of multi-drug resistant tuberculosis (MDR-TB). Therefore, the objective of the present study was to investigate the steady state plasma concentrations and the PK of ofloxacin, one of the drugs used in the treatment of MDR-TB in patients infected with MDR-TB and patients with MDR-TB co-infected with HIV.

Methods:
Blood samples were collected, after informed and written consent, between January and July 2008 before and at different times over 24 hours after ofloxacin oral administration. For the determination of ofloxacin plasma concentrations, the liquid chromatography coupled with mass spectrometry (LC/MS) analysis method was used.

Results and discussion:
Eight patients (3 female and 5 male) were involved in this study. Five of them were HIV positive and 3 HIV negative. The mean age (±SD) was 33.2±12.75 yrs. LC/MS method was validated over a concentration range of 0.1-10 µg/ml. The lower limit of ofloxacin detection was 0.05µg/ml, while the lower limit of quantification was 0.1µg/ml. The response was linear over the range used with a mean recovery of 97.6%. Ofloxacin peak was well separated at a retention time of 9.6 minutes. The pharmacokinetic parameters obtained were presented as mean ± standard deviation. The peak concentration of ofloxacin (C_{max}) was 4.71± 2.27 µg/ml. It occurred at 3±1.29 hours (T_{max}) after ofloxacin oral administration. The mean area under the plasma concentration-time curve over 24 hours (AUC_{0-24}) and the mean area under the plasma concentration-time curve from zero to infinity (AUC_{0-\infty}) were 68.8±42.61 µg/ml.hr and 91.93±76.86 µg/ml.hr, respectively. Ofloxacin distributed widely with a mean volume of distribution (V_d) of 2.77±1.16 L/kg and it was eliminated with a mean total clearance rate of 0.27±0.25 L/hr/kg. Ofloxacin mean half-life (T_{1/2}) was 9.55±4.69 hours and the mean mean residence time (MRT) was 15.12± 6.59 hours. Compared with the findings from previous studies, ofloxacin PK was altered in MDR-TB patients with or without HIV co-infection. The AUCs and C_{max} were reduced, while T_{1/2} and T_{max} were prolonged. This suggests that, both the rate and the extent of ofloxacin absorption were decreased. Furthermore, ofloxacin was highly eliminated in patients, which may be related to the altered liver function in this group of patients.

Conclusion:
Further studies investigating the effect of HIV, liver and kidney dysfunctions on ofloxacin PK are recommended in larger number of patients infected with MDR-TB. Furthermore therapeutic drug monitoring is required in order to make sure that ofloxacin plasma levels are maintained within the normal therapeutic plasma levels in this group of patients.
POSTER SESSION 2

PG2 27  A High Performance Liquid Chromatography method for the quantitative analysis of atazanavir in human plasma

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Purpose:

Atazanavir (ATZ) is a Protease Inhibitor (PI) recently registered in South Africa. It exhibits non-linear pharmacokinetics and is a known substrate of cytochrome P450 3A4 (CYP3A4) and the drug efflux transporter, P-glycoprotein (P-gp). These properties raise the susceptibility of ATZ to pharmacokinetic interactions with food and other medicines which have the ability to modulate the activities of CYP3A4 and P-gp. Such interactions may lead to sub-therapeutic and/or toxic plasma concentrations of ATZ. Since HIV/AIDS patients in South Africa usually take a plethora of conventional and possibly complementary or African traditional medicines (ATMs), therapeutic drug monitoring of ATZ could play a vital role in the management of those taking this PI. Several HPLC methods for the quantitative analysis of ATZ in plasma have been described in the literature. However, most of these published methods are complicated by the use of solid-phase extraction or gradient elution or long run times. The purpose of this work was to develop and validate a simple, cost-effective HPLC method, for specific application to a clinical study investigating the effect of some ATMs on the pharmacokinetics of ATZ in healthy male volunteers.

Methods:

Liquid-liquid extraction with hexane/ethyl acetate (50/50) was used to extract plasma samples alkalised with 2 M sodium carbonate, after addition of the internal standard (IS), diazepam. The extracts were evaporated under nitrogen and the residues reconstituted with mobile phase. Isocratic chromatographic separation was achieved using a Phenomenex® Luna C18 (2) (5 μm, 150 X 2.0 mm i.d.) column, protected by a Phenomenex® Luna C18 guard column (4 mm × 2.0 mm i.d.), both maintained at a temperature of 30 °C. Ultraviolet detection was used at a wavelength of 210 nm. The mobile phase consisted of 55 % 10 mM formate buffer (pH 3) and 45 % acetonitrile. Aliquots of 20 µl were injected onto the column at a flow rate of 0.3 ml/min. The method was validated with respect to selectivity, linearity, accuracy, precision and recovery according to the guidelines set out by the U.S. Food and Drug Administration.

Results:

The IS and ATZ eluted at 7 and 8.5 minutes respectively, with a total run time of 10 minutes. There was no interference of ATZ and IS by endogenous compounds. Calibration plots constructed in the range 0.1 μg/ml, the lower limit of quantification (LLOQ) to 10 μg/ml, the upper limit of quantification (ULOQ), using an unweighted least squares regression analysis were found to be linear (R² = 0.9996 ± 0.000436). Intra- and inter-day accuracy was between 100.7-108.3 % and 100.5-102.1 % respectively and intra- and inter-day precision between 1.39-3.92 % and 4.01-6.58 %, respectively. The average extraction recovery was 93.6-101.3 % for ATZ and 95.8 % for the IS.
In vivo drug content analysis and histopathological evaluation of an intravaginal polymeric platform in a pig model

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Purpose:
To determine the drug concentration in both plasma and vaginal tissue in a pig model inserted with a zidovudine (AZT)-loaded Intravaginal Polymeric Platform (IPP) for the prophylaxis and/or prevention of HIV and STIs. In addition, histopathological and morphological evaluation was undertaken on the vaginal mucosa to assess the potential for toxicity.

Methods:
Biocompatible polymers namely PLGA, ethylcellulose (EC) and PAA were blended with model drug AZT, compressed into caplet shaped IPPs and then intravaginally inserted into the pig model. Fifteen pigs divided into 3 groups (control, placebo and AZT-loaded IPPs) of 5 pigs each were employed for this study. The IPPs were inserted into the posterior fornix of the vagina (group 2 and 3) using a novel applicator under anaesthesia. Plasma samples (10mL) were taken from the jugular vein of each pig at day 0, 3, 7, 14, 21 and 28. AZT was extracted via Solid Phase Extraction (SPE) and subjected to Ultra Performance Liquid Chromatography (UPLC) analysis using a phenyl column (1.7µm; 2.1×50mm). A gradient method was used with a binary mobile phase (water/acetonitrile) varying from 60:40 at t₀, 5:95 at t₁-2.6min, and 60:40 at t₃.5-3.6min with an injection volume of 2µL, a flow rate=0.5mL/min and UV detection set at 267nm. At day 28 each pig was euthanized to remove the vaginal tissue which was digested with subtilisin, extracted and then subjected to AZT content analysis using UPLC. Histopathological and morphological evaluation involved assessment of the epithelial histological lesions, the lamina propria, as well as submucosa and vaginal wall.

Results and Discussion
AZT content in the porcine vaginal tissue from day 0-28 ranged between 56-67% (N=5) indicating that AZT was substantially retained within the vaginal tissue. This was attributed to the high matrix integrity of the IPP that was imposed by the networked microstructure that was formed by the hydrophobic and hydrophilic polymers employed. This finding correlates (R²=0.99) with the results obtained from AZT content analysis in blood samples where AZT content was minimal and ranged between 22-37% (N=5). Histopathological and morphological evaluation revealed that hyperplasia, exocytosis, exudates on the mucosal surface, ulceration, polymorphonuclear infiltration and perivascular ranged from negative to mild-moderate levels.

Conclusions
AZT concentration in the plasma and vaginal tissue as well as the histopathological and morphological findings suggests that the developed IPP may be suitable for intravaginal drug delivery.
PG2 29  Pheroid™ technology enhance bioavailability of antituberculosis drugs in mice

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Introduction:
Current TB treatment regimes suffer from drawbacks such as long, tedious treatments and adverse side effects due to long exposure to anti-bacterials such as rifampicin, ethambutol, pyrazinamide and isoniazid. Inappropriate use of treatment regimes result in rapid spreading of the disease, drug resistant TB strains, and often death of patients. An urgent need exists for shorter treatment regimes and fewer side effects, or ultimately both.

Pheroid™ technology possesses characteristics that may lead to the administration of a lower concentration of drugs, as well as a shortened exposure and therefore the possibility to reduce side effects. Supra-bioavailability of existing anti-tuberculostatics formulated in the Pheroid™ drug carrier system when compared to both a commercially available treatment and the pure pharmaceutical actives, could result in the incorporation of Pheroid™ technology in an improved dosage form.

Methodology:
Female BALB/c pathogen free mice at an average mass of 20 g were purchased from South African Vaccine Producers (Sandringham). Animals were housed under standard conditions at the Experimental Animal Centre of the North-West University (Potchefstroom Campus).

The following 4 test formulations containing comparable amounts of rifampicin, ethambutol, pyrazinamide and isoniazid (as prescribed by the WHO) were administered via oral gavage: a) commercially available dosage form Rifafour®-e275; b) the active compounds formulated in Pheroid™ vesicles produced from pro-Pheroid™ test formulation c) the same free pharmaceutical actives dissolved/suspended in distilled water and d) the active compounds dissolved/suspended in pro-Pheroid™ formulation.

Mice were anesthetised and whole blood collected from hearts at given times to obtain a drug plasma profile. Plasma was collected, snap freezed in liquid nitrogen and send to an independent accredited laboratory where plasma levels of the four antituberculosis drugs were determined. Statistic analysis of the drug plasma levels obtained for the test formulations was performed by the NWU statistical services.

Conclusions:
Formulation of the four active compounds in Pheroid™ vesicles improved the bioavailability between 9 and 32 % when compared to the free drugs. The Pheroid™ vesicles furthermore improved bioavailability between 33 % and 85% when compared to the commercially available treatment. The pro-Pheroid™ formulation showed an improvement in bioavailability of 74 % and 84% when compared to the commercially available and free drugs respectively. This study therefore shows enhancement of bioavailability of existing anti-tuberculostatics when formulated in the Pheroid™ drug carrier system vs. both a commercially available dosage form and the pure pharmaceutical actives, suggesting that: dosages can be significantly decreased when using Pheroid™ technology for delivery of rifampicin, ethambutol, pyrazinamide and isoniazid in the treatment of tuberculosis and the resultant lower dosages will result in less side effects and improved compliance.
PG2 30  *In vitro* antimalarial efficacy enhancement of mefloquine and artemether with Pheroid™ technology

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**Purpose:**

Malaria, a burden on the health of humankind since 1880, is a parasitic disease caused by a unicellular parasite called *Plasmodium*. Malaria causes more than a million deaths each year. Apart from the high mortality rate and the total number of malaria cases reported each year, the increasing emergence of drug resistance against the first line antimalarial drugs are also cause for distress. For the purpose of this *in vitro* study, the antimalarial drugs, mefloquine and artemether, were entrapped into the Pheroid™ drug delivery system to determine the possible enhancement of the efficacy of both these drugs against *Plasmodium falciparum*. The Pheroid™ delivery system is a formulation consisting of plant and essential fatty acids.

**Methods:**

Mefloquine and artemether were each entrapped for 24 hours in separate diluted Pheroid™ formulations. These Pheroid™ formulations were compared to control groups where the drugs were individually dissolved in sterile water for injection. *In vitro* cultures of the chloroquine sensitive 3D7 *P. falciparum* strain were added to pre-dosed cell culture plates and incubated for 72 hours at 37°C. Final drug concentrations of mefloquine in the cell culture plates ranged from 0-400 nM and artemether from 0-200 nM. Samples were prepared in duplicate. Parasite growth was analyzed using a DNA-recognition method with To-pro stain on the FACSCalibur (flow cytometry). Data obtained from the experiments were processed to calculate the EC$_{50}$ values for further use in experiments to combine these two drugs.

**Results:**

Parasite growth decreased significantly in all the treatment groups. The mean EC$_{50}$ value for mefloquine in the control group was 72.5 ± 0.18 nM. For the Pheroid™ group the mean EC$_{50}$ value was 53.5 ± 0.085 nM. This relates to a 37.5 % decrease in the EC$_{50}$ values.

In agreement with the experimental data obtained with mefloquine, the mean EC$_{50}$ value in the control group for artemether was 57.5 ± 0.275 nM. Artemether’s corresponding mean EC$_{50}$ value for the Pheroid™ group was 22 ± 0.10 nM. This accounts for a 161 % decrease in EC$_{50}$ values.

The use of Pheroid™ technology and its enhancement of the efficacy of both drugs reveal the ideal prospect to combine antimalarial drugs with drug delivery systems for further enhancement of its activity in its aim to overcome drug resistant parasites. This approach may lead to new combined drug formulations for the treatment of malaria.
PG2 31  Buccal permeability enhancement of didanosine using Aloe Vera gel: Histological and microscopical evaluations

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Purpose:
The epithelium of the buccal mucosa acts as a barrier to the permeation of drugs for buccal delivery. Drug Permeability can be improved by the use of penetration enhancers; thus effective and safe enhancers need to be identified. Aloe Vera gel (AVGel) has been reported as a potential intestinal (Chen et al 2008) and skin (Cole et al 2007) enhancer; however data on its buccal permeability is yet to be reported. This study aimed to determine the buccal permeability enhancement properties of AVGel and to evaluate the histological effects on the mucosal tissue.

Methods:
Ethical approval (University of KwaZulu-Natal Ref: 028/09/Animal) was obtained for this study. ddI was studied using modified Franz cells and porcine buccal mucosa with Phosphate Buffer Saline (PBS) pH 7.4 at 37 °C. Varying concentrations of AVGel (0.5, 1.0, and 2.0 %w/v) were incorporated in the ddI/PBS donor compartment (20mg/mL). Buccal mucosae were divided into three portions, each treated with PBS, ddI/PBS and ddI/PBS/AVGel in concentrations stated above, at 37 °C over six hours. A minimum of three replicates was conducted for all experiments. Preliminary investigations of the mucosae were performed using Light and Transmission Electron Microscopy (TEM). The mucosae were cut into pieces not exceeding 0.5mm, and placed in a 2.5% glutaraldehyde solution buffered to pH 7.2 and fixed for 24 hours at 4°C. Tissue was processed and embedded in epoxy resin for electron microscopy using standard protocols. Ultrathin sections were contrasted with uranyl acetate and lead citrate and viewed with a JEOL 1010 TEM.

Results:
The initial flux value of ddI (20 mg/mL) increased with AVGel (0.5%w/v). Increase in the concentrations of AVGel from 0.5 to 2.0 %w/v led to a significant increase (ANOVA p<0.05) in the flux value of ddI. Control cells showed normal plasmalemma and desmosomes, mitochondria appeared dense with well-developed cristae and nuclei showed regular nuclear envelopes and evenly dispersed chromatin. PBS and ddI/PBS treated mucosae showed similar ultrastructure. However, in the AVGel (0.5%w/v) treated mucosa; some cells showed mitochondrial clearings and increased intercellular spaces. Extended endoplasmic reticulum profiles and an abundance of ribosomes suggested increased protein synthesis. Increased signs of cellular damage were evident in AVGel 1.0 and 2.0%w/v treated mucosae. Cells showed increased plasma membrane crenulation and intercellular spacing, mitochondria appeared translucent and showed little internal detail. Nuclear envelopes appeared distended and chromatin compacted and unevenly dispersed. In spite of this, desmosome structure remained constant throughout all treatments.
PG2 32  

**In vitro transbuccal delivery of an antiretroviral drug: effect of donor concentrations on didanosine permeation**

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**Purpose:**

Didanosine (ddl) is an antiretroviral drug, with limitations including short half-life, low bioavailability due to severe degradation in the gastrointestinal (GI) tract and extensive first pass hepatic metabolism (Li and Chan 1999). Advantages of the buccal route include bypass of first pass hepatic metabolism and the avoidance of GI degradation. Buccal administration, therefore, could be an alternative route for ddl. A need for studies on the buccal permeability properties of ddl is essential for eventual formulation into a suitable buccal delivery system. This study aimed to examine the effect of donor concentrations on the transbuccal permeation of ddl.

**Methods:**

**Ethical Clearance:** Application was submitted to the Animal Ethics Committee, University of KwaZulu-Natal (UKZN). Ethical approval (Ref: 028/09/Animal) was obtained for this study.

**Permeation Study:** In vitro permeation of ddl was studied using vertical, glass Franz diffusion cells (PermeGear Inc. Hellertown, PA, USA), and porcine buccal mucosa with Phosphate Buffer Saline (PBS) pH 7.4 at 37°C. Varying concentrations of ddl (5, 10, 15 and 20 mg/mL) were investigated. Samples were collected at predetermined time intervals over six hours, and subsequently analyzed by UV Spectroscopy ($\lambda_{\text{max}} = 250\text{nm}$, UV - 1650PC Shimadzu Japan). The total amount of drug permeated through the mucosa was determined and plotted as a function of time. Steady state flux value was calculated for each drug concentration using linear regression analysis (Microsoft Office Excel 2003). Permeability coefficient was determined using the calculated flux value. A minimum of three replicates was conducted for all experimental conditions.

**Statistical analysis:** Permeation data was analyzed using SPSS version 15 (Chicago, USA).

**Results:**

The steady state flux of ddl at pH 7.4 increased with the increase in donor concentration. The flux value of the initial donor concentration (5 mg/mL) was 25.94 ±1.35 $\mu$g/cm$^2$ hr. Increase in the donor concentrations from 5 to 20 mg/mL led to a significant increase ($p< 0.05$) in the flux values for ddl. The flux value for ddl (20 mg/mL) was 71.57 ±3.12 $\mu$g/cm$^2$ hr. Permeability coefficients for the donor concentrations 5, 10, 15 and 20 mg/mL were determined as 5.18 ±0.27, 4.98 ±0.91, 3.82 ±0.33 and 3.58 ±0.16 x10$^{-3}$cm/hr respectively. Results showed a linear relationship ($R^2 = 0.9557$, $p<0.05$) between the steady state flux and the donor concentrations of ddl. In conclusion, permeation study demonstrated that the \textit{in vitro} transbuccal delivery of ddl is dependent on the donor concentrations.

**Acknowledgements:**

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Validating SeDeM in theory and practice

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Purpose:
The SeDeM method is a new method used in tablet pre-formulation to study the characteristics of active pharmaceutical ingredients (API’s) for direct compression. This method identifies the parameters of the powder that may need to be altered to enhance direct compression. It provides reliable and reproducible results for the galenic characterization of substances (Suñé-Negre et al., 2008:1029). Properties such as compressibility and flowability were investigated using the SeDeM method. The excipients selected for this study were: microcrystalline cellulose (Avicel PH 101®, Avicel PH 102®), dicalcium phosphate dihydrate (Calipharm®) and spray dried lactose (Lactopress®). The aim of this study was to validate the SeDeM method using four well known excipients with documented properties.

Methods:
The procedure for the galenic characterization required that the parameters of the SeDeM diagram should be determined (Perez et al, 2006). These parameters were determined using standard pharmacopoeial methods. The following parameters were determined: bulk density (Da), tapped density (Dc), inter-particle porosity (Ie), Carr’s index (IC ), cohesion index (Icd), Hausner’s ratio (IH), angle of repose (a), flowability (t’”), loss on drying (%HR), hygroscopicity (%H), percentage of particles <50µm and the homogeneity index (Iθ).

Results:
The SeDeM method confirmed the properties of the excipients tested except for one grade of microcrystalline cellulose (Avicel pH 101®). The good compression index (IGC) for Avicel pH101 was below the specified limit, thus it is a poorly compressible substance for direct compression. The following problematic parameters were identified for the excipients studied:

Avicel pH 101 - bulk density, inter-particle porosity, flowability and loss on drying.
Avicel pH 102 - bulk density, loss on drying and homogeneity index.
Calipharm – cohesion index, Hausner's ratio, loss on drying, flowability and homogeneity index.
Lactopress – cohesion index, Carr's index and inter-particle porosity.

Identification of problematic parameters allows for the manipulation of the material to improve the excipients compressibility by direct compression.

Conclusion:
The SeDeM method was able to confirm seven of the eight properties investigated using the different materials. This study proves that the SeDeM method is a reliable tool for investigating substances for direct compression.
A novel method for the determination of bioavailability of topical cream formulations containing clotrimazole (1%)

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Purpose:
Conventional approaches for the assessment of bioavailability (BA) cannot be used for topical products. Systemic concentrations following the application of a topical product are generally very low and often not measurable. Moreover, such measurements are inappropriate since the site of action is not the systemic circulation but the skin tissue. Furthermore, the active ingredient is not intended to be absorbed into the systemic circulation. Hence, the assessment of BA of topical products presents a formidable challenge. In view of these difficulties, medicine regulatory agencies generally require that clinical trials in patients be undertaken. Such studies are extremely expensive and time-consuming.

A novel approach, the Residual Method, is presented for the determination of BA of topical products. The method involves measurement of the difference between the amount of clotrimazole (CLZ) applied to the skin as a topical cream formulation and the amount subsequently recovered following removal of the cream from the application site after a predetermined dose duration. It is based on the assumption that the difference is the amount of clotrimazole which has penetrated into the skin and thus considered to be available at the site of action.

Methods and Results:
CLZ is an imidazole antifungal compound that is used topically as a 1% cream to treat cutaneous fungal infections such as candidiasis, dermatophytoses and tinea vesicolor. Eight 2x2cm sites were marked on the volar aspect of the arm of each volunteer and ≈15mg of Canesten® 1% topical cream was applied to each site. The various dose durations were 0.25, 0.5, 1, 2, 4, 6 or 8 hours after application and subsequently the product was carefully removed from the sites using a cotton swab. CLZ was extracted from the swab using methanol and the amount of CLZ was determined quantitatively using a validated HPLC method.

When the mean amount (n=7) of CLZ in the skin was plotted against time, the resulting curve was fitted to an Emax model with an R² value = 0.9825 and an ED50 = 0.68hrs.

Conclusion:
The Residual Method provides a simple, non-invasive and cost effective means for determining the bioavailability of topical CLZ formulations. The relatively low variability of this method makes it a promising tool for the assessment of bioequivalence.

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PG2 35  Encapsulation of essential oils within a polymeric liposomal formulation for enhancement of antimicrobial efficacy

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Purpose
Essential oils and their constituents are known to possess antimicrobial activity; however their inherent volatility is a limiting hurdle. In order to effectively exploit the antimicrobial efficacy of essential oils, encapsulation within polymeric liposomal systems was undertaken.

Methods
Artemisia afra, Eucalyptus globulus and Melaleuca alternifolia were incorporated into diastearoyl phosphatidylcholine (DSPC) and diastearoyl phosphatidylethanolamine (DSPE) liposomes employing a reverse phase evaporation methodology. In order to further enhance the stability of the formulations, a polyelectrolyte coating was applied via the layer-by-layer (LbL) self-deposition technique. The encapsulated and non-encapsulated essential oils were inoculated with four pathogens: Candida albicans (ATCC 10231), Escherichia coli, ATCC 8739, Pseudomonas aeruginosa (ATCC 9027) and Staphylococcus aureus (ATCC 6538). Minimum inhibitory concentrations (MICs) were compared to observe whether the antimicrobial efficacy was improved via encapsulation. Fractional inhibitory concentrations were calculated in order to determine synergistic interactions between liposomes and essential oils.

Results
Phospholipids are known to display anti-microbial activity without demonstrating significant mammalian toxicity or skin irritation effects. The interactive effects of the liposomal formulation and the incorporated essential oil were thus elaborated on. Synergy between liposomes and Artemisia afra against Candida albicans was observed. Eucalyptus globulus entrapped within liposomes showed synergy against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus; however antagonism was observed against Candida albicans. Melaleuca alternifolia encapsulated within liposomes demonstrated synergy against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus, and no interaction was observed against Candida albicans. In general, microbial growth was inhibited at significantly lower concentrations for the encapsulated formulations than for the non-encapsulated oils.

Conclusions
This investigation served as a stepping stone towards the promotion of the antimicrobial use of essential oils. The added benefits are that essential oils not only provide effective antimicrobial efficacy, but also promote a ‘greener’ consumerism. Within liposomes, they will enhance dermatocosmetic properties and increase the marketing image of the final product. Studies are underway to conclusively describe the effect conferred by polymeric coating of the liposomal formulations.
Differential Scanning Calorimetry studies of nicotinic acid

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Purpose:
Differential scanning Calorimetry (DSC) is a technique which is used to identify the thermal properties of pharmaceutical active ingredients which are important during formulation. In this study DSC was used to determine the compatibility between nicotinic acid, which is an anti-hyperlipidimic agent and excipients proposed for the formulation. The compatibilities were determined by interpreting the thermal transitions of nicotinic acid.

Methods:
Experiments were conducted using a DSC Q20; (AMS Laboratory Technologies, Johannesburg, South Africa). Samples were analysed under a nitrogen atmosphere at a flow rate of 50 ml/min. A temperature range of 25 ºC to 410 ºC was used with a 10 ºC ramp.

Nicotinic acid and the excipients (chitosan, calcium sulfate, sodium sulfate, tri-polyphosphate, stearic acid) were analysed individually by weighing (0.5-1.0 mg) into T-zero® aluminium pans. The pans were closed with a hermetic lid and a hole made in the lid to allow the escape of any volatile components released from the samples. Thermograms obtained were characterized by determining peak maximum temperature which indicates the melting point of the compound. During compatibility studies 1:1 binary mixtures of the drug with each excipient analysed.

Thermograms obtained from both the runs are analysed using TA Universal Analysis® software, (AMS Laboratory Technologies, Johannesburg, South Africa). Thermograms obtained were analysed based on shifts, disappearance or appearance of a new peak. In case of peak shift, any shift more than 10 ºC shift was considered as an indicator of possible interaction.

Results:
The thermogram of nicotinic acid showed an endothermic peak at 237.22 ºC which corresponds to the melting point of nicotinic acid. From the thermograms of the binary mixtures a peak shift of more than 10 ºC was observed with chitosan, stearic acid and tri-polyphosphate. Sodium sulfate and calcium sulfate were found to be compatible with nicotinic acid because the thermograms did not indicate any interactions. DSC is not conclusive therefore; samples will be subjected to short term accelerated studies followed by HPLC analysis to supplement DSC results.
Ex-vivo assessment of the drug release from a rapidly disintegrating mono-layered oramucosal wafer delivery system

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Purpose
This study aimed to assess the influence of critical formulation variables on the efficacy of a Wafer Delivery System (WDS) for the rapid delivery of drug through the oramucosa into the systemic circulation.

Methods
Preparation of the WDS: Stock solutions of PAA (0.5-1.5% w/v) and a cellulosic polymer (4-6% w/v) were homogenously combined in a 2:1 ratio along with diphenhydramine (DPH), sodium starch glycolate (SSG) (1-2% w/v) and β-cyclodextrin in accordance with a Box-Behnken Design template. Solutions were transferred into pre-oiled moulds and frozen for 24 hours and then lyophilized with a freeze phase set at -60°C and a pressure of 25mtorr for 48 hours. Drug Entrapment Efficiency (DEE): Wafers were completely dissolved in 20mL of simulated saliva and analyzed via UV Spectroscopy to determine the quantity of drug contained in each WDS to quantify drug content. Drug release: Conducted using Franz Diffusion Cells. Freshly excised porcine buccal mucosa was placed between the donor and receiver compartments at 37°C, containing 2mL of simulated saliva (pH 6.75) and simulated plasma (pH 7.4), respectively. Samples from the receiver compartment were drawn at 20, 60, 180, 300, 600, 1800, 3600 and 5400 seconds, with an equal quantity of simulated plasma replaced after each sampling point. The concentration of drug in the receiver compartment was measured using UV spectroscopy. Disintegration testing: Performed using a Texture Analyzer (TA) (Stable Microsystems, UK), fitted with a 5kg loadcell (N=10). WDSs were attached to the flat-ended cylindrical probe of the TA and lowered at a constant pre-determined force into 5mL of simulated saliva (pH 6.75, 37°C). Disintegration rate and the distance travelled by the probe were monitored with typical Time-Distance profiles generated as well as linear regression.

Results and Discussion
Fifteen batches of porous opaque white WDSs were produced (16mm diameter, 5 mm thick). Average DEE was calculated as being 72.96±14.32% (N=75). The relatively high standard of deviation is a result of the differing ratios of matrix constituents which affect the drug entrapment capacity of the WDS. Average drug release was determined to be 86.32±20.37% (N=45). Formulations containing lower concentrations of HPC and higher concentrations of SSG exhibited near-complete drug release within the first 60 seconds of testing. This was further corroborated via disintegration testing. Formulations displaying rapid and complete drug-release also displayed rapid disintegration ranging from 9-20 seconds. Average disintegration of all wafers tested was determined to be 29.33±15.91s (N=45).

Conclusions
Based on these results, it can be concluded that the mono-layered WDS is able to rapidly and effectively deliver drug through the oramucosa.
PG2 38  Formulation and *in-vitro* assessment of sustained release minocycline capsules

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**Purpose:**
Although minocycline has a relatively long plasma half-life, modified-release pharmaceutical dosage forms may be of benefit in reducing the incidence and/or severity of adverse events associated with minocycline. Rapid dissolution of minocycline has been associated with higher incidences and greater severity of vestibular effects. Therefore decreasing the dissolution rate of minocycline may reduce the incidence and severity of vestibular disturbances. The purpose of this study was to develop and assess the dissolution characteristics of sustained release capsule formulations of minocycline.

**Method:**
Hydroxypropyl cellulose (HPC) and high viscosity grade hydroxypropyl methylcellulose (HPMC) and lipids such as glyceryl palmitostearate (Precirol®) and glyceryl behenate (Compritol®) were investigated as potential release retarding agents for inclusion in minocycline sustained release formulations. Powder formulations for capsule filling contained minocycline hydrochloride equivalent to 100 mg minocycline base, 10-50% w/w of the hydrophilic polymer under investigation and microcrystalline cellulose as filler. Powders were prepared by geometric dilution and passed through a 315 µm sieve before filling into size 0 hard gelatin capsules. Capsule fill materials with 30-50% w/w lipid content and 100 mg minocycline base were prepared by dispersing minocycline hydrochloride into a molten lipid. After allowing the mixture to set the materials were size-reduced and passed through a 1 mm sieve before filling into size 0 hard gelatin capsules. In vitro release characteristics were assessed using USP Apparatus 1 with stirring at a rate of 50 rpm at 37 ºC± 0.5 in 900 ml de-ionized water as dissolution medium. Samples were analyzed using a validated HPLC-UV method.

**Results:**
HPMC (50% w/w) exhibited the greatest effect on dissolution rates with only 40.6% minocycline being released over a 6 hour-period whereas 96 % minocycline was released over the same time period when HPC (50% w/w) was used in the formulation. The use of Compritol® (50% w/w) ensured greater retardation of release than when Precirol® (50% w/w) was used. Only 8% minocycline was released over a 6 hour-period from the Compritol® formulation whereas 100% minocycline was released over 3 hours when Precirol® was included in the formulation.

**Conclusion:**
The use of both hydrophilic polymers and lipids resulted in the successful retardation of minocycline release from capsule formulations. The use of Compritol® and HPMC exhibited the greatest effect on the dissolution rate of minocycline from these formulations. Ongoing investigations are underway to optimize the rate of minocycline release from capsules formulations.

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The effect of electromagnetic radiation in the mobile phone range on the behaviour of the rat

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Electromagnetic radiation (EMR) is emitted from electromagnetic fields that surround power lines, household appliances and mobile phones. Research has shown that there are connections between EMR exposure and cancer and also that exposure to EMR may result in structural damage to neurons. In a study by Salford et al. (2003) the authors demonstrated the presence of strongly stained areas in the brains of rats that were exposed to mobile phone EMR. These darker neurons were particularly prevalent in the hippocampal area of the brain. The aim of our study was to further investigate the effects of EMR. Since the hippocampus is involved in learning and memory and emotional states, we hypothesised that EMR will have a negative impact on the subject's mood and ability to learn. We subsequently performed behavioural, histological and biochemical tests on exposed and unexposed male and female rats to determine the effects of EMR on learning and memory, emotional states and corticosterone levels. We found no significant differences in the spatial memory test, and morphological assessment of the brain also yielded non-significant differences between the groups. However, in some exposed animals there were decreased locomotor activity, increased grooming and a tendency of increased basal corticosterone levels. These findings suggested that EMR exposure may lead to abnormal brain functioning.
Evaluation of electrospun polymeric membranes for potential rate-modulated drug delivery

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Purpose
This study evaluated the process of electrospinning for the development of a micro-fibrous polymeric membrane system and the potential for rate-modulated drug delivery.

Methods
Electrospinning: Polymers including polyvinyl alcohol (PVA) and poly(ethylene oxide) (PEO) were investigated for electrospun membrane formation. Solutions of PVA and PEO (10-30% w/v) were prepared. A plasticizer was incorporated, as well as varying concentrations of polymer solvents such as water and a water: propan-2-ol solvent system, were investigated. Drug-loading was attempted by dissolving model drug, diphenhydramine HCl (DPH) in the polymeric solution prior to electrospinning. Polymer solutions were subjected to a predetermined voltage and allowed to electrospin for a time period sufficient to produce acceptable membranes.

Microscopic evaluation: Electrospun fibres underwent microscopic evaluation to assess fibre morphology, size and consistency.

Fourier Transform Infrared Spectroscopy (FTIR): FTIR spectroscopy was performed in order to assess any constituent interactions in the formulation due to electrospinning.

Drug Entrapment Efficacy (DEE): DEE studies were conducted on the fibres and quantified using a standard curve generated for DPH.

Results and Discussion
Plasticizer addition resulted in a higher elasticity and strength of membranes. Of the polymers selected for electrospinning, fibres spun from PEO resulted in poorly formed membranes compared to PVA. The physicomechanical properties of membranes prepared using different solvents differed vastly. With water as the solvent, resilient membranes were prepared with relatively smaller fibre sizes, whereas the fibre sizes of water: propan-2-ol membranes were inconsistent and were prone to rupture. Fibres produced were micrometer in size. Parallel, nonwoven and uniform fibre membranes were formed from PVA while PEO solutions formed woven membranes with inconsistent fibre sizes. An ideal voltage applied to the polymer solution during electrospinning promoted adequate elongation of the fibres resulting in smaller fibre diameters as seen with the PVA membranes. Electrospinning produced no significant changes on the polymer as demonstrated by the FTIR spectra generated, where the polymer backbone remained intact upon exposure to the voltage associated with electrospinning. Studies indicated that the DEE value was significantly higher with PVA as compared to PEO (93.5% vs. 54% for PVA and PEO respectively), thus indicating that PVA possessed a greater potential for entrapping DPH due to its hydrophilic nature.

Conclusions
Electrospinning produced no significant physicochemical changes to the properties of the polymers selected. Furthermore, electrospun membranes showed relatively superior drug entrapment and fibres were found to have an adequate size range for modulated drug delivery.
Background and Objectives

The treatment of *Mycobacterium Tuberculosis* requires the use of multiple drug containing regimens where rifampicin (RIF) is used as part of the first line regimens. Nonetheless RIF is known to have highly variable absorption and to induce its own metabolism. These characteristics, coupled with potential drug-drug interactions and low RIF concentrations, may increase the likelihood of treatment failure and the emergence of drug resistance. The primary objective of this pharmacokinetic analysis was to determine the population pharmacokinetics of RIF at pre-induced and fully auto-induced state (steady state) amongst African patients with pulmonary tuberculosis using nonlinear mixed-effects modelling. Subsequent to this, possible drug-drug interactions with gatifloxacin may be assessed. Additionally, population PK models will be developed for the remaining drugs used within the study’s multi-drug regimens.

Methods

Pulmonary tuberculosis infected adult patients (n = 101) received once daily doses of either 450mg (below 50 kg) or 600 mg (above 50 kg) of RIF together with isoniazid, pyrazinamide and ethambutol for 6 days of the week. Three blood samples per patient were taken for pharmacokinetic determination after the first dose (pre-induction) and repeated after approximately 28 days (steady state). 562 RIF concentration levels (from 101 patients) were determined in plasma using high performance liquid chromatography coupled to tandem mass spectrometry. A semi-mechanistic pharmacokinetic model incorporating an enzyme turn over model to address RIF auto-inductive properties, together with a multiple dosing transit absorption compartment model to describe the drugs highly variable absorption was developed using the first order conditional method in NONMEM. Due to the data being collected only at pre-induced and induced state, the enzyme turn-over half-life was fixed to approximately 24 hours (kENZ fixed to 0.029 h⁻¹) reaching steady state in approximately one week (7).

Results

Allowing the model to assume differences in parameters between occasions CL/F was predicted around 2 and a half fold higher at steady state compared to the pre-induced state. In addition rifampicin appeared as a potent inducer with an estimated EC50 of 0.329 mg/L.
PG2 42 Physicomechanical and physicochemical analysis of novel chemically modified lyophilized polymeric matrices

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Purpose:
The purpose of this study was to formulate chemically modified matrices of natural and synthetic polymers with the aim of reducing premature solvation of the polysaccharide polymer and in turn reducing upper gastrointestinal release of drug.

Methods:

Synthesis of modified matrices: Three formulations of various combinations of polysaccharide polymer (CHT and/or pectin) and polyacrylamide (PAAm) were synthesized. The DPH-loaded polymer blends were stirred until homogenous and initiation of radical formation was permitted with the addition of SPS and TEMED. Aliquots (1mL) of each blend were pipetted into pre-lubricated cylindrical moulds, frozen at -70°C for 24 hours and subsequently lyophilized. In vitro release studies: Drug release studies were conducted in the BioDiss apparatus in SGF (pH 1.2; ±37°C) for 2 hours and SIF (pH 6.8; ±37°C) for 4 hours. Samples (5mL) were withdrawn after each hour and subsequently analyzed by UV spectroscopy at a wavelength of 254nm. Drug Entrapment Efficiency (DEE): Matrices were completely dissolved in 100mL of simulated gastric fluid (SGF) (pH 1.2; 37°C) and analyzed via UV spectrophotometry to determine DPH content. Physicomechanical analysis: Matrix resilience (MR) was determined based on a force-time profile acquired by a textural analyzer. Matrix hardness (MH) and deformation energy (DE) was determined based on a force-distance profile for each formulation. Fourier Transmission Infrared Spectroscopy (FTIR): FTIR was performed on all native polymers involved in matrix formulation as well as on lyophilized matrices as a means of validating the successful synthesis of a modified polymeric matrix.

Results
CHT-PAAm matrices only began releasing DPH after 2 hours of dissolution studies with less than 40% of DPH release after 6 hours. Pectin-PAAm matrices began releasing DPH immediately; however the rate of drug release was sustained with less than 40% DPH release in the 6 hours. The combination matrix of Pectin-CHT-PAAm showed the least favorable release profile as complete drug release was achieved in only 4 hours. CHT-PAAm and Pectin-PAAm matrices showed a greater resilience (6.1% and 5.3% respectively) than the combination matrix (3.9%). The MH and DE were also greater in matrices of CHT-PAAm and Pectin-PAAm compared to the combination matrix. FTIR spectra validated the formation of chemically modified matrices with distinct bands not present in the native polymers e.g. the formation of a primary aliphatic alcohol represented by the band between 3500-3100cm⁻¹ for Pectin-PAAm matrices.

Conclusions
All matrices remained intact after 6 hours of dissolution studies and displayed varying physicochemical and physicomechanical properties dependent on the polymers employed.
UV Spectrophotometric method for the identification and determination of solubility of nevirapine in water, methanol and 0.1 N HCl

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Background:
Nevirapine is an anti-retroviral used in the treatment of HIV infection. It is a non-nucleoside reverse transcriptase inhibitor of the dipyridodiazepinone class.
Although complete solubility and permeability data for nevirapine are lacking, the FDA classifies it as a Class II (high permeability, low solubility) drug.

Purpose:
UV spectrophotometry was investigated as a fast, reliable and cost-effective alternative to HPLC for the determination of nevirapine content in water, methanol and 0.1 N HCl. The solubility of anhydrous nevirapine, as well as the hemi-hydrated form, was to be determined for each of these solvents at 37°C, because little is currently known about the exact solubility of nevirapine in various solvents.

Methods:
Nine solutions, of different concentrations, were made for each solvent and UV absorbance was read at the wavelength of maximum absorbance. These data were used to construct a standard curve for nevirapine in each solvent.
For each solvent and nevirapine form, 6 sealed test tubes were prepared with solvent and an excess of drug powder. Solubility was determined after a 24-hour period of agitation at 37°C.

Results:
The spectrum of nevirapine, dissolved in either water or methanol, showed a peak of maximum absorbance at 283 nm. If 0.1 N HCl is used as solvent, the nevirapine peak of maximum absorbance shifts to 313 nm. This peak-shift associated with solvents can be a useful method for nevirapine identification, especially if more sophisticated equipment is not available.
Although both forms are approximately 40 times more soluble in 0.1 N HCl than in water, the anhydrous form is about 1.7 times more soluble than the hemi-hydrate in each of these aqueous media. Methanol is the exception, in that both forms are equally soluble.

Conclusion:
A fast and reliable UV spectrophotometric method was developed for the quantitative determination of nevirapine in water, methanol and 0.1 N HCl. It was found that qualitative determination is also possible, due to a peak-shift observed with 0.1 N HCl. The solubility, for both the anhydrous and hemi-hydrated forms of nevirapine, was determined in each of the three solvents at 37°C.
Drug addiction has become an alarming global phenomenon and affects the lives of both young and old. Methamphetamine abuse in particular, has shown a high incidence in South Africa. Central to the neurobiology of addiction is the dopaminergic system in the brain, specifically the mesolimbic dopaminergic system. This system consists of fibres originating in the ventral tegmental area and terminating in the nucleus accumbens, two areas involved in reward related learning and behaviour. The development of addictive behaviour correlates with classical conditioning, which is a learnt response. The present study thought it relevant to focus on vasopressin, a neurotransmitter that plays a vital role in both social behaviour and learning and memory. The conditioning place preference paradigm was employed to investigate if the administration of the selective vasopressin V1a receptor antagonist, Relcovaptan, would reduce methamphetamine-induced place preference behaviour. Intraperitoneal injections were administered twice daily to animals divided into three groups; saline, methamphetamine, and Relcovaptan with methamphetamine. Using a two-compartment place preference box the addictive behaviour associated with methamphetamine use was evaluated. Our data showed a positive place preference performance upon administration of methamphetamine which was partially prevented by the vasopressin V1a receptor antagonist Relcovaptan.
Introduction and background:

Stability plays an important role in the development of a new drug product. HPLC is considered a stability indicating method of analysis. It is widely used in the pharmaceutical industry for the quantitation of small organic molecules.

Previous stability studies conducted on Pheroid™-based drug products, indicated problems with generating reliable stability data. The methods used to analyze these formulations were validated for the API only. Results were inconclusive in most cases and the need developed for a HPLC method that takes the Pheroid™-delivery system into account.

Objectives:

The purpose of this study was to determine how the changes, that occur in the Pheroid™-delivery system, under accelerated storage conditions, influence HPLC analysis.

Method:

Pheroid™ microsponges, with no API, were prepared and stored for a period of three months at 5°C, 25°C+60%RH, 30°C+65%RH and 40°C+75%RH. Monthly HPLC analyses were done, using existing methods for mefloquine and artesunate.

Results:

The following changes occurred in accordance with time, increased temperature and humidity: The number of detectable compounds increased. Longer runtimes became necessary to elute all of the compounds. Peak areas showed an upward trend. Solubility in the sample solvent, namely methanol, decreased. Consequently, not all of the samples could be analyzed by means of HPLC. Physical signs of instability, like discoloration and creaming, were noted.

Conclusion:

Stability of the Pheroid™-based delivery system may be in question when considering the results obtained. Further investigation of the Pheroid™ formula is recommended.

The HPLC analysis methods for Pheroid™-based formulations also pose room for improvement. Since the Pheroid™ seems to change significantly over time, a pilot study should be done to develop a suitable and reliable HPLC method for a specific API, before the commencement of a stability study.
Purpose:

The southern parts of Africa have the highest prevalence of HIV-infected people and South Africa is the country with the highest number of infections in the world. There is still no cure for AIDS, but anti-HIV medicine can prolong and enhance the quality of life of an HIV infected person. Pheroid™ technology is a patented delivery system consisting of mainly plant and essential fatty acids. The entrapment of an active within the Pheroid™ would generally provide a safer, more effective formulation than the active alone.

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay is a widely used quantitative colorimetric assay to measure the viability of cells. When analysing the potential of the Pheroid™ to deliver antiretroviral agents on M7-Luc T-lymphocyte cells in vitro, conflicting results were obtained. Incubation with Pheroid™ showed increased cell viability according to the MTT viability assay although decreased cell numbers were observed using trypan blue staining and a microscope. The direct reductive potential of the Pheroid™ was studied in a cell-free experiment to evaluate any interference with the MTT viability assay.

Methods:

M7-Luc cells infected with a clade C human immunodeficiency virus (SW7-TCLA) were used in this assay. A stock solution of abacavir (ABC) and a 0.033 % (w/v) solution of Pheroid™ were prepared and diluted with growth medium. The cells were grown in a 96 well plate in the presence and absence of ABC and/or the Pheroid™ formulation. Cell viability was analyzed on day four, using trypan blue and MTT. Trypan blue staining was used to stain the non-viable cells which were observed using an inverted microscope. MTT (5 mg/ml in phosphate buffered saline) was added in a 10 % (v/v) ratio to the cell suspension. The plate was then incubated in an incubator at 37°C (5 % CO₂) for two hours. A mixture of 20% (w/v) sodium dodecyl sulphate (SDS) and 50% (v/v) dimethylformamide (DMF) in distilled water were added in a 1:1 ratio to the supernatant in each well. The colour reaction was measured at wavelengths of 570 nm and 690 nm after 24 hours using a microplate reader. Viability data is presented as the percentage of viable cells in the experimental groups, compared to the negative control group.

Results:

The cell-free Pheroid™ reduced the MTT to the dark blue formazan and produced results higher than the control. If the cell-free Pheroid™ values were subtracted from the values obtained from the ABC entrapped Pheroid™ within the M7-Luc cells it resulted in very poor cell viability. This reduced cell viability portrayed the true cell count rendered by the trypan blue staining. The artificially enhanced cell viability was found to be the result of the presence of anti-oxidants in the Pheroid™. Vitamin E, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) was found to have the ability to reduce MTT to the formazan crystals which might be misinterpreted as enhanced cell viability. This study therefore shows that the MTT viability assay is not a feasible assay for determining cell viability when anti-oxidants are present.
PG2 47  The preparation and characterisation of novel polymorphic forms of efavirenz

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Objective:
The aim of this study was to prepare new crystal forms of efavirenz by the recrystallisation of the raw material from organic solvents, and to determine the physico-chemical properties of these forms.

Methods:
Efavirenz raw material was recrystallised from methanol, ethanol, n-propanol, iso-propanol, n-butanol, water, tetrahydrofurane (THF), dimethylformamide (DMF), ethyl acetate, chloroform, acetonitrile, dioxane, n-heptane, toluene, 50% n-heptane/50% ethanol mixture, 50% toluene/50% ethanol mixture and dichloromethane. Various techniques were used to characterise the different polymorphic forms of efavirenz: differential scanning calorimetry (DSC), thermo gravimetric analysis (TGA), infra-red spectroscopy (IR), X-ray powder diffraction (XRPD), hot stage microscopy (HSM) and Karl Fischer (KF).

Results:
Five crystalline polymorphic forms of efavirenz are currently described in literature: Forms 1, 2, 3, 4 and 5 are recrystallised from mixed solvent systems and are identified with XRPD and DSC. Thermodynamically, Form 1 is the most stable and a suitable recrystallisation medium is heptane or a mixture of THF and Heptane. Heating of Forms 2, 3, 4 and 5 results in transformation to Form 1.

A total of 15 recrystallisation products were prepared. Seven of these were identified as Form 1, one as Form 3, two as Form 4 and five did not correspond with known data for previously described forms.

Conclusion:
Apart from the forms already described in literature, at least five new crystal forms, having different physico-chemical properties, were prepared and characterized according to differences shown in the DSC, TGA, IR, XRPD, HSM and KF results.
PG2 48 The antimalarial properties of metronidazole thiosemicarbazone analogues

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Introduction:
Ninety percent of malaria cases reported in sub-Saharan Africa are due to the Plasmodium falciparum parasite. For decades malaria chemotherapy has hinged on a limited range of drugs, with the parasite developing resistance to some. This has initiated an urgent search for novel compounds with unique mechanisms of action against plasmodia species. Metronidazole is commonly used against bacterial and protozoal infections by causing DNA damage through free radical damage. Thiosemicarbazones are a small class of compounds which have shown to have profound antiviral, antifungal, antitumour and antimalarial effects.

Aims:
To examine the effects of a series of metronidazole-thiosemicarbazone analogues on the in vitro growth of P. falciparum, elucidate a possible mechanism of action, as well as assess the toxicity of these compounds.

Methods:
Metronidazole thiosemicarbazone analogues were synthesized wherein the thioamide moiety was substituted by different cyclic and aromatic amines. The P. falciparum chloroquine-sensitive strain (3D7) was maintained continuously in culture. For assessment of antimalarial activity, the tritiated hypoxanthine incorporation assay was employed. From the data generated the concentration required to inhibit parasite growth by 50% (IC50 value) was obtained. To determine a possible mechanism of action, the effects of the compounds on _-haematin formation was examined. To assess the toxicity of these compounds a red blood cell toxicity assay was performed.

Results:
The results obtained showed that all but one of the compounds inhibited parasite growth, with IC50 values below 10μM. Results from the _-haematin assay indicated that all but two of the compounds were able to completely inhibit _-haematin formation by an average of 95% at a mole:mole ratio of less than one for drug:haemin. Some of the compounds inhibited _-haematin formation as effectively as quinine. Red blood cell toxicity results indicated negligible amounts of red blood cell lysis and were comparable to those of quinine and metronidazole alone.

Overall, (1E)-1-(4-((E)-2-(1-(2-hydroxyethyl)-5-nitro-1H-imidazol-2-yl)vinyl)benzylidene)-4-cyclooctylthiosemicarbazide (Y1) and (1E)-4-(2-chlorobenzyl)-1-(4-((E)-2-(1-(2-hydroxyethyl)-5-nitro-1H-imidazol-2-yl)vinyl)benzylidene)thiosemicarbazide (Y3) were the most active, with IC50 values of 2.99 ± 0.10μM and 2.89 ± 0.09μM respectively, as well as possessing the best safety profile.

Conclusion:
The above results indicate that these compounds show promising antimalarial activity, but require further attention to determine their combined interaction when used with classic antimalarials.

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The National Research Fund, Medical Faculty Research Endowment Fund, Department of Pharmacy and Pharmacology, University of the Witwatersrand, Belgium Technical Cooperation.
The effect of gum arabic on the physicomechanical properties and drug release behaviour of thermo-responsive poly(methyl-vinyl-ether) gels

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Purpose

This investigation sought to determine the effect of Gum Arabic (GA) on gel strength and drug release behaviour of thermosensitive poly(methyl-vinyl-ether) (PMVE) hydrogels. Characterisation of the interactions occurring between the polymers at the molecular level was characterised. As the implant formed is intended for the prolonged treatment of solid tumours, conditions were selected to mimic those found at a tumour site.

Methods:

Preparation of solutions: PMVE solutions were diluted to a 0.25% w/v solution. CaCl₂ was added to 20mL of PMVE in varying concentrations (0.05-0.3M) with or without the addition of GA (4% w/v).

Textural Analysis: PBS (pH 6.75) was heated to 37±0.5°C and 3mL of the polymeric blend was immersed for 10 seconds. Gel strength and compressibility were then determined using a textural analyser (TA.XTplus, Stable Microsystems, UK). All tests were conducted in triplicate.

Fourier-Transform Infrared Spectroscopy (FTIR): Polymeric blends were examined using FTIR spectrophotometry.

Drug release: Folic acid (model drug) was added to prepared polymeric blends in the concentration of 1mg/mL. Solutions were agitated until the folic acid was evenly suspended. The formulation (5mL) was injected into a dialysis bag (MWCO: 2kDa) which had been prefilled with 3mL of PBS (pH 6.75). The dialysis bag was then immersed in a vessel containing 60mL PBS (pH 6.75) and placed in an orbital shaker bath (37±0.5°C; 25rpm). Samples (4mL) were drawn at 0.5, 1.5, 3.0, 4.5, 6.0, 24, 36 and 60 hours and replaced with pre-warmed buffer to maintain sink conditions. Drug content was assayed by UV spectroscopy at 280nm.

Results:

Textural analysis: The CaCl₂ containing gels were superiorly robust and the area under the curve for these gels were greater than those of PMVE gels without any additives. The addition of GA to the CaCl₂ containing gels resulted in gels that were weaker.

FTIR: The profiles for the combinations of polymers showed no interaction between either the polymers, or polymer-salt or polymers-drug combinations.

Drug Release: Sustained release of folic acid was achieved from all the formulations. The PMVE-CaCl₂ gels released drug more slowly than the PMVE gels, and release was slowed even further with the addition of GA. Less than 20% of drug was released from the PMVE-CaCl₂ and PMVE-CaCl₂-GA formulation after 20 hours and approximately 35% of drug had been released from the PMVE gels at the same time point.

Conclusions:

The addition of CaCl₂ to PMVE formed gels that were harder and released drug at a slower rate than the PMVE gels with no additives. The addition of GA to CaCl₂-PMVE gels retarded drug release further, but the gels formed were weaker indicating that the gel strength did not correlate with drug release from the formulation.
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