NZIC Chemistry IN NEW ZEALAND

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The Cover

Cover photography by Matt Walters, School of Biological Sciences, University of Canterbury

New Zealand Institute of Chemistry

supporting chemical sciences

October News

Comment from the President

As I'm sure previous Presidents will attest, visiting the various Branches is, perhaps, the most pleasurable of Presidential duties. I have now visited most Branches and it has been great to meet many old friends as well as more recently appointed chemists working in industry and academia. I am particularly grateful to members for their hospitality.

As part of my study leave this year, I spent a month in the UK and Singapore. The contrast between Singapore and NZ could not be more dramatic. The Singaporean Government is putting huge resources into education and research, that leaves NZ far behind. It is possible to get a feeling for the importance of education in this environment when a well-known multinational restaurant chain finds it necessary to produce glossy posters requesting that students refrain from studying there between 6 am and 12 midnight! The NZ Government's talk of a *knowledge economy* is largely talk and little action, despite the sterling efforts of some of our top scientists in lobbying; it seems that little will change in the forseeable future. Indeed, Roger Field's headline article on university funding in the current NZVCC's *NewsLetter* makes depressing reading. Unfortunately, this is is not really news any more; the long term lack of funding for education and research struggles to even register on the radar.

Some Council news: After many years, *Alan* ***Happer*** has decided to step down as the Editor of our publication CHEMNZ. On behalf of Council, I would like to thank Alan for generously giving his time for this publication. *Peter* ***Hodder*** has agreed to take on the role, and I would like to take this opportunity to encourage Members to submit an article on any topic that is likely to be of interest to the wider chemical community, especially schools. Peter will be more than happy to discuss the suitability of any proposed topic with you; his e-mail address is: *Peter.Hodder@vuw.ac.nz*

Lastly, time for some light-hearted chemistry; I recently came across a fascinating paper published in the *Journal of Organic Chemistry* (**2008**, *68*, 8750-8766) by Prof James Tour and co-worker Stephanie Chanteau of Rice University in Houston. This paper describes syntheses of a range of anthropomorphic molecules, called NanoPutians, which include NanoKid, NanoScholar, NanoBallet Dancer, among many others. These are two-nanometre sized molecules, synthesised using a first-rate array of techniques common in modern synthetic organic chemistry. Will these easily-recognised compounds ever find practical applications? Probably not, but this is a great piece of fundamental research, that you should peruse and admire.

Bill Henderson
President

The 2008 International Year of Planet Earth paper in this issue is that by Smith and Te Kanawa on traditional colourants – see page 127.

Prizes

The NZIC Council is pleased to announce the following prizes.

Fonterra Prize for Applied and Industrial Chemistry; *Simon* ***Hall***, Massey University for work on the development and commercialization of rechargeable zinc batteries.

Maurice Wilkins Prize for academic research; *Henrik* ***Kjaergaard***, Otago University for work on the computational chemistry and spectroscopy of small molecule clusters relevant to atmospheric chemistry

ABA Books Denis Hogan Prize for Chemical Education; *Craig* ***Steed***, Freyberg High School, for outstanding contributions to high school chemistry programmes and development and implementation of NCEA chemistry.

International News

In the latest round of journal impact factors the *Australian Journal of Chemistry* has again climbed, this time to 2.4 (from 1.9 last year). It is now close behind the RSC *New Journal of Chemistry* (2.7) - and higher than the Swiss, Japanese, and Canadian chemistry journals. Members should keep AJC in mind when deciding where to publish.

Conferences

The **2008 NZIC Conference** is to be held in Dunedin from Nov. 30 to Dec 4. Brief details were circulated with the July issue but a 75th jubilee celebration is planned. More information is available at: *www.otago.ac.nz/nzic*

New Fellows

Drs *Aston* ***PARTRIDGE***, *Simon* ***HALL*** and *Trevor* ***KITSON*** of the Manawatu Branch were elected to Fellowship at the September Council meeting. Council and the Branch offer their warmest congratulations.

Communicator of the Year

Council has accepted the *Chemistry in New Zealand* ***Communicator of the Year*** award as one of its centrally funded awards. It is for that poster at the NZIC biennial conference which, in the view of the selection panel (President, a Vice-President and the Editor or their nominees) has the most visual appeal. The award is valued at $200 plus a framed certificate and is open to all poster papers presented.

AGM

The NZIC AGM will be held on Tuesday 2 December 2008 at 12.30 pm in

the St David Lecture Theatre Complex (Cnr. St David Street and Cumberland Street), the University of Otago.

BRANCH NEWS

AUCKLAND

The Branch reinstated its Annual Dinner which was held at *Truffles*, the AUT training restaurant in late July; it was a well attended and lively evening. Other events have included a site visit to the highly automated laboratories of the NZ Racing Laboratory Services, hosted by Dr *Geoff* ***Beresford*** and a very interesting seminar by Dr *Laura* ***Nicolau*** (University of Auckland) who spoke about the unique aroma characteristics of NZ Sauvignon Blanc. There were plenty of excellent wines to test before and during this last event.

University of Auckland

Norrie ***Pearce***, *Elizabeth* ***Chia***, *Michael* ***Berridge***, *George* ***Clark***, *Jacquie* ***Harper***, *Leslie* ***Larsen***, *Elisabeth* ***Maas***, *Michael* ***Page***, *Nigel* ***Perry***, *Victoria* ***Webb***, and *Brent* ***Copp*** have won the Arthur E. Schwarting and Jack L. Beal Award for best paper in the *Journal of Natural Products* for their paper: *Anti-inflammatory Thiazine Alkaloids Isolated from the New Zealand Ascidian Aplidium Sp.: Inhibitors of the Neutrophil Respiratory Burst in a Model of Gouty Arthritis* (**2007**, *70*, 936-940). The award was established in 2001 by the ACS and the Foundation Board of the American Society of Pharmacognosy. *Lynley* ***Crawford*** (PhD student, Tamaki Campus), was among the finalists at the MacDiarmid Young Scientists awards held on 14 August.

In early July, the Chemistry Department took part in the Science Faculty *Incredible Science Day*. Similar to previous years, contributions included Magic Shows (by Prof *Douglas* ***Russell*** and Drs *David* ***Ware*** and *Gordon* ***Miskelly***), glass-blowing displays, and the ever popular slime-making.

On a sad note, Chemistry Department said farewell to A/Prof *Hicham* ***Idriss***, who has taken up a post at Aberdeen, UK.

MANAWATU

The Branch hosted NZIC President, *Bill* ***Henderson***, on August 14. As part of his visit, Bill experienced the diverse nature of chemistry in the Manawatu with site visits to Fonterra and New Zealand Pharmaceuticals. He gave his address later at Massey's Turitea campus.

In July a cooperative of NZIC members ran a tutorial day for students who are to sit the Year 13 Scholarship exams later in the year. This was part of a wider three-day programme run by Massey University's College of Education. The chemistry programme, coordinated by *Adrian* ***Jull***, consisted of classroom and laboratory sessions and featured a presentation by *Suzanne* ***Boniface*** of the Wellington Branch. Local schools showed a strong interest and the programme received plenty of positive feedback from the students.

2008 Quiz night

The annual Branch quiz was held on the 19th of August at Massey University. This year saw a much stronger turn-out of school teams who outnumbered the adults as they made up only three teams on the night.

The questions, prepared by quiz masters *Paul* ***Plieger*** and *Ghislaine* ***Cousins***, caused much hilarity - as did many of the answers! The answers to the super duper pooper scooper question, when the school teams were asked to name the piece of labware pictured, were especially amusing.

The organizers thank the quiz masters, their happy helpers, *Adrian* ***Jull*** and *Liam* ***McBride***, and all the sponsors for creating a thoroughly enjoyable evening. Congratulations to the winning school team of Year 11 students from ***Palmerston North Boys' High School***, who showed that age was no barrier to success.

Massey University

Pranav ***Karmwar*** has spent 6 months with *Shane* ***Telfer*** and *Mark* ***Waterland*** loading BODIPY and other dyes into nanoparticle systems and Shane gave an invited talk at the Philadelphia ACS meeting on recent work on complexes of dipyrrin ligands. Pranav left Massey in September for PhD studies with *Keith* ***Gordon*** and *Thomas* ***Rades*** at Otago. *Islah-****u-Din***, a recipient of an HEC Scholarship from the Pakistan Government, is to join the Waterland group and will begin his PhD studies in 2009. *Carl* ***Otter*** joined the Telfer/Waterland group as a Massey-funded postdoctoral fellow, earlier in the year and is currently developing methods for using coordination chemistry to fabricate nanoparticle dimers. MSc student *Serena* ***Smalley*** (student member of the Branch Commitee) and BSc(Hons) student *Hilary* ***Corkran*** have been awarded NZ Federation of Graduate Women scholarships.

OTAGO

The Branch organized two events in August. One was a visit from the NZIC Pesident, *Bill* ***Henderson*** (Waikato), who delivered an enjoyable and informative presentation about using mass spectrometry to study platinum sulfide and selenide complexes. Bill presented Fellowship certificates to recently elected *Kim* ***Currie*** and *Thomas* ***Rades***. The Branch also organized a tour of the Musselburgh Pump Station and Tahuna Water Treatment Plant in Dunedin. About 30 members donned hard hats and latex gloves in order to get close-up views of raw sewage, Olympic-pool sized sedimentation tanks, and biofilters. The evening ended with pizza and beer on the St. Clair Esplanade.

The upcoming NZIC biennial conference entitled *Chemistry and the Biosphere* will be held at the University of Otago between November 30 and December 4. It will feature distinguished plenary lecturers and a workshop commemorating the centenary of the award in 1908 (Dec. 10) of the Nobel Prize in Chemistry to Ernest Rutherford. *Keith* ***Hunter*** is chair of the organizing committee. More information can be obtained from: *http://www.otago.ac.nz/nzic*

Chemistry Department University of Otago

Nigel ***Lucas***, the Department's latest lecturer, arrived in July and has

quickly integrated into the Department. ***Nigel*** obtained his BSc (Hons) and PhD (2002) from ANU working with Mark Humphrey. Following postdoctoral research at New South Wales and the Max Planck Institute for Polymer Science (Germany) as an Alexander von Humboldt Research Fellow, he returned in 2005 to take an ARC Postdoctoral Fellowship at Sydney University. *Nigel*'s research interests span the synthesis and properties of carbon-rich molecular materials, supramolecular interactions and self-assembly, organometallic chemistry and catalysis, and crystallography.

The Department was very successful in the latest FRST funding round with grants made to *Steve* ***Moratti***, *Lyall* ***Hanton***, *Jim* ***Simpson***, *Brian* ***Robinson***, and *John* ***McAdam*** for work on smart gels, to *Robin* ***Smith*** and *David* ***Larsen*** for pharmaceutical development, and to *Eng* ***Tan***, *Guy* ***Jamison***, and *Sally* ***Brooker*** for a project on the intelligent delivery of chemicals to the brain; *Christina* ***McGraw*** received a 3-year FRST Postdoctoral Fellowship. The goal of her project is to develop pH-controlled culture systems to study the impact of ocean acidification on marine organisms of economic, cultural, and environmental importance to NZ.

The Department has recently purchased several instruments: *Sally* ***Brooker*** obtained a 57Fe Mössbauer Spectrometer through the MacDiarmid CAPEX round that has arrived and will be installed and tested by the manufacturer before this appears in print. *Guy* ***Jameson***, the Department Mössbauer expert, will be in action on the instrument quite soon. The Department's 18-month-old Bruker microTOFq (high resolution Quadrapole/Time of Flight) mass spectrometer has had a Dionex HPLC system to link to it. It will be used for routine work to aid those in the synthesis labs to monitor their reactions. It will also be used to quantify and identify chemicals found in aquatic humic sediments. This purchase will help with enquiries related to identification of unknowns that are regularly made. Finally, *Robert* ***Alumbaugh*** reports that the Chemistry Teaching Labs are excited to have acquired an Agilent 8453 UV-Vis spectrophotometer with PDA detector, a Dionex Ultimate 3000 HPLC with a PDA detector, and a Varian 380-LC Evaporative Light-Scattering Detector.

A number of renovation projects have been completed. *Guy* ***Jamison***'s research group recently moved into their new PC2 lab that has separate write-up space. *Russell* ***Frew***'s group moved into a completely renovated third floor lab. Two-thirds of the central second floor lab was gutted in August to receive new flooring, fume hoods, ventilated cupboards, and benches. A central 10-person write-up room that looks into both ends of the lab, is also being constructed. The remaining third of that lab will be tackled next.

In August, the Department co-hosted with Botany a workshop on ocean acidification that brought together researchers from the university, NIWA and MFish. The workshop was scheduled around the visit of *John* ***Raven*** from the University of Dundee, who chaired the RS working group on ocean acidification.

Among the various visitors to the Department, *Petra* ***van Koningsbruggen*** (Gröningen, Netherlands) returned for another week-long visit with *Sally* ***Brooker*** and gave a seminar featuring work that *Andy* ***Noble*** (ex-Brookers Bunch) has been doing in her laboratories.

Guy ***Jamison*** spoke at the European Conference on Biological Inorganic Chemistry (Wroclaw, Poland) in September. *Henrik* ***Kjaergaard*** gave a plenary talk at the 9th Informal Conference on Atmospheric and Molecular Science (Helsingør, Denmark) in June, and PhD student, *Ben* ***Miller*** (Kjaergaard group) has been selected as one of the three successful applicants to attend the Cheiron synchrotron summer school at Spring-8 in September; the trip is funded by the NZ Synchrotron Support Programme. *Kimberly* ***Hageman*** presented her work at the Environmental Toxicology and Chemistry Conference in Sydney in August. *Benji* ***Compton*** [supervisors *Rex* ***Weavers*** and *Lesley* ***Larsen*** (Crop & Food Research)], has submitted his PhD thesis on the chemical synthesis of anthocyanins and started working for IRL in September. Lesley will be using Benji's methods to produce isotopically-labeled anthocyanins for animal metabolism studies. *Joseph* ***Lane***, who recently completed his PhD in the Kjaergaard group, has become an RAO (Request Authority Officer) for Bestgrid (Broadband enabled Science and Technology Grid).

Nigel ***Perry*** (Crop & Food Research) returned in late August from a visit to the US, where he visited the Monell Chemical Senses Center in Philadelphia. He is planning collaboration with a researcher from this Center who works on chemesthesis. He also attended the ACS Conference in Philadelphia, which had a strong session on flavor chemistry that included *Terry* ***Acree***'s definition of flavor as a function of taste, odor, chemesthesis and time; the psychological response to odour labelling, *e.g.* isovaleric acid labeled as cheddar cheese evoked positive responses, but a negative one when defined as body odor. *Peter* ***Schieberle*** (Munich) showed that most cooked foods had the same few key odor compounds; and presentations that included new taste enhancers and supressors, with one test using a *lickometer* where trained rats were screening for bitter blockers with a high throughput.

The MacDiarmid Institute AMN4 conference organising team is in an exciting phase of the organising process as the invited speakers and abstracts are confirmed. For more information on this mid-February 2009 conference see: *http://macdiarmid.ac.nz/amn-4/*

WAIKATO

The first ever Branch meeting to be held at the Waikato Institute of Technology (Wintec) took place on a rather stormy winter's night. Two short presentations were made by Wintec staff. *Miruna* ***Petcu***, gave a seminar entitled *Anaesthetic Monitor based on Molecularly Imprinted Polymers* and *Stephen* ***Harlow***, gave a seminar entitled *E-learning and Chemistry*.

University of Waikato

Bill ***Henderson*** has returned from study leave when he spent two weeks at Durham University with Paul Low and two weeks at the National University of Singapore (NUS) with Andy Hor, but the rest of the leave was based here catching up with writing and finishing off some projects.

We congratulate *Karen* ***Love*** on winning the 2008 Masters Level Award in the *Adding Value to Nature* category at the MacDiarmid Young Scientists of the year ceremony. Her MSc research was carried out jointly at SCION with *Robert* ***Franich*** and *John* ***Lloyd*** and at Waikato with *Brian* ***Nicholson***. She successfully developed a technique for impregnating the cell walls of wood fibres with silica, for use in composites and for further derivatization of the surfaces. Karen is now employed at SCION to further develop her findings, but is currently on vacation in Europe; she plans to have three-months of research at Karlstad University in Sweden early next year. *Kelly* ***Kilpin*** and *Bevan* ***Jarman*** presented their PhD research results at the 23rd ICOMC conference in Rennes in July, followed by a holiday in Europe.

The annual Analytical Chemistry Competition run by the Chemistry Department for Year 13 students was held in mid-June. A total of 24 four-person teams entered this year, with students coming from around the Waikato and Bay of Plenty regions, with some extras from Auckland and Taranaki. The task was to analyse $ZnSO_4$ both gravimetrically (SO_4^{2-}) and volumetrically (EDTA for Zn^{2+}) and hence deduce the level of hydration in the crystal. Despite the demands and time constraints some excellent results were achieved. The prizes went to:

1st: Sared Heart Girls College (*Claire Burnett*, *Naomi O'Connell*, *Gabrielle Bisschops* and *Rebecca Evans*)

2nd: Fairfield College (*Prineshan Moodley*, *Ryan McRae*, *Grace Ng* and *Erica Prentice*)

3rd: St Johns College (*Ron Blaza*, *Isaac Hayes*, *Timothy Miller* and *Jozef Kamp*)

4th: Fraser High School (*Matthew Bennett*, *Lavinia Raj*, *Hae Won Kim* and *Jared Lindsey*)

5th: Putaruru College (*Nom Mpande*, *Jake McCarthy*, *Kelly Carter* and *Alex Naea*).

Overall the competition allowed 100 keen Year 13 chemists to spend a day in university laboratories and mix with peers from other schools. It also provided an opportunity for the teachers who accompanied the students, to meet with each other and University chemists. Financial support from Hill Laboratories generously sponsored the prizes and resources, the Waikato Branch (for funding the lunches) and the Waikato Chemistry Department (for facilities and staff time) are acknowledged with thanks.

NIWA

Michael ***Ahrens*** has accepted a faculty position at the Universidad de Bogota Jorge Tadeo Lozano, commencing January 2009. In addition to lecturing and starting research, *Michael*'s role will be to help the university build an MSc programme in Marine Sciences. He is planning to leave from NIWA at the end of the year, but will continue research collaboration with colleagues in NZ on biomarkers, emerging contaminants, and contaminant bioavailability. *Hilke* ***Giles*** has returned from her visits to the BioGeochemical Systems Dynamics (Utrecht University Geochemical Research Group) and Unisense (Aarhus, Denmark) and will be applying her skills in sediment geochemical profiling on lake sediments in Antarctica this summer. *Bob* ***Wilcock*** attended the Dairy Australia catchment workshop and gave a joint presentation with Ross Monaghan (AgResearch) on the NZ study focussing on land-water linkages in five dairying catchments.

Craig ***Depree*** recently published a research report for Land Transport NZ regarding the potential to stabilise and reuse road-derived sediment. The research was presented at the NZ Water & Waste Association 2008 Stormwater conference and at the International Symposium on Sediment Management (I2SM) in Lille (France); he only arrived in Lille three hours before his presentation on day two of the conference due to a *bird strike* that delayed his flight in Los Angeles by 30 hours. Following the conference, it was onto a brief stop in Norwich to sample many fine British ales, before visiting Dr *Claire* ***Hellio***'s marine biology group at the University of Portsmouth.

WELLINGTON

In July *NanoScience, Nanotechnology and Energy* formed the basis of the Branch meeting with an excellent lecture by Prof *James R* ***Heath*** (Caltech) who challenged us in reducing the cost of energy. This breaks down into several individual issues: harvesting energy, energy conversion, storing energy, transporting energy, and increasing the energy efficiency of the things we do. For almost all of these tasks, nanotechnology-enabled solutions are now at the forefront of the relevant science. He discussed only the issue of energy conversion, focussing on thermoelectric materials – one which converts between thermal energy and electrical energy – that can be considered as an engine with no moving parts.

The August meeting provided the annual presidential address from Prof *Bill* ***Henderson*** (Waikato) that explored his research activities in the form of *Chemistry by mass spectrometry – studies of the reactivity of platinum sulfide and selenide complexes*.

The Branch again sponsored prizes for best chemistry exhibits in the junior and senior sections of the Wellington Science Fair held at VUW's School of Chemical and Physical Sciences over the August 13-16 period.

Carina Chemical Laboratories Ltd

Some of our readers may have seen the August 5 report by Craig Borley in *The New Zealand Herald* covering Padraig Harrington's winning of the British Golf Open. The item praised Harrington saying that Greg Norman's remarkable run at this year's British Open golf tournament was stopped, in part, by New Zealand! How so?

Heading into the tournament, Harrington was nursing a sprained right wrist (from use of an impact bag) and put his chances of finishing at just 50%. To assist overcoming the injury, two unique NZ anti-inflammatory gels were applied to the offending wrist. The gels, Eze and All Black & Blue, were licensed to NZ biotech company Nemidon by their inventor and formulator *Ian* ***Miller*** of Carina Chemical Laboratories. They are based on a unique carrier gel derived from seaweed mixtures. It is insoluble in cold water and, because of its molecular structure, water readily passes in and out of it; it delivers the active ingredients to the inflamed areas far more quickly than conventional products. The gels were used in tandem by Harrington's doctor, Dale Richardson.

He has also used them on other top golfers, including Michael Campbell, Davis Love III, Nick Faldo and Thomas Bjorn, and notes that he has found that they are not only the best-quality soft-tissue therapy products on the market, but that they offer a perfect therapeutic range to deal with most soft-tissue injuries. Ian Miller initiated work on the gel some 15 years ago and the product has been on the market for eight years.

Victoria University

The Marine Natural Products Group under *Peter* ***Northcote***'s direction has seen three of its members graduate and move on. *Katie* ***Dowle*** gained her MSc with distinction and is now working in the Otago Medical School (in Wellington) until she starts her medical degree next year. Two of the local Branch student stalwarts, *Joanne* ***Wonjar*** and *Wendy* ***Popplewell***, have successfully defended their PhD studies and are now working overseas, Joanne with VUW graduate *Steve* ***Kent*** at the University of Chicago, and Wendy with *Mike* ***Davies-Coleman*** at Rhodes University in South Africa. *Teck* ***Lim*** successfully defended his PhD thesis in September on *the synthesis and properties of indium metal nanoparticles, InP nanowires and* Zn_3P_2 *nanoparticles*. He is remaining with *Richard* ***Tilley***'s group as a research fellow for some months. *Brendan* ***Burkett*** is becoming accustomed to his new role in Singapore but, for the benefit of our readers, he has agreed to continue his cartoon series ChemScrapes in this Journal.

Recent visitors to the School of Chemical & Physical Sciences have included Prof *Peter* ***Griffiths*** (University of Idaho) who spoke on the *Detection and quantification of atmospheric molecules by open-path Fourier transform spectrometry*, Dr *Craig* ***Milestone*** (Environment Canada) on *The road to reducing the environmental impact of the pulp and paper industry – from sludge to coloured effluent to masculinised female fish*, and *Peter* ***McLeish*** who gave a fascinating illustrated lecture *An Art & Science collaboration based on Red Sprites & the Polar Regions* supported by SCINEMA- Australia's Science Film Festival - and the Canadian High Commission in Wellington. He is an international multi-media artist whose work has been presented all over the world with over 20 grants and/or support from different branches of the Canadian and/or Quebec Governments between 1991 and 2008.

PACIFICHEM 2010 News Update

Honolulu, Hawaii 15-20 December 2010

The Pacifichem 2010 Congress will present the latest research in the core topic areas of chemistry, feature multidisciplinary programming, and highlight chemistry's impact on society. For more information see: *http://www.pacifichem.org*

Symposium proposals submitted in Round 1 that closed on April 15 resulted in 151 symposia being accepted into the technical program. Round 2 of symposium proposals opened on 1 August ***and closes on 30 November 2008***. Before submitting a proposal, please review the Pacifichem 2010 Technical Symposia at *http://www.pacifichem.org/symposiumproposals* in order to avoid an overlap with currently accepted symposia.

To submit a new symposium proposal in Round 2, please go to the WebSymposium System and follow the directions on the *Create Account* tab. After you have set up a new symposium submission account, you may access your WebSymposium on the *Login* tab. Send any questions about the current technical program or the proposal submission process to *pacifichem@acs.org*

Prof. Rob Smith (*rajsmith@chemistry.otago.ac.nz*, phone 03 479 7924) is the NZ and NZIC representative on the Pacifichem organizing committee. He would be very pleased to answer any questions or comments about organizing a symposium for Pacifichem 2010.

Some Traditional Colourants of Maori and other Cultures

Gerald Smith[a] and Rangi Te Kanawa[b]

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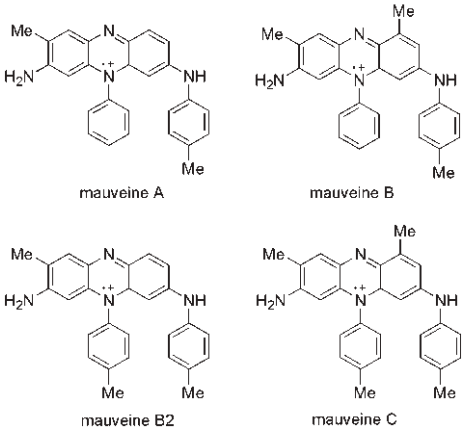
A Global History of Dyes and Pigments

We live in a world of colour. Today, materials can be decorated in an almost limitless range of colours. The colourants used come in two forms; either as dyes or pigments. Dyes are water soluble chemicals whereas pigments are fine, coloured particles dispersed in a liquid medium which 'dries' after application to the substrate, *i.e.* it solidifies by polymerization, gelation or protein denaturation and *not* by evaporation of the solvent.

The explosion in the number of colourants was due to the advent of synthetic chemistry and the person often credited with being the father of modern organic chemistry is William Perkin who, in 1856, synthesized a mauve dye from aniline which is a component of coal tar. It was known as *mauveine*, as aniline purple, and Perkin's mauve. The actual structure of the dye proved difficult to determine and was confirmed only in 1994 to be a mixture of four related aromatics differing only in the number and location of the methyl groups (Chart 1).[1]

Chart 1. The structures that comprise mauveine

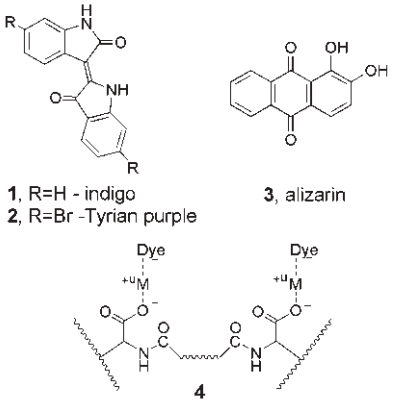


For thousands of years prior to this, dyers relied on dyes extracted from plants, insects and marine organisms. Painters had used variously coloured powders of metal-containing ores and minerals dispersed in 'drying' oils, such as the fatty acids extracted from the seeds of some plants or animals.With the profusion of artificial colourants so familiar to us today, we forget the ingenuity of our ancestors from many cultures who discovered how certain coloured natural substances could be fixed or bound to objects, to give a durable finish that resisted removal by water, *wash-fast*, or fading by exposure to light – *light-fast*.

The very first colourants used by man, perhaps ten or fifteen thousand years ago, were iron oxides, charcoal and chalk rubbed onto a surface or deposited as a suspension in water. As textile fibres and weaving developed, so the technology associated with dyeing grew in sophistication. It was discovered that cellulosic fibres obtained from plants such as linen and cotton could be coloured to give a reasonably fast finish with *vat dyes*, such as indigo **1**, which is still used today in blue denim clothing, and Tyrian purple **2** extracted from molluscs (Chart 2). This famous purple dye was the most valuable commodity in the ancient world and was traded widely throughout the Near East and Europe; it generated the wealth that enabled the Phoenician Empire to flourish during the 2nd and 1st millennia BC. The first large industry producing a chemical was the extraction of the dibromoindigo dye from certain types of mollusc. It is estimated that 10,000 of these shellfish were required to produce just one gram of dye. Vast middens of crushed molluscs have been excavated at sites around the Mediterranean providing evidence of the existence of substantial purple dye-extraction operations. The chemistry underlying the use of these vat dyes is complex. It requires an initial reduction of the indigo (or thioindigo) molecule by anaerobic fermentation to a water soluble form and then after it has been applied to the textile aerial oxidation provides the insoluble form.

Another class of natural dye is the anthraquinones. The best known of these is madder, which comes from the root of the madder plant, and consists of a mixture of many closely related anthraquinones, the principal one being alizarin **3** (Chart 2).[2] Such molecules are water-soluble in their glycosidic form (with sugar groups attached) but, because of their flat molecular profile, they can intercalate cellulosic structures and be *locked* in place within the fibre by hydrogen bonding between the hydroxyl groups of the cellulose and those of the hydroxyquinone. Alizirin was first synthesised in Germany in 1868. To make the synthetic version of the dye water-soluble the molecule is sulfonated. Such dyes, when they are used without a metal ion mordant, are known as *substantive* or *direct* dyes. Traces of madder, applied as a direct dye, have been found on the linen cloth used to wrap Egyptian mummies including that of Tutankhamun, *ca*. 1350 BC.[3]

Chart 2. Some historic dyes



4, mordant-attached dye to celluloe fibre (schematic)

Cellulosic fibres from plants are very much more difficult to dye than the proteinaceous fibres of animals where metal ion mordants can be employed to fix the dye molecule to the fibres. These cationic mordants fix dyes by binding to the abundant anionic carboxylic acid groups, associated with the glutamic and aspartic acid residues present in proteins and simultaneously having ionizable polyphenolic and negatively charged dye ligands such as flavonoids and quinones attached as shown by **4** (see Chart 2). Anthraquinones can be used as direct dyes when used on cellulosics but with the advent of white wool, they became almost exclusively used as metal ion mordant dyes because these gave more brilliant colours.

The liganded dye molecules form charge-transfer complexes with the metal ions and these absorb strongly in the visible region of the spectrum and therefore are highly coloured. The positions of their absorptions depend on redox potentials and hence on the nature of the metal ion. As a result, a number of different colours can be obtained from a given dye molecule by using different metal ions. The earliest and most commonly used metal ion was aluminium but iron, copper, tin, lead, and zinc were also used. Until fifty years ago, the British Army's scarlet or *red coat* tunics were dyed with a mordant dye formed between the cochineal anthraquinones (obtained from an insect) and tin ions. Thus, the first great revolution in dyeing and associated technologies was when, in the second half of the 1st millennium BC, the Romans succeeded in breeding sheep with white fleeces thereby providing a plentiful source of proteinaceous fibre for dyeing with mordant dyes. Prior to that, the Bronze and Iron Age sheep had dark wool that was unsatisfactory for dyeing because the colour did not show up against the dark background.

The chemistry of the natural dyes and pigments used by Maori prior to European contact, and the relationship between these colourants and those used by other cultures throughout the world to decorate their heritage objects follows.[4] Problems with the stability of the colourants are discussed as well as treatments that have been employed to retard their degradation.

Maori Dyes

Prior to European contact, NZ had no hair or fur-bearing animals apart from the Polynesian dog, kuri. The Maori's principal textile fibre, harakeke, was obtained from the leaves of *Phormium tenax* (from Latin - strong basket) which is a cellulosic fibre with morphology very similar to that of linen – hence the common name for the plant of the New Zealand flax. The other main fibre used was from the *Cordyline* (cabbage tree) genus. These cellulosic fibres limited the classes of dye molecules that could be satisfactorily fixed to such fibres. Although many NZ plants contain compounds such as anthraquinones and flavonoids (from Latin *flavus* - yellow), these usually require a metal ion mordant and therefore are unsuitable for dyeing cellulosic *P. tenax*.

Indigo-bearing plants appear to be rare, if present at all in NZ, and so the only vat dye that could have been available would have been the purple dye from the indigenous mollusc. However, there does not appear to be any record of its use by Maori. There were four colours of fibres used by Maori weavers;[5] undyed, and three principal dyes: black - iron-tannate: yellow - Raurekau bark, and red/brown - Tanekaha bark.

Undyed fibres

There is a wide variation in the colour of the fibres extracted from *P. tenax* ranging from white to yellow/brown depending on the plant variety. Maori weavers prized the white fibres to enhance the contrast of the patterns in the garments they wove and it is probable that certain varieties were selected for this accordingly.

Work carried out in our laboratory has found that some varieties of *P. tenax* contain a number of substituted coumarins,[6] a number of hydroxylated derivatives which emit strong blue fluorescence when excited with radiation in the near UV region of the spectrum; they have been used commercially as optical whiteners and brighteners.[7,8] However, they are very photochemically active and have been shown to contribute to the photoyellowing and tendering of textile fibres to which they are applied.[8] It has been established that reactive oxygen species such as hydrogen peroxide are produced by irradiation of wet wool containing these fluorescent whiteners.[9] We have observed that a number of varieties of *P. tenax* exhibit fluorescence at 450 nm which is excited maximally at 340 nm. This emission is consistent with fluorescence originating from naturally occurring hydroxycoumarins. Further, irradiation of wet, undyed *P. tenax* fibres with UV radiation from 360-400 nm results in the formation of hydrogen peroxide and is accompanied by yellowing of the fibres.[10]

In unpublished work from our laboratory,[10] it has been found that treatment of *P. tenax* fibres with alum (potassium aluminium sulfate) resulted in significant colour changes. Soaking in aqueous alum solution at ambient temperature created a yellow chromophore which reflectance spectroscopy showed to have an absorption maximum at 430 nm. There was also a featureless, but diminishing absorption extending to 600 nm. This is attributed to complexes formed between aluminium ions and polyphenolics in the fibre.[11] Subsequent rinsing of these fibres in hot water for 30 min at 90ºC removed the coloured complexes and produced a very white fibre. The reflectance spectrum of the fibres treated in this way showed no detectable absorption in the visible region of the spectrum beyond ~400 nm. Apparently the aluminium complexes are soluble in hot water and extract the natural chromophores from the fibre. There are deposits of alunite, the hydrated potassium aluminium sulfate ore, in volcanic/geothermal areas of NZ, *e.g.* the Taupo volcanic zone, the *Alum Lake* and *Alum Cave* at Orakeikorako near Wairakei.[12] In this context it is interesting to note reports that a Maori chief from the Taupo region wore a startlingly white cloak woven from *P. tenax* fibres at the signing of the Treaty of Waitangi in 1840.[13]

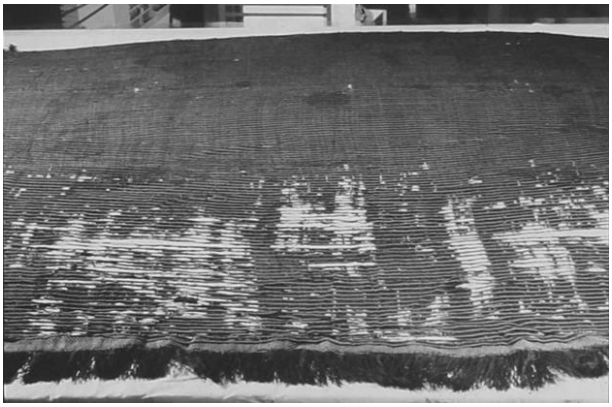
Black dye

Black dye is a charge-transfer complex formed between iron(III) and catechol groups of tannins as shown by **5** (Chart 3). The tannins were obtained from the bark of cer-

tain trees; the gallotannins from hinau bark and the condensed tannins from a mixture of manuka and kanuka. The two types of tannin produce slightly different hues; the gallotannins give a deep bluish-black whereas the condensed tannins produce slightly less intense blacks and with a greenish hue.[14]

The method used by the Maori weavers for black dyeing was to soak the *P. tenax* fibres or muka (extracted from the leaf by scraping off the green epidermal layer) in an infusion of bark that had been simmered in water for several hours. The fibres were dried without rinsing and a coating of tannin is formed on the fibres. The fibres were then covered in a fine-textured mud, *paru* - which has a high iron content - for several hours. The excess mud was removed and the fibres, thoroughly rinsed with cold water to remove the remaining paru, were then exposed to sunlight to develop the black colour.[5]

Piu piu (waist garment) with degraded black fibres

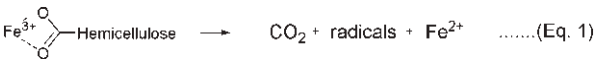


Loss of black dyed warp fibres in traditional Maori cloak

A number of cultures have independently discovered the same type of black dyestuff based upon an iron-tannin complex, although the source of iron and tannin differed depending on the local materials. The ancient Egyptians used it as ink on papyrus and linen. It was used extensively both as a dye and as ink on wool, cotton, linen, paper and parchment in Europe for many centuries; it was used to write Magna Carta. It was used as a dye in Mali, in Africa, in South America, and throughout Polynesia.

When it has been applied to textile fibres or paper, it slowly degrades the underlying substrate that eventually disintegrates. Not only is there a loss of mechanical strength and flexibility but the dye also fades. The chemistry of this degradation is not fully understood. Our research team and colleagues overseas have been studying the process in order to find ways of arresting it, or at least slowing it down. It has been established that the deterioration of the fibre involves water and oxygen, and it is accelerated by acid.[15] As ageing and degradation proceed, the substrate evolves acetic acid and this further exacerbates degradation.[16] It is interesting to note that when iron-tannate inks were used on parchment - animal skin that has been treated with lime - there is no noticeable degradation, *e.g*. Magna Carta. Presumably, acids produced during degradation are neutralized by lime residue in the parchment. Conservators at the British Museum, which has a large collection of black dyed woven *P. tenax* dating back to Captain Cook's visits to NZ, have been experimenting with weak alkaline reagents such as magnesium bicarbonate to neutralise the acid formed during ageing of the *P. tenax*.[17]

We have found that accelerated thermal ageing of black-dyed *P. tenax* results in the evolution of carbon dioxide that is linked to the degradation of the fibre. We believe that excess iron(III) in the dyed material is complexed (or bound) to carboxylic acid groups associated with the hemicellulose component of the fibre and that these decarboxylate with the formation of CO_2 and reactive free radicals (Eq 1). The radicals can cleave the cellulosic polymer backbone resulting in its fragmentation and a consequent loss of the mechanical integrity of the fibre.[18]



One strategy to avoid this degradative chemistry has been to give the fibres a second treatment with tannin after dyeing to scavenge the excess iron(III) that would otherwise bind to the carboxylic acid groups. This approach is very effective and slows the degradation of black-dyed fibres. Furthermore, it has the advantage that it does not involve the introduction of any materials that are foreign to the traditional dye process.[18]

In mediaeval times, the problem of the degradation of textile fibres by black iron – tannin dye was well recognized and prompted the Doge of Venice to ban its use to dye wool.[19] However, it was later found that pretreatment of cloth with indigo provided some stabilization of the black-dyed fibres and a 1581 act of the English parliament required dyers to dye a *ground* of indigo on wool cloth before the application of iron tannate dye.[20] In Germany, as far back as the late 18th century, mention is made of an iron-tannate ink containing indigo, the blue dye.[21] Stephens is credited with the invention of *blue-black* ink in 1832 and this contains an indigo additive; Stephens and Arnold started manufacture of this ink in the early 1830s.[22] One benefit of including indigo was its stabilization of the ink. Like the catechol and gallo moieties of tannin, indigo forms a strong complex with iron(II) and -(III).[23] It removes iron ions bound to the carboxylates associated with the hemicellulose component of the paper fibres and, consequently the degradation that would otherwise result from decarboxylation. The English poet,

John Clare, wrote in 1832 that he had produced his own iron-tannate ink containing indigo and made his paper from birch bark.[24] Although this paper is very white, it has an extremely high content of a pectic acid, 4-*O*-methylglucuronoxylan, a characteristic of trees of the *Betula* family,[25] and is therefore subject to extensive hydrolytic deacetylation accompanied by the release of acetic acid that catalyses the degradation of the ink. Indeed, the smell of vinegar has been reported from Clare's manuscripts and the ink is badly degraded.[26] In this case the acetic acid appears to have negated the stabilizing activity of indigo.

A similar approach to preserving iron-tannate inks has been employed by present-day conservators at the Netherlands Institute for Cultural Heritage. They have used salts of phytic acid, polyphosphates, to complex and scavenge excess iron(III) that could otherwise initiate degradative decarboxylation reactions in the fibrous substrate.[27]

Maori Golden Yellow Dye

When raw muka is placed in a boiling infusion of the bark stripped from raurekau (*Coprosma areolate*) for several hours, the fibres are dyed to a rich golden yellow. The dye molecules in raurekau belong to the anthraquinone family, as described above for madder. Although most anthraquinones are red at neutral pH, those present in raurekau give a more yellow hue. The principal anthraquinone in raurekau has been identified[28] as morindone **6** (Chart 3) the same compound found in the plant family *Morinda* of SE Asia. When applied to *P. tenax* in the way described above, it produces a very good wash and a light-fast finish.

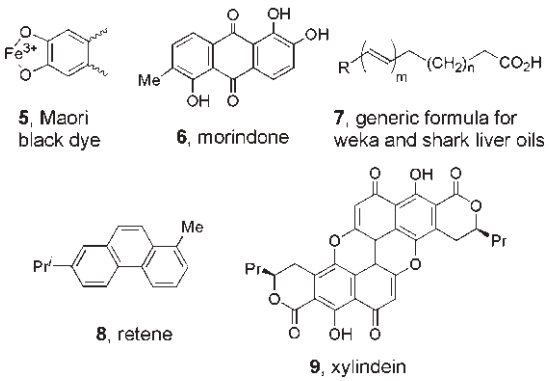
Red/Brown Dyes

Tanekaha (*Phyllocladus trichomanoides*) bark produces a tan colour. It is collected and treated in the same manner as that for hinau bark. Muka is soaked in the tannin solution for 12 hours whereupon the fibres are a rusty-brown colour. They are removed from the tannin solution, rubbed into warm wood ash (alkaline potassium compounds) while still moist and then exposed to sunlight for up to an hour before being thoroughly washed and left to dry.[5]

The bark of tanekaha is rich in a class of condensed tannin known as the proanthocyanidins. The development of colour with hot alkaline wood ash suggests that polyphenolics are being converted to the red anthocyanidin products widely found in plant material. This colour gradually fades in time to a pale tan, but the intensity and hue can be restored by further treatment with dilute alkali. The quinonoid oxidation products of phlobaphenes, familiar in the vegetable tanning of leather, are also believed to contribute to the red colour of this dyestuff.[29]

Herries Beattie's ethanological records *Traditional Lifeways of the South Island Maori*[30] is an invaluable collection of description of technologies, methods and customs of pre-20th century Maori. As well as the dyes already discussed, he notes that a red dye that could be used as an ink was extracted from the kokihi plant. However, it could not be used as a textile dye because it was not wash-fast. Similarly the yellow flavonoid dyes obtained from puriri cannot be fixed to cellulosic fibres.

Chart 3. Some Maori dyes, paints and pigments



5, Maori black dye

6, morindone

7, generic formula for weka and shark liver oils

8, retene

9, xylindein

Paints and Pigments

A range of coloured paints was prepared from inorganic and organic pigments dispersed in a drying oil as a vehicle for their application. The vehicles used by Maori were shark liver oil or weka oil. These drying oils are polyunsaturated fatty acids of generic formula **7** (Chart 3) that polymerize to a solid by free radical chain reactions. These types of oils were, and still are, the basis of Western easel painting. The best known and oldest of these is linseed oil, extracted from the seed of the linen plant, and it was used by the ancient Egyptians. Other drying oils that have been used were from the poppyseed and the walnut.

The involvement of double bonds in the *drying* reaction means the rate of polymerization (or drying) depends on the number of double bonds present. Also, the presence of various metal ions and metal oxides, *e.g*. lead oxide in lead paints, causes decarboxylation of the fatty acids and the generation of free radicals, and they are therefore useful promoters of drying (Eq. 2). Conversely, some organic pigments contain aromatic residues that are free radical scavengers and terminate the polymerization thereby inhibiting solidification. Examples of such pigments are the sooty materials produced by burning the terpenes in resinous woods and gums. These dark/black pigments are a mixture of carbon (charcoal) with partially reduced and aromatized terpenes such as retene **8** (Chart 3). These dark pigments were much favoured in various schools of European easel painting for their lustrous and translucent finish, *e.g*. Rubens and Van Dyck,[31] but because of their slow drying, small quantities of lead oxide were sometimes added to accelerate the polymerization of the oil vehicle.

$$M^{n+} + ROOH \rightarrow M^{(n-1)+} + ROO^{\bullet} + H^{+}$$

$$\text{or } M^{n+} + ROOH \rightarrow M^{(n-1)+} + R^{\bullet} + CO_2 + H^{+} \text{ .....(Eq. 2)}$$

The pigments and paints used by Maori before European contact were similar to those used by other cultures. Their black paint, like that just described in Europe, was made from soot collected on the leaves of harakeke from burning resinous woods such as rimu and mixing with shark liver oil or weka oil.[32] This paint was used from the Archaic Maori period in rock art on the walls of limestone shelters in the South Island dating from as early as the 15th century. Maori painters also extensively used ferric oxide minerals to produce pigments with colours ranging from yellow ochre to red ochre. The yellow goethite is a hydoxy/hydrated ferric oxide and can be converted to the red haematite, Fe_2O_3 by roasting the yellow ore.[32] Research

in our laboratory is seeking to confirm textual and anecdotal reports of materials processing being practised by pre-European-contact Maori in the production of their red pigment from goethite. In some areas, where they were available, bluish clays – ferric phosphates - were also used as pigments.

An interesting organic blue pigment reported by Beattie[30] was obtained from decaying inner wood of the tawai. The dry dust, which is probably a tetrahydroxynaphthalene oligimer derived from a fungus growing on the wood, was moistened with water and then mixed with a drying oil. It was applied to fibres as a fluid suspension as for a dye, or thickened and used as paint.[29] A green colourant which is also derived from the NZ fungus awheto, *Cordyceps robertsii*, is that traditionally used in tattooing (moko).[34] Green fungal secondary metabolites are produced as intermediates in the fungal melanin biosynthetic pathway by polymerization of tetrahydroxynaphthalene. One of these green compounds has been identified as xylindein **9** (Chart 3) produced by fungi of the *Chlorosplenium* genus.[31]

Back to the Future

The emphasis of this paper has been the history of traditional dyes and pigments. There are intriguing parallels in the chemistries of the materials used by pre-European-contact Maori and the colourants used by other civilizations before the advent of synthetic dyes. From locally available raw materials peoples of different cultures independently discovered the same classes of chemicals and used them to decorate their textiles and other objects. However, it is instructive with present day concerns about environmental pollution, to reverse the perspective from the past to the future and close with a brief discussion about dye auxiliary agents.

Although it is fair comment that dyeing took a giant leap forward in the middle of the 19th century with the synthesis of dyestuffs, commercial dyeing has the rather tarnished image of the *dark satanic mills* of 200 years ago. For example, it was found that chromium was a particularly effective mordanting agent and it became, and still is, widely used in this capacity. However, it is very toxic and now its discharge is tightly controlled. Similarly, while chlorination of many fibres significantly improves the fixing of dyes to the fibres, the toxic organohalides produced are unacceptable; as is the discharge of unfixed dyestuffs into waterways. In our quest for a *green and pleasant land*, finding ways of cleaning up the dye process has become an industrial imperative. The global market for auxiliary agents that improves the fixing of dyes is ~$NZ 3 billion p.a. and is growing at a rate of 6.5% p.a., driven largely by stringent environmental regulations and reducing energy costs. Without disclosing the details, there is a step used by Maori to produce the black dye that has the potential to be developed into an environmentally benign way of priming natural fibres for the more efficient uptake and fixing of commercial dyes.

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Article

Meat and Wool New Zealand Limited Consortia Research

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Introduction

Meat & Wool New Zealand Limited (M&WNZ) is funded by livestock producers through levies on all beef, sheep and goats slaughtered and wool sold. The income is used to maintain and extend trade access for NZ wool and red meat, and increase its preference both domestically and internationally, and to fund research and development that will help improve NZ farm returns.

On-farm, M&WNZ research looks to support farmers through regional initiatives, education programs and applied research and development in animal health, farm productivity and the environment. Off-farm, M&WNZ works in collaboration with industry and Government organisations, investing in six research consortia, each of which address a number of critical issues for the development of NZ's Beef and Sheep Industry. The consortia are legal entities, with Boards of Directors, who source their scientific expertise from leading NZ providers to ensure the best returns for their shareholders. Largely agriculture and biology-based, the consortia draw on a number of different disciplines to underpin their research, including genetics, animal husbandry, engineering, and chemistry. This review outlines the general work of some of the consortia funded by M&WNZ, including aspects of their chemical research. These consortia are Pastoral Green House Gases Research (PGgRC), Meat Biologics Research (MBC), Ovita, MIRINZ Inc., and Johne's Disease Research. M&WNZ also funds research in wool.

Ovita® - Sheep Genomics

Ovita's goal is to support the sheep industry in developing biological tools that enable farmers to make choices that will best meet the demands made of them, be they geographical, environmental, or market-driven. The key focus is sheep genomics, an area in which Ovita has world-leading expertise. Genetic techniques can be used to address the incidence of parasites and disease, meat yield, and meat quality in animal livestock. The team at Ovita is developing selection tools for the sheep industry based on well characterized traits and proteins discovered from decades of sheep-breeding in NZ.

The organization has successfully commercialised a number of its genetic products for the farming sector through its spin-out company *Catapult Genetics*. Established in June 2006 and recently purchased by Pfizer, Catapult's product list includes:

• Inverdale®, a naturally occurring gene that increases sheep fertility. The Inverdale® DNA test allows farmers to preferentially select animals carrying the Inverdale® gene, and integrate the gene into their breeding programs.

• Shepherd®, which profiles DNA from sires, dams and lambs to generate pedigree and family information. When interfaced with breeding software Shepherd® is used to predict breeding values and genetic indexes. Breeding values are the measure of the genetic value of an animal for a specific trait, while genetic indexes measure the ability of an animal to transmit its genes to the next generation.

• Loin-MAX® and MyoMAX®, DNA tests for selecting animals with increased muscle size. Increased muscling results in less carcass fat and an improved carcass weight compared to animals of the same live-weight and genetic background.

• i-Scan®, a DNA test for Microphthalmia. This is a recessive gene disorder that causes developmental malformation in the eyes of affected lambs and results in blindness. Microphthalmia only occurs when a lamb inherits damaged copies of the gene from both parents. If only one parent has the gene then the animal is a carrier, with normal eye development and function, but capable of passing the damaged gene to the next generation. Blind progeny are an indication of multiple carriers in a flock, for which i-SCAN® can test and guide farmers in breeding decisions to help eliminate the disorder.

• Worm-STAR®, a tool for parasite management. It helps to identify parasite-resistant animals and slow the development of drench resistance.

At the core of Ovita's future developments are SNP (pronounced *snip*) Chips. SNP's are single nucleotide polymorphisms or small genetic changes occurring within a DNA sequence. They occur infrequently - less than 1% of the time in the human population - and are considered most significant when associated with the small percentage of DNA sequences that code for the production of proteins. These have the greatest potential for altering protein biological function. The SNP acts as a marker, allowing segments of DNA to be traced over many generations. The SNP chip brings together thousands of these markers together on one device. The technology can then be used to accurately select animals for traits that are important for efficiency and profitability, including disease resistance, meat quality attributes, maternal ability, and other factors that are not easily measured in an animal until later life. Breeding values (as noted above, the measure of the genetic value of an animal for a specific trait) are traditionally determined over multiple generations. However, SNP's have the ability to accurately predict breeding values for new-born animals, for which no previous data exist. The long term prospects for the application of this technology are extremely promising, offering the potential to help eradicate diseases such as facial eczema,

reduce drenching frequencies, significantly increase lamb survival rates, and (ultimately) the overall quality of NZ lamb meat.

Wool Research

M&WNZ investment in wool is focused on maintaining the profile of NZ wool with international users of the fibre. With textile processors having an ever increasing array of fibre options to produce their products with, a key strategy for M&WNZ is to keep wool as a viable textile fibre choice. Ensuring a continuous stream of new innovation in wool products is core to the future of wool as a fibre. Our investment is focused on maximizing wool fibre quality, developing technologies that enable new and improved performance wool products to be introduced to the market, and addressing areas of market risk, including research to improve lightfastness, insect resistance, and chemical residue identification.

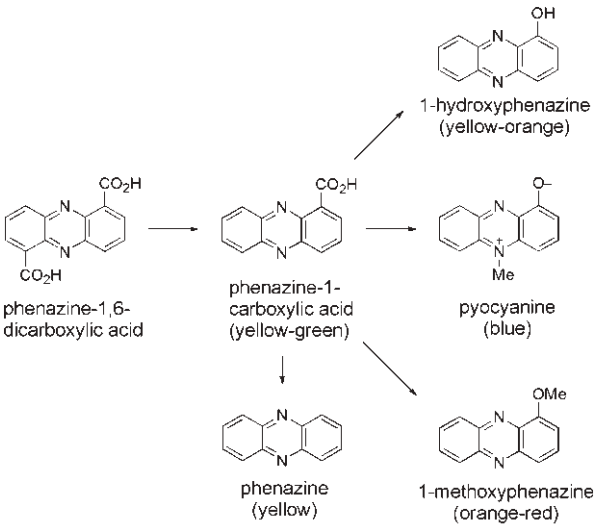
Wool Discolouration

Prior to dyeing and processing, wool is washed in hot water and detergent to remove the non-wool contaminants. This process is known as scouring. The quality and value of the wool is detrimentally affected by the presence of colour that is not extracted from wool during the scouring process.[1] Intense yellow discoloration on a sheep is often associated with warm humid temperatures when fungal and bacterial organisms thrive in the wool fleece. This colour is not removed by conventional scouring methods. Knowing the origin and identity of the compounds causing the discoloration is vital in understanding how to treat the discolored wool. Scientists at AgResearch found the yellow discoloration to be associated with the wool cuticle and identified the compounds responsible as phenazine-based chromophores, including 1-hydroxyphenazine, pyocyanine, and phenazine-1-carboxylic acid (Scheme 1). Phenazines form the structural basis of the azine dyes such as eurhodines, indulines, and safranines. There are over fifty naturally occurring phenazines, whose microbial production is limited to a few bacterial genera, the most prominent of which is *Pseudomonas*. *Pseudomonas aeruginosa* is a widely studied phenazine-producing bacterium that is a ubiquitous member of wool fleece microflora. It is often the dominant species when the fleece is wet and is thought to be associated with the ovine dermatological condition, fleece rot. Biosynthesis of a range of coloured phenazines, including those identified in the wool cuticle, has been proposed to start with phenazine-1,6-dicarboxylic acid, as shown in Scheme 1.

Having identified the chromophores responsible for fleece discolouration, the researchers are now investigating commercially viable treatments for removing the phenazine-related colour from the wool. Simple treatments, such as reduction with sodium hydrogen sulfite both pre- and post-scour, have already been demonstrated as effective, causing substantial improvements in whiteness of the wool without any observable damage to the fibre.

Metal Free Wool Dyeing

With some synthetic fibers guaranteeing up to ten years lightfastness or fade resistance, the challenge to improve

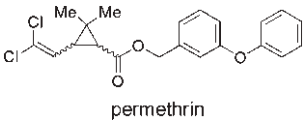


Scheme 1. Phenazine Chromophore Biosynthetic Pathway

lightfastness of woolen fibers is crucial for NZ wool in international markets. Metal-free dying techniques, developed by scientists at AgResearch, are a significant breakthrough for the industry.[2] To enhance the lightfastness of conventional woolen dyes, small amounts of cobalt- and chromium-containing dyes are traditionally used for colouring carpets. The new dyes do not contain heavy metals but form pigments inside the wool fibres to achieve significantly higher lightfast qualities than conventional wool dyes. They are also more environmentally friendly than their predecessors, an important attribute for effluent discharges from wool mills. The research program has progressed through concept and experimental stages to full-scale industrial trials in Europe, with plans for two major European companies to launch the product commercially in mid-2008 if the trials are successful.

Insect Resistance

A problem unique to wool fibre is its susceptibility to attack by certain moths and beetles.[2] The broad-spectrum insecticide, permethrin, which has traditionally been used to treat woolen carpets for insect resistance, is no longer environmentally acceptable as it is highly toxic to aquatic organisms, killing the invertebrates and affecting the growth of insects that fish feed on. Strict international effluent discharge standards for the insecticide are having a profound impact on the industry, particularly in European cities where multiple carpet mills can be discharging effluent to one sewage treatment works. Developing a long-term non-insecticidal treatment was important in maintaining the carpet industry's international competiveness.



permethrin

Again AgResearch Limited scientists have met this challenge and developed a new set of carpet protection agents. Their first initiative was the development of a new generation insecticide effective in producing insect-resistant carpets, but with a low environmental impact on waterways. Mystox is now in commercial development with Catomance Technologies Ltd., a UK based industrial technology company. More recently, the team at AgResearch

has taken a different approach by creating two biodegradable treatments for carpets that are not insecticides. These treatments bind to wool fibre in much the same way as a dye would and prevent the insects from eating the carpet, but they do not kill them. The treatments do not break down in light as insecticides do, and once they have been shown to be fast with regard to shampooing and light exposure, have the potential to be successful commercial products for insect resistance. Spin-off benefits have also resulted. The agents used in the treatments are also excellent dye bath leveling agents (they ensure the fibres dye evenly without streaking) and may, in addition, have potential applications in the apparel industry.

Nanotechnology

Nanotechnology[3] is being used to create high performance textiles for the wool industry. Inert nanoparticles attach themselves to wool fibres, increasing fibre friction and blocking dirt. *Lanasan NCF* (Nano Carpet Finish) developed in 2005 by scientists at AgResearch and commercialised with Swiss multinational Clariant is one such treatment. The carpet is treated with the nanoparticles during the dyeing or final yarn washing stage to produce a high performance carpet with strong yarn and a stable pile. Manufacturers see an 80% improvement in yarn strength that reduces shedding, fuzzing and pilling, and therefore the need to vacuum. The tiny particles attach themselves to the wool, making the pile more robust and increasing the ability to withstand short-term wear by up to 20% and doubling long-term resistance. The particles also prevent soil from attaching itself to the carpet, improving the soil resistance of the final product.

There are also options to apply nanotechnology to other textiles, with high performance clothing and upholstery applications currently under development. In one particular application gold nano-particles are being used as stable colour-fast colourants on wool. As the colour reflected from a gold nano particle is highly dependent on the size and shape of the particle, red, purple, yellow, green, and blue colours can be created by controlling the finish on the textile. While organic dyes break down in sunlight, gold-dyed fibres benefit from gold's relatively inert properties and show improved fade resistance.

Pastoral Greenhouse Gas Research Consortium (PGgRC)

Greenhouse gases (GHGs) and their role in global climate change is extremely topical, in NZ and globally. It is widely acknowledged that human activities are altering the composition of the atmosphere. In NZ 48% of GHG emissions come from agriculture. The Pastoral Greenhouse Gas Research Consortium (PGgRC) represents a key investment by the NZ livestock and pastoral industries in mitigating GHG emissions from the agricultural sector. The consortium aims to decrease the total emissions of greenhouse gases in NZ 10% by 2013 (relative to 2004), estimated to be a 4 Mt reduction in the agricultural GHG emissions.

Two greenhouse gases are associated with pastoral agriculture: methane and nitrous oxide. The majority of methane is belched from ruminant animals as a by-product of rumen fermentation, while nitrous oxide is formed as part of the nitrogen cycle in pastures when soil conditions are anaerobic, particularly in winter when soil moisture and water tables are high (Fig. 1).

Fig. 1. Measuring gas emissions from sheep

Methane Reduction

Methane is the by-product of the digestion of forage by micro-organisms in the rumen of an animal. Digestion produces hydrogen, which is converted by methanogens in the gut to methane. Therefore, the inhibition or elimination of methanogens is a major focus of the methane GHG reduction strategy for pastoral animals. This presents a significant scientific challenge as altering the ruminal ecosystem to reduce methane emission can have profound effects on animal health, farm management and animal productivity. Developing successful intervention strategies to reduce methane emissions depends heavily on understanding rumen ecology and the parameters that influence animal productivity. The PGgRC research programme includes research in genetic sequencing and identification of methanogen species and populations, dietary effects on ruminant methane, the effect of animal variation on methane production, development of a methanogen vaccine, and the identification of a chemical inhibitor for methane production.

A significant breakthrough for PGgRC in 2008 has been the mapping of the genetic sequence of the methanogen, *Methanobrevibacter Ruminantium*, a world first for the NZ team (Fig. 2).[3] The microbe is a member of a major group of rumen methanogens, having more than 2200 genes, but at 3 million bases in size is considered of medium size. The genome sequencing research project aims to identify new genes and proteins that can be used to target and inhibit methanogens, without decreasing animal productivity or affecting the many other microbes that are beneficial to the rumen and digestion. While there is still a long way to go to reduce methane emissions, closing this genomic sequence is seen as an important piece of a complex puzzle.

Nitrous Oxide Emission Reduction

The greatest source of nitrous oxide emissions result from the dung and urine deposits of grazing animals (94%), while the remainder comes from nitrogenous fertilisers. Nitrous oxide is formed by the actions of soil bacteria, during either the oxidation of ammonium ions to nitrate or the reduction of nitrate to nitrogen gas. Mitigation strate-

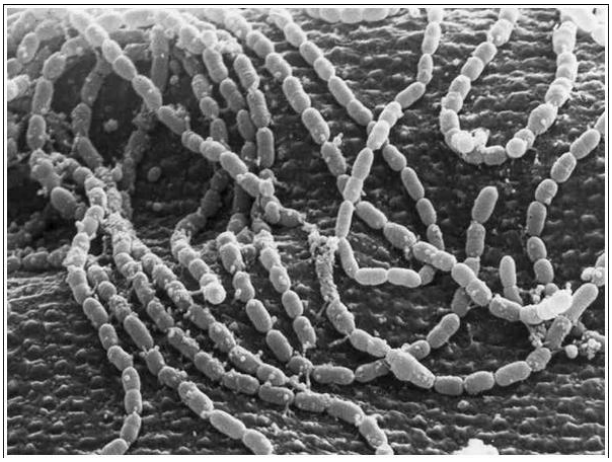
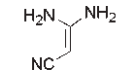


Fig. 2. Methanogen string

gies for nitrous oxide include both on-farm management and science-based solutions. Removing animals from wet pasture and collecting their excreta for later application to dry pasture, controlling the amount of nitrogen added to the soil from fertiliser, and ensuring farmers use low nitrogen feed supplements all actively reduce nitrogen accumulation in the soil. Farm system management tools are invaluable in applying these strategies, with programmes such as OVERSEER™ providing practical nutrient budgeting models for use on-farm. Nitrification inhibitors, compounds that inhibit the conversion of ammonium to nitrate, provide a science-based nitrous oxide mitigation strategy. One nitrification inhibitor, Dicyandiamide (DCD) has successfully passed proof-of-concept tests in short-term, small- to farm-scale testing and has been commercially available for several years. Research continues on proving the long term effects of inhibitors and to accurately account for the effect of this mitigation strategy at paddock scale.



dicyandiamide
DCD

Meat Biologics Research Consortium (MBC)

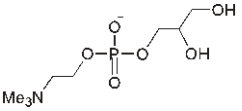
The Meat Biologics Research Consortium was established in 2002 to develop novel nutraceuticals, functional foods and health supplements from red meat or red meat co-products. These bioactives are targeted for use by humans for general health and well-being, and have been developed around specific, strategically selected, market opportunities, ultimately aimed to increase the value of NZ meat. The consortium is currently focused on a small number of products that show potential for commercial development, including a treatment for iron deficiency and a protein supplement.

Iron Deficiency

The WHO estimates that 1.3 billion people in both developed and developing countries suffer from iron deficiency.[4] Those at risk include people suffering from chronic parasite infestation, weaning babies, young adolescent girls, and women in menstruation. Iron deficiency is a widespread, generic issue. The use of red meat to enhance iron uptake and bioavailability has been well established in the scientific literature[5] and has come to be known as the *meat factor*.

A team at Massey University, funded by MBC, is one of several groups globally that are attempting to identify the active compound in meat which is responsible for enhancing iron absorption from the diet. Chemical fractionation procedures are complemented by an array of *in vivo* and *in vitro* testing that guide the team to a successful conclusion.

The hunt for the meat factor has spanned 40 years of research. Despite extensive studies demonstrating the enhancing effect of meat on iron absorption, the mechanism of the effect remains controversial and none of the research has unequivocally identified the active component or components responsible to date. Fatty acids,[6] peptides arising from the proteolytic digestion of meat[7] and the amino acids histidine, cysteine, and to a lesser extent, lysine[8] have all been implicated as playing a role in the meat effect. The most recent publication in the area has identified L-α-glycerophosphocholine (L-alpha) as an ingredient contributing to iron uptake from the diet.[9] L-alpha was isolated from digested meat samples following separation by fast protein liquid chromatography and identification using a combination of mass spectrometry, nuclear magnetic resonance and HPLC. When added to vegetarian lasagna, L-alpha has been shown to increase iron absorption in women of child-bearing age with low iron stores.



L-α-glycerophosphocholine

Protein Supplements

Protein is an essential part of the human diet and is required for cell maintenance and repair and a wide range of bodily functions. Low consumption of protein may lead to muscle wasting and increased susceptibility to infection. In particular, in physiological states such as malnutrition in the elderly, sports nutrition for endurance training, weight training or body building, pregnancy, or malnutrition, the provision of a high quality protein supplement with the appropriate balance of amino acids can be a significant aid to protein retention or synthesis.

Protein supplements can be provided either as native protein or with the protein already partially digested (hydrolysed). Digestion typically begins in the stomach by pepsin and is continued by trypsin and chymotrypsin in the intestine. The protein is broken down progressively, until – on reaching the ileum – most of the protein is in the form of amino acids or small di- or tripeptides. These amino acids and small di- and tripeptides are then absorbed by the cells lining the gastrointestinal tract. Once inside the cell most of the absorbed di- and tripeptides are digested into amino acids by cytoplasmic peptidases and exported from the cell into blood. Thus provision of protein already in the form of the small peptides ready for absorption can enhance both the degree of and speed of absorption.

As well as displaying rapid and complete absorption, a good protein supplement should provide the body with essential amino acids in balanced proportions. Overall protein synthesis will be limited if any particular essential amino acid is present at a lower concentration than that required for protein synthesis. Amino acids should also be bioavailable so that their absorption achieves the required balance in the body.

A wide range of protein supplements is available commercially, many of which are sourced from casein. In order to make an impact in the protein market, MBC is developing a range of highly characterised meat proteins with potential applications in the personalized food arena, where foods are tailored to meet the individual preferences and health needs of a consumer. The meat hydrolysates are high quality, highly digestible forms of amino acids, which have an ideal composition very similar to that of human muscle. With the growing global trend to providing consumers with scientifically supported foods that address specific nutritional and health issues, MBC's range of meat hydrolysates can be tailored to provide the correct balance of amino acids for consumers, be they elderly patients or professional body builders.

Johnes Disease Consortium

Johne's disease (pronounced *yo-knees*) is a contagious, chronic and sometimes fatal infection that affects the small intestine of ruminant animals including cattle, sheep, goats, and deer. Discovered in 1905 by the German bacteriologist and veterinarian Heinrich A. Johne, the disease is caused by the bacterium *Mycobacterium paratuberculosis* and is widespread throughout the environment in many countries. In NZ the bacterium has been isolated from a range of wildlife, including rabbits, hedgehogs, ferrets, hares, cats and gulls. Overseas, animals such as foxes and various marsupials have been shown to carry the bacteria. The disease is spread by animals eating infected pastures and drinking from infected waterways; infection of an animal normally occurring shortly after birth. The onset of symptoms, however, may not occur until an animal is between 2 and 6 years old. As there are limited diagnostic tests available for Johne's disease, farmers and vets often have to rely on verification of infection post-mortem. Johne's is progressive; starting with diarrhea and wasting and leading to the animal becoming increasingly emaciated and dying from dehydration and severe malnutrition. The effect of the disease is costly, with impacts on animal health and production estimated to cost up to $88 million each year in this country.

The Johne's Disease Research Consortium (JDRC) has been developed to unite and accelerate research into the disease in NZ aiming to develop efficient and effective tools to control and reduce its prevalence. Four significant developments are the focus of JDRC research. The first is to develop diagnostic tools for farmers and vets that enable identification of infected animals. Leading on from this is effective management systems for on-farm control of infected herds. The third and fourth developments are preventative in focus, looking to create a new vaccine and identify possible gene markers for resistance to the bacteria. Vaccines are currently available for Johne's but these can have side effects and may affect the result of tuberculosis testing. Genetics, however, may hold the long term key to this disease if researchers are able to identify a gene-marker that does not compromise production and which will allow farmer to select for Johne's resistant stock.

MIRINZ Inc. - Meat Processing

MIRINZ Food Technology and Research (MIRINZ Inc) aims to increase the profitability of NZ meat processors by focusing on increasing the value of the animal carcass and accompanying quantity of sales, while reducing the costs associated with meat processing. It is possible to increase the returns from an animal carcass by upgrading the worth of low value meat cuts. It is well accepted that the degree of animal stress prior to slaughter influences meat quality, but quality is most significantly affected by the post-slaughter processing of the carcass, with the effects of pH and meat cooling accounting for up to 70% of the final quality of a cut.

During processing, various changes take place in the biochemical and structural attributes of muscle tissue in the meat, especially when meat transforms from a pre-rigor mortis to a post-rigor mortis state. During rigor mortis a carcass stiffens as muscles shorten and the muscle pH falls. Good quality meat is associated with lower muscle pH, as at high pH meat is darker and less desirable to customers, and also runs the risk of increased meat spoilage. The application of an electric current to the carcass after slaughter (electrical stimulation) reduces the pH of the muscle quickly and hastens the onset of rigor mortis, optimising meat quality. Smart Stimulation is an on-line measurement tool and tailored stimulation system co-funded by MIRINZ Inc. and Meat and Livestock Australia (MLA) to improve pH control on the processing chain. The system has the ability to measure the pH of the carcass easily and efficiently and, as a result, administer a tailored electrical input to the carcass to ensure consistent meat quality. MIRINZ Inc. is also co-funding the development of an NMR-based online tool to measure pH further down the processing chain to predict tenderness, with initial trials showing promise.

Tenderness can also be affected by stretching meat prior to rigor mortis. Stretching improves tenderness and also positively influences the colour of the meat and reduces drip loss. Drip retention is important for retaining tenderness and juiciness in a cut of meat. The Boa Meat Stretcher is a new device for stretching meat, which was developed with co-funding from MIRINZ Inc. Industry trials of a *pre-production unit*, have successfully improved the shape and tenderness of lower quality meat cuts.

Through such initiatives and other automation technologies under development MIRINZ Inc. is helping to bring improved productivity and value to the NZ meat industry. MIRINZ Inc. also has a role in maintaining existing markets for NZ meat. While meat has traditionally been shipped to international markets frozen, there is a high demand for fresh, chilled, NZ product. Such meat must be protected from spoilage by organisms and pathogens or risk rejection by importers. One new initiative under de-

velopment by is the addition of lactic acid bacteria (LABs) to meat during chilling. *Brochothrix thermosphacta* is the predominant spoilage organism in chilled raw meats. As the organism grows at temperatures between 0°C and 30°C and thrives in depleted aerobic conditions, refrigeration and vacuum packing provide ideal conditions for growth. Lactic acid bacteria have the ability to inhibit spoilage by growing faster than undesirable organisms, such as *Brochothrix thermosphacta*. This work will help secure the future of NZs excellent international safety reputation.

Conclusion

The agricultural sector plays a lead role in underpinning the NZ economy. Meat and wool exports earned us approximately $6000 million in 2006-07, so ensuring that we remain at the forefront of scientific advancements in agriculture is vital for our economy. Through its participation in Research Consortia, M&WNZ is actively investing in the future of the NZ beef and sheep industry. That support extends across a broad range of activities, but is consistent in its approach, securing first class research from the country's scientific community for the benefit of the NZ farmer and the economy as a whole.

Acknowledgements

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New Zealand Science Scene

Big Future in Vaccines

New adjuvants, the helpers in making vaccines effective, are currently being worked on at Industrial Research Limited in Lower Hutt.

Science teams led by Gavin Painter and Phill Rendle, have discovered two new compounds they believe could be effective adjuvants. The ideal adjuvant needs to be potent, non-toxic, water soluble, biodegradable and stable.

The possible compounds currently being worked on by IRL come from a bacteria and a plant. The first works by cell-mediated immunity. A synthetic version of the plant derivative has been developed with Otago University's School of Pharmacy.

The word adjuvant comes from the Latin verb adjuvare meaning *to help*. Often without the help of adjuvants, the immune response to a vaccine is small. Vaccines are composed of one or more antigens and one or more adjuvants. The adjuvants enhance the immune recognition of the antigens and increase the immune system's ability to make antigen-specific antibodies. They can also, among other things, reduce the need for booster vaccines.

There are only a few adjuvants licensed for human use. Many of the common ones are aluminium salts first used in the 1930s. Adjuvants are a rapidly growing area of research. This is because more is understood about immunological mechanisms and there are new technologies helping the research. Also, organisations like The World Health Organisation have set ambitious goals for using vaccination in disease control.

An effective adjuvant can mean a cheaper vaccine because less active ingredients need to be included for it to trigger a response. It can also be used to improve vaccines that previously failed in trials.

Gavin Painter and Phill Rendle using IRL's newly upgraded spectrometer to analyse adjuvant samples.

It is possible in the future there maybe vaccines for chronic diseases like cancers and auto immune diseases and for diseases that affect thousands around the world such as malaria or tuberculosis. A vaccine for cancer is already being developed in a collaboration between IRL, Grow Wellington, Victoria Link and the Malaghan Institute. IRL is providing an adjuvant.

Article

In Search of Biological Activity

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There are many reasons why a chemist might become interested in a new molecule - it may present an opportunity to develop or apply new synthetic methodology, give new insights into fundamental aspects of structure, or it may have a valuable function. Of these functions, one of the most quoted is *biological activity*. Yet for all the interest in biological activity, it is a poorly understood and highly complex concept. Discussed below are some of the ways of viewing biological activity at the cellular level, and an exploration of some of the methodologies now available to determine the underlying biological mechanisms involved.

An introduction to biological activity

A living cell contains an intricate set of interacting pathways. In a highly simplified view, at its centre is a nucleus which contains the nucleic acid DNA that carries information coded in the form of a sequence of the nitrogen-rich bases adenine, cytosine, guanine and thymine. This stored information encodes for the production of proteins. All cells in the human body contain the same DNA but those in the brain are distinguished from those in the toes by the parts of the DNA information utilized for the production of proteins. Proteins are produced through a process that starts with copying the relevant coding section of DNA into a related nucleic acid, RNA. This RNA copy initially carries sections present in the original DNA sequence that are interruptions of the protein coding sequence; these non-coding regions are excised and the RNA is spliced together to create a molecule which carries only protein coding sequence. The RNA is then checked for obvious errors and then used as a template for protein production. By controlling the sections of DNA that are copied into RNA, and the rate at which the RNA molecules are used for the production of proteins, a cell can take on the form required by its environment. It can also respond to changes in that environment, which can include treatment with a biologically active molecule.

The biological activity of a compound reported in the chemical literature is commonly measured by one of three methods – ability to inhibit an isolated protein target, a gross impact on the growth of cells in culture, or the impact on a disease, *e.g.* anticancer, antibiotic, immunosuppressant. Each of these provides a valid and useful way to understand how a small molecule interacts with a biological system, but the bigger picture can be so much more interesting and open up whole new avenues for research.

Determining the activity of a compound against a single, isolated protein target is an important way of comparing a series of compounds. Generally, it can be undertaken in high throughput on large compound sets and, in the hands of a medicinal chemist, can be used to guide the production of new compounds with improved properties through structure-activity relationship analysis. Measuring activity in this way has been the backbone of many research and development programmes. However, it presupposes that the functional target of the molecule is known, and that it is the only significant target. What is becoming clearer as more and more detailed studies into the mode of action are undertaken is that many compounds, potentially the majority of compounds with biological activity, have a set of biological targets through which they function, and not the ideal single target.

For example, it is well known that caffeine (**1**; Chart 1) is a psychostimulant that it exerts its effect through adenosine receptors. However, the way this effect is mediated by different adenosine receptor subtypes, or dimers of receptor subtypes, is still a matter of investigation.[1] The biological activity of caffeine does not stop there, however, but impacts on cellular processes including cell growth and DNA synthesis. In one of the more eloquent descriptions of a compound having multiple activities through disparate targets, Kaufmann *et al*. note *Caffeine occupies an important niche in the cell cycle checkpoint field. Not only does it help bleary-eyed scientists concentrate on their experiments, it directly inhibits the checkpoint kinases, ATM and ATR*.[2] Whilst this discovery may sound of only esoteric interest, such action of caffeine allows a cell to progress through its cycle while carrying damaged DNA; it avoids the checkpoint in which the damage would be repaired or the cell committed to death. This checkpoint is designed not only to ensure that harmful DNA mutations are not propagated into future generations of cells, but also it aids in providing resistance to tumours from radiotherapy and DNA damaging therapeutic agents. Thus, caffeine could be seen as sensitising tumours towards a number of therapeutic interventions. However, the concentration of caffeine required to inhibit the activity of its checkpoint targets is *ca*. 500-fold higher than that which gives its psychostimulatory effects on the adenosine receptors. Kaufmann's team can therefore use caffeine to help them stay awake to the wee small hours (studying checkpoint kinases) without putting themselves at risk of acquisition of DNA mutation.

Whilst discussing caffeine, it is clear that only one particular target set, the adenosine receptors, are the realistic targets in the body. For other molecules this is not so clear. Many clinical agents, even though ascribed as having the same mode of action through a common primary target, have discernible *flavours* of activity through secondary targets. This is exemplified by the anti-leukemic compound imatinib (**2**; Chart 1) [Gleevec® (Glivec® Europe/Australia) as its mesylate salt] and its analogues. A number of these drugs were specifically developed as imatinib analogues and yet each compound has its unique range of targets. This includes one analogue that binds to

over 30 different kinases and another with a functional target from a quite unrelated set of proteins.[3]

Caffeine and imatinib are but two examples from many that illustrate the growing literature on the promiscuity of drug-like compounds. The human genome is believed to contain approximately 25,000 genes that hold the information which becomes translated into proteins. However, the RNA copy of the coding DNA is commonly manipulated by the cell on the way to the production of proteins, allowing a gene to code for multiple protein variants from the same gene. Following that, the proteins can undergo different modifications that alter their functions. The take home message from this is clear: A human cell has the capacity to produce close to 100,000 different proteins, and many-fold more functionally distinct variants beyond that once the amino acid backbone becomes decorated with sugars, lipids, phosphates and other moieties. It is little surprising that, when offered this panoply of interesting targets, a drug will find more than one with which it can interact. Indeed, it would be astounding should a drug *only* find one target.

Thus, defining biological activity becomes a difficult task. It is necessary to identify how many proteins your molecule can interact with, and which of those interactions are truly responsible for any observed activity. This understanding also confounds the traditional medicinal chemistry approach to structure activity analysis since, in developing an analogue of your lead compound to improve its interaction with one target, its interactions with the potential remaining 99,999 proteins will also be changing - and the chance that your new compound will promiscuously acquire a new target becomes a significant probability.

How to determine biologically relevant targets

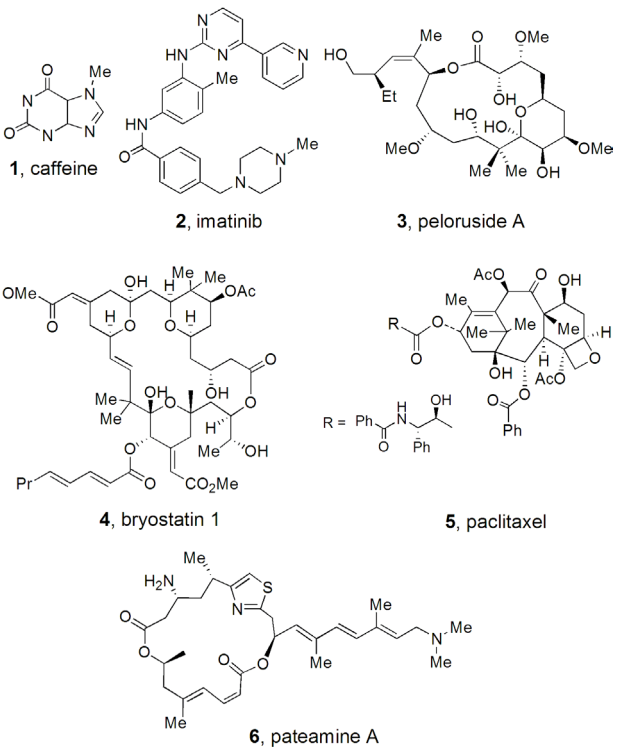
Unfortunately there is no single technique that can be used to reveal the target(s) of a biologically active compound. Probing biological activity is rather more like playing Cluedo. In this classic board game, players have to identify how Dr Black was killed, based on a set of six murder weapons, nine rooms, and six possible perpetrators. Cellular Cluedo is far more interesting! Whilst we know the *who* (our biologically active molecule), we do not know the *how* (which set of the ~100,000 proteins) or the *where* (which cellular compartment the drug reached). Consequently, there are far more possibilities in Cellular Cluedo than the 324 provided by the board game, and the questions asked have to be carefully planned. Even then, the evidence might be circumstantial only.

Two compounds, peloruside and pateamine, serve here to illustrate some of the methods available to identify cellular targets. These compounds come from the laboratory of the marine natural products group at Victoria University, led by Peter Northcote.

Peloruside A – serendipity in target discovery

Peloruside A (**3**; Chart 1) was isolated by Linden West in Peter Northcote's laboratory, and first reported in 2000.[4] Aside from its interesting and novel structure, early work

Chart 1



revealed that it was a highly potent toxin to mammalian cells, leading to cell death at low nanomolar concentrations.

In itself, this toxicity was insufficient to make **3** an interesting molecule as there are many toxins in the world that are every bit as lethal. John Miller and then PhD student, Kylie Hood, set about defining the mode of action of **3** at Victoria's School of Biological Sciences. Whilst a daunting task, structural analysis suggested a similarity between **3** and bryostatin (**4**; Chart 1), a compound with a well-characterized mode of action. After more than a year of careful work, Kylie conclusively demonstrated that **3** and **4** did *not* share the same target.[5] Thus, one target down, 99,999 to go! Good luck then came to Kylie's aid as she stumbled upon a picture of cells treated with paclitaxel (Taxol®, **5**) and noticed similarities between those cells and the cells she had treated with **3**. She then showed that, despite no common structural features, peloruside (**3**), like **5**, stabilizes microtubules.[6] A microtubule is a dynamic assembly of tubulin proteins, which forms a rigid rod that a cell uses during cell division to help pull copies of DNA apart. Many well-established anti-cancer drugs, including **5**, interfere with microtubules.

The serendipitous discovery of a functional target of **3** is but part of the story. Peloruside only has a future as a drug if it has a point of difference from existing drugs that bind to tubulin. In 2004 we were able to show that **3** has a binding site on tubulin that is different from paclitaxel,[7] and thus peloruside can be used synergistically with paclitaxel.[8]

The story of **3** serves to demonstrate that even when the target is known, there is more to discover about the biological activity of a compound; the location of the binding site and interaction with the cells' systems for dealing with foreign substances are important. There is one fur-

ther twist to this story. In work soon to be published, Anja Wilmes (with Miller and Jordan) has used proteomics (the study of the proteins produced by a cell under specific conditions) to investigate further the difference between peloruside and paclitaxel. While a human cell may have the potential to produce up to 100,000 different proteins, a particular cell only needs to produce a subset of these at any one time; this is referred to as its proteome. By looking at the proteome of cells with and without drug treatment, one can see which proteins the cell is calling upon to respond to the drug. Many of these proteins will be directly related to the drug's mode of action, *e.g*. many of the responding proteins in the experiment with **3** interact with tubulin. However, even though **3** and **5** appear to share a common mode of action (stabilising of microtubules) the proteome responses to the two compounds have relatively little in common. Our working hypothesis is that the differences point to as yet uncharacterized targets of either peloruside or paclitaxel.

Planning target discovery

As the discovery of the target of **3** was serendipitous, it seemed reasonable to develop and exploit more systematic tools for such discovery. Four questions need addressing in order to be confident that the mode of action of a compound has been identified:

1. What characterizes the cellular response to the compound?
2. What cellular components *can* it interact with?
3. Which of these components does it interact with *functionally*?
4. Is it possible to validate the link between the proposed target and the cellular response?

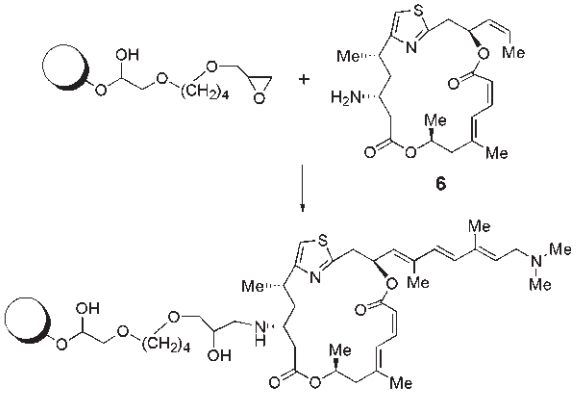
Pateamine – target discovery by design

Pateamine A (**6**; Chart 1) is found within the same species of sponge that produces peloruside (**3**), and was first reported[9] from the Blunt and Munro laboratory in 1991. Like **3**, **6** is a potent toxin and a suggestion soon arose that pateamine had immunosuppressive activity.[10] Romo initiated a study probing the structure activity relationships of **6** and its analogues, but noted *Further analysis and understanding of these results in regard to their relevance for protein ligand interactions must await structural characterization of the interactions of the putative cellular protein receptor(s) with these PatA derivatives*.[11]

Although Romo's group remained interested in **6** for several years, it attracted little interest elsewhere, being one amongst a myriad of known toxins. The lack of a characterized target, the complexity of Romo's structure activity data, and its availability within our laboratories made **6** an ideal candidate to use in building a repertoire of target discovery skills. What follows stems from talented student, James Matthews who considered the questions proposed above. It shows how answers to these questions assisted in defining the biological activity of **6**.

1. What characterizes the cellular response to 6? - It has a number of biological activities associated with it as it is antiviral, antifungal and immunosuppressive. The key to these activities is its potent toxicity; it induces death in a number of cell lines at concentration in the very low nM range.[9,12] We have confirmed the relationship between the toxicity of **6** and its proposed mode of action, protein synthesis inhibition. If tritiated amino acids are introduced into the broth in which cells are grown, the radioactivity is incorporated into proteins. When cells are provided with both **6** and a tritiated amino acid, radioactivity is ***not*** incorporated; protein biosynthesis is stopped. This inhibition ***precedes*** the processes of cell death indicating that loss of protein biosynthesis is the cause, and not a consequence, of cell death.

2. What cellular components can 6 interact with? - Unpublished work within the Northcote group and Romo's structure activity analysis[11] both indicated that the $-NH_2$ moiety of **6** could be derivatized with little impact on global activity and provided a handle for chemical modification. Under mild basic conditions, **6** is alkylated with an epoxy-terminated agarose gel (Scheme 1). Cells, grown in culture were lysed (burst open) to provide a solution rich in cellular proteins, although lacking proteins remaining associated with cell membranes. This lysate was passed through agarose-immobilized **6** and led to the proteins that bind **6** being preferentially retained by the column. These proteins were subsequently eluted and their identity determined (MS methods) as actin, β-tubulin, and the charmingly named eukaryotic initiation factor 4A (eIF4A).



Scheme 1. Generating an affinity matrix with pateamine (**6**).

Using affinity chromatography to identify a target in this way has three weaknesses. Firstly, where the target is a membrane-bound protein it would not be isolated. Secondly, functionally-relevant binding proteins might be swamped by other proteins present in high concentrations within cells, and thirdly, the very act of derivatizing the compound may change its affinity for its cellular target. Two of these possibilities were evident from our results. No membrane associated proteins had been found and both actin and tubulin are present in very large amounts in cells. Realising the chance of important target information being lost, the functionally of **6** was tested for interaction with any of these three proteins.

3. Which of these components does 6 interact with functionally? - Actin and tubulin are important proteins in cells and contribute to the cytoskeleton - the protein filaments which give a cell its structure. To provide these filaments, monomeric actin or tubulin proteins assemble

into long fibres to give actin filaments or microtubules, respectively. Drugs that interact functionally with actin or tubulin generally change the position of the equilibrium between the protein monomers and filament/microtubule. Tubulin polymerization assays show that **6** has essentially no impact on microtubule formation or dissociation, even at concentrations many thousand fold in excess of those that lead to cell death. Also **6** is able to perturb actin polymerization, but again only at very high concentrations, albeit exhibiting a greater effect at lower concentrations than required for tubulin.

The third putative target, eIF4A, proved interesting. Collaboration Pelletier (McGill University) showed that **6** perturbs the function of eIF4A proteins at much lower concentration than was required for an effect with actin or tubulin.[13] Interestingly, **6** *stimulates* the activity of eIF4A in isolated systems. It is not until eIF4A activity is determined in its cellular context that inhibition becomes apparent – a salutary lesson on the risks of using isolated proteins to determine biological activity.

4. Can the link between the proposed target and the cellular response be validated? - Discovery of eIF4A as a potential target of **6** through the affinity chromatography analysis was very exciting. The DNA code is transcribed in the nucleus of a cell and creates a mobile RNA molecule that is used as a template for the production of a protein. The conversion of the nucleic acid sequence of RNA into the protein's amino acid sequence is *translation*. It is complex and requires a number of proteins to recognize an appropriate RNA molecule and then assemble into a functional machine, called a ribosome. The mammalian ribosome is an assemblage of over 80 proteins and large dedicated RNA molecules.[14] What is salient here is that eIF4A is one of the key proteins involved in ensuring that the ribosome is brought to the start of the coding section of an RNA molecule in eukaryotes (higher organisms), and thereby initiating translation. Any loss of eIF4A function will result in cessation of the majority of protein synthesis.

For **6**, this combination of a cellular activity dominated by protein synthesis inhibition and its ability to bind to and inhibit eIF4A make a compelling case that eIF4A is the primary target. However, while compelling, the evidence is somewhat circumstantial. Additional confidence that eIF4A is the primary target of **6** came by using yeast as a model organism. It was grown in increasing concentrations of **6** over many generations, eventually, to give colonies that could grow in concentrations of **6** lethal to normal yeast. Each of these yeast colonies could have acquired resistance to **6** through a number of mechanisms but, by using some elegant molecular biology, James copied only the gene that codes for eIF4A from the resistant yeast colonies. These eIF4A genes were sequenced and each was found to contain the same mutation. Furthermore, when this gene was placed into normal yeast, it became resistant to **6**. These yeast cells were not trained to cope with high concentrations of **6** so that the only route for their resistance is through the new copy of the eIF4A gene. This proves that eIF4A is the primary functional target of pateamine.

There is one further complication relating the interaction of **6** with eIF4A. Humans carry three variants of eIF4A, two of which (eIF4A1 and eIF4A2) fulfil essentially identical roles to each other in translation initiation. The third variant, imaginatively denoted eIF4A3, has an entirely different role. This protein is involved in checking RNA for errors in a process called nonsense-mediated decay. This removes faulty RNA molecules before they are used as a template for the production of proteins. Nonsense-mediated decay is an essential process in cells, and interfering with it leads to cell death. We have shown that **6** can bind to eIF4A3 and inhibit its activity. However, it is not clear just how significant this inhibition is to the biological activity.

In the time that it has been studied, **6** has moved from being a mildly interesting toxin with a range of valuable activities to yet another toxic protein synthesis inhibitor, to one that interacts with a number of cellular targets, including actin (albeit weakly), the eIF4As associated with initiation of protein synthesis and, in humans, the eIF4A3 protein central to mediating the error checking of RNA. Not surprisingly, structural modification of **6** will alter its affinity for these targets, and even allow it to interact with completely new partners in a cell.

Finally, it needs to be noted that **6** was the first drug-like molecule shown to interact with the eIF4A proteins opening up this protein as a new drug target. As a result of these studies and that presented subsequently by Liu,[15] marine natural product **6** has attracted substantial international interest and become the target of review articles in two quite disparate publications, ACS's *Chemical Biology* and *Chemical and Engineering News*.[16]

Upcoming developments in identifying biological activity

This discussion has highlighted a number of valuable tools, old and new, in identifying the target of a biologically active compound. These include simple microscopy, affinity chromatography, classical biochemical assays with an isolated target, proteomics, and developing and sequencing resistant strains. However, this is but a glimpse of the approaches now available to identify drug targets. Many groups use transcriptomics (the study of RNA levels in a cell using microarray technology), which should be representative of the proteins that a cell is intending to make in response to a compound. The emerging technique of chemical genetics allows for understanding the interaction of a biologically active compound with a cell on a gene-by-gene basis.[17] Using this technique with **6** has opened up new avenues for exploration in both the mode of action of the compound and in understanding some fundamental cell biology. In addition to using chemical genetics to understand the detail of the biological interactions of the substrate, it can be used to show what other compounds it shares a mode of action with. The growing database of compounds with well characterized gene-by-gene interaction profiles allows the results from a new compound to be clustered with those whose mode of action is known and provide insight into the function of the new compound.[18]

The yeast biology community have provided many tools that are useful in tracing biological activity. Yeast is a simple organism, having about a quarter of the number of genes that are found in humans. However, the majority of the cellular processes found in yeast are also found in humans, and *vice versa*, and much of the detail of what we know about how our cells function comes from study in yeast. This combination of relative simplicity and shared fundamental pathways makes yeast a surprisingly good model organism for biological activity research. Amongst the available tools are the yeast gene deletion libraries that provide the basis of chemical genetics [17] and the yeast green fluorescent protein (GFP) library.[19] GFP is a fluorescent protein produced by the bioluminescent jellyfish *Aequorea victoria*. The GFP library contains over 4,000 yeast strains. Each strain has the genetic code for the green fluorescent protein inserted into a different gene, so that when transcribed and translated the protein produced has a GFP marker at its carboxyl terminal. Using this powerful tool, the location of each of these proteins within the cell can be identified.[19]

Although apparently not exciting enough for the world of chemistry it has profound implications. To date, biological activity at the cellular level has been largely limited to answering questions like *Does it kill cells?* and *Does it inhibit this enzyme*. Use of the yeast GFP library allows far more subtle biological effects to be revealed than simple toxicity. Image recognition software and powerful automated microscopes allow almost any change that we choose to follow within a cell to be monitored. In combination with chemical genetics profiling, almost all biological activities can be revealed. For example, Fig. 1 shows four microscopy images with a) and b) the *before and after* treatment with dithiothreitol. These show both upregulation and relocalization of a protein that is drawn upon to assist with the protein unfolding the dithiothreitol causes. Panels c) and d) show the upregulation of metallothionein, a copper binding protein, in response to treatment with Cu^{2+} ions. For both treatments, the yeast cells seen in the micrographs are healthy, but the treatments cause marked biological activity revealed by the responses in key proteins. This ability to pick up subtle changes in abundance and location for almost any protein in a cell is a tremendous step forward in assay methods.

Those who make or isolate new molecules will frequently have experienced the disappointment of poor biological activity from their compounds. Usually, all that has been learned is that the compound interacts weakly with just one of the proteins in a cell or is not particularly toxic. This is no bad thing as many good drugs are not outstandingly toxic either! Phillip von Hohenheim, born in 1493 and later known as Paracelsus, had a mixed impact on scientific development with his ideas often clouded by the beliefs and practices of his day. He is reputed to be the originator of the name zinc and is recognized for his contributions to pharmacology and toxicology. He stated *All things are poison and nothing is without poison, only the dose permits something not to be poisonous*.[20] Were biological activity analysis limited to studying toxicity, we would be little further forward than the practitioners

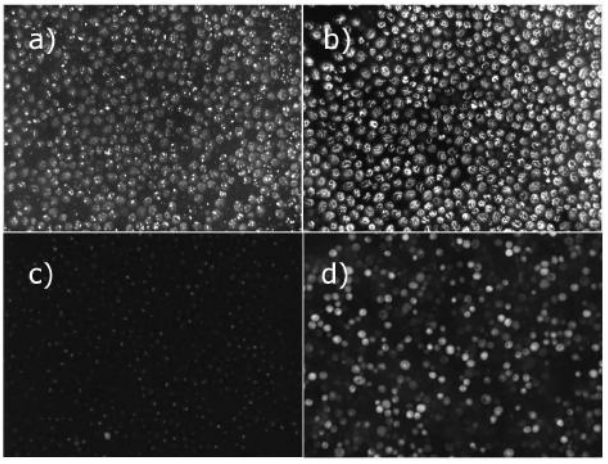


Fig. 1. Changes in the localization of selected proteins in yeast on treatment with dithiothreitol (a and b) and copper (c and d); images reproduced with permission, courtesy of Peter Bircham, School of Biological Sciences, VUW and Dr David Maass, ESR Ltd.

of the early 16th century. Below the dose that achieves toxicity lies a panoply of other, potentially beneficial, biological effects, *e.g.* as is seen with sub-lethal doses of caffeine. The yeast GFP library allows the visualization of any number of these effects.

Conclusion

Biological activity extends well beyond the inhibition of a single, isolated protein target or toxicity in cell culture. Identifying how a compound acts within a cell is possible using a range of classical and modern methodologies. Biological sciences are in an era of investigating interacting networks, and large scale high-throughput analyses – the -omics era. Genomics, transcriptomics, and proteomics can all be used in one way or another to look holistically at what a biologically active compound does, and to determine or infer a target or targets responsible for the activity. There are more emerging technologies, such as the use of following protein localization in a cell, that will reveal subtle cellular responses to compounds at sub-lethal levels. As Paracelsus reminds us, everything is a toxin, it is the subtle responses that are likely to make a compound functionally useful.

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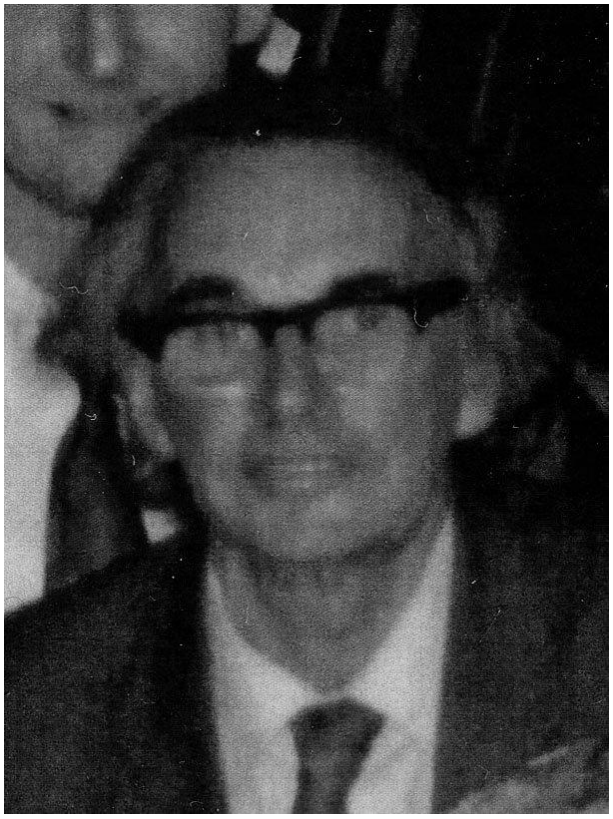
Walter Sidney Metcalf (1928-2008)



Walter Metcalf, a long-time member of the Chemistry Department at Canterbury University, passed away on Thursday July 24. He arrived at Canterbury University College (now the Arts Centre), from what is now Victoria University of Wellington, in 1954 as a senior lecturer in charge of physical chemistry, replacing Hugh Parton who had just moved to Otago. He retired as a Reader 21 years later.

Though not driven to publish prolifically in his own right, Walter initiated into original research a number of people whose careers provide ample testimony to the blindingly obvious truth that universities are about more than just putting your name into print. Amongst these are *Robin* ***Clark*** (FRS), *Leon* ***Phillips***, *Sally* ***Page***, *Murray* ***McEwan***, *Ward* ***Robinson***, *David* ***Natusch*** (Rhodes Scholar) and the late *Terry* ***Quickenden*** (a unique and eccentric academic stimulator in the Metcalf tradition). His early career at Victoria involved *Hugh* ***Melhuish***, who subsequently joined DSIR and became NZ's foremost physical photochemist, and *Laird* ***Ward***. Laird made his career in US industry but remained loyally fond of Walter, for whom he had synthesized many large round-bottomed flasks of brilliantly fluorescing organic liquids.

Walter was an unorthodox and often inspiring lecturer who, on one occasion, cleared everybody out of the chemistry lecture room with an *aluminium-powder-plus-finely-ground-iodine-plus-a-few-drops-of-water* demonstration that produced rather more iodine vapour than anticipated, and on another occasion (a beautiful spring morning) gave a lucid and highly memorable lecture on Förster non-radiative energy transfer in solution while leading the class of six or seven honours students on a hike around Lake Victoria and the botanic gardens. His main research field was photochemistry, for which work he won the RSNZ T.K. Sidey Medal. He did his DPhil at Oxford University with E.J. Bowen, author of *Chemical Aspects of Light* which was one of the first books on photochemistry, and subject of an oft-repeated rhyme: Says E.J. Bowen, '*I always empty my pipette by blowin*'. In later years Walter branched out into calcium metabolism.

Walter also had a bachelor's degree in music (his instrument was the viola), which he obtained simultaneously with his first science degree. He had wanted only to sit in on a few courses but his scholarship would not cover the fees for a course that was not aimed at gaining a degree, so he went ahead and completed the degree. Subsequently, he played in a quartet with friends and became chief rescuer of old violins and cellos for use by pupils of the Christchurch School of Instrumental Music.

Sally Page has commented that she only began to appreciate the quality of the advice given to her by Walter when she had to advise her own graduate students at UC London. Ward Robinson comments that: *I was privileged to participate in a modest MSc project with Walter and this had a lot to do with my reaching for the research career which is still thrilling me 48 years later. I am sure we all would want to record our deep appreciation of all the different ways in which his long life impacted upon us*, and Leon Phillips adds: *Walter was a Quaker, an exceptionally kind person and an enthusiastic individualist on almost any topic; I miss him*.

Leon Phillips & Ward Robinson

Article

Letting Your Hair Down with Party Pills

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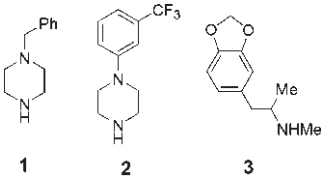
Over the past few years several piperazine analogue drugs have caught the attention of the public, authorities and media in NZ, with many questions being raised about both their safety and legal status. Two of the more common drugs in this class are 1-benzylpiperazine (BZP, **1**) and *m*-trifluoromethylphenylpiperazine (TFMPP, **2**). With comparisons being made to amphetamine and ecstasy (methylenedioxymethamphetamine – MDMA, **3**) they have been classed as stimulants, reported and advertised as giving feelings of euphoria, alertness, and a desire to socialise.[1] Side effects include a *hangover* (similar to that of alcohol), dry mouth and urine retention. The legal status of these drugs varies throughout the world and until recently little scientific research had been conducted on their use as recreational drugs. Until April 2008 the purchase and use of *Party Pills* containing BZP and TFMPP was legal in NZ, providing a unique opportunity for their study.



1 **2** **3**

The detection of *Party Pill* and other recreational drugs is important in workplace and roadside drug testing, forensic casework, and court disputes, *e.g.* child custody cases. From a forensic toxicology perspective it is essential to have a method that is simple, robust, accurate and reproducible. Traditionally gas chromatography mass spectrometry (GC-MS) has been the method of choice for the types of analyses mentioned above and it has an extensive history of use in the forensic field. GC-MS, however, requires time-consuming sample preparation or is otherwise limited to volatile compounds.[2] Liquid chromatography tandem mass spectrometry (LC-MS/MS) allows the detection and quantification of many different drugs in biological matrices and often requires less sample preparation. Indeed, according to the review by Maurer,[3] LC-MS/MS has the potential to become the *golden standard* for forensic and clinical analysis.

The ESR Forensic Toxicology group at Kenepuru have developed several LC-MS/MS methods for the analysis of prescription drugs and drugs of abuse. Included in this suite of analyses are two methods for the detection of prescription sedatives, amphetamine-type stimulants, and opiates in hair. The first of these methods simultaneously detects and quantifies drugs such as the amphetamines: **3** and methamphetamine (MA, *N*-methyl-1-phenyl-2-propanamine) and the opiates morphine, heroin (6-acetylmorphine, 6-MAM), and codeine. The second method detects and quantifies sedatives such as the benzodiazepine type drugs: 7-aminoclonazepam, temazepam, oxazepam, alprazolam, diazepam, and the non-benzodiazepine sedative zopiclone.

The adaptation of the amphetamines/opiates method to include the detection and quanititation of BZP (**1**) in hair using LC-MS/MS was undertaken by Natasha Lucas at ESR as part of her Waikato MSc studies. The assay was validated according to current best practice for quantitative methods following guidelines promulgated by the Society of Hair Testing, an international group of hair analysis experts.

Hair testing is becoming common, especially in workplace drug testing, drug facilitated sexual assaults, and child custody disputes. In some European countries hair tests are used to determine abstinence from drug use before driving licences are reissued following bans for driving under the influence of drugs. Although analysis of hair cannot determine the exact time of exposure, or the level of impairment, it does offer long-term exposure information not offered by other biological specimens, such as blood and urine.

There are many different forms of samples that can be collected for use in drug analysis. These include blood (and its components, *e.g.* plasma, serum), urine, oral fluid (saliva), sebum, hair, nails, and skin. Specimens of hair and nails can be useful for determining drug-use patterns. Examples of uses of such specimens include workplace drug testing (hair, sweat, oral fluid), criminal investigations (hair, sweat, oral fluid), child custody disputes and divorce cases (hair), and roadside testing for drug-impaired drivers (oral fluid).[4] The use of hair, nails and oral fluid for drug testing is becoming more popular because it is non-invasive. Fig.1 shows the various biological specimens and the time period in which they are useful for the detection of drugs. Blood and oral fluid are useful for immediate detection, and can give an indication of levels of impairment in a user. Urine can allow detection (depending on the drug) for days. Hair and nails are proving valuable as they can give a history of a person's drug use, although they cannot give information on the level of impairment, or the precise time of ingestion.

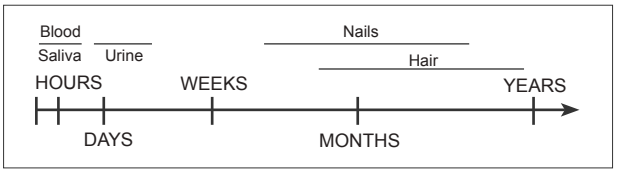


Fig. 1. Timeline of detection of drugs in biological specimens

Urine is the most widely used biological sample for toxicological analysis. Urine demonstrates use of a drug but gives little or no idea of when the drug was taken or the magnitude of any pharmacological effect, and therefore cannot be used to determine the level of impairment. Urine analysis can also take a long time, as there is often a need to identify metabolites owing to extensive transformation of the drug during metabolism. It is possible to detect drugs/metabolites in urine from hours until days after use.

Blood has many advantages as a biological matrix, as it can provide information about distribution, metabolism, and pharmacokinetics, and it can be used to measure drug levels almost immediately after administration.[5] Plasma is often the most common choice for blood analysis, although in autopsy cases plasma often cannot be obtained and so whole blood is used. With major advances in sample preparation, chromatography, and detectors, the use of whole blood as a matrix for quantification and identification has become widespread.[5]

Testing of hair for toxins began with heavy metals in the 1950's and was extended in the late 1970's with methods for the analysis of drugs being published. It is proving valuable in toxicological analysis, as it can give an idea of a person's drug history (months to years; see Fig. 1) and is also non-invasive. It is particularly useful in cases such as suspected drug-facilitated sexual assaults where a urine or blood sample was not taken soon enough.[6]

The methods of incorporation of drugs in hair are still rather unclear. The simplest explanation is by passive transfer from the blood into the hair follicle during formation. However, the multi-compartmental model[7] seems to be the most widely accepted.[7,8] This comprises:

- *Passive Transfer:* This occurs via passive diffusion from the blood stream into growing hair cells at the base of the follicle. The drug is retained in the interior of the hair (medulla) during keratogeneisis (formation of keratin, hardening of hair). Hair grows approximately one cm every 28 days[8] so that drugs deposited into the hair will be found approximately one cm from the scalp one month after the drug was taken.
- *Transfer from Sweat and Sebum:* Drugs are transferred into the hair after formation through sweat and sebum. Drugs have been found in sweat in higher concentrations than are found in the blood, so this offers an explanation to the higher concentrations sometimes found in the hair.[7]
- *Transfer from External Environment:* Drugs are passed into the hair from the environment. This could be through air, water or hair treatments (dying, perming, *etc*.). Drugs such as amphetamine, cannabis, heroin and cocaine are often smoked and hence transferred into the hair.
- *Intradermal Transfer:* Very lipid-soluble drugs such as tetrahydrocannibinol (THC, cannabis) are deposited into skin layers and transferred to hair.
- *Transfer from Melanin:* Drugs could possibly bind to melanin-related sites in the skin, which could result in drug uptake in the hair.

For analysis using hair, decontamination of the surface of the hair is a vital step. It is important to clear the hair of drugs deposited via air or hair treatments as mentioned in the multi-compartmental model above. The reviews by Boumba *et al*.[8] and Pragst, and Musshoff and their groups[9] give comprehensive overviews of the methods of drug incorporation into hair mentioned above, as well as the structure of hair and methods of drug detection.

Determining a person's drug history is important in drug-related deaths, as it can help establish whether or not they were a chronic or naive user, and this can often shed some light on why they might have died. Also, detection in hair can help determine whether someone may have been using particular drugs at the time of an earlier (criminal) incident. This can help support or refute other information. Other applications of hair analysis involve child custody disputes and drink spiking incidents.[6]

In the last year ESR has analyzed 80 samples for amphetamines, 25 for opiates and 17 for BZP. The majority of these analyses were undertaken to allow parents to prove abstinence from drug use and gain access to their children. A summary of results is presented in Table 1. The range of BZP concentrations detected in the hair was similar to that found for the amphetamines, from around 0.4 to 37 ng/mg of hair analyzed. There has been no work done as yet to directly correlate the amount of BZP found in the hair to the amount of drug taken. In all cases when a hair sample is taken from a living person, a drug use questionnaire is completed. From this information we anticipated a positive test for BZP in only 2 out of the 7 positive tests, whilst no one volunteered the use of illegal drugs.

Table 1. Drug analyses performed by ESR over 12 months

Drug	No of Tests	Positives	% Positive
Party Pills - BZP	17	7	41 %
Methamphetamine	80	32	40 %
MDMA	80	6	7.5 %
Codeine	25	7	28 %
Morphinea	25	3	12 %
Heroin (6-MAM)[a]	25	3	12 %

[a]The heroin marker 6-MAM was present in all cases where morphine was detected.

Whilst the number of hair cases/samples analyzed for BZP is small by comparison with amphetamines, the percentage of positive tests is similar to that of methamphetamine. It would be anticipated that prior to its reclassification, BZP would have been used amongst the general population more widely than methamphetamine. Following reclassification, we anticipate more requests to analyze hair samples for BZP.

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Article

Synthesis and Applications of Nanoparticles and Quantum Dots

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Introduction

Materials with extremely small size have been intensively pursued by researchers in the last two decades.[1] Owing to their small size, these materials can have unique optical, magnetic, and chemical properties that differ remarkably from their bulk counterpart. They have been a strong driving force behind nanoscience and nanotechnology.

A nanoparticle is typically defined as a particle between 1 and 100 nm in size.[2] However, size-dependent properties are most pronounced for very small particles, smaller than about 10-20 nm. In most case, these nanoscale properties are lost when particles reach a size of 40–50 nm,[3] for example, with quantum confinement of electrons and the critical size for a single magnetic domain. More recently, with the development in the strategies of anisotropic growth, a nanomaterial was interpreted as one with at least one dimension ranging from 1 to 100 nm and these include nanoparticles, nanorods, nanowires, nanobelts, and nanodisks.

Much nanomaterial research has been driven by a need to reduce the size of electronic components and devices. However, with the advent of nanotechnology and the interdisciplinary nature of scientists who call themselves nanotechnologist, new applications are being investigated between the traditional scientific fields of chemistry, biology and physics; medical imaging applications of quantum dots provide a typical example of this. Moreover, with the development of characterization techniques capable of atomic resolution, it has become possible to determine the structure of nanomaterials and gain a greater understanding of how nanomaterials grow. High resolution transmission electron microscopy (HRTEM) now provides a routine means to image nanoparticles and characterize their crystallinity, size and shape, and due to the atomic level of the resolution, obtain the (atomic) crystal structure as well.

Solution Phase Synthesis

The synthesis of nanoparticles is largely dominated by two preparatory methods. There is a physical approach based upon vapour deposition and a chemical approach of solution phase synthesis. Solution or liquid phase syntheses requires the reaction of appropriate starting materials, *e.g.* the reduction of metal ions or the decomposition of a single organometallic precursor, in the presence of a surfactant or polymer that prevents the particles growing and aggregating into larger sizes. It is the nucleation and growth of the nanoparticles in the presence of the surfactant (and the subtle manipulation of other reaction conditions including reaction time, temperature and concentrations) that allows for control of nanoparticle size and shape, and thus properties.

Silicon Quantum Dots

Research in the field of semiconductor nanocrystals synthesized in the liquid phase has been intense since the formation of CdSe quantum dots in 1993 by Murray *et al*.[4] Like CdSe quantum dots, silicon particles have an intense visible luminescence owing to quantum confinement, as first reported in porous silicon by Canham.[5] The most promising applications of these species include biological imaging agents,[6] and opto-electronic and photovoltaic devices.[7] The main difference between solution-made quantum dots and those from vapour phase deposition is that the former are capped or coated by surface molecules. Thus, applications best suited to solution synthesized quantum dots are those where the presence of a surface coating is advantageous as opposed to a hindrance, as is the case with biological applications.[6]

Quantum dots are becoming popular replacements for dyes in biological fluorescence imaging due to their superior stability against photobleaching. To date, considerable emphasis has been placed on using CdSe quantum dots with a ZnS shell as biological chromophores since they emit light that can be tuned throughout the visible spectrum.[4] However, concerns have been raised about the toxicity of quantum dots in living systems. This toxicity can arise from two sources a) the quantum dot core, and b) and the capping molecule.[8,9] A seminal study by Derfus *et al*. showed that quantum dots with a CdSe core and *without* a ZnS shell were toxic to liver cells after exposure to UV light.[8] However, the likely biocompatibility of silicon makes photoluminescent quantum dots with a silicon core ideal candidates for biological fluorescence imaging; they should eliminate the toxicology problems of those with a CdSe core.[8] For quantum dots to be used as fluorescence imaging agents in biological systems, surface capping is critically important in producing particles that are hydrophilic and biologically compatible.

A key initial aim of our VUW research was to form silicon nanoparticles with a smaller particle size distribution than previously reported. To do this we elected to use powerful hydride reducing agents. The synthesis of highly monodispersed particles is significant because the electronic, and thus optical, properties are highly dependent on the particle size.[10] Silicon is an indirect band gap material that shows remarkable changes in its optical properties when the particle size is reduced below 10 nm. Silicon in its bulk form is an extremely important semiconductor material for electronic applications. Critical to the future technological development of silicon are investigations of its nanoscale properties. In the bulk, silicon is an indirect band gap material with a phonon-mediated transition that leads to near-IR photoluminescence with poor efficiency.[10] Quantum confinement in silicon nanocrystals increases the probability of radiative recombination via direct band gap transitions and

this can lead to enhanced photoluminescence efficiency in the visible spectrum.[10] In silicon this requires the physical dimensions of the nanocrystals to be on the order of, or less than the bulk exciton Bohr radius of 4 nm.[10] It is also essential that full characterization and a detailed understanding of the optical properties of these silicon nanocrystals be obtained before any exploitation of their optical properties is undertaken. Such characterization can only be performed on monodispersed samples, and in terms of applications, semiconductor nanoparticles for use as LEDs and biological chromophores must be of uniform size to give sharp emission.

Syntheses of Silicon Quantum Dots

The liquid phase syntheses of nanocrystals use surfactant molecules to interact with particles as they grow. This interaction inhibits growth and limits the final particle size. The formation of silicon nanoparticles must be performed under rigorously anhydrous conditions otherwise the silicon is oxidized to silica.Although a wide range of surfactant molecules is available, silicon nanoparticle formation requires surfactants that dissolve in anhydrous organic solvents. Typical here are combinations that include TOAB (tetraoctyl ammonium bromide) in toluene and the ethoxylated alcohol, pentaethylene glycol mono-dodecyl ether (C12E5) in hexane. The syntheses were carried out in an argon atmosphere in a dry box with oxygen levels below 10 ppm at all times to prevent silicon oxidation.

The silicon source material is $SiCl_4$, which is readily reduced by hydride reducing agents. The use of hydride reducing agents produces silicon particles with surfaces terminated by silicon-hydrogen bonds.[11] The Si-H surface bond can then be reacted with organic molecules carrying a terminal π bond to produce both hydrophobic and hydrophilic surface types as illustrated in Fig. 1. The reaction is catalyzed by platinum and produce silicon particles whose surfaces are capped by strong covalent Si-C bonds. This approach offers options and advantages unavailable to chloride-terminated particle surfaces by reaction with Grignard reagents[12] or from formation of Si-O-R bonds as employed in earlier work.[13] The Grignard approach provides silicon particles whose surfaces are capped with alkyl chains without other functionality. However, if particles with surfaces suitable for use in biological applications are required the limited availability and the synthetic difficulty of producing suitable Grignard reagents become important. The alternative method of terminating particle surfaces with Si-O-R surface bonds is problematic because the polar Si-O bonds have a major impact on the electronic charge distribution in the quantum dots produced; particle photoluminescence in the visible region is reduced.[13] Capping with Si-C bonds has provided silicon quantum dots with a variety of surfaces that now include hydrophobic and hydrophilic particles.[14]

Biological Imaging

One application of silicon quantum dots is as imaging agents for cell biology. This application is illustrated by Fig. 2 with Vero cells. It shows the fluorescence images from Vero cells with the inclusion of silicon quantum dots transfected into the cytosol. The fluorescence illuminating

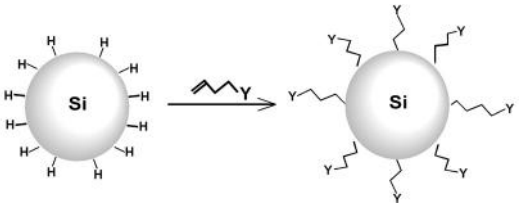

Fig. 1. Stylized schematic for the formation of silicon quantum dots with polar or non-polar terminal groups.

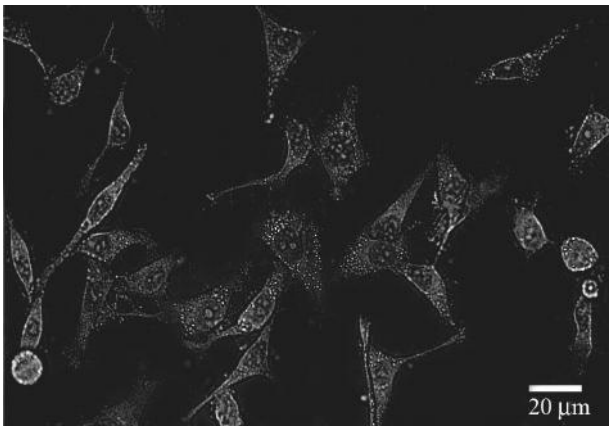

Fig. 2. Fluorescence microscope images of Vero cells with Si quantum dots transfected inside the cytosol; inset: Si quantum dots fluorescence under UV radiation.

the cells comes from direct band gap emission from the silicon quantum dots in Vero cells illustrates the use of hydrophilic silicon quantum dots as biological fluorescence imaging agents.[11]

The results show that silicon nanoparticles can be synthesized in controlled size in micelles and with surfaces that are defined from reactions with C=C compounds. The particles emit light in the blue region of the visible spectrum and are suitable as fluorescent markers in cell imaging. Further research in the field of nanoscale silicon is needed in both the design of synthetic protocols and to gain an improved understanding of the fundamental physics involved. Future chemical challenges include scaling up the syntheses to enable low cost production of monodispersed (+/- 0.2 nm) silicon quantum dots in the 1 to 4 nm regime so that they are commercially available for widespread use. For biological and opto-electronic applications control of the surface properties is essential. Methods that allow the attachment of suitable biomolecules, such as drugs and antibodies as well as conducting materials are needed. A far greater understanding of the physics of nanoscale silicon is needed and a study of monodispersed samples and computer modeling is also vital at both fundamental and applied levels.[15]

Particles of Anisotropic Shape

Cadmium Sulfide

Nanoparticles synthesized in solution are generally spherical due to surface tension making a sphere as the most thermodynamically stable shape. The shape-controlled synthesis of non-spherical nanoparticles in solution can be achieved under through control of both kinetic and ther-

modynamic conditions. Control through reaction kinetics typically results in *pod-like growth* and branched structures; generally, it can be understood as selected growth off different nanocrystal facets. In the thermodynamically controlled regime, the energies of the different terminating facets on the surface of the final particle produced are critical, and typically highly faceted cubic or triangular prismatic nanocrystals result. Recently, there has been considerable interest in the strategies for making shape-controlled nanoparticles as both chemical and physical properties are shape-dependent.

In all cases, the shape of the resultant nanocrystal has a key relationship to the inherent shape of the unit cell of the crystal structure. An example is the ability to synthesize highly crystalline flat triangular/hexagonal CdS nanocrystals and cubic CdS nanocrystals. The CdS nanocrystals are synthesized by injecting a solution of sulfur dissolved in oleyamine [octadec-9-enamine, $Me(CH_2)_7CH=CH(CH_2)_8NH_2$] into a hot solution of $CdCl_2$ also in oleyamine and then reacted for 1 h. The temperature of the $CdCl_2$/oleylamine solution during the sulfur addition, and the temperature at which the nanocrystals are ultimately grown controls the shape and size. Purification is by precipitation with ethanol, centrifugation, and re-dispersal in hexane.[16]

Fig. 3 shows the transmission electron microscopic (TEM) image of the 20 nm CdS nanocrystals formed with an injection and subsequent growth temperature of 160 °C. The nanocrystals have a relatively uniform size of 20 nm and consist of squares and triangles. A selected area electron diffraction (SAED) pattern was obtained and showed both the cubic (zinc blende) and the hexagonal (wurtzite) CdS structures to be present in the nanocrystals. All the CdS nanocrystals were highly crystalline and electron diffraction from single hexagonal/triangular crystals displayed diffraction spots that matched the hexagonal wurtzite crystal structure suggesting that all the nanocrystals of this shape are wurtzite. Electron diffraction patterns from single cubic CdS nanocrystal displayed diffraction spots that matched the cubic zinc blende structure and all the cubic CdS nanocrystals displayed the same electron diffraction pattern. This confirms that the cubic nanocrystals adopt a cubic crystal structure and that the triangular/hexagonal nanocrystals adopt a hexagonal crystal structure and that the ultimate shape of the nanocrystals is governed by the basic crystal structure adopted. This provides a simple illustration how the crystal structure can effect the final shape and morphology of a nanocrystal.

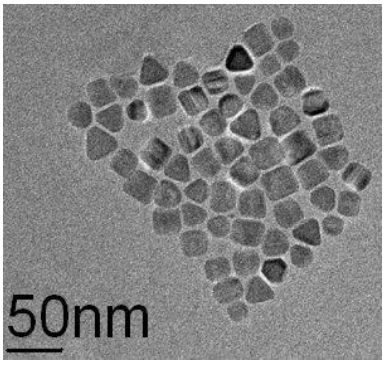

Fig. 3. Transmission electron microscopic image of 20 nm CdS nanocrystals

Platinum

The platinum metal adopts the face centered cubic (fcc) crystal structure. As a nanoparticle, it is a fundamental industrial catalyst for reducing pollutant gases from the exhausts of automobiles, producing hydrogen from methane, and in the direct methanol fuel cell. Spherical Pt nanoparticles have been extensively investigated for their unique catalytic properties which depend on the surface area of the particle. Faceted, non-spherical Pt nanoparticles were first described in the late 1980s and their catalytic performance has attracted considerable interest. Systematic studies of the catalytic performance of Pt nanoparticles with different morphologies demonstrate that activity and selectivity in catalytic processes are very dependent upon which facets terminate the surface nanoparticle.[17] For example, it has been demonstrated that Pt nanoparticles terminated by {1,1,1} facets exhibit higher catalytic activities than spherical or other faceted particles.[18] Thus, shape-dependent catalytic properties have been a key driver in research into the shape-controlled synthesis of Pt nanoparticles.

The general protocol of solution phase synthesis of Pt nanoparticles involves the reduction of a Pt-containing precursor in an organic solvent in the presence of a surfactant. While the preparation of Pt nanoparticles of various sizes has been successfully achieved, shape-controlled synthesis is still a challenge. In our experiments the syntheses of Pt nanoparticles were carried out in a pressure reaction vessel which is also known as a Fischer-Porter bottle. Typically, a platinum precursor [$Pt(acac)_2$] and a surfactant are dissolved in an organic solvent, transferred to a Fischer-Porter bottle, that is then evacuated prior and filled with hydrogen gas. The gas-filled bottle containing the reaction solution is then placed in an oven and held at constant temperature for the required reaction time, removed, cooled to room temperature, and the product purified by precipitation. The products were then re-dispersed in a non-polar solvent to give a stable colloidal suspension for characterization in a TEM.

The shape of a synthesized Pt nanoparticle is determined by a number of factors, and the impact of concentration, surfactant concentration, and reaction temperature and time have been assessed. These investigations also facilitated the optimization of the reaction conditions for the preparation of monodispersed particles. Of all the variables, precursor concentration was found to be most critical to the morphologies of the particles formed with toluene solutions of 0.05 M and 0.005 M [$Pt(acac)_2$] producing different shaped particles. Synthesis at a relatively low precursor concentration of 0.005 M produced polydispersed Pt nanocubes (Fig. 4) whose size distribution ranged from 10 nm to more than 30 nm and aspect ratio ranged from 1 (cubes) to 2 (rectangular prisms).[18] Due to the broad size distribution and non-uniform shape these nanocubes do not form long-range assemblies. The rates of nucleation and growth under these conditions are comparable and competitive, and lead to nuclei forming throughout the reaction mixture giving polydispersed nanoparticles. In comparison to the nanocubes obtained from use of high precursor concentrations, these low concentration nanocubes have sharpely faceted edges and a uniform contrast over the cube.

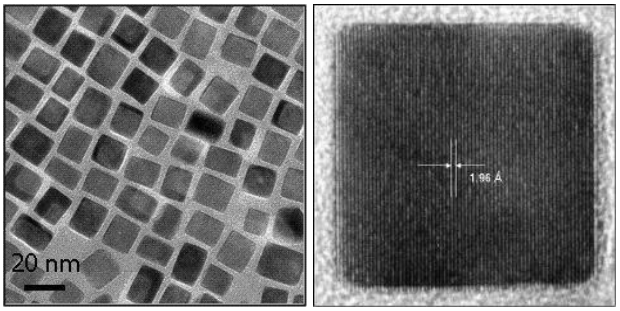

Fig. 4. TEM images of Pt nanocubes synthesized at low precursor concentration at low (left) and high (right) resolution.

Experiments with relatively high (0.05 M) precursor concentration produce highly monodispersed *Pt-filled octapod nanocubes*.[18] As can be seen in Fig. 5, the Pt filled octapod nanocubes have a very narrow size distribution and a uniform shape. These particles were so monodispersed that they arranged themselves into ordered assemblies. The monodispersity achieved during the formation of the filled octapod nanocubes compared to the faceted nanocubes can be understood by considering the growth kinetics. The growth at high concentrations occurs rapidly with the formation of a high concentration of nuclei throughout the reaction mixture. The growth of these nuclei is very fast rapidly producing monodispersed filled octapods uniformly throughout the reaction solution.

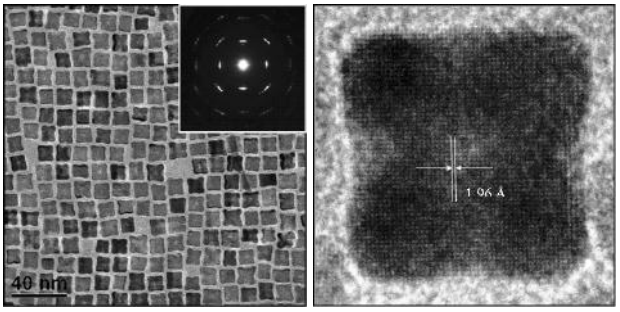

Fig. 5. TEM image of a of Pt-filled octapod nanocube monolayer assembly synthesized at high precursor concentration with (inset) selected area electron diffraction (left), and HRTEM image of a single filled octapod nanocube (right).

Closer examination of the HRTEM image of the single filled octapod nanocube (Fig. 5) shows that the Pt nanocubes are not perfect cubes synthesized when prepared from high concentrations of the precursor. The cubes no longer have sharply faceted edges and have a lighter contrast in the center of the cube and the middle areas of each edge. This light contrast means that these areas of the cube have fewer Pt atoms than the areas of dark contrast. Moreover, the distance between two neighbour parallel atomic lattice fringes is 1.96 Å and the fringes are at 90° to each other corresponding to the {2,0,0} lattice planes of Pt.

Mechanism of Growth of Platinum Nanocubes

The different morphologies observed with changing Pt precursor concentrations can be understood by considering the growth mechanism of the particles. At low concentrations most of the particles are polydispersed faceted cubes with their final shape originating from the thermodynamic stability of the of facets on the surface of the cube.[18] Conversely, at high concentration, kinetic control is dominant and it is the different growth rates on {1,1,1} and {1,0,0} facets that need to be considered to give filled octapod nanocube morphology.[18]

Formation of Faceted Nanocubes - For an fcc crystal the surface energy (γ) of different crystal faces is in the order: $\gamma(110) > \gamma(100) > \gamma(111)$.[18] At low precursor concentrations, low concentrations of Pt atoms are produced and this makes the particles grow relatively slowly from nuclei. The driving force for the cubic shape under these conditions is to form faceted surfaces that minimize the final surface energy of the particles. From calculations of small clusters in vacuum, particles of polyhedral shapes bound by both {1,0,0} and {1,1,1} faces are expected to have the lowest energy. However, under these solution conditions, it is possible that faceted cubes bound by solely {1,0,0} faces have lower energy compared to other polyhedral shapes due to a stronger surfactant-to-platinum interaction of the {1,0,0} faces compared to {1,1,1} faces (Fig 6).

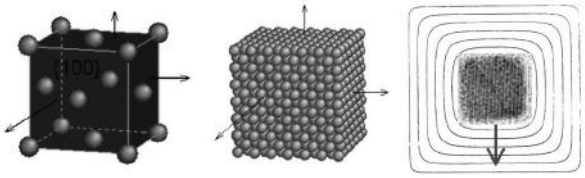

Fig. 6. Growth mechanism of {1,0,0} faceted nanocubes

Formation of Octapods - At high concentrations of the Pt precursor, the reduced platinum atoms are present in greater quantities, resulting in a faster growth of the nanoparticles. Under these conditions kinetic control dominates and particles with highly branched structures are produced.[18] A fundamental and basic shape for Pt nuclei is the cuboctahedron depicted in Fig. 7a-d. Cuboctahedra are defined when atoms at the edges of the unit cell are bound by eight {1,1,1} and six {1,0,0} planes; growth can occur on either facet with the rate of reduction on the surfaces controlling the final shape.[18] If the growth rate (G) for the {1,1,1} plane is significantly larger than that for the {1,0,0} then the nuclei will grow to become an octapod as illustrated in Fig. 7e,f.

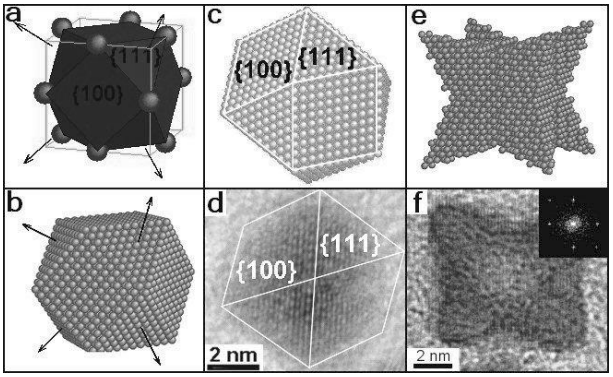

Fig. 7. Initial growth of filled octapods (a-d) and formation of octapods (e,f)

Outlook

In solution, the precise reason why growth on {1,1,1} facet of the Pt nanoparticles can be much faster than {1,0,0} growth is difficult to understand.[19] In solution a complex

mixture of surfactant, solvent, metal precursor and other ions are present, and all have been found to affect the growth of nanocrystals. However, at this point in time and, in general, with nanocrystals made in solution, the precise roles of the various species are uncertain and continue to provide a significant fundamental scientific challenge to researchers. On an applied level, currently, only *ca*. 10% of nanoparticles such as platinum are active in catalytic converters in automobiles. Clearly, increasing this level of activity through the formation of faceted particles remains an exciting challenge.

Acknowledgements

The author wishes to thank Prof Yamamoto, Akiyoshi Hoshino, Amane Shiohara and Jamie Warner for contributing to Si quantum dot research, Jamie Warner for work on CdS nanocrystals, and Jintian Ren for work on Pt nanocrystals. Funding from FRST and the MacDiarmid Institute of Advanced Materials and Nanotechnology is gratefully acknowledged.

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New Zealand Science Scene Continued

Wine Drinkers Benefit from Yeast's Clever Strategy

Why exactly does yeast turn grape juice into wine?

It seems a simple question and scientists at the University of Auckland have been looking at the environmental engineering carried out by the yeast.

Many organisms modify their environment but the evolutionary effect has not been measured before.

Saccharomyces cerevisiae, the yeast that converts sugar to alcohol, is present in low levels on grapes from the field. The yeast creates a warm, high alcohol, low oxygen environment. This environment is toxic to all other yeasts and microbes, which means *S. cerevisiae* can rapidly multiply during the fermentation process.

The process is less efficient for the yeast than to metabolise the fruit sugar completely to water and carbon dioxide. But it is the sole survivor due to the environment it creates by releasing ethanol and heat.

Environmental engineering is a concept originally described by Darwin. 2009 will the 200th anniversary of Darwin's birth and the 150th anniversary of the publication of *On the Origin of Species*. Today scientists are still following up his pioneering work.

You can read the full paper by Matthew R. Goddard, published in the latest issue of *Ecology* at the following link. *www.esapubs.org/esapubs/journals/ecology.htm*

All the use of a Transmission Electron Microscope without Owning one

It is now possible to use some of the most advanced transmission-electron microscopes in the country without having to travel to AgResearch's Lincoln campus.

The KAREN network is providing the opportunity. By using video-conferencing and tele-instrumentation, scientists can operate the microscopes via the network.

The KAREN network is the high capacity, ultra high-speed telecommunications network, involving tertiary institutions and research organizations around New Zealand.

AgResearch's microscopes include an instrument that can be used for electron tomography, which has a wide application in biological, medical and material sciences.

Science and Technology Co-operation Agreement Reached

In July the New Zealand government signed a science and technology co-operation agreement with the European Union.

Such agreements have been signed with 32 other countries. It replaces the administrative arrangement New Zealand used to have with the European Community that concluded in 1991.

The agreement is supposed to help extend participation by New Zealand researchers into fields of common interest like health, the environment and information technologies. It is also to help the involvement of European researchers in New Zealand research activities.

The press release from the European Union stated; "New Zealand has a productive and high-performing research and development RS&T system by international standards. The New Zealand government is committed to strengthening the RS&T system so it can better support and accelerate economic and social development and enhance the quality of the environment."

The agreement should be in place by the end of the year once it has been fully ratified by the European Union.

Supercritical Fluid Processing of Organic Compounds

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Introduction

Supercritical fluids have sometimes been heralded as enabling revolutionary formulation and synthesis opportunities because of their unique flow and solvent properties. Apart from a well established supercritical fluid extraction industry, the number of applications that has been successfully commercialized to date is somewhat more modest than the degree of research interest would suggest, but there are areas where exciting progress towards commercialization has been made.

A fluid that is above its critical temperature does indeed have distinctive properties. By changing the pressure, the density of the fluid can be varied continuously from a gas-like density to a liquid-like density without a phase change occurring. However, the flow and transport properties of the fluid remain close to that of a gas; thus, it is possible to have both a high solvent capacity (high density) and high gas-like mass transfer and diffusivity rates.

These properties have been used to advantage in the supercritical fluid extraction industry for at least 30 years, for example in de-caffeination of coffee beans and extraction of hops. More recently, there has been interest in using these properties for material formulation and synthesis. The continuously variable solvent properties allow for fractionation of different species, or control of molecular weight in polymerization,[1] by selecting the conditions under which they precipitate from solution. The lack of a phase change, or surface tension, is ideal for uniform and controlled mixing or deposition of layers down to the nanoscale. Moreover, their high transport properties are good for impregnation, rapid supersaturation, and for systems in which reaction rate is limited by mass transfer. By mixing different fluids it is also possible to create solvent systems with a wide range of properties that can be *tuned*, *e.g.* polarity, hydrogen-bonding behaviour, conductivity, and miscibility or immiscibility with different solvents.[2]

There is a substantial number of emerging applications in inorganic chemistry, but this article is focused on organic applications, and, more particularly, on organic applications in natural bio-actives, food, and cosmetics, that are of interest to the supercritical fluid processing group at Industrial Research Ltd. (IRL). Applications in these areas tend to limit the useful range of supercritical fluids to those that are non-toxic and have low critical temperatures, *e.g.* carbon dioxide, light hydrocarbons, and ethers. Many of these fluids are also gases at atmospheric pressure, enabling easy removal of the fluid from products. Alcohols are often used as useful co-solvents, particularly with carbon dioxide, for modifying the polarity.[3]

Particle Formation Techniques

The physical form of bioactive consumer products, such as pharmaceuticals and nutritional foods, influences many properties including the bio-availability and stability of the product, as well as ease of storage, handling and consumption. Particle size reduction to sub-micron size is advantageous for improving surface area and bio-availability, particularly for poorly water-soluble compounds, and controlled sizes in the 1-5 μm range are ideal for inhalation dosing of drugs. It is important for many thermally labile compounds to be processed gently and at low temperature to prevent degradation or conformational changes. The low processing temperature of supercritical fluid systems and the low solvent residues that can be obtained give an advantage in some cases over alternative approaches such as milling, spray-drying, and solvent crystallization.[4]

Supercritical fluid processing methods can be categorized according to whether the supercritical fluid is used as a solvent or antisolvent, and whether the supercritical fluid forms a continuous or distributed phase, as shown in Fig. 1. As a continuous phase solvent, dissolved compounds are precipitated by rapidly decreasing the pressure through a nozzle - the rapid expansion of supercritical solution process (RESS). At the lower pressure the solute is no longer soluble, and the rapid supersaturation and expansion of the fluid enables fine distributed nucleation, and production of a solvent-free product, without high temperatures. This process was described as early as 1984,[5] and examples of compounds processed include Naproxen, Lovastatin, Cyclosporin A,[6] and salicylic acid.[7] Improved dissolution rates are observed, but widespread use of this approach is limited by the low solubility of most compounds in carbon dioxide. Using polar co-solvents or polar supercritical fluids offer viable alternatives, *e.g.* acetaminophen produced by the RESS process using near-critical dimethyl ether (DME) as the solvent is shown in Fig. 2.[8] Acetaminophen solubility of up to 15 g/kg in DME is several orders of magnitude higher than in CO_2.

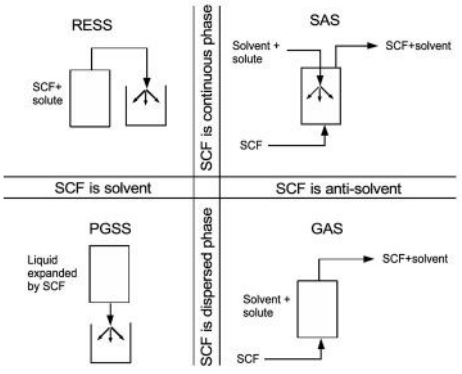

Fig. 1. Schematic of typical contacting arrangements for supercritical fluid (SCF) particle formation and coating

The PGSS process (particles from gas saturated solution - see Fig. 1) works by dissolving the supercritical fluid into the product to reduce the viscosity and, in some cases, the melting point. The *gas-saturated* liquid is then expanded, releasing the dissolved gas, causing atomization and

Joule-Thompson cooling that helps to solidify the product. This process has a much higher throughput than the more dilute RESS process, making it more economically viable, and giving it a wider range of useful applications, particularly with polymeric materials. However, the process is limited to liquid or liquefiable feed materials.

Because of the low solubility of many compounds in CO_2, it is an effective antisolvent for many systems, as in the SAS (supercritical anti-solvent), and GAS (gas anti-solvent) processes (see Fig. 1). In the SAS process, a solution is contacted with the supercritical fluid where the solvent is highly soluble in the supercritical fluid but the solute is not. The solvent expands into the supercritical fluid until the solute is no longer soluble and a solid precipitate forms. The key advantages are the low temperatures used, the rapid mass transfer and fine particle nucleation that occurs, the improved range of compounds that can be processed compared to the RESS process, and the higher solubilities that can be achieved by choosing an appropriate solvent. Many compounds, including insulin, hydrocortisone, lysozyme, albumin,[4] and plant and dairy products,[9-11] have been processed. Fig. 3 shows an example of particles of K_2CO_3 formed by this process, by precipitation (and reaction) of KOH with CO_2 from solution in EtOH. In the GAS process, the solution forms the continuous phase and the supercritical antisolvent is introduced, gradually decreasing the solvent capacity of the solution until particles precipitate. Further washing with the supercritical fluid can remove the majority of the solvent from the system. This has been used for many systems, including proteins, but it is a batch process with more limited control over the particle size.

The major disadvantage of the antisolvent process is the presence of residual solvent in the product. Suitable solvents for use with CO_2 include alcohols, but solvents that are less desirable because of their toxicity, such as CH_2Cl_2 and Me_2SO (DMSO), have often been used. Use of other supercritical fluids is possible, including C_2H_6 and NH_3,[4] and DME can be used to precipitate compounds directly from aqueous solution,[11] as shown in Fig. 4 for particles of bovine serum albumin produced by the SAS process.

Variations of these types of process can be employed to form more complex systems, including the use of emulsions and sonication to aid phase dispersion. Surfactants and stabilizers can be added to limit re-agglomeration or deterioration of particles that are formed. More extensive reviews of all of these particle formation processes and their applications have been published elsewhere.[2,4,12-15]

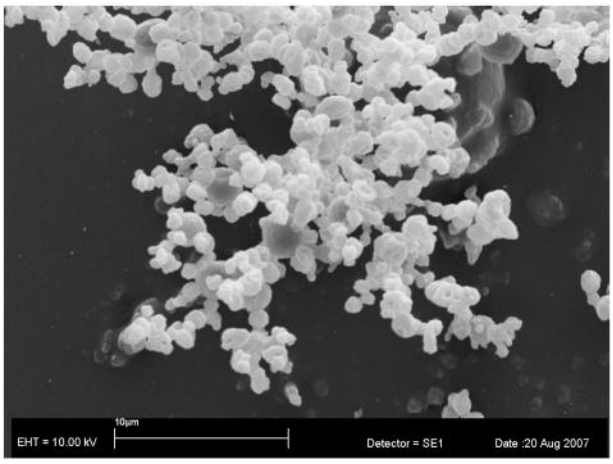

Fig. 2. Acetaminophen particles formed by the RESS process from solution in near-critical dimethyl ether

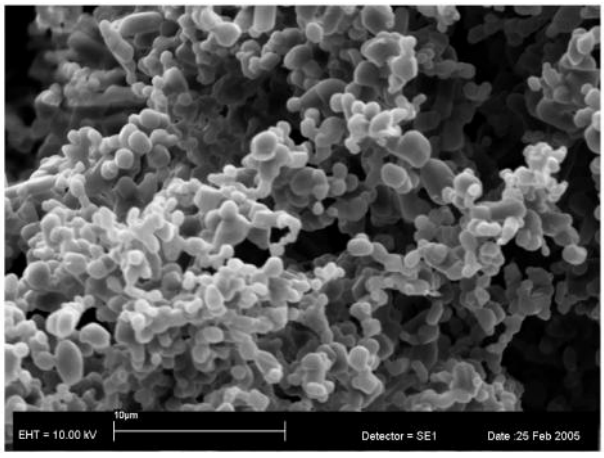

Fig. 3. K_2CO_3 formed by SAS precipitation of KOH from solution in ethanol into supercritical CO_2

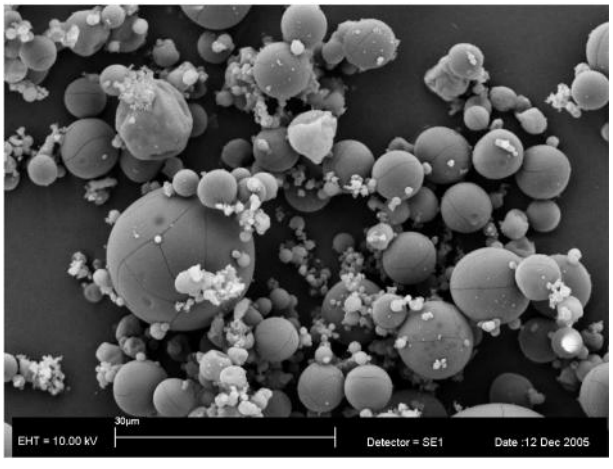

Fig. 4. Bovine serum albumin particles from aqueous solution using DME as antisolvent

Particle Coating/Composites

Considerable research has been carried out using supercritical fluids for particle coating applications. In organic systems this is largely driven by applications in drug delivery, where the aim is to develop a stable highly bioavailable product with a controlled release rate, or to incorporate the drug within compounds that enable targeted delivery.[12] Applications in food, cosmetics, and agricultural products also exist.[16] Expected benefits of supercritical processing technologies over conventional methods include improved particle size and coating control, low temperature processing, and the elimination or reduction in organic solvent use. Composite materials can be formed by surface coating, encapsulation, impregnation, or blending.

If the coating material is soluble in the supercritical fluid, then a RESS- or PGSS-type process can be used. Polymers that are suited to processing using CO_2 include siloxanes, fluoropolymers, and biopolymers such as polylactic acid (PLA) and polyglycolic acid. Polypropylene, polystyrene,

and polymethylmethacrylate have been processed using pentane and propane as the supercritical fluid.[13] Examples of studies include PLA/lovastatin and PLA/naproxen systems where both the drug and polymer are co-precipitated, although control over the distribution of the components can be difficult using this process. Drugs like nifedipine and felodipine that are liquefied by CO_2 have been coated with polyethyleneglycol by a PGSS process. Polymer coating can also be achieved by expanding polymers that are dissolved, or plasticized, by the supercritical fluid with a suspension of pre-formed drug particulates. Lipid coatings, generally soluble in CO_2, have also been used. Impregnation of polymers or other porous substrates is effective using a supercritical solvent to diffuse compounds into the matrix, *e.g.* ibuprofen into α-lactose, or β-cyclodextrin,[17] or angiogenic growth factors into poly(lactide-co-glycolide). The use of DME as the supercritical solvent has been described by Perrut *et al*.[15] for formation of drug/polymer composites.

A greater number of processing options are available using supercritical fluids as an antisolvent. Poorly soluble compounds and polar molecules can be processed with a wide range of coatings including polymers, cyclodextrins, lactose and chitosan, as well as inorganics such as magnetite[18] for use in magnetic field directed drug delivery. Compounds,including insulin and DNA have been successfully encapsulated and stabilized using these methods.[6] CO_2 antisolvent systems are generally used with DMSO or CH_2Cl_2 solvents, and residues of these solvents in the product detract from the other benefits of supercritical fluid processing. Limited work with other supercritical fluid antisolvents has been carried out.[10,16,19]

Developing New Supercritical Fluid Technologies

Research carried out at IRL, has shown that DME can be used as an effective antisolvent for proteins in aqueous solution, and for water soluble coating compounds. This solvent is non-toxic and there are currently applications in process for registration of it with regulatory bodies in the EU and NZ for use with food products. The high vapour pressure of DME results in negligible residues – levels that we have been unable to detect in typical organic substrates with a measurement resolution of better than 1 ppm. In composite particles produced by co-precipitation of an Amano Lipase enzyme with β-cyclodextrin (Fig. 5), the enzymatic activity was retained, and no DME residue was recorded in the product.

The flammability of DME may have contributed to the limited range of research and development carried out because of the dedicated research facilities required. However, at an industrial level, processing standards for flammable solvents are well established and there are no practical barriers to their commercial use. In aqueous-based processing, or using combinations of inert fluids such as CO_2 with the flammable solvent, the flammability is suppressed.

An important factor in the selection and design of supercritical fluid processing systems is developing an understanding of their solvent properties and phase behaviour. Research at IRL and elsewhere enables characterization of supercritical fluid solvent systems. Phase behaviour can be studied either in variable volume pressure cells to measure saturation and dew points, or by sampling phases held in equilibrium and determining their composition.[20] Solid solubilities can often be modelled with simple correlations.[21] Liquid and gas phase separations can be correlated reliably using equation of state models,[22,23] but they have limited applicability for highly polar compounds, longer chain molecules, or highly complexing systems. More involved interaction models, or the use of molecular dynamics modeling has been applied in these cases with some success. Our recently measures phase boundaries for the dimethyl ether/water system are shown in Fig. 6 at 333 K. Modelling using the Peng Robinson equation of state[23] with corrections described by Wong and Sandler,[24] fits both the liquid and vapour phase boundaries well. The presence of a small liquid-liquid-vapour region is also reliably predicted.

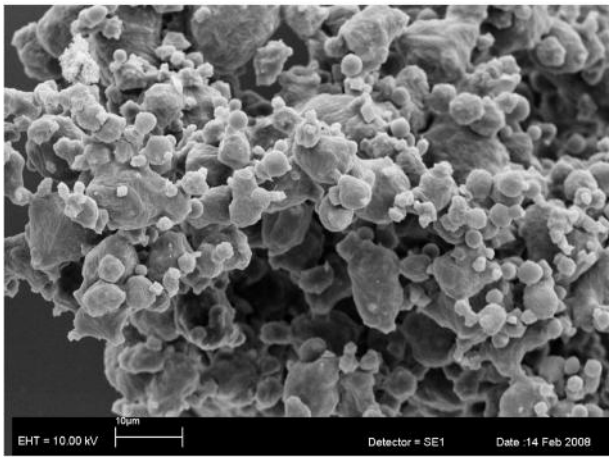

Fig. 5. Composite β-cyclodextrin and Amano Lipase precipitated from aqueous solution using DME as an antisolvent; enzyme activity maintained at >90% of preprocessing levels; DME residues not detected (<1 ppm)

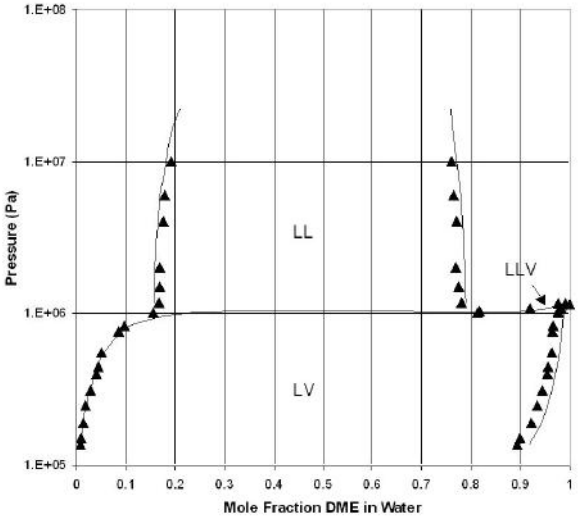

Fig. 6. Phase boundaries for DME and water at 323 K;. solid line: curve fit using the Peng-Robinson equation of state with mixing rules according to Wong and Sandler

Other solvent properties, including di-electric properties, can be measured[25] and Fig. 7 shows the variation in permittivity of CO_2 and DME mixtures with pressure and composition. A range of properties from non-polar to the polarity of pure DME can be obtained by varying pressure, temperature, or composition.

It is also important to be able to demonstrate that new processing technologies can be scaled reliably,[26] and tested for commercial viability. IRL has developed a range of scales of operation, including pilot scale equipment that can be used to generate commercial samples and engineering design and costing information. A portable pilot scale plant (Fig. 8) is also available for lease, enabling production of test products in regulated food or pharmaceutical facilities.

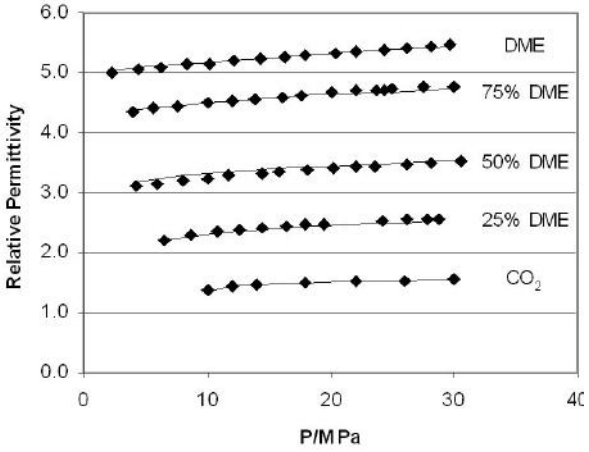

Fig. 7. Relative permittivity of carbon dioxide and dimethyl-ether (DME) mixtures at 313 K

Fig. 8. Portable pilot scale supercritical fluid processing plant

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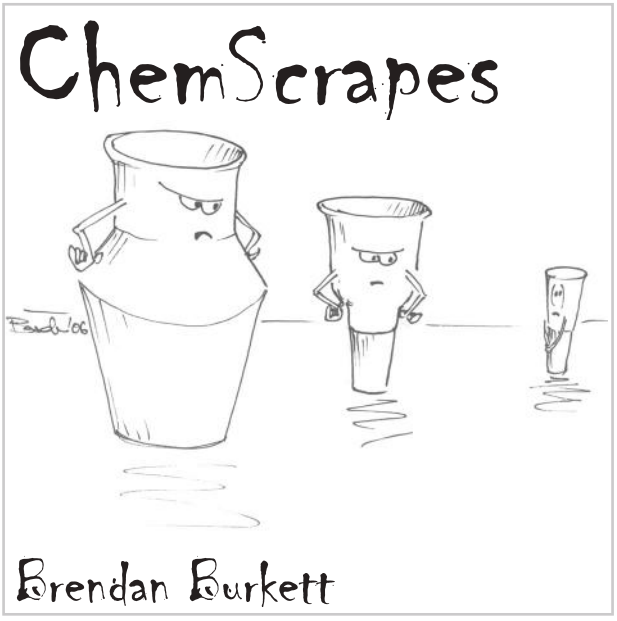

From Small Rings to Big Things: Benzocyclobutenes and High Performance Polymers

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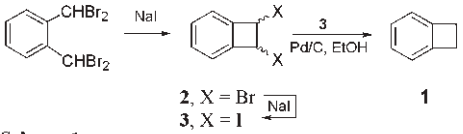
The first report of a four-membered ring being fused across adjacent positions of benzene appeared in the 1909 doctoral thesis of Hans Finkelstein (*Über ein Derivat des Benzocyklobutans*) at the University of Strasbourg.[1] The subsequent paper that encompasses his dibromide **2** was published in 1910 in *Berichte der Deutschen Chemischen Gesellschaft*,[2] but Finkelstein's main concern lay in the replacement of bromides and chlorides by iodide in organic halides employing NaI in acetone (in which NaI is soluble but NaCl and NaBr are insoluble), a classical transformation known as the Finkelstein reaction. Benzocyclobutenes (BCBs) attracted no further attention until 1956 when Cava and Napier confirmed the original synthesis[3] and transformed **2** into parent **1** *via* diiodide **3**, itself formed by Finkelstein reaction (Scheme 1).

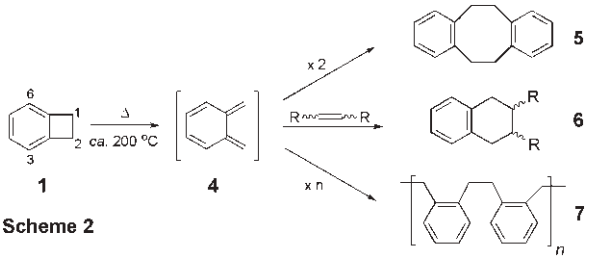


Scheme 1

Following this disclosure, BCBs became the subject of many reports (> 500 to 1986) that encompassed details of their synthesis, the chemistry of the strained ring system and the physicochemical properties recorded. Although some 40 patents were registered during this time (and a further 100 added during the next ten years), the fifth decade following Cava and Napier's epic communication[3] (1996-2005) had more than 400 patents registered. The total now represents close to 30 % of the benzocyclobutene literature available on Scifinder®.

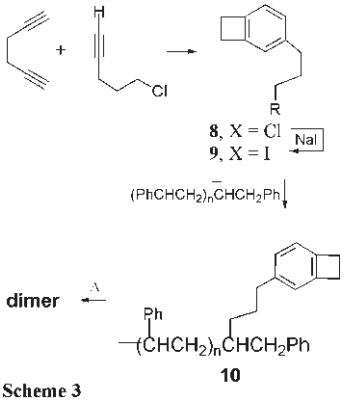
The volume of literature is clear in implying that **1** is an important entity and one must ask what is so significant about the humble C_8H_8 bicyclic hydrocarbon **1** to make it so attractive. The answer is simple and lies in its thermal chemistry. At *ca*. 200 °C, **1** undergoes electrocyclic ring-opening to the reactive quinodimethane **4**. This either dimerizes to dibenzocyclooctadiene **5** or yields poly-*o*-xylene **7** (Scheme 2), each with regeneration of the benzene ring. Although the dimerization is thermodynamically favoured, the presence of a separate and independent π bond – a dienophile - induces Diels-Alder cycloaddition at a temperature some 100 °C lower and a tetrahydronaphthalene, *e.g*. **6**, is formed under kinetic control. Thus, by comparison, the formation of **5** (or **7**) is inhibited[4] compared with the diversion of **4** to a cyclohexane and this makes BCBs of major significance in the polymer industry.

Appropriately functionalized BCBs have use in the termination of polymerizations and give a polymer that is *end-capped* with an intact BCB moiety.[4] This occurs with,



Scheme 2

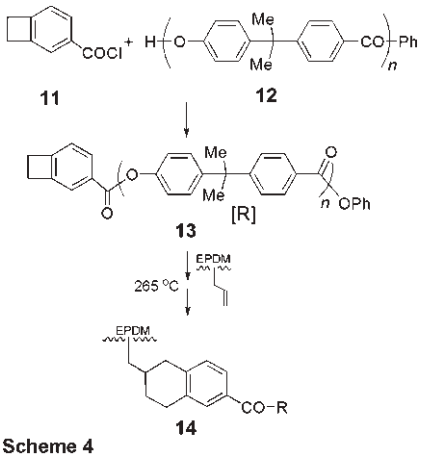
e.g. 4-(3-iodopropyl)- **9** and 4-benzocyclobutenoyl chloride **11**. Iodide **9** is easily prepared by metal catalysed cyclotrimerization to **8** that employs hexa-1,5-diyne and 5-chloropent-1-yne (Scheme 3), which then undergoes iodide-for-chloride replacement under Finkelstein conditions.[4] When **9** is used to terminate the anionic polymerization of styrene, an end-capped polystyrene **10** is formed as shown in Scheme 3.[5] Refluxing in triisopropylbenzene (236 °C) causes the BCB ring of this to open and dimerize to the dibenzocyclooctadiene analogue of **5**; the molar mass is doubled from a little fewer than 25,000 to close to 50,000 g/mol, a process is known as *chain extension*. Should the polymer be capped at both ends by BCB, then further polymerization can result in a new polymer of indefinite length. It is important to note is that these polymer extensions occur thermally without the evolution of by-products.



Scheme 3

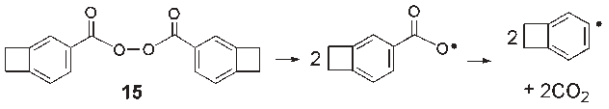
In a similar way, acid chloride **11** efficiently end-caps the phenolic benzoate polymer **12** to give polyacrylate chains **13**, almost all of which carry a BCB head group (Scheme 4).[6] At 265 °C the BCB ring of **13** opens and, in the presence of an alkene π bond, the quinodimethane cycloadds, *e.g*. with an EPDM (*e*thylene *p*ropylene *d*iene *m*onomer) rubber[7] to give, after compression moulding, a grafted polymer as shown stylized by **14** in Scheme 4. A variety of such polyacrylate thermoplastic elastomers have been prepared.[6]

Just as benzoyl peroxide (PhCO-O-O-COPh) initiates radical polymerization, so does benzocyclobutenyl per-



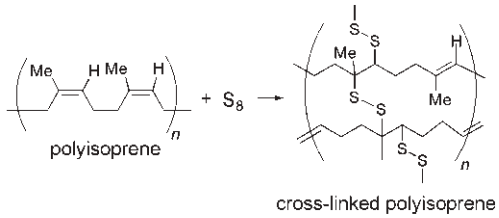
Scheme 4

oxide **15** (Scheme 5) and when used as radical initiator for styrene it can provide polymers with BCB moieties randomly attached to the chain and at the ends.[8] Heating above 240 °C effects BCB ring opening and results in both chain extension and *chain branching*.



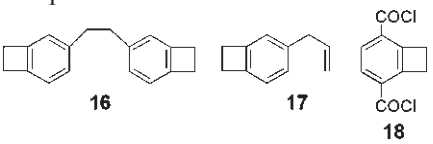
Scheme 5

The ability to cross-link polymers and enhance their strength has been known since the mid-1800s and the times of Charles Goodyear. Thus, *while boiling gum with sulfur on his wife's cooking stove, Goodyear let fall a lump of the material on the hot stove, and it immediately vulcanized. He deduced through reasoning and further experiments that the more sulfur added to the mixture, the stiffer the material. Goodyear finally discovered that by using pressurized steam for four to six hours at 270 °F, he could produce the most uniform results*.[9] Sulfur bonds to alkene sites in polyisoprene (natural rubber), brittle at low temperatures and sticky when heated, to give a cross-linked product with enhanced strength, rigidity and value (Scheme 6).[10] A patent for vulcanization of rubber with sulfur was granted not simply to Goodyear in the USA (1844) but in the previous year to Hancock in England.[9] The beneficial effects of organic accelerators in the cross linking process were not discovered until after the 1906 work of Oenslager.[11] In seeking ways to vulcanize cheaper (wild) rubbers as rapidly as those of high grade, he found that aniline and *p*-nitrosodimethylaniline accelerated the process. These became the first accelerants used in vulcanization but they were soon replaced by the less harmful thiocarbanilide [$(PhNH)_2CS$], which also gave the advantage of requiring less sulfur.

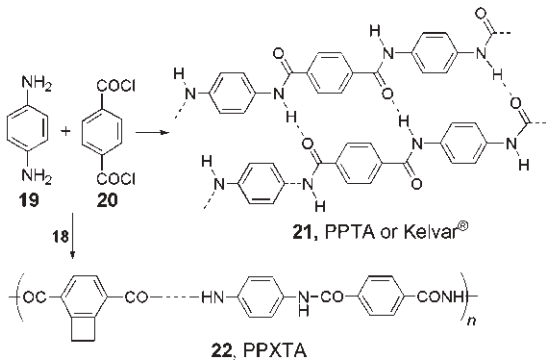


Scheme 6

Similar vulcanization of styrene-butadiene rubber (SBR) can be brought about by cross-linking with bis(benzocyclobutenyl)ethane **16**. Upon heating, the quinodimethane entities formed add to residual π bonds of the polymer chain giving a product, which, depending upon the amount of BCB used, can have enhanced properties compared with a sulfur cross-linked equivalent.[12] A polymer developed by the Goodyear Tyre and Rubber Company is produced from hex-1-ene and 5-methylhexa-1,4-diene[13] and known as *Hexsyn*. In its vulcanized form it found biomedical usage, *e.g*. in totally synthetic hearts, hip prostheses and artificial finger joints. A perceived disadvantage of the material was in the slight toxicity of the vulcanizing agents used in its production. However, the polymer can be cross-linked with BCB substrates of which 4-allylbenzocyclobutene **17** is most efficacious,[14] and when used as a co-monomer in *Hexsyn*, even in the presence of carbon black, it gives rise to cross-linked material from Diels-Alder cycloadditions (*cf*. Scheme 2). The mechanical properties of the sulfur and BCB vulcanized materials are comparable and the BCB-containing materials were accepted as non-toxic for *in vivo* use.[15]

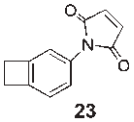


Fibres made from poly(*p*-phenyleneterephthalamide) **21** (PPTA or Kelvar®) developed by DuPont have high tensile but poor compressive strength because the polymer chains buckle. Thus, the polymer chains need to carry a reactive group that is dormant during the initial stages of fibre processing but which can be triggered into a reactive state later, *i.e*. cross-link chemistry that can be in initiated during a post-spinning heat treatment. Incorporation of the BCB diacid dichloride **18** (known as XTA-Cl) in controlled amounts with the polymer monomers, terephthaloyl dichloride **19** and *p*-phenylenediamine **20** (Scheme 7), provides material **22** (PPXTA) that has the BCB randomly incorporated. It differs from PPTA only by the presence of some four-membered rings, and thus retains the favourable mechanical properties of PPTA. Cross-linking of PPXTA fibres occurs *above* 350 °C presumably by quinodimethane dimerization. While this is well below the cracking temperature of the polymer, it is some 100 °C above the temperature usually needed to open the four-membered ring. Despite this, the temperature needed for processing is much lower (*ca*. 80 °C lower)[16] and it leads to fibres that take almost double the strain before they kink. The copolymer fibres exhibit increased resistance to creep and lateral deformation after heat treatment.[17]



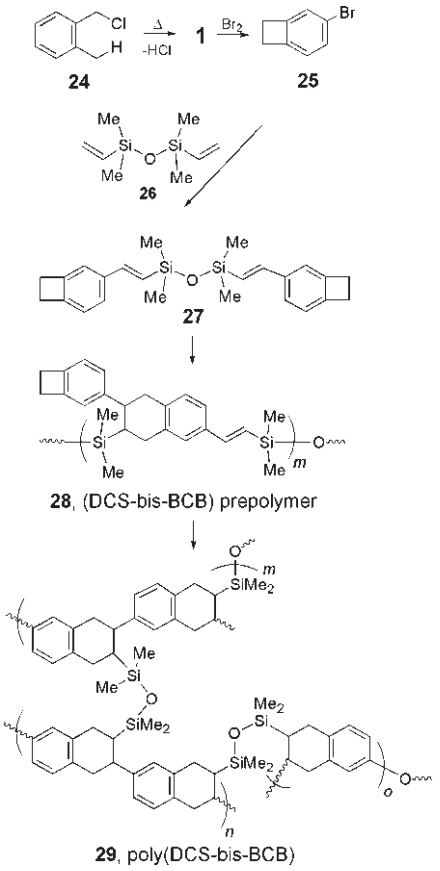
Scheme 7

The family of BCBs itself provides a series of thermally polymerizable monomers that can be classified into two groups.[18] The first encompasses molecules that contain only benzocyclobutene moieties while the second has monomers that contain sites of unsaturation elsewhere in the molecule. The monomers can be *partially polymerized* in a process termed B-staging, where the molar mass of the monomer (or monomers) is advanced by heating to give a *prepolymer* that can be processed into a desired form before completing the polymerization with final curing. Such polymerizations provide processing advantages for various composite fabrication techniques. Those monomers that contain multiple benzocyclobutene moieties, optionally with separate sites of unsaturation, transform into multifunctional network junctions when the thermosets are fully cured – the four-membered ring opens to the *o*-quinodimethane and it adds to a site of unsaturation on the same or a different chain, *cf*. **1** → **6** (Scheme 2). As an example, 3-*N*-maleimidobenzocyclobutene **23** thermally polymerizes to a substantially linear polymer with a high glass transition temperature (T_g). Thus, BCB polymers encompass materials that have properties ranging from high T_g thermosets to those of substantially linear thermoplastics. Some polymers exhibit an excellent retention of their room temperature mechanical properties to at least 200-250 °C, qualifying them as high performance polymers for high-temperature coatings with applications in the aerospace industry. Other polymers have outstanding electrical properties, including very low dielectric constants and water pickup that make them useful in electronic applications.[18] The performance enhancements seen from this technology come in part from the thin film polymeric dielectrics used in their fabrication. Polymer performance is based on the complex interrelationship between such properties as adhesion, stress, moisture absorption, and thermal and chemical stability and the inherent electrical and mechanical properties.



23

These last properties have triggered considerable attention to BCB-incorporated polymers. In the late 1980s, the Dow Chemical Company began to develop a series of BCB-based polymers with low dielectric constants for use in microelectronic packaging and interconnect applications.[19] The early studies provided commercially successful products in, *e.g*. the fabrication of gallium arsenide (GaAs) integrated circuits, bumping and redistribution,[20] and for planarization and isolation in flat panel display materials.[21] A commercial synthesis of **1** from pyrolysis of α-chloro-*o*-xylene **24**, subsequent bromination[22] to **25**, and then coupling with divinyltetramethylsiloxane **26** gave **27**, known as DVS-bis-BCB (Scheme 7).[23] This monomer, which can be purified to the ppb level if needed,[19] is of immense value and was the first BCB introduced for application in microelectronics.[20] On heating, **27** can be B-staged to prepolymer **28** without production of volatile side-products through Diels-Alder cycloaddition of the quinodimethane to a vinylic group of another molecule. Product **28** has excellent film-forming properties, shows less than 5 % shrinkage on cure and has a relatively low molar mass as applied, and gives > 90 % planarization from a single application. It can be processed and then cured at a later stage to give a product, *e.g*. **29**, with very desirable electrical, thermal, and planarization properties.[20] These properties, coupled with the low absorption of water and low dielectric constant make it superior to *e.g*. polyimides.



Scheme 8

The widespread use of polymers as insulating layers in microelectronic structures is relatively recent. As electronic devices become smaller, there is a continuing desire to increase the circuit density in electronic components, *e.g*. integrated circuits, multichip modules and the like, without degrading electrical performance, and to increase the speed of signal propagation in these components. One method of accomplishing these goals is to reduce the dielectric constant of the polymer used as the interlayer insulating material in the components. BCB-based polymers find use for this and in stabilizing the surface and preventing oxidation.

Dow has formulated solutions of B-staged BCB prepolymer **28** and commercialized them as a series of resins marketed as Cyclotene®™. The products are specifically engineered to meet industrial microelectronic needs for extendible, integratable dielectrics. Cyclotene resins exhibit < 5 % shrinkage on cure and have a relatively low molar mass as applied and result in > 90 % planarization from a single application.[20] Cyclotene resin is currently being used to planarize thin film transistor (TFT) flat panel display plates, which allows subsequent indium tin oxide (ITO) deposition on a completely planarized surface. This results in a larger viewing angle and low-

er power operation. The 3000 series of dry-etch resins finds use in applications where film thicknesses are controllable from 1.0 to 26.0 μm. Dow Cyclotene®™ 3022 is a low permittivity insulator developed in response to the need for faster integrated circuits with higher packing densities. The 4000 series are photosensitive resins (Photo-BCB)[24] ideal for wafer level applications where a thin dielectric layer is required, or where a protective layer is needed for passivation or chemical resistance. They contain photosensitive additives to render the formulation photoimageable, are active to broad band UV radiation, and are negative acting dielectrics (the unexposed material is removed during solvent development). Different formulations provide for differing film thicknesses (Cyclotene®™ 4024-40: 3.5-7.5 μm; 4026-46: 7-14 μm) and processing is similar to that for other negative photoresists; the present Cyclotene®™ range of polymers finds extensive use.[25]

Various approaches have been adopted to enhance the ductility of DVS-bis-BCB polymers,[26] one of the more recent being addition of a styrene-butadiene-styrene (SBS) triblock copolymer.[27] This consists of polystyrene sequences (or blocks) at each end of the chain and a polybutadiene sequence in the centre. The polystyrene end-blocks of adjacent chains collect together in small *domains*, so that clusters of polystyrene are distributed through a network of polybutadiene. Such a structure makes SBS a thermoplastic elastomer with the elasticity and resilience of polybutadiene along with the permanence of the fixed ends. When the DVS-bis-BCB monomer **27** was B-staged with SBS added, the prepolymer had advantageous properties. Moreover, a high proportion of SBS could be used, thereby reducing the cost of the final product. Fracture toughness improved without any notable loss of the poly(DVS-bis-BCB) properties.[27]

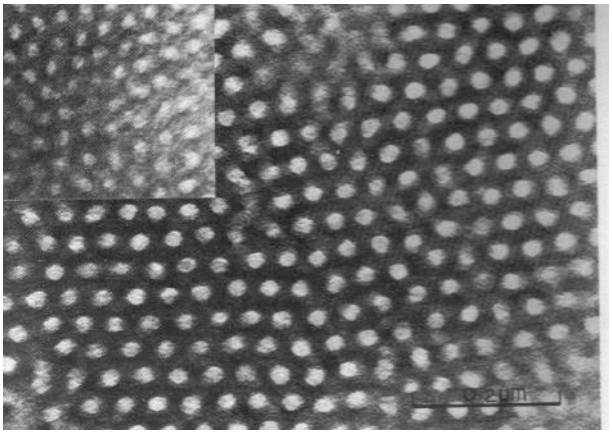


Fig. 1. An early transmission electron micrograph of an SBS triblock copolymer (from *http://en.wikipedia.org/wiki/Image:Sbs_block_copolymer.jpg*; accessed 12 May 2008).

Current research has focussed on the evaluation of BCB-bonded and thinned wafer stacks for three-dimensional integration.[28] A fully wafer-level packaged single-crystal silicon multiple ported microelectromechanical (MEM) switch using benzocyclobutene as a packaging adhesive layer has appeared.[29] Finally, a self-priming and photosensitive aqueous-base developable BCB dielectric material curable in air has been made from **27** and BCB-acrylic acid. Whether cured in nitrogen or in air, the formulation produces a film with optical, electrical, thermal, and mechanical properties desired for many microelectronic applications, such as packaging applications and a planarization layer or insulation layer in display applications.[30]

As pointed out in the earlier articles in this series,[31] the recognition, use, and transformation of small ring organics into materials of industrial importance has come from initially non-targeted, curiosity-driven research.

Acknowledgements

I am grateful to Leslie Hatfield and Dan Scheck of the Dow Chemical Company for assistance and helpful comments. Dr. Guy Whippler of the Université Louis Pasteur, Strasbourg kindly provided the information on Finkelstein's doctoral thesis.

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Dates of Note

Oct 10 is the 75th anniversary of issue of US Pat. 1,929,453 *Synthetic Rubber-like Composition and Method of Making Same* to *Waldo* ***Semon***. It was for his method of making plasticized PVC, now known simply as vinyl. Initially, hard and difficult to form into useful articles, Semon found a way to make it in a rubber-like form that involved dissolving the polymerized vinyl halide in a non-volatile organic solvent at elevated temperature. On cooling a stiff rubbery gel appears. It is also the 75th anniversary of the marketing of the first detergent containing a surfactant (*Dreft*) by Procter & Gamble. Additionally, *Earle* ***Dickson***, the inventor of the Band-Aid, was born on this day in 1892.

Oct 12 marks 300 years since the birth of *Vincenzo* ***Dandolo***, the Italian pharmacist, natural scientist, writer and statesman. He studied chemistry at the University of Padua, and championed new scientific theories, especially those of Lavoisier. He was committed to the advancement of secondary education in general and to health care in particular.

Oct 15 is the 400th birthday of *Evangelista* ***Torricelli*** whose barometer experiment with quicksilver filling a tube then inverted into a dish of mercury created a vacuum. Oct 20 is the 25th anniversary of the redefinition of the metre. Originally based on one ten-millionth of the distance from the North Pole to the equator, it was re-established as the distance that light travels in a vacuum in 1/299,792,458 sec.

Oct 21 is the 175th anniversary of the birth of *Alfred* ***Nobel***. On the same day in 1895 ***Linde*** reported the liquefaction of air. The 22nd of October represents the 165th anniversary of *Stephen* ***Babcoc***, the American agricultural chemist, regarded as the father of scientific dairying. In 1880 he developed his (Babcock) test of measuring the butterfat content of milk. It consists of liberating the fat globules by dissolving the casein in a strong acid and then separating the fat by means of a centrifuge. The test discouraged milk adulteration. It also provided for the first time an adequate standard by which fair payment for milk could be determined, stimulated improvement of dairy production, and aided in factory manufacture of cheese and butter. This day also marks 70 years since *Chester F.* ***Carlson*** demonstrated xerography for the first time.

Isidor ***Traube***, the German physical chemist who founded capillary chemistry and whose research on liquids advanced knowledge of critical temperature, died 65 years ago (27 Oct 1943). On the same day in 1873, farmer *Joseph F.* ***Glidden*** applied for a patent on barbed wire. The barbs were cut from sheet metal and were inserted between two wires which were twisted considerably more than with today's common design.

Oct 29 is the 85th birthday of *Carl* ***Djerassi***, noted for establishing physical methods for determining organic molecular structure and for contributions to synthetic organic chemistry. This day also marks the 50th anniversary of the first coronary angiogram, performed by Dr. *F. Mason* ***Sones***, *Jr.*, a pediatric cardiologist at the Cleveland Clinic in Ohio, USA.

On Oct 30, 1888, the first US patent for a ballpoint pen (No. 392,046) was issued to *John J.* ***Loud*** of Weymouth, MA, whist the 31st marks the 5th anniversary of the US Food and Drug administration releasing its summary findings that cloned farm animals and their offspring pose little scientific risk to the food supply. The 31st also marks 120 years since ***Dunlop*** patented pneumatic bicycle tyres.

Nov 4 marks 135 years since the first US patent for a gold tooth crown was issued to Dr *John B.* ***Beers*** of San Francisco while the 7th is the 120th anniversary of the birth of *Chandrasekhara Venkata* ***Raman***, recipient of the 1930 Nobel Prize for Physics for his 1928 discovery of what is now known as Raman scattering. On Nov 7, 100 years ago, Prof *Ernest* ***Rutherford*** announced in London that he had isolated a single atom of matter.

Ernst Otto ***Fischer*** who, with Sir *Geoffrey* ***Wilkinson***, solved the structure of ferrocene and received the 1973 Nobel Prize, was born on Nov. 10, 1918. The day also marks the 25th anniversary of then student *Fred* ***Cohen*** providing information of the first documented computer virus, created as an experiment in computer security.

Nov 13 marks 115 years since the birth of *Edward Adelbert* ***Doisy***, the American biochemist who discovered and synthesized vitamin K; he shared the 1943 Nobel Prize for Physiology or Medicine with Henrik Dam. The 14th is also the anniversary of the death of French chemist *Nicolas-Louis* ***Vauquelin*** (1829) who discovered chromium

and beryllium (1797/8). Nov 15 marks 125 years since *Thomas* ***Edison*** received a patent for his two-element vacuum tube, the forerunner of the vacuum tube rectifier.

Nov 16 is the 65th birthday of *James W*. ***Mitchell*** who, with his collaborators at Bell Labs, pioneered the development of XRF methods for part per billion (ppb) trace element analyses. The 20th marks the anniversary of *F. W*. ***Aston***'s death – he received the 1922 Nobel Prize in chemistry for development of the mass spectrometer. Additionally, the 20th is the 85th anniversary of the issue of a patent for the automatic traffic signal.

Nov 21 is the 225th anniversary of man's first flight. In 1783, *Jean Francois Pilatre* ***de Rozier***, a professor of physics and chemistry, and the Marquis *Francois Laurant* ***d'Arlandes*** lifted their hot-air balloon from a royal palace in the Bois de Boulogne (Paris) and flew almost 6 miles in 25 min. The 22nd marks 65 years since *Wolfgang* ***Ostwald***, a founder of colloid chemistry, died.

Nov 26th is 110 years since the birth of *Karl* ***Ziegler*** who shared the 1963 Nobel Prize with *Giulio* ***Natta*** for discoveries in the field of high polymers. 150 years ago on Nov 28 Baronet Sir *Robert* ***Hadfield*** was born; he was the British metallurgist who developed manganese steel in 1882. The Polaroid land camera went on sale on this same day 60 years ago.

John ***Mercer*** found that when cotton is treated with caustic chemicals, it became thicker and shorter and hence stronger and shrink-resistant. The cotton was then more easily dyed and could be given an attractive silk-like lustre. This *mercerization process* is still in use today. He died on Nov 30 in 1866.

Dec 3 is the 75th birthday of *Paul* ***Crutzen***, the 1995 Chemistry Nobel Laureate who showed that N_2O accelerates the destruction of stratospheric ozone. *Joseph Louis* ***Gay-Lussac*** was born 230 years ago on Dec 6; in 1805 he demonstrated the 2:1 volume ratio of H_2 to O_2 to form water.

Dec 9 is noted for the births of *Fritz* ***Haber*** 140 years ago (he received the 1918 Nobel Prize for his ammonia synthesis), *Claude Louis* ***Berthollet*** (1748) who was responsible for the law of mass action, and *Carl Wilhelm* ***Scheele*** (1742) who discovered oxygen in 1772.

Dec 10th is the 100th anniversary of receipt of the 1908 Nobel Prize in Chemistry by *Ernest* ***Rutherford***.

Dec 11 is the 75th anniversary of the death of *Georges* ***Friedel***, the French crystallographer who formulated basic laws concerning the external morphology and internal structure of crystals. On this day 10 years ago the entire genetic blueprint of the tiny nematode worm, *Caenorhabditis elegans*, was reported in *Science*.

Dec 15 marks the birth dates of Wairarapa-born *Maurice* ***Wilkins*** and Sir *Peter* ***Buck*** (originally Te Rangi Hiroa) the Maori anthropologist, physician, scholar, writer, and politician. He was born the son of a Maori chiefess and an Irish father in Urenui. This day is also the 50th anniversary of *Wolfgang* ***Pauli***'s (Pauli Exclusion Principle) death and the 38th of Sir Earnest Marsden (1970).

Dec 16 is the 100th anniversary of the birth of *Willard Frank* ***Libby*** who introduced ^{14}C dating (1960 Nobel Prize) to radiochemistry, while the 17th marks 230 years since Sir *Humphry* ***Davy***'s birth; he discovered the alkaline earth metals calcium, strontium, barium, and magnesium 200 years ago in 1808.

Dec 22 is the 170th anniversary of *Vladimir Vasilyevich* ***Markovnikov***'s birth; his electrophilic addition rule was enunciated in 1869. He was the first to synthesize four- (1879) and seven-membered (1889) rings. It is also 180 years since the birth of *William Hyde* ***Wollaston*** who discovered Pd and Pt and was first to provide the latter in malleable and ductile form.

70 years ago, on 31 Dec 1938, the first breath testing device for car drivers was officially introduced in Indianapolis and termed the *drunkometer*.

1 Jan 2009 marks 176 years since the existence of the *ethyl radical* was proposed in the *Dublin Journal of Medical and Chemical Sciences* by *Robert* ***Kane***. Initially a subject of ridicule, he was given appropriate credit after Justus Liebig gave credibility to the concept. The editor acknowledges that he missed the 175th last year!

Jan 4 is the 200th anniversary of the birth of *Louis* ***Braile*** who developed the tactile form of printing and writing now known as Braille; the 8th marks the 125th anniversary of the first US patent for the tanning of hides with a metallic salt (US Pat. 291784/5).

Jan 9 marks 140 years since the *Richard Wilhelm Heinrich* ***Abegg*** proposed a theory of valency and 40 years since the first test flight of the supersonic Concorde. Jan 10 marks 60 years of 7 inch, 45 rpm records – they were introduced by RCA in the US on this day in 1949 and play up to 8 min of sound per side.

Paul ***Vieille***, the French inventor of smokeless powder (*Poudre B*), that revolutionized small gun and rifle usage, died 65 years ago (14 Jan 1944). Jan 15 is the 250th anniversary of the opening of the British Museum in 1759, the world's oldest public national museum.

Knowing your right hand from your left

It is well known that enantiomers of chemical compounds may possess different physiological properties. A recent Court of Appeal decision from the United Kingdom, *H. Lundbeck A/S v. Generics*,[1] discusses the patentability of a purified enantiomer when the racemate is already known.

The Circumstances

In 1979 Lundbeck obtained a patent in the UK for the antidepressant drug citalopram. The patent specification disclosed a racemic mixture which had antidepressant effects. Following the expiry of this patent in 1999, several generic pharmaceutical manufacturers began producing competing versions of the citalopram racemate.

In 1987 Lundbeck had derived a novel method for preparing the separate (+) and (-) enantiomers of citalopram. Lundbeck discovered that the antidepressant effects of citalopram were mediated by the (+) enantiomer, and in 1989 patented a method to produce the (+) enantiomer (escitalopram). The novelty and inventiveness of Lundbeck's claim directed to the new method of producing the (+) enantiomer has never been challenged. However, in addition to the method claim, the 1989 patent also included claims directed to the (+) enantiomer itself and pharmaceutical compositions containing the (+) enantiomer, and it is these claims that have been contested.

The Issue

Several generic drug manufacturers applied to the UK High Court to have the 1989 patent revoked on the grounds that Lundbeck was simply repatenting citalopram (or at least the active ingredient of citalopram) in an attempt to extend its patent protection beyond the term of the original 1979 patent. The UK High Court agreed and held that the claims to the (+) enantiomer and pharmaceutical composition were invalid. The court found that by 1988 medicinal chemists with knowledge of racemic mixture of a compound would routinely attempt to investigate its enantiomers. The (+) enantiomer was therefore known and all Lundbeck had invented was a new method of producing it. Lundbeck had effectively, by claiming the (+) enantiomer by itself, obtained a patent which granted it a monopoly covering the (+) enantiomer – regardless of the method used to produce it. In other words, the Court found that Lundbeck had obtained more than it had invented and it limited the patent accordingly.

The High Court considered that in order to claim the (+) enantiomer, it would be necessary for Lundbeck to have disclosed information in the 1989 patent relating to every possible method of producing the (+) enantiomer. This rather narrow interpretation suggests that any claims to a novel compound may be invalid, if an alternative method of manufacturing the compound - which has not been disclosed in the patent specification - is discovered at a later date.

The Appeal Decision

Lundbeck appealed and the Court of Appeal overturned the High Court ruling. The Court of Appeal stated that the claims to the purified (+) enantiomer of citalopram were valid because the patent specification adequately taught the skilled person how to make the (+) enantiomer. In other words, the purified (+) enantiomer was itself a significant contribution to the art and because Lundbeck was the first to produce the (+) enantiomer, it was entitled to claim the associated rights.

What does this decision mean?

This decision provides an incentive for developing a method of manufacturing previously unavailable products. If a new method of synthesising a product, *i.e.* separation of previously inseparable enantiomers, is devised which provides an improved product, *i.e.* a single enantiomer which was previously unattainable, it may be possible to obtain broad patent rights to that product which extend beyond the methods disclosed in the original patent specification.

A reminder: if you have any queries regarding patents or patent ownership, or indeed any form of intellectual property, please direct them to:

Patent Proze

Baldwins Intellectual Property

PO Box 852, Wellington

Email: *email@baldwins.com*

1. *H. Lundbeck A/S v. Generics (UK) Ltd. & Ors*, [2008] EWCA Civ 311.

Blair Hesp and Jarrod Ward of Baldwins Intellectual Property specialise in chemistry and biotechnology patents. Blair joined Baldwins in 2006 and Jarrod in 2007. Blair has a PhD in pharmacology from the University of Otago as well as a NZDipBus with a management focus. Jarrod obtained his PhD in chemistry from the University of Auckland in early 2008. Blair and Jarrod are both currently studying towards law degrees and registration as patent attorneys.

Conference Calendar

The 11th Asian Workshop on First-Principles Electronic Structure Calculations, Kaohsiung, Taiwan, 3-5 November 2008.

Further details available at the website: http://asian11.phys.nsysu.edu.tw/aes11/

Functional Foods 2008: Functional Foods and Edible Oils - the Future, Auckland, 12-13 November 2008.

Further details available at the website: www.foodworks.co.nz/ffoods/

Geosciences '08, Wellington, 23-26 November 2008.

Further details available at the website: www.victoria.ac.nz/geosciences08/

30APS, 30th Australasian Polymer Symposium, Melbourne, Australia, 30 November - 4 December 2008.

Further details available at the website: www.30aps.org.au/

RACI ChemEd08, Fremantle, Australia, 29 November – 3 December 2008.

Further details available at the website: www.raci-chemed08.org/

NZIC Conference, Chemistry and the Biosphere, Dunedin, 30 November – 4 December 2008.

Joint conference with NZSBMB & NZSPB. Further details available at the website: www.otago.ac.nz/nzic

RACI Organic 08, Wrest Point, Hobart, Australia, 7-12 December 2008.

Further details available at the website: www.organic08.org/

2nd International Symposium on Organic Chemistry, Sofia, Bulgaria, 13-16 December 2008.

Further details available at the website: www.organic2008.bg-conferences.org/

IC08, Inorganic Chemistry Conference, Christchurch, New Zealand, 14-18 December 2008.

Further details available at the website: www.chem.canterbury.ac.nz/ic08/

ICMSAO '09, Third International Conference on Modeling, Simulation and Applied Optimization, Sharjah, United Arab Emirates, 20-22 January 2009.

Further details available at the website: www.aus.edu/conferences/icmsao09/index.php

ANZSMS22, The 22nd Biennial Australia & New Zealand Society for Mass Spectrometry Conference, Sydney, Australia, 27-30 January 2009.

Further details available at the website: www.mmb.usyd.edu.au/ANZSMS22/

SCM-4, Fourth International Symposium on Separation and Characterization of Natural and Synthetic Macromolecules, Amsterdam, Netherlands, 28-30 January 2009.

Further details available at the website: www.ordibo.be/scm/

AMN4, 4th MacDiarmid Institute for Advanced Materials and Nanotechnology Conference, University of Otago, 8-12 February 2009.

Further details available at the website: http://macdiarmid.ac.nz/events/amn-4.php

Gordon Research Conferences, Gaseous Ions; Structures, Energetics & Reactions, Galveston, Texas, USA, 1-6 March 2009.

Further details available at the website: www.grc.org/programs.aspx?year=2009&program=gaseous

PITTCON 2009, 60th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Chicago, Illinois, USA, 8-13 March 2009.

Further details available at the website: www.pittcon.org/

MCR 2009, Fourth International Conference on Multi-Component Reactions and Related Chemistry, Ekaterinburg, Russia, 24-28 May 2009.

Further details available at the website: www.mcr2009.ru/

EuCheMZ COMC XVIII, Conference on Organometallic Chemistry, Goteborg, Sweden, 22-25 June 2009.

Further details available at the website: www.chemsoc.se/sidor/KK/comc18/index.htm

13th JCQC, International Congress of Quantum Chemistry, Helsinki, Finland, 22-27 June 2009.

Further details available at the website: www.helsinki.fi/kemia/icqc/

ICCC39, 39th International Conference on Coordination Chemistry, Adelaide, Australia, 25-30 July 2010.

ICCC39 will encompass all aspects of coordination chemistry through plenary, keynote and section lectures and poster presentations.

NZIC Annual General Meeting

The NZIC AGM will take place in the St David Lecture Theatre Complex of the University of Otago, Dunedin on **Tuesday 2 December 2008** at **12.30 pm** (Cnr. St David Street and Cumberland Street).

AGENDA

1. Minutes of the 2007 AGM held at the University of Canterbury on Wednesday 12 Dec 2007
2. Matters arising
3. Announcement of prize winners
4. Financial report - including the auditor's report
5. Election of officers
   - President
   - 1st Vice-President
   - 2nd Vice-President
   - Treasurer
   - Honorary General Secretary
6. Any Other Business

Nominations for the Officers of Council close with NZIC administration on 31 October 2008.

Council News

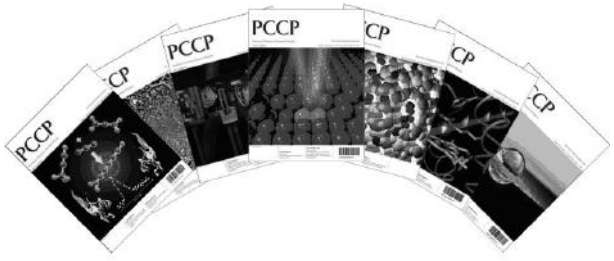
Council and Executive have, this year, been considering proposals to become partners in two Journals; *Physical Chemistry Chemical Physics* (PCCP) published by a partnership of European Chemical Societies and *Chemistry – An Asian Journal* (CAJ) published by Wiley's. After receiving clarification on a number of issues Council resolved, at its September meeting, to accept the invitation to join both publishing agreements. In both cases NZIC benefits in a profit share based on NZ author contributions to the journals.

PCCP already has a good reputation and, while CAJ is relatively new, Council felt that joining the publishing agreement would help maintain and lift our profile in the Asia-Pacific region. A welcome message from PCCP is printed below.

PCCP Welcomes NZIC as a New Owner Society

Physical Chemistry Chemical Physics (PCCP) is an international journal renowned as the journal of choice for fast publication of high quality research in the broad areas of physical chemistry, chemical physics and biophysical chemistry.

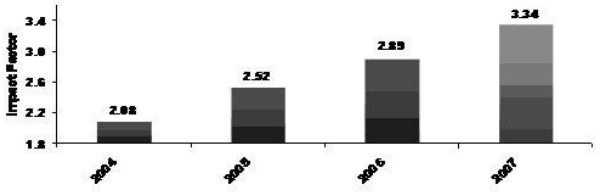
PCCP is owned and run by fifteen international learned and professional societies, and published by the Royal Society of Chemistry on a not-for-profit basis. We are now delighted to welcome the **New Zealand Institute of Chemistry** as our sixteenth owner society. Now, publishing in PCCP benefits your society! As an Owner Society, NZIC receives a royalty every time the journal publishes an article by a researcher based in New Zealand.



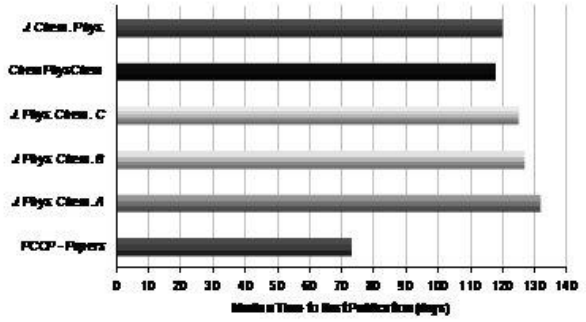
Key benefits of publishing in PCCP

• High quality and high impact research

The latest citation data released by Thomson ISI in June 2008 reveal that PCCP's Impact Factor has risen by over 15% to its highest ever value of **3.34**. This represents an enormous 60% increase over the past three years



• Professional and friendly editorial services, with rigorous yet fair peer review, and rapid publication times. We are by far the quickest general physical chemistry journal, with average times to publication just 70 days from receipt!



• Flexibility. There are no author charges, and authors may use free colour in papers. Submissions welcomed in almost any layout and electronic format.

• Extensive promotion of published articles, and truly international readership.

For more information about PCCP please visit our website: *www.rsc.org/pccp* Manuscripts can be submitted either *via* the website, or as an email attachment to *pccp@rsc.org*

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