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January News

The 2009 Officers of NZIC elected at the AGM in Dunedin are:

President:	**Prof *John* Spencer**	(Victoria University)
1st Vice-President:	**Dr *Mark* Waterland**	(Massey University, PN)
2nd Vice-President:	**Dr *Gordon* Rewcastle**	(Auckland University)
Hon. Gen. Secretary:	*Richard* **Rendle**	(Christchurch)
Treasurer:	**Dr *Colin* Freeman**	(Canterbury University)

Dr ***Peter* Hodder** has taken over the editorship of the NZIC publication CHEM NZ. It is likely to appear re-named as CHEMED NZ.

About the President

John Spencer received his BSc (Hons.) and PhD (1971) degrees from the University of Otago. His PhD research, on the synthesis and structure of cobalt carbonyl cluster molecules, was directed by Dr Brian Robinson. In 1970 he was appointed Junior Fellow at the University of Bristol, where he worked with Professor F. G. A. Stone and Dr Michael Green on the organometallic chemistry of the late transition metals. John moved through several research positions at Bristol and became Lecturer in Inorganic Chemistry in 1977. In 1985 he was appointed Professor of Inorganic Chemistry at the University of Salford and for four years between 1989 and 1993 he also served as Pro-Vice-Chancellor. In 1996 John returned to New Zealand to take up the post of Professor of Chemistry at Victoria University of Wellington where he is currently Head of the School of Chemical and Physical Sciences and Deputy Dean of Science. He has served on the Wellington Branch of the NZIC and was Branch Chairman between 1999 and 2001. During his term he took the opportunity to promote chemistry in secondary schools by supporting the work of secondary teachers. His research interests are in organometallic chemistry and homogeneous catalysis. He has a particular interest in controlling the reactivity of transition metals through ligand design, and in the activation of σ bonds in small molecules using transition metals.

NZIC Conferences

At the time of going to press, there were some 280 registrants for the biennial NZIC Conference in Dunedin. A full report of the event will appear in the April issue.

NZIC Conference 2011

The Waikato Branch has accepted Council's invitation to host the next NZIC Conference that will most likely take place in 2011 so as not to clash with Pacifichem 2010. *Michèle* ***Prinsep*** has accepted appointment as Conference Chairperson and *Michael* ***Mucalo*** is Treasurer. More details will appear in future issues.

RSNZ

Prof *David* ***Parry,*** Distinguished Professor of Biophysics at Massey University, Palmerston North, was awarded the 2008 RSNZ Rutherford Medal at the Science Honours Dinner held on November 11.

New Fellows

Profs *Terry* ***Collins*** (Carnegie Mellon University, Pittsburgh) and *John* ***Miners*** (Flinders University, Adelaide) have been elected to Honorary Fellowship of the Royal Society of New Zealand. Terry, an Auckland graduate (PhD with Warren Roper), is world renowned for his *green chemistry* (*This Journal*, **2001**, *65*(3), 15-17) while John, (PhD Victoria and Ted Harvey's last student – see p.41) has moved from organic chemistry and is a noted clinical pharmacologist.

Among the ten new NZ Fellows elected on November 5 were A/Prof *Jim* ***McQuillan*** (Otago) and Dr *Peter* ***Tyler*** (IRL). Jim is best know for his co-discovery of *Surface Enhanced Raman Spectroscopy* and Peter has made world-recognised contributions to synthetic organic chemistry in the direct pursuit of improved human health.

2008 Science Honours Dinner

Prof *David* ***Parry*** (Massey University) was bestowed with the 2008

Rutherford Medal at this now annual event. NZIC utilized the opportunity of the dinner to award the 2008 Fonterra Prize for Industrial & Applied Research to A/Prof *Simon* ***Hall*** (Massey University) for his work on the development and commercialization of rechargeable zinc batteries. The award was made by then 1-VP and current NZIC President *John* ***Spencer***.

Simon Hall accepting the Fonterra Industrial & Applied Chemistry Award from John Spencer (images courtesy RSNZ)

NZAS Awards

Two chemists were among the recipients of the 2008 NZ Association of Scientists awards. Dr *Graeme* ***Gainsford*** (IRL) was awarded the Shorland Medal for a lifetime contribution to original research, for his extensive studies involving X-Ray crystallography, while Dr *Yeap* ***Foo*** received the organization's Marsden Medal. He has thirty-eight years of research with IRL as an organic chemist studying complex plant tannins and identifying their structures.

The NZAS research medal went to Dr *Ulrich* ***Zuelicke*** of Massey University's IFS for his work into the theory of new electronic devices at the nanometre scale. The Communicator award was made to Prof *Ian* ***Spellerberg*** of Lincoln University.

News from the RSC

It would seem that the Royal Society of Chemistry is culinary capable! It recently made the bold proclaimation that Yorkshire puddings simply aren't up to scratch unless they rise to four inches or higher. With the collaboration of Dr *John* ***Emsley*** – chemist, RSC author, and proud Yorkshireman – our sister organization established that the perfect Yorkshire pud stands at just over four inches, with a light, fluffy texture and crisp exterior. A more detailed report and the detailed recipe can be found at: *http://prospect.rsc.org/blogs/rsc/2008/11/12/jon/yorkshire-puddings-must-rise-four-inches-or-higher-rule-the-chemists*

BRANCH NEWS

AUCKLAND

University of Auckland – Chemistry

Drs *David* ***Barker*** and *Andrew* ***Dingley*** were successful in securing Marsden Funding from RSNZ. David obtained $100,000 for each of 3 years for his research on HIF-1 inhibitors; the project is titled *Hunting for HIV-1 inhibitors: synthesis of Manassantin B, a potent, non-cytotoxic HIF-1 inhibitor*, while Andrew's project *Protein dynamics is the key to the regulation of cytokine-induced intracellular signalling* secured $740,000 per year for 3 years.

Prof *Laurence* ***Melton*** was elected as one of 25 new Fellows of the International Academy of Food Science and Technology (IAFoST) at the World Congress of Food Science and Technology in Shanghai. Laurence is Director of the University's Food Science Programme and his research focuses on the microstructure of plant foods, particularly the changes in texture that occur during freezing, canning or drying. He also has an interest in how proteins and polysaccharides interact in food.

An Australian Scientific Glassblowers Symposium was held on 7th November hosted by Mr Mike Wadsworth and Mr Alistair Mead. The affair was a wild success with glassblowers from NZ, Australia, Germany and the USA attending. Our lads absconded with two of the four competition trophies, Alistair for best presentation of his glass *Beam Engine*, and Mike for best practical workshop demonstration. The Symposium wound up with a Dinner and Awards evening at the Viaduct.

CANTERBURY

The Branch meetings continue to be well attended. In July a combined NZ Institute of Food Science and Technology and NZIC meeting was held at ESR. A large crowd appreciated Keith Woodford's talk *The Science and Politics of A1 and A2 milk*. Also in July a beer tasting sponsored by *Three Boys Brewery* was very popular. The President's talk featured in August, and in September Michael Taylor, ESR, explained blood spatter analysis to a gruesomely fascinated audience. In October, this year's *Trivia and Truffles* was enhanced by the use of smart visuals, music clips and an electronic score board. Our thanks to *Marie* ***Squire*** and *Chris* ***Fitchett*** for these, and thanks also to our quiz master *Michael* ***Edmonds***. Twenty one teams enjoyed an evening of hilarity and tense competition pondering over questions that sometimes had a less than tenuous link to chemistry. Contestants came from overseas, ESR, Chemistry, Biochemistry, Management, Chemical Engineering (a first!), Physics, and Student Services. The results were:

1st Manganesium (*Jan-Yves* ***Ruzicka***, *Michael* ***Hunter***, *Rachel* ***Hanover-O'Connor***)

2nd Past Due Date (*Jan* ***Wikaira***, *Don* ***McNickle***, *Sim* ***Wikaira***)

3rd Victory is (mela)mine! (*Sam* ***Edwards***, *Paul* ***Wilson***, *Brett* ***Davis***)

4th Sparge My Phlogiston (*Henry* ***Toombs-Ruane***, *Tammie* ***Cookson***, *Chris* ***Hawes***)

Best Artwork – 210P (*Phil* ***Emnet***, *Paul* ***Kruger***, *Kiran* ***Dayaram***)

Best team names – Victory is (mela)mine! (*Sam* ***Edwards***, *Paul* ***Wilson***, *Brett* ***Davis***) and Amide (S)-touch (*Aidan* ***Harrison***, *Tim* ***Allison***, *David* ***Tran***)

Spot prizes – *Rebecca* ***Hurrell***, *Jessica* ***Baker*** and *Graham* ***Townsend***

Mystery Problem – Sparge My Phlogiston (*Henry* ***Toombs-Ruane***, *Tammie* ***Cookson***, *Chris* ***Hawes***)

First Entry – Manganesium (*Jan-Yves* ***Ruzicka***, *Michael* ***Hunter***, *Rachel* ***Hanover-O'Connor***)

Best wrong answer was (Q: How many doctors in *Shortland Street* have been murdered by the evil pharmaceutical company?) – ***Not enough!***

CPIT

The Year 11 chemistry competition had 23 teams from 13 schools across Canterbury, ranging from Ashburton in the south to Rangiora and Kaiapoi in the North. Teams of three students completed activities in qualitative inorganic analysis and use of a pH meter, in a limited time. The winning team was from *Lincoln High School*, with *Hillmorton High School* runner-up. A comparable competition for Year 10 students was held in late November and was sponsored, in part, by The Canterbury Science Teachers' Association.

University of Canterbury

Professor *John* ***Blunt*** who retired at the end of 2008, has been awarded the title of Professor Emeritus. Dr *Chris* ***Fitchett*** was welcomed as a lecturer to the Department. Chris is interested in the synthesis of large planar heterocycles, the investigation of their properties and their interaction with metal atoms. He is also interested in the development of charge density studies using X-ray crystallography. Chris comes back to the Department following several short stints as lecturer, X-ray technician, laboratory supervisor, and postdoctoral fellow at Trinity College (Dublin) and James Cook University (Townsville).

Craig ***Tennant***'s appointment as an Adjunct Senior Fellow has been extended for another three-year term. *Andrew* ***Gross***, is a new PhD student under the supervision of *Alison* ***Downard***. Originally from Ipswich he graduated from the University of Surrey with a Master's degree in analytical chemistry, and worked for a year at Hill Laboratories (Hamilton).

We have had two Erskine visitors: Professor *Peter* ***Griffiths***, from the University of Idaho, whose research interests are in FTIR and Raman Spectroscopies, and remote sensing and environmental applications. Dr *Robert* ***Raja***, currently Reader in Chemistry at Southampton, has a focus on discovery and design of novel nanoporous and mesoporous solids for application as single-site heterogeneous catalysts in chemical, fine-chemical, pharmaceutical and environmental technologies, using energy-renewable feedstocks and sustainable processes. Sabbatical visitors have been *Andy* ***Sykes*** (University of South Dakota) and *Don* ***Hastie*** (York University, Toronto). Andy is working with *Richard* ***Hartshorn*** and *Alison* ***Downard*** on mutually interesting topics in the realm of inorganic, organic and electrochemistry. Don was collaborating with *Murray* ***McEwan***.

The past months have seen a number of visiting students with us. *Wiradej* ***Thongsuwan*** has joined the group of *Vladimir* ***Golovko*** for a one year research project as a part of his PhD in Materials Science at Chiang Mai University (Thailand). *Jérémie* ***Gatignol*** is visiting from Saint-Bardoux, near Lyon (SE France). Jérémie is in the second year of graduate studies at the National Graduate School of Chemistry (ENSCCF) in Clermont-Ferrand. He is hosted by *Jim* ***Coxon***. *Nurul* ***Aqmar***, a visiting post-graduate student from MARA, the University of Technology in Malaysia, is working on collaborative research with Profs *Tony* ***Cole***, *Murray* ***Munro*** and *John* ***Blunt***. *Lisa* ***Roeder***, from Germany, has been working with *Peter* ***Steel***. After working with the Marine Natural Products group, *Suwannee* ***Saisan*** has returned to Thailand.

Don ***House*** recently made his annual visit to us and ex-student Prof *Ray* ***Butcher*** (Howard University, Washington) spent six weeks with us. *Andrew* ***Abell*** has also visited.

Professor *Ian* ***Shaw***, has announced his resignation as PVC – College of Science; he will become a Professor in the Department of Chemistry, where he will conduct research into human exposure to environmental chemicals and teach environmental chemistry and biochemistry.

Outreach Activities have included *Info Days*, *Science of Air* and the *Golden Key Youth Forum*. *Vladimir* ***Golovko*** energetically led a large group of visitors in a tour around the Department; *Chris* ***Hawes***, *Henry* ***Tombes-Ruane***, *Rob* ***Stainthorpe*** and *Archna* ***Tandon*** received praise and thanks for their contributions to the Science of Air day in November, and *Paul* ***Kruger*** received compliments for *an excellent presentation that made quite an impression on the students* at the *Youth Forum*.

Student successes include: *David* ***Garrett*** who won the inaugural IBM Visiting Scholarship for this year. He is currently undertaking a 10-week placement at the IBM Almaden Research Center in San Jose CA on a generous stipend of NZ$20,000. *Wanting* ***Jiao*** has recently embarked on a molecular modelling study of calpain interactions under the supervision of *Emily* ***Parker*** and *Jim* ***Coxon*** under a Top Achiever Doctoral Scholarship. *Francine* ***Smith*** has been awarded an ESR/BIC PhD scholarship; she is working on the metabolites of cyanobacteria under the supervision of *Sally* ***Gaw***. Students who have been awarded UC Summer Scholarships are: *Chris* ***Hawes*** (with *Paul* ***Kruger***), *Rosanna* ***Archer*** (*Owen* ***Curnow***) and *Lauren* ***Pinfold*** (*Sally* ***Gaw*** and *Marie* ***Squire***). *Jan-Yves* ***Ruzicka*** has been awarded a joint Chemistry-Engineering Summer Scholarship on *Electrospinning of cobalt colloid – PVP nanofibre assemblies for novel catalysis applications* [with *Mark* ***Staiger*** (Eng.) and *Vladimir* ***Golovko*** (Chem.)]. College of Science Summer Research Scholarships go to *Jackie* ***Knobloch***, a 300-level student in Chemistry, who will work on metal-sulfide nanoparticles with *Vladimir* ***Golovko*** and *Andy* ***Pratt***. *Jayne* ***Gulbransen***, a 400-level honours student, will study the imidazole frameworks for new colossal aromatic molecules with *Chris* ***Fitchett***.

PhD completions include: *David* ***Bones*** (*Liquid Aerosol Photochemistry – Leon* ***Phillips***/*Colin* ***Freeman***); he is now working with Prof *Sergey* ***Nizkorodov*** (UC-Irvine) on

atmospheric and aerosol chemistry; *Justine* ***Cottam*** (*Studies in Metallo-supramolecular Chemistry* – Prof *Peter* ***Steel***; *Neroli* ***Ayling*** (*Photochemistry of Coordination Complexes in the Aging of Dyed Fibres – Richard* ***Hartshorn***).

Paul ***Kruger*** has recently heard that two of his papers hold citation records: *Anion Recognition in Organic and Aqueous Media Using Luminescent and Colorimetric Sensors* (*Coord. Chem. Rev.* **2006**, *250*, 3094-3117 – Elsevier) and *The role of acid in the formation of hydrogen-bonded networks featuring 4,4 -dicarboxy-2,2-bipyridine (H_2dcbp): Synthesis, structural and magnetic characterisation of {[Cu(H_2dcbp)Cl_2]H_2O}$_2$ and [Cu(H_2dcbp)(NO_3)$_2$(H_2O)]* (*CrystEngComm*. **2005**, *7*, 90. The first *Nature* publication of this century by a departmental staffer came from *Vladimir* ***Golovko*** on selective oxidation involving gold nanoparticles. The article warranted an additional commentary by *Wayne* ***Goodman*** (*Nature*, **2008**, *454*, 948-949) entitled *Precious Little Catalyst*.

Congratulations go to UC Adjunct Professor *Richard* ***Keene*** (Townsville), *Grant* ***Collins*** (Adelaide) and *Peter* ***Steel*** who have recently been granted three year ARC funding the *Development of New Materials Based on Multinuclear Ruthenium Complexes* and to *Richard* ***Hartshorn*** and *Ken* ***Morrison*** (Chemical and Process Engineering) who have been awarded Bright Ideas Funding regarding a research project regarding the *Minimising of Bio-film Build-up*.

The Department sponsored prizes in the Year 9+ Technology section at the Canterbury-Westland School's Science and Technology Fair. The 1st Prize was won by *Emil* ***Martin*** (*Shower Boss* – also Senior 2nd prize Environment Canterbury Award); Joint 3rd *Katelyn* ***Thorn*** (*Breathe Ezy Mask*) and *Jake* ***Eastwood*** (*Hove Fatboy Hover*). The students were all from Lincoln High. The NZIC Branch awards for the best Chemistry-related exhibits went to *Brendan* ***Chin*** 1st (Cobham Intermediate – *From Dirty Water to Clean*), 2nd equal *Liam* ***Mciver*** and *Callum* ***Dow*** (Kirkwood Intermediate – *Graffiti, is it a Problem in Your Neighbourhood?*) and *Savannnah* ***Adams*** (St. Margaret's – *Walking on Water*) and *Sankari* ***Ganeshan*** (Lincoln High – *Going with the Flow of the Avon River*).

MANAWATU

Massey has just seen a MacDiarmid Institute-funded *Rigaku Spider* X-ray diffractometer installed. It is designed for the structural analysis of small molecule and protein crystals and powder samples. It is connected to a previously unused port of Geoff Jameson's micro-focus rotating anode X-ray generator. The high beam intensity and curved image plate detector will allow challenging problems involving small and/or weakly diffracting crystals to be tackled. Interested users are invited to contact *Shane* ***Telfer*** for further information.

Marco ***Wenzel*** joined the Plieger group earlier this year as a Massey-funded post-doctoral fellow and is currently developing new ligands for metal salt extractants. PhD student *Karl* ***Shaffer*** is continuing to develop small cation chelators and is hoping to head back to Los Alamos National Laboratory later next year for further beryllium studies. *Quintin* ***Knapp*** is completing the last of his anion titration studies prior to writing up for MSc. *Paul* ***Plieger*** recently visited and gave a talk at Auckland University, a day after swimming across the Auckland harbour with 1400 other people and some very big Orca whales (did I mention they were big?). Massey will be hosting *Peter* ***Tasker*** for a couple of days prior to IC'08 in early December.

*Islah-**u-Din*** has completed a post-graduate diploma in Science and will be working with *Jeff* ***Tallon*** at IRL over the summer period, before beginning his PhD studies with *Mark* ***Waterland***. Islah's study is supported by the Higher Education Commission of Pakistan. At the annual Massey-Victoria Student symposium (at VUW), *Hilary* ***Corkran*** was awarded the best talk of the day, for her work on coordination complexes of paracyclophanes, supervised by *Shane* ***Telfer***, *Gareth* ***Rowlands*** and ***Mark Waterland*** – some suggest that she probably deserves another award for surviving the year with three supervisors.

Phil ***Bunker*** from the Steacie Institute for Molecular Sciences, Ottawa visited on November 29 and gave a talk on the structure and symmetry of the benzene dimer. *Steve* ***Kirk*** successfully passed his PhD oral exam. Steve studied with *Andrew* ***Brodie*** and *Eric* ***Ainscough*** and is currently fabricating carbon nanotubes with *Ashton* ***Partridge*** with whom *Gaile* ***Dombroski*** and Helen ***Hsu*** have begun PhD studies.

Sivakumar ***Balakrishnan*** joined *Shane* ***Telfer***'s group as MacDiarmid Institute-funded post-doctoral fellow in late November 27. Sivakumar has a background in materials chemistry and joins Massey from the CSIRO in Melbourne.

OTAGO

The Branch AGM elected Drs *Julian* ***Eaton-Rye*** its 2009 Branch Chairperson, *Guy* ***Jamison*** as Secretary and *James* ***Crowley*** as Treasurer.

The Branch sponsored a highly successful High School Quiz Night for the fifth year in a row. The quiz, held in the Hutton Theatre at the Otago Museum in September, once again had teams from almost every school in Dunedin and from several schools in Otago and Southland fill the venue. Fifty teams competed for the coveted *Bunsen Burner of Wisdom* and this year it was won by a team called *So-di-um come here often?* (Kavanagh College). After a tense tie-breaker between four teams, *Bunsens on Fire* (Otago Girls High) came second and *The Oxidising Agent Squad* (also Kavanagh College) were third. It was

Caption: Winners of the Otago/Southland High School Quiz Night Competition holding their prize, the Bunsen Burner of Wisdom

great to see a number of teams who had competed in 2007 return for this year's quiz. Thanks to a new sponsor this year, *Silicon Graphics Inc*., several prizes were awarded for the Chemical Haikus. An excellent night was had by all. Many thanks go to the staff and students of the Chemistry Department who helped out and to the loyal sponsors, *Poppas Pizzas*, *The University Bookshop*, *The Otago Museum*, and *Silicon Graphics*.

University Chemistry Department

Russell ***Frew***, *Dave* ***Larsen***, and *Barrie* ***Peake*** learned in October that they will become Associate Professors in February next. As noted elsewhere A/Prof *Jim* ***McQuillan*** was elected FRSNZ in November for his landmark work on Surface Enhanced Raman Spectroscopy. *Sally* ***Brooker***, who was elected in 2007, recently attended a celebration reception for newly elected Fellows. Di McCarthy hosted the occasion and Carolyn Burns presented the certificates and lapel badges. Sally's research group, resplendent in suits, did their supervisor proud.

Jim ***Simpson*** was recently appointed as Editor of *Acta Crystallographica* Section E. *Lyall* ***Hanton*** and *Allan* ***Blackman*** were among ten lecturers to receive Otago Top Teaching Awards in 2008. The awards are administered by the Otago Student Association and provide students the opportunity to cast votes for their best lecturers.

The Department was very successful in recent funding rounds. *Sally* ***Brooker*** obtained a Marsden Grant of $815,000 over three years for the project *Designer dimetallic spin crossover complexes: developing tuneability, cooperativity and hierarchical organisation*. *John* ***van Klink***, *Nigel* ***Perry***, and their collaborator *Kevin* ***Gould*** from Victoria University received a Marsden Grant for $777,000 over three years for their project entitled *Aposematic colouration in plants: honest signals of chemical defences & influences on herbivore fitness*. 2009 OU Research Grants went to *Allan* ***Blackman***, *Philip* ***Boyd***, *Russell* ***Frew***, *Keith* ***Gordon***, *Kimberly* ***Hageman***, *Guy* ***Jameson***, *Henrik* ***Kjaergaard***, *Sylvia* ***Sander***, *James* ***Crowley***, *Nigel* ***Lucas***, and their many associated investigators.

Martine ***Poffet*** (post-doctoral fellow with *Keith* ***Hunter***) won the 2008 University of Fribourg (Switzerland) Environment Award for her PhD project on *Thermal Runaway of dried sewage sludge in storage tanks*.

Henrik ***Kjaergaard*** gave an invited lecture entitled *Molecular spectroscopy of water dimer* and *Joseph* ***Lane*** presented a poster entitled *Calculated electronic transitions in the water ammonia complex* at the World Association of Theoretically Oriented Chemists Conference (Sydney, Sept. 2008). *Mikkel Bo* ***Hansen*** from the Lundbeck Center for Theoretical Chemistry (Aarhus, Denmark) and *Priyanka* ***Bagaria*** (Birla Institute of Technology and Science, Pilani, India) have recently spent time in Henrik's group as visiting researchers. *Ben* ***Miller***, Joseph, and Henrik went to the Australian Synchrotron Facility in Melbourne last August to continue their high resolution FT-IR spectra of deuterated acetylacetone.

Julia ***Rinck***, a PhD student with Professor *Annie* ***Powell*** (Karlsruhe, Germany), joined *Sally* ***Brooker***'s group in November for four months of collaborative research. Annie will also be visiting in February. A new postdoctoral, *Laszlo* ***Mercs***, will join Brooker's Bunch in March after he completes his PhD with Professor *Martin* ***Albrecht*** (Fribourg, Switzerland). *Nick* ***White*** completed his hugely productive research assistantship and is now on his big OE until he starts his PhD overseas in mid-2009. Current Brooker's Bunch members are thrilled to have moved into the new group office space that they will share with *James* ***Crowley***'s group – now when the music stops, they will all have a desk to sit at! This office lies between, and looks into, both of the renovated laboratory areas that were completed prior to the Christmas break.

The Crop & Food Research Plant Extracts Research Unit staff, located in the Chemistry Department, are excited about their December merger with HortResearch. This brings together a large group of chemists with mutual interests in areas such as flavours, insect attractants, and bioactives in foods.

The GoTrace Global Origin and Traceability Symposium is scheduled for 30 June – 2 July 2009 at the Dunedin Centre. It will bring together scientists and businesses working in the areas of verification of origin and traceability with producers, exporters and consumer groups, and provide the first forum in NZ and Australia for delegates to examine and discuss the requirements of international markets, the development of new technologies, and the applications of these technologies to a wide-range of food and other products. *Russell* ***Frew*** is to be one of four keynote speakers.

WAIKATO

Branch Officers 2009

Chairperson & Secretary: Ms *Marisa* ***Till***

Treasurer: Dr *Michael* ***Mucalo***

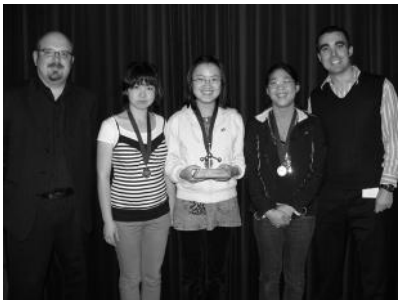
Chemistry Department University of Waikato

Peter ***Morris***, the longest-standing member of the Chemistry Department, has recently retired. Peter was appointed in 1970, the first year that Chemistry was taught at Waikato, joining founding members *Alec* ***Wilson*** and *Malcolm* ***Carr***, both of whom had transferred from Victoria. Peter played a key role in developing general and physical chemistry in the somewhat frantic early stages when cows on the ex-Ruakura site still roamed outside makeshift laboratories, and new practical classes were often preceded by hurried day-before trips to Auckland's Chemistry Department to borrow essential equipment. Peter had completed a PhD with the late *Bob* ***Hay*** at Victoria and a postdoctoral with Bruce Martin in Virginia. His research at Waikato centred mainly on metal ion catalysis of amino acid ester hydrolysis. Peter was well recognised for his dedication to teaching the rigours of physical chemistry in the most palatable way to successive generations of less and less mathematically

prepared students. This brought high accolades from even the most hardened of students, and he leaves a hole that will be very hard to fill. He will remain on his magnificent Matangi Road gully property, which he and his wife Margaret have worked tirelessly to break in from a gorse-filled gully over 25 years. Experimenting with the best types of plants for restoration, and developing his own nursery along the way, Peter has received much recognition and a major environmental award for restoration of the gully, which was protected by a QEII covenant in 2006. In retirement Peter will doubtless remain the sought-after expert on restoration in gully-riddled Hamilton, and take advantage of all the extra time freed up from university duties to further his gully work.

The annual Chemquest quiz, now in its 12th year, was recently held in the Department. A total of 62 teams coming from the greater Waikato region and Bay of Plenty participated. As usual, this was a fun-filled evening for students studying NCEA level 2 Chemistry, with the traditional variety of chemistry-based questions ranging from Periodic Puzzlers, music-based questions, smells and chemical demonstrations.

The winners *Steele's Trio* (Hillcrest High – *Lily* ***Zhang***, *Ann* ***Yu*** and *Kejia* ***Wang***), received the James & Wells trophy plus $150 cash. 2nd prize went to *TBC Repz* (Tauranga Boys College), 3rd prize to *Triawesome Sulfate* (Hamilton Boys' High), 4th to *In it for the moles* (Fairfield College), and 5th equal were *Aluminium Nitrate* (Waikato Diocesan for Girls) and *Team Boris* (Hamilton Boys' High). Prizes were generously sponsored by *James & Wells Intellectual Property* and *Hill Laboratories*, as well as the WU's *School of Science and Engineering*. Question masters were *Richard* ***Coll*** and *Bill* ***Henderson***, with *Brian* ***Nicholson*** and *Lyndsay* ***Main*** the chief judges, assisted by *Pat* ***Gread***, *Kelly* ***Kilpin*** and *Bevan* ***Jarman***. Others thanked for their participation in running the event are *John* ***Little***, *Jannine* ***Rhodes***, *Wendy* ***Jackson***, *Annie* ***Barker***, *Jenny* ***Stockdill***, *Jonathan* ***Puddick***, *Hannah* ***Lerke***, *Simon* ***Addison*** and *Peter* ***Wilson***.



The winning team, *Steele's Trio* (Hillcrest High School) with sponsors Martin Lovell (Hill Laboratories, right) and Jared Millar (James & Wells, left)

Bill **Henderson** delivered his presidential talks to all the Branches concluding with his home Branch Waikato. *Michèle* ***Prinsep*** visited Singapore and the UK last August and September as part of her study leave. In Singapore she visited colleagues at the National University and Nanyang Technological University and delivered a seminar and some graduate lectures. In the UK she visited Chris Moody at Nottingham, Claire Helio at Portsmouth and Jo Porter at Aberystwyth to discuss collaborative research and deliver seminars. *Michael* ***Mucalo*** attended the Zing Nanomaterials Conference in Playa del Carmen, Mexico in December. His paper *An overview of IR spectroscopic, mass spectrometric and surface analytical techniques used in the in situ or ex situ studies of precious metal colloids* dealt with aspects of his colloid research spanning the last 18 years.

There are a number of students working in the Department over the summer period on Waikato scholarships. *Stefan* ***Smith*** working on carbene complexes, *Michael* ***Scott*** on main group chemistry and *Ashleigh* ***Richards*** on gold chemistry, are all under the supervision of *Bill* ***Henderson***. *Maria* ***Revell*** is pursuing LC-MS studies of marine natural products with *Michèle* ***Prinsep***, and *Jacob* ***Jaine*** will be looking at growth of hydroxyapatite on cholesterol with *Michael* ***Mucalo***. *Greer* ***Tanner-Dempsay*** is working on properties of biocarbons in conjunction with Prof *Michael* ***Antal*** *Jr* of the Hawaii Natural Energy Institute and Dr *Ajit* ***Sarmah*** of Landcare and *Nicole* ***Cameron*** is working on LAICPMS of possum teeth in relationship to geographical origin of samples. *Katie* ***de Lange*** is working on IC of organic ions in hot springs water with *Nancy* ***Hinman*** from the University of Montana. The latter three students are under the supervision of *Merilyn* ***Manley-Harris***.

Dr *Graham* ***Saunders*** has accepted a senior lectureship in inorganic chemistry replacing *Derek* ***Smith*** who is about to retire. Graham completed his doctorate at Oxford working with M. L. H. Green and subsequently had post-doctoral positions at Auckland (Warren Roper) and Leicester (J. Holloway and E. Hope) before taking up a lectureship at Queens University, Belfast. He has broad research interests with recent emphasis on applications of fluorinated compounds in organometallic chemistry, and as substrates for super-hydrophobic surfaces.

NIWA

In September, Mike Stewart and Cindy Baker hosted a visiting scientist, Sandy Scott, from The Centre for Environment, Fisheries and Aquaculture Science (CEFAS – UK). Sandy is a world renowned fish endocrinologist who has pioneered techniques for the detection and identification of steroid pheromones in fish. The work with Sandy has allowed us to close in on the physiologically active sex pheromones produced by the female redfin perch (*Perca fluviatilis*), an exotic fish species in NZ waters. *Bob* ***Wilcock*** attended the International Water Association conference on diffuse pollution (DIPCON 2008) in Khon Kaen (Thailand) last August, where he chaired sessions and gave a paper on *Climate Change Mitigation for Agriculture: Water Quality Benefits and Costs*. Greg Olsen spent four weeks in North America last September-October visiting several laboratories (USGS, USEPA, Southern Nevada Water Authority, Colorado State University, Hollings Marine Laboratory - NOAA) studying methodologies for emerging contaminant analysis.

WELLINGTON

Members of the Branch were saddened by news of the death of NZIC stalwart *Ted* ***Harvey*** on October 20. A full obituary appears elsewhere in this issue. On a brighter note we were

delighted to see two VUW graduates, the last student Ted supervised, Prof *John* ***Miners***, and Dr *Peter* ***Tyler*** of IRL elected Hon. FRSNZ and FRSNZ, respectively. We also congratulate Drs *Graeme* ***Gainsford*** and *Yeap* ***Foo*** on the receipt of the NZAS Shorland and Marsden medals, respectively. Prof *Jim* ***Johnston*** was the Science and Technology category winner in the annual '*Wellingtonian of the Year Awards*' – congratulations Jim.

The September meeting comprised a lecture by *Mike* ***Berridge*** (Malaghan Institute of Medical Research) addressing the issues *of the Cancer Questions, Stem Cells, Chemistry & Cures*. His discourse provided an excellent over-view of the state of cancer research in the Malaghan Institute. Based upon essentially unchanged cancer death rates for 55 years, the molecular and cellular basis of cancer and whether it is a curable disease were addressed, So was the extent to which cancer is a preventable disease. A focus of the Malaghan work is cancer cure through the development of therapeutic vaccines and the broad application of this personalised medicine approach to cancer treatment. This opens up new opportunities for interdisciplinary translational research and development involving cell and molecular biologists, chemists, immunologists and clinicians.

Mike started work in Wellington as a Malaghan Fellow jointly at Wellington Hospital and Victoria University investigating the stem cell origins of blood in 1976. The Fellowship became part of the Wellington Cancer & Medical Research Institute when it was constituted in 1978 and when renamed as the Malaghan Institute of Medical Research. Since then, the Cancer Cell and Molecular Biology group has pursued numerous research interests concerned with cancer and inflammatory diseases, and is currently poised to immunologically target the cancer stem cell.

The October meeting, a planned site visit to the Building Research Association of NZ (BRANZ) had to be cancelled due to insufficient numbers – a rare event in the history of Wellington meetings. However, in November the Branch held its AGM followed by a lecture *Proof of Life' Sciences in NZ: Ventures and Value Creation* from Prof *Mark J*. ***Ahn***, (Chair, Science & Technology Entrepreneurship, VUW and Principal at Pukana Partners Ltd., which provides strategic consulting to life science companies) In his lecture he asked the questions *Can science be a business? How will the global credit crunch affect research funding for life science businesses and venture creation in NZ?*

Emerging entrepreneurial high growth ventures, financed with venture or risk capital, seek to quickly create or capture large markets, scale rapidly, and are chronically under-resourced. As such, a thriving economic ecosystem is required to enhance new venture success due to high failure rates, technological complexity, and market risk. Prof Ahn reviewed key mega forces impacting upon science-led venture creation, criteria for successful science-led ventures, and unique challenges and opportunities facing technology firms competing from a NZ context. He did this in a question and answer style to get audience involvement but the entire concept is based upon technological development of good science rather than the generation of good science.

The Branch AGM elected Dr *Peter* ***Hodder*** as its 2009 Chairperson, with Drs *Joanne* ***Harvey*** as secretary and *Suzanne* ***Boniface*** as continuing treasurer (e-mail: all are at *given name. family name@vuw.ac.nz*).

Victoria University

Ashna ***Khan*** has successfully completed her MSc degree working with Dr Burkett on the synthesis of novel [5.5.7]-fused tricyclic hydrocarbons and *Lynton* ***Baird*** has completed his PhD study on the synthesis of Aigialomycin D with Drs *Joanne* ***Harvey*** and *Paul* ***Teesdale-Spittle***. Dr *Peter* ***Northcote*** continues his whirlwind tours of places distant having recently been in the US for discussions with the pharmaceuticals company involved in the development of his selected marine natural products, in Tonga to seek government approvals for the collection of samples for his Tongan MSc student *Taitusi* ***Taufa***, and in Japan at the Riken conference on Biological Chemistry where he gave an invited presentation *Spectroscopically Directed Isolation of Marine Natural Products.*

Recent visitors have included Drs *Gareth* ***Rowlands*** (Massey, Palmerston North) and *Rob* ***Keyzers*** who gave seminars on *Basic Instincts: sulfoxides, allylation and planar chirality* and *Marine Natural Products; new compounds for pharmaceutical discovery*. Rob, a former Northcote student, returned to NZ briefly following appointment to the Food Futures Flagship, CSIRO Plant Industries in South Australia. Dr *Chris* ***Braddock*** (Imperial College, London) visited the Northcote group in December and provided the School with a seminar on his synthetic studies directed towards marine natural products entitled: Biosynthetically-inspired chemical synthesis of complex halogenated marine natural products.

Inhibitors of Phosphatidylinositol 3-kinases: The Next Wave of Anti-Cancer Drugs?

Gordon W. Rewcastle and William A. Denny

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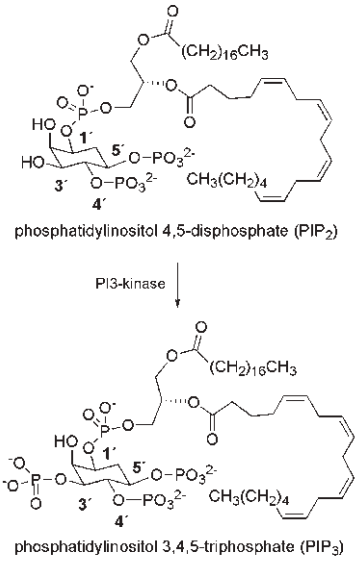
Phosphatidylinositol 3-kinases (PI3Ks) are a family of lipid kinase enzymes, which catalyse the phosphorylation of the 3′-hydroxyl position of the inositol ring of phosphatidylinositol 4,5-diphosphate (PIP_2) to give the messenger molecule phosphatidylinositol 3,4,5-triphosphate (PIP_3) (Scheme 1). This then participates in a variety of physiological processes, including cell growth and differentiation.[1] The PI3Ks are divided into three classes (I-III) based on their structure, mode of regulation, and substrate specificity. Class 1A PI3Ks are comprised of three isoforms (p110*α*, p110*β* and p110*δ*) that share a common regulatory subunit (p85) activated by signals from receptor protein tyrosine kinases, while the Class IB PI3K (p110γ) is structurally similar but lacks a regulatory subunit, and is activated by G protein-coupled receptors.[2] The pathway through p110α is the most frequently activated signalling pathway in human cancer, and its corresponding gene (*PIK3CA*) undergoes amplification in tumours, with activating *PIK3CA* mutations being relatively common in late-stage colon, brain, breast, and gastric cancers.[3,4]

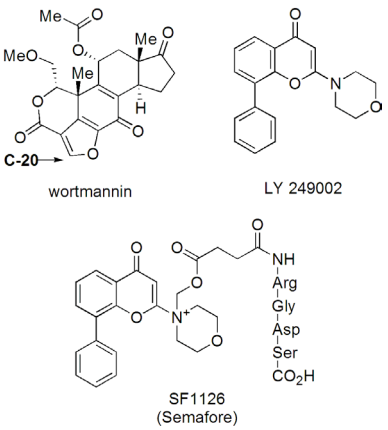


Scheme 1. PI3K phosphorylation of phosphatidylinositol 4,5-disphosphate (PIP_2).

Early investigations into the mechanism of PI3K inhibition were aided by two compounds, the fungal natural product wortmannin, first isolated[5] from *Penicillium wortmanni* in 1957, and the synthetic inhibitor LY294002 (Chart 1), which was first synthesized by Eli Lilly in the early nineties.[6]

Wortmannin is a potent and irreversible inhibitor in which the furan ring adds to the amino group of a lysine residue in the ATP binding pocket of PI3K giving an enamine at C20. X-ray studies with the p110γ isoform confirmed that this is with the amino group of Lys-833 and they also showed an H-bond between the C17 carbonyl oxygen and the backbone NH of Val-882.[2] However, since similar amino acid residues are found in all of the PI3K isoforms, wortmannin shows very poor isoform selectivity, and displays considerable liver toxicity at low doses in animal studies. Several wortmannin analogues have been prepared in an attempt to reduce this toxicity[7] but, since they all function as prodrugs of wortmannin itself, they show no advantage in terms of PI3K selectivity.

Chart 1



LY294002 binds reversibly with moderate potency and has proved useful as a tool due to its stability. It was the first synthetic PI3K inhibitor to have its complex with PI3Kγ structurally elucidated.[2] The morpholine oxygen makes an H-bond with the backbone amide NH of Val-882, the same residue that forms an H-bond with wortmannin and, in fact, this is a much conserved interaction that is now known to be shared by all current PI3K inhibitors and ATP itself. LY294002 is too insoluble for investigation as a drug, although a prodrug derivative, SF 1126 (Chart 1) has now entered human clinical trials as a pan-PI3K inhibitor, targeting cell growth, proliferation and angiogenesis.[8]

The issue of isoform selectivity is potentially important since each of the isoforms have a suite of significant biological effects; the p110*β* isoform is important in thrombus formation, while the p110*δ* and p110γ isoforms are important in aspects of inflammation. However, despite high-quality crystal structure data on both the α- and γ-isoforms, obtaining compounds with high selectivity for p110α has proved difficult. This is illustrated (Table 1) by the IC_{50} values (concentration of drug for 50% inhibition of PIP2 phosphorylation) by LY294002, wortmannin, and the first of the other new PI3K inhibitors that have

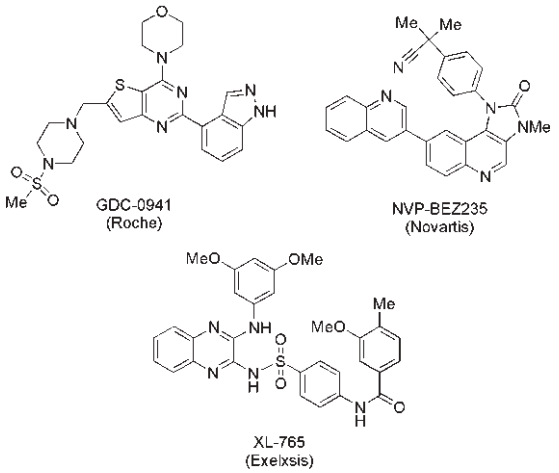
begun clinical trial; GDC-0941, NVP-Bez235, XL-765 (Chart 2).

Table 1. Isoform selectivity of PI3K inhibitors.

Compound	IC_{50} (nM) p110			ratio
	-α	-β	-δ	β/α
Wortmannin	~4	~4	~4	~1
LY294002	800	1000	700	1.2
SF-1126	NA*	NA*	NA*	NA*
GDC-0941	3	33	11	11
NVP-Bez235	20	160	12	8.0
XL-765	13	113	43	8.7

*NA - not applicable; prodrug

Chart 2



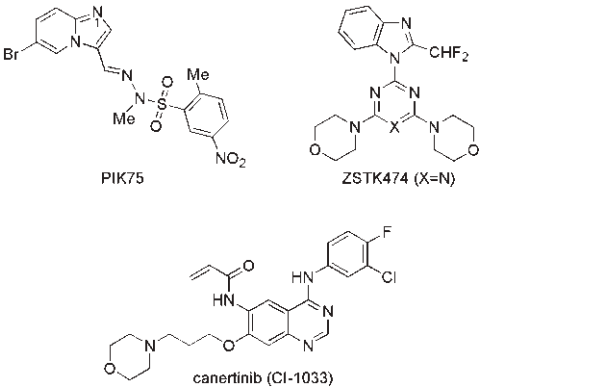
GDC-0941 (Genentech/Roche) is the result of much study with other morpholine-containing analogues of LY294002, and is currently undergoing Phase I human cancer clinical trials.[9] Heteroaromatic nitrogen atoms can also participate in hydrogen bonding to the NH of Val-882 and examples of this class include the Phase I clinical agent NVP-Bez235 (Novartis),[10] where it is believed that the primary H-bond is via the quinoline nitrogen of the imidazo[4,5-*c*]quinoline core of the molecule. Another azaheterocycle that is reported[11] to have entered clinical studies for the treatment of solid tumours is the quinoxaline derivative XL-765 (Chart 2), although few details are available. There is, therefore, high interest in the development of PI3K inhibitors as anticancer agents,[4,11,12] although most of the current compounds are pan-inhibitors, rather than specific inhibitors of p110*α*, the PI3K isoform most often mutated in human cancers.

From its outset in 2005, our programme has thus been focused on the development of more selective inhibitors of p110*α* as anticancer drugs, and began by studying the literature to see where we could make positive improvements.[13] We started with the imidazo[1,2-*a*]pyridine derivative PIK75 (Chart 3), which is a moderately selective inhibitor of p110*α* compared to the other Class I PI3K isoforms (p110*β*, p110δ and p110γ) (Table 2),[14] and is also active in human cancer xenograft models.[15] Our systematic study of the changes to the imidazopyridine chromophore indicated tight structure-activity relationships (SAR),[16] but did lead us to a new chromophore that had both high potency and much higher selectivity for p110*α*. A patent application has been filed on this new class of inhibitor,[17] and we are continuing to optimise the structure. To date, we have been able to retain high potency and improve p110α selectivity.

Table 2. Inhibitory effects of PIK75 on PI3K isoforms.

Compound	IC_{50} (nM) p110			ratio
	-α	-β	-δ	β/α
PIK75	20	300	1000	15

Chart 3



In order to try and rationalize the high p110*α* specificity of the imidazo[1,2-*a*]pyridines and the new chromophore, we studied the binding mode of the known inhibitor PIK75 to p110*α* using a molecular modelling approach.[16] For this work it was necessary to develop a p110*α* homology model,[16,18] since prior to December 2007 structural data were available only for the p110γ isoform.[2] We used the high level of sequence identity shared across the PI3K isoforms around the ATP binding cleft to develop this model.[16,18] As expected, the primary interaction involves an H-bond between the *N*-1 of PIK75 and the backbone NH of Val-851 (equivalent to Val-882 in p110γ) but, in addition, a possible hydrogen bonding interaction between one of the oxygen atoms of the sulfonyl group and the NH of a histidine residue (His-855) was identified.[16] Since this histidine is unique to the p110*α* isoform, it was proposed that the additional interaction could account for the high selectivity of PIK75 against p110*α*. However, in December 2007 the structure for the full length human p110*α* catalytic subunit in conjunction with a portion of its p85*α* regulatory subunit was published,[19] and demonstrated that while most of the ATP binding site residues had a similar 3D structure, there were some notable differences at certain positions. Significantly, the most notable difference from our homology model related to His-855 which was *tied-back* due to an H-bond with Asp-925 and therefore not accessible to the sulfonyl oxygen atoms of PIK75. Whether this is a crystallization artefact or a real phenomenon remains to be determined, but in the interim we are developing a refined model based on this new data.[20]

The second literature lead that we investigated in detail, was the dimorpholino-1,3,5-triazine derivative ZSTK474 (X=N; Chart 3) that is reported to be a reversible and non-selective PI3K inhibitor, but with excellent oral activity against human xenografts in mice.[21,22] This is a very competitive field, with a Japanese patent application filed by

Zenyaku Kogyo Kabushiki Kaisha[23] in 2006 that covers both the triazine and its 2-pyrimidine derivatives (X=CH), where the second morpholine has been replaced by a piperazine group, and a suite of 12 patent applications from AstraZeneca covering a variety of different morpholine replacements, and all three possible pyrimidine isomers.[24] We modelled the binding of ZSTK474 (X=N) in the ATP-binding site of the p110γ crystal structure,[25] and identified a binding mode in which the key H-bond with the NH of Val-882 was with the oxygen atom of one of the morpholine groups, rather than with the benzimidazole nitrogen as proposed,[22] with the latter nitrogen actually H-bonding to the NH_2 group of Lys-833 (the amino group responsible for the irreversible interaction with wortmannin). Our binding model allowed us to design new analogues that are not predicted by the published model, and enabled us to identify several potent new lead structures.

With the exception of wortmannin and its analogues, all of the approaches discussed so far have involved reversible PI3K inhibitors that must compete with ATP for binding in the catalytic site of the enzyme. Irreversible inhibitors have advantages in that they allow for longer-term inhibition of the enzyme, promising greater therapeutic effect, while allowing for longer times between treatments, as shown by the erbB irreversible inhibitor canertinib (CI-1033; Chart 3) that we developed earlier to Phase II clinical trial.[26] Thus, our aim was to develop compounds able to bind irreversibly to the p110*α* site, but only reversibly to the other isoforms. Such specific p110*α* irreversible inhibitors should have better therapeutic potential than pan-PI3K irreversible inhibitors based on wortmannin. Preliminary results suggest this approach is feasible.[27]

Our work in the PI 3-kinase area began in 2005 with in-house funding and support from the government-funded Maurice Wilkins Centre for Molecular Biodiscovery. A successful 2006 HRC grant application, coupled with 2007 support from Auckland's Faculty Research Development Fund, enabled sufficient results to be obtained for the commercialization arm of the University (Auckland UniServices Ltd.) to set up the spinout company *Pathway Therapeutics Ltd*., which has recently successfully raised $A10 million from two Australian-based venture capital companies, CM Capital Investments (Brisbane) and GBS Venture Partners (Melbourne), and the new Trans-Tasman Commercialisation Fund.

Our initial PI3K research team consisted of the authors with cell biologists Bruce Baguley and Elaine Marshall, and biochemist Peter Shepherd. More recent additions to the team include chemistry PhD student Andrew Marshall, biologist Claire Chaussade, molecular modellers Raphael Frederick and Jack Flanagan, pharmacologist Phil Kestell, and technicians Claire Mawson and Mindy Chao. New additions to the team resulting from the Pathway funding are chemists Swarna Gamage, Anna Giddens and Sophia Tsang, and five technical positions are to be filled.

The pharmaceutical development of PI3K inhibitors has taken great strides during the last five years. Several compounds are now in clinical trial, and large amounts of structural and biological data are becoming available. We are hopeful that the future will see even better therapeutic results being achieved with more selective inhibitors.

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Article

Twisting Fate: Ring Torsions and Photochemistry in Aryl-X=Y-Aryl Systems (X,Y = P, C, N)

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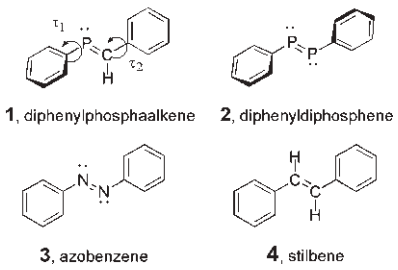
[b]Department of Chemistry, Case Western Reserve University, Cleveland Ohio, USA.

Over the past 30 years or so, the design and synthesis of molecules has been transformed by chemists increasingly interested in imparting controllable functionality to their molecular architectures. One of the most widely exploited control processes is $E \rightarrow Z$ photoisomerization, particularly in azobenzenoid (Ar-N=N-Ar) and stilbenoid (ArCH=CHAr) molecules. Molecular switches, memory elements, capacitors, sensors, modulators of liquid crystal optical properties, organic light-emitting diodes (OLEDs), and other photon-driven materials have been explored with success.[1] The patent literature abounds with hundreds of photoisomerization-based ideas, from portable body warmers[2] to solar energy collectors and storage elements.[3]

As development of these systems continues, the exploration of the relatively untravelled regions of the periodic table in search of new molecules, materials and reactions continues. Phosphorus was long thought to eschew multiple bonds. In fact, this *double bond rule* once applied to all elements beyond the first row of the periodic table, where carbon, nitrogen and oxygen are so prolific in forming multiple bonds. In 1981, however, Yoshifuji and co-workers reported the first stable diphosphene (P=P) molecule.[4] Since then, well over 100 compounds with multiple bonds involving atoms such as Si, Ge and As have been reported.

Multiple bonds among heavier main group elements tend to exhibit higher reactivity than their carbon and nitrogen cousins. However, with sufficiently bulky ligands, multiple bonds involving P, As, Si, Ge, and others can be successfully stabilized kinetically and studied. Knowledge of the synthesis, structures, coordination chemistry and reactivity of these compounds is at a relatively advanced stage, and has been reviewed recently.[5-9] Less thoroughly investigated, but equally important for understanding and exploiting photocontrollable behaviour, is the photochemistry and photophysics of these systems.

Chart 1



Recently, we began a productive collaboration with a group that synthesizes diphosphenes (P=P) and phosphaalkenes (C=P) for use in photochemically active materials. As a natural first step towards a thorough understanding of their photochemistry and photophysics, we applied both theoretical chemistry and ultrafast spectroscopy to study the ground and electronic excited states of some of these promising new molecules. Our previous studies have shown that the phenyl twist angle in aryl-substituted diphosphenes has a significant impact upon the ordering of the frontier orbitals and the oscillator strength of the major ultraviolet-visible (UV-vis) absorption transitions.[10] We have now extended those studies to aryl-substituted phosphaalkenes, and compare both the ground state isomerization (Fig. 1) and phenyl twist barriers (Fig. 2) to those of P=P, C=C ,and N=N analogs. All of the model compounds studied here (Chart 1) either exist and undergo photoisomerization (stilbene[11], azobenzene[12]) or possess experimental analogs known to undergo photoisomerization.[13-16] The results shed light on important differences in bonding among the main group elements, and help build a useful framework within which to interpret ongoing and future photochemical and photophysical research.

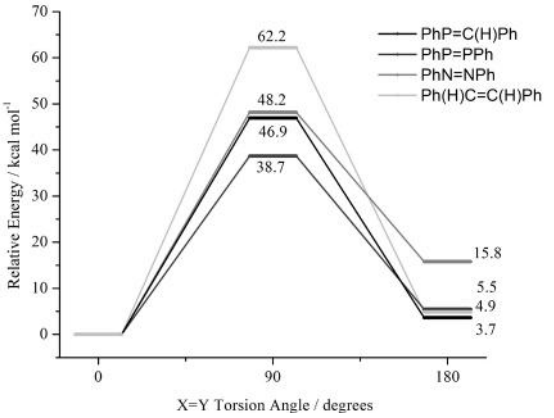


Fig. 1. ΔE(*E-Z*) and rotational barriers for torsions about the central X=Y double bond, where X,Y = C, N, P; calculations at the B3LYP/6-31+G** level.

Computational Methods

All computations were performed with the Gaussian 03 (rev.D.01) software package[17] with density functional theory (DFT) using the B3LYP hybrid functional[18] and 6-31+G** basis set.[19] This level of theory was chosen as a reasonable balance between computational expense and accuracy. The discussion herein focuses upon ground state structures and energies, and vertical transitions to lower-lying excited states, and not chemical reactivity, *i.e.* no bond breaking or forming processes, and no

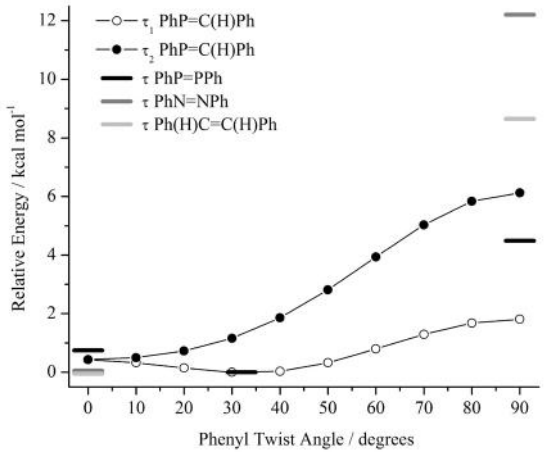


Fig. 2. Rotational barriers for torsions about the X-Ph single bond (X = C, N, P); calculations at the B3LYP/6-31+G** level with τ_1 referring to torsion about the P-Ph bond and τ_2 to torsion about the C-Ph bond.

intermolecular interactions. The B3LYP hybrid functional, by far the most widely used in recent times, performs well for the target outcomes in main-group chemical systems of similar sizes and complexity.[20]

Many experimental diphosphenes and phosphaalkenes attach phenyl-based bulky groups to the phosphorus (and carbon) atoms to kinetically stabilize the double bond. PhP=CHPh was thus chosen to include authentic molecular features such as conjugation and π-interactions, and to approximate steric interactions. Ground state structures were calculated for PhP=CHP with appropriate constraints on the phenyl ring twisting coordinates, followed by time dependent DFT calculations (TDDFT)for the excitation energies, *i.e.* TDDFT-B3LYP/6 31+G**//B3LYP/6 31+G**. The potential energy surface was calculated at 10° intervals along the τ_1 (C=P-Ph) and τ_2 (P=CHPh) twist coordinates (see Fig. 1). The ground state (S_0) *E* (*trans*) to *Z* (*cis*) isomerization barrier was also calculated. The results are compared to findings from previous studies on PhP=PPh, PhCH=CHPh, and PhN=NPh at the same level of theory.

Aryl-X=Y-Aryl Bonding

Background

One area of critical interest is the nature of the heavier main-group multiple bond and its effect upon structures, properties, and reactivity.[5,6,7,21,22] From the relative energies of the σ and π bonds in homonuclear double-bonded species (Table 1),[5,24] first row atoms clearly behave differently from those in lower rows of the periodic table. While the π-bond is of comparable or greater strength than the σ-bond for C, N and O, heavier main group elements have relatively weak π bonds. While chemists tend to think of the first long row of the periodic table as the well-behaved one, it is actually the anomaly.[21] Carbon is perhaps the epitome in this respect with nearly equal σ and π bond strengths (σ/π ratio = 1.14; the others vary from 1.41 for P to 1.73 for S) and this is the origin of the diversity of the stable compounds that it forms.

Table 1. Relative σ/π bond energies for homonuclear diatomics (kcal/mol)[a]

C=C 80/70	**N=N** 38/94	**O=O** 35/83
Si=Si 46/28	**P=P** 48/34	**S=S** 64/37
Ge=Ge 39/26	**As=As** 42/29	**Se=Se** 50/30

[a]Data taken from reference 21.

The next closest σ/π ratio to carbon belongs to phosphorus. This is a manifestation of the increasingly apparent diagonal relationships in the periodic table. Phosphorus compounds often behave more like their carbon analogs than their directly vertical nitrogen neighbors.[25] Similarly, carbon resembles phosphorus more than silicon in many ways. For example, the electronegativities of carbon and phosphorus are comparable at 2.5 and 2.2, but quite different from those of Si (1.7) and N (3.1).

A classic analysis of bonding in heavier main group elements invokes relatively poor hybridization of orbitals to account for differences between first and later row compounds, including the greater propensity of first row elements to form multiple bonds, and for heavier elements to form hypervalent compounds.[21] The commonly assumed fixed relationship between the degree of hybridization, *i.e.* s and p contributions to the molecular orbitals, and bond angles is not justified beyond n = 2.[22] So why do heavier main group elements hybridize more poorly? Efficient hybridization occurs when the constituent atomic orbitals have similar energies and large spatial overlap. The relative energies of the atomic orbitals actually favour *more* hybridization for the heavier elements. The culprit is the very different valence s and p radial probability distributions for the first row compared to the others with s and p valence orbitals have approximately the same radial extent; the heavier elements have the valence p orbital extend significantly beyond its s orbital partner. Thus, the degree of hybridization decreases and the lone pair adopts more s character going down the periodic column.

Aryl-X=Y-Aryl

The geometries predicted by the level of DFT used by us are remarkably good. For example, the C=P bond length calculated (1.695 Å) compares favourably to that in the crystal structure of (*E*)-(4-Br-2,6-$Mes_2C_6H_2$)-P=CH(4-BrC_6H_4) (1.682 Å), as do the P-C and C-C bond lengths and bond angles in the C-C=P-C central unit.[13] Similarly, the calculated P=P bond length of 2.058 Å is quite similar to the experimental values of 2.046 Å and 1.985 Å for bis(2,4,6-$Bu^t_3C_6H_2$)diphosphene[4,24] and bis(2,6-$Mes_2C_6H_3$)diphosphene,[26] respectively; bond angles also are reproduced well.

The *E*-isomer is the lower energy structure for all four molecules studied (Fig. 1). (*Z*)-PhP=CHPh is destabilized the least, with ΔE = 3.7 kcal/mol; (*E*)-PhN=NPh is destabilized the most relative to its (*E*)-isomer (ΔE = 15.8 kcal/mol). The barriers to rotation about the double bond can provide an estimate to the π bond strength, though the

results here point to some weaknesses inherent in using DFT for such evaluations. Fig. 1 indicates that the weakest π bond is found in the P=P molecule. The strongest is predicted to be C=C by a significant margin in contrast to what is predicted in Table 1. Finally, the π bond in PhP=CHPh is predicted to be of similar strength to that of PhN=NPh. Thermal isomerization rates have been observed for a few diphosphenes ($Z \rightarrow E$: $\Delta G‡$ ~20 kcal/mol at 0 °C,[16] $\Delta H‡$ = 29.5 ± 1.4 kcal/mol, and $\Delta S‡$ = 38 ± 6 cal/mol/K for a similar diphosphene[14]). The relatively large activation entropy may be due to steric crowding that is relieved by isomerization. Niecke *et al*.[27] have synthesized a diphosphene that equilibrates at 25 °C to a 6:11 mixture of *Z*- and *E*-forms, with thermal isomerization rates for both processes of ~10^{-7}/s.The barrier height calculated here for the diphosphene is qualitatively consistent with these experimental measurements, though the experimental systems have much bulkier ligands.

Unfortunately, the order of the barrier heights for isomerization calculated by B3LYP is not consistent with the π bond strengths presented in Table 1. Most likely this is due to well-known difficulties in calculating the transition states of these molecules with single-reference methods; the transition states possess considerable biradical character.[28] CASPT2//CASSCF calculations are underway in our lab to better address these issues, and to examine the features of the ground state potential surface with greater fidelity. Of additional interest to the ground state rotational barrier is the thermal inversion coordinate and energetics. We are also using complete active space calculations to evaluate the excited states along these reaction coordinates.

The π orbitals of the phenyl ring substituents have the capability of interacting with the central double bond, to varying extents, in these compounds. The barrier height to twisting the phenyl ring gives an indication, albeit qualitatively, of the extent of π delocalization across the molecule (Fig. 2). The potential energy surface along the >C=PPh torsion (τ_1) and -P=CHPh torsion (τ_2) angles is shown in detail. Endpoint values of the other species discussed in this study are provided for ease of comparison.[10]

The ground state *E*-isomers of both PhN=NPh and PhCH=CHPh have planar minimum energy configurations of C_{2h} symmetry. In contrast, the Aryl-X=Y=Aryl molecules containing phosphorus are not planar. The phenyl ring attached to the P atom is twisted between 30º and 35º for both PhP=PPh and PhP=CHPh. In the latter species, there is a slight phenyl twist (6º) about the C-Ph bond as well.

The lowest energy structures of these molecules reflect an intramolecular balance between the conjugation of the π system across the molecule that drives towards molecular planarity, and steric repulsions that induce non-planar configurations. In this case, phenyl twisting relieves repulsions between the large third-shell phosphorus lone pairs and phenyl *ortho*-hydrogens. These steric repulsions are further exacerbated by the small (ligand)-P=X angle (103º in both molecules), that is a consequence of the less effective sp^2 hybridization in heavier main group systems, as discussed above. The equivalent angles in PhCH=CHPh and PhN=NPh are much closer to 120º, as is the (ligand)-C=P angle in the phosphaalkene studied here (127º). The energies at $\tau = 90º$, where the phenyl ring is orthogonal to the central X=Y bond, indicate that the conjugation across the molecule is reduced for the phosphorus-containing molecules as well. In experimentally characterized diphosphenes and phosphaalkenes, the use of bulky groups to protect the reactive double bond leads to a wide range of phenyl twist angles, most of which are greater than 45º. Our calculations confirm the suggestion that phenyl twisting provides an energetically thrifty path along which the molecule can relieve steric stresses. Furthermore, the small barriers indicate that at room temperature, the P-phenyl substructure samples a wide range of twist angles in solution.

Frontier Orbitals, UV-vis Transitions, and the Phenyl Twist

Previous studies in our group have found that the photochemically important order and energetic splitting between the two occupied frontier orbitals is exquisitely sensitive to twisting of the phenyl substituent rings relative to the C-P=P-C plane of a diphosphene.[10] The transition energies and intensities of the first and second singlet states (S_1 and S_2) also depend upon the phenyl twist angle in diphosphenes and the possibility of analogous behaviour in the PhP=CHPh system demanded examination.

The situation in PhP=CHPh is a bit more complicated as more orbitals become involved and the degrees of freedom (τ_1 and τ_2) for the two phenyl ring twists are no longer identical. In the fully planar PhP=CHPh, the HOMO is a π– orbital, with bonding character across the P=C and an antibonding relationship between the P=C and the phenyl rings (Fig. 3). The next two orbitals are e_{1g} π orbitals

π-(e_{1g}+e_{1g})	e_{1g}	e_{1g}	n	π*+(e_{1g}-e_{1g})
(HOMO)	(HOMO-1)	(HOMO-2)	(HOMO-3)	(HOMO-4)

Fig. 3. The molecular orbitals most important for the $S_1 \leftarrow S_0$ and $S_2 \leftarrow S_0$ transitions; calculations at the B3LYP/6-31+G** level.

localized on the phenyl rings; they are largely insensitive to the distortions cause by the phenyl twists. The n-orbital associated with the P-atom is HOMO-3. The last orbital of interest here is the HOMO-4, a π*+ orbital that has antibonding character at the P=C double bond, and bonding interactions with the phenyl rings.

As the phenyl ring attached to the P-atom is twisted, the π– and π*+ orbitals are significantly stabilized whilst the n orbital is destabilized. At planarity (0º twist), the n and π*+ orbitals are within 0.2 eV of one another (Fig.4). However, when orthogonal (90º twist), they have moved to *ca*. 1 eV difference in energy, with the π*+ being the energetically much more stable orbital. Furthermore, the π– (HOMO) and n orbitals have moved considerably closer in energy to one another, though π– is still the HOMO by about 0.8 eV. The π– orbital remains the HOMO throughout the full range of τ_1 distortion from 0º to 90º. The P=CHPh moiety was held planar throughout this rotation for all calculations.

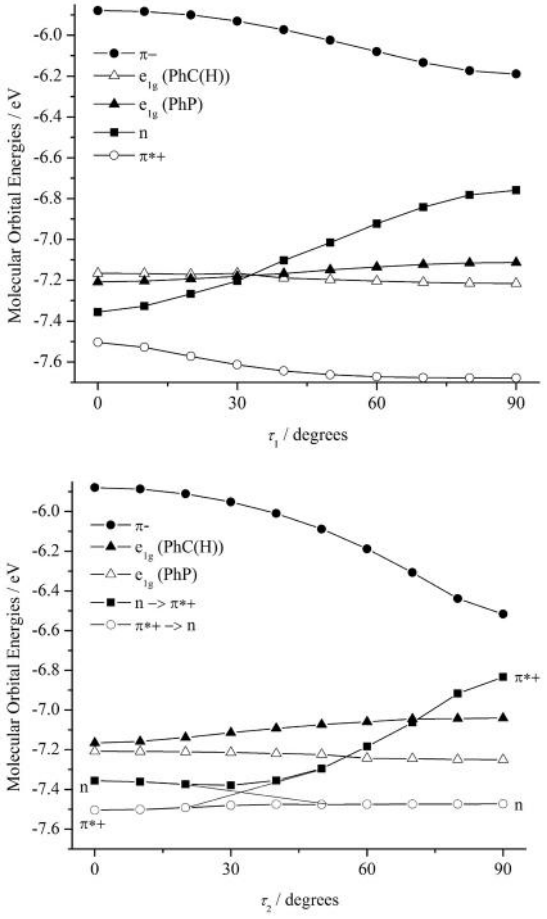


Fig. 4. Molecular orbital energies vs τ_1 and τ_2; calculations at the B3LYP/6-31+G** level.

The TDDFT-B3LYP/6-31+G**//B3LYP/6-31+G** calculations illustrate the impact of twisting the phenyl groups upon the two lowest energy electronic excitations (Fig. 5). For the planar molecule, the S_1 state is assigned to a π-π* transition (3.42 eV; 362.5 nm; f = 0.611), and the S_2 to the n-π* (4.042 eV; 306.7 nm; f = 0.037).[13] Under the point group symmetry of the molecule, the n-π* is formally forbidden. However, it gains intensity by mixing with the nearby π-π* orbital. The experimental electronic absorption spectrum for a phosphaalkene with aryl groups at the C and P positions shows a broad peak at about 350 nm, in excellent agreement with the calculations.

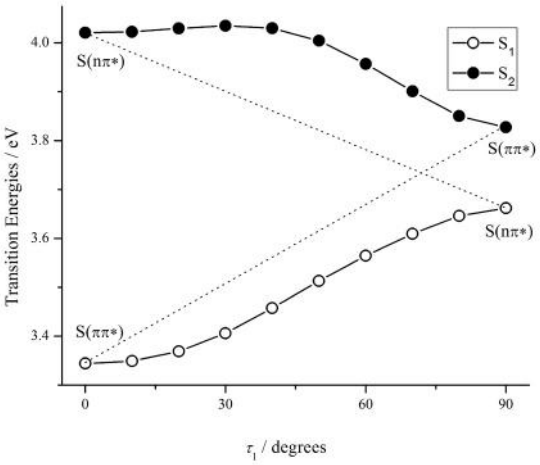


Fig. 5. Excited state (S_1 and S_2) energies *vs* τ_1 and τ_2; calculations at the TDDFT/6-31+G**//DFT/6-31+G** level.

Twisting the phenyl ring attached to the P atom leads to enhanced interaction between the two transitions, and eventually a swapping of the order of the states at $\tau_1 \sim$ 70º. In highly distorted systems, then, n-π* is expected to become the lower energy state but this may be difficult to see experimentally as it is expected to have the weaker intensity. Twisting the =CHPh phenyl ring at the other end of the molecule (τ_2) while holding the PhP=C architecture planar leads to somewhat different behavior. The most significant dissimilarity is a strong mixing between the n and π*+ orbitals that begins at around τ_2= 20º. By τ_2 = 90º, the orbitals have nearly exchanged places. The HOMO-1 orbital is now mostly a π*+ orbital with strong polarization to the phosphorus lone pair, while HOMO-4 is dominated by phosphorus n contributions that are strongly polarized towards the phenyl ring. When the phenyl and central C=P units are orthogonal, the π– (HOMO) and π*+ (HOMO-1) orbitals are within a few tenths of an eV of one another.

In addition to the mixing that occurs during phenyl rotation at the carbon, Fig. 4 also shows that HOMO stabilization is more dramatic for distortion along τ_2 than for displacement along τ_1. That is, twisting the phenyl ring attached to the carbon has a larger impact upon π-bonding between the C and P than does twisting the phenyl ring attached to the phosphorus. This effect is probably due to the uneven contributions of carbon and phosphorus to the π-bonding interaction. The impact on the UV-vis absorption spectrum of twisting along τ_2 is significantly more complicated than that observed for the phenyl bound to the phosphorus (not shown), and is not yet fully understood. The S_2 (nπ*) state appears to have an avoided crossing with S_3 whilst the S_3 state (e_{1g}π*) is in the 0º < τ_2 < 50º region before it crosses the S_2 (nπ*) state ($\tau_2 \sim$ 75º) to become the HOMO when the phenyl ring is fully orthogonal. The S_1 and S_2 transitions mix, and eventually exchange their order so that at τ_2 = 90º the S_1 state is nπ* in nature. At about the same phenyl twist angle, the S_1 (ππ*) transition shifts to shorter wavelengths and eventually crosses the state just above it. Up until now this

complex behaviour has not been thoroughly interpreted, and studies are underway to gain further insight.

One important and unique feature of diphosphene systems is that the S_1 state does not primarily reflect an excitation from HOMO to LUMO.[10] In the phosphaalkene model studied here, this effect is not observed, save for the larger phenyl twist angles. In the diphosphenes, the effect is due largely to stabilization of S_1 through participation by transitions from the lower energy phenyl ring π orbitals to the π* LUMO. As one might expect, this interaction is quite sensitive to the twist angle of the phenyl ring. In addition, the changes in the UV-vis absorption spectrum caused by phenyl twisting and predicted by the DFT and TDDFT computations of diphosphenes are mirrored in experimental observations across a wide variety of aryl-substituted diphosphene molecules.

Speculations Concerning Phenyl Twists and Photochemical Fates

It is well known that the frontier orbitals play a significant role in determining the chemical and physical fate of photoexcited molecules.[29] Thus, the potential impact of the phenyl twist upon diphosphene and phosphaalkene photochemistry is significant. These molecules exhibit a rich diversity of photoprocesses. Diphosphenes have been shown to undergo many photoinduced reactions, including dimerization, metathesis, intramolecular ring formation, and cleavage.[8,13-16,27-30] Of particular interest is the photoactivated *E*-to-*Z* isomerization reaction that has attracted so much attention for stilbenes and azobenzenes. Clearly, photoinduced isomerization occurs in some diphosphenes, and the *Z*-isomer of Mes*P=PMes* has been trapped by irradiation of the *E*-isomer under low temperature conditions.[14,15] However, it is not clear whether all diphosphenes photoisomerize, how the isomerization occurs, or its role in subsequent photochemistry.[14] Phosphaalkenes also exhibit diverse photochemical behaviour, most notably in undergoing photoisomerization.[13]

The most natural approach to understanding these exciting new systems would be to build upon the extensive body of literature exploring stilbene and azobenzene photoisomerization. However, what these previous studies mean for diphosphene and phosphaalkene photochemistry is unclear. The cognate electronic configurations of nitrogen and phosphorus suggest that photoisomerization in diphosphene is likely to mirror that of azobenzene. However, the diagonal relationships in the periodic table engender many surprising similarities between the properties of carbon and phosphorus compounds.[25] There is evidence of this in the present study as the barrier to torsion about the central double bond follows the nice trend of PhCH=CHPh > PhP=CHPh > PhP=PPh. Similarly, in diphosphenes the thermal isomerization barrier for inversion is significantly higher than that for rotation,[10,28] while just the opposite is true for azobenzenes.

The relatively poor hybridization in heavier main group compounds, and the differences in relative π and σ bond strengths discussed above, offer uncertainty in predictions based upon similarities to compounds derived from the first long row of the periodic table. While these issues have been considered extensively in reactivity and structure studies, their photochemical and photophysical consequences remain to be addressed. How does one think about photochemistry in molecules where *e.g.* a π bond can be weaker than a σ bond? What rules govern excited state geometries, barriers, and dynamics? What are typical excited state lifetimes, and what affects them? How do the photophysics and photochemistry of these systems relate to C=C- and N=N- counterparts? Do the diagonal relationships of the periodic table extend also to photodynamics? The studies reported here and elsewhere represent an important first step in addressing these critical issues.

Acknowledgment

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Dates of Note

Jan 17 marks the 50th anniversary of full energy release by the first synchrotron, which was installed at the Radiation Laboratory, Berkeley. On 19 Jan 1894, Prof *James* ***Dewar*** exhibited several properties of liquid air, and produced solid air, at the meeting of the Royal Institution. Jan 20 marks the 175th anniversary of the birth of *Adolf* ***Frank*** who invented the brown coloured beer bottle to preserve the ale.

Jan 22 marks the day 70 years ago when the uranium atom was split for the first time using the Columbia University cyclotron in New York; it marked the start of the Manhattan Project that gave us the atom bomb.

German-born *John* ***Polanyi***, who shared the 1986 Nobel Prize for Chemistry (with Dudley R. Herschbach and Yuan T. Lee) for contributions to the *development of a new field of research in chemistry – reaction dynamics*, has his 80th birthday on Jan 23. Jan 25 is the 35th anniversary of ***Barnard***'s pioneering transplant of the first human heart.

100 years ago on Jan 26 *Alexander* ***King***, the Scottish chemist who pioneered in environmental awareness, was born. He warned of the dangers to the environment from extensive industrial development. Jan 29 marks the 75th anniversary of *Fritz* ***Haber***'s death. He won the 1918 Nobel Prize for Chemistry (1918) for his ammonia synthesis. The 30th marks 50 years since the first use of a pacemaker.

On Feb 1 1959, Texas Instruments was issued a patent on the integrated circuit. *Dmitry Ivanovich* ***Mendeleev*** was born 175 years ago on Feb 8 while Feb 13 marks 175 years since the birth of *Heinrich* ***Caro***, Technical Director of Badische Anilin & Soda Fabrik; he commercialized alizarin and indigo amongst others. *James* ***Cook*** died on Feb 14, 230 years ago.

The 150th anniversary of *Svante August* ***Arrhenius***'s birth is on Feb 19; he received the 1903 Nobel Chemistry Prize. Sir *Earnest* ***Marsden*** was born on the same day in 1889.

Johannes Nicolaus ***Brønsted***, the Danish chemist known for the acid-base concept, was born 130 years ago on Feb 22 and *Heinrich* ***Hertz*** the same day in 1857. It is also the day in 1828 that *Friederich* ***Wöhler*** informed Berzelius that he had synthesized urea. Feb 23 marks 55 years since the first mass inoculation with Salk polio vaccine was performed.

Feb 25 is the 10th anniversary of *Glen T*. ***Seaborg***'s death; he was co-recipient of the 1951 Nobel Prize for Chemistry. The day also marks the birth of *H. H*. ***Dow***, founder of Dow Chemical Company in 1866 and 180 years since the birth of *Levi* ***Strauss***. Feb 27 marks 130 years since the discovery of saccharin, the artificial sweetener.

The first push-button telephone was put to test on 2 Mar 1959. The American biochemist *Elmer* ***McCollum***, who originated the letter system of naming vitamins, was born on 3 Mar 1879 while *Gerhard* ***Hertzberg*** died 10 years ago this day.

Dmitry ***Mendeleev*** published his first version of the periodic table on Mar 6, 1899. ***F. M***. ***Crafts***, of Friedel-Crafts fame, was born on Mar 8, 1839; ***Friedel*** was born on Mar 12, 1832. Mar 14 marks 130 years since the birth of *Albert* ***Einstein*** and Mar 15 is the 5th anniversary of *John* ***Pople***'s death; he devised the Gaussian suite of programmes.

Sir *Derek* ***Barton*** died 11 years ago on Mar 16. Mar 20, 75 years ago, saw the first test of a practical radar apparatus. On Mar 21, 1925, *Wolfgang* ***Pauli*** published his exclusion principle. 23 Mar 1989 saw the cold fusion announcement by electrochemists *Martin* ***Fleischmann*** and *Stan* ***Pons***.

Mar 24 marks the 125th anniversary of the birth of *Peter* ***Debye***, the Dutch physical chemist most noted for dipole moments and the unit of measurement named after him. *Johann* ***Döbereiner*** died on the same day in 1849; his observation of similarities among certain elements anticipated the development of the periodic system.

1 Apr deaths include *François-Marie* ***Raoult*** (1901) and Dame *Kathleen* ***Lonsdale*** (1971) and Apr 3 has to be noted as the 75th anniversary of the granting of a British patent to *Percy* ***Shaw*** for the *catseye road marker*. 6 Apr 1954 was the first day of sale of the TV dinner.

10 Apr marks the 55th anniversary of ***Woodward*** and ***von Doering***'s synthesis of quinine. The 12th is the 125th birth date of *Otto* ***Meyerhoff*** who is known for his work on carbohydrate metabolism and the working muscle; he gained the 1922 Nobel Prize for medicine.

Apr 14 would have marked the 80th birthday of *Alan* ***MacDiarmid***.

Article

The Oxidation of Red and White Wines and its Impact on Wine Aroma

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Introduction

The oxidation of wines has quite different consequences for red and white varieties, although the underlying chemistry is similar.[1,2] Oxygen additions are usually required in the maturation of red wines prior to bottling, to enhance wine quality (through the removal of unwanted aromas), to stabilize colour and to improve mouth feel, but it is difficult to predict the optimum level of oxygen exposure. On the other hand, oxygen additions seldom improve white wines where preservation of fruity aromas is sought, and where oxidative browning can detract from the appearance of the wine. This article summarizes the chemistry behind wine oxidation with a focus upon polyphenol-mediated processes and how these impact upon aromas in red and white wines.

Oxygen in Wine

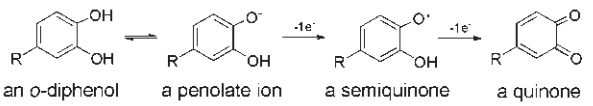
It is inevitable that wines are exposed to O_2 at various stages of production. Air-saturated wine can take up to 6 mL/L (8.6 mg/L) of O_2 at room temperature, with greater solubility at a lower temperature. Larger doses are supplied to red wines during deliberate pump-overs, while slower rates of O_2 ingress occur for wines in barrels. For example, while mixing wines from different casks was found to raise the O_2 concentration to around 1.8 mg/L, racking of a wine at 15-20 °C produced an O_2 concentration of 0.4 mg/L, but this value increased three-fold when the temperature of racking was lowered to 10 °C.[3] An alternative to barrel aging is the new technology of micro-oxygenation now commonly used with red wines. This involves continuous, slow bubbling of oxygen into the wine for several weeks at a rate of a few mL of O_2/L of wine per month. Under these conditions the dissolved O_2 has been measured at 0.2 to 0.25 mg/L.[4]

Once a wine is bottled it might be expected that oxygen is largely excluded, but wine closures vary considerably in how much O_2 they allow into the wine. Synthetic plastic corks allow the entry of larger amounts of O_2 to enter the wine than natural corks and screw caps and are thus best suited for wines that are to be consumed soon after bottling. The effects of closure type upon the colour and aroma in trials on red and white wines are referred to below. The conditions used for bottling are also very important, as the small headspace above the bottled wine can contain a few mg of O_2,[5] equivalent to several months of the oxygen entry through the closure, unless a special vacuum or inert gas system is used on the bottling line.

The Oxidation of Wine Polyphenols

There are many organic compounds in wine that are potential targets for oxidation processes. These range from ethanol itself through to various acids [tartaric acid (**1**) being the major wine acid – see Chart 1 and aroma compounds, but these are not, in fact, the main initial substrates of oxidation. An important finding in the research undertaken by Vernon Singleton (UC Davis) in the 1970s, was that ethanol oxidizes to acetaldehyde at a significant rate only through the coupled oxidation of readily oxidizable polyphenols such as caffeic acid (**2**, typical of white wine hydroxycinnamic acids) and catechin (**3**, a flavanol at high levels in red wine – see Chart 1).[6] Without these polyphenols ethanol and tartaric acid are remarkably stable to oxidation. The oxidation of polyphenols generates a strong oxidant, presumed to be H_2O_2, that can oxidize other substances in wine such as ethanol.

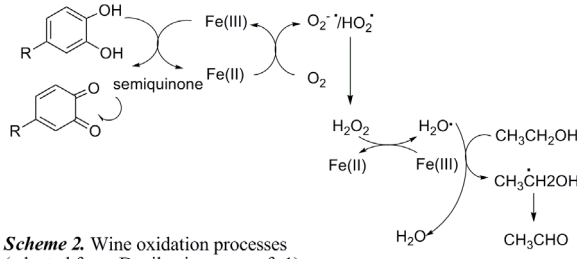
Wine polyphenols containing a 1,2-diphenol (an *o*-catechol group), such as **2** and **3**, can be oxidized through to quinone forms easily as shown in Scheme 1. Model studies have shown that in solution this process is more rapid at a higher pH, due to a higher percentage of the phenolate that reacts with oxygen.[7] Only a small proportion of phenolate ions are expected at wine pH (pK_a polyphenols *ca*. 9-10), but many more will be present in a pH 4 wine than a pH 3 wine, consistent with higher pH wines being more susceptible to oxidation problems. It has also been shown that one of the subsequent reactions of the quinones formed is with remaining polyphenols and leads to brown products, but the process regenerates the catechol group making it available for further oxidation. Overall, more oxygen is taken up than would be expected given the original number of polyphenol molecules present.



Scheme 1. Oxidation of polyphenols

Oxygen itself is a triplet, and requires activation of some form before it can be reduced progressively to hydroperoxyl radical ($HO_2^{\bullet}$), hydrogen peroxide (H_2O_2), the hydroxyl radical ($OH^{\bullet}$), and eventually H_2O. In wines, the activation of oxygen is thought to involve catalysts, particularly iron and copper as these complex O_2 and facilitate the oxidation process with polyphenols (Scheme 2).[8] In the coupled oxidation process, Fe(II) converts H_2O_2 to the very reactive $OH^{\bullet}$ (the Fenton reaction) that oxidizes most organic compounds, including ethanol to acetaldehyde and glycerol to glyceraldehyde, *etc*.[9]

Polyphenols containing a 1,2-diphenol (an *o*-catechol moiety) or a 1,2,3-triphenol (a galloyl group) are the most easily oxidized, and show the lowest oxidation-reduction potentials in a model wine solution measured at a glassy carbon electrode.[10] The current peak in cyclic voltam-



Scheme 2. Wine oxidation processes (adapted from Danilewicz - see ref. 1)

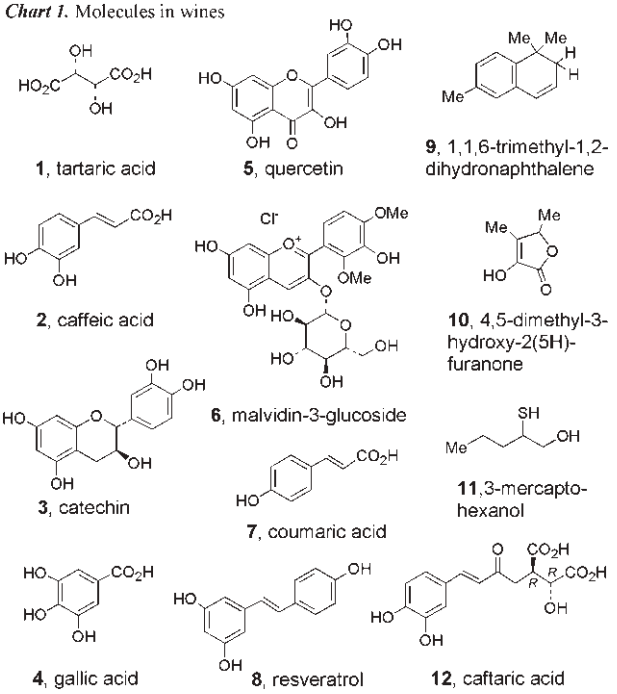
mograms for common wine polyphenols such as **2**, **3**, or gallic acid (**4**), and quercetin (**5**; Chart 1) is seen at a similar potential, *ca*. 0.4 V (*vs*. Ag/AgCl), as is the main current peak for diluted red and white wines. This further confirms that such polyphenols are the main initial substrates in wine oxidation.[11] Integration of the current peak can quantify the level of catechol- and galloyl-containing polyphenols in wine.[10-12] Further compounds, such as the malvidin anthocyanins (see **6**), the major coloured species in red wines, and compounds with more isolated phenolic groups, such as *p*-coumaric acid (**7**) and resveratrol (**8**; Chart 1), are oxidized at higher potentials. However, despite their lower ease-of-oxidation, anthocyanins such as malvidin-3-glucoside (**6**) degrade faster in wine than, *e.g.* **2** or **7**, the catechol-containing hydroxycinnamic acids,[13,14] as other reactions involving the anthocyanins come into play, including the formation of bridges between the polyphenol moieties.

The aldehydes produced by coupled polyphenol oxidation, and through yeast activity, have important roles in wine aging. They provide links between various flavonoid polyphenols (including anthocyanins) to produce new polymeric pigments that, explain the change in red wine hue with age.[15] These components are often more stable than the anthocyanins that they are formed from and are resistant to bleaching by the bisulfite added as a wine preservative. There is considerable current interest in the way in which anthocyanins combine with wine tannins (larger oligomeric and polymeric polyphenols made up of catechin-type units) and lower the astringent effect of the tannins. Such studies help explain the *softening* of red wine astringency with age, an important area of sensory science where the underlying chemistry is still poorly understood.

Oxidation and Effects on Wine Aroma

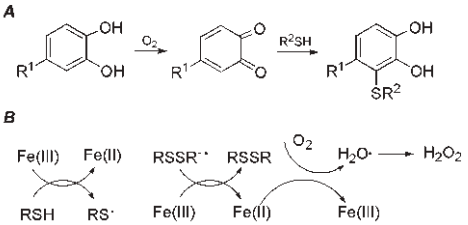
A range of off-odours can be formed from wine oxidation.[16-18] At low concentrations these may add to the complexity of a wine, but as these increase they begin to detract from wine quality. Some examples of the compounds associated with sensory terms for aged wines such as *farm-feed* and *woody-like* include phenylacetaldehyde ($PhCH_2CHO$), 3-(methylthio)propionaldehyde ($MeSCH_2CH_2CHO$), 1,1,6-trimethyl-1,2-dihydronaphthalene (**9**; responsible for the *kerosene* odour in aged Riesling) and 4,5-dimethyl-3-hydroxy-2(5*H*)-furanone (**10**).[17] At the same time, the concentration of acetaldehyde itself does not always increase markedly during wine oxidation experiments, and it is recognised that many important wine oxidation aromas remain to be identified.[16]

Chart 1. Molecules in wines



Alongside the production of new odours, wine oxidation can lead to the removal of existing aroma compounds, particularly those containing sulfur. This can be a positive development, as many sulfur-containing compounds produce unwanted aromas reminiscent of rubber or cooked cabbage.[19] Winemaking processes involving the introduction of O_2 to wine (as in racking) provide the first means for their removal, while fining with copper salts is also used. At the same time, there are sulfur-containing compounds present that add to the varietal character of the wine, but these may be lost through oxidation processes. These include 3-mercaptohexanol (3MH, **11**) which provides important grapefruit and passion fruit-type aromas in Sauvignon Blanc and other wines.[20]

One mechanism proposed for the removal of sulfur-containing compounds is by reaction with the quinones formed during polyphenol oxidation (Scheme 3A). Experiments exposing catechol-containing polyphenols to oxygen show losses of **11** consistent with a polyphenol-mediated oxidation mechanism.[21,22] The oxidation of thiols to disulfides (Scheme 3B) has also been suggested as a possible pathway.[19,23,24] In one recent survey of wines of different ages, the tendency towards higher levels of dimethyl disulfide (MeSSMe) and diethyl disulfide (EtSSEt) in the older wines was seen as implicating disulfide formation during aging.[25] The rapid reaction of the thiol-containing amino acid cysteine in the presence of O_2, Fe(II) and Cu(II) has also been ascribed to the metal-catalysed oxidation of thiols as shown in Scheme 3B.[22] However, while the addition of O_2 was seen to lower the concentrations of methane and ethane thiols in a micro-oxygenation study, no disulfides were seen.[26] Thiols with low sensory thresholds potentially can be released from disulfide forms by reduction with bisulfites in wine,[27] or through the hydrolysis of thioacetates.[24] However, there is a lack of experimental data on the effects of oxidation upon sulfur-containing compounds, and research is being undertaken in this area at the University of Auckland.



Scheme 3. Oxidation of S-containing compounds in wine; ***A:*** polyphenol-mediated; ***B:*** metal-catalyzed thiol oxidation (adapted from Danilewicz - see ref. 24)

Influence of Wine Antioxidants

In addition to controlling the rate of O_2 entry into a wine, winemakers can make use of antioxidants to control oxidation, using those already present in the grape juice, such as glutathione, or through added SO_2 (bisulfite in solution) and ascorbic acid. SO_2 is almost universally used in modern winemaking at levels of 20 mg/L or more of free SO_2 (and to 100 mg/L or more of total SO_2 once forms bound to acetaldehyde and other compounds are included). Sulfites are added to grape juice to inhibit the rapid oxidation caused by polyphenol oxidase activity.[28] Here it can act as a scavenger of H_2O_2 formed from further oxidation processes, but it does not react rapidly with O_2 itself.[1] On the other hand, SO_2 has a further role in the rapid reduction of oxidized polyphenols,[29] thus removing polyphenol quinones from further browning and aroma degradation processes.

Related protection is provided in grape juice and young wines by the presence of free glutathione at 30 to 100 mg/L with the actual concentration being dependent upon the pressing conditions used.[30] An important role for glutathione in white grape juice is to react with the quinone formed from the main hydroxycinnamic acid, caftaric acid (**12**), to produce an S glutathionyl caftaric acid, which is more stable to enzymatic oxidation and limits the browning of the juice.[28] Glutathione also appears to have a protective role in wines by reacting with oxidized polyphenols in preference to varietal aroma compounds such as thiol **11**, or other polyphenols.[31]

There has been some interest in finding replacements for SO_2 additions in winemaking owing to potential health-problems in sensitive individuals and ascorbic acid has been considered. As the dienol moiety is readily oxidized[1] by O_2, it can be used for its direct removal, a role that is not ascribed to SO_2 or glutathione. However, ascorbic acid additions to wine have a controversial history in that certain pro-oxidative effects have been observed and ascribed to the formation of H_2O_2 or other reactive oxygen species following the initial antioxidant activity. This is analogous to the polyphenol oxidation of Scheme 2. In model studies, ascorbic acid was shown to rapidly form acetaldehyde in ethanolic solutions, a process that could be slowed but not completely eliminated through SO_2 additions,[6] and a change from anti-oxidative to pro-oxidative activity has been seen after a certain time in accelerated aging trials.[32] On the other hand, wine storage trials have shown mixed results regarding added ascorbic acid, with some trials showing little benefit to wine browning from the addition.[33] In other trials, such as a three year trial on Chardonnay and Riesling at the Australian Wine Research Institute (AWRI) in Adelaide, wines without ascorbic acid additions were browner, and the additions either led to no difference in aroma or to less oxidized and more fruity aromas, with little change in SO_2 levels.

Red Wine Oxidation

Red wines contain polyphenols at a higher concentration (1 to 5 g/L) than white wines, particularly much higher levels of the anthocyanin flavonoids responsible for colour and astringency (flavanol oligomers and polymers). Some of the established effects of O_2 additions to red wine include a decrease in certain smaller polyphenols and an increase in red polymeric pigments, alongside a loss of sulfites.[34] Several recent reports on the effects of micro-oxygenation in red wines have confirmed the loss of monomeric anthocyanins and other polyphenols, along with the enhanced formation of polymeric pigments (resistant to SO_2 bleaching), often with an increase in wine colour density.[13,14,35,36] Further changes in red wine pigments have included the formation of ethyl-bridged compounds associated with the acetaldehyde released during wine oxidation processes,[35,37] while a build up of acetaldehyde has been recorded in the later stages of regular micro-oxygenation,[38] and during an electrochemical micro-oxygenation approach.[39] Overall, micro-oxygenation has been shown to increase the rate of a range of red wine aging processes, allowing wines to be prepared for bottling in a shorter period.[40] A further influence on the rate of oxidative changes during micro-oxygenation is the level of SO_2 in the wine. We have tracked the development of polymeric pigments from monomeric anthocyanins during a sixteen week treatment of a Merlot wine at an O_2 exposure of 10 mL/L/month, and observed that these processes are severely restricted as more SO_2 is added to the wines (Fig. 1).[14]

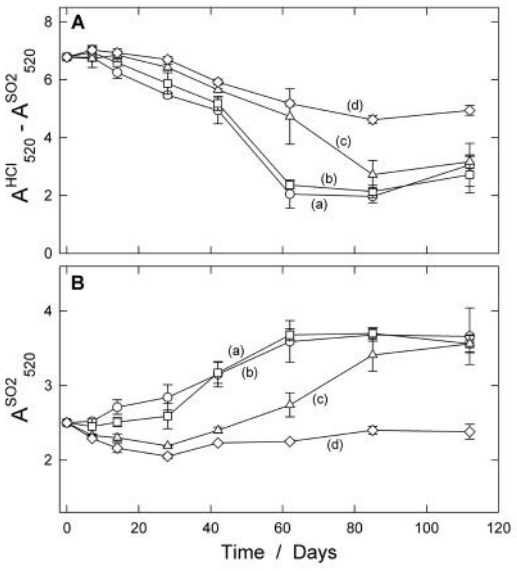


Fig. 1. Loss of monomeric anthocyanins given by the spectrophotometric measure (A^{HCl}_{520} - $A^{SO_2}_{520}$), and increase in non-bleachable (mainly polymeric) pigments ($A^{SO_2}_{520}$) during the micro-oxygenation of a red wine with different SO_2 additions: (a) 0, (b) 50, (c) 100, (d) 200 mg/L (n = 3).

The influence of red wine oxygenation upon aroma compounds and wine sensory properties has been more difficult to confirm compared to effects on wine colour. Micro-oxygenation is promoted as a technique that lowers unwanted vegetative characters in wines and elevates varietal, fruity aromas,[41] but the limited reports in this area show little change in levels of fruity esters, short chain fatty acids, or floral terpenes[42] while, in a separate report, the intensity of the berry/plum character and overall wine quality both fell in the micro-oxygenated wines.[13] Trends in aroma profiles have also been observed in wine closure trials with both white and red wines undertaken at the AWRI. In a three year closure trial on a Cabernet Sauvignon wine, that with the greatest air headspace showed significant losses of SO_2 soon after bottling and developed a higher *oxidized* aroma score.[43] Conversely, the wine under screw cap with the smallest air headspace showed the smallest loss of SO_2 and recorded higher, but not dominating, *struck flint/rubber* aromas. This shows how different wines can develop in the bottle according to the choice of wine closure and bottling procedures.

White Wine Oxidation

White wines contain lower levels of polyphenols (0.2-0.5 g/L), mainly hydroxycinnamic acids, *e.g.* **2** and **7**, but these remain very important for oxidation issues centred around wine browning and losses in varietal aroma. The low concentrations of flavonoids such as catechin (**3**) and quercetin (**5**) glycoside remain important particularly for wine browning and are more prevalent in musts exposed to longer skin contact times and harder pressings.[7,30] Tests on browning rates with different wines have shown varying results with respect to the importance of phenolic content, SO_2 level, pH, and metal content.[44]

Wine closure trials at the AWRI have again shown interesting trends in aroma development in the bottle. In the trial on the Chardonnay and Riesling wines referred to above, a higher rate of O_2 ingress through a synthetic closure led to lower levels of SO_2, higher browning and more advanced oxidized aromas.[45] By contrast, the limited O_2 ingress for wines under screw cap and cork, or for storage in glass ampoules, led to lower rates of browning and lower SO_2 levels, low oxidized characters, but again a discernable *struck flint/rubber* aroma for the screw cap and ampoule wines. This relates to the low oxygen ingress combined with the presence of certain sulfur-containing precursors at bottling.

For NZ Sauvignon Blanc, we have examined the effect of storage conditions on the decline in compounds responsible for the passion fruit and citrus aromas, particularly 3MH (**11**) and its acetate 3MHA.[20,46] Across sixteen Sauvignon Blanc wines bottled at the wine research hall in Auckland, under both cork and screw cap closures, a steady increase in absorbance at 420 nm (a widely used measure of wine browning) was seen (Fig. 2).[47] The rate of browning was greater under the cork closure, but this can be related more to the method of bottling at the University (which allows more O_2 into the wine than does a commercial operation) rather than to properties of the closure. The development of the two aroma compounds was very different, with 3MHA declining to very low levels over the first year in the bottle (Fig. 3), regardless of the closure type. This confirms the need to drink this wine young while such fruity aromas are at their most intense. A different aging pattern is shown by 3MH (**11**) and, in many cases, its concentration increased over the first three months in the bottle, likely due to hydrolysis of its acetate. A decline in level then follows with longer storage (Fig. 4). The 32% average decrease in **11** under cork versus a 21% average decrease under screw cap across the sixteen wines, matched the higher level of (oxidative) browning under the cork closure, related to conditions at bottling for this particular trial.

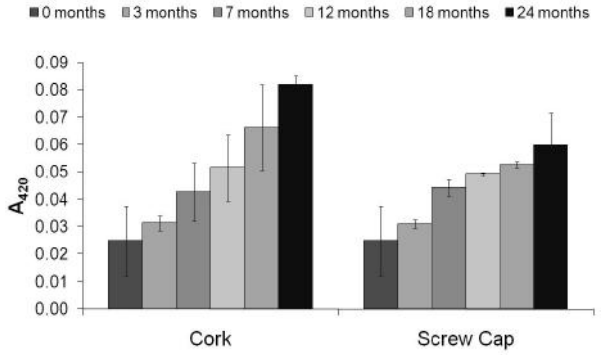


Fig. 2. Typical increase in 420 nm absorbance (browning) for a Marlborough Sauvignon Blanc in the bottle (n = 3).

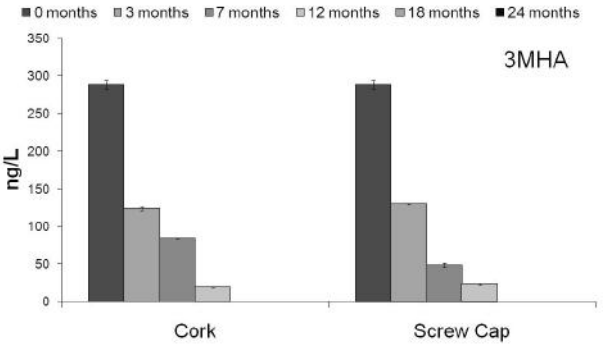


Fig. 3. Typical loss in 3-mercaptohexanol acetate (3MHA) for a Marlborough Sauvignon Blanc in the bottle (n = 3) (same wine as for Figs. 2 and 4).

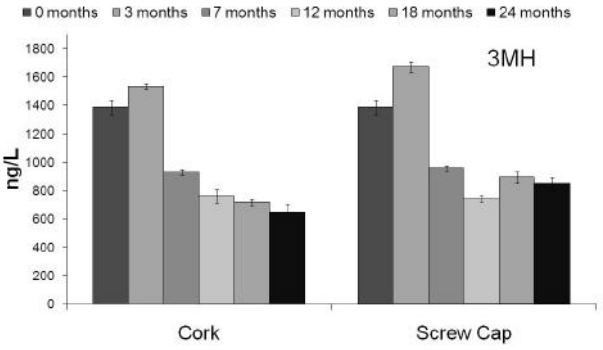


Fig. 4. Typical evolution of 3MH (**11**) for a Marlborough Sauvignon Blanc in the bottle (n = 3).

Final Remarks

The chemistry underlying wine oxidation processes has developed considerably over the past 10-20 years, and the role of polyphenol-mediated oxidation processes is a fea-

ture of this chemistry. The implications for red and white winemaking continue to grow and reveal both positive and negative contributions of O_2 for wine quality. Integrating chemical analyses with sensory studies remains an important area in the study of wine oxidation processes and it needs to progress. At the same time, a more detailed study of the chemical interactions between aroma compounds and oxidized polyphenols is needed to better appreciate the complexity, which makes wine such an interesting, and enjoyable, chemical matrix.

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Studying Interactions with Biological Membranes using Neutron Scattering

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Introduction

Biological membranes, especially cellular membranes, play an exceptionally important role in most physiological processes. They function as the primary gate-keeper for the cell, and through their interaction with proteins or other biological molecules are essential in regulating the relationship between a cell and its surroundings and display a rich variety of structure and function. However, a cell membrane is a difficult entity to characterize at the molecular level. The cellular membrane is extremely complex and consists of a mixture of lipids, proteins and sugars. It has interacting functions that are not fully understood as illustrated by the complexity of cholesterol behaviour in lipid membranes.[1] Moreover, the cellular membrane is an intrinsically fluid and highly disordered system, which relies on this disorder for its physiological functioning. Thus, it is not well suited to crystallography studies. Indeed, the highly asymmetric environment of a cellular membrane, characterized by a strongly hydrophobic interior with fully hydrated hydrophilic headgroups forming the exterior, is difficult to reproduce in a crystal. Of the many proteins known to be integral to cellular membranes only a small number have been crystallized, and this number is growing much more slowly than for soluble proteins.[2]

Biological membranes are also fragile and consist of a very small percentage of the total mass in a cellular system, so surface sensitive techniques are essential. There has been considerable development of techniques capable of measuring bilayer membrane model systems, including Atomic Force Microscopy (AFM), electrochemical methods (especially Electrical Impedance Spectroscopy – EIS), ellipsometry, Quartz Crystal Microbalances, reflectance IR measurements, or through neutron and X-ray scattering.

Of these, neutron scattering is particularly well-suited to the investigation of the structures of membrane systems, and is currently being developed internationally to take advantage of a range of new, brighter neutron sources and instruments. To date, the principal obstacle to neutron scattering has been the limited access to neutron sources. The two major types of neutron sources are restricted to large-scale experimental centres. The first type is a reactor-based neutron source, where the operation of a research reactor (of much lower power output than a typical nuclear power station) is optimized so that the normal neutron radiation is maximized, while heat production is minimized. The other type of source is a spallation neutron source, in which pulses of particles (typically protons) are accelerated into a heavy metal target, literally chipping (*spalling* in mining parlance) neutrons free. Europe has traditionally dominated neutron-scattering based science, and the current two brightest sources of neutrons are there – the ILL (a reactor in the south of France)[3] and ISIS (a spallation source near Oxford, UK).[4] Recently, however, there has been a surge in the development of neutron sources, with new facilities recently opened or opening in the US, Japan, Germany and, more locally, in Sydney.[5] The proximity of the new research reactor in Sydney, which goes into user operation for most of its instruments early in 2009, makes a sustained program of research into biological membrane interactions using neutrons much more feasible in New Zealand.[5,6]

Neutron Scattering

The principles of neutron scattering are relatively straight forward, and similar to those of light or X-ray scattering. An intense beam of highly collimated neutrons shines upon a sample, and the fluctuations in the intensity of the scattered beam is measured as a function of either the scattering angle or the energy of the incoming beam (Fig. 1). In reflection mode, the scattering is from a surface flat and smooth enough to give rise to specular reflection. The reflectivity gives information about the nature of the interfacial region, specifically picking up on scattering length density changes perpendicular to the surface (which can be related to physical density changes).[7] In small angle scattering mode, on the other hand, the scattering is from a bulk sample and information is gained about density fluctuations in the bulk – such as the number, structure and shape of particles in solution. Extracting the information from the measured scattering is well-developed for both techniques,[8] and shares much with light and X-ray measurements. That said, there are a number of particular features of neutron scattering that make it well-suited to probing biological questions.

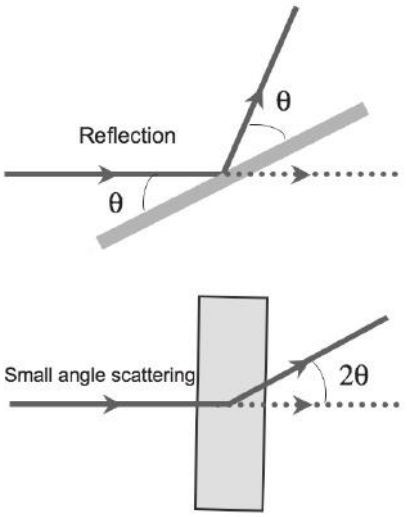

Fig. 1. Scattering geometries commonly used in neutron and X-ray scattering

The first is that the measurement is sensitive to interactions between the nuclei of atoms in the material probed

and the neutrons, rather than the electron cloud (as is the case for X-rays). The strength of this interaction is measured through a quantity known as the *scattering length* of the nucleus, which varies in an erratic fashion across the periodic table and may differ significantly even for isotopes of the same element. This measure is difficult to predict from first principles, but has been well measured for most common isotopes of biological interest.[9] Table 1 shows that the isotopes of hydrogen interact strongly with neutrons relative to other biologically relevant elements – this means that hydrogen atoms in biological structures tend to be well-defined by neutron scattering, in sharp contrast to X-ray scattering where the scattering from H is very weak. But perhaps the most important feature is that ^{1}H and ^{2}H (D) have very different scattering lengths. The scattering length difference between ^{1}H and ^{2}H give rise to the possibility of using deuterium labelling to highlight features of interest in biological structures, a particularly non-invasive and simple labelling method. The H/D scattering length difference also enables measurements of a single substrate that differ only in the scattering contrast due to the solvent isotopic composition, *e.g.* measurements made in H_2O and D_2O. By constrained modelling of these multiple measurements the unavoidable ambiguity in the measurements owing to the loss of scattering phases, common to all scattering techniques, can be reduced.

Table 1. Bound coherent scattering lengths (*b*) for some common isotopes of interest to biology[a]

Isotope	^{1}H	^{2}H (D)	^{12}C	^{14}N	^{16}O
$b/10^{-5}$Å	–3.74	6.67	^{12}C	9.37	5.81

[a]Data taken from ref. 9.

Other advantages of neutron scattering are based on the rather weak interaction of neutrons with matter. This means that the neutron beams are highly penetrating, and are able to be used in situations that involve elaborate sample environments. Examples are measurements made at the solid/liquid interface, where the neutron beam must pass through thick silicon substrates; or in cases when samples are subjected to high pressures, magnetic fields or temperatures. This also means that the neutron beams do not perturb or damage the samples – which is of particular importance in delicate biological systems.

Neutron Reflection from Model Membrane Systems

The complexity of the natural cellular membrane, and consequent difficulty of making suitable measurements on it, have led many research groups to develop suitable model membrane systems that mimic biological ones (biomimetic membranes), current methods of which have been reviewed.[10] These can be broadly characterized as vesicle-based bulk systems, or some form of flat lipid mono- or bi-layer.

As mentioned above, neutron reflection is particularly well suited to the study of flat and smooth surfaces. This includes systems such as Langmuir monolayers on water that have long been studied using X-ray reflectometry. Another type of model system that has grown significantly over recent years is the use of solid supported bilayer membranes.[11] Solid support lends robustness to model membrane systems. This enables long-term or sequential measurements to be made on a single surface, and ultimately is necessary for the development of biosensor applications. In turn, the development of good model membrane systems allows the study of the interactions between biological membranes and other proteins/peptides or other chemical interactions to be better characterized in physiologically relevant and non-crystalline conditions. Solid-supported membranes can be prepared in several different ways, including vesicle rupture on a clean surface, through Langmuir/Blodgett and/or Langmuir/Schaeffer film deposition, or through use of a suitable molecule anchor which tethers the bilayer to the substrate. Such a tether gives added stability to the model membrane, but comes at the cost of reducing the mobility of the molecules in the inner and outer membrane leaflets, and may reduce the free space available between the membrane and the solid surface.[11]

Our tethered bilayer system is an example of the power of neutron reflectometry to give structural information about such a system.[12] It is based on a synthetic lipid, constructed of two alkyl tails connected to a hydrophilic hexaethylene oxide *spacer* which is functionalized with a thiol to permit the formation of self-assembled monolayers on gold (Fig. 2). A complete bilayer membrane was formed by rapid solvent exchange, which involves the deposition of a concentrated lipid solution in ethanol flushed away rapidly by aqueous buffer.[13] Neutron reflectometry showed not only that the layer was complete, and gave parameters of the bilayer thickness and separation from the surface, but also showed that the tether molecules could be reduced to as little as 20% of the inner leaflet of the membrane without compromising the completeness of the layer. This reduction in the tethering density was found to be necessary to hydrate the inner leaflet of the membrane. Previously, this hydration had been inferred but not determined by electrochemical measurements – the use of isotopic labelling of the water permits the location and density of the water in the tether region to be directly measured.[14]

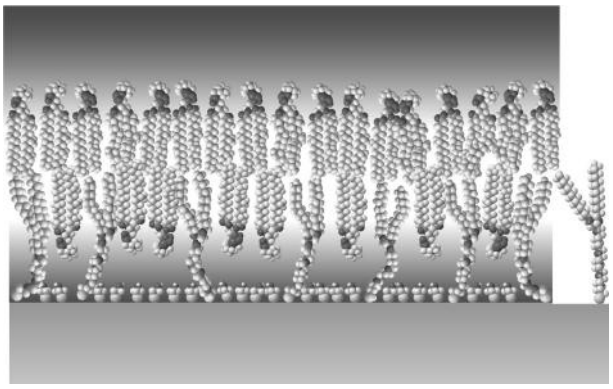

Fig. 2. A membrane system tethered to a flat and smooth gold surface suitable for characterisation by neutron reflectometry, with the synthetic tethering lipid pictured to the right. The synthetic lipid consists of two 14-carbon alkane chains, joined through ether linkages to a glycerol molecule functionalized in the 1-position with a hexaethylene glycol that is attached to the gold surface through a thiol. The spacing between tethers in the inner leaflet is controlled by the co-adsorption of small mercaptoethanol molecules to the surface. The bilayer membrane is completed with phospholipid molecules (reprinted with permission from ref. 12. Copyright American Institute of Physics 2007).

This membrane now has been used to test the membrane interactions with pore-forming and toxin proteins[15] and to give insight into the mechanism of Alzheimer's β-amyloid oligomer toxicity.[16] Each of these cases takes advantage of the ability of neutron scattering to use multiple solution isotopic contrasts to reduce ambiguity, and to sequentially measure change in the membrane structure after in situ changes to the membrane environment, *e.g.* pH, temperature.

Neutron Diffraction from Biomembrane Stacks

A special case of neutron reflection occurs when there is a semi-crystalline membrane bilayer stack formed on the solid-support (Fig. 3). These stacks are formed relatively simply through the slow evaporation of lipid-containing solvent solutions on the solid surface. They consist of thousands of bilayer repeats aligned on the surface; they show crystalline periodicity perpendicular to the interface, but usually remain disordered laterally. These stacks can be hydrated in highly humid atmospheres, and are measured in the absence of solvent, allowing X-ray and neutron measurements to be made. The constructive interference resulting from the scattering from multiple layers gives Bragg peaks, capable of providing detailed information about the perpendicular structure of the phospholipid bilayer within each bilayer repeat, to sub-nanometre resolution.

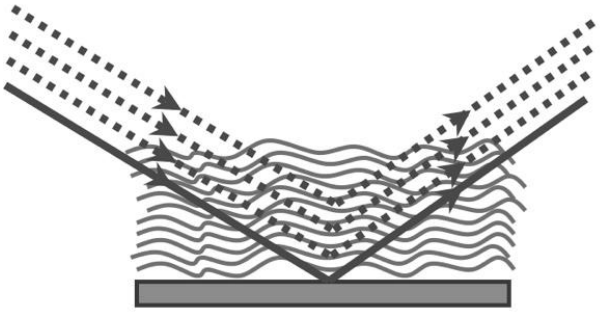

Fig. 3. Diffraction arising from a stack of bilayer membranes aligned on a solid support. The constructive interference from scattering from each of the membranes gives rise to Bragg peaks. Note that the membranes are still fluid, and laterally inhomogeneous even if they display crystallinity perpendicular to the surface.

This method has been used to great effect in understanding the behaviour of the lipids themselves in biological membranes, including their native thickness, degrees of interdigitation, and phase transitions with temperature.[17] The method also allows the resolution of the position and orientation of small additives to the biological membrane, such as cholesterol in biological membranes or fragments of membrane active proteins.[17] An unfortunate weakness of the method, however, is that there is very limited space between bilayer repeats (*ca*. 1 nm), meaning that it is not possible to incorporate larger membrane proteins that may extend beyond the phospholipid surface. It is also extremely difficult to achieve 100 % relative humidity around the bilayer to properly simulate physiological conditions.

Small Angle Neutron Scattering

The final major method of gaining structural information about model membranes using neutron scattering is small angle neutron scattering (SANS). The method, which probes the scattering from particles in bulk solution, is widely used for studying the structures of soluble proteins, and protein assemblies and is normally used in a complementary fashion to X-ray small-angle scattering (SAXS).[18] Biological membrane models used for SANS are typically phospholipid vesicles,[19] although some limited work has also focussed on the formation of planar *bicelles* formed from the mixing of a long and short-chain phospholipid (which allows experimental overlap with NMR).[20] The size resolution of SANS/SAXS is ~1–100 nm, meaning that experiments focus on the membrane rather than on the whole vesicle. An extension to SANS – ultra-small angle neutron scattering, USANS – is currently proposed for the Sydney reactor that will be able to resolve much larger structures to address this issue. As this is a solution measurement, there is no steric limitation to the size of proteins incorporated to the membrane, as in the case of neutron diffraction; and the samples are again relatively simple to form. The curvature of the vesicle also gives rise to features, such as asymmetric lipid distribution across the membrane, that are inhibited in planar geometries.

Conclusions

The strengths of neutron scattering in studying biological membrane systems, coupled with the forthcoming availability of neutrons at the OPAL research reactor in Sydney, means that the application of neutron methods to biological problems in NZ should significantly increase over the coming years. This work can build on the extensive international efforts to develop biomimetic membrane systems appropriate for structural characterization using neutrons and X-rays, and is expected to augment NZ's existing strengths in biological membrane systems.

Acknowledgement

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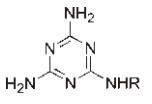
Melamine Food Contamination

It is impossible to know how many children have been affected by the melamine found in milk powder that occurred in 2008. Melamine ingestion is not a disease so the Chinese government is not required to notify how many have suffered. The BBC in October said more than 53,000 children had become ill from the contaminated milk powder. Other media reports suggest the number could be as great as 300,000.

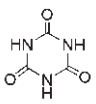
This is not the first melamine contamination problem to arise from China. In 2007, melamine was found in Chinese wheat gluten sold to the USA as a pet food thickener. This contamination led to cats and dogs becoming ill in the United States and Canada.

Melamine is not used by the body and is a very small molecule. In small amounts it is absorbed through the intestinal tract, goes to the kidney and is passed out in the urine. However in higher concentrations it can crystallise into kidney stones. This is what happened to some of the infants fed the milk and some of the pets in the 2007 case.

During the pet food contamination cyanuric acid, ammelin and ammelide were also found in the wheat gluten. Cyanuric acid can cause crystals to develop in the kidney tubules. At the end of October, Reuters reported some of the infants, who had drunk the melamine contaminated milk, were found to have developed crystals in their kidney tubules and these appeared to be cyanuric acid.



melamine: R = H
cyromazine:
R = cyclopropyl



cyanuric acid

After the first story broke about the milk powder contamination, many countries carried out extensive testing on other Chinese products. Among a number of reports, Canada reported melamine present in Mengniu Strawberry Flavour Sour Milk, Vietnam reported melamine present in Singapore's Pokka corporation Melon Milk, Cappuccino Coffee and Milk Coffee Europe and Gold Nutritionals Master Gaine Powdered Milk. This product uses milk powder from New Zealand but also has additives from Thailand. In mid-October Malaysian authorities found melamine above the safety standard, in ammonium bicarbonate imported from China. The high levels were first found in two brands of biscuits, the contamination was traced to ammonium bicarbonate (used as a raising agent). In late October, The New York Times reported that Hong Kong had found high levels of melamine in eggs imported from China. The levels were above the safety standards but not as high as that found in the contaminated milk.

Allowable concentrations of melamine in food products varies around the world. Vietnamese regulations require no melamine to be present in food. New Zealand regulations allow for 2.5 ppm in food products and 1 ppm in infant formulas. The allowance for some melamine in food products is because of possible leaching from food packaging or processing. New Zealand's Tatua Cooperative suspended exports in late 2008 of lactoferrin protein powder after low levels of melamine were found in the product. The levels were under the safety standards but Tatua were working to find the source. Low levels were also found in Westland Milk Product's lactoferrin. Westland used the same processing technology as Tatua.

The US FDA has published an interim safety/risk assessment on melamine and structural analogues and for melamine has established a tolerable daily intake (TDI) of 0.63 mg per kg of body weight per day. The European Food Safety Authority has published a provisional statement for melamine of a TDI of 0.5 mg per kg of body weight per day.

Why was melamine in the Chinese milk powder in such large concentrations in the first place? Melamine (2,4,6 triamino-*s*-triazine), contains six nitrogen atoms. If total nitrogen content measurement is used to equate to protein content, adding a high nitrogen substance, like melamine, appears to increase protein content. This means the product can be diluted but appear undiluted if the protein content is still within the range expected. At this stage it is hard to know when the contamination occurred. There are a number of theories as well as the dilution theory, another possibility is the milk came from undernourished cows so would not have passed the protein content test. According to the Wall Street Journal some Chinese farmers say that for years *protein powder* has been used to help milk from undernourished cows meet quality checks. It is also possible for melamine to enter the food chain from insecticides containing cyromazine used by farmers.

Continued on page 33...

Development of Low-Cost Ozone Measurement Instruments Suitable for Use in an Air Quality Monitoring Network

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Introduction

Air quality concerns every individual and impacts directly on health and productivity. Air quality is inherently variable in time and space. Concentrations of air pollutants are primarily determined by the balance between emissions rates, chemical formation and the ability of the atmosphere to transport and disperse pollutants. In the troposphere, ozone (O_3) is formed as a secondary pollutant within urban and industrial plumes that may extend many hundreds of kilometres downwind of the source area. As the world population becomes increasingly industrialized and urbanised there is a need to improve our understanding of the processes influencing air quality in order to minimise population exposure.

Attempts to measure O_3 distribution have been limited by the instrumentation available. Conventional instruments are based on chemiluminescence methodologies which are bulky, expensive, and require AC power and temperature-controlled enclosures. This limits their deployment in the field. Monitoring sites are chosen typically to be representative of *average* or background concentrations in the region and not influenced by local sources of pollution. At this scale, sparsely distributed instruments are used to provide information about pollutant gas accumulation and transportation on a regional or district scale, and data are used to inform local authorities if pollutant levels in their areas are within or are exceeding acceptable levels. However, there remains considerable debate about the intra-region and particularly intra-urban homogeneity of pollutant concentrations due to variations in source strength, meteorology, topography, and location of monitoring sites.[1]

At urban to local scales the temporal and spatial heterogeneity of emission patterns, local wind flow patterns, and the complexity of the urban surface results in complex dispersion pathways at local scales within urban areas. This can lead to strong gradients in vertical and horizontal pollutant concentration.[2] In urban areas, people spend a substantial component of their outdoor time near busy roadways and intersections while commuting to work (on bicycles or foot), employed at local shops or cafes, or using the pavement space for retail or recreational activities.[3] Although individuals may not remain in this environment for more than a few hours each day, the prevalence of local pollutant hot spots often results in significant exposure. Even in densely populated areas of economically advanced countries, instrument spatial densities are at most 1 or 2 per square kilometer, as is the case with the London Air Quality Network (LAQN).[4] A limited number of fixed monitoring stations may not provide a good indication of personal exposure to pollutants, so hindering the establishment of strong relations between pollutant concentrations and meteorology[5] or health outcomes,[6] Such limitations have led to attempts to deduce the pollutant burden from measurement of traffic density, emission inventories and dispersion modeling.[7]

There is, therefore, a great need for the introduction of portable, low-cost ozone monitors that can easily be deployed in a high density network. The results from such a network will help inform the understanding of pollutant dispersion pathways and human exposure. Such data are essential to the formation of coherent air pollution abatement strategies. The work described in this article is part of an effort directed at enabling high spatial density air quality monitoring through the development of suitable low-cost instrumentation.

Gas sensors based on conductivity changes of heated semiconducting oxides[8] are widely used for industrial safety monitoring. While there are reports in the literature of gas-sensitive resistors being used for ambient atmospheric monitoring, there is a perception that the devices suffer from effects such as drifts of zero and calibration slope, and cross-sensitivities to other gases making them unsuitable for quantitative, long-term atmosphere measurement. However, we have shown that with careful attention to detail, based on a thorough understanding of the mechanisms underpinning device operation, these perceived deficiencies can be overcome. This article presents the design principles for low-cost instruments based upon heated metal oxide sensors for the reliable measurement of part-per-billion (ppb) levels of ozone in the atmosphere. Long-term performance data are presented, and common failure modes and their diagnosis are described.

Semiconducting Oxides and Ozone Measurement

The very large conductance response at temperatures in the range 300–550 °C of highly porous layers of WO_3 to the introduction of ppb concentrations of ozone in air (Fig. 1) has been described and discussed in detail,[9,10] and there are preliminary reports in the literature describing the use of such sensors in ozone measurement instruments.[11,12] The response mechanism deduced[9,10] is given as Scheme 1. It has been shown that the conductance of the porous sensor assembly can adequately be described as a parallel equivalent circuit, with one element (the *interface*

element, representing the surface zone of the individual grains of the material) being dependent on the ozone concentration, and the other (the *bulk* element, representing the interior of the individual grains) being independent of the ozone concentration but dependent on oxygen partial pressure. Long-term drifts in baseline, which have been significant issues dogging the first attempts to develop monitoring instruments based on WO_3 sensor elements,[12] could be understood as resulting from the slow exchange of oxygen vacancies between interface and bulk elements. The predicted variation of conductivity with ozone concentration (Eq. 1) fitted the data well. The conductance difference in the presence and absence of ozone (Eq. 1) did not vary over several months of operation, consistent with the prediction of the assumed mechanism and equivalent circuit.

$$\sigma_{(O_{3-0})} - \sigma = \frac{aP_{O_3}}{bP_{O_3}+1} \qquad \text{(Eq. 1)}$$

Response Model basics

- Conductivity controlled by oxygen vacancies.

$$X \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} V + \tfrac{1}{2}O_2 \qquad V + O_3 \xrightarrow{k_2} X$$

X = unperturbed lattice
V = neutral species (ion pair of oxygen vacancy + reduced lattice tungsten ion)

- Charge carriers produced by thermal excitation of electrons from reduced tungsten ions into the conduction band.

Charge carrier and therefore conductivity changes in response to changes in oxygen vacancy concentration at the interface (determined by $[O_3]$).

Scheme 1

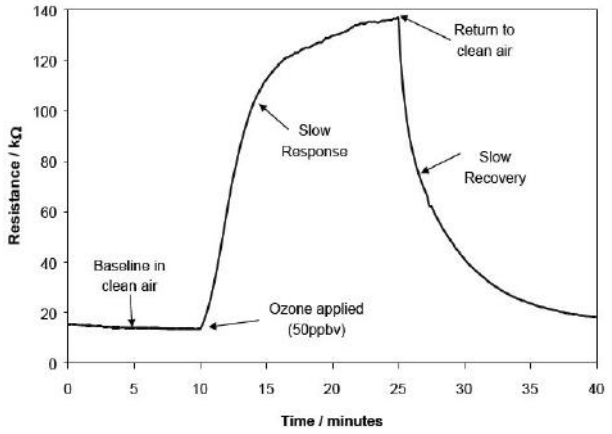

Fig. 1. Resistance transient of a tungstic oxide sensor element in response to the application and removal ozone in air

Instrument Design and Operation

The atmosphere monitoring instrument has been developed from a successful commercial implementation of the design principles in a hand-held device (Fig. 2). Several thousand of these instruments have been sold and have operated successfully over several years. The atmosphere monitoring instrument design reflects the accrued operational experience. Because O_3 is such a reactive gas readily decomposed on surfaces (particularly if traces of organic matter are present), it is well-known to take care in the choice of materials for housings and pipework, and also in calibration.

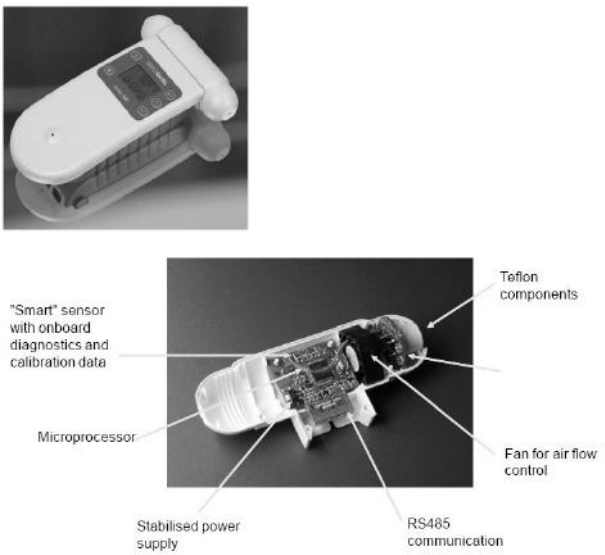

Fig. 2. Commercial implementation of a hand-held ozone meter utilizing a WO_3 sensor element (copyright Aeroqual Ltd., reproduced with permission)

Sensor Fabrication

The sensor substrate is a 2 x 2 x 0.250 mm alumina tile with a meandering platinum heater track printed on one side, and inter-digitated gold electrodes printed on the other. A thin (*ca*. 90 μm) layer of WO_3 is screen printed over the gold electrodes, creating the sensing element (Fig. 3). The sensor is held inside a sealed plastic housing with an inlet and outlet nozzle to allow sample gas to be drawn over the sensor at a controlled rate. Precise control of the operating temperature is essential to instrument precision and stability, simply because the oxide conductivity varies strongly with temperature: the activation energy is about 0.5 eV. The method employed for temperature control incorporates the platinum heater track (which has a well defined resistance-temperature relationship) into a Wheatstone bridge circuit whereby the out-of-balance signal across the two arms of the bridge is used to regulate the current through the sensor heater, thus controlling the heater temperature by keeping its resistance constant.[11] The sensor resistance is determined with a simple DC measurement, with the potential difference across the sensor controlled at 0.1 V. Higher potential differences across the sensor were found to cause an excessive resistance drift. The sensor housing is a cylindrical plastic enclosure

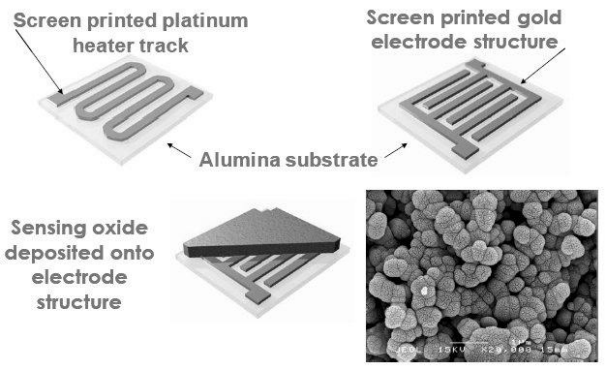

Fig. 3. Construction of the sensing element and micrograph showing grain structure; scale bar 1 μm

with four bonding posts in the base from which the sensor is suspended freely in the air by fine platinum connecting wires.

Instrument Operation

The two key problems to resolve were to overcome the limitations imposed by the very slow ozone response that is evident in Fig. 1 and to find a way to determine the zero ozone signal, so that the Eq. 1 could be applied and the zero drift compensated. The means adopted to resolve these issues have been crucial to the development of a successful operating procedure. The first problem, that of the slow response, was solved by the use of a temperature stepping regime.[9] At sufficiently high temperature, the ozone signal becomes essentially zero. The idea is that the surface comes rapidly to equilibrium with O_2 in the air, establishing a surface oxygen vacancy concentration that is repeatable, being dependent only on the temperature and the oxygen partial pressure. Thus, a step to a sufficiently high temperature establishes a repeatable initial condition for a subsequent step back to a lower temperature for the measurement. The theory[9,10] shows a clear and simple relationship between the initial rate of change of conductance and the ozone concentration, provided that the initial surface oxygen vacancy concentration is repeatably defined. Since the temperature control is accurate, the rate of change of conductance can be inferred from the conductance measured a fixed (short) time after the step. The choice of temperature and time is a trade-off between signal and cycle time. The upper temperature is also limited by the stability of WO_3, which will sublime at a significant rate at high temperature, especially in the presence of a high vapour pressure of water. This sublimation changes the microstructure of the sensing layer and hence the sensitivity. Loss of WO_3 from the sensor would ultimately lead to a loss of electrical continuity in the sensing layer and hence failure of the device.

The solution to the second problem, namely that of continually determining the zero ozone conductances, came from measurement of the dependence of the signal on the air flow rate over the sensor. The signal was independent of the air flow rate if this was sufficiently large but fell to zero if the air flow rate was decreased.[8] The explanation is that O_3 is rapidly decomposed on plastic surfaces, especially if these are warm; it is also rapidly decomposed in air if the temperature is sufficiently high. There is a thermal boundary layer just outside the sensing layer, and the power dissipated by the sensor warms the plastic housing. Both effects mean that the sensor signal is strongly dependent on the air flow rate over the sensor. Thus, a periodic zero measurement can be obtained by dropping the flow rate across the sensor to zero. Implementing a periodic zero flow condition, during which the sensor temperature is cycled between the high and low temperature states, provides a continuous measurement of the sensor baseline resistance. In the final implementation in the instrument, the high and low sensor temperatures are 600 ^oC and 520 ^oC, respectively, which gives a good compromise of stability of microstructure of the sensing layer, sensitivity, and required cycle time. An entire measurement cycle lasts one minute and comprises a zero flow condition at high and low temperatures and a high- flow condition at high and low temperatures. The low temperature conductance difference between high and low air flow states, measured a fixed time after the temperature step, follows Eq. 1.

Cycling of the air flow rate is obtained by drawing air across the sensor using an electrically-modulated fan or pump sealed against the base of the sensor housing, which has a hole drilled through it, centred on the sensor and with diameter just greater than the sensor diagonal. The entry face of the sensor housing is fitted with a tapered nozzle that directs the air flow onto the sensor. The sensor is protected from dust by a fine stainless steel mesh and a porous PTFE filter. All other components in contact with the gas before the sensor element are made from PTFE.

Air Quality Monitoring Instrument Construction

The instrument design for long-term air quality monitoring is essentially the same as that of the hand-held commercial instrument. The main differences are in the provision of a more reliable and accurate air pump, in the data handling and communication, in the housing, and in the provision of diagnostics for confirming reliability of the data. The instrument has been designed in two blocks: a sensor management block and a data management block. The sensor management block holds the calibration information and sensor identity. It manages the heater and air flow, makes the resistance measurement, and converts the result to concentration using the calibration information. The data management block controls the acquisition rate, acquires the concentration data, and stores and presents the output. These components are shown in (Fig. 4). The device is housed in an enclosure appropriate for its intended application.

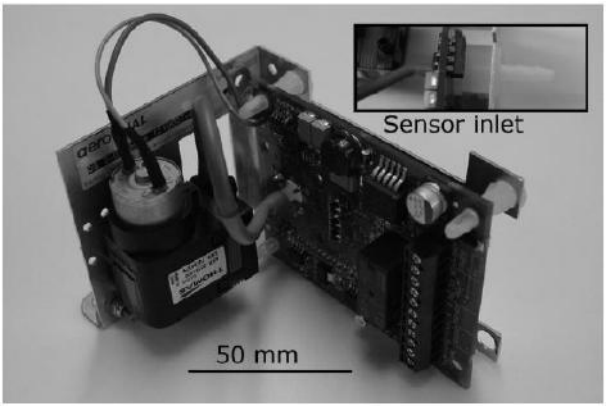

Fig. 4. Ozone instrument module for air quality monitoring (copyright Aeroqual Ltd., reproduced with permission)

Calibration

A significant part of the cost of any instrument is that incurred in calibration. As the capital cost of the instrument decreases so the calibration cost becomes more significant. Thus, a *low cost* instrument necessarily requires a *low cost* calibration procedure. We have split the calibration task into two parts: *linearization* and *calibration refinement*. The linearization part is a laboratory calibration using a restricted number of different O_3 concentrations, that can be performed reliably on a large number of instruments at any

one time, and whose purpose is to derive the parameters for the fitted curve of conductance vs concentration such that the imposed concentrations are reproduced with a precision of ±5% at worst. The derived calibration function linearises the output from the sensor, delivering a result measured in gas concentration units. The calibration refinement step comprises measurement in the atmosphere alongside a reference analyser for 24-48 hours. The idea is to use the natural variability of the atmosphere to sample the full range of concentration required, and to take advantage of the fact that the semiconductor-based instruments are small, rugged and portable. As we will demonstrate, sensor-indicated concentration is a linear function of analyser-indicated concentration, so that a simple linear correction factor can be applied to the laboratory calibration in order to derive an accurate result for the atmosphere measurement. After calibration refinement, the instruments would be moved to the desired location.

Linearization

Previous work described a burn-in phenomenon for WO_3 sensors for ozone[9] that was attributed to effects of a reaction of O_3 with traces of organic matter remaining from the sensor fabrication. The complete sensor modules (sensors inside their housing with air flow control and the control electronics) were run at the sensor operating temperature for 2 weeks in ambient air, prior to linearization. The use of an adequate burn-in time before calibration has been important to the success of the instruments described in this paper. Ambient air was scrubbed of hydrocarbons and NO_2 by passage through a packed bed of activated charcoal, then introduced to a 1.5 m^3 measurement chamber constructed of Perspex; there was no humidity control. The complete sensor modules were mounted on a stainless steel rack in the centre of this chamber. The air inside the chamber was circulated by a small fan and continuously sampled from the centre of the chamber, close to the sensor modules being linearized, to a spectrophotometric ozone analyser. An ozone generator inside the chamber was controlled from the ozone analyser output, to maintain a fixed concentration of O_3 inside the chamber around the sensor modules. This concentration was held in sequence at 0, 50, 100 and 150 ppb, for 20 min at each concentration before the measurement sequence was initiated on each module. As noted in the above, the sequence was a step from high to low sensor temperature at high and low air flow rate over the sensing element. Sensor resistances at the end of the low-temperature step were recorded at low and high air pump rate. Rather than an exact inversion of equation 1 to derive O_3 concentration from conductance difference, a quadratic fit to the conductance difference between high and low sensor fan speed was found adequate empirically to linearize the sensor output. Fig. 5 shows the distribution of deviations between set concentration and concentration inferred from the fit, for a typical set of 60 sensor heads. The variability stems from the sensor microstructure varying from one device to another, the underlying model is a greatly simplified one for the effect of microstructure on the response, and the use of a quadratic fitting function to derive concentration from measured conductance is itself an approximation. Sensor modules that showed average deviations greater than ±5% were rejected for this application.

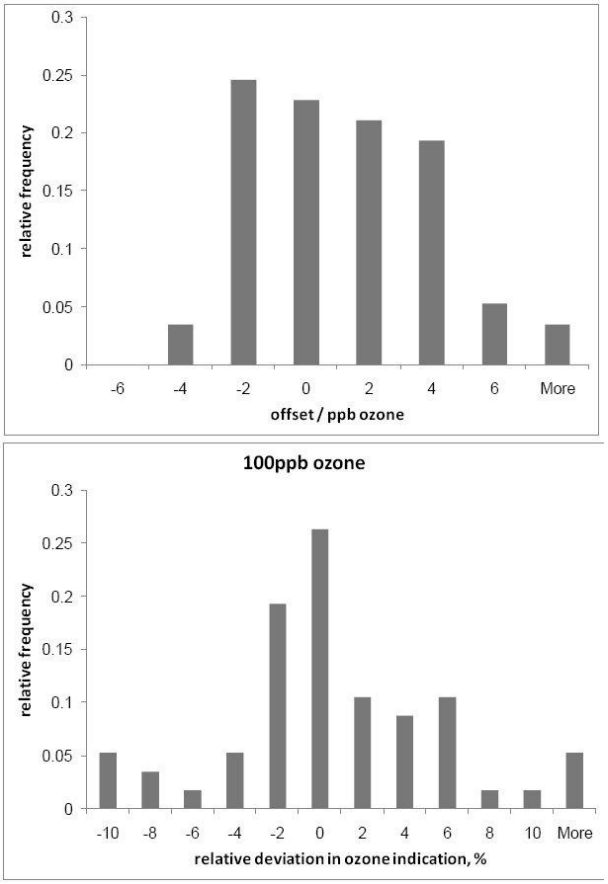

Fig. 5. Distribution of deviations in instrument indication following initial linearization

Field Calibration Refinement

An air monitoring station was established in a pod protruding some 6 m above the 7th floor of the physics building on the Auckland campus. This site is at a high point of the Auckland central business district near heavy traffic and is exposed to wind from all sides. A sample manifold comprising 15 m of 150 mm diameter PVC pipe and an in-line fan draws air from outside on one side of the building and exhausts to the other side at a rate of *ca*. 90 L/sec (see Fig. 6). A reference analyzer (Thermo 49 C) and the semiconductor-based ozone instruments sampled outside air from this manifold, *via* PTFE tubing, each making a measurement every minute. The data, logged on a networked computer, were recorded as a tab delimited text file with a line of data being written every minute. A new file was automatically created at the start of each day. Sample air temperature and humidity, rainfall, wind speed and direction, and room temperature and humidity were monitored during the sampling period. The sensor baseline resistance, derived from the low-flow, low-temperature measurement, and the heater current and potential difference for each semiconductor instrument were also recorded.

As the levels of ozone were fairly low in the Auckland region at the time, O_3 was introduced into the sample manifold, using the generator connected to a timer, for two hours between 2 and 4 a.m. every morning. This provided elevated O_3 levels to extend the calibration and test range. The calibration refinement phase was the first

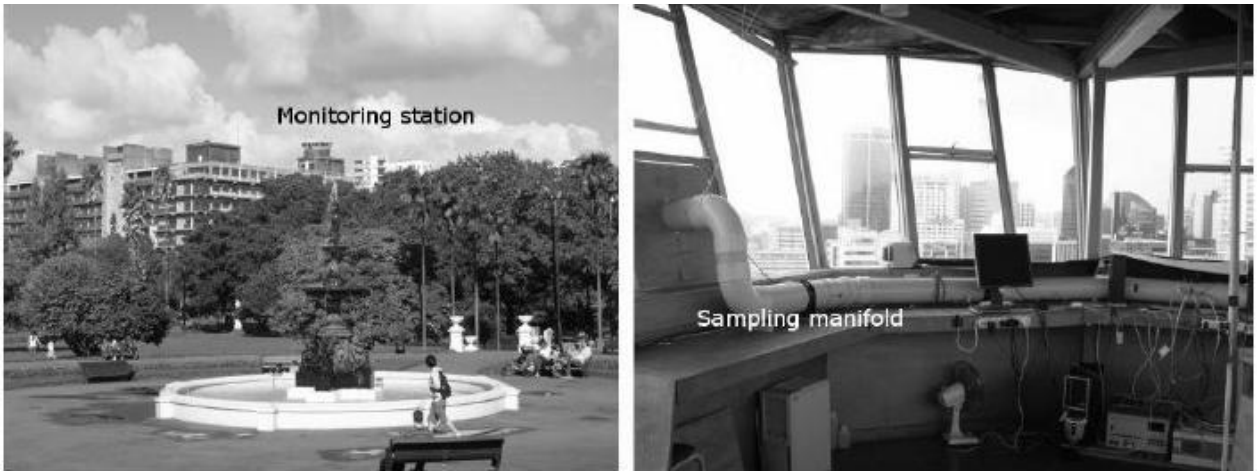

Fig. 6. Left; Image of monitoring station from ground level; right: image from inside monitoring station showing sampling manifold and exit port

period of 72 h of operation, while data from the final 48 h was used. A linear correlation function was calculated for each sensor against the reference analyzer. The example of Fig. 7 illustrates that the initial sensor output linearization procedure was adequately accurate. The standard error of estimate of the analyser indication derived from the semiconductor instrument indication using linear correlation, for all 5 instruments, was 2 ppb (n=3000). Fig. 8 shows the superposition of the time series of the analyser indication and the semiconductor instrument indication corrected by the linear correlation function.

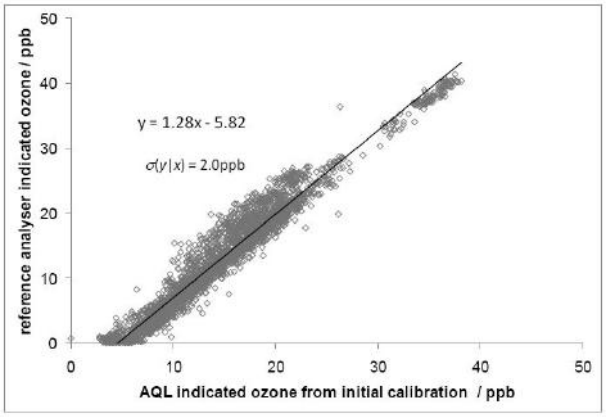

Fig. 7. Calibration refinement: linearized module output predicting the reference analyser indication, over 48 h

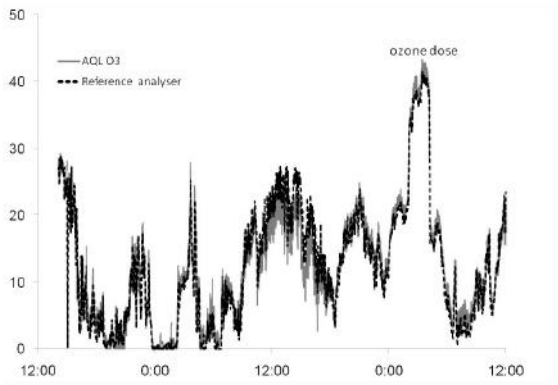

Fig. 8. Calibration refinement: linearized and *in-situ* calibrated module output tracking the reference analyser over a 48 h period

Field Measurements

Following the field calibration, the semiconductor-based instruments were left running continuously for a period of approximately 6 weeks with no intervention. The evolution of the slope and intercept of the linear correlation of analyser indication with semiconductor instrument indication is shown in Fig. 9. The evolution with time of the mean over each day of the difference between semiconductor instrument reading and analyser reading, each measured every minute, is shown in Fig. 10. A small and slow variation in the indication of the spectrophotometric reference analyser cannot be ruled out, giving an apparently correlated small drift of all of the semiconductor instruments. In addition

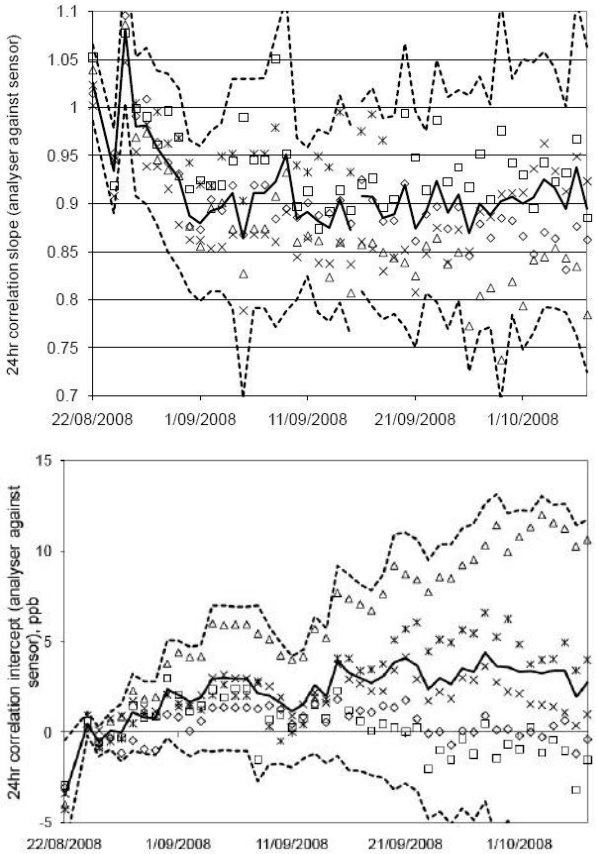

Fig. 9. Evolution over time of the 24 h correlation slope and intercept, between sensor module and reference analyser. Solid line is the mean of the 5 sensor modules; dashed lines are ±2 standard deviations

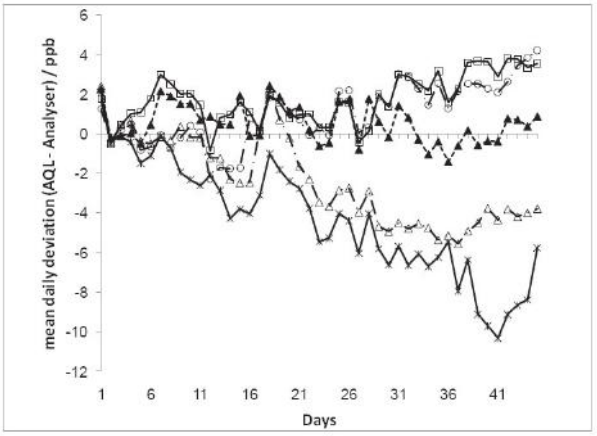

Fig. 10. Evolution over time of the mean over each 24 h of the difference between the measurements made each minute by the sensor module and reference analyser, for 5 sensor modules

to this effect, there is some evidence that the result from one of the semiconductor instruments had drifted to a small additional extent over the 6 week period. It is clear however, that the small, simple low-cost semiconductor-based instruments reproduced the measurement of the spectrophotometer to within 10 ppb O_3 over this extended period of unattended operation. They meet the requirement for deployment in an air quality measurement network.

Failure Modes and Diagnostics

For confidence in the reliability of the data, a necessary condition for deployment of instruments into a monitoring network is that common failure modes are identified and means for recognizing failures are delineated. Three main cases of failures of the semiconductor-based instrument have been identified in the present work.

Pump Degradation: Diminution of Air Flow Rate over the Sensor

Control of the air flow over the sensor is critical to the correct operation of the instrument. Fig. 11 shows the variation in indicated O_3 concentration with alteration of the volume flow rate of air under the high-flow condition. If the flow rate is too high, then the convective heat loss from the device exceeds the power available from the heater driver circuit, and the sensor temperature falls. This results in a higher ozone signal. If the flow rate is too low, then the signal falls. There is a range of flow rates where the indicated signal is independent of flow: this is the desirable control condition for the instrument; Fig. 11 illustrates the effects. An O_3 concentration of approximately 100 ppb was maintained in an enclosed chamber. The concentration was monitored with a reference O_3 analyzer and a semiconductor ozone instrument with adjustable flow control. This instrument had been calibrated previously at a flow rate of 200 mL/min. The ratio of the reported O_3 concentration from the semiconductor instrument to that of the analyzer was determined for flow rate into the semiconductor instrument ranging between 20 and 1600 mL/min.

The air flow can change as a result of degradation of the air pump during continuous operation; the result would be an under-reading of the instrument. A simple diagnostic is to measure the current supplied to the heater and the potential difference across it. The derived heater resistance confirms that the sensor is operating at the correct temperature and that this temperature is stable. The derived heater power dissipation indicates variations in the air flow rate, since the heater functions as a simple hot-wire anemometer. Fig. 12 shows an example where this simple diagnostic has uncovered a pump failure, with the associated under-reading of the O_3 concentration.

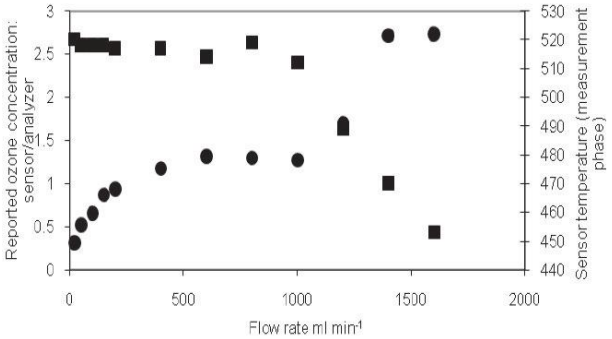

Fig. 11. Effect of gas flow rate on module indication and sensor temperature;. (●) ratio of the module-reported ozone concentration to that of an optical reference analyzer; (■) Sensor temperature in °C

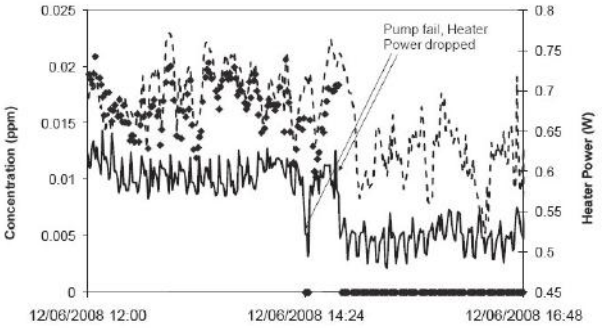

Fig. 12. Effect of pump failure on the ozone module signal, and its detection by heater power measurement. (♦) module indication; (—) heater power; (- - -) reference analyser indication

Dirt Accumulation: Ozone Decomposition on Dirt Deposited in the Inlet Filter

With long-term operation, particulates from the atmosphere accumulate on the PTFE and stainless steel filters protecting the sensor. Such material is catalytically active for O_3 decomposition and it also clogs the filter and reduces the air flow rate. The result is that the sensor underreads. Fig. 13 illustrates the effects for instruments where the filters were visibly dirty.

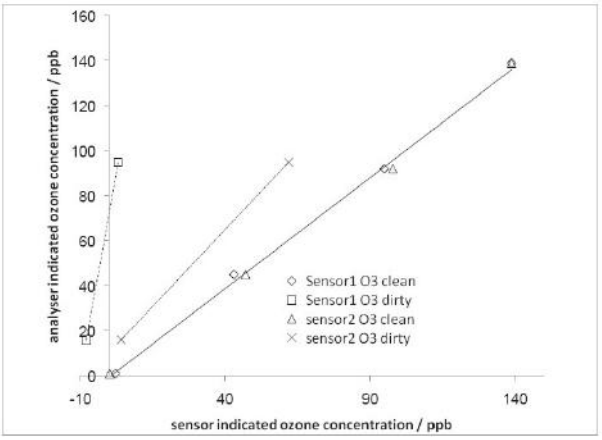

Fig. 13. Effect of dirt on the sensor filter on the module-indicated ozone concentration

Sensor Failure in High-Humidity Environments

WO_3 sublimes at elevated temperatures. We have observed that the effect is enhanced if the water vapour pressure is high, and it can become significant at the upper operating temperature of the sensor. The result is that the sensor grows whiskers of WO_3 thus allowing the microstructure of the sensor element to change slowly over time. The assumption behind the application of Eq. 1 is that the microstructure remains unchanged over time. Thus, a slow variation in microstructure over time would give rise to a drift in the indicated O_3 concentration. As the sublimation proceeds, then over a period of months to years (dependent upon the atmospheric humidity) the baseline resistance can rise to such an extent that the measurement moves beyond the range of the sensor electronics.

A variation in microstructure should be detectable by a variation in the zero-ozone resistance of the sensor. This number is available from the low-flow resistance measurement. Indeed, Fig. 14 shows that the variation in baseline resistance can be correlated with a slow drift of the signal that is most sensitively detected by a small drift in the intercept of the 24 h correlation between reference analyser and sensor-based instrument.

Fig. 14. Variation of sensor baseline resistance over time when resistance rose and module ozone signal began to change significantly: baseline resistance and intercept of the linear correlation between reference analyser and module against time

Conclusion

A low-cost instrument for monitoring ozone in the atmosphere, with estimation error (2σ) ±4 ppb compared to a spectrophotometric reference analyser over the range 0-100 ppb O_3 has been demonstrated. The instrument has a measurement cycle time of 1 min and is based on the resistance changes of a heated semiconducting oxide. Common failure mechanisms and means for diagnosing these have been described. The device has performance suitable for use in long-term atmosphere measurement, with remote diagnostics and minimal maintenance requirement.

Acknowledgement

This work is supported by the Foundation for Research Science and Technology, New Zealand.

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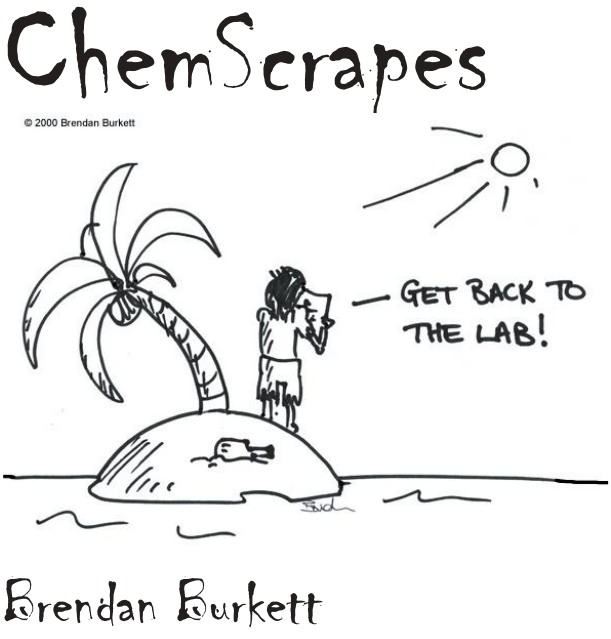
Continued from page 26.

Melamine and similar substances like cyanuric acid can be detected by a number of methods including ELISA, gas chromatography, mass spectrometry, and high pressure liquid chromatography. But the real issue surrounding this food contamination scare is not about just these substances but the ease in a globalised economy, with which many countries can be affected by one country's mistake – whether it has been done deliberately or in ignorance. How will testing of products evolve to overcome what appears to be ongoing problems? How can effective and affordable testing regimes be put in place for the plethora of products from toys to food ingredients and finished food products when the possible contaminants could be anything? Is this a risk we just have to live with in our modern world or is it something humans have always lived with and today it just catches us by surprise as we put our trust in quality control? In November the US Food and Drug Administration opened their first office outside of the United States in China.



Article

From Small Rings to Big Things: Fruit Ripening, Floral Display and Cyclopropenes*

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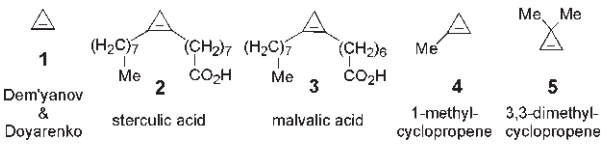
*Dedicated to Bede Squire FRCS who, fortunately, left the NZ native bushes and trees until after his retirement.

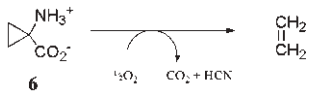
Introduction

Cyclopropene chemistry first graced the chemical literature by way of assumptions for the presence of symmetrical derivatives in works by Wolff and Merling as referred to by Franz Feist[1] when he reported the isolation of the 3-methyl-1,2-dicarboxylic acid in 1893. The paper, on cumulene ring decomposition, includes a 1,3-dehydrobromination that provides the diacid in direct analogy to the preparation of cyclopropane some ten years earlier.[1] Another twenty years passed before the parent, gaseous C_3H_4 hydrocarbon **1** was recognised, initially in a Russian publication,[2] and, more widely, one year later in *Berichte*.[3] Following these reports and the synthesis of various derivatives over the ensuing thirty years, the naturally occurring sterculic and malvalic acids were confirmed as **2** and **3** (Chart 1) in the early to mid-1950s.[4] The latter half of the last century saw the essential chemistry of the class of compounds explored, but it is only in the last fifteen years that the commercial importance of 1-methylcyclopropene[5] (**4**) and other alkyl homologues, *e.g.* **5**, has been recognized and implemented.

Chart 1



The occurrence and biological activity of cyclopropane derivatives in nature is well known[4,6] with all green plant matter containing 1-aminocyclopropane carboxylic acid (**6**, known also as ACC). It is converted by ACC oxidase to ethylene,[7] an important plant hormone. Environmental and endogenous signals regulate the biosynthesis of ethylene primarily through differential expression of ACC synthase genes whose activity controls the rate of production; its regulation is the key to controlling ethylene biosynthesis, the final step of which involves a radical process (Scheme 1),[8]



Scheme 1

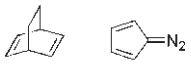
Recognition that ethylene causes premature senescence (ageing) and defoliation of plants dates to the nineteenth century when several reports of leaks of illuminating gas in greenhouses and near trees appeared.[9] Neljubov, a Russian plant physiologist, recorded etiolated pea seedlings (ones with small yellow leaves) growing horizontally in his laboratory but upright in outside air. He showed that the abnormal growth habit was caused by contaminating illuminating gas and went on to prove, in 1901, that the active principle in the gas was ethylene. Chemical proof that plants produce ethylene dates back some 75 years,[9] since which time the regulation of a multitude of plant processes by ethylene has been recognised. Sometimes ethylene is called the *death hormone* because it promotes the ripening and aging of many fruits and flowers.

As a fruit approaches maturity a *climacteric* (critical) event takes place and, during the build-up to this, ethylene production increases within the fruit and cell respiration rises. The production of the ethylene is associated with the expression of hundreds of genes that influence various ripening related changes, *e.g.* in fruit colour and sugar release; it marks the end of fruit maturation and the beginning of fruit senescence. For fruits that are food, the climacteric event equates to the peak of edible ripeness when the best texture and taste are present. After the event, fruit is more susceptible to fungal infection and begins to degrade. The climacteric fruits include apples, apricots, bananas, melons and tomatoes while citrus fruit, grapes and strawberries are non-climacteric as they ripen without ethylene and the associated respiration bursts. Many fruits are picked prior to full ripening because ripened fruits do not ship well. Thus, bananas are picked when green, shipped, and may be subsequently artificially ripened by treatment with ethylene to mimic the natural process. Left alone, bananas actually produce enough ethylene to accelerate ripening in other fruits when placed next to them.

Cut flowers such as the carnation and geranium, as well as a range of plants, can become stressed during transport and suffer as a result of the ethylene they produce – it reduces significantly the floral display and the consequent shelf-life. Thus, ethylene production can cause economic loss in the market place for growers, suppliers, and florists alike and for this reason such flowers are cooled prior to packing and kept between 1 and 4 °C during shipment. It is not surprising, therefore, that there has been a search for compounds that delay the onset of fruit ripening and extend the shelf-life of cut flowers.

Efforts to understand the effects that ethylene has on plant materials began by storing fruits under controlled atmospheres and assessing the changes that took place.[10] They then moved on to examine the nature of the ethylene binding site. Many compounds interact with the receptor, some reversibly, *e.g.* 2,5-norbornadiene (**7**), and some irreversibly, *e.g.* diazocyclopentadiene (**8**).[10,11] An assumption that the site involves coordination of ethylene by copper(I) has been confirmed, and studies have found that of several transition metals, only silver(I) mimicks the effect of copper, nicely consistent with the similarities of the two ions. Silver

thus proved capable of replacing copper and interacting with ethylene, but not in transducing the signal to downstream effectors.[9,12] Hence, the possible use of silver ion on an industrial scale to prolong flower life emerged and was taken seriously once silver thiosulfate became available.[10] However, usage of a heavy metal, coupled with the associated contamination concerns led researchers, notably Sisler and his colleagues, to seek organic analogues of ethylene that would do the same job.[10-14]



7 **8**

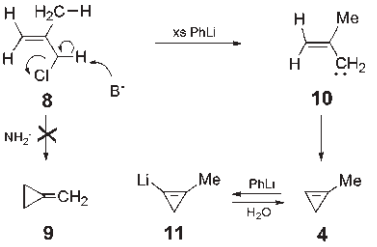
Cyclopropenes

There are two approaches by which the effects of ethylene can be minimised. The first seeks to prevent (or minimise) ethylene synthesis while the second aims to block the ethylene binding site completely. Inhibiting ethylene synthesis is the least effective option because ethylene from other sources is still able to bind and impact. In comparison, a blocking of the binding site prevents all ethylene from binding irrespective of its source such that fruits, plants and flowers remain in a state of limbo until the block is removed and ethylene production recommences - and this is independent of whether the ethylene source were endogenous or exogenous.

Work from the 1960's had shown that not only CO_2, but also CO and the lower members of the alkene family are ethylene antagonists and, of the last group, the longer chain members are inactive.[15] This led to the belief that activity was inversely related to molecular size and the idea that likely inhibitors of ethylene perception would be analogues of ethylene with comparable, but by no means identical spatial requirements. Thus cyclopropene and its simple alkyl derivatives received early attention[10,11,14,15] and the discoveries made stem in large measure from the laboratories of Sisler and Blakenship at North Carolina State University.

What was found in the mid-1990's was that cyclopropenes **1**, **4**, and the 3,3-dimethyl derivative **5** (Chart 1) are especially effective blockers of the ethylene binding site and prevent the physiological action of ethylene for extended periods of time.[13] After a 24 hour exposure to a concentration of **4** as low as 0.5 nL/L (1 mL in 2,000,000 L), carnation flowers (*Dianthus caryophyllus*) are protected for several days against the effects of ethylene, and 0.7 nL/L of **1** or **4** will prevent the ripening of banana (*Musa sapientum*) for 12 days at 24 °C after which they then ripen normally. By comparison the dimethylated analogue **5** has higher spatial demand; it requires a concentration 1000 times greater and then only provides a 7 day protection. Methylcyclopropene **4** is more easily handled than parent **1** and, as it appeared to be non-toxic at the concentrations that are active, its future use in regulating the ripening of fruits and preventing the deleterious effects of ethylene in plants and flowers seemed assured.[12] Cyclopropene **4** is now used in a number of horticultural products that are registered in more than 26 countries.

The first report of 1-methylcyclopropene (**4**) came from Fisher and Applequist[5] in 1965. These authors found that attempts to produce methylenecyclopropane **9** by 1,3-dehydrochlorination of 2-chloromethylpropene **8** with sodamide gave instead the endocyclic cyclopropene **4** (Scheme 2). The cyclization proceeds by way of α-elimination to vinylcarbene **10**, which then self inserts. The gaseous product was condensed in a dry ice trap and formally characterized. Improvements were made to the procedure in 1971 from use of phenyllithium as base[16,17] and exclusion of lithium bromide and non-PhLi bases, *e.g.* lithium alkoxide. Yields of **4** were in the 60-80% range when crystalline phenyllithium in ether was used. However, with excess of PhLi the cyclopropene is transformed into its 1-lithio salt **11** and this is stable for months in ether in the freezer.[17] With water **4** is rapidly regenerated and Sisler has provided a convenient laboratory procedure for this.[13]



Scheme 2

Sisler and Blakenship[14,18] acquired the first patent for commercial applications of **4** in the USA but had Floralife Inc. in South Carolina commercialize for the floral market with later development for fruits and vegetables planned.[19] A preparation approved by the US Environmental Protection Agency in 1999 for plants and flowers is sold as EthylBloc™. Rohm and Haas Company recognized the opportunity to purchase a product with a very favourable safety profile, formed subsidiary AgroFresh Inc. to develop **4** for food production, and purchased the rights for development in fruits and vegetables from Floralife in 1999. The development led to the subsiduary trading SmartFresh℠,[20] principally for the apple industry, following EPA approval in 2002. Although FloraLife was aquired by Rohm and Hass in 2006, it was sold to Smithers-Oasis Co. (a manufacturer of floral foam and related products) a year later. Since then, AgroFresh have provided a new liquid formulation of **4** under the Invinsa™ label[21] for use in stress protection of crops in the field. Their March 2008 press release says that they plan to have the technology commercial within two years and direct it to major field crops including canola, corn, cotton, rice, soybean, sunflower and wheat. Although set to trade independently, the Rohm and Hass company was taken over by The Dow Chemical Company in July 2008.

Because **4** is a gas under normal conditions commercial development demanded a simpler, easier, and more convenient synthesis, and then the means to get the product to market. Here, the advances in supramolecular chemistry dominate. Encapsulation of **4** can be brought about by hosts such as the cyclodextrins, crown ethers, zeolites, *etc*., and patents for these have appeared.[22,23] Use of α-cyclodextrin gives rise to a powder (from which subsequent tablet formulation is presumed) that is ideal for shipping and handling. On addition to water under normal conditions, the host-guest complex releases gaseous **4** within 20-30 min.[11] The original US patent[23] involved a batch synthesis and encapsulation of **4**. However, patent rights were granted to Rohm and Haas for a continuous process in 2005 (applica-

tion 2002).[22] In this the α-elimination from chloropropene **8** is brought about using a slurry of sodium amide in mineral oil with added hexamethyldisilazane ($Me_3Si\text{-}NH\text{-}SiMe_3$) which when mixed with **8** generates gaseous cyclopropene **4**. After purification, the cyclopropene is bubbled into a solution of cyclodextrin to precipitate the inclusion complex which is filtered off.[22]

The marketed formulation, *SmartFresh*SM, is applied to apples soon after harvest in a cone-like device within storage rooms. The treatment takes one day and leaves no detectable residue. As a side benefit, this treatment often replaces the need for diphenylamine ($PhNH_2$) to control the postharvest disorder, scald; the toxicity of **4** is less than that of the amine. SmartFreshSM was approved by the European Union in 2005 and is used in some 26 countries including NZ. Rohm and Haas gained ERMA approval for importation of **4** within the SmartFreshSM technology in late 2003. The much more recent Invinsa™ is a sprayable liquid formulation of **4** and the first product to specifically protect crop yield during extended periods of high temperature, mild-to-moderate drought, and other crop stresses. Although still under development, it is approved in Chile and Argentina and US regulatory approval was expected during 2008; it is to be marketed in a joint venture between AgroFresh and Syngenta AG.

SmartFreshSM has reaped a number of awards for providing fresher fruit and it is now used on apples, apricots, avocados, bananas, cantaloupe melons, kiwifruit, mangoes, nectarines, papayas, peaches, pears, plums, persimmons and tomatoes. Of course critics are plentiful. The use of **4** came under scrutiny in 2005 when it was revealed that the ripening of apples was being delayed by up to a year so that consumers purchased year-old produce without being aware of it; that similar storage lengths had been standard industry practice for many years was not mentioned. However, SmartFreshSM was given a *balanced* (author emphasis) account in the *Dining and Wine* section of the *New York Times* by David Karp[24] under the headline *Puff the Magic Preservative: Lasting Crunch, but Less Scent*. Despite some concerns about the effects of very low levels impurities,[25] the safety, toxicity, and environmental profiles of **4** are exceptionally favourable, and no death or clinical signs of systemic toxicology were seen.[26] The impact of **4** on the nutritional content of fruits is still under investigation although fruits appear to maintain antioxidant and vitamin levels.

The effective concentration of **4** needed varies widely with the commodity concerned,[11] and the temperature and the method of application, but the uses to which it continues to be put have placed 1-methylcyclopropene **4** among the significant players in the agrochemicals market. At the present time, something in excess of 60% of the apples sold on the North American market have been treated with SmartFreshSM, while cantaloupes imported into the US from Central America can be harvested riper and stay firm and free from decay after treatment with SmartFreshSM. The use of **4** is widespread. It is applied to substantial quantities of Asian pears in Korea, on South African and Chilean avocados and Chilean kiwis sent to Europe, and on small lots of plums in France and Chile.

In 2003 the series of 1-alkyl substituted cyclopropenes from methyl to decyl were prepared and assessed as ethylene antagonists for banana.[27] Each member of the series showed activity but, quite unexpectedly, from the 1-butyl derivative onwards *the effective minimum concentration fell below that of 1-methylcyclopropene*. The 12 day protection period of **4** was attained by 1-ethyl-, 1-propyl-, and 1-butylcyclopropene at ambient temperature (22-23 °C), but only from use of higher concentrations (4–6 nL/L *vs* 0.7 nL/L). However, 1-pentyl- was effective for 14 days, 1-hexyl- for 20 days, 1-heptyl- 21 days, 1-octyl- 25 days, 1-nonyl- 35 days, and 1-decylcyclopropene for 36 days. These results were achieved using concentrations of 0.3 – 0.5 nL/L and *lower* than the 0.7 nL needed for **4**; 1-decylcyclopropene gave the longest protection from ripening at the lowest concentrations (0.3 nL/L).[27] Some 20 cyclopropenes were assessed in a 2006 study reported in the Russian literature[28] and some of these compounds extended the exhibition life of cut mini-carnation flowers as well as delaying senescence of bean leaves.

During 2008 a further study employing a variety of 1-alkyl substituted cyclopropenes was reported.[29] This encompassed testing their potency to inhibit ethylene-induced plant processes on a variety of climacteric fruit like avocado and tomato, on ethylene-induced growth modification in etiolated pea seedlings, and on abscission in citrus leaf explants. Fruits responded differently than other plant organs to the same inhibitor, indicating possible differences in characteristics and availability of the ethylene receptors in the various tissues. The potency of the inhibitors was markedly dependent on their molecular structure and size, but each inhibitor gave the highest potency when treatment was prior to the onset of ethylene action. All of the fruits resumed normal ripening after recovery from the inhibition, a crucial factor when considering putative inhibitors for practical use.[29] It would seem from these studies that the cyclopropene moiety of the 1-alkyl derivatives blocks the binding site and that the long alkyl chain fits into the available volume and exerts little to no effect. Whether any of these more complex cyclopropenes will make it to the market remains to be seen, but any development will demand a simple synthesis and delivery mode as well as retaining the minimal toxicological impact of 1-methylcyclopropene (**4**).

There has been much recent activity in the Asian scientific communities with a number of reviews on the uses and applications of **4** appearing.[30] Thus, treatment of varieties of pears delayed fruit ripening and prolonged storage periods whilst keeping fruit quality and palatability.[31] It also inhibited the occurrence of melanoderma and black heart of pear.[32] The number of patent applications for formulations and uses of **4** in these countries has also increased over the last four years.

The strawberry typifies non-climacteric fruits but its ripening is not yet well understood and the role of ethylene is unclear. Recent studies have included use of **4** in the four different ripening stages (green, white, pink and fully ripe) of the fruit. As **4** specifically blocks the ethylene signal transduction pathway, its use enables the indirect identification of genes activated by ethylene.[33]

Those who prize organic and/or seasonal produce may well find that the premium prices increase in parallel to the use of **4**. However, the complete loss of **4** after removal of the fruit from containment is apparently encouraging AgroFresh to seek US National Organic Standards Board to allow fruits treated with SmartFresh[SM] to be labeled as *organic*.

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Patent Proze
But what does it actually do?

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The first Court decision from the United Kingdom to consider the validity of a gene sequence patent, *Eli Lilly* vs. *Human Genome Sciences*,[1] has accentuated the need for an invention to have a practical purpose.

The Background of the Decision

Patents are granted for inventions which are considered inventive and to be useful to society. Patents are not granted for mere discoveries. So, whilst the synthesis of a new compound may be the result of a significant investment, the compound itself cannot be patented without knowledge of a potential use, or more specifically, a commercial application. Similarly, while it is often possible to patent a gene sequence - even if identical to a gene found in its natural environment - the function or some commercial use for the gene sequence must also be disclosed.

In 2005, Human Genome Sciences (HGS) was granted a European patent covering the Neutrokine-α protein, the DNA sequence encoding it, antibodies binding to it, and corresponding pharmaceutical and diagnostic compositions. Neutrokine-α is a member of the TNF (Tumour

Necrosis Factor) ligand superfamily which has generated significant interest due to the role the ligands play in inflammation. With so many diseases associated with inflammation this family was a highly valuable target.

HGS-identified Neutrokine-α using bioinformatics – a process where computers are used to compare sequences and identify genes of interest by their similarity to previously identified and characterised genes. The patent application included a long description of predicted activities and uses for Neutrokine-α based on the genetic similarity to other members of the ligand family, which in some cases were not well understood at the time. However, the application contained no experimental evidence to support the asserted uses.

The Judgement

In 2007, Eli Lilly applied to revoke the patent on the grounds, amongst others, that there was a lack of industrial application (also referred to as utility, usefulness, or industrial applicability). The UK High Court agreed with Eli Lilly and revoked the patent. The court was of the opinion that the practical purpose was not clearly contained in the application in relation to what was known about the area of research at the time.

In coming to its decision, the Court set out some useful guidelines as to how to determine if an invention meets the *practical purpose* requirement. Highlights of the guidelines can be briefly summarised as follows:

1. The purpose need not be for profit;
2. it does not need to be explicitly laid out in the patent application but should be derivable by a person knowledgeable in the field taking into account what is known at the time of filing the patent;
3. the requirement will not be met if what is described is merely an interesting research result that might yield a yet to be identified purpose - a speculative indication of possible objectives is not sufficient;
4. if a substance is disclosed and its function is essential for human health, then the identification of the substance having that function will immediately suggest a practical application; and
5. it is not a bar to patentability that the invention has been found by bioinformatic techniques, although this may have a bearing on how a person knowledgeable in the field would understand what was described in the specification.

Specific, Substantial and Credible

Similar principles apply in other countries. For example, in the US patents are granted only for inventions which have a *specific, substantial, and credible* use.

That is:

- The use must be *specific* rather than generic; in this case a long and diverse list of functions was provided, some of which were contradictory, and therefore the purpose was not considered to be specific;
- a *substantial* use is an *immediate real world use* - if further research is necessary to identify a specific use, as in the present case, the purpose is not substantial; and
- the use must be *credible* to a person knowledgeable in the field of the technology, for example, a perpetual motion machine is unlikely to meet this requirement.

The Situation in New Zealand

In New Zealand, a third party can challenge a patent on the ground the invention is not useful. However, usefulness of the invention is not assessed during the examination phase before the Intellectual Property Office. Amendments to the legislation seem set to change this. The much delayed and anticipated draft Patents Bill sets out five criteria which must be met by a patentable invention, one of which is that it must be *useful*. It also goes on to define an invention as being *useful* if it has a *specific, credible, and substantial utility*. It seems likely that New Zealand Patent Examiners will be expected to examine patent applications for a practical purpose in the future.

In conclusion, a patent application must demonstrate a practical purpose for the invention. As one Judge put it in a previous judgement: *a patent is not a hunting license. It is not a reward for the search, but compensation for its successful conclusion*.

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] Eli Lilly *vs*. Human Genome Sciences, Inc. [2008] EWHC 1903 (Pat), 31 July 2008.

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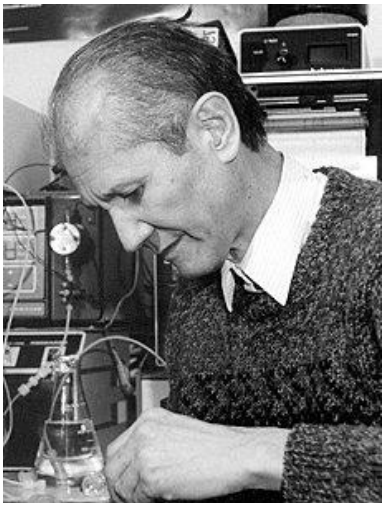
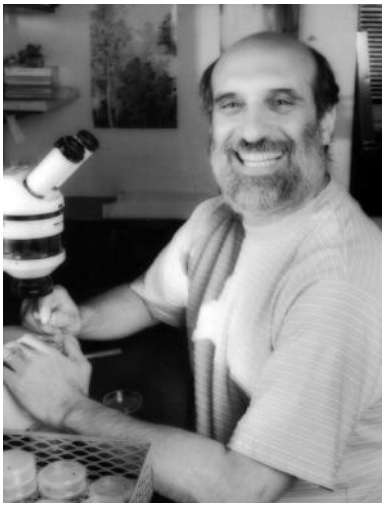
The 2008 Nobel Prize for Chemistry





L-R:Professors Osamu Shimomura, Martin Chalfie and Roger Tsien*

*Images used with permission from Boston University (Shimomura: *www.bu.edu/today/world/2008/10/08/bu-prof-wins-nobel-prize-chemistry*), Martin Chalfie (*www.columbia.edu/cu/biology/faculty/chalfie*), and UC San Diego (*http://ucsdnews.ucsd.edu/newsrel/science/NobelPrize08.asp* courtesy UC San Diego).

The 2008 Nobel Prize for Chemistry was awarded for the discovery and development of the green fluorescent protein (GFP). It rewards the initial discovery and a series of important developments that have led to its use as a tagging tool in bioscience. The Laureates were Osamu Shimomura, an Emeritus Professor of the Marine Biological Laboratory and Boston University Medical School, Boston; Martin Chalfie, Professor of Biological Sciences at New York's Columbia University; and Roger Y. Tsien, who is Professor and Investigator at the Howard Hughes Medical Institute at UC-San Diego.

The remarkable, brightly glowing, green fluorescent protein was first observed in the jellyfish, *Aequorea victoria* in 1962 by Shimomura. Since then, it has become one of the most important tools used in contemporary bioscience as it provides a means to monitor processes that were previously invisible, such as the development of nerve cells in the brain or how cancer cells spread. By using DNA technology, researchers can now connect GFP to other interesting, but otherwise invisible, proteins. The glowing marker allows them to watch the movements, positions and interactions of the tagged proteins. They can also follow the fate of various cells with the help of GFP, such as nerve cell damage during Alzheimer's disease or how insulin-producing β cells are created in the pancreas of a growing embryo. In one spectacular experiment, researchers succeeded in tagging different nerve cells in the brain of a mouse giving a kaleidoscope of colours.

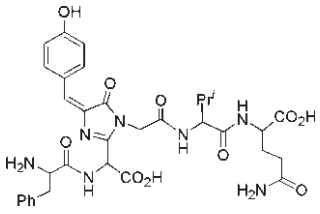
Green fluorescent protein is now a standard tool for thousands of researchers the world over. The story of its discovery has its origins in Japan in the years after WWII. Osamu Shimomura's education was disrupted by the war and the devastation caused by the atom bomb. Nonetheless, in 1955 he was employed as an assistant by Professor Yashimasa Hirata at Nagoya University and put to work on a seemingly impossible project – to discover what made the remains of a crushed mollusc, *Cypridina*, glow when it was moistened with water. That Hirata gave the inexperienced assistant such a difficult task may seem strange as leading American researchers had tried for a long time to isolate the material.

In 1956, and against all odds, Shimomura had the material in his hand. It was a protein that glowed 37,000 times more brightly than the crushed mollusc. After publishing his results, Shimomura was recruited to Princeton University by Frank Johnson. Professor Hirata was generous in seeing to it that Shimomura, despite not being a doctoral student, was awarded a PhD from Nagoya .

In Princeton, Shimomura began to study another naturally luminescent material. This time it was from the beautiful jellyfish *Aequorea victoria* (Fig. 1) that lives in the sea off the Washington coast of NW America and whose outer edge glows green when the jellyfish is agitated. During the summer of 1961, Shimomura and Johnson gathered jellyfish in San Juan Island's Friday Harbor. They cut off the edges of the jellyfish and pressed them through a filter to get what they termed a *squeezate*. One day when Shimomura poured some of the squeezate into the sink, it flashed brightly. He realised that there was seawater in the sink and that it was the ions present that had caused the chemical reaction. Strangely enough, the flash of light was not green like the edges of the jellyfish, but blue. The raw material gathered that summer was taken back to Princeton and within a few months they had isolated a few milligrams of the blue luminescent material from the liquid. It was a protein that they named *aequorin*.

In the 1962 publication that described the process by which aequorin was obtained,[1] Shimomura and Johnson also mentioned that they had isolated a protein that was slightly greenish in sunlight, yellowish in the light from a light bulb, and fluorescent green under UV light. This was the first time that anyone had described GFP. Shimo-

mura and Johnson called it the *green protein*, later to be named the *green fluorescent protein* by Morin and Hastings.[2] GFP contains 238 amino acids[3] and residues 65-67 (Ser-Tyr-Gly) spontaneously[4] form the fluorescent *p*-hydroxybenzylideneimidazolinone chromophore (shown below) in the primary structure.[5] The excitation spectrum has a dominant maximum at about 400 nm with a much smaller absorbance *ca*. 470 nm, while the emission maximum is a sharp at *ca*. 505 nm with a shoulder at 540 nm, it is green.[6]



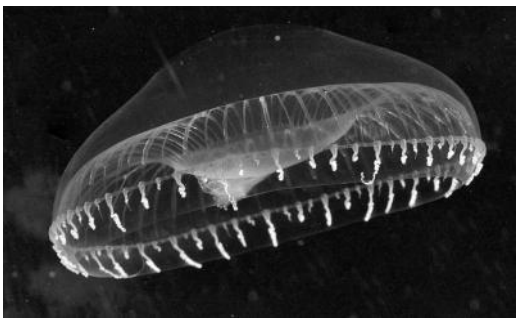


Fig. 1. *Aequorea victoria* from *http://en.wikipedia.org/wiki/Image:Aequorea4.jpg*; copyright S. S. Blakely

In jellyfish, the GFP chromophore simply transforms blue light from aequorin into green allowing it (and aequorin) to glow in different colours. GFP is revolutionary in that it does this alone in contrast to aequorin and other bioluminescent proteins, which require a continuous supply of energy rich-molecules. Irradiation of GFP with UV or blue light activates the GFP directly inside a cell where it glows green.

Chalfie had spent some time studying the millimetre-long roundworm *Caenorhabditis elegans* (Fig. 2), one of the most frequently studied organisms in the world. Although it consists of only 959 cells, it has a brain, grows old and mates. In addition, a third of the roundworm's genes are related to human genes. Last but not least, *C. elegans* is transparent making it easy to study its organs under an ordinary microscope. Chalfie heard about GFP in a 1988 seminar dealing with bioluminescence and he recognised that it would be a fantastic tool for mapping the roundworm. It would act as a glowing green signal for various activities in the roundworm's cells.

Fig. 2. Caenorhabditis elegans from *http://en.wikipedia.org/wiki/Image:Adult_Caenorhabditis_elegans.jpg*

To test his ideas, Chalfie needed to locate the gene for GFP in the genome of *Aequorea victoria*; he found that Douglas Prasher (Woods Hole Oceanographic Institution, Falmouth, MA) had already started the search. Prasher was asked if he would get in touch if he succeeded in cloning the gene, which he did. The GFP gene was sent to Chalfie who, in turn, had a graduate student attempt to get *E. coli* to produce GFP. Success came in about one month and the bacteria glowed green when irradiated with UV light.[7]

This discovery forms the basis of today's revolutionary use of GFP. However, the discovery was quite unexpected at the time as it was generally accepted that naturally fluorescing molecules and pigments were produced in several steps in the cells with each step requiring a protein to control the chemical production. Many believed that several proteins were needed to produce the chromophore in GFP, but Chalfie's experiment showed that this premise was wrong. The only protein needed is GFP. In the next step, Chalfie placed the gene behind a promoter that is active in six touch receptor neurons in roundworm *C. elegans* and saw the neurons glowing bright green. The image was displayed on the cover of *Science* in February 1994.[7]

GFP is generally non-toxic and can be expressed to high levels in different organisms with minor effects on their physiology.[7] Furthermore, when the gene for GFP is fused to the gene of a protein to be studied in an organism, the expressed protein of interest retains its normal activity; moreover, GFP retains its fluorescence. Thus, the location, movement, and other activities of the studied protein can be followed by microscopic monitoring of the GFP fluorescence.[8] Taken together, the remarkable and unexpected properties of GFP from *Aequorea victoria* are essential to its usefulness in studies of dynamic processes in living cells at the molecular level.

Roger Tsien's greatest contribution to the GFP revolution was to extend the palette by providing new colours that glowed longer and with increased intensity. To begin with, he charted how the GFP chromophore is formed chemically in the 238-amino-acid-long GFP protein. It was known that the three amino acids in position 65–67 of GFP react with each other to form the chromophore. He accounted for the reaction by showing that oxygen but no other protein is needed.[6] With the aid of DNA technology, Tsien then exchanged various amino acids in different parts of GFP to generate proteins that absorbed and emitted in other parts of the spectrum. By altering the amino acid composition, Tsien was able to develop variants of GFP that emit more strongly and with different colours such as cyan, blue and yellow.[6,8] Use of these now allows researchers to mark different proteins in different colours to see their interactions. Despite these advances, Tsien was unable to produce a red colour. Since red light is the best for penetration of biological tissue, it is especially useful in the study of cells and organs inside the body. Mikhail Matz and Sergei Lukyanov became involved in the GFP revolution by finding GFP-like proteins in fluorescent corals. They identified six more proteins; one red, one blue and the rest green.[9]

The desired red protein was designated DsRED from the coral *Discosoma*.[9] Unfortunately, it was larger and heavier than GFP. It consists of four amino acid chains instead of one, thus making it of less use as a fluorescent tag.[10] Tsien redesigned DsRED such that the protein is now stable and fluoresces as a single amino acid chain that can easily be connected to other proteins.[11]

From this smaller protein, Tsien's group also developed proteins with mouth-watering names like mPlum, mCherry, mStrawberry, mOrange and mCitrine, according to the colour they glowed.[12] Several other researchers and companies have also contributed new colours to this growing palette. Today, some forty-six years after Shimomura first wrote about the green fluorescent protein, there is a kaleidoscope of analogues that transmit all the colours of the rainbow.

Three of the proteins have been used recently in a spectacular experiment. Mice were genetically modified to produce varying amounts of yellow, cyan and red within the nerve cells of their brain. The result was a mouse brain that glowed with the colours of the rainbow. The researchers could follow nerve fibres from individual cells in the dense network in the brain. The experiment was termed *the brainbow*.[13]

One mystery yet remains to be solved. Why does the jellyfish *Aequorea victoria* shine? Many organisms living in the sea use light from biofluorescent proteins to confuse their enemies, to attract food or to tempt a partner. But no one yet knows which has caused *Aequorea victoria* to evolve aequorin and GFP.

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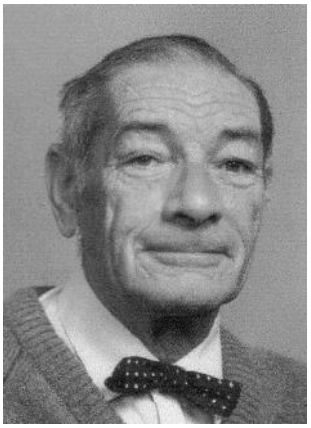
Brian Halton

(Compiled from material freely available; see: *htpp://nobelprize.org*)

Obituary

William Edward (Ted) Harvey 1925-2008

October 20 saw the passing of an icon in New Zealand Chemistry and the Institute. Ted Harvey was known nationally and internationally as the face of Chemistry in NZ from the nineteen years that he served as our Honorary General Secretary, his bowtie a hallmark. He became Assistant Secretary in 1956 and Secretary from 1957-1975. His worldwide dealings with our sister societies and their officers, and the chemistry visitors hosted by NZIC were paper-based, occasionally by telephone (e-mail and fax were not available then), and in person. Indeed, whenever he returned from an overseas trip he would tell us that he had met up again with … – and the list of distinguished chemists would roll off his tongue. On completion of his secretarial service in August 1975 he became 2nd Vice-President and subsequently President for the 1977-78 year, a role that he fulfilled with distinction. Subsequently he was elected to Honorary Fellowship.



Ted was born in Auckland in 1925 and attended Epsom Normal School and Auckland Grammar, gaining third place nationally in his Scholarship examinations after John Ziman and Bob Tizard (formerly Professor of Physics at Bristol University, and politician, minister and Deputy Prime-Minister, respectively). His MSc degree was with Professor Lindsay Briggs at Auckland University after which he moved to Cambridge for PhD study with Alexander (Lord) Todd. He then took a postdoctoral position with Holger Erdtman in Stockholm that lasted for two years and this was his most prolific research period generating five publications. His return to NZ was

to the Dominion Laboratory in Sydney Street where he met Helen (Barr), his wife of almost 52 years. In 1953 he accepted appointment to Victoria University of Wellington as a lecturer in chemistry under the headship of Prof. Stanley Slater and remained at the institution for the rest of his working days. He arrived when the Department was housed in the Hunter Building and was one of its inhabitants for five years before moving to the new *Easterfield Building* on Kelburn Parade when it opened in 1958. He was also Warden of the VUW hostel for men, Weir House, at that time and his term (1956-59) left lively memories.

He rose through the ranks of academia to become Reader in 1967 and Associate Professor in 1969. Ted had a first-rate pair of hands and was adept and astute at the bench but did not publish much of his work. The author recalls him being quite distressed one day having found data in a reputable journal that was wrong – it differed from his own that made markedly more sense – but the matter went no further. He had few research students but his last, John Miners, distinguished himself and is now Professor and Head of the Department of Clinical Pharmacology at Flinders University of South Australia and a 2008 Hon. FRSNZ. He taught generations of undergraduates, always taking a load commensurate with his colleagues, and he made a lasting impression on them. As Bill Jordan (Biological Sciences, VUW) said: '*Ted was always supportive and his teaching enhanced my passion for organic chemistry*'.

Ted's role in the Chemistry Department saw him take charge of technician training and be the interface for the general staff to the Administration. He ensured that the demands of the Technician Certification Authority were met and that every trainee technician spent the requisite period of time in each of the laboratories of the Department, thus gaining an ability to work in one area and provide trained back-up in another. This duty and the training syllabi involved him with Wellington Polytechnic (as it was) and he served also on its Council as the VUW representative from 1971 until the end of 1988. Additional to this, he had charge of the chemical stores, the ordering, and the provision of chemicals within the allocated budget. This was at the time when few materials came by air-freight and there was a single annual order that took from 3-6 months to come by boat. The annual order was the largest single exercise in purchasing and provided the chemical supplies needed throughout the university with discounted prices negotiated from the bulk order made. The undergraduate laboratories rarely ran out of supplies and few academic staff could complain of the services he provided, but then in those days, the store housed a good range of chemicals and Ted added some new and potentially useful materials each year.

Ted was always supportive of his colleagues and insistent that *organic chemistry* got its fair share of everything – some might suggest more! Undoubtedly, he was active in ensuring that the area received an established professorship to follow the mid-1960's appointments in inorganic (James Duncan) and physical (John Tomlinson). The position was advertised and an appointment made, with Robin Ferrier arriving in late 1970. During the early-to-mid-1970's Ted spent more and more of his time with administrative duties such that in 1978 he accepted appointment as Acting Registrar whilst retaining many of his duties in chemistry. The position was made permanent in 1979 and he formally left the Chemistry Department to be in administration until his retirement at the end of 1989. As Registrar he excelled, being as flexible as possible –as John Prebble (Law, VUW) said '*he even allowed me to swap a half-secretary for VUW's first laser printer'*. This, with its associated cables, allowed Law at VUW to become the first non-science department in an Australasian university to have a network!

As an individual, Ted formulated his views from the available information and was forceful with them. Equally, if he did not feel that your opinion or needs were valid the now legion '*you haven't a snowball's chance in hell*' would be proffered. His demeanour in the undergraduate laboratory was somewhat severe and likely would not be tolerated in today's climate. Yet every student knew precisely where they stood and what they needed to do; if they did not have the knowledge or skill to achieve a task he would painstakingly take them through the exercise. Ted was a master at the bench and few left his laboratory courses without a marked increase in ability. He returned as demonstrator to 1st-year after retirement where he was much admired and respected.

On a personal basis, Ted was the first academic I was introduced to on my arrival at VUW in September 1968. I was told that Ted would look after me and he did, likely in more ways than he ever imagined. His dedication to first-rate bench work, his assertion that professional matters rated highly, and his willingness to assist were immeasurable. That I have spent so much of my time on Institute matters has its basis in the professionalism that he and Denis Hogan inspired. He maintained his contacts with the Institute throughout his retirement usually attending Branch meetings, always cheerful and always helpful.

Away from work, his earlier days saw him active in tennis and hockey. As the years passed he took to hockey refereeing, then, later in the Saturday afternoon he would provide match reports on Radio 2ZB. In later times he continued with tennis, taking up golf only in retirement; he was an adept bridge player. He served many terms on the VUW Staff Club Committee, was its barman and cracked many a keg in the Rankine Brown facility.

Ted Harvey became a legend in his own lifetime, had a candle with more ends than could be counted and burnt them all. But above all he was a dedicated and loyal servant to his profession. He is survived by his chemist wife Helen, and son Matthew. We will miss him.

Brian Halton

(Sources: Author, Matthew Harvey, David Weatherburn and VUW Calendars 1950-90; photographs courtesy of the J.C. Beaglehole Room Archive Victoria University (left), and the Harvey family (right); used with permission)

Grants and Scholarships

Grants and Scholarships currently available for your application.

International Conference Fund

This fund is provided by the Minister of Research, Science and Technology and administered by the Royal Society of New Zealand. It is funding to assist organizations and institutions to host major international conferences in New Zealand. There is no closing date for applications.

For further information see the website: *http://www.rsnz.org/funding/int_conf/*

Seed Funding

The Royal Society of New Zealand has a limited amount of funding to assist organizations setting up meetings, workshops or symposia. There is no closing date for applications.

For further information see the website: *http://www.rsnz.org/funding/int_conf/*

Conference Assistance Programme

This programme provides assistance for bidding to host an international conference in New Zealand. This can include discounted airfares to travel to present bid, help to write bid documents and accompanying marketing material as well as other assistance.

For further details see the website: *http://www.conventionsnz.com/cap.aspx*

Foundation of Research, Science and Technology

The Foundation has a number of on-demand schemes that provides funding to enable businesses to develop new research and development projects. There are a number of different options available.

The following website has a table with how often these on-demand schemes are considered for funding and further details about the various schemes.

http://www.frst.govt.nz/investframe/process/ondemand

Enterprise Development Grants for Market Development

This fund is intended to help a business enter a new export market or new activity in an existing export market. Co-funding of up to 50% is offered.

For further details see the website: *http://www.nzte.govt.nz/section/14187.aspx*

Enterprise Development Grants for Capability Building

These grants help a business reach its growth potential by co-funding the cost of increasing skills and expertise. The funding can be used to purchase expert help and advice needed to undertake a significant new project.

For further details see the website: *http://www.nzte.govt.nz/section/14185.aspx*

NZ Science and Technology Postdoctoral Fellowship Scheme

These fellowships are for those with a doctoral degree. Funding is $61,000 per annum for up to three years and up to $30,000 per annum for research related costs. The Request for Proposals for the 2009 investment round will be released in February with a likely closing date in April.

For further details see the website: *http://www.frst.govt.nz/funding/students/postdoc*

Te Tipu Putaiao Fellowships

These fellowships are for students completing masters, doctorate or postdoctoral work in a science, engineering or technology discipline. All research proposals must demonstrate relevance and contribute to one of the four Vision Matauranga Research Themes. Funding depends on the qualification, ranging from $10,000 stipend for a masters to $61,000 for a post doctorate.

The Request for Proposal for the 2009 investment round will be released in February with a likely closing date in April.

For further details see the website: *http://www.frst.govt.nz/funding/students/TTP*

RSNZ Travel Grants

This provides for $1,000 to assist students, undertaking full-time PhD study in science, to attend their first overseas scientific conference (excluding any conferences they have been to in Australia). Closing date for applications is 1 March 2009.

For further details and an application form see the website: *http://www.royalsociety.org.nz/Site/International/travel/default.aspx*

Bilateral Research Activities Programme involving Germany

This fund is for researchers wishing to establish or further collaborative projects with their German counterparts. It provides funding to travel to Germany and for local costs. Priority is given to applications in health, food/agriculture biotechnology, environment, marine and Antarctic research areas.

For further details and an application form see the website: *http://www.royalsociety.org.nz/site/funding/germany/default.aspx*

Shirtcliffe Fellowship

This fellowship is to assist graduate students of outstanding ability and character to continue their studies. The Fellowship is for up to three years at a value of $5,000 per year. Closing date for applications is 1 March 2009.

For further details, criteria and an application form see the website: *http://www.nzvcc.ac.nz*

Vernon Willey Trust Fellowship

This fellowship is for research and education relating to production, processing and marketing of wool and the general developing and improvement of the sheep and wool industry. Closing date is 15 March 2009.

For further detail see the website: *http://www.meatandwoolnz.com/*

Continued next page...

Conference Calendar

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: *http://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: *http://www.pittcon.org/*

TRACE 5th Annual meeting and conference, Freising, Germany 1-3 April 2009

New methods and systems for confirming the origin of food including Isotope ratio mass spectrometry. Further details available at the website: *http://trace.eu.org/je/germany/*

Chemical BioPhysics Symposium, Toronto, Canada, 24-26 April 2009

Further details available at the website: *http://www.chem.utoronto.ca/symposium/biophys/2009/*

EUCHEM Conference on Stereochemistry, The 44th Burgenstock Conference, Brunnen, Switzerland, 17-22 May 2009

Further details available at the website: *http://www.stereochemistry-buergenstock.ch/*

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

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

2nd International Congress on Green Process Engineering, Venice, Italy, 14-17 June 2009

A conference on Green Process Engineering – environmentally conscious design of chemical processes. Further details available at the website: *http://www.gpe-epic2009.org/*

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

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

XXII Conference on Advances in Organic Synthesis, Karpacz, Poland 8-12 July 2009

Further details available at the website: *http://www.icho.edu.pl/aos2009/index.html*

ESOC 2009, 16th European Symposium on Organic Chemistry, Prague, Czech Republic. 12-16 July 2009

Further details available at the website: *http://www.esoc2009.com/*

EPF'09 European Polymer Congress, Graz, Austria 12-17 July 2009

A conference covering all aspects of polymer science. Further details available at the website: *http://www.epf09.org/*

21st International Symposium: Synthesis in Organic Chemistry, University of Oxford, United Kingdom, 20-23 July 2009

Further details available at the website: *http://www.rsc.org/ConferencesAndEvents/RSCConferences/OS09/index.asp*

13th International Conference on the Application of Density Functional Theory in Chemistry and Physics, Lyon France 31 August - 4 September 2009

Further details available at the website: *http://www.dft09.org/*

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.

Grants and Scholarships Continued...

Maori Education Trust Professions Scholarship

This scholarship is available to Maori students undertaking research at post-graduate level in a profession where very few Maori are represented. It is valued at $5,000. Closing date for applications, 1 May 2009.

For further details see the website: *http://www.maori-education.org.nz/sch/post_grad.html*

Roy Watling Mitchell Prestigious Professions Scholarship

This scholarship is available to a Maori graduate with a record of academic excellence and the proven ability to complete postgraduate studies in New Zealand. It is valued at $5,000. Closing date for applications, 1 May 2009.

For further details see the website: *http://www.maori-education.org.nz/sch/post_grad.html*

New Zealand Postgraduate Study Abroad Award

This award is available to postgraduate students enrolled in either Doctoral or Master's degree programmes at a New Zealand institution, whose research would benefit from up to six months of study or research overseas. The value of the award is up to $10,000 depending on the proposed project. Closing date for application is 1 May 2009.

For further details see the website: *http://www.newzealandeducated.com/int/en/institutions_courses/scholarships/outgoing/new_zealand_postgraduate_study_abroad_award*