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## Comment from the President



It was a pleasure to chair my first NZIC Council meeting in Wellington on Friday 20 February, where we examined a range of issues from Pacifichem, to enhanced engagement with the RSC, to improvement of our web interface, and much more. I'll pick up on one or two of these issues shortly. As a fun counterpoint, our Council meeting was held against the back-

drop of the New Zealand cricket team crushing the England team at the Wellington Stadium over the other side of our capital city. Who knows what will have happened by the time this text gets to print!

First, in this issue we celebrate the lives and contributions of two unique individuals who made their marks in very different ways in support of Chemistry in New Zealand.

Betty Wignall was Administrative Secretary of the NZIC for 15 years and worked tirelessly with the Canterbury Branch in particular over many years. She graciously leaves the tangible legacy of a scholarship to support our Canterbury PhD Chemistry students, established in 2011.

Tony Woolhouse was a gifted synthetic organic chemist and a Fellow of our Institute. All who knew Tony (including myself from the early 70s) recognised his ability to combine the highest quality science delivery with the most extraordinary flair and charisma.

I want to draw your attention to two issues active in the 2015 NZ Chemistry scene that featured at our February Council meeting.

Pacifichem 2015 will be held in Hawaii this December, with abstract submissions open until early April. With the next domestic NZIC conference scheduled for August 2016 in Queenstown (to be hosted by Massey) the Pacifichem conference represents the largest and most ac-

cessible major international conference on our revolving chemistry calendar and should be a primary conference focus for many of our chemists for 2015. I do encourage you to participate and raise the flag for New Zealand chemistry. As well as the value to our researchers there is actually a small subtext here since our national chemistry body does capture a small royalty from any Pacifichem profits proportioned to our attendance. You would have to agree that, aside from the professional value, Hawaii has an agreeably pleasant climate for a conference in December.

The Chemical Education 2015 conference is to be held this year in partnership with the Biology Educators' Association of New Zealand (BEANZ) at Victoria University from 5-8 July. The conference focus is "Moving Forward: Pathways and Partnerships for Biology and Chemistry Learning" and targets our Chemistry and Biology educators (see: <http://biolivechemed.nz/>). The NZIC is supporting both this conference and also what promises to be a unique chemistry experience for many: Dr Peter Wothers 'Chemistry of Light' extravaganza, with 5 performances to be held in Wellington (the last of which will be held during the opening ceremony for BioLive/Chem Ed 2015). There are also 3 performances to be held at Palmerston North. Peter Wothers (Cambridge Uni) is a brilliant science communicator. Check out his RSC lectures on YouTube.

Finally, let me introduce our new NZIC office bearers for 2015: our 1<sup>st</sup> Vice-President is Associate Professor Paul Pleiger from the Manawatu Branch and Massey University (who is also co-chair for the 2016 NZIC conference) and our 2<sup>nd</sup> Vice President is Professor Penny Brothers from the Auckland Branch. It was abundantly clear during our Council meeting that these experienced research leaders have ideas and drive that will bring great benefit to the aims and outputs of our Institute over the next few years.

Ian Brown  
Callaghan Innovation  
NZIC President 2015

## From the Editor



I am pleased to report that the problem I mentioned in January of having more material than we could publish has been repeated for April! It is very encouraging to have again received additional unsolicited contributions. Thank you to all the authors who were happy to have their contributions held

over to the next issue, including our regular *unremembered chemists* and *patent proze* items which will return in July.

This issue features Otago students past and present, the latter having participated in the annual student essay

competition. As you will notice from the biographical details, the essays have been written by students who are at various stages of their degree programmes. It is excellent to see these submissions from our young future scientists and of course it gives them valuable experience in scientific writing. Well done to all of the entrants.

We also have two articles from Auckland - on antimicrobial peptides and aquatic geochemistry - that were held over from the previous issue, a chemical perspective on the recreational use of geothermal waters in NZ, a review of a DVD on Ernest Rutherford and an article on the topic of air pollution which might provoke some controversy - and perhaps generate some Letters to the Editor? Enjoy the April issue.

Cath Nicholson

# New Zealand Institute of Chemistry

## supporting chemical sciences

### April News

#### Branch News

##### AUCKLAND

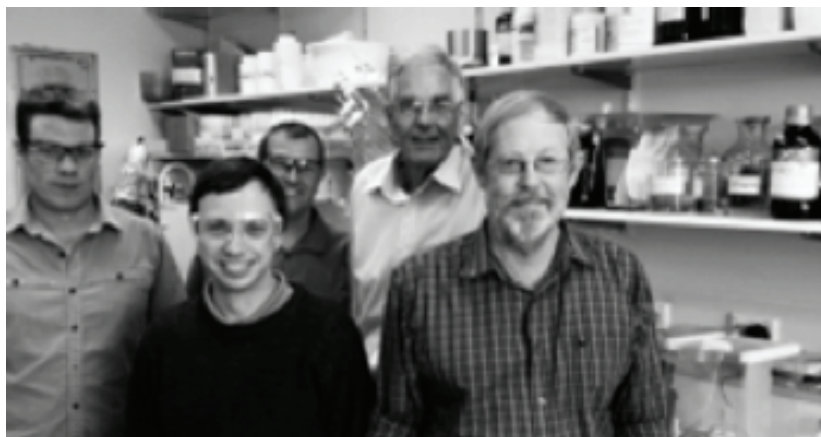
The Auckland Branch held its AGM in November 2014 and planned several activities for 2015 including two events centered on postgraduate students. The AGM was followed by a lecture entitled *Bringing drugs to clinical trial from a university setting* by Professor Bill Denny.

##### *The University of Auckland*

The School of Chemical Sciences celebrated the 100<sup>th</sup> anniversary of the establishment of the Department of Chemistry, now the School of Chemical Sciences. The SCS Centenary Celebrations were held on 13-14 March. News and highlights from the celebrations will be shared in the July issue of the journal.

University of Auckland researchers were crucial in the development of a potential tuberculosis drug (TBA-354) that is the first to advance to Phase One clinical trials in six years as was announced by the TB Alliance in New York in February 2015. TBA-354 emerged from the studies conducted by the TB Alliance in collaboration with the University of Auckland and University of Illinois-Chicago.

"Our chemistry team has worked on this since 2006 when the TB Alliance approached us to help with this project," says Professor **Bill Denny**, director of the Auckland Cancer Society Research Centre and a Principal Investigator in the Maurice Wilkins Centre at the University of Auckland. "We made several hundred compounds, from which TBA-354 was selected for clinical development in 2011. It's a validation of our work designing this compound to create a new and improved drug for the treatment of tuberculosis." A group of five researchers from the University of Auckland has worked on the project, with the chemistry led by Associate Professor **Brian Palmer**. The team is now working on another drug devel-



The successful TB drug development team from the Auckland Cancer Society Research Centre. From left: Senior Research Fellows Dr Hamish Sutherland, Dr Andrew Thompson, and Dr Adrian Blaser with Distinguished Professor Bill Denny and Associate Professor Brian Palmer.

opment project with the TB Alliance.

The School of Chemical Sciences was well represented at AMN7 (Advanced Materials and Nanotechnology Conference) held in Nelson in February with keynote (Professor **Kevin Smith** and Associate Professor **Cather Simpson**) and plenary (Professor **Jadranka Travas-Sejdic**) lectures delivered by staff. SCS students (**Julie Kho**, **Nina Novikova** and **Nihan Aydemir**) won highly commended poster awards.

##### *The New Zealand Institute of Advanced Study (NZIAS), Massey University, Auckland*

**Thomas Fallon** recently joined Massey University Auckland as the new lecturer in organic chemistry. His work is focused around fluxional molecules such as bullvalene. Thomas received his PhD from ANU in Canberra and joined the organic chemistry group of **Martin Oestreich** at the Technical University of Berlin as an Alexander von Humboldt Fellow.

**Peter Schwerdtfeger** has been invited to deliver the Källen Lecture series in theoretical physics at the Lund University in Sweden.

##### CANTERBURY

A combined NZIC-UC chemistry

seminar entitled *Ionic liquids for sustainable chemistry: applications in the chemical-, materials-, electro- and bio- sciences* was given in the Department by Professor **Douglas R. MacFarlane**, RSC Australasian Lecturer 2014, School of Chemistry, Monash University, Victoria, Australia on 11 December 2014 (<http://www.chem.monash.edu.au/ionicliquids>). The original seminar advertisement, which includes biographical notes, can be found at: <http://www.chem.canterbury.ac.nz/news/seminars/2014/McFarlane,%20Douglas.pdf>

##### *Postgraduate Student Research Showcase*

The Department of Chemistry held its 4<sup>th</sup> *Postgraduate Student Research Showcase* on 20 February. This was an afternoon event that celebrated the past and prospective research endeavours of our 2<sup>nd</sup> year postgraduate students (PhD). There were six excellent presentations during two sessions, given by **Fiona Given**, **Kajitha Suthagar**, **Nathaniel Gunby**, **Vivek Poonthiyil**, **Marat Sibae** and **Chris Burn**.

At the conclusion of the Showcase, the judges (Sally, Vlad, Matt and Alexander) deliberated for quite some time to decide the winner of

the *Ralph H. Earle Jr. Seminar Prize*. This prize results from a generous bequest given to the Department by the late *Ralph H. Earle Jr.*, (Ralph was a Postdoctoral Fellow in the Department during 1965), because of his strong belief that chemists should appreciate the importance of being able to verbally communicate their subject. The Prize is awarded annually for the best review seminar presentation given in the Department of Chemistry by a second-year postgraduate student. The Prize was awarded to Vivek Poonthiyil from the Fairbanks group for his presentation entitled *Synthesis and applications of gold and glycogold nanoparticles*.

There was also a RSC prize which was shared by Nathaniel Gunby and Marat Sibaev. Each and every presentation was superb and the quality of the delivery and research contained within them would allow them to sit comfortably within the programme of any international meeting. The students were a true credit to themselves, their research groups and to the Department.

Following the presentations there was a drinks reception sponsored by the Canterbury Branch of NZIC and ChemSoc. Many thanks to those who helped in the organisation and general set up of the day, and for washing up afterwards!

### University of Canterbury

Awards and appointments

Congratulations to Dr *Deborah Crittenden*, Dr *Sally Gaw* and Dr *Vladimir Golovko* who have been promoted to Senior Lecturer.

Congratulations to *Anna Farquhar* (working with Professor *Alison Downard* and Dr *Paula Brooksby*) who won the 'greatest potential impact' poster prize at the MacDiarmid Institute Investigator/Student/Postdoc meeting.

Congratulations to *Rohul Adnan* who successfully defended his PhD thesis on 17 December 2014. External examiner Professor *Jim Johnson* (VUW) was impressed by the quality of the presented work and number of publications (six) in Q1 and Q2 journals co-authored by Rohul.

Mr *David Young* (Kruger Research Group) has received the Don Shanks Award announced at the Royal Australian Chemical Institute's National Congress in Adelaide, 7-12 December 2014. This award recognises outstanding performance in research within a current PhD candidature and is made under the auspices of the RACI Inorganic division. David's research talk and poster were entitled *Examining the modular nature of zinc metal-organic macrocycles*.

Professor *Paul Kruger* and Dr *Sarah Masters* were both successful in the recent Dumont d'Urville Awards announced by The Royal Society of New Zealand in conjunction with the Ministry of Business, Innovation and Employment, and the French Ministry of National Education, Higher Education and Research. The broad purpose of the arrangement is to promote and support scientific and technological cooperation between New Zealand and French researchers in the public, non-governmental and private sectors. Paul's project was entitled *Switchable molecular magnetic materials* and is in collaboration with Professor *Corine Mathoniere* (IC-MCB University of Bordeaux). Sarah's project *Synthesis & characterisation by electron diffraction of new compounds for hydrogen storage* is in collaboration with Professor *Jean-Claude Guilleman* (ENSCR, Rennes).

Congratulations also (again) to Dr Sarah Masters who was successful in the recent Royal Society of Chemistry (UK) Research Fund Award with a project entitled *Development of gas-dynamic virtual nozzles to harness ultrabright electron sources: enabling single molecule protein structure determination*.

### MANAWATU

Members of the Manawatu Branch are organising a special event at Massey University to celebrate 2015 being the International Year of Light. Together with our colleagues in the Wellington Branch, we are arranging for Peter Wothers, MBE, from the University of Cambridge to bring his award-winning public demonstration lecture on the *Chemistry of light* to New Zealand. Peter has a reputation

as one of the most thrilling science lecturers in the UK and is regularly interviewed on television. He has run chemistry demonstrations for school-aged children for over a decade. Peter was awarded the 2011 President's Award by the Royal Society of Chemistry for his outreach activities. In 2014 he made the UK Science Council's list of 100 leading practising scientists. Peter will be giving his public demonstration in Palmerston North on the 9<sup>th</sup> and 10<sup>th</sup> of July. His action-packed shows will involve oil lamps used by the Romans, luminol and gun cotton explosions. Further news about this event will be posted shortly on the NZIC website. For more information, please contact *Mark Waterland* (M.Waterland@massey.ac.nz) or *Catherine Whitby* (C.P.Whitby@massey.ac.nz).

### Massey University, Institute of Fundamental Sciences

Dr *Andrew Wadsworth* (Auckland University) visited the laboratory of Dr *Vyacheslav V. Filichev* as part of an ongoing collaboration with Distinguished Professor *Margaret Brimble* and Professor *David Williams* on cell-penetrating peptide-DNA conjugates for 2 weeks in December 2014.

Professor Reuben Harris (Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, USA) presented a seminar entitled *Virus restriction and cancer mutation by the APOBEC family of DNA cytosine deaminases* on February 19. He visited the laboratories of Dr *Elena Harjes* and Dr Filichev as part of collaborative efforts to find inhibitors of APOBEC enzymes.

*Luke Liu*, a PhD student in Associate Professor *Shane Telfer's* group, has been awarded a Chinese Government Award for Outstanding PhD Students. This award is for Chinese students undertaking their PhD overseas. Around 500 students, including just three in New Zealand, were selected this year across all disciplines. The assessment is based on the students' publication records, reference letters, oral / poster presentations at international conferences as well as their contributions to their field.

In a somewhat belated announce-

ment, Associate Professor Shane Telfer has been awarded a full Marsden Grant (\$750k over three years) for a project entitled *Ordered multicomponent metal-organic frameworks*.

Associate Professor **Paul Plieger's** group welcomes **Becky Severinsen** as a new chemistry Honours student working on the chemistry of quinoquinolines and welcomes back **Joseph Corrigan** as a PGDipSci student working on the single molecule magnets project for 2015.

## OTAGO

Over summer the Otago Branch ran their second student essay competition, with the winners awarded cash prizes and their articles published in Chemistry in New Zealand. The 1st prize winner was **Richard Lamb** (Honours student 2014) with his essay *Misassigned natural products*. The 2nd place prize was awarded to **Alistair Richardson** (3<sup>rd</sup> year student 2014) who wrote about *Hot new chemistry from a kiwi pepper tree*. Both of these articles, along with others from present and past members of the University of Otago, are published in this issue.

## University of Otago, Department of Chemistry

Brookers Bunch has welcomed **Hannah Davidson** back as a PhD student, working on the design and synthesis of greener catalysts for a range of polymerisations. We also congratulate Hannah on winning both the Joseph and Emma Mellor Prize for the leading student of 400-level chemistry and the P. K. Grant Prize for experimental skill and research methodology at 400 level. **Sally Brooker**, her postdoc **Humphrey Feltham**, and PhD students **Reece Miller**, **Ross Hogue** and **Santi Rodriguez** participated in the Advanced Materials and Nanotechnology Conference (AMN7) in Nelson, with talks presented by Sally, Humphrey and Reece, and posters by Ross and Santi. Thanks to AMN7, in the following week we hosted four visitors at Otago so were treated to a series of absolutely fantastic department seminars, by Professors **Jeff Long** (Berkeley), **Linda Doerr** (Boston), **Richard Eisenberg** (Rochester) and **Eugenio Coronado** (Valencia). The Department has taken delivery of a Quantum Design Versalab instrument (3 tesla, cryogen-free physical property measure-

ment system) which was installed in late February.

Associate Professor **Michael Schramm** from the Department of Chemistry and Biochemistry, California State University, Long Beach visited the Crowley group in January. Michael's research efforts utilise principles of molecular recognition to design and prepare modular alpha-helical peptidomimetic libraries. Additionally, these principles are being directed at the preparation and study of synthetic small molecule membrane transporters, the topic of a seminar Michael presented during his visit.

A number of staff and students from the Department travelled to Adelaide in December for the RACI National Congress. **Lyall Hanton**, **Bill Hawkins**, **Nigel Lucas**, **Christopher Larsen** (Gordon and Lucas groups) and **Reece Miller** (Brooker group) all presented short research seminars, in Reece's case as a student finalist in the RACI-NZIC Stranks Awards. **Pauleen Bandedeen** and **Lisa Bucke** greeted the conference attendees at the Campbell Microanalytical Laboratory's booth amongst the trade exhibitors.



Otago Branch chair Guy Jameson presents prize cheques to the student essay competition winners (top) Richard Lamb and (bottom) Alistair Richardson.



Professor Eugenio Coronado (Valencia), Dr Neil Dille (Quantum Design) and Professor Sally Brooker with the new Versalab magnetometer. Photo by Ian Stewart, February 2015.

**WAIKATO**

The Waikato Branch Committee for 2015 is as follows:

Chairperson: Michael Mucalo

Treasurer: Michael Mucalo

Secretary: Raymond Onyekachi

Council Delegate: Jo Lane

Chemistry Education Group Representative: Jo Lane

Student Representative: Raymond Onyekachi

Student Liaison Representative: Bill Henderson

Branch Editor: Michèle Prinsep

Committee Members: John McDonald-Wharry, Brian Nicholson, Michèle Prinsep

**University of Waikato**

**Liam Mackintosh** has been working on organogold(III) chemistry over the summer with **Bill Henderson**. A number of Masters and Honours students are about to submit theses and dissertations and these will be reported on in the next issue of the journal. Chemistry welcomes three new staff. **Karla Watson** has been appointed as the technician in charge of the first year laboratory, **Tom Featonby** has been appointed as a research technician, primarily in charge of the mass spectrometer suite and **David Richens** has been appointed as a new academic to a teaching position with responsibility for a wide range of lectures.

**WELLINGTON**

December 11 2014 saw Dr **Miranda Li Wu**, the 2013 President of the American Chemical Society, meet with staff and present a Branch lecture on *Partners for progress and prosperity: celebrating diversity together!* Today she is a global ambassador for the ACS advocating for science and education. Her lecture covered the importance of diversity and inclusivity in science to solve global challenges and outlined her presidential theme of working together as *partners for progress and prosperity*. Marinda received her BS from The Ohio State University and PhD in inorganic chemistry from the University of Illinois in 1976. She

spent more than thirty years working in the chemical industry, many for Dow Chemical R&D and Dow Plastics Marketing where she forged partnerships between industry, education, government and communities.

**ESR News**

Dr **Helen Poulsen** attended the 2<sup>nd</sup> annual post-mortem toxicology course on interpretation of forensic toxicology run by the Center for Forensic Science and Research, Philadelphia, USA in the last week of January.

ESR-Forensic is currently in the final stages of implementing a new LIM system, a major undertaking for the entire business group.

**The Ferrier Institute**

The memorial service for former staff member Dr **Tony Woolhouse** (for a full obituary see elsewhere in this issue) was held on January 23 at Old Saint Paul's. It proved to be a notable gathering of Wellington scientists, harriers and cyclists encompassing Tony's life and career. A memorial cabinet is to be placed in the Ferrier Institute.

**Victoria University – SCPS**

The Annual Chemistry Teachers' Day, organised by Dr **Suzanne Boniface** was held on November 27 2014. It was attended by ca. 70 teachers from the greater Wellington region and from Nelson. The morning session began with two VUW speakers: Paul Teesdale-Spittle speaking on *Using chemistry to cure disease which* provided insight not just into the chemistry of potential drugs for cancer cure but also ideas for how to link this chemistry to the organic chemistry classroom. Justin Hodgkiss addressed *Tomorrow's smart materials: from hormone detectors to plastic solar cells* and looked at smart new materials with special properties that are being created at VUW at the interface of chemistry, physics, and biology. One material being formulated is for exquisitely sensitive biosensors that can detect the hormone estrogen and another, a new class of solar cell that can be printed from special plastic materials. These were followed by talks from local industries on the *Chemistry of*

*chocolate and coffee* (Chemistry of Coffee – Flight Coffee and Chemistry of Chocolate – Wellington Chocolate Factory).

Teachers shared examples of good practice with an emphasis on using technology and two RSNZ Teacher Fellows shared what they had learned from their time at SCPS. There was then a forum which allowed teachers to discuss current issues in chemistry teaching.

Following lunch there were workshops on process-oriented guided-inquiry Learning (POGIL), spectroscopy, and teaching scholarship chemistry. The day concluded following discussion on the 2014 examinations.

Dr **Richard Tilley** resigned his position in the SCPS and left on April 2 to take up a new position as Director of the Electron Microscopy Unit and Professor of Chemistry at the University of New South Wales, Sydney. We wish him and his family much success across the ditch. One of his former students, **Alec LaGrow**, returned from his King Abdullah University of Science and Technology postdoctoral in Saudi Arabia and delivered a seminar entitled: *Controlling size in the production of platinum alloy nano-octahedra* while home. He has taken up a second postdoctoral in the electron microscopy unit at York University (UK). In his seminar, Alec outlined why platinum alloys have undergone extensive research and in particular their shape controlled catalytic facet-dependent enhancements in catalytic activity and selectivity. He discussed his recent work on the continuous shape-controlled synthesis of platinum alloys sized from 1.5 – 16 nm by selecting the appropriate precursor and reaction time. Halide precursors in non-polar reaction conditions allow for particle size tuning from the halide ligand used and the amount of it present.

Dr **Rob Keyzers** was awarded one of the Victoria Teaching Excellence Awards for 2014.

Dr Lise-Marie Lacroix (Maitre de conférences, Université de Toulouse) visited the School on February 20, during her time at the Ferrier Insti-

tute. Her lecture: *Synthesis of metallic nanoparticles (NPs) exhibiting complex shapes: the versatility of liquid phase synthesis* was delivered to a large fascinated audience. She described the group's syntheses of nanoparticles of Fe, Pt and Au exhibiting peculiar morphologies. The use of transmission electron microscopy (TEM) and magnetic measurements allowed for the synthesis of metallic FeCo nanoparticles from organometallic precursors under dihydrogen and bulk magnetisation was obtained with the alloy NPs. She went on to describe the Pt 3 and 5-fold stars or dendrites obtained after reduction of chloride salts in the presence of oleylamine that are single

crystals with well-defined crystallographic faces of the fcc structure. Finally, she presented her ultrathin gold NWs, prepared by reduction of  $\text{HAuCl}_4$  in oleylamine, that have size homogeneity (diameter 1.7 nm, micrometer length), a unique 1D feature that confers remarkable conductivity properties such as quantum phenomena at room temperature. Any study of the electronic properties of single NW remains a technological challenge.

The MacDiarmid Institute for Advanced Materials and Nanotechnology's biennial meeting (AMN-7) was held in Nelson over February 9-13. Victoria staff and students made a

significant contribution.

Last year Suzanne Boniface visited ANU to observe Dr Peter Wothers from the University of Cambridge present his *Chemistry of light* show. This is an impressive 80 minute show that tells the history of light from a chemistry perspective. Wellington Branch is now bringing Peter to NZ and he will present his show as part of the International Year of Light NZ programme in Wellington (3 – 5 July) and Palmerston North (9 – 10 July). Peter's final show in Wellington will be at the opening of the ChemEd and BioLive teachers' conference to be held at Victoria University in the July school holidays.

## Single cell transcriptomes: how low can you go?

Chris Harris

Centre for Translational Cancer Research, Department of Biochemistry, University of Otago, PO Box 56, Dunedin 9054 (email: [harch909@student.otago.ac.nz](mailto:harch909@student.otago.ac.nz))

**Keywords:** *single cell, transcriptome, PCR, linear amplification*



Chris graduated with an LLB and BSc(Hons) in 2013 from the University of Otago. He is currently completing an MSc at Otago focussed on studying single cancer cells with the view to improving diagnostic tests. Chris hopes to continue his studies to improve outcomes for cancer patients.

enous mass of cells with different sub-populations that often co-operate to evade therapies and our natural defences.<sup>1-3</sup> There may be a small number of cells within a tumour that are integral to driving uncontrolled growth, (e.g. cancer stem cells). We need to study these cell types at a single cell transcriptome level to understand what they are doing and how to treat them.

In the last two years, efficient methods for studying individual cells at the transcriptome level have been developed. The importance of studying single cell transcriptomes to further our understanding of cancer and biology in general warrants greater understanding and improvement of these methods.

### Introduction

Biologists have been studying the cell since the discovery of this indivisible building block of life in 1665 by Robert Hooke. They are the basic units of structure and reproduction. Their importance is self-evident; however, the technology to study life at this indivisible level in great detail has only emerged recently.

The morphology of single cells has been well characterised, but their inner workings less so. The important instructions for the cell to make its products (e.g. proteins) are made of DNA and are stored in the nucleus. The DNA instructions (genome) cannot be moved outside the nucleus to the cellular factories, so copies of the instructions need to be made for delivery. These copies are made from a molecule called messenger RNA (mRNA). All the mRNA in a cell at any one time is called the 'transcriptome'. Studying the transcriptome, rather than the genome, will enable us to determine what the cell is actively producing and potentially in what quantities. For example, cancer happens because of mutations in DNA, but not all DNA mutations cause cancer. Studying the transcriptome will allow us to determine the functional impact of DNA mutations.

In the last four years, the advent of next-generation sequencing (e.g. RNA-seq) combined with whole transcriptome amplification methods have enabled us to study massive quantitative data sets. These methods have been largely applied to whole tissues rather than individual cells. When looking at the characteristics of any kind of population – people or cells – studying them as a whole, as opposed to individuals, can leave out important information. For example, a rugby team might score 20 points, but this gives no indication that one player scores 80% of those points.

Similarly, studying cells at an individual level can reveal clinically important extremes. Tumours often begin from a single initiator cell and grow to become a heterog-

### The challenge of single cell transcriptome amplification

It has been demonstrated that the average gene expression across a population of cells is significantly biased by a small sub-population of cells with high expression.<sup>4</sup> Specific transcripts can vary as much as 1000 fold between presumably equivalent single cells.

A single cell contains about 1-50 pg of RNA.<sup>5</sup> Diluting this amount of RNA in 10 µl of water (the volume used in some single cell reactions) is roughly the equivalent of sprinkling less than a pinch of salt in an Olympic-size swimming pool. Furthermore, only 1-5% of the total RNA is mRNA (0.01-2.5 pg per cell). It is not yet possible to sequence mRNA directly from a single cell. The mRNA needs to be amplified approximately 10<sup>7</sup> fold to be analysed with RNA-seq or microarray technology. Two broad amplification strategies have been described:

- Exponential amplification through a **reverse transcription (RT) - polymerase chain reaction (PCR)** based method
- Linear amplification through **RT - *in vitro* transcription (IVT)** or **phi29** based methods.

The first PCR method was described by Norman Iscove and the first IVT method was described by James Eberwine, both around 20 years ago.<sup>6,7</sup> Since that time, several variations of these strategies have been developed. Most of them are able to detect the expression of thousands of genes in single cells. They all attempt to optimise four elements for effective single cell transcriptome analysis:

1. High sensitivity (i.e. amplification of very limited) potentially very degraded, starting RNA amounts. This is particularly relevant for clinical samples.
2. Reproducibility between cells of the same type.
3. Coverage of all mRNA fragments, including rare species.

4. Fidelity of amplification (i.e. the ability to preserve the relative abundance and content of the starting mRNA population).

Many single-cell studies focus on reproducibility, whereas fewer consider fidelity. This is surprising, given the entire purpose of the amplification exercise is to determine the quantity of each mRNA species the cell is in fact producing. A couple of studies have compared amplified mRNA to unamplified mRNA to evaluate the fidelity of single cell methods. One evaluated both a PCR and IVT method<sup>8</sup> and the other focused on IVT.<sup>9</sup> Although the amplified and unamplified mRNA may be from the same source, determining fidelity from this comparison is significantly limited by sampling error. The challenge is to develop a method to determine what mRNA is in fact amplified.

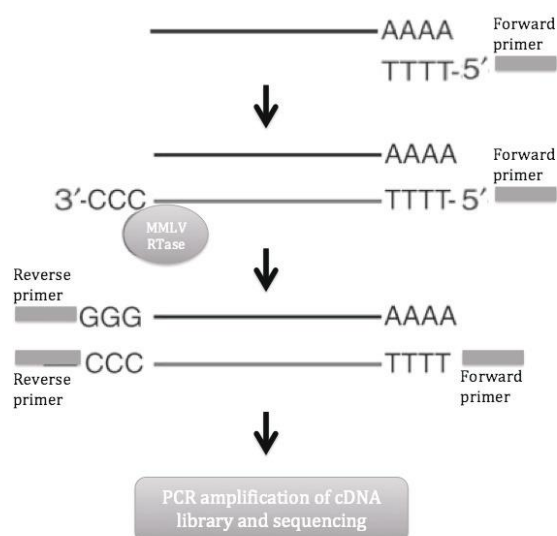
Islam *et al.* determined the absolute quantity of original mRNA transcripts by tagging each mRNA molecule with a unique molecular identifier (random 5bp sequence) before amplification.<sup>10</sup> The number of original molecules for each mRNA species should correspond to the number of unique molecular identifiers present in the amplified population for each species. For example, if only two kinds of unique molecular identifier are present among 10,000 amplified copies of a specific mRNA molecule, that means there were only two copies of the mRNA molecule to begin with.

### Amplifying the single cell transcriptome by PCR

The first step of all current amplification methods involves converting the mature mRNA into complementary DNA (cDNA) by reverse transcriptase (RTase). The RTase is primed from a synthesised poly-thymine oligonucleotide (oligo dT) primer annealed to the poly-adenine (poly A) tail of the mRNA (Fig. 1). This synthesised oligonucleotide contains the forward PCR primer. The reverse primer can be attached to the 5' end of the cDNA in a variety of ways.

A procedure was developed by Chenchik *et al.* (1998) to add a reverse PCR primer to the 5' end of cDNA with the help of the 'template-switch' (TS) effect.<sup>11</sup> This method takes advantage of the ability of Moloney Murine Leukemia Virus (MMLV) reverse transcriptase to add a few non-templated cytosines to the 3' end of the newly synthesised cDNA strand. This usually occurs when the reverse transcriptase reaches the capped 5' end of the mRNA. An oligonucleotide containing the reverse PCR primer sequence with three guanines on its 3' end is added to the reaction (the template switch oligonucleotide).

Several laboratories have taken advantage of the MMLV RT enzyme 'template switch' phenomenon, while adding their own modifications. One of the simpler methods is SMART-seq 2, developed by Picelli *et al.* (2014).<sup>12</sup> A similar method called single-cell tagged reverse transcription (STRT) was developed previously by Islam *et al.* (2011), but notably is able to analyse dozens of single cells in a single PCR.<sup>10</sup> This is possible because Islam *et al.* tag a unique barcode to the template switch oligonucleotide for each sample, enabling the samples to be distin-



**Fig. 1.** The addition of three cytosines to the 5' end of the cDNA by the RTase enables an oligonucleotide with the reverse primer attached to three guanines to base pair with the cDNA.

guished in downstream analysis. Multiplexing will be a necessary feature in methods designed to elucidate the nature of heterogeneity of populations of cells, requiring the analysis of thousands of cells.

### PCR by poly A tailing methods

An alternative to the template switching approach is the ligation of a poly A tail to the 3' end of the newly synthesised cDNA template by a terminal deoxynucleotidyl-transferase (TdT).<sup>13</sup> An oligo dT with the reverse primer sequence is then able to base pair to this poly A tail attached to the 3' cDNA end. This approach is more flexible than the MMLV capped switch approach given that it does not require the cDNA template to extend to the capped 5' end of the RNA. This is potentially useful for degraded RNA samples that have lost a large proportion of capped ends. However, it requires more reaction steps compared to template switching methods, potentially increasing the level of technical noise. Poly A tailing techniques such as Quartz-seq by Sasagawa *et al.* demonstrate impressive reproducibility.<sup>14</sup> Quartz-seq produced a Pearson correlation constant (PCC) of 0.93 when performed on triplicate diluted RNA samples (10 pg) from mouse embryonic stem cells. It remains to be seen how this method directly compares to SMART-seq 2.

### PCR problems and proposed solutions

According to a prevalent hypothesis in the literature, as the number of PCR cycles increase, the fidelity decreases as a result of the exponential increase in stochastic amplification and differing efficiencies for various template lengths and abundances.<sup>14</sup> Iscove *et al.* (2002) tested this hypothesis by comparing the amplified cDNA from an IVT linear amplification method and poly A tailing exponential amplification with unamplified RNA sample (10 µg).<sup>15</sup> Contrary to expectations, they found that the exponential amplification better preserved the relative abundance of mRNA transcripts than a single round of linear amplification. Further investigation is required to confirm this finding.

The TS approach has significant intrinsic problems relating to the production of background templates. This background 'noise' can consist of amplification of small artefacts produced by interruption of first strand synthesis and concatenation of TS oligonucleotides.<sup>16</sup> First strand interruption can be caused by the TS oligo hybridising to the first strand cDNA by sequence complementarity, before it has reached the 5' cap. Plessy *et al.* (2013) discourage first strand interruption by adding a 6- nucleotide long spacer between the barcode and the ribo-guanosines in the TS oligonucleotide.<sup>17</sup> Concatenation occurs when the MMLV RTase enzyme continues to add cytosines to the end of the complementary strand to the TS oligo. This results in a continuous string of linked TS oligos. Kapteyn *et al.* (2010) resolve concatenation simply by adding blocking isomeric bases to the 5' end of the TS oligo, preventing any extension from that end.<sup>18</sup>

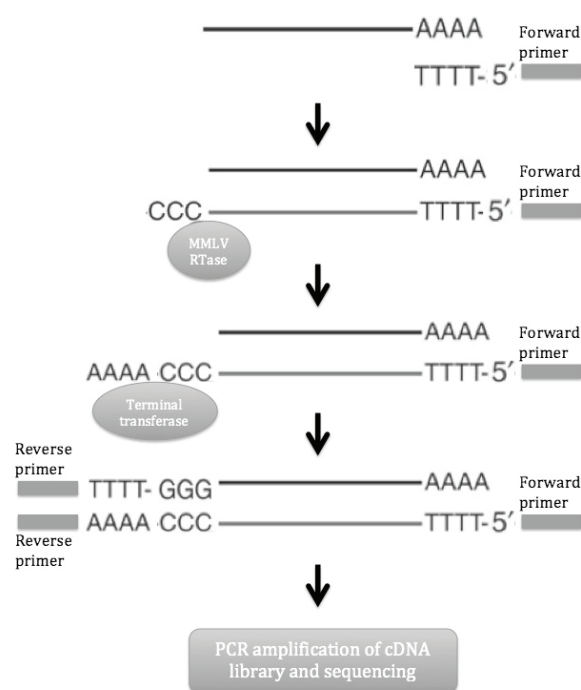
A TS approach combining elements of both MMLV RTase enzyme cytosine addition and poly A tailing was devised by Schmidt and Mueller (1999).<sup>19</sup> The poly A tail is ligated to the non-templated cytosines (Fig. 2). A TS oligonucleotide with a 3' string of ribo-thymines as well as ribo-guanines is required to base pair to the cDNA 3' overhang. This is designed to make it more difficult for the TS oligo to prematurely base pair and cause first strand interruption. Another approach incorporates a 'locked nucleic acid' (LNA) on the ribo-guanine end of the TS oligo.<sup>20</sup> Picelli reported that this doubled the cDNA yield compared to the conventional ribo-guanine ends when the SMART-seq 2 protocol was used. It was speculated that LNA increases the efficiency of TS oligo binding to the cytosine cap, probably because LNA:DNA base pairs have greater thermal stability.

Template switch by poly A tailing also has intrinsic byproduct issues. The TdT modifies the RT primer to produce <200 bp fragments.<sup>13</sup> Sasagawa *et al.* completely eliminated these byproducts by a combination of minimal oligo dT concentration, exonuclease I treatment, restricted poly A tailing and an optimised suppression PCR, all in a single tube reaction.<sup>14</sup> Interestingly, Sasagawa *et al.* found that topoisomerase V treatment alone could suppress byproduct synthesis. Topoisomerase V relaxes supercoiled DNA by cleaving and rotating a single strand in a double helix, and then ligating it back together. It is not known how it reduces byproduct synthesis in this case.

#### PCR without template switching or poly A tailing

While template switching remains an attractive option for PCR, whether by 'template switching' or poly A tailing, there are notable alternatives where a TS oligo is unnecessary.

Designed-primer seq (DP-seq) involves a PCR with a suite of specific primers that are designed to amplify the majority of the transcriptome.<sup>21</sup> Bhargava *et al.* (2013) developed an algorithm to identify 44 primer sequences that preferentially amplified unique transcripts of a reference mouse transcriptome, while minimising mis-priming and primer dimerisation. This enabled an RT reaction using the RT primer only, but required a more complicated PCR process. The PCR consisted of a 37°C Klenow polymerase



**Fig. 2.** A terminal transferase, or TdT, adds a poly A tail to the cytosines already added by the MMLV RTase. This combination increases the binding specificity by the complementary bases tagged to a reverse primer for PCR.

incubation step, followed by a PCR step with a 72°C extension, in order to amplify partially hybridised primers.

Bhargava *et al.* were able to amplify more than 80% of the transcriptome from 50 pg of mouse embryonic stem cell (mESC) RNA. Amplifying samples smaller than 50 pg resulted in a substantial increase of technical noise from PCR artefacts and significant deviations in the observed expression of low copy genes. The dominant bias was due to primers being blocked by cDNA secondary structure. This was despite efforts to predict secondary structure using UNAFold software. The designed primer method is better able to quantify rare transcripts than random primer methods and it has a high technical quantitative reproducibility ( $R^2=0.86$  from 50 pg RNA samples).

#### Amplifying the single cell transcriptome by linear methods

The second broad category of amplifying cDNA transcribed from mRNA is by linear methods. Linear amplification methods tend to only amplify a few hundred base pairs of template. This has several theoretical advantages over exponential amplification by PCR. PCR amplification is most efficient over small regions of a few hundred base pairs, so that longer cDNA strands are not amplified to the same degree as shorter strands. The differential PCR amplification of a 1 kb cDNA fragment compared to a 6kb fragment can be as much as 1300 fold after only 25 cycles.<sup>22</sup> Also, very small sub-populations of RNA can miss being amplified in PCR, and those that are amplified can become over-represented exponentially.<sup>23,24</sup> Therefore, in theory, linear amplification methods are better at preserving the relative abundances of mRNA species in the original sample.

Linear amplification methods for single cells have been

largely based on the T7 RNA polymerase (T7) and the phi29 DNA polymerase (phi29), both derived from bacteriophages. The T7 catalyses the formation of first strand anti-sense RNA (aRNA) from template cDNA (known as *in vitro* transcription or IVT). T7 can amplify RNA up to 1000 fold in one round of amplification.<sup>25</sup> It has the advantage of being extremely specific to a T7 promoter sequence. Limitations include the low processivity of T7 (only synthesises up to 1500bp of RNA per binding event,<sup>26</sup> though this isn't necessarily relevant for quantification) and a requirement for double stranded promoter sequence. This method is also unsuitable for the sense strand probes of spotted oligo microarrays because the aRNA needs to be converted back to sense strand cDNA.<sup>27</sup>

### T7 RNA polymerase methods

CEL-seq (Cell Expression by Linear amplification and Sequencing) is a T7 polymerase method developed by Hashimshony *et al.* (2012).<sup>28</sup> IVT on single cells has an inherent limitation in that at least 400 pg of starting RNA is required. Hashimshony *et al.* remove this problem by pooling samples that have been barcoded with a unique sequence in the oligo dT after the RT, while also providing a multiplex capability similar to the PCR STRT method. Hashimshony's group compared the data gathered from their method with the data from Islam's PCR method, and found their linear method was more reproducible, amplified more genes than STRT, and was better able to distinguish gene expression levels between different cell types.

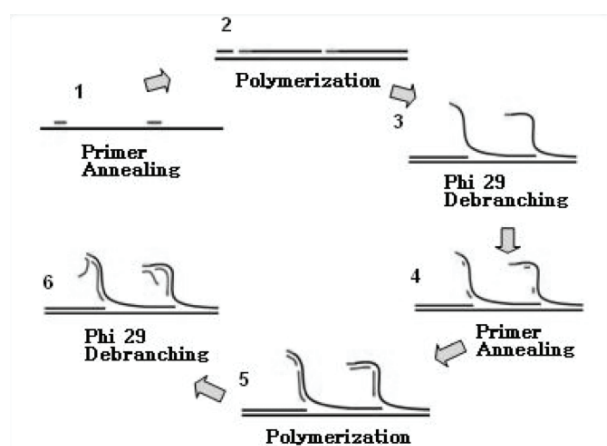
The Chum-RNA method is another T7 IVT method developed by Tougan *et al.* (2008) that uses a different approach to overcome the 400 pg hurdle.<sup>29</sup> Key enzymes involved in the IVT reaction, such as RTase, T7 RNA polymerase, DNA polymerase I and DNA ligase, require at least 1  $\mu$ M of substrate to work efficiently. This is about one million times more than what is available from a single cell. Tougan *et al.* resolve this by adding dummy or 'Chum' RNA (41bp strands including a poly A tail) to the single cell amount of RNA. This allows the enzymes to be in contact with sufficient substrate, enabling efficient enzyme reaction. 'Chum' refers to the 'friendly' action of the RNA.<sup>29</sup> The whole RNA population is converted to cDNA and amplified efficiently, including the single cell RNA. The dummy cDNA population is then selectively removed by column chromatography, leaving the population of interest.

The Chum-RNA method allows the amplification of down to 0.49 femtograms of mRNA (or 730 molecules of RNA), corresponding to a sub-population of mRNA in a single cell. This is the lowest amount of RNA that has been amplified by any single cell method, demonstrating the Chum-RNA's impressive sensitivity. Fluorescent labeling and microarray analysis showed that this method did not introduce any significant bias. The Pearson correlation of gene expression between a single cell and 1 million cells was 0.98, potentially indicating strong fidelity.

### phi29 DNA polymerase methods

In contrast to T7 polymerase, phi29 polymerase synthesises DNA from cDNA and is characterised by the high-

est processivity among known DNA polymerases (70kb insertions per binding event).<sup>30</sup> It is also characterised by its ability to amplify DNA by multiple strand displacement. Multiple strand displacement begins with random primers annealing to the template to initiate polymerisation by phi29. When phi29 reaches the next primer on the strand, it causes the newly formed double strand to become single stranded at that point, i.e. it becomes displaced (Fig. 3). This allows further primer annealing to occur on the displaced, newly synthesised, strand. The process repeats on the newly synthesised strand, resulting in a hyper-branched network of polymerisation and a high yield of product. The Klenow polymerase used in DP-seq also has strand displacement activity.



**Fig. 3.** The phi29 DNA polymerase elongates DNA from the primer annealed to the cDNA template strand (steps 1 and 2). Once the polymerase reaches an adjacent primer, the new strand is partially displaced (debranched) from the template (step 3). This enables more primers to anneal to the newly synthesised strand (step 4) and further DNA polymerisation occurs using the new strand as the template (step 5). This process is repeated.

Pan *et al.* (2012) developed the phi29-mRNA amplification (PMA) method that was adapted from their whole genome amplification procedure.<sup>31</sup> The cDNA is circularised by intra-molecular ligation before amplification by phi29. This enables multiple strand displacement and amplification to occur in a rolling circle, so that both ends of the cDNA are captured (Fig. 4).

Pan *et al.* used PMA to detect ~5000 transcripts from a single cell from an erythroleukemic cell line, with coverage of most or all codons. However, PMA was unable to detect many low abundance transcripts. The low abundance sequences that were picked up were sometimes missing their 3' ends. This suggests these transcripts were being lost before or after cDNA circularisation because of incomplete ligation. Although PMA is less sensitive than an exponential amplification method, it gives full-length transcripts. This may be important for delineating novel splice variants.

## Discussion

There are a multitude of methods currently available to amplify the transcriptome of single cells. Only a few of the more mainstream methods have been discussed here and there are many others.<sup>32</sup> The next steps will be to determine systematically which methods have the best sensitivity, coverage, reproducibility, and fidel-

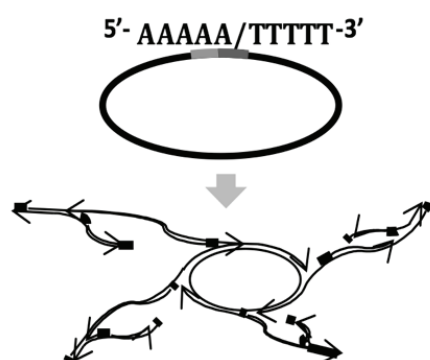


Fig. 4. The cDNA template is circularised, and strand displacement with further primer annealing for phi29 DNA polymerase initiation occurs in the same fashion as in Fig. 3.

ity, and how to improve upon these elements. Very few studies attempt to quantify biological stochasticity and stochasticity caused by technical error.<sup>33</sup>

All of these methods are limited to amplifying poly-adenylated RNA transcripts, i.e. predominantly mRNA. Future methods should encompass non poly-adenylated mature RNA species that have a role in gene expression regulation and carcinogenesis, such as micro-RNA.<sup>34</sup> Poly A tailing to these non poly-adenylated molecules is a possible solution. Improving sensitivity will also be important for studying long non-coding RNA with important regulatory roles, despite being present in low numbers.<sup>35</sup>

Expense and labour are also factors often overlooked in the literature. It may be necessary to sequence the transcriptome of hundreds or thousands of cells from a single tissue and the costs and labour quickly skyrocket. The future will likely be in automated microfluidic lab-on-a-chip methods, such as those developed by Fluidigm<sup>®</sup> that use a PCR based approach.<sup>36</sup> The Fluidigm<sup>®</sup> C1<sup>™</sup> Single-Cell Auto Prep System is capable of processing up to 96 individual cells at a time. Linear amplification methods tend to be more time consuming than PCR-based methods, and therefore perhaps less suited to clinical use.<sup>23</sup>

As single cell transcriptome amplification methods improve, so will our understanding of biological processes involved in carcinogenesis and organ or embryo development. We will only reach a fuller understanding of these processes when we go beyond poly-adenylated transcripts to all cellular transcripts. It is hoped that one day these methods will be applied in the clinic to improve the sensitivity and specificity of diagnosis, determine prognosis and improve the treatment of cancer and other diseases.

### Acknowledgements

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### References

1. Nowell, P. C. *Science* **1976**, *194*, 23–28.
2. Merlo, L. M.; Pepper, J. W.; Reid, B. J.; Maley, C. C. *Nat. Rev. Cancer* **2006**, *6*, 924–935.
3. Axelrod R.; Axelrod D. E.; Pienta K. J. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 13474–13479.

4. Bengtsson M.; Ståhlberg A.; Rorsman P.; Kubista M. *Genome Res.* **2005**, *15*, 1388–1392.
5. Boon W.C.; Petkovic-Duran K.; Zhu Y.; Manasseh R.; Horne M. K.; Aumann T. D. *J. Vis. Exp.* **2011**, *11*, e3144.
6. Brady, G.; Barbara, M.; Iscove, N. *Methods Mol. Cell. Biol.* **1990**, *2*, 17–25.
7. Eberwine J.; Yeh H.; Miyashiro K.; Cao Y.; Nair S.; Finnell R.; Zettel M.; Coleman P. *Proc. Natl. Acad. Sci.* **1992**, *89*, 3010–3014.
8. Iscove, N. N.; Barbara, M.; Gu, M.; Gibson, M.; Modi, C.; Winegarden, N. *Nat. Biotechnol.* **2002**, *20*, 940–943.
9. Subkhankulova, T.; Livesey, F. J. *Genome Biol.* **2006**, *7*, R18.
- Islam, S.; Zeisel, A.; Joost, S.; Manno, G.; Zajac, P.; Kasper, M.; Lönnberg, P.; Linnarsson, S. *Nat. Methods* **2014**, *11*, 163–166.
11. Chenchik, A.; Zhu, Y.; Diatchenko, L.; Li, R.; Hill, J.; Siebert, P. In *RT-PCR Methods for Gene Cloning and Analysis* (Ed.: Siebert, P.; Larriek, J.), BioTechniques Books, MA, 1998, 305–319.
12. Picelli, S.; Faridani, O. R.; Björklund, A. K.; Winberg, G.; Sagasser, S.; Sandberg, R. *Nat. Protoc.* **2014**, *9*, 171–181.
13. Tang, F.; Barbacioru, C.; Nordman, E.; Li, B.; Xu, N.; Bashkirov, V. I.; Lao, K.; Surani, M. A. *Nat Protoc.* **2010**, *5*, 516–535.
14. Sasagawa, Y.; Nikaido, I.; Hayashi, T.; Danno, H.; Uno, K. D.; Imai, T.; Ueda, H. R. *Genome Biol.* **2013**, *14*, R31.
15. Iscove, N. N.; Barbara, M.; Gu, M.; Gibson, M.; Modi, C.; Winegarden, N. *Nat. Biotechnol.* **2002**, *20*, 940–943.
16. Tang, D. T.; Plessy, C.; Salimullah, M.; Suzuki, A. M.; Calligaris, R.; Gustincich, S.; Carninci, P. *Nucleic Acids Res.* **2013**, *41*, e44.
17. Plessy, C.; Desbois, L.; Fujii, T.; Carninci, P. *Bioessays* **2013**, *35*, 131–140.
18. Kapteyn, J.; He, R.; McDowell, E. T.; Gang, D. R. *BMC Genomics* **2010**, *11*, 413.
19. Schmidt, W.; Mueller, M. *Nucleic Acids Res.* **1999**, *27*, e31.
20. Picelli, S.; Björklund, A. K.; Faridani, O. R.; Sagasser, S.; Winberg, G.; Sandberg, R. *Nat. Methods* **2013**, *10*, 1096–1098.
21. Bhargava, V.; Ko, P.; Willems, E.; Mercola, M.; Subramaniam, S. *Cell Rep.* **2013**, *3*, e1740.
22. Jeffreys, A. J.; Wilson, V.; Neumann, R.; Keytel, J. *Nucleic Acids Res.* **1988**, *16*, 10953–10971.
23. Day, R.; McNoe, L.; Macknight, R. *Int. J. Plant Genomics* **2007**, *2007*, e61028.
24. Lauss, M.; Vierlinger, K.; Weinhaeusel, A.; Szameit, S.; Kaserer, K.; Noehammer, C. *Virchows Arch.* **2007**, *451*, 1019–1029.
25. Wilhelm, J.; Muyal, J.; Best, J.; Kwapiszewska, G.; Stein, M.; Seeger, W.; Bohle, R.; Fink, L. *Clin. Chem.* **2006**, *52*, 1161–1167.
26. Skinner, G.; Baumann, C.; Quinn, D.; Molloy, J.; Hoggett, J. *J. Biol. Chem.* **2004**, *279*, 3239–323244.
27. Goff, L.; Bowers, J.; Schwalm, J.; Howerton, K.; Getts, R.; Hart, R. *BMC Genomics* **2004**, *5*, e76.
28. Hashimshony, T.; Wagner, F.; Sher, N.; Yanai, I. *Cell Rep.* **2012**, *2*, 666–673.
29. Tougan, T.; Okuzaki, D.; Nojima, H. *Nucleic Acids Res.* **2008**, *36*, e92.
30. Blanco, L.; Bernad, A.; Lázaro, J.; Martín, G.; Garmendia, C.; Salas, M. *J. Biol. Chem.* **1989**, *264*, 8935–8940.
31. Pan, X.; Durrett, R. E.; Zhu, H.; Tanaka, Y.; Li, Y.; Zi, X.; Marjani, S. L.; Euskirchen, G.; Ma, C.; Lamotte, R. H.; Park, I.-H. H.; Snyder, M. P.; Mason, C. E.; Weissman, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 594–599.
32. Saliba, A.-E.; Westermann, A.; Gorski, S.; Vogel, J. *Nucleic Acids Res.* **2014**, gku555.
33. Marinov, G.; Williams, B.; McCue, K.; Schroth, G.; Gertz, J.; Myers, R.; Wold, B. *Genome Res.* **2014**, *24*, 496–510.
34. Saini, H. K.; Griffiths-Jones, S.; Enright, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 17719–17724.
35. Derrien, T.; Johnson, R.; Bussotti, G.; Tanzer A. *Genome Res.* **2012**, *22*, 1775–1789.
36. Streets, A.; Zhang, X.; Cao, C.; Pang, Y.; Wu, X.; Xiong, L.; Yang, L.; Fu, Y.; Zhao, L.; Tang, F.; Huang, Y. *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111*, 7048–7053.

# The application of membrane technology to post-combustion separation of carbon dioxide from coal-fired power plant flue gas

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**Keywords:** *membrane technology, post-combustion carbon dioxide capture*



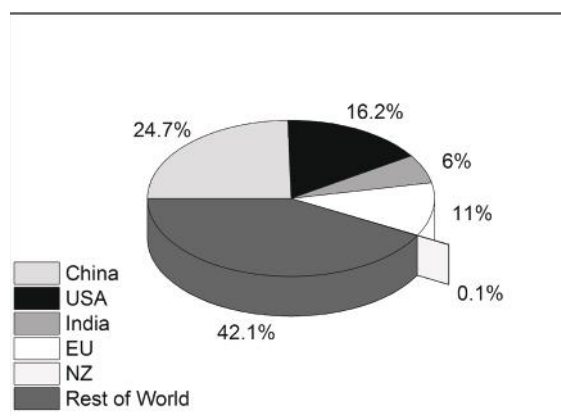
Matthew received his BSc(Hons) (2008) and PhD (2012) in inorganic chemistry at the University of Otago. Since 2012 he has worked as a postdoctoral associate at the University of Colorado Boulder, split between the Chemistry and Chemical Engineering Departments. During this time he was part of the academic-industrial team which successfully completed a \$3.8 million DOE ARPA-E project relevant to post-combustion carbon dioxide capture by producing the ~6000 GPU membrane described in this article. He has also taken part in numerous collaborative projects regarding materials for olefin/paraffin separation, magnetic alignment of lyotropic liquid crystals, metal-ion-containing ionic liquids, materials for carbon dioxide/methane separations, and barrier materials for chemical warfare agents. Matthew is currently working on a second DOE ARPA-E project related to carbon dioxide/nitrogen separation technology. His future research interests include fundamental and directly applicable science in the field of inorganic/organic materials chemistry.

## The convenience and cost of coal power

Coal-fired power plants are extremely useful sources of electricity. Coal is an abundant and cheap source of energy for electricity generation. It has relatively few alternate uses when compared to other fossil fuels such as petroleum and natural gas. Coal-fired power plants can be located close to areas of major electricity consumption, like population or industrial centres, to minimise losses or interruptions in the transmission of electricity. Furthermore, coal-fired power plants are reliable and their output can be increased at short notice, allowing them to cope with surges in demand.

New Zealand is fortunate to have significant sources of renewable energy, including wind, geothermal, and hydroelectric.<sup>1</sup> However, the Huntly power plant plays a strategic role in meeting electricity needs, responding to surges in demand, and avoiding transmission losses. The Huntly plant runs using a mixture of coal and natural gas and is capable of generating 20% of NZ's electricity needs, but in doing so produces over 50% of New Zealand's carbon dioxide gas emissions from electricity generation. The coal units at Huntly were estimated to produce 1600 kT of CO<sub>2</sub> in 2010,<sup>2</sup> approximately 5% of New Zealand's total CO<sub>2</sub> emissions for that year.<sup>3</sup> If Huntly were an average, 600 MW<sub>e</sub> coal-fired power plant (which it is not),<sup>4</sup> it would emit 11,000 tons of CO<sub>2</sub> per day<sup>5</sup> - approximately 13% of New Zealand's total CO<sub>2</sub> emissions in 2010.<sup>3</sup>

Globally, coal plays a much greater role than it does in New Zealand. Many industrialised and developing countries are heavily reliant on coal for electricity generation. Incidentally, countries with some of the largest coal deposits are also the highest emitters of CO<sub>2</sub>. These are China (25%), the United States (16%),<sup>6</sup> and India (6%) - accounting for 47% of total world CO<sub>2</sub> emissions in 2010 (Fig. 1).<sup>3</sup> A 2007 study led by the Massachusetts Institute



**Fig. 1.** The relative contributions of countries to global CO<sub>2</sub> emissions in 2010<sup>3</sup>

of Technology determined that CO<sub>2</sub> emissions from coal-fired power plants account for almost 40% of annual global anthropogenic CO<sub>2</sub> emissions.<sup>7</sup>

Research on climate change and ocean acidification has made it abundantly clear that there will be significant environmental, economic, and social costs to generating and releasing large amounts of CO<sub>2</sub> into the atmosphere.<sup>8</sup> Being able to separate and capture CO<sub>2</sub> from large point sources, such as coal-fired power plants, would significantly slow the rate that anthropogenic CO<sub>2</sub> is released into the atmosphere. This would help to alleviate, or at least delay, the worst effects of climate change.

From a political standpoint, it is difficult to reconcile the clash between socioeconomic and environmental factors. Socioeconomic concerns are immediate, in that people want to use a lot of electricity in the present, and the consequences of a lack of electricity are obvious. In contrast, environmental factors are diffuse, intangible, futuristic, even overwhelming. This makes them difficult to conceptualise, understand, and appreciate.

The largest reduction in CO<sub>2</sub> emissions would be accomplished by a significant change in human behaviour. However, without an immediate and easily-understandable threat, dramatic changes are unlikely to occur. Alternatively, the development of a technological solution to the problem is particularly desirable. This would result in minimal increases to electricity availability and price, while significantly reducing CO<sub>2</sub> emissions. Allowing such 'consequence free' growth would neither impose economic pressure on, nor necessitate significant behavioural changes by, the average person.

The availability of an economically viable technological solution may provide the impetus for international political action with tangible outcomes. Hopefully, a legally binding international agreement to reduce CO<sub>2</sub> emissions will be reached during the United Nations Framework Convention on Climate Change meeting in Paris in November-December 2015. It is likely that consideration of *how* any agreed targets will be achieved will play an important role in the formulation of any agreement.

Since coal-fired power plants are responsible for such a large proportion of global CO<sub>2</sub> emissions, it would be sensible to design a CO<sub>2</sub> capture technology with that application in mind. So, where could an economically viable technology for limiting CO<sub>2</sub> emissions fit into a coal-fired power plant?

### Options for CO<sub>2</sub> capture

Currently there are three proposed strategies for capturing CO<sub>2</sub> from a coal-fired power plant.<sup>7</sup> *Oxy-fuel combustion* requires separating oxygen from air (mainly nitrogen). Burning coal with 95% O<sub>2</sub> would result in CO<sub>2</sub> being the major product of combustion, making it easy to capture. *Pre-combustion capture* requires converting coal into hydrogen and CO<sub>2</sub>, then removing the CO<sub>2</sub> before burning the hydrogen to generate electricity. *Post-combustion capture* would mean that coal is burnt as normal and the CO<sub>2</sub> is then separated from a waste stream (*flue gas*) composed largely of N<sub>2</sub> (Table 1).

**Table 1.** Example gas compositions for coal-fired and natural gas power plants

Component	Coal-fired power plant flue gas (%)	Natural gas power plant flue gas (%)
N <sub>2</sub>	77	74
CO <sub>2</sub>	13	8
H <sub>2</sub> O	6	14
O <sub>2</sub>	4	4
NO <sub>x</sub>	420 ppm	70 ppm
SO <sub>2</sub>	420 ppm	N/A
CO	50 ppm	300 ppm

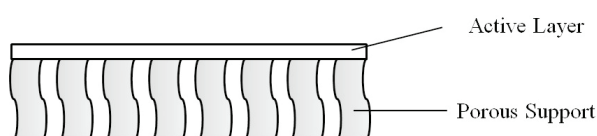
In principle, a *post-combustion* capture system can be retrofitted into existing plants and plant designs. Retrofitting is less costly and faster to implement than the alternate technologies which would require major alterations to process and plant designs.

For the *post-combustion capture* of CO<sub>2</sub>, the United States Department of Energy (DOE) has set 'cost for capture'

targets of \$40 per ton of CO<sub>2</sub> by the year 2020-2025 and \$10 per ton of CO<sub>2</sub> by the year 2030-2035.<sup>9</sup> Membranes are one technology that could be applied to achieving these goals, so let's discover what they are and how they could be used.

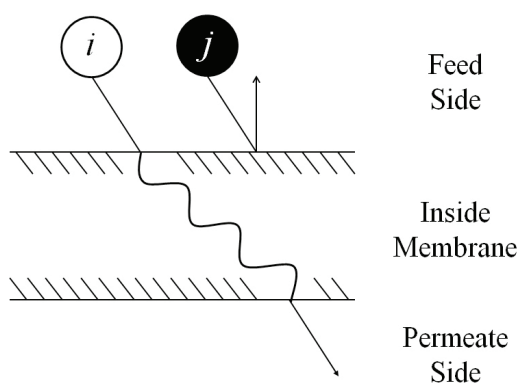
### How membranes work

For the purpose of this article, a membrane will be defined as a material used to partition two volumes. In practice, a membrane will be made of a 'selective' or 'active' layer, comprised of a dense polymeric material, typically 100 to 1000 nm thick (Fig. 2). The active layer is referred to as the *membrane material* and is responsible for separating different gases. Because the active layer is so thin, it sits atop a porous support to provide the membrane with mechanical strength.



**Fig. 2.** A cartoon representation of a composite membrane

A membrane can be thought of as a doorway between two rooms, but on a molecular scale that analogy is not correct. In dense polymeric membranes, transport through the membrane occurs via the solution-diffusion mechanism (Fig. 3).<sup>10</sup> To get from one volume, the *feed side*, to the other volume, the *permeate side*, gas *i* must cross the membrane. To do so, it dissolves into the membrane at the feed side. The gas molecule then diffuses across the membrane,<sup>11</sup> driven by a concentration gradient such as partial pressure. Upon reaching the permeate side, gas *i* will desorb from the membrane and escape into the permeate volume.



**Fig. 3.** A simplified depiction of gas *i* moving across a dense polymeric membrane by the solution-diffusion mechanism. It is important to note that gas *j* will actually move across the membrane using the same mechanism as gas *i*, albeit at a significantly slower rate. Modified from reference 12.

### Important membrane properties

The defining features of a membrane are *permeability*, *permeance*, and *selectivity*.<sup>13</sup>

*Permeability* reflects the ability of a gas to transport through the active layer. Permeability always refers to a specific gas, therefore will be written as "the permeability of gas *i* is...". As shown in Equation 1, permeability

reflects the ability of a gas to dissolve into the material (solubility) and diffuse through it (diffusivity). Permeability is typically reported in units of 'barre',<sup>14</sup> named after New Zealander Dr. Richard M. Barrer.

*Permeance* describes the rate at which a gas moves across a membrane area. This means that membranes with high permeance will process gas faster, or require less membrane area, than a membrane with low permeance. As can be seen in Equation 2, a higher permeance membrane can be obtained by reducing the thickness of the active layer. Permeance is typically reported in 'Gas Permeation Units (GPU)'.<sup>15</sup>

*Selectivity* is the ratio of how permeable the material is to two different gases (in this case *i* and *j*). *Ideal selectivity* refers to the permeability ratio of two different gases when they are tested *individually*, i.e. as single gas measurements, rather than as a mixture of gases.

$$\text{Permeability}_i = \text{Diffusibility}_i \cdot \text{Solubility}_i \quad (1)$$

$$\text{Permeance}_i = \frac{\text{Permeability}_i}{\text{Active Layer Thickness}} \quad (2)$$

$$\text{Selectivity } (\alpha_{i/j}) = \frac{\text{Permeability } (i)}{\text{Permeability } (j)} \quad (3)$$

**Equations 1-3.** The *permeability* of gas *i* is described as the product of its diffusivity and solubility coefficients inside the membrane material. The *permeance* of gas *i* is determined by the permeability and thickness of the membrane material. The *selectivity* of a membrane is determined by the relative permeabilities of gases *i* and *j*.

To apply a membrane in the real world, other factors also need to be considered.<sup>12</sup> For example, can the material be processed into defect-free thin (ca. 100 nm) films, are the properties of the thin film the same as a thick film, how does the material perform with a mixed-gas or 'real' feed, is the membrane physically and chemically stable enough to retain performance over time, and is the material expensive to make?

For the moment, we shall ignore these rather pressing issues. What then are the *permeance* and *selectivity* properties required to economically separate CO<sub>2</sub> from flue gas?

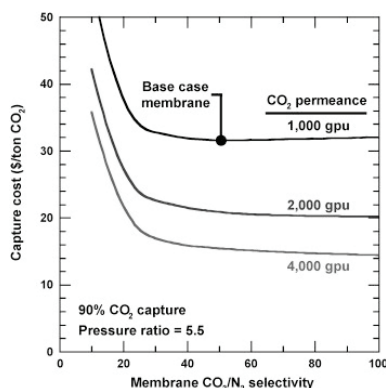
### Membrane requirements for separating CO<sub>2</sub> from flue gas

When considering the application of any academic idea to the real world, or perhaps even before starting to generate ideas, it is important to consider what the real world actually needs.

In 2010, the 'Membrane Technology and Research company' (MTR) published a definitive article evaluating the applicability of several membrane process designs to the capture of CO<sub>2</sub> flue gas from coal-fired power plants.<sup>5</sup>

Fig. 4 shows MTR's modelling results for the best of the multi-stage processes they considered. They demonstrate the effect of increasing CO<sub>2</sub>/N<sub>2</sub> selectivity on the cost of CO<sub>2</sub> capture for three potential membranes with permeances of 1000, 2000, and 4000 GPU, respec-

tively. It is interesting to note that the cost of capture is much lower when the permeance of the membrane is increased, but levels off after a CO<sub>2</sub>/N<sub>2</sub> selectivity of 30.



**Fig. 4.** The effect of membrane *permeance* and *selectivity* on the cost of capturing CO<sub>2</sub> from the flue gas of a coal-fired power plant<sup>5</sup>

Using the conditions MTR assumed for processing flue gas, the *maximum* possible concentration of CO<sub>2</sub> on the permeate side is 73 mol%.<sup>16,17</sup> This is true *no matter how selective the membrane is!* But, because 27 mol% of N<sub>2</sub> must also be collected, and the permeance of N<sub>2</sub> is inversely proportional to the selectivity, a larger membrane area will be required to permeate the N<sub>2</sub>. Therefore, as the selectivity of the membrane approaches infinity so does the amount of membrane area required. That would be quite expensive!

The levelling off of capture costs with increasing selectivity therefore represents the compromise between a more efficient process and increasing the amount of membrane area required to treat the gas.<sup>18</sup>

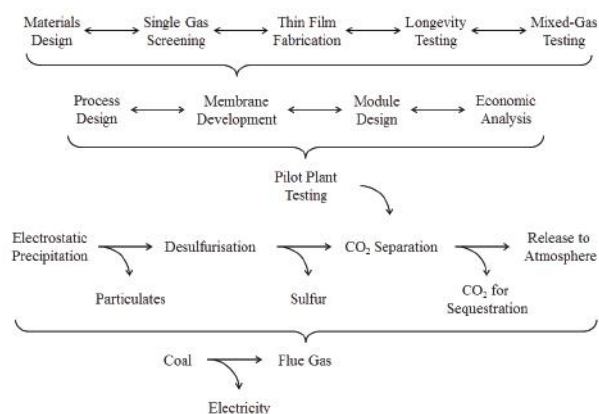
In conclusion, we know that *high CO<sub>2</sub> permeance* is the key parameter for achieving economic CO<sub>2</sub> separation from flue gas. It implies that modest CO<sub>2</sub>/N<sub>2</sub> selectivities of 20-40 are acceptable and that CO<sub>2</sub>/N<sub>2</sub> selectivities over 30 will make little difference to the cost of CO<sub>2</sub> capture.

Now that we have an idea of our criteria, are there any membranes which could achieve our goals?

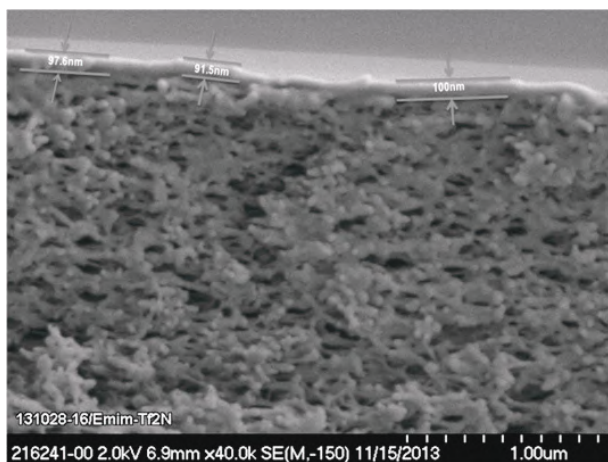
### State-of-the-art membrane technology

When assessing state-of-the-art technology it is important to consider where the technology lies along its developmental pathway. This is because companies, which are quaquary by nature, need to balance the financial risk of paying to adopt a new technology against the potential economic value that technology could provide. In the case of CO<sub>2</sub> capture from flue gas, the risk would be in a technology unproven to operate over a financially justifiable period, and the economic value in adopting membrane technology could be in avoiding legislatively imposed penalties or fines for releasing CO<sub>2</sub> into the atmosphere. To contextualise the development pathway of membrane technology for an example post-combustion CO<sub>2</sub> capture process, a flow chart is presented in Fig. 5.

Recently, a collaboration between the University of Colorado Boulder and the 3M Company produced a membrane with a permeance of 6100 ± 400 GPU and a CO<sub>2</sub>/



**Fig. 5.** A brief consideration of where membrane technology could fit into an example post-combustion CO<sub>2</sub> capture process and the developmental pathway required to make that a reality



**Fig. 6.** A scanning electron microscope (SEM) image of a membrane showing the  $96 \pm 4 \mu\text{m}$  active layer on top of a porous support. Reprinted with permission from Ind. Eng. Chem. Res., 53 (51), 20064-20067. Copyright 2014 American Chemical Society.<sup>19</sup>

N<sub>2</sub> selectivity of  $22 \pm 2$  - corresponding to a potential capture cost of \$15 per ton of CO<sub>2</sub>.<sup>19</sup> This membrane has an approximately 100 nm thick active layer comprised of ionic liquid and poly(ionic liquid) (Fig. 6), prepared using a two-step coating process.<sup>20</sup> The preparation of this membrane is a significant achievement, but it is at a very early stage of development.

MTR currently has a set of membranes named 'Polaris™' going through pilot plant testing.<sup>21</sup> The composition of Polaris™ is a closely guarded secret. The original 'Polaris™' has a CO<sub>2</sub> permeance of 1000 GPU and a CO<sub>2</sub>/N<sub>2</sub> selectivity of 50 - corresponding to a potential capture cost of \$30 per ton of CO<sub>2</sub>. In 2014, an advanced iteration of Polaris™ was disclosed to have a CO<sub>2</sub> permeance of 3000 GPU and a CO<sub>2</sub>/N<sub>2</sub> selectivity of 50 - corresponding to a potential capture cost of \$20 per ton of CO<sub>2</sub>.<sup>22</sup> These capture costs are well under the DOE target of \$40 per ton of CO<sub>2</sub> by the year 2020-2025.

In 2010, a pilot-scale membrane unit containing the original Polaris™ was reported to have lasted for 45 days of continuous operation at the Cholla power plant in Arizona with no decrease in performance.<sup>21</sup> In 2014, a second generation Polaris™ membrane (2000 GPU) was reported to have 60-70% increased capacity over the first genera-

tion system.<sup>22</sup> These results are extremely promising and hopefully this technology will soon be available for commercialisation.

### What to do with the captured CO<sub>2</sub>?

Assuming that separation technologies are successfully developed and implemented, what can be done with the captured CO<sub>2</sub>? Worldwide there are options like burying the CO<sub>2</sub> in stable geological formations deep within the earth, such as depleted natural gas reservoirs, or using CO<sub>2</sub> to enhance oil recovery from petroleum wells.

What would be good options for New Zealand?

Perhaps the Maui gas fields would provide a suitable site for CO<sub>2</sub> sequestration? However, the geology of New Zealand is relatively volatile and it is difficult to predict the long-term stability of deep sites. Should leaks occur from a deep sea site like Maui would it be worse to have directly injected CO<sub>2</sub> into the ocean rather than releasing it into the atmosphere?

Recycling CO<sub>2</sub> for applications such as carbonation of drinks, fire extinguishers, and synthesis of chemical feed stocks seems like an attractive option. Perhaps an effective solar and algae-based process can be developed to recycle CO<sub>2</sub> into biofuels? However, recycling would likely only account for a small fraction of the total CO<sub>2</sub> produced and may or may not add feasibility to the economics of CO<sub>2</sub> capture.

### Outlook

Contrasting the convenience of coal-fired power plants with the consequences of climate change and ocean acidification paints an unattractive picture. Similarly, the juxtaposition of immediate and futuristic socioeconomic consequences presents a difficult political problem.

In this context, a technological development that will delay or mitigate future environmental, economic, and social consequences, but avoid imposing significant socioeconomic penalties in the present, is a very attractive option.

Membrane technology appears to be a promising candidate in the short-term. However, CO<sub>2</sub> emissions are just one contributor to global climate change, and climate change is just one problem facing the world. How humanity faces its problems and advances culturally and technologically will be determined in part by your choices and actions. Sometimes the choice to do nothing will have the most significant impact of all - so choose wisely and act courageously. Ka kite anō.

### Acknowledgements

Thanks to M. E. Damour and W. M. McDanel for valuable discussions regarding the article.

### References and notes

1. Energy supply and demand report card. <http://www.mfe.govt.nz/more/environmental-reporting/energy/energy-demand-indicator/energy-supply-and-demand> (accessed 29/1/2015).
2. Gordon, R., Carbon from Huntly Power Station. Public Affairs Manager (Genesis Energy): 2015.

3. CO<sub>2</sub> emissions (kt). [http://data.worldbank.org/indicator/EN.ATM.CO2E.KT/countries?order=wbapi\\_data\\_value\\_2010+wbapi\\_data\\_value+wbapi\\_data\\_value-first&sort=desc](http://data.worldbank.org/indicator/EN.ATM.CO2E.KT/countries?order=wbapi_data_value_2010+wbapi_data_value+wbapi_data_value-first&sort=desc) (accessed 29/1/2015).
4. Huntly Plant Description. <https://www.genesisenergy.co.nz/huntly-power-station-plant-description> (accessed 29/1/2015).
5. Merkel, T. C.; Lin, H.; Wei, X.; Baker, R. J. *Membr. Sci.* **2010**, *359*, 126-139.
6. U.S. CO<sub>2</sub> emissions and electricity generation from coal are projected to decrease significantly in the coming years. This is due to the increase in hydraulic fracturing, which has allowed the replacement of coal-fired plants.
7. The Future of Coal. <http://web.mit.edu/coal/> (accessed 29/1/2015).
8. (a) Gluckman, P. D., New Zealand's changing climate and oceans: The impact of human activity and implications for the future Office of the Prime Minister's Science Advisory Committee: New Zealand, 2013; (b) Parry, M. L.; Canziani, O. F.; Palutikof, J. P.; van der Linden, P. J.; Hanson, C. E.; (eds). IPCC, 2007: Climate Change 2007: Working Group II: Impacts, Adaption and Vulnerability 2007.
9. Carbon Capture Technology Program Plan. U.S. Department of Energy: <http://www.netl.doe.gov/File%20Library/Research/Coal/carbon%20capture/Program-Plan-Carbon-Capture-2013.pdf>, 2013.
10. Wijmans, J. G.; Baker, R. W. *J. Membr. Sci.* **1995**, *107*, 1-21.
11. During diffusion, the gas molecule occupies transient cavities of up to 5 Å in diameter. These cavities are constantly opening and closing, due to the random motion of polymeric chains in the membrane material. Where the cavities are permanent a 'size-sieving' model is better used to describe transport across the membrane.
12. Baker, R. W.; Low, B. T. *Macromolecules* **2014**, *47*, 6999-7013.
13. Baker, R. W., *Membrane Technology and Applications*. 3rd Edition ed.; John Wiley & Sons: 2012.
14.  $1 \text{ barrer} = 1 \times 10^{-10} \frac{(\text{cm}^3_{\text{gas}} (\text{STP}) \cdot \text{cm})}{(\text{cm}^2 \cdot \text{s} \cdot \text{cmHg})} = 0.33 \times 10^{-15} \frac{(\text{mol}_{\text{gas}} \cdot \text{m}_{\text{thickness}})}{(\text{m}^2_{\text{area}} \cdot \text{s} \cdot \text{Pa}_{\text{gas}})}$ .
15.  $1 \text{ GPU} = 1 \times 10^{-6} \frac{(\text{cm}^3_{\text{gas}} (\text{STP}))}{(\text{cm}^2 \cdot \text{s} \cdot \text{cmHg})} = 0.33 \times 10^{-15} \frac{(\text{mol}_{\text{gas}})}{(\text{m}^2_{\text{area}} \cdot \text{s} \cdot \text{Pa}_{\text{gas}})}$ .
16. Huang, Y.; Merkel, T. C.; Baker, R. W. *J. Membr. Sci.* **2014**, *463*, 33-40.
17. Assuming that the starting CO<sub>2</sub> concentration in flue gas is 13% and the total pressure drop across the membrane is 5.5:1. To have a concentration gradient across the membrane:  $\text{Pressure}_{(\text{feed})} \cdot \text{Concentration of CO}_2(\text{feed}) \geq \text{Pressure}_{(\text{permeate})} \cdot \text{Concentration of CO}_2(\text{permeate})$ .
18. This implies that the capture of CO<sub>2</sub> from a natural gas-fired power plant is a more difficult problem due to the lower starting concentration of CO<sub>2</sub> in the flue gas (Table 1).
19. Zhou, J.; Mok, M. M.; Cowan, M. G.; McDanel, W. M.; Carlisle, T. K.; Gin, D. L.; Noble, R. D. *Ind. Eng. Chem. Res.* **2014**, *53*, 20064-20067.
20. Carlisle, T. K.; McDanel, W. M.; Cowan, M. G.; Noble, R. D.; Gin, D. L. *Chem. Mater.* **2014**, *26*, 1294-1296.
21. Amo, K.; Baker, R.; Firat, B.; He, Z.; Lin, H.; Merkel, T.; Pande, S.; Wei, X.; Wijmans, H. A Membrane Process to Capture CO<sub>2</sub> from Coal-Fired Power Plant Flue Gas. <http://www.netl.doe.gov/File%20Library/Research/Coal/ewr/co2/Xiaotong-Wei---NT0005312-and-NT43085.pdf> (accessed 29/1/2015).
22. Amo, K.; He, Z.; Huang, I.; Kaschemekat, J.; Merkel, T.; Pande, S.; Wei, X.; White, S.; Seshadri, P.; Farzan, H. Pilot Testing of a Membrane System for Post-Combustion CO<sub>2</sub> Capture. <http://www.netl.doe.gov/File%20Library/Events/2014/2014%20NETL%20CO2%20Capture/T-Merkel-MTR-Pilot-Testing-of-a-Membrane-System.pdf>.

## Betty Wignall 1927-2014



Betty Wignall, the former Administrative Secretary of the NZIC, died on 20 December 2014 at Archer Home, Christchurch. She was born Ngaire Elisabeth Barlow in Sumner on 27 March 1927. Betty studied medical intermediate in Christchurch in about 1945 before completing a home science degree at Otago University. Following this Betty taught science (plus clothing) at Waikato Diocesan School for Girls for three years (around 1948-51). After a year spent working at the Hammersmith Hospital laboratory in London, Betty returned to NZ where she continued with lab work, briefly for a private lab in Christchurch and then for four years with the DSIR (now ESR) in Wellington, working in the water lab. It was there that she met the late Denis Hogan, the NZIC Honorary General Secretary and Registrar. She

returned to Christchurch to marry, but after her divorce, Betty started working for the NZIC in the newly created post of Administrative Secretary working with Denis in 1974. During that time the Institute's membership records were computerised with the assistance of Robert Maclagan. Membership invoices and membership lists were produced. The system that was created was considered superior to those of a number of other professional organisations. This was in the days of punched cards and before computer terminals. Betty's service to the Institute went well beyond the call of duty. She spent 15 years in this part-time role. Her hard work for the Institute was characterised by both loyalty and friendliness. While not a chemist she continued her association with chemistry. Her daughter Anne is a well-known chemical educator. More recently, Betty generously established a scholarship for a PhD student in chemistry at the University of Canterbury - the Betty Wignall Scholarship in Chemistry - something really appreciated in these days of low student funding, which is being used to contribute to the fees of a PhD student. She is survived by her children Trevor, Anne, and Keith.

Contributed by Robert Maclagan

## Misassigned Natural Products

Richard A. Lamb

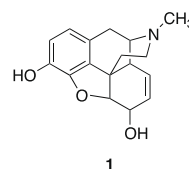
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**Keywords:** medicinal natural products, structural characterisation, organic synthesis



Richard attended Timaru Boys High School before moving to Dunedin to study at the University of Otago, graduating with a BSc(Hons) in 2014. Richard plans to begin his PhD studies under the supervision of Dr Bill Hawkins this year working on the total synthesis of bioactive natural products.

an improved understanding of reactivity and the three-dimensional nature of organic compounds.<sup>4</sup> To borrow words from Doering: "In the beginning, the isolation of chemicals from natural sources provided an unceasing stimulus to the creation and development of science".<sup>5,6</sup>



**Fig. 1.** Morphine

Throughout history, before drug discovery and modern chemistry, natural products have been used to treat illness. Cocktails composed of nature's ingredients such as herbs, animal products or inorganic materials, perhaps intertwined with witchcraft, mysticism, astrology or religion, were used to effect these treatments. Early treatments were recorded and documented, eventually leading to a disciplined scientific description of natural materials that could be used in medicine. As scientific knowledge grew and improved, the active constituents from these remedies were isolated, characterised and subsequently synthesised in the laboratory.<sup>1</sup>

The more active and selective of these natural products, derivatives of, or compounds inspired by natural products, have become many of the drugs used by people every day. In fact, natural product-derived pharmaceuticals constitute about half of those used in the clinic.<sup>2</sup> As such, considerable research and interest is dedicated to the investigation of these architecturally diverse and structurally complex molecules, which have been shaped by evolution and nature over the course of millions of years. These compounds are often decorated with multiple ring systems, stereocentres and unique and imaginative structural motifs. Not only do these compounds meet an unfulfilled need for new drugs, but techniques and methodologies associated with detecting biological activity, isolation and purification, characterisation, synthesis and biological evaluation for these compounds can also be discovered and improved.

A classic example of such a natural product is morphine (**1**, Fig. 1), which was extracted from the opium poppy plant by Friedrich Serturmer and the first alkaloid ever extracted from a plant source.<sup>3</sup> Of course, the discovery of this powerful analgesic that is used in the clinic every day all around the world was of huge benefit to mankind, but the 122 year delay between its isolation and structural elucidation saw equally important advancements in chemical synthesis and characterisation, as well as

In the 19<sup>th</sup> century and the early half of the 20<sup>th</sup> century, before the arsenal of spectroscopic and other characterisation techniques of today, chemical synthesis was the only way in which the structural determination of natural products could be carried out. Molecular architecture was revealed through meticulous and laborious derivatisation and degradation, assuming large enough quantities of the compound in question could be obtained. Depending nearly solely on chemical synthesis as a means of carrying out structural elucidation was fraught with errors and limitations. A classic example of one of these early structural misassignments was made in the 1920s by two researchers in Germany, Weiland and Windaus, who proposed the structural motifs of a number of steroids including cholesterol (**2**, Fig. 2). Although they were awarded the Nobel Prize for this work, the inaccuracies associated with their work can instantly be recognised today and their mistakes were corrected in 1932 through the use of X-ray crystallography and thus establishing the correct steroid core structure (**3**, Fig. 2).

Nowadays, with a whole host of more powerful, accurate and less time-consuming techniques such as multi-dimensional NMR and X-ray crystallography, structural assignments of natural products have become much more accurate, practical and relatively rapid. However, in a review published by Nicolaou *et al.* in 2005,<sup>5</sup> the case is made that chemical synthesis still has an important role to play in the structural elucidation of natural products. Between January 1990 and April 2004, there have been well over 300 cases of structural misassignments reported in the scientific literature. These structural revisions cover virtually every compound class and include not only stereochemical misassignments, but also extend to include complete constitutional changes. Nicolaou *et al.* point out that these mistakes can be attributed to the fact that every structural elucidation technique has its own inherent weaknesses, some of which cannot be resolved even when every other tool is applied.

For example, while X-ray crystallography is generally seen as an infallible technique, complications can arise when investigating functional groups lacking hydrogen atoms. As X-ray crystallography uses electron density to map out the position of atoms, it is unable to reliably reveal the location of hydrogen atoms. This can make differentiating between functional groups devoid of hydrogen atoms difficult, for example, between N-H groups and O atoms. NMR spectroscopy, despite being an extremely powerful tool for structure elucidation, can demonstrate weaknesses especially when there are insufficient hydrogen atoms to correlate  $^{13}\text{C}$  and  $^1\text{H}$  resonances.

One example of a misassigned natural product is kinamycin C. It was originally isolated from *Streptomyces murayamaensis* in 1973, and was shown to possess antibacterial activity against mainly gram-positive bacteria. The structure of this compound was originally assigned using a whole battery of techniques including mass spectrometry, X-ray crystallography, NMR, UV-vis, and IR spectroscopies, as well as chemical derivatisation and degradation.<sup>6</sup> It was not until 21 years later that it was realised the cyano group on **4** (Fig. 3) was actually a diazo group as in **5** (Fig. 3), which was confirmed by 2D NMR spectroscopy<sup>7</sup> and chemical synthesis.<sup>8</sup>

Another example of a structural misassignment was diazomide A, an unusual halogenated cyclic peptide with potent *in vitro* cytotoxicity.<sup>9</sup> The compound was isolated from the ascidian (sea squirt) *Diazonia chilensis* and was originally assigned as **6** (Fig. 4) using a combination of NMR spectroscopy experiments as well as X-ray crystallography. It was not until a decade later that the compound identical to the structure proposed in 1991 was synthesised.<sup>10</sup> Upon comparing the analytical data of the synthetic compound **6** to the naturally occurring compound it was found they were not identical and upon further analysis the structure was revised to **7** (Fig. 4).

Since 2005, there have been plenty more cases where the structure of natural products have been revised following synthetic studies. Azaspiracid-1 is a natural product isolated from *Mytilus edulis* (a species of mussel) and was discovered after at least eight people fell ill following its consumption.<sup>11</sup> The marine toxin was originally assigned using multi-dimensional NMR experiments and mass spectrometry, and featured two spiro ring domains, a cyclic amine and a carboxylic acid (**8**, Fig. 5). The challenge of synthesising azaspiracid-1 was taken up by the Nicolaou group and its completion revealed the original spectra of the natural product did not match that of the synthetic target.<sup>12</sup> Following degradation of the neurotoxin into three different compounds, the analytical data was matched with synthetically derived fragments. A new structure **9** (Fig. 5) was subsequently proposed, which was ultimately confirmed through its total synthesis.<sup>13</sup>

More recently, the structure of cinbotolide, a natural product isolated from the phytopathogen *Botrytis cinerea* has also been revised following synthetic studies.<sup>14</sup> The originally proposed structure **10a** is shown in Fig. 6. Again, the spectra of a synthetic analogue (**10b**) having significant differences to that of the natural prod-

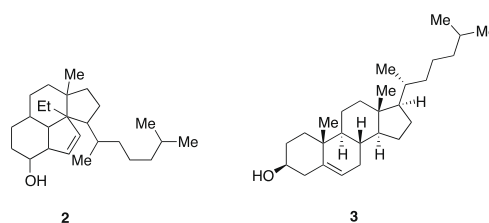


Fig. 2. Originally proposed (**2**) and correct (**3**) structure of cholesterol

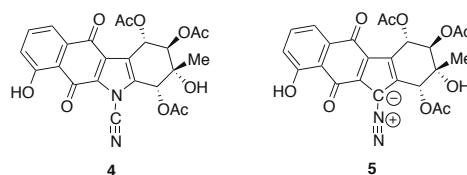


Fig. 3. Original (**4**) and revised (**5**) structure of kinamycin C

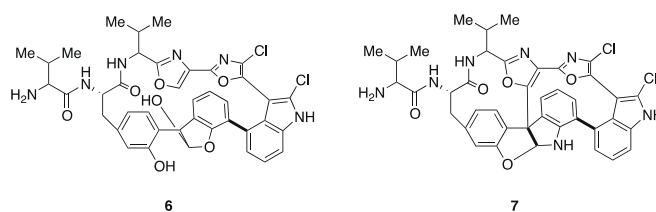


Fig. 4. Original (**6**) and revised (**7**) structure of diazomide A.

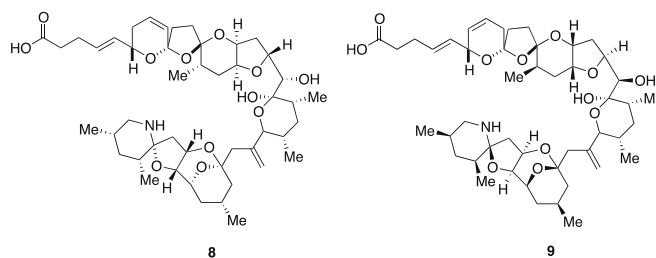


Fig. 5. Original (**8**) and revised (**9**) structure of azaspiracid-1

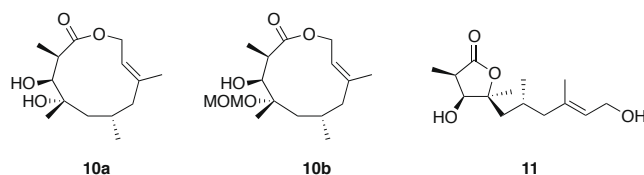


Fig. 6. Originally proposed structure (**10a**), synthetic methoxy-methyl (MOM) protected ether analogue (**10b**) and revised (**11**) structure of cinbotolide

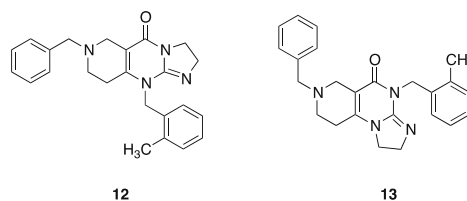


Fig. 7. Original (**12**) and revised (**13**) structure of TIC10

uct prompted further investigation into the correct structure of the compound. Quantities of the natural product were obtained from a *B. cinerea* mutant that overproduced the polyketide. Following a series of chemical transformations, multi-dimensional NMR and IR spectroscopy experiments, a revised structure (**11**, Fig. 6) was proposed and ultimately confirmed through an asymmetric total synthesis.

Another example of a structural misassignment was uncovered in 2014 following the synthesis of TIC10. This compound is not a natural product, but the story illustrates the potential financial and health risks that structural misassignments could incur. TIC10 was originally discovered by a group from Pennsylvania State University through a search of a free National Cancer Institute (NCI) database. It was found to induce apoptosis by promoting the expression of a tumour suppressor protein called TRAIL and to be efficacious *in vivo* and *in vitro* against glioblastomas, prostate cancer, sarcomas, melanoma and lymphomas.<sup>15</sup>

After the compound had been patented and licensed to a pharmaceutical company and clinical trials initiated, another research group from the Scripps Research Institute in California became interested in the same compound in the context of anticancer-combination therapy. After synthesising the compound they found the previously patented structure **12** (Fig. 7) to be biologically inactive.<sup>16</sup> In order to address this disparity, the second group obtained the repository compound from the NCI and found it to be biologically active. Following 2D NMR spectroscopy and X-ray crystallography experiments, they showed that the patented structure had been misassigned and the correct, biologically active compound (**13**, Fig. 7) was actually a constitutional isomer of the originally patented structure. As a result, the patent and clinical trials have been called into question and the two research groups and pharmaceutical companies could be led into an unprecedented legal case.

The invention and continual improvement of characterisation and isolation techniques has seen a marked improvement in the accuracy and speed with which new natural products can be discovered. However, based on the examples above, as well as hundreds of other cases, mistakes can still occur with far reaching consequences as seen with TIC10. The value of organic synthesis as a means of obtaining these scarce and valuable compounds for biological evaluation and drug discovery, often accompanied by important advances in the field of organic chemistry, is unparalleled. These examples underscore the idea that the structural intricacy, connectivity and reactivity of these interesting and complex compounds can only fully be appreciated through the act of physically making them.

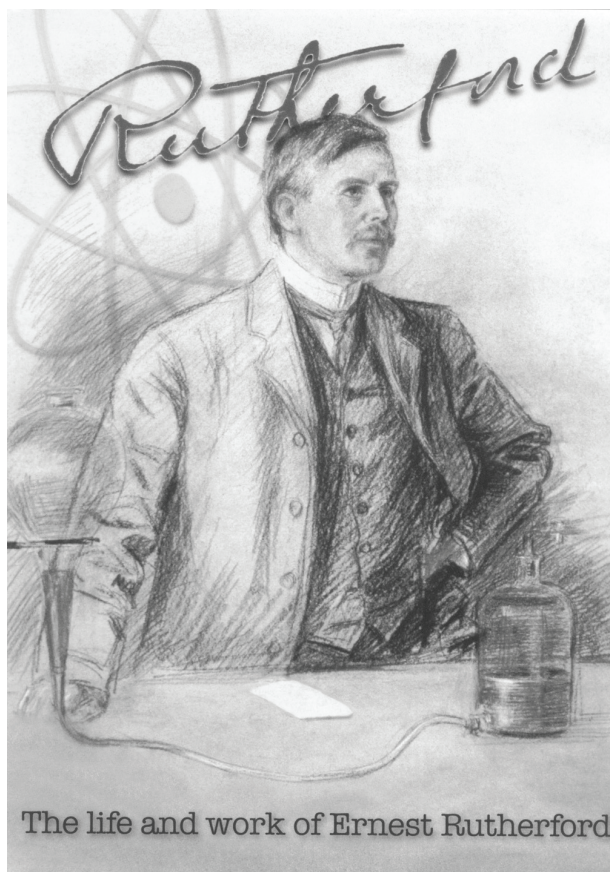
### Acknowledgements

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### References

1. Dewick, P. M. *Medicinal Natural Products: A Biosynthetic Approach*, John Wiley & Sons, Ltd: Chichester, 2<sup>nd</sup> ed. 2001, 1-5.
2. Clark, A. *Pharm. Res.* **1996**, *13*, 1133-1141.
3. Sneader, W. In *Drug Discovery*, John Wiley & Sons, Ltd, 2006, 88-105.
4. Couteur, P. L.; Burreson, J. *Napoleon's Buttons: 17 Molecules that Changed History*. Jeremy P. Tarcher/Penguin, 2004.
5. Nicolaou, K. C.; Snyder, S. A. *Angew. Chem. Int. Ed.* **2005**, *44*, 1012-1044.
6. Omura, S.; Nakagawa, A.; Yamada, H.; Hata, T.; Furusaki, A.; Watanabe, T. *Chem. Pharm. Bull.* **1973**, *21*, 931-940.
7. Gould, S. J.; Tamayo, N.; Melville, C. R.; Cone, M. C. *J. Am. Chem. Soc.* **1994**, *116*, 2207-2208.
8. Mithani, S.; Weeratunga, G.; Taylor, N. J.; Dmitrienko, G. I. *J. Am. Chem. Soc.* **1994**, *116*, 2209-2210.
9. Lindquist, N.; Fenical, W.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1991**, *113*, 2303-2304.
10. Li, J.; Jeong, S.; Esser, L.; Harran, P. G. *Angew. Chem. Int. Ed.* **2001**, *40*, 4765-4769.
11. Satake, M.; Ofuji, K.; Naoki, H.; James, K. J.; Furey, A.; McMahon, T.; Silke, J.; Yasumoto, T. *J. Am. Chem. Soc.* **1998**, *120*, 9967-9968.
12. (a) Nicolaou, K. C.; Pihko, P. M.; Bernal, F.; Frederick, M. O.; Qian, W.; Uesaka, N.; Diedrichs, N.; Hinrichs, J.; Koftis, T. V.; Loizidou, E.; Petrovic, G.; Rodriguez, M.; Sarlah, D.; Zou, N. *J. Am. Chem. Soc.* **2006**, *128*, 2244-2257; (b) Nicolaou, K. C.; Chen, D. Y. K.; Li, Y.; Uesaka, N.; Petrovic, G.; Koftis, T. V.; Bernal, F.; Frederick, M. O.; Govindasamy, M.; Ling, T.; Pihko, P. M.; Tang, W.; Vyskocil, S. *J. Am. Chem. Soc.* **2006**, *128*, 2258-2267.
13. Nicolaou, K. C.; Koftis, T. V.; Vyskocil, S.; Petrovic, G.; Tang, W.; Frederick, M. O.; Chen, D. Y. K.; Li, Y.; Ling, T.; Yamada, Y. M. A. *J. Am. Chem. Soc.* **2006**, *128*, 2859-2872.
14. Botubol, J. M.; Durán-Peña, M. J.; Macías-Sánchez, A. J.; Hanson, J. R.; Collado, I. G.; Hernández-Galán, R. *J. Org. Chem.* **2014**, *79*, 11349-11358.
15. Allen, J. E.; Krigsfeld, G.; Mayes, P. A.; Patel, L.; Dicker, D. T.; Patel, A. S.; Dolloff, N. G.; Messaris, E.; Scata, K. A.; Wang, W.; Zhou, J.-Y.; Wu, G. S.; El-Deiry, W. S. *Sci. Transl. Med.* **2013**, *5*, 171ra17.
16. Jacob, N. T.; Lockner, J. W.; Kravchenko, V. V.; Janda, K. D. *Angew. Chem. Int. Ed.* **2014**, *53*, 6628-6631.

## DVD review: Rutherford - The life and work of Ernest Rutherford



This is a three hour DVD in three parts, which my wife and I watched as three separate episodes with a few days between each.

**Part 1: The Apprentice.** This covers Rutherford's early years to his first stint at Cambridge University.

**Part 2: The Alchemist.** From Cambridge, Rutherford took a position at McGill University in Montreal which had a (then) state-of-the-art physics laboratory. *The Alchemist* covers his time there and, after nine years, his subsequent return to England and Manchester University. The title comes from one of his many achievements, that of changing one element into another (nitrogen into oxygen) – the long sought after goal of the alchemists – and don't mention transmutation.

**Part 3: The Statesman.** In 1919 Rutherford succeeded J. J. Thomson as Head of the Cavendish Laboratory at Cam-

bridge and remained there until his death in 1937. *The Statesman* covers this period.

Watching this DVD in instalments is not an issue as Parts two and three start with an introduction summarising "the story so far".

The production moves at a satisfying pace, is well compiled with photos, story links from John Campbell and others, short re-enactments of events and demonstrations of some of Rutherford's key experiments. Strong impressions are the influence of inspired teachers at all levels of his education and the – at times – fortuitous provision of funding without which the Rutherford genius may not have flourished.

He was ahead of his time with his encouragement of women to take up science and his willingness to allow students to take the credit for work they had done and discoveries made while under his supervision.

The respect that historians, curators, succeeding academic staff members, fellow Nobel Laureates, and in the final part, students that worked under him, had for Rutherford comes through strongly.

He comes across as a down-to-earth inspired practitioner with an incisive mind, a sense of humour and also a loving family man who was devoted to his wife, doted on his daughter Eileen and was very conscious of the importance of taking a break from the stresses of work.

Regardless of what you think you know about the work of Lord Ernest Rutherford, the story told in this DVD will open your mind to aspects that you were unaware of and will enhance those parts you thought you did know about. Until you see the story as a whole, his impact on the development of our ideas on the structure of the atom can easily be underestimated.

This DVD is highly recommended viewing, a must see for any proud Kiwi (science background or not – my wife found it fascinating) and continues the promotion of one of the greatest New Zealanders who ever lived and who has, until recently, been somewhat in the shadow of other great New Zealanders. The DVD is obtainable from [www.rutherford.org.nz](http://www.rutherford.org.nz) and costs \$40.

Reviewed by Richard Rendle

## Solid phase extraction

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**Keywords:** *analytical chemistry, chromatography, sample preparation*



Christie is visiting the University of Otago as a summer research intern from the University of Victoria, Canada, where she is about to enter her final year of undergraduate chemistry studies towards a BSc(Hons) degree. Her research at Otago with Dr Kim Hagemann concerns a multi-residue pesticide analysis of regional honey bee populations.

versed phase SPE aims to remove nonpolar analytes from a polar matrix. A hydrophobic solid phase is used to retain the analytes and organic solvents are used for elution. Normal phase SPE uses a polar solid phase to extract polar compounds from a nonpolar sample matrix. Typically a solvent that is more polar than the sample's original matrix is then used to elute the analytes. Ion exchange SPE is used for compounds that are charged when in a solution. In this case, sample pH may be adjusted before extraction and organic solvents are used for elution.

Solid phase extraction sorbents are available in a variety of formats: contained in cartridges, in columns similar to syringe barrels, in disks, or in bulk. Typical sorbents are based on either functionalised silica or polymers, but carbon nanotubes, biosorbents, and nanoparticles have also recently come into use.<sup>6</sup>

Typical materials for column housings are made of glass or polypropylene with the sorbent contained by polyethylene, stainless steel, or Teflon frits. Samples may be extracted using individual columns (Fig. 1), vacuum manifolds accommodating 12-24 samples, using a vacuum flask assembly, or large volume samplers. When selecting the appropriate solid phase extraction mode and equipment, the sample volume, matrix, and anticipated concentration of analytes must all be considered.

### General procedure for solid phase extraction

There are four typical steps for solid phase extraction: sorbent conditioning, sample loading, washing, and elution (Fig. 2). Once the extraction is complete, the eluate is ready for instrumental analysis.

The conditioning step prepares the sorbent by making it compatible with the liquid solution, promoting better surface contact, and removing any impurities or contaminants present. Typically, a volume of 5-60 mL of solvent<sup>7</sup> is adequate for a sorbent in an SPE tube or disk.

Once the sorbent has been conditioned, the sample is quantitatively transferred onto the column and allowed to pass through using vacuum, applied pressure, or a pump. The flow rate depends on the analytes, column dimensions, and the sorbent particle size. In all cases flow rate should be kept reasonably constant and, although dropwise flow is ideal, flow rates from 2-50 mL/min are typical.<sup>7</sup> As the sample passes through the column, the analytes are retained while undesired matrix components pass through.

Once the sample has passed through the column the sorbent is washed by passing a carefully chosen solution or solvent through the column. The aim of this step is to re-

### Introduction

Sample preparation is a critical step in the analysis of environmental and biological samples. This transformation of the bulk sample to a form suitable for analysis has been estimated to take 80% of the typical total analysis time.<sup>1</sup> In analytical chemistry, an important part of sample preparation is the quantitative extraction of the compounds of interest (analytes) from the other components of the sample (the matrix) which may interfere with instrumental analysis.

Historically, liquid-liquid extraction has been widely used in analytical chemistry for the extraction of analytes from environmental samples. In recent decades, however, solid phase extraction (SPE) has rapidly developed as a useful alternative technique. In solid phase extraction, a liquid sample is passed through sorbent particles (the solid phase) to which the analytes have a greater affinity than the bulk liquid. The analytes are selectively retained by the sorbent and subsequently extracted from those particles by elution with an appropriate liquid solvent. This method of extraction simplifies the analysis through removal of much of the sample matrix.<sup>2</sup>

Although the technique of solid phase extraction was first applied experimentally in the late 1940s,<sup>3</sup> the developments leading to its widespread use and adoption into current analytical methods started in the 1970s. Solid phase extraction techniques were initially used to concentrate small amounts of organic pollutants from water, but its use has now extended to a wide variety of matrices including serum, blood, urine, milk, oils, sediments, soils, plant and animal tissues, and pharmaceutical preparations.<sup>1,4,5</sup>

### Solid phase extraction modes and sorbents

The main modes of solid phase extraction are reversed phase, normal phase, and ion exchange. These methods differ in how the compound of interest is retained. Re-

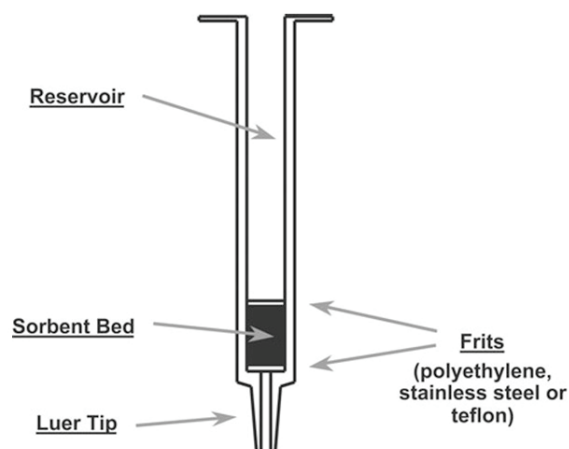


Fig. 1. Typical SPE column [adapted from ref. 7]

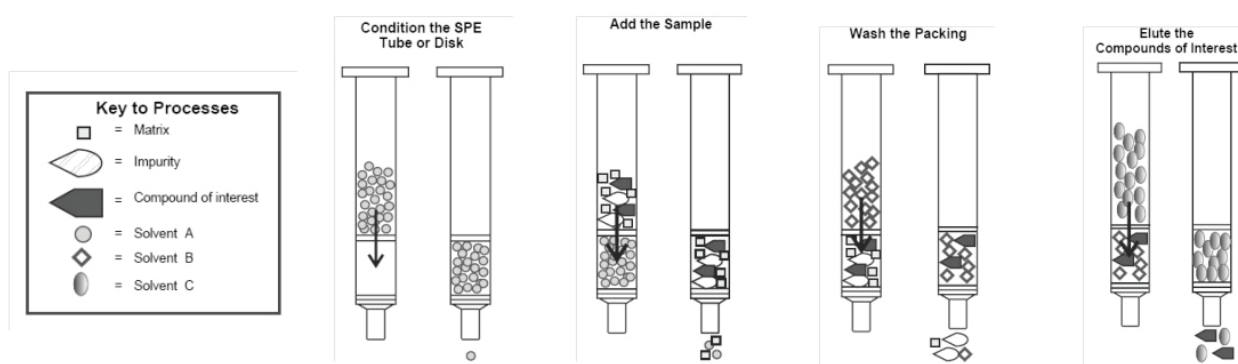


Fig. 2. The solid phase extraction process [adapted from ref. 5]

move undesired matrix components while retaining the analytes. Typically, volumes of 5-60 mL of solvent<sup>7</sup> for SPE tubes or disks are used for the wash step.

The final step in the extraction process is to recover the analytes using an elution solvent. The analytes are removed from the sorbent and returned to a liquid phase appropriate for analysis while undesired components, which were not removed in the wash step, are left behind. The elution solvent, typically 200  $\mu$ l to 10 mL of an organic liquid,<sup>7</sup> is added to the column and collected. A well-chosen elution solvent will use as small a volume as possible to completely extract the analytes from the solid phase.

Prior to extraction, additional sample pretreatments such as pH adjustment, filtration or addition of organic solvents may be required to enhance the retention of the analytes on the solid phase. The sample volume, matrix, sorbent type, and analytes will dictate the necessary sample preparation.

### Advantages of solid phase extraction

Solid phase extraction has a number of advantages over liquid-liquid extraction that have led to its rapid development and increasing usefulness as a sample preparation technique. These benefits include faster, less labour intensive sample manipulation, reduced solvent use, and higher concentration factors.<sup>1,4,5</sup>

Rapid, easily performed extractions make solid phase extraction an attractive alternative to liquid-liquid extraction. Large volume samples can easily be accommodated, multiple extractions can be carried out simultaneously, and the process can be readily automated. Liquid-liquid extraction, by contrast, requires significant and labourious manipulation of the sample, the automation of which is difficult.

Reduced solvent use is another advantage of solid phase extraction. The large volumes of organic solvents required for liquid-liquid extraction pose issues concerning its appropriate disposal, analyst exposure, and potential contamination of sample extracts. Solid phase extraction uses significantly less organic solvents than liquid-liquid extraction, reducing both costs and exposure.

Solid phase extraction achieves a concentration of analytes due to the small elution volumes used for extraction from the sorbent material. Liquid-liquid extraction concentration factors (a measure of how much more concentrated an analyte is in the extract than in the sample) are limited by the volume ratio of the sample and solvent. Concentration factors of 1000 or more are possible using solid phase extraction, where a highly efficient liquid-liquid extraction may only achieve a concentration of 100.<sup>8</sup>

### SPE in action

While solid phase extraction may be used as a stand-

alone method for sample preparation, in recent years it has also proven to be useful as a pre-concentration step in other analytical procedures. For example, capillary electrophoresis (CE) is a valuable analytical method which provides high resolution separation with small sample volumes, but has low sensitivity when used with relatively dilute samples. Coupling solid phase extraction with capillary electrophoresis allows for the simultaneous concentration and clean-up of large sample volumes before injection on the CE instrument, resulting in improved sensitivity and lower detection limits.<sup>9</sup> This particular application of solid phase extraction has been used in capillary electrophoretic analyses of acidic pharmaceuticals in river water,<sup>10</sup> the detection of amines in wine,<sup>11</sup> the determination of insulin derivatives in biological fluids,<sup>12</sup> antioxidants in olive oil,<sup>13</sup> and melamine residues in milk.<sup>14</sup>

### Conclusions

Solid phase extraction techniques, methods, and materials are continually being refined and developed. Innovations in sorbent materials and miniaturisation techniques such as solid phase microextraction and hyphenated capillary electrophoresis,<sup>10</sup> liquid chromatography,<sup>15</sup> and NMR techniques<sup>16</sup> indicate that solid phase extraction will continue to offer new solutions to the challenges facing analytical chemists.

### References

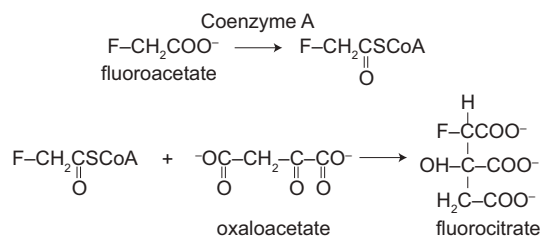
1. Buszewski, B.; Szultka, M. *Crit. Rev. Anal. Chem.* **2012**, *42*, 198-213.

- Harris, D. *Quantitative Chemical Analysis*, W.H. Freeman and Company: New York, 8<sup>th</sup> edn. 2010.
- Liška, I. *J. Chromatogr. A* **2000**, *885*, 3-16.
- Mitra, S. *Sample Preparation Techniques in Analytical Chemistry*, John Wiley & Sons, Inc.: Hoboken, New Jersey, 2003.
- Supelco, *Guide to Solid Phase Extraction*, Bulletin 910, Sigma-Aldrich Co.: 1998.
- Wan Ibrahim, W. A.; Abd Ali, L. I.; Sulaiman, A.; Sanagi, M. M.; Aboul-Enein, H. Y. *Crit. Rev. Anal. Chem.* **2014**, *44*, 233-254.
- Fritz, J. S. *Analytical Solid-Phase Extraction*, Wiley-VCH: New York, 1999.
- Biotage, [www.biotage.se](http://www.biotage.se) (accessed 1/09/2012).
- Puig, P.; Borrull, F.; Calull, M.; Aguilar, C. *Anal. Chim. Acta* **2008**, *616*, 1-18.
- Maijón, I.; Borrull, F.; Calull, M.; Aguilar, C. *Electrophoresis* **2011**, *32*, 2114-2122.
- Arce, L.; Ríos, A.; Valcárcel, M. *J. Chromatogr. A* **1998**, *803*, 249-260.
- Visser, N. F. C.; Van Harmelen, M.; Lingeman, H.; Irth, H. *J. Pharm. Biomed. Anal.* **2003**, *33*, 451-462.
- Nevado, J. J. B.; Robledo, V. R.; Callado, C. S. C. *Food Chem.* **2012**, *133*, 497-504.
- Sun, H.; Liu, N.; Wang, L.; He, P. *J. Chromatogr. Sci.* **2010**, *48*, 848-853.
- Christophoridou, S.; Dais, P.; Tseng, L. I. H.; Spraul, M., *J. Agric. Food Chem.* **2005**, *53*, 4667-4679.
- Jaroszewski, J. W. *Planta Med.* **2005**, *71*, 795-802.

## 1080

The chemical name for 1080 is sodium monofluoroacetate,  $\text{FCH}_2\text{CO}_2\text{Na}$ . The trade name 1080 comes from its product number in a laboratory catalogue. It occurs naturally in some plants where it acts as a deterrent against browsing animals and insects. Its effectiveness in killing rats has been known since 1942.

1080 acts by entering the citric acid cycle producing fluorocitrate instead of citrate.



Fluorocitrate interferes with the action of enzymes, particularly acotinase, a critical enzyme in the Krebs cycle, inhibiting the energy production of cells. The end result is that the body's vital organs can no longer function. Herbivores usually die of heart failure, whereas carnivores are more likely to die of respiratory failure.

1080 is toxic to many animals and invertebrates. Dogs, cats and pigs appear to be most susceptible to poisoning. The lethal dose to humans is 2 – 10 mg/kg

Sodium fluoroacetate is readily soluble in water. It does not accumulate in tissues.

New Zealand uses 80 % of world production of 1080. Because New Zealand does not have native animals it is seen as less of a risk to indigenous species than in other countries.

It is seen as the most effective way of controlling possum which defoliate forests and carry bovine tuberculosis, and rats and stoats which attack native birds.

As with the widespread use of any poison there are significant counter-arguments to its use.

### Sources of further information

- [http://en.wikipedia.org/wiki/Sodium\\_fluoroacetate](http://en.wikipedia.org/wiki/Sodium_fluoroacetate)
- <http://www.1080facts.co.nz>
- [http://en.wikipedia.org/wiki/1080\\_usage\\_in\\_New\\_Zealand](http://en.wikipedia.org/wiki/1080_usage_in_New_Zealand)
- [http://en.wikipedia.org/wiki/Sodium\\_fluoroacetate](http://en.wikipedia.org/wiki/Sodium_fluoroacetate)
- <http://ban1080.co.nz/1080-facts/>

## Hot new chemistry from a kiwi pepper tree

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**Keywords:** *kawakawa*, *natural products*, *alkaloids*



Originally from Christchurch, where he attended the Christchurch Rudolf Steiner School, Alistair moved to Dunedin for undergraduate study. Having recently completed a Bachelor of Science majoring in both chemistry and food science, he will be studying towards an Honours degree in chemistry throughout 2015. He has also worked closely with Plant & Food Research, having participated in their summer studentship programme working with the Chemistry Team at the Palmerston North site. This has led to working with the Plant Extracts Group, Plant & Food Research at the University of Otago, with whom he will complete the research component of his Honours degree.

### Introduction

Prior to the colonisation of New Zealand by European immigrants, Māori had developed an extensive knowledge of the native plants and their uses.<sup>1</sup> While plants such as mānuka (*Leptospermum scoparium*) and rimu (*Dacrydium cupressinum*) were primarily used for crafting weapons and canoes, many other native species were valued for their medicinal properties.<sup>1</sup> Harakeke (*Phormium tenax*) and koromiko (*Hebe stricta* and *H. salicifolia*) have a host of uses in treating the sick or injured.<sup>1,2</sup> Diarrhoea, sore throats and open wounds can all be cured using different parts of these plants prepared in a variety of different ways.<sup>2</sup> Harakeke has an added bonus, as it may also be used to stitch together the skin when treating more serious cuts.<sup>1</sup> However, perhaps one of the most prominent native plants in Māori medicine is kawakawa (*Macropiper excelsum*).

Kawakawa is most commonly found growing in shady areas along the coastline of the North Island and upper South Island.<sup>1</sup> The small shrub-like tree can be identified by its shiny green leaves and small yellow fruit (Fig. 1), both of which were exploited by Māori for their significant healing properties.<sup>1</sup> Steaming or boiling the leaves was a common treatment for stomach pain and, after the Europeans arrived, gonorrhoea.<sup>1</sup> Poultices made from the leaves and bark could be applied to cuts and wounds as well as inflamed areas, to reduce swelling and prevent infection.<sup>2</sup> As well as being a crucial part of Māori medicine, kawakawa has significant spiritual meaning, generally associated with the cycle of life and death.<sup>1</sup> A sprig of kawakawa is seen as a good luck charm and as such is present at many traditional Māori ceremonies from birth and naming to funerals.<sup>1</sup>

More recent uses of kawakawa have been in the food industry.<sup>3</sup> Leaves of the plant have been used to give the characteristic peppery flavour and aroma to Taakawa beer (Fig. 2).<sup>3</sup> The fact that kawakawa provides a hint of pepper is not unexpected given that it is sometimes re-



**Fig. 1.** Leaves (left) and fruit (right) of kawakawa (*Macropiper excelsum*) (from <https://www.flickr.com/photos/fos-tert/5385589463/in/photostream/> with permission).

ferred to as 'pepper tree'.<sup>1</sup> Other species belonging to the *Piperaceae* family are responsible for providing the original pepper flavours.<sup>4</sup> These include *Piper nigrum*, which is more commonly known as black pepper.<sup>4</sup> The fruits of kawakawa and *Piper nigrum* even exhibit some similarities in their appearance. The fact that kawakawa has such a plethora of beneficial properties to both pharmaceutical and food applications, provides a basis to investigate the chemistry of this species and determine the source of its biological activity.



**Fig. 2.** Taakawa Indigenous Ale of Aotearoa, flavoured using kawakawa leaves and fruit of *Piper nigrum*.

## Chemistry of kawakawa leaves

Research into the chemical composition and biological activity of kawakawa is limited. The majority of this research has focused on the leaves of the plant and little is known regarding the fruit or roots.<sup>5</sup> The most significant findings have been the isolation and identification of two known bioactive molecules, myristicin and diyangambin (Fig. 3), from the leaves.<sup>5</sup>

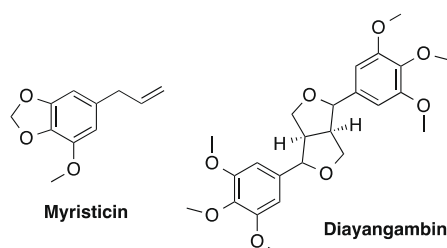


Fig. 3. Chemical structures of myristicin and diyangambin

Myristicin is the more common of the two and can also be found in other plant sources including nutmeg, parsley and dill.<sup>6</sup> The biological activity of myristicin has been well studied and helps to explain the medicinal properties of kawakawa. Not only is myristicin an anti-inflammatory and an anti-microbial agent but is also hepatoprotective, helping to prevent damage to liver cells.<sup>6,7</sup> Furthermore, myristicin is a known psychoactive drug and precursor to the psychedelic drug MDMA, a transformation which some have suggested may even occur during metabolism in the body.<sup>8</sup> This may well explain the symptoms described by those who have overindulged in beverages made from kawakawa leaves or root.<sup>1</sup> As if myristicin did not already have sufficient bioactivity it has also been found to act as an effective insecticide.<sup>1</sup> This activity has been utilised to keep away insects by burning kawakawa branches around food storage pits.<sup>1</sup>

Conversely, diyangambin is a much more rare natural product, but it also has significant bioactivity.<sup>9</sup> Not only does diyangambin exhibit anti-inflammatory activity, but it is also an immunosuppressive agent.<sup>9</sup> Drugs that utilise immunosuppressive agents such as diyangambin are used to treat several conditions in which the body's immune system requires down-regulation. This includes patients whose body rejects an organ transplant as well as those who suffer autoimmune diseases such as rheumatoid arthritis.<sup>9</sup>

## The unknown fruit: recent research

The presence of myristicin and diyangambin accounts for the medicinal properties of kawakawa leaves. However, the chemical composition and activity of the fruit is largely unknown, as are the compounds responsible for its characteristic flavour profile. A research team from Plant & Food Research at the University of Otago investigated the chemistry of kawakawa fruits and discovered a complex series of alkaloids.

The first significant finding was made by Otago undergraduate student Jeremy Lei who isolated a rare bioactive alkaloid, known as piperchabamide A (Fig. 4), from kawakawa fruits. Piperchabamide A was originally iso-

lated from another member of the Piperaceae family, *Piper chaba*, and since then has only been identified in a handful of other natural sources.<sup>10</sup> Extracts of the *P. chaba* have been shown to have gastroprotective properties, but the specific activity of piperchabamide A is largely unexplored.<sup>10</sup>

Further investigation into the chemistry of the fruits by Elaine Burgess of the Plant & Food Research team revealed that piperchabamide A was far from being the only alkaloid present. Amongst the compounds isolated were two new alkaloids (Fig. 4), referred to as compounds **1** and **2**. Since both compounds are previously unreported there is no information available regarding their properties, including any potential biological activity.

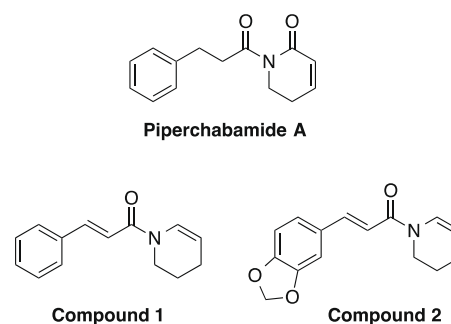


Fig. 4. Chemical structures of specific alkaloids isolated from the fruits of kawakawa (*Macropiper excelsum*)

One of the few things known about the new compounds is the presence of a restricted conformational exchange phenomenon (Fig. 5). This interesting property was identified when characterising the molecules and was investigated further as part of an undergraduate research project undertaken by the author. The phenomenon was first observed when using NMR spectroscopy to determine the structure of the molecule. It was noticed that the hydrogen atoms of the nitrogen-containing ring (Fig. 5) were represented by two peaks, rather than the expected single peak. This suggests that these hydrogen atoms experience two different environments, something that could be explained by the presence of two different conformations.

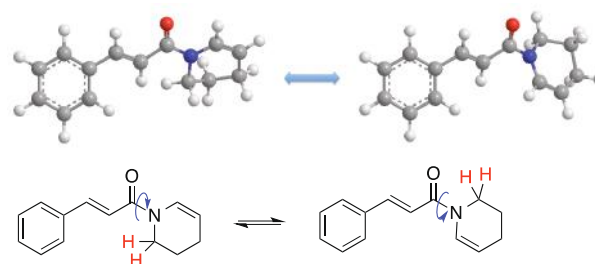


Fig. 5. Conformational exchange phenomenon exhibited by new alkaloids from kawakawa: 3D depiction (top) and schematic representation (bottom)

By using more advanced NMR techniques it was found that two conformations were possible due to rotation about the amide bond (Fig. 5). Due to the partial double bond character of the amide linkage, the rotation is restricted at low temperatures as an energy input is required for the rotation to occur. At high temperatures,

rotation, and therefore exchange between conformations, occurs much more rapidly. Again this effect may be observed using variable temperature NMR. At low temperatures two distinct signals are seen, one for each conformation as interconversion is very slow, whereas at higher temperatures the conversion rate is more rapid and the signals become broad and less defined (Fig. 6). This is not only interesting from a purely academic point of view as it may also have implications regarding the biological activity. For example, one conformation may be able to bind to a receptor in the body, while the other may not bind due to the relative orientation of groups.

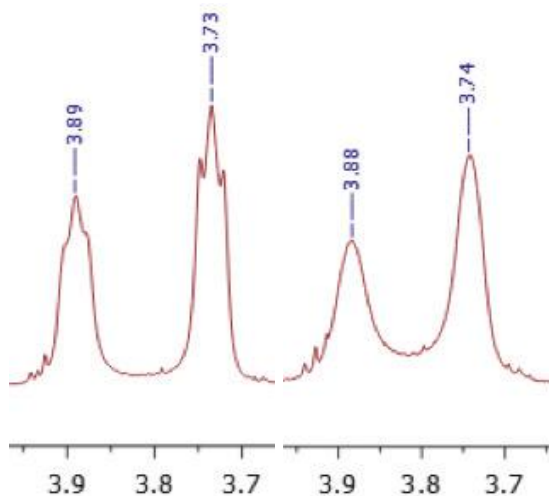


Fig. 6.  $^1\text{H}$  NMR signals for protons highlighted in Fig. 5 at room temperature (left) and at  $40^\circ\text{C}$  (right)

The newly discovered alkaloids are also of great interest due to the fact that they display structural similarities to several biologically significant alkaloids. One such compound is piperine (Fig. 7), an alkaloid responsible for the pungency of black pepper.<sup>4</sup> As well as providing flavour, piperine has been the focus of studies investigating its efficacy as an anxiolytic (anti-anxiety) and sleep-inducing agent.<sup>4</sup> Currently in the spotlight for its ability to selectively kill cancer cells, piperlongumine (Fig. 7) is another alkaloid with many similarities to the newly discovered kawakawa compounds.<sup>11</sup> It has been proposed that the activity of piperlongumine is caused by a combination of two mechanisms.<sup>11</sup> The first is an elevation of reactive oxygen species (ROS) in the cell, putting the cell under an oxidative stress.<sup>11</sup> The second involves the binding of piperlongumine to the cells' glutathione, an antioxidant which prevents cell damage by ROS.<sup>11</sup> Binding of piperlongumine to glutathione renders it incapable of performing its function in the cell.<sup>11</sup> The combination of these effects is enough to cause irreversible damage and induces cell death.<sup>11</sup> The most significant feature of piperlongumine's activity is that it is selective for cancer cells, leaving healthy cells unaffected.<sup>11</sup> While it is easy to postulate on the potential activity of the new alkaloids based on structural similarities to highly bioactive molecules, any reported activity must be based on thorough testing.

The University of Otago Plant & Food Research team is working in conjunction with Sarah Baird from the Department of Pharmacology and Toxicology to determine

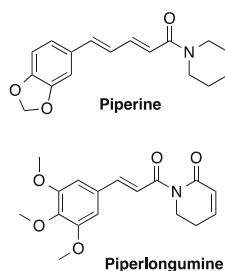


Fig. 7. Chemical structures of biologically significant alkaloids, piperine (top) and piperlongumine (bottom)

whether the new compounds are in fact bioactive. Cytotoxicity assays on the alkaloids isolated from kawakawa fruits will provide information as to whether there is biological activity present, and a series of related synthetic alkaloids will also be studied alongside the natural samples. The preparation of the synthetic analogues continues to be investigated with Bill Hawkins (Department of Chemistry, University of Otago) and aims to provide more insight into which parts of the alkaloid are responsible for their activity. Furthermore, the synthesis of the kawakawa compounds affords larger quantities of the compounds as they have a tendency to degrade over time, leaving little or no material for the relevant bioassays.

## Conclusions

Kawakawa fruits have proven to be an interesting research topic with much more depth than was initially anticipated. The identification of new compounds provides exciting potential for use in pharmaceutical, perfume or food applications.

## Acknowledgements

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## References

- Riley, M. *Maori Healing and Herbal*, Viking Sevenses N.Z. Ltd: Paraparaumu, 1994.
- Nga Tipu Whakaorange database, <http://maoriplantuse.landcareresearch.co.nz>, Records 1119, 1080 and 1068 (accessed 13/01/2015).
- Indigenous Ale of Aotearoa (2011) <http://www.taakawa.com/> (accessed 5/10/2011).
- Wimmer, L.; Schönbauer, D.; Pakfeifer, P.; Schöffmann, A.; Khom, S.; Hering, S.; Mihovilovic, M. D. *Org. Biomol. Chem.* **2015**, *13*, 990-994.
- Russell, G. B.; Fenemore, P. G. *Phytochemistry* **1973**, *12*, 1799-1803.
- Morita, T.; Jinno, K.; Kawagishi, H.; Arimoto, Y.; Suganuma, H.; Inakuma, T.; Sugiyama, K. *J. Agric. Food Chem.* **2003**, *51*, 1560-1565.
- Kuete, V.; Krusche, B.; Youns, M.; Voukeng, I.; Fankam, A. G.; Tankeo, S.; Lacmata, S.; Efferth, T. *J. Ethnopharmacol.* **2011**, *134*, 803-812.
- Shulgin, A. T. *J. Psychedel. Drug.* **1976**, *8*, 167-169.
- De Leon, E. J.; Olmedo, D. A.; Solis, P. N.; Gupta, M. P.; Terencio, M. C. *Planta Med.* **2002**, *68*, 1128.
- Matsuda, H.; Ninomiya, K.; Morikawa, T.; Yasuda, D.; Yamaguchi, I.; Yoshikawa, M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2038-2042.
- Adams, D. J.; Dai, M. J.; Pellegrino, G.; Wagner, B. K.; Stern, A. M.; Shamji, A. F.; Schreiber, S. L. *P. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 15115.

## Membrane lytic antimicrobial lipopeptides

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**Keywords:** antibiotic resistance, antimicrobial peptides, membrane lysis, therapeutic potential, colorimetric assay



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### Introduction

The development of antibiotics to treat life-threatening infections has been one of the greatest achievements of modern medicine. However, the appearance of penicillin resistant *Staphylococci* almost concomitantly with the introduction of penicillin into the market makes the problem of antibiotic resistance as old as antibiotics. Since then several more bacteria have become resistant to old and newer generations of antibiotics. In the context of antibiotic resistance, multidrug-resistance (MDR) refers to “non-susceptibility to at least one agent in three or more antimicrobial classes”, whereas extensively drug-resistant (XDR) is defined as “nonsusceptibility to at least one antimicrobial agent in all but two or less antimicrobial classes”.<sup>1</sup> The lack of new antibiotics to treat fatal infections caused by MDR bacteria, particularly *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* has become a serious global concern. The World Health Organisation has referred to antimicrobial resistance as a “problem so serious that it threatens the achievements of modern medicine”.<sup>2</sup> There is an urgent need to fill the gap created because of the difference in pace between the rapid emergence of “superbugs” and the lack of new antibiotics to combat these. Antimicrobial agents with novel chemical structures and mechanism of action different to conventional antibiotics are important in this regard. Antimicrobial peptides (AMPs) are promising therapeutics to address the challenge of antibiotic

resistance. AMPs are part of the innate immune system in almost all forms of life from microbes to humans. The majority of AMPs are cationic in nature which make them selective to microbial cell membranes having high concentrations of anionic phospholipids as opposed to mammalian and plant cell membranes which are mostly zwitter-ionic in nature. AMPs show broad spectrum antimicrobial activity, including against Gram positive and Gram negative bacteria, fungi, viruses and parasites. Recent research also highlights the anticancer activities of AMPs.<sup>3,4</sup> The therapeutic potential of peptides is now receiving the pharmaceutical industry’s attention with focus shifting towards the development of these as marketed drugs<sup>5</sup> (Table 1). Unlike conventional antibiotics, such as streptomycin, development of resistance to AMPs is thought to be highly improbable, mainly because of their unique mechanism of antibacterial action. Since the main target of AMPs is the microbial membrane, bacteria (or other microbes) would have to significantly alter the structure of their membrane, to gain resistance to AMPs, which is highly unlikely.

This is a short review of a sub-class of AMPs, namely the lipopeptide antibiotics, particularly daptomycin and polymyxins (currently in clinical use) and octapeptides which have the potential to follow, based on literature evidence and our own recent research.

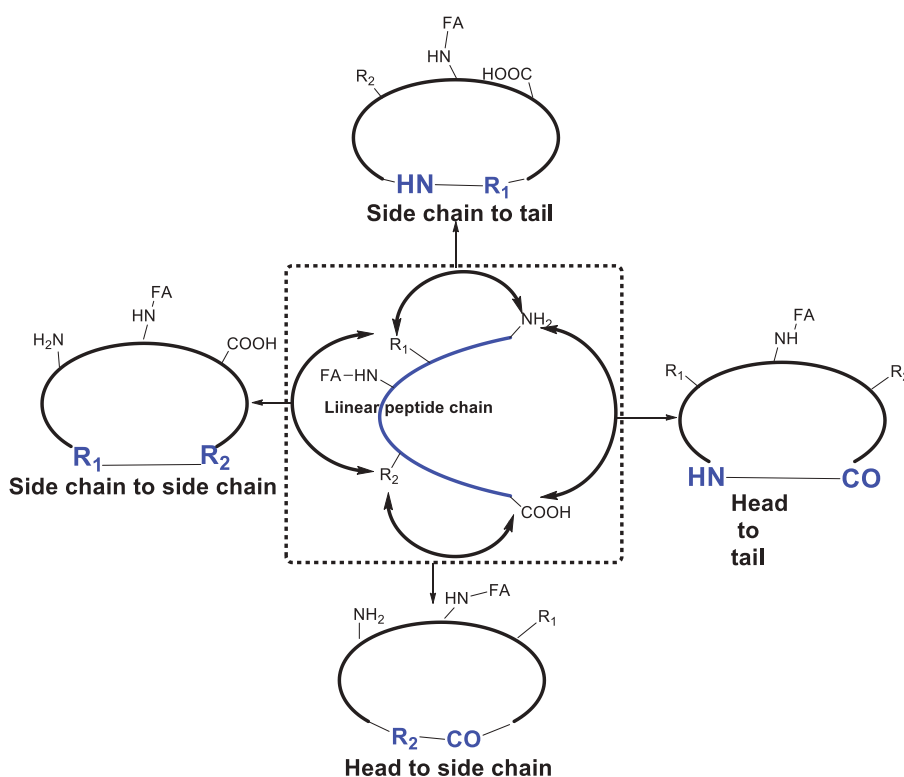
## Lipopeptides

Natural lipopeptides are synthesised via non-ribosomal peptide synthases (NRPS) and carry long-chain fatty acids on their N-termini.<sup>6</sup> The peptide component of lipopeptides can be cyclic or linear chains of amino acids, generally ranging in length to about 15 residues. The ring can be a lactone, a lactam or mediated by a disulphide bridge. In principle, lactam formation can happen between the N- and C-termini (head-to-tail, in which case the lipidation would involve side chain amine function-

ality); between the carboxyl terminus and a side chain amine group or the N-terminus and side chain carboxyl group. Lactonisation usually involves the side chain hydroxyl groups in either Ser or Thr and, either the side chain or the C-terminal, COOH. The different possibilities for macrocyclisation in lipopeptides are shown schematically in Fig. 1. Primary structures of polymyxin B and daptomycin, as examples of macrolactam and macrolactone rings, are shown in Fig. 2.

**Table 1.** Peptide-based antimicrobials already approved for clinical use or as food preservatives as well as those in different stages of clinical trials

Name	Structural Classification	Application	Stage of Development
Gramicidin S	Cyclic peptide	Used topically against Gram positive and negative pathogens	Clinically used
Oritavancin	Glycopeptide	Used topically against Gram positive pathogens	Approved in 2014
Dalbavancin	Lipoglycopeptide	Used in intravenous form against Gram positive skin infections	Approved in 2014
Vancomycin	Glycopeptide	Used in intravenous form against Gram positive pathogens	Clinically used
Daptomycin	Cyclic lipopeptide	Used in intravenous form against Gram positive pathogens	Clinically used
Polymyxin B	Cyclic lipopeptide	Used as treatment against Gram negative pathogens	Clinically used
Colistin	Cyclic lipopeptide	Treatment against lung infection in cystic fibrosis	Clinically used
Nisin	Lantibiotic	Used as a food preservative	Commercially used food preservative
Pexiganan acetate	Cationic peptide	Diabetic foot ulcers	Phase III
CB-315	Lipopeptide Cationic peptide	Treatment against <i>C. difficile</i>	Phase III
P-113	(Histatin derivative)	Mouthwash against candidiasis	Completed Phase II
IB-367	Cationic peptide (Protegrin analogue)	Used as aerosol in cystic fibrosis patients with chronic respiratory infections	Phase II



**Fig. 1.** Four possible ways a peptide/lipopeptide can be constrained into a macrocycle.<sup>30</sup>

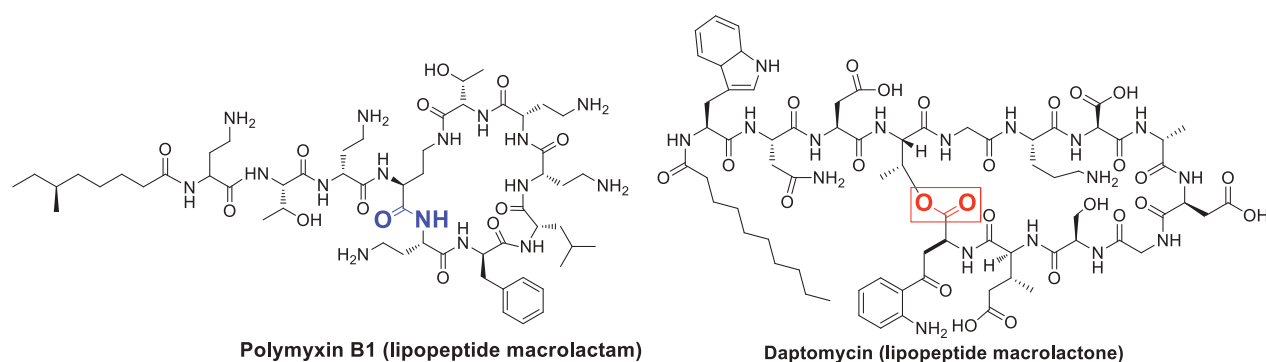


Fig. 2. Chemical structures of polymyxin B1 and daptomycin, depicting the lactam and lactone modes of cyclisation.

Lipopeptides are structurally diverse and are produced by a wide range of bacterial genera such as *Bacillus*, *Streptomyces* and *Pseudomonas* as well as certain fungi, e.g. *Aspergillus*.<sup>6,7</sup> Their structural diversity implies their varied roles are also unique to the producing organism. Most naturally occurring lipopeptides are secondary metabolites produced by soil bacteria. Examples of naturally occurring lipopeptides include polymyxins,<sup>8,9</sup> octapeptins,<sup>10</sup> brevistin,<sup>11</sup> stendomycin,<sup>12</sup> polypeptin,<sup>13</sup> cerexin,<sup>14</sup> iturins,<sup>15</sup> surfactins,<sup>16</sup> fengycins,<sup>17</sup> fusaricidins,<sup>18</sup> tridecaptin,<sup>19,20</sup> etc. The oldest amongst these are polymyxins produced by the soil bacterium *Bacillus polymyxa*.<sup>8,9,21,22</sup> Lipopeptides have entered the stage for the fight against antimicrobial diseases with the FDA approval of daptomycin<sup>23</sup> for the treatment of serious infections caused by Gram positive bacteria and the revival of interest in the previously abandoned polymyxins for clinical use to treat life threatening infections caused by Gram negative bacteria.<sup>24</sup> Lipopeptides are also known for their surfactant activities.<sup>16,25</sup> Table 2 lists the widely known lipopeptides and the microorganisms susceptible to them.

### Brief discussion on synthesis of cyclic peptides

The use of orthogonal protecting groups and linkers cleavable under mild conditions are paramount to the solid phase syntheses of cyclic peptides. The specific strategy for a particular synthesis will depend on the nature of the ring (lactone, lactam or disulphide bridge), length of the peptide chain, and, for lipopeptides, the nature and position of the lipid group(s). 2-chlorotriethylchloride resin generates protected peptides following mild acid cleavage which can then be used in solution phase cyclisation reactions.<sup>26</sup> Macrocyclisations need to be carried out under high dilution to facilitate intramolecular cyclisation against intermolecular polymerisation. Syntheses of linear and cyclic lipopeptides have been carried out following solid and solution phase strategies in the literature.<sup>27,28,29</sup> There are several articles in recent literature where the syntheses of cyclic peptides, cyclic depsipeptides as well cyclic lipopeptides (lactam, lactone and disulphide bridge) are discussed.<sup>28,30,31</sup> A general strategy for the synthesis of cyclic lipopeptides with a macrolactam ring, using a combination of solid and solution phase techniques is shown in Scheme 1.

### Current clinically used lipopeptide antibiotics

#### Daptomycin

Daptomycin (Fig. 2) belongs to A21798C, a complex of acidic lipopeptides produced by *Streptomyces roseosporus* using non-ribosomal peptide synthetases.<sup>32</sup> Closely related to daptomycin are the cyclic lipodepsipeptides A54145<sup>33</sup> and calcium-dependent antibiotic (CAD)<sup>34</sup> as well as the cyclic lipopeptides amphomycin,<sup>35</sup> firulimycin,<sup>36</sup> lasportomycins<sup>37</sup> and glycinocins<sup>38</sup> produced by various streptomyces strains.

Daptomycin was approved for the treatment of infections caused by Gram positive bacteria, particularly methicillin resistant *Staphylococcus aureus*.<sup>23</sup> The presence of several aspartic acids and one glutamic acid makes daptomycin anionic in nature. However it binds to Ca<sup>2+</sup> and functions as a cationic peptide through electrostatic interactions with the negatively charged phospholipids on cytoplasmic membranes of Gram positive bacteria.<sup>39-41</sup> Literature reports are contradictory about the exact sequence in which bacterial cell death occurs, with views presented of membrane depolarisation being the cause of cell death as well as a consequence of cell death.<sup>39,42</sup> The spectrum of activity of daptomycin includes several clinical isolates belonging to methicillin resistant *S. aureus* strains, penicillin resistant *S. pneumonia* as well as vancomycin resistant *enterococci*.<sup>43</sup> However, daptomycin does not penetrate the cell membranes of Gram negative bacteria, and hence cannot be used against such infections.<sup>41,44</sup>

#### Polymyxins

Polymyxins, discovered almost 7 decades ago from the soil bacteria *Paenibacillus (Bacillus) polymyxa*, are bactericidal cyclic lipopeptides active against multidrug resistant Gram negative bacteria.<sup>8,21,45</sup> Polymyxin B (PMB) and polymyxin E (colistin) are the two clinically used polymyxins. Both have a decapeptide core with 5 copies of  $\alpha$ , $\gamma$ -diamino butyric acid. They are N-terminally acylated with (*S*)-6-methyloctanoic acid and (*S*)-6-methylheptanoic acid in polymyxin B and E respectively. Additionally D-Phe<sup>6</sup> in polymyxin B is replaced by D-Leu<sup>6</sup> in polymyxin E. Dab<sup>4</sup> side chain NH forms an intramolecular cyclic lactam with the carboxy terminal Thr<sup>10</sup> resulting in a 23-membered ring (Fig. 2). Despite entering the clinic decades ago, polymyxins were abandoned in the early 80s because of nephrotoxicity concerns and re-

Table 2. Common naturally occurring lipopeptides

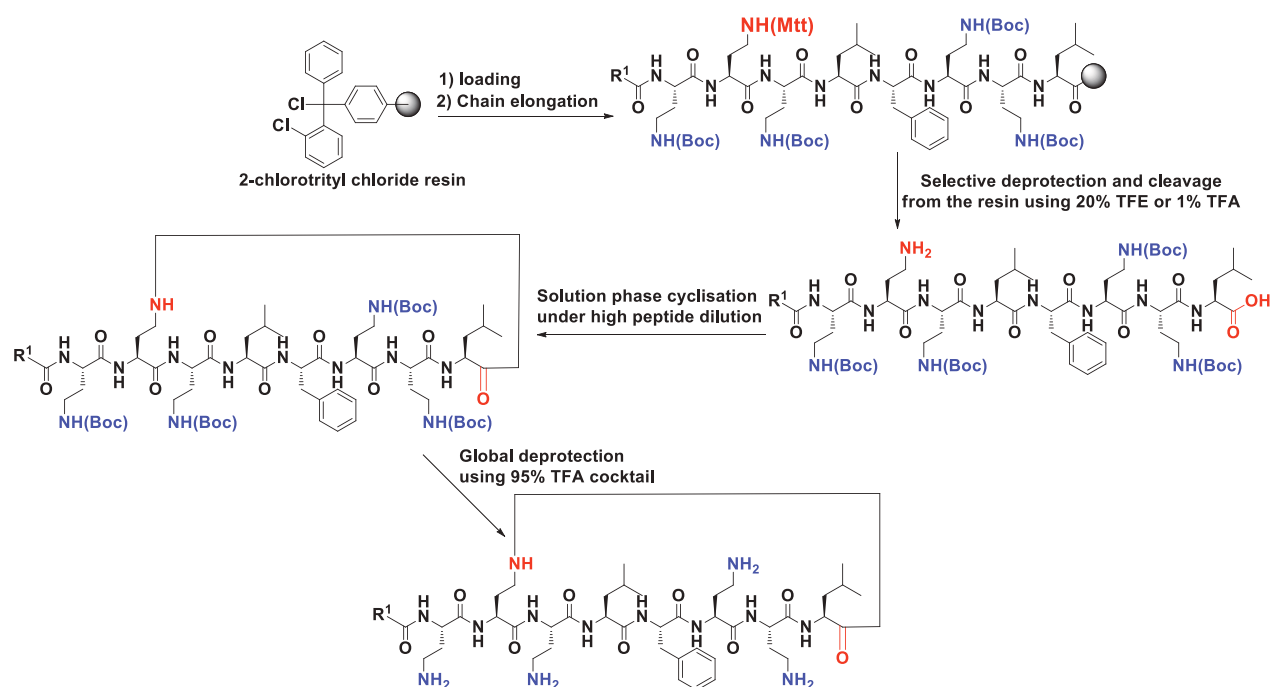
Type	Source	Susceptible strains	Ref.
<b>Lactam</b>			
Polymyxin	<i>Paenibacillus polymyxa</i>	Gram negative ( <i>P.aeruginosa</i> , <i>A.baumannii</i> , and <i>K.pneumoniae</i> )	8
Octapeptin	<i>Bacillus</i> and <i>Paenibacillus</i> sp	Various Gram negative ( <i>P.aeruginosa</i> , <i>A.baumannii</i> and <i>K.pneumoniae</i> ), positive ( <i>S.aureus</i> ) and fungi ( <i>C.albicans</i> )	10
Polypeptin	<i>Paenibacillus</i> sp	Various Gram negative ( <i>P.aeruginosa</i> ), positive (methicillin-resistant <i>S.aureus</i> ) and fungi ( <i>F.graminearum</i> )	67
Friulimicins	<i>Actinoplanes friuliensis</i>	Gram positive (methicillin-resistant <i>S.aureus</i> and vancomycin-resistant <i>Enterococcus</i> )	36
Marihysin A	<i>Bacillus marinus B</i>	Low broad spectrum activity against plant pathogens	68
Laspartomycin	<i>Streptomyces viridochromogenes</i>	Gram positive (methicillin-resistant <i>S. aureus</i> and vancomycin-resistant <i>S. aureus</i> )	37
Echinocandins	<i>Aspergillus nidulans</i> var	Antifungal ( <i>C. albicans</i> and <i>Aspergillus</i> sp)	69
Iturins	<i>Bacillus subtilis</i> and <i>Bacillus amyloliquefacien</i>	Antifungal ( <i>Aspergillus</i> sp, <i>Penicillium</i> and <i>Pyricularia</i> spp)	70
Bacillomycin	<i>B. subtilis</i>	Antifungal ( <i>Aspergillus</i> sp, <i>C. albicans</i> )	71
<b>Lactone (Depsipeptide)</b>			
Daptomycin	<i>Streptomyces roseosporus</i>	Gram positive (methicillin-resistant <i>S. aureus</i> and vancomycin-resistant <i>S. aureus</i> )	32
Surfactin	<i>B. subtilis</i>	Gram positive ( <i>Enterococcus faecalis</i> ) and Gram negative ( <i>E.coli</i> )	16
Fengycin	<i>B. subtilis</i>	Antifungal ( <i>Pyricularia oryzae</i> and <i>Curvularia lunata</i> )	17
Empedopeptin	<i>Empedobacter haloabium</i>	Gram positive (methicillin-resistant <i>S.aureus</i> and penicillin-resistant <i>Streptococcus pneumoniae</i> )	72
Tripropeptin	<i>Lysobacter</i> Sp	Gram positive (methicillin-resistant <i>S. aureus</i> , penicillin-resistant <i>Streptococcus pneumoniae</i> and vancomycin-resistant <i>Enterococcus</i> )	73
Calcium dependent antibiotics (CDAs)	<i>S. coelicolor</i> A3	Gram positive in presence of calcium ( <i>S. aureus</i> )	34
Brevistin	<i>B. brevis</i>	Gram positive ( <i>S. aureus</i> and <i>Streptococcus pneumoniae</i> )	11
Fusaricidins	<i>Paenibacillus polymyxa</i>	Antifungal ( <i>Fusarium</i> and <i>Aspergillus</i> ) and Gram positive ( <i>S. aureus</i> )	74
<b>Linear</b>			
Cerexin	<i>Bacillus cereus</i>	<i>S. aureus</i> and <i>S. pneumoniae</i>	14
Tridecaptin	<i>Paenibacillus polymyxa</i>	<i>K. pneumoniae</i> and <i>E. cloacae</i>	19
Tauramadine	<i>Brevibacillus laterosporus</i>	<i>Enterococcus</i> sp	75
Dragomide	<i>Lyngbya majuscula</i>	<i>Plasmodium falciparum</i> , <i>Leishmania donovani</i> and <i>Trypanosoma cruzi</i>	76

placed by aminoglycosides that were considered to be less toxic. The rapid emergence of *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Enterococci* resistant to  $\beta$ -lactams, fluoroquinolones and aminoglycosides, has led to rejuvenated interest in polymyxins for the treatment of such serious infections.<sup>24</sup> Currently polymyxins are being used as the last line of defence against otherwise untreatable infections caused by Gram negative bacteria.

#### Mechanism of action and spectrum of antibacterial activity

Lipopolysaccharides (LPS), the structural components of Gram negative outer bacterial membranes, are composed of the highly conserved amphiphilic lipid moi-

ety, "lipid A".<sup>46</sup> PMB binds to the lipid A component of LPS, disrupting the outer membrane which eventually causes inner membrane permeabilisation as well.<sup>47</sup> PMB has been shown to displace  $Mg^{2+}$  and  $Ca^{2+}$  from cation binding sites.<sup>48</sup> LPS binding has been directly linked to anti-endotoxin activity of PMB.<sup>49</sup> Polymyxin B and colistin are active against all important nosocomial pathogens, namely *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas aeruginosa*, and *Acinetobacter*.<sup>50</sup> PMB is administered as it is, but colistin is administered as a prodrug colistin methanesulfonate.<sup>51</sup> Primary clinical use of colistin is in the treatment of lung infections caused by *P. aeruginosa* in cystic fibrosis patients. *P. aeruginosa* is known to be a metabolically dynamic pathogen. It has been reported that metabolically active *P.*



**Scheme 1.** General strategy for the synthesis of a cyclic lipopeptide macrolactam formed between a side chain  $\text{NH}_2$  group and the C-terminus. Abbreviations: Mtt - 4-methyltrityl; Boc - tert-butyloxycarbonyl;  $\text{R}^1$ - fatty acid

*aeruginosa* biofilm cells are resistant to colistin, while metabolically inactive ones are sensitive to the drug.<sup>52</sup> Resistance to polymyxin has been reported in *Acinetobacter baumannii*.<sup>53</sup> Resistant bacteria have been found to alter the lipid A structure leading to weak binding by PMB.<sup>54</sup> The interaction between PMB and LPS has been studied by various biophysical techniques including high resolution NMR and circular dichroism.<sup>55</sup> The five diamino butyric acid residues together with the hydrophobic components (N-terminal fatty acid and the D-Phe-Leu dipeptide moiety) have been shown to be important for the antibacterial activity and LPS binding of polymyxin B.<sup>29,56</sup>

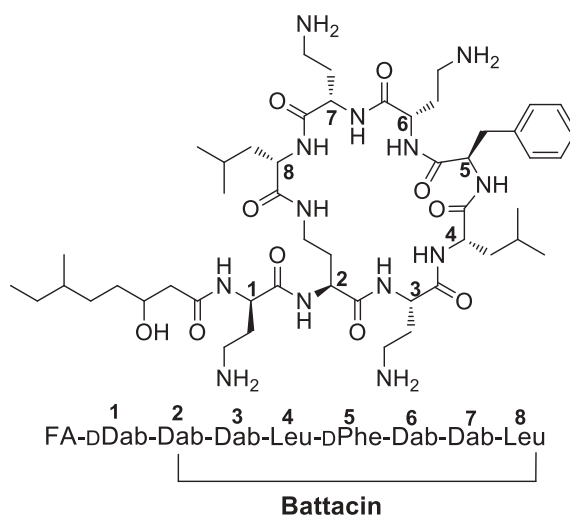
### Nephrotoxicity of polymyxins

The strong cationic nature of polymyxins contributes to their nephrotoxicity, resulting in damage of renal proximal tubuli. To minimise the nephrotoxic effect, polymyxin analogues with fewer positive charges (the NAB series with 3 positive charges) have been developed.<sup>57</sup> The NAB compounds did not induce necrosis in porcine renal proximal tubular LLC-PK1 cells even at 1 mM, whereas polymyxin B elicited 50% necrosis at 0.5 mM.<sup>58</sup> Table 3 lists the sequence and level of nephrotoxicity observed in PMB, colistin and their synthetic derivatives. Endotoxin (lipopolysaccharide) the structural component of Gram negative outer bacterial membrane is a pathogenicity factor involved in septic shock which leads to organ failure and death in critically ill patients. Unfortunately, the anti-endotoxin activity of PMB is over-shadowed by its nephrotoxicity.

### Octapeptins

Octapeptins are another class of cyclic lipopeptides produced by the soil bacteria *Panabacellius* species.<sup>10</sup> As is evident from the name, the peptide component of octapeptins is made up of eight amino acids and the N-terminus carries a  $\beta$ -hydroxy fatty acid (similar to poly-

myxin B6), which can be either a straight or branched chain of carbon atoms. The protein amino acids, leucine and phenylalanine, together with the non-protein amino acid  $\alpha$ , $\gamma$ -diaminobutyric acid, constitute the peptide part. Octapeptins can be considered as truncated polymyxins, however both have similar amino acid compositions with a high percentage of  $\alpha$ , $\gamma$ -diaminobutyric acid and an N-terminal fatty acid of similar length. Unlike polymyxins, octapeptins are active against both Gram positive and Gram negative bacteria.<sup>59</sup> Octapeptins have been classified into four sub-classes A-D.<sup>60,61</sup> Battacin (Fig. 3) is a novel cyclic lipopeptide belonging to the octapeptin B class reported in 2012.<sup>61</sup> Similar to other octapeptins, battacin has a high percentage of  $\alpha$ , $\gamma$ -diaminobutyric acid. The only protein amino acid found in battacin is leucine (residues 4 and 8). Battacin also has one D-Phe residue (position 5). Battacin has been proposed to have an LD sequence (Leu<sup>4</sup>-DPhe<sup>5</sup>)<sup>61</sup>, unlike other octapeptins and all polymyxins which are known to have a DL sequence. This is intriguing and will have to be verified us-



**Fig. 3.** Chemical structure and sequence of battacin. FA: fatty acid

**Table 3.** Sequences of polymyxin and related lipopeptides showing net positive charge and nephrotoxicity potential<sup>58</sup>

Lipopeptide	Sequence	Charge	Nephrotoxicity
Polymyxin B	MHA/MOA-Dab-Thr-Dab-cy[Dab-Dab- <sub>D</sub> Phe-Leu-Dab-Dab-Thr]	5	+++
Collistin	MHA/MOA-Dab-Thr-Dab-cy[Dab-Dab- <sub>D</sub> Leu-Leu-Dab-Dab-Thr]	5	+++
CB-182,804	2-CPAC-Dab-Thr-Dab-cy[Dab-Dab- <sub>D</sub> Phe-Leu-Dab-Dab-Thr]	5	++
NAB739	OA-Thr- <sub>D</sub> Ser-cy[Dab-Dab- <sub>D</sub> Phe-Leu-Dab-Dab-Thr]	3	+
NAB7061	OA-Thr-Abu-cy[Dab-Dab- <sub>D</sub> Phe-Leu-Dab-Dab-Thr]	3	+
NAB741	Ac-Thr- <sub>D</sub> Ser-cy[Dab-Dab- <sub>D</sub> Phe-Leu-Dab-Dab-Thr]	3	+

Abbreviations and symbols: MHA/MOA - mixture of methyl octanoyl and methyl heptanoyl, 2-CPAC - 2-chloro-phenylaminocarbonyl, Dab - diaminobutyryl, Abu - aminobutyryl, OA - octanoyl, Ac - acetyl.

+++ : 50 % necrosis of porcine renal proximal tubular LLC-pk1 cells at 0.5 mM

++ : necrosis potential considered high, but no data available on % necrosis

+ : <10 % necrosis of porcine renal proximal tubular LLC-pk1 cells at 0.5 mM

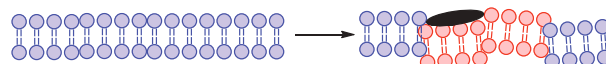
ing chemical synthesis. Battacin has a better therapeutic index than polymyxin B with high potency against MDR and XDR pathogens and low level of cytotoxicity. These characteristics make battacin, and probably other octapeptides, ideal candidates for development as future antibiotics against MDR Gram negative pathogens, against which polymyxins are not ideal because of nephrotoxicity and neurotoxicity. Our recent research has identified synthetic battacin derivatives more potent than battacin as well as having a broader spectrum of antimicrobial activity.<sup>62</sup>

The following section describes a simple membrane lytic assay to quickly determine the ability of antimicrobial agents to lyse bacterial membranes and puts this in the context of our own results using a library of battacin peptides.

### Membrane lytic assay

Given the rapid and alarming increase in antibiotic resistance and the urgent need for novel antimicrobials with a mechanism of action different to conventional antibiotics, a simple but reliable assay for the detection of such compounds is important. Detection of biological molecules following colour changes with the naked eye is particularly attractive. Vesicles, composed of phospholipids and polymerised diacetylene, mimic cell membranes and serve as a biomimetic sensing platform.<sup>63</sup> Lipid/polydiacetylene (PDA) vesicles have recently been utilised for the detection of bacteria based on colour changes induced by membrane active amphiphilic molecules produced by these organisms.<sup>64</sup> Dimyristoylphosphatidylcholine-polydiacetylene (DMPC-PDA) vesicles provide an excellent system to mimic the bacterial membrane and exhibit an intense blue color upon irradiation with UV light which immediately changes to red when the vesicular structure is disrupted.<sup>65</sup> A representative diagram depicting such a scenario is shown in Fig. 4. Differences in colour changes induced by native and analogue peptides

have been correlated to their observed antibacterial activities and the extent of membrane permeabilities.



**Fig. 4.** Representation of membrane perturbation induced by antimicrobial agents being detected as colour changes. An ordered membrane is shown on the left hand side in blue. The black oval represents a potential antimicrobial agent, which upon binding to the membrane causes structural perturbations, detected as immediate colour changes from blue to red.










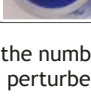
### Evaluation of membrane lytic activity of a synthetic battacin library

A library of battacin peptides was synthesised following the general protocol shown in Scheme 1. DMPC-PDA vesicles prepared following literature protocols<sup>63,65</sup> were used to assess the ability of the battacin peptides to cause lysis of bacterial membranes following the assay referred to above. Streptomycin and a known membrane lytic antimicrobial peptide (pexiganan) were used as the controls. All compounds were used at 0.1 mM concentration in the membrane lytic assay. Results are shown in Table 4. As expected, pexiganan, a well-known membrane-lytic peptide showed an intense colorimetric response from blue to red immediately on addition to the vesicles. Streptomycin, despite being bactericidal, did not show any color change, which is in accordance with its known mechanism of action that does not involve membrane perturbation. The observed colorimetric responses of the battacin library correlated well with their observed antimicrobial potencies (minimal inhibitory concentrations: MICs). Battacin analogues with MICs < 100 mM showed clear membrane perturbation as confirmed by the immediate color change observed on addition to the vesicles, whereas those analogues with MICs > 500 mM did not induce any membrane perturbation.

### Summary

Development of biologically active peptides is an active

Table 4. Membrane lytic activity of battacin library

Peptide	MIC	Colour of the well <sup>a</sup>	Membrane lysis
GZ3.55	~1000 $\mu$ M		No
GZ3.40	~500 $\mu$ M		No
GZ3.19	~500 $\mu$ M		No
GZ3.15	~50 $\mu$ M		Yes
GZ3.37	~20 $\mu$ M		Yes
GZ3.26	~20 $\mu$ M		Yes
GZ3.38	~100 $\mu$ M		Yes
Pexiganan	~5 $\mu$ M		Yes
Streptomycin sulphate	~1 $\mu$ M		No
Negative control	-		-

<sup>a</sup>The numbers inside the coloured wells in column 3 are the numbers of the corresponding wells in the 96-well assay. Blue indicates unperturbed membrane structure whereas red indicates perturbed membranes. (See Fig. 4 for a schematic representation).

area of research in our laboratory at the University of Auckland. Our previous work in this area has resulted in short synthetic AMPs with potency against PSA, the kiwi fruit pathogen.<sup>66</sup> Work is currently in progress towards the chemical syntheses, characterisation and bioassay analysis of battacin diastereomers in an effort to establish the stereochemistry of the natural product and to develop potent analogues with a broader spectrum of antimicrobial activity.

### Acknowledgements

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### References

- Magiorakos, A. P.; Srinivasan, A.; Carey, R. B.; Carmeli, Y.; Falagas, M. E.; Giske, C. G.; Harbarth, S.; Hindler, J. F.; Kahlmeter, G.; Olsson-Liljequist, B.; Paterson, D. L.; Rice, L. B.; Stelling, J.; Struelens, M. J.; Vatopoulos, A.; Weber, J. T.; Monnet, D. L. *Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **2012**, *18*, 268-281.
- www.who.int/drugresistance/documents/surveillancereport/en/ (accessed October 5, 2014).
- Zaiou, M. *J. Mol. Med.* **2007**, *85*, 317-329.
- Jenssen, H.; Hamill, P.; Hancock, R. E. W. *Clin. Microbiol. Rev.* **2006**, *19*, 491-511.
- Rossi, L. M.; Rangasamy, P.; Zhan, J.; Qiu, X. Q.; Wu, G. Y. *J. Pharm. Sci.-US* **2008**, *97*, 1060-1070; Marx, V. *Chem. Eng. News* **2005**, *83*, 17; Vlieghe, P.; Lisowski, V.; Martinez, J.; Khrestchatsky, M. *Drug Discov. Today* **2010**, *15*, 40-56.
- Strieker, M.; Marahiel, M. A. *Chembiochem.* **2009**, *10*, 607-616.
- Bonmatin, J. M.; Laprevote, O.; Peypoux, F. *Comb. Chem. High T. Scr.* **2003**, *6*, 541-556; Mandal, S. M.; Barbosa, A. E. A. D.; Franco, O. L. *Biotechnol. Adv.* **2013**, *31*, 338-345.
- Benedict, R. G.; Langlykke, A. F. *J. Bacteriol.* **1947**, *54*, 24-25.
- Wilkinson, S.; Lowe, L. A. *Nature* **1966**, *212*, 311.
- Meyers, E.; Parker, W. L.; Brown, W. E.; Linnett, P.; Stroming, J. *Ann. New York Acad. Sci.* **1974**, *235*, 493-501.
- Shoji, J.; Kato, T. *J. Antibiot.* **1976**, *29*, 380-389.
- Bodanszk.M.; Izdebski, J.; Muramats.I. *J. Am. Chem. Soc.* **1969**, *91*, 2351-2358.
- Sogn, J. A. *J. Med. Chem.* **1976**, *19*, 1228-1231.
- Shoji, J.; Hino, H. *J. Antibiot.* **1975**, *28*, 60-63.
- Magetdana, R.; Peypoux, F. *Toxicology* **1994**, *87*, 151-174.
- Arima, K.; Kakinuma, A.; Tamura, G. *Biochem. Bioph. Res. Co.* **1968**, *31*, 488-494.
- Vanittanakom, N.; Loeffler, W.; Koch, U.; Jung, G. *J. Antibiot.* **1986**, *39*, 888-901.
- Nakajima, N.; Koyama, Y.; Chihara, S. *J. Antibiot.* **1972**, *25*, 243; Kurusu, K.; Ohba, K.; Arai, T.; Fukushima, K. *J. Antibiot.* **1987**, *40*, 1506-1514; Kajimura, Y.; Kaneda, M. *J. Antibiot.* **1997**, *50*, 220-228.
- Kato, T.; Hino, H.; Shoji, J. *J. Antibiot.* **1978**, *31*, 652-661.
- Shoji, J.; Hino, H.; Sakazaki, R.; Kato, T.; Wakisaka, Y.; Mayama, M.; Matsuura, S.; Miwa, H. *J. Antibiot.* **1978**, *31*, 646-651; Kato, T.; Sakazaki, R.; Hino, H.; Shoji, J. *J. Antibiot.* **1979**, *32*, 305-312.

21. Ainsworth, G. C.; Brown, A. M.; Brownlee, G. *Nature* **1947**, *160*, 263-263.
22. Storm, D. R.; Rosenthal, K. S.; Swanson, P. E. *Ann. Rev. Biochem.* **1977**, *46*, 723-763.
23. Kirkpatrick, P.; Raja, A.; LaBonte, J.; Lebbos, J. *Nature rev. Drug discovery* **2003**, *2*, 943-944; Sauermaun, R.; Rothenburger, M.; Graninger, W.; Joukhadar, C. *Pharmacology* **2008**, *81*, 79-91.
24. Evans, M. E.; Feola, D. J.; Rapp, R. P. *Annals Pharmacother.* **1999**, *33*, 960-967.
25. Nakano, M. M.; Corbell, N.; Besson, J.; Zuber, P. *Molecular & General Genetics*: **1992**, *232*, 313-321; Raaijmakers, J. M.; De Bruijn, I.; Nybroe, O.; Ongena, M. *FEMS Microbiol. Rev.* **2010**, *34*, 1037-1062.
26. Barlos, K.; Chatzi, O.; Gatos, D.; Stavropoulos, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 513-520.
27. Ludolph, B.; Eisele, F.; Waldmann, H. J. *Am. Chem. Soc.* **2002**, *124*, 5954-5955; Kragol, G.; Lumbierres, M.; Palomo, J. M.; Waldmann, H. *Angew. Chem. Int. Ed.* **2004**, *43*, 5839-5842; Malesevic, M.; Strijowski, U.; Bachle, D.; Sewald, N. *J Biotechnol.* **2004**, *112*, 73-77.
28. Vila, S.; Badosa, E.; Montesinos, E.; Feliu, L.; Planas, M. *Org. Biomol. Chem.* **2013**, *11*, 3365-3374.
29. Tsubery, H.; Ofek, I.; Cohen, S.; Fridkin, M. *J. Med. Chem.* **2000**, *43*, 3085-3092; Tsubery, H.; Ofek, I.; Cohen, S.; Fridkin, M. *Peptides* **2001**, *22*, 1675-1681.
30. White, C. J.; Yudin, A. K. *Nat. Chem.* **2011**, *3*, 509-524.
31. Lam, H. Y.; Zhang, Y. F.; Liu, H.; Xu, J. C.; Wong, C. T. T.; Xu, C.; Li, X. C. *J. Am. Chem. Soc.* **2013**, *135*, 6272-6279; De Vleeschouwer, M.; Sinnaeve, D.; Van den Begin, J.; Coenye, T.; Martins, J. C.; Madder, A. *Chem. Eur. J.* **2014**, *20*, 7766-7775.
32. Debono, M.; Barnhart, M.; Carrell, C. B.; Hoffmann, J. A.; Ocolowitz, J. L.; Abbott, B. J.; Fukuda, D. S.; Hamill, R. L.; Biemann, K.; Herlihy, W. C. *J. Antibiot.* **1987**, *40*, 761-777.
33. Boeck, L. D.; Papiska, H. R.; Wetzell, R. W.; Mynderse, J. S.; Fukuda, D. S.; Mertz, F. P.; Berry, D. M. *J. Antibiot.* **1990**, *43*, 587-593; Fukuda, D. S.; Mynderse, J. S.; Baker, P. J.; Berry, D. M.; Boeck, L. D.; Yao, R. C.; Mertz, F. P.; Nakatsukasa, W. M.; Mabe, J.; Ott, J.; Counter, F. T.; Ensminger, P. W.; Allen, N. E.; Alborn, W. E.; Hobbs, J. N. *J. Antibiot.* **1990**, *43*, 623-633.
34. Lakey, J. H.; Lea, E. J. A.; Rudd, B. A. M.; Wright, H. M.; Hopwood, D. A. *J. Gen. Microbiol.* **1983**, *129*, 3565-3573.
35. Heinemann, B.; Kaplan, M. A.; Muir, R. D.; Hooper, I. R. *Antibiot. Chemother.* **1953**, *3*, 1239-1242.
36. Aretz, W.; Meiwes, J.; Seibert, G.; Vobis, G.; Wink, J. *J. Antibiot.* **2000**, *53*, 807-815.
37. Naganawa, H.; Hamada, M.; Maeda, K.; Okami, Y.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1968**, *21*, 55-62.
38. Kong, F. M.; Carter, G. T. *J. Antibiot.* **2003**, *56*, 557-564.
39. Jung, D.; Rozek, A.; Okon, M.; Hancock, R. E. *Chem. Biol.* **2004**, *11*, 949-957.
40. Baltz, R. H.; Miao, V.; Wrigley, S. K. *Nat. Prod. Rep.* **2005**, *22*, 717-741.
41. Silverman, J. A.; Perlmutter, N. G.; Shapiro, H. M. *Antimicrob. Agents Ch.* **2003**, *47*, 2538-2544.
42. Allen, N. E.; Alborn, W. E.; Hobbs, J. N. *Antimicrob. Agents Ch.* **1991**, *35*, 2639-2642.
43. Tally, F. P.; Zeckel, M.; Wasilewski, M. M.; Carini, C.; Berman, C. L.; Drusano, G. L.; Oleson, F. B., Jr. *Expert opinion investig. drugs* **1999**, *8*, 1223-1238.
44. Carpenter, C. F.; Chambers, H. F. *Clin. Infect. Dis.* **2004**, *38*, 994-1000.
45. Jones, T. S. *Annals New York Acad. Sci.* **1949**, *51*, 909-916.
46. Hancock, R. E. W. *Trends Microbiol.* **1997**, *5*, 37-42.
47. Morrison, D. C.; Jacobs, D. M. *Immunochemistry* **1976**, *13*, 813-818; Velkov, T.; Thompson, P. E.; Nation, R. L.; Li, J. *J. Med. Chem.* **2010**, *53*, 1898-1916.
48. Groisman, E. A.; Kayser, J.; Soncini, F. C. *J. Bacteriol.* **1997**, *179*, 7040-7045.
49. Rifkind, D. J. *Bacteriol.* **1967**, *93*, 1463; Warren, H. S.; Kania, S. A.; Siber, G. R. *Antimicrob. Agents Ch.* **1985**, *28*, 107-112.
50. Landman, D.; Georgescu, C.; Martin, D. A.; Quale, J. *Clin. Microbiol. Rev.* **2008**, *21*, 449-465.
51. Bergen, P. J.; Li, J.; Rayner, C. R.; Nation, R. L. *Antimicrob. Agents Chemother.* **2006**, *50*, 1953-1958.
52. Pamp, S. J.; Gjermansen, M.; Johansen, H. K.; Tolker-Nielsen, T. *Mol. Microbiol.* **2008**, *68*, 223-240.
53. Li, J.; Nation, R. L.; Turnidge, J. D.; Milne, R. W.; Coulthard, K.; Rayner, C. R.; Paterson, D. L. *The Lancet. Infectious diseases* **2006**, *6*, 589-601; Li, J.; Rayner, C. R.; Nation, R. L.; Owen, R. J.; Spelman, D.; Tan, K. E.; Liolios, L. *Antimicrob. Agents Chemother.* **2006**, *50*, 2946-2950.
54. Hase, S.; Rietschel, E. T. *Eur. J. Biochem.* **1977**, *75*, 23-34; Sidorczyk, Z.; Zahringer, U.; Rietschel, E. T. *Eur. J. Biochem.* **1983**, *137*, 15-22.
55. Bruch, M. D.; Cajal, Y.; Koh, J. T.; Jain, M. K. *J. Am. Chem. Soc.* **1999**, *121*, 11993-12004; Meredith, J. J.; Dufour, A.; Bruch, M. D. *J. Phys. Chem. B* **2009**, *113*, 544-551; Pristovsek, P.; Kidric, J. *J. Med. Chem.* **1999**, *42*, 4604-4613; Pristovsek, P.; Kidric, J. *Curr. Top. Med. Chem.* **2004**, *4*, 1185-1201.
56. Clausell, A.; Garcia-Subirats, M.; Pujol, M.; Busquets, M. A.; Rabanal, F.; Cajal, Y. *J. Phys. Chem. B* **2007**, *111*, 551-563.
57. Vaara, M.; Fox, J.; Loidl, G.; Siikanen, O.; Apajalahti, J.; Hansen, F.; Fridmodt-Moller, N.; Nagai, J.; Takano, M.; Vaara, T. *Antimicrobial. Agents Chemother.* **2008**, *52*, 3229-3236; Vaara, M.; Vaara, T. *Peptides* **2010**, *31*, 2318-2321; Vaara, M. *Curr. Opin Microbiol.* **2010**, *13*, 574-581.
58. Mingeot-Leclercq, M. P.; Tulkens, P. M.; Denamur, S.; Vaara, T.; Vaara, M. *Peptides* **2012**, *35*, 248-252.
59. Kato, T.; Shoji, J. *J. Antibiot.* **1976**, *29*, 1339-1340; Meyers, E.; Parker, W. L.; Brown, W. E. *J. Antibiot.* **1976**, *29*, 1241-1242.
60. Terabe, S.; Konaka, R.; Shoji, J. *Chromatography A* **1979**, *173*, 313-320; Sugawara, K.; Yonemoto, T.; Konishi, M.; Matsumoto, K.; Miyaki, T.; Kawaguchi, H. *J. Antibiot.* **1983**, *36*, 634-638; Kato, T.; Shoji, T. *J. Antibiot.* **1980**, *33*, 186-191.
61. Qian, C. D.; Wu, X. C.; Teng, Y.; Zhao, W. P.; Li, O.; Fang, S. G.; Huang, Z. H.; Gao, H. C. *Antimicrob. Agents Chemother.* **2012**, *56*, 1458-1465.
62. De Zoysa, G. H.; Cameron, A. J.; Hegde, V. V.; Raghobama, S.; Sarojini, V. *J. Med. Chem.* **2015**, *58*, 625-639.
63. Jelinek, R.; Kolusheva, S. *Top. Curr. Chem.* **2007**, *277*, 155-180.
64. Rangin, M.; Basu, A. *J. Am. Chem. Soc.* **2004**, *126*, 5038-5039.
65. Kolusheva, S.; Boyer, L.; Jelinek, R. *Nat. Biotechnol.* **2000**, *18*, 225-227.
66. Cameron, A.; De Zoysa, G. H.; Sarojini, V. *Biopolymers* **2014**, *102*, 88-96.
67. Howell, S. F. *J. Biol. Chem.* **1950**, *186*, 863-877.
68. Liu, R. F.; Zhang, D. J.; Li, Y. G.; Tao, L. M.; Tian, L. *Helv. Chim. Acta* **2010**, *93*, 2419-2425.
69. Nyfeler, R.; Keller-Schierlein, W. *Helv. Chim. Acta* **1974**, *57*, 2459-2477.
70. Peypoux, F.; Guinand, M.; Michel, G.; Delcambe, L.; Das, B. C.; Lederer, E. *Biochemistry-US* **1978**, *17*, 3992-3996.
71. Peypoux, F.; Pommier, M. T.; Das, B. C.; Besson, F.; Delcambe, L.; Michel, G. *J. Antibiot.* **1984**, *37*, 1600-1604.
72. Konishi, M.; Sugawara, K.; Hanada, M.; Tomita, K.; Tomatsu, K.; Miyaki, T.; Kawaguchi, H.; Buck, R. E.; More, C.; Rossomano, V. Z. *J. Antibiot.* **1984**, *37*, 949-957.
73. Hashizume, H.; Igarashi, M.; Hattori, S.; Hori, M.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **2001**, *54*, 1054-1059.
74. Kajimura, Y.; Kaneda, M. *J. Antibiot.* **1996**, *49*, 129-135.
75. Desjardine, K.; Pereira, A.; Wright, H.; Matainaho, T.; Kelly, M.; Andersen, R. J. *J. Nat. Prod.* **2007**, *70*, 1850-1853.
76. Balunas, M. J.; Lington, R. G.; Tidgewell, K.; Fenner, A. M.; Urena, L. D.; Della Togna, G.; Kyle, D. E.; Gerwick, W. H. *J. Nat. Prod.* **2010**, *73*, 60-66.

# Spectroscopic studies of oxide interfaces in aquatic geochemical systems

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## Setting the scene

Geochemistry has been described as the study of the chemical forces that determine the distribution of elements in the Earth.<sup>1</sup> Even though 95 % of the volume of the Earth's crust is comprised of close-packed oxygen ions, there is a rich diversity of minerals based on the variety of cations and the numerous ways these cations can be arranged within the oxygen ion lattice. Silicon is the main cation with a crustal abundance of 28 % (by mass) followed by Al, Fe, Ca, Mg, Na, and K with crustal abundance in mass % of 8.4, 5.1, 4.6, 2.7, 2.4 and 1.6 respectively.<sup>2</sup> Carbon, which is so prominent in so much chemistry, doesn't even make the 1<sup>st</sup> XV in crustal abundance.

Silicate phases make up 90 % of the Earth's crust and, with few exceptions, contain SiO<sub>4</sub> tetrahedra. These tetrahedra exist with varying degrees of polymerisation via Si-O-Si linkages where a given Si is designated as Q<sup>n</sup> where n is the number of Si-O-Si linkages present. Silicate minerals range from having isolated SiO<sub>4</sub><sup>4-</sup> anions, the Q<sup>0</sup> orthosilicates, to those with polymerisation in three dimensions, the Q<sup>4</sup> tectosilicates. The silicon-oxygen bond has unusual characteristics and warrants some introduction. Unlike the ubiquitous ketones in carbon chemistry, the first silanone compound (i.e. with a Si=O) was only recently reported.<sup>3</sup> The instability of Si=O explains the marked difference in the properties of CO<sub>2</sub> and SiO<sub>2</sub>. The Si-O single bond has been subject to much historical debate<sup>4</sup> and even for the simple molecule of disiloxane (H<sub>3</sub>Si-O-SiH<sub>3</sub>) a full explanation for the anomalous basicity compared to its carbon analogue, dimethyl ether, was only recently reported and illustrates some interesting chemistry.<sup>5</sup> For H<sub>3</sub>X-O-XH<sub>3</sub>, the lower electronegativity of Si than C explains the larger X-O-X bond angle when X = Si, as expected from Bent's rule whereby less electronegative substituents bind to hybrid orbitals with less p character. However, the lower basicity of the oxygen in H<sub>3</sub>S-O-SiH<sub>3</sub> than H<sub>3</sub>C-O-CH<sub>3</sub> is unexpected from the difference in electronegativity but arises because the larger X-O-X bond angle for X = Si enhances vicinal hyperconjugation which removes electron density from the oxygen.<sup>5</sup>

Geochemistry covers extremely diverse conditions and processes that occur on phenomenal scales in time and space. In the fiery bowels of the Earth, the formation of assemblages of minerals (i.e. rocks) occurs as magma cools and the distribution of the elements depend on subtle chemistry relating to the stability and miscibility of phases at high temperatures and pressures. The mountain tops are the other extreme of both temperature and the life cycle of rock. Tectonic forces thrust rocks up into the weather where they meet their fate under

the influences of O<sub>2</sub> and water which, by a combination of physical, chemical and biological weathering, eventually converts rock to soils. Subduction returns weathered surface material to the furnace and completes the rock cycle process.

## Weathering, iron oxides and H<sub>4</sub>SiO<sub>4</sub>

The distribution of elements during weathering of minerals is determined by the aqueous solubility of the cations. Of the dominant cations in the crust, the Group I and II cations are extremely soluble, while exposure to 0.2 atm of O<sub>2</sub> results in the Fe<sup>2+</sup> cation in silicate minerals being oxidised to Fe<sup>3+</sup>. In water at pH 7 and at 25 °C, the solubility of Si<sup>4+</sup> (as H<sub>4</sub>SiO<sub>4</sub> or silicic acid) with respect to an amorphous SiO<sub>2</sub> phase is 1.8 mM while the solubilities of Al<sup>3+</sup> and Fe<sup>3+</sup> with respect to amorphous oxyhydroxide phases are respectively 7.9 mM and 0.28 nM.<sup>6</sup> Therefore, the weathering process ultimately converts silicate minerals to the oxyhydroxides of Al<sup>3+</sup> and Fe<sup>3+</sup> with the aluminosilicate clays being intermediate phases. Given the economic importance of iron and aluminium oxides, the difference in the New Zealand and Australian economies is in some ways a difference in the extent of weathering reactions.

The iron oxide phases give the weathered solids of the Earth (i.e. the soils and sediments, their characteristic brown, yellow and red colours). In addition, the iron oxides often play an important role in the aqueous geochemistry of many cationic, anionic, and neutral species, including many environmentally important trace elements. This was elegantly demonstrated by John Aggett at the University of Auckland who determined the phase association of arsenic accumulated on lake sediments using an EDTA solution to cause the slow dissolution of the metal oxide phases in the sediment over 48 h.<sup>7</sup> A comparison of the dissolution rates of As and the major solid phase cations (Fe, Al, Mn and Ca) demonstrated a clear phase association of the solid phase As with the iron oxides in the sediment. This was one of the early definitive indicators of the importance of iron oxides in determining arsenic mobility in aquatic systems which has far reaching implications for global population health.<sup>8</sup> In general, it is found that anions which are the conjugate bases of strong acids (such as nitrate and chloride) have a low affinity for metal oxides, while weak acids and their conjugate bases, such as H<sub>4</sub>SiO<sub>4</sub>, HAsO<sub>4</sub><sup>2-</sup> and HPO<sub>4</sub><sup>2-</sup>, have a high affinity for metal oxide surfaces.

Silicic acid derives from the weathering reactions which produce the iron oxides and typical concentrations of H<sub>4</sub>SiO<sub>4</sub> in aqueous systems (≈ 0.1 to 1 mM) are higher

than those of many other adsorbing ligands. For this reason iron oxides in weathered systems can contain large amounts of silicon.<sup>9</sup> The presence of adsorbed  $\text{H}_4\text{SiO}_4$  affects many aspects of the geochemistry of iron oxides including the Point of Zero Charge,<sup>10</sup> phase stability, morphology,<sup>11-13</sup> and colloid stability.<sup>14</sup> Understanding the chemistry of  $\text{H}_4\text{SiO}_4$  on iron oxide surfaces is therefore important in numerous systems including catalytic supports,<sup>15</sup> corrosion science,<sup>16</sup> and wastewater treatment,<sup>17</sup> in addition to many natural aquatic systems.<sup>18</sup>

Before discussing the *in situ* infrared spectroscopy of  $\text{H}_4\text{SiO}_4$  chemistry at the aqueous-iron oxide interface it is necessary to consider the  $\text{H}_4\text{SiO}_4$  species in aqueous solution. A strong feature of  $\text{H}_4\text{SiO}_4$  chemistry in aquatic systems is a high propensity for polymerisation via Si-O-Si linkages. In concentrated silicate solutions at high pH a total of 48 different silicate anions are identified and range from isolated monomers to a complex array of cyclic and cage structures.<sup>19</sup> Early spectroscopic and potentiometric studies indicated that oligomeric silicates were significant species in the aqueous chemistry of  $\text{H}_4\text{SiO}_4$  at neutral pH<sup>20,21</sup> but more recent work has shown that monomeric  $\text{H}_4\text{SiO}_4$  is more than 99 % of the total amount of Si in solution at neutral pH.<sup>22</sup> Another important aspect of  $\text{H}_4\text{SiO}_4$  aquatic chemistry is the limit on solubility. Quartz is the least soluble  $\text{SiO}_2$  phase with a solubility product ( $K_{sp}$ ) of  $10^{-4}$  meaning that a system with 0.1 mM  $\text{H}_4\text{SiO}_4$  is saturated with respect to quartz. However, quartz does not precipitate at ambient temperatures for kinetic reasons in aquatic systems and  $\text{H}_4\text{SiO}_4$  solubility is limited to 1.8 mM by precipitation of an amorphous  $\text{SiO}_2$  phase.

Infrared spectroscopy is particularly useful for the study of  $\text{H}_4\text{SiO}_4$  chemistry on the surfaces of iron oxides because the shape of the Si-O stretching modes,  $\nu(\text{Si-O})$ , for the sorbed  $\text{H}_4\text{SiO}_4$  provides insight on the symmetry of the surface complex<sup>23</sup> while the position of the Si-O stretching modes provides insight on the degree of silicate polymerisation.<sup>24,25</sup> However, water is a very strong absorber of IR radiation and the applicability of IR to the study of the metal oxide-water interface has been dramatically improved by the advent of Attenuated Total Reflectance IR (ATRIR) which provides a sampling method with the required short path length.

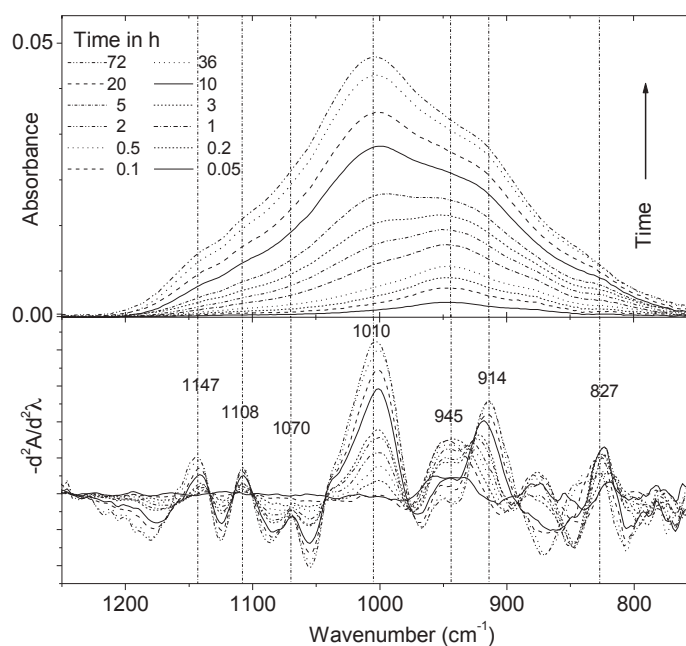
### Some ATRIR spectra

The most prominent feature in the ATRIR spectrum of  $\text{H}_4\text{SiO}_4$  in solution is a symmetrical band at  $939\text{ cm}^{-1}$  due to the asymmetric Si-O stretching mode. There is also a weak and broad Si-O-H bending mode at  $1100\text{ cm}^{-1}$  while the O-Si-O bending modes occur below the wavelength limit of the ATR crystal and the O-H stretching modes are not apparent due to the strong absorbance by  $\text{H}_2\text{O}$ . In the Raman spectrum of  $\text{H}_4\text{SiO}_4$  in solution there is one band at  $787\text{ cm}^{-1}$  due to the symmetric Si-O stretching mode. An interesting feature

of these spectra is the shift in band position upon deuteration. For  $\text{D}_4\text{SiO}_4$  in  $\text{D}_2\text{O}$  the symmetrical Si-O stretch shifts to lower frequency as expected from Hooke's law and the increase in mass given that the H(D) is displaced with the oxygen in the stretching modes. However, the frequency of the asymmetrical stretch in the ATRIR spectrum makes a counter-intuitive shift to higher frequency.<sup>26</sup> A computational study to decipher the reasons for this counterintuitive shift revealed that deuteration caused the Si-O-D bending modes to shift to lower frequency allowing them to couple with the Si-O stretching mode. This coupling is responsible for the unexpected blue shift of the stretching modes.<sup>26</sup>

To measure the *in situ* infrared spectra of  $\text{H}_4\text{SiO}_4$  chemistry at the aqueous-iron oxide interface, a few microliters of an iron oxide suspension is deposited on the ATRIR crystal. The water is allowed to dry and the oxide phase adheres to the surface of the crystal and a flow cell is clamped over the crystal. Initially water at pH 11 is pumped through the flow cell which causes any carbonate on the oxides surface to desorb as evidenced by negative peaks at  $1350$  and  $1467\text{ cm}^{-1}$  that grow over time until all carbonate has been removed. After this time a NaCl electrolyte solution at the desired pH and ionic strength is pumped through the cell to equilibrate the oxide under the desired set of conditions. A background ATRIR spectrum is recorded and then the desired concentration of  $\text{H}_4\text{SiO}_4$  is added to the electrolyte which is pumped over the oxide and ATRIR spectra are recorded over time as the oxide reacts with the  $\text{H}_4\text{SiO}_4$ .

Typical spectra demonstrating the main features of the system are shown in Fig. 1 for a system where the poorly ordered iron oxide ferrihydrite reacts with  $0.91\text{ mM}$   $\text{H}_4\text{SiO}_4$  at pH 4. The absorbance in the  $\nu(\text{Si-O})$  region of the spectra increases over time as the surface coverage



**Fig 1.** Top: The ATRIR spectra measured over time as  $0.91\text{ mM}$   $\text{H}_4\text{SiO}_4$  in  $0.01\text{M}$  NaCl at pH 4 reacts with a ferrihydrite film. Bottom: The negative second derivatives (Savitsky-Golay 2<sup>nd</sup> derivatives, 15 point, order 3) of the spectra in the upper panel.

(termed  $G_{Si}$ ) increases but even after 3 minutes of reaction time the absorbance in the  $n(\text{Si-O})$  region of the spectrum is  $> 20$  times larger than the IR absorbance of the  $\text{H}_4\text{SiO}_4$  in solution. This occurs because the ferrihydrite film on the ATRIR crystal surface is located in the few micrometres of the IR attenuating beam and demonstrates the concentration of  $\text{H}_4\text{SiO}_4$  associated with the ferrihydrite surface compared to the same volume of water. The spectra collected immediately after the introduction of  $\text{H}_4\text{SiO}_4$  at low  $G_{Si}$  had a maximum at  $\sim 945 \text{ cm}^{-1}$  with a shoulder at  $\sim 880 \text{ cm}^{-1}$  (clearly seen in the second derivatives of these spectra). The position and shape of the spectra with  $\text{H}_4\text{SiO}_4$  on ferrihydrite at low  $G_{Si}$  is indicative of a monomeric adsorbed silicic acid species. Compared to the IR spectra of  $\text{H}_4\text{SiO}_4$  in solution with one  $n(\text{Si-O})$  band at  $939 \text{ cm}^{-1}$ , the  $\text{H}_4\text{SiO}_4$  symmetry is lowered by adsorption and spectra are consistent with a bidentate mode of attachment where the  $\text{SiO}_4$  tetrahedra share corners with two edge-sharing Fe octahedra (Fig. 2). This is also consistent with Fe K-edge extended X-ray absorption fine structure studies of Fe(III) hydrolysed in the presence of  $\text{H}_4\text{SiO}_4$ .<sup>27</sup>

As the  $G_{Si}$  on ferrihydrite increases an ATRIR band at  $1010 \text{ cm}^{-1}$  grows and becomes the dominant feature in the spectra. In the second derivatives of these spectra there are bands located at 827, 914, 1070, 1108 and  $1147 \text{ cm}^{-1}$  that are associated with the main feature at  $1010 \text{ cm}^{-1}$ . The shift in the (Si-O) to higher wavenumber as  $G_{Si}$  increases is indicative of  $\text{H}_4\text{SiO}_4$  polymerisation. However, the regular position of the band maxima in the second derivative spectra over time is quite striking and indicates that there is a single silicate species that develops as surface coverage increases and dominates the surface chemistry at high surface coverage. Comparing the shape of the IR spectra of  $\text{H}_4\text{SiO}_4$  on ferrihydrite at high  $G_{Si}$  to the IR spectra from silicate minerals with known anion structures provides some constraints on the structure of this polymerised interfacial silicate. Silicate minerals that have dimeric silicate anions ( $\text{Si}_2\text{O}_7^{6-}$ ) have only one  $n(\text{Si-O})$  band at  $> 1000 \text{ cm}^{-1}$  which is due to the bridging Si-O-Si band. In more condensed silicates, the proportion of bridging Si-O-Si bonds increases at the expense of non-bridging Si-O bonds, the number of Si-O-Si stretching modes at  $>1000 \text{ cm}^{-1}$  increases, and the maximum absorbance shifts to higher wavenumber. Cyclic silicates have been proposed as possible oligomeric species on iron oxide surfaces but they have strongly absorbing IR ring deformation modes between  $\sim 740$  and  $600 \text{ cm}^{-1}$  that were not observed in this work. Silicates with polymerisation occurring in three dimensions have  $n(\text{Si-O})$  modes at  $\sim 1100 \text{ cm}^{-1}$  such as quartz and the amorphous  $\text{SiO}_{2(\text{am})}$  phase with their strongest bands at 1090 and  $1110 \text{ cm}^{-1}$ , respectively. The formation of a specific silicate oligomer on the ferrihydrite could be rationalised if the arrangement of adsorption sites on the ferrihydrite caused adjacent sorbed  $\text{H}_4\text{SiO}_4$  monomers to be held in an orientation that is conducive to the formation of a condensed silicate species. All data are consistent with a model whereby a linear trimeric silicate species is formed on the iron oxide surface by the insertion of a  $\text{H}_4\text{SiO}_4$  molecule from the solution phase between two suitably orientated adjacent

sorbed monomers as illustrated in Fig. 2.

### Solid state $^{29}\text{Si}$ NMR spectra

Solid state  $^{29}\text{Si}$  NMR is a useful tool in the study of the structure of silicates because Si with different  $Q^n$  have distinct chemical shifts. Typical  $^{29}\text{Si}$  shifts are  $-70 \pm 4 \text{ ppm}$  for isolated  $\text{SiO}_4$  tetrahedra ( $Q^0$ ),  $-80 \pm 3 \text{ ppm}$  for  $Q^1$  such as dimers or the ends of linear chains,  $-87 \pm 1 \text{ ppm}$  for  $Q^2$  such as in single silicate chains,  $-98 \pm 1 \text{ ppm}$  for  $Q^3$  such as in silicate sheets and lastly  $-109 \pm 2 \text{ ppm}$  for  $Q^4$ .<sup>28</sup> However, the iron oxides are not amenable to NMR studies so an X-ray amorphous  $\text{TiO}_2$  phase was produced by the hydrolysis of  $\text{Ti}(\text{OEt})_4$ .<sup>29</sup> The  $\text{TiO}_2$  and ferrihydrite phases are both composed of particles of approximately 2 nm diameter with very little long range order. The ATRIR spectra of  $\text{H}_4\text{SiO}_4$  reacting on the  $\text{TiO}_2$  surface showed the same progression from monomeric sorbed  $\text{H}_4\text{SiO}_4$  to a polymerised species with the same spectral features as observed on the ferrihydrite.

A sample of  $\text{TiO}_2$  was prepared with high  $G_{Si}$  using  $\text{H}_4\text{SiO}_4$  solution prepared from  $^{29}\text{Si}$ . The main peak in the NMR spectrum of this sample (Fig. 3) occurs at  $-87 \text{ ppm}$  and there are clearly shoulders on either side of this peak, at  $-79.8$  and  $-95.7 \text{ ppm}$ . There is a low intensity tail extending on the more negative side of the peak at  $-105 \text{ ppm}$ . The  $^{29}\text{Si}$  NMR peak position for a  $Q^n$  Si depends primarily on the value of  $n$  but for Si with the same  $Q^n$  the peak position becomes more negative as the charge densities of the cations to which the silicates are associated increases.<sup>30,31</sup> Therefore to assign the peaks in the spectrum it is necessary to refer to materials where silicates are coordinated with  $\text{Ti}^{4+}$  cations. The peak at  $-79.8 \text{ ppm}$  is within the range observed for  $Q^0$  Si including the orthosilicate mineral titanite  $\text{CaTiSiO}_5$  ( $-79.6 \text{ ppm}$ ). Similarly the peak at  $-95.7 \text{ ppm}$  is within the range for  $Q^2$  Si in sol-gel prepared mixed  $\text{TiO}_2$ - $\text{SiO}_2$  oxides ( $-92$  to  $-96 \text{ ppm}$ )<sup>32,33</sup> and in the cyclosilicate mineral benitoite ( $-94.2$ ).<sup>30</sup> Only one reference value for  $Q^1$  Si was found which was for a mixed  $\text{TiO}_2$ - $\text{SiO}_2$  oxide ( $-84 \text{ ppm}$ )<sup>32</sup> and it is clear that the peak at  $-87 \text{ ppm}$  is  $Q^1$ . The tail at  $-105 \text{ ppm}$  indicates a small amount of silicate with  $Q^3$ - $Q^4$  but the peak is too weak to allow for definitive peak fitting. The NMR spectrum qualitatively agrees with the proposed model for  $\text{H}_4\text{SiO}_4$  polymerisation at an oxide surface. The  $Q^0$  peak corresponds to the monomeric adsorbed  $\text{H}_4\text{SiO}_4$ , while the  $Q^1$  and  $Q^2$  peaks correspond to terminal and middle Si in a linear silicate oligomer. The ratio of  $Q^1$  and  $Q^2$  peak areas would be 1:0.5 for a linear trimer which is in reasonable agreement with the measured value of 1:0.4.

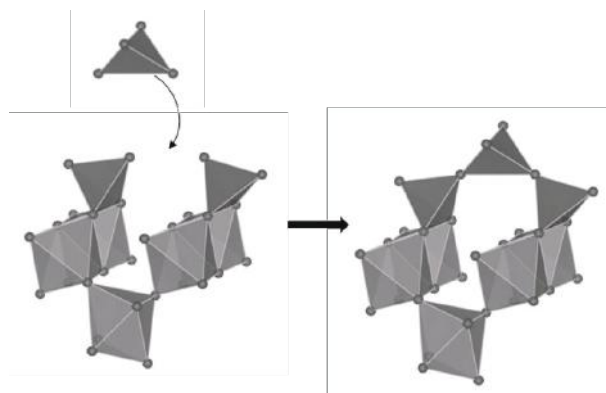
### Structural interpretation

The only condensed silicate species that is consistent with the ATRIR and  $^{29}\text{Si}$  NMR spectra is a linear trimeric species. Furthermore the very similar ATRIR spectra for  $\text{H}_4\text{SiO}_4$  condensation of ferrihydrite and amorphous  $\text{TiO}_2$  suggests that the same silicate oligomerisation product is formed on the surface of two different poorly ordered oxides. This implies that this is a general phenomenon whereby bidentate silicate monomers on an oxide surface are disposed towards forming linear trimers by condensation reactions involving two terminal Si-OH groups.

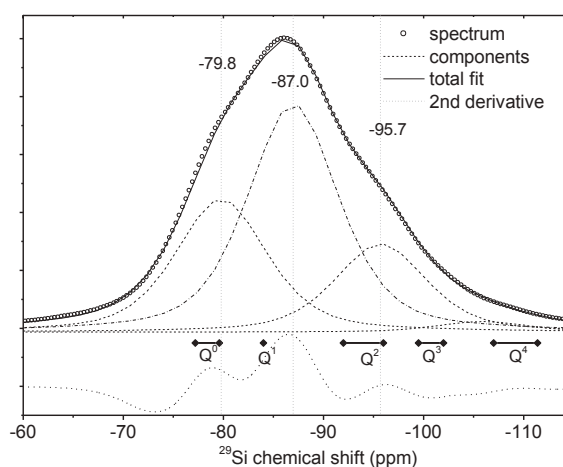
In this section we demonstrate that this mechanism is structurally reasonable based on geometric constraints of silicate polymerisation and the structural model for amorphous  $\text{TiO}_2$  that was developed by Zhang *et al.*<sup>29</sup> and for which the atomic coordinates were kindly provided.

In this structural model the  $\text{TiO}_2$  particles contain 123 Ti atoms, 246 O atoms and have an irregular “potato” shape. The particles have a diameter of 2 nm with a small strained “anatase-like” core and a highly distorted shell. The average Ti coordination number was 5.3 due to the truncation of the many  $\text{TiO}_6$  octahedra on the particle surface. In an aqueous suspension the surface will have a large number of terminal Ti–OH groups and there were 67 Ti ions on the particle surface that had a coordination number less than six and these Ti ions were considered to be surface active. The geometric constraints for surface silicate species were based on the range of Si–O bond distances and angles observed in silicate minerals. Liebau<sup>34</sup> gives Si–O distances between 1.57 and 1.72 Å, O–Si–O angles between 98 and 122° and Si–O–Si angles between 120 and 180°. The average distance between nearest neighbour surface Ti atoms is  $3.1 \pm 0.2$  Å and 64 of the 67 surface active Ti ions have an adjacent site sufficiently close to be bridged by a  $\text{H}_4\text{SiO}_4$  tetrahedra. To form a linear trimeric silicate requires adjacent monomers with an appropriate distance and orientation for insertion of a molecule of  $\text{H}_4\text{SiO}_4$  in solution. Based on geometric constraints 54 of the surface Ti ions could be part of a site for linear trimer formation. This model of the surface is depicted in Fig. 4 which shows trimers can form on most of the particle surface. From this argument it would appear to be reasonable that oligomers can dominate the surface at high  $G_{\text{Si}}$ .

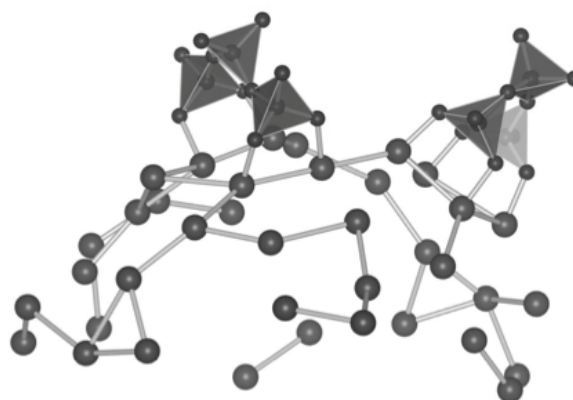
Finally, we discuss the observation that the  $\text{TiO}_2$  surface is not predisposed to the formation of three dimensional polymers. Fig. 4 shows the relationship between adjacent adsorbed trimers. It is clear that adjacent trimers are orientated away from each other because of the high curvature of the surface of the small diameter  $\text{TiO}_2$  particle. The distance between terminal hydroxide groups on the adjacent trimers range from 8 to 12 Å. This is too large to be bridged by a  $\text{H}_4\text{SiO}_4$  molecule in solution and this prevents the formation of higher order silicate polymers. The similarity in the  $\text{H}_4\text{SiO}_4$  oligomerisation chemistry on the poorly ordered oxides of both  $\text{Ti}^{\text{IV}}$  and  $\text{Fe}^{\text{III}}$  suggests the proposed interpretation of silicate oligomerisation may be more generally applicable to disordered surfaces, such as ferrihydrite. From simple geometric arguments nanometer sized oxide particles will have both a high curvature and also a high surface concentration of coordinatively unsaturated cations that will produce surface hydroxyl groups in water. These are the basic features of the proposed silicate oligomerisation model. While various structural models for ferrihydrite have been proposed and refuted, the  $\text{TiO}_{2(\text{am})}$  model indicates that nanometer sized particles with crystalline cores will tend to have a highly strained and disordered surface because of the constraints of high surface curvature. The primary particle size of ferrihydrite is 2–3 nm which is comparable to that of  $\text{TiO}_{2(\text{am})}$ . In addition, the proposed concentra-



**Fig 2.** Model for the formation of surface oligomeric silicate species on an iron oxide surface. Tetrahedra are  $\text{SiO}_4$  and octahedra are  $\text{FeO}_6$ .



**Fig 3.** The  $^{29}\text{Si}$  solid state NMR spectrum of  $\text{H}_4^{29}\text{SiO}_4$  on  $\text{TiO}_2$  at high  $G_{\text{Si}}$ . Reference values are for mineralogical samples and  $\text{TiO}_2$ - $\text{SiO}_2$  mixed phases.



**Fig. 4.** The arrangement of Ti ions with coordination number < 6 on the surface of a  $\text{TiO}_{2(\text{am})}$  particle. Just the top half of the particle is shown. Lines between spheres depict sites where adjacent Ti ions are sufficiently close to allow bidentate coordination to  $\text{SiO}_4$  tetrahedra. The tetrahedra in the figure depict two adjacent interfacial silicate trimers.

tion of monomer adsorption sites are comparable on the two oxides i.e. 0.26 per Ti for  $\text{TiO}_{2(\text{am})}$  and 0.2 per Fe for ferrihydrite. These structural and morphological similarities may explain the similar silicate surface chemistry on the ferrihydrite and  $\text{TiO}_{2(\text{am})}$ .

## References

1. Goldschmidt, V. M. *Nature* **1929**, *124*, 15-17.
2. Rudnick, R. L.; Fountain, D. M. *Rev. Geophys.* **1995**, *33*, 267-309.
3. Filippou, A. C.; Baars, B.; Chernov, O.; Lebedev, Y. N.; Schnakenburg, G. *Angew. Chem. Int. Ed.* **2014**, *53*, 565-570.
4. Pauling, L. *Am. Miner.* **1980**, *65*, 321-323.
5. Weinhold, F.; West, R. *Organometallics* **2011**, *30*, 5815-5824.
6. Gustafsson, J. P. *Geoderma* **2006**, *136*, 320-330.
7. Aggett, J.; Roberts, L. S. *Env. Sci. Tech.* **1986**, *20*, 183-186.
8. Smith, A. H.; Lingas, E. O.; Rahmen, M. *Bull. WHO* **2000**, *78*, 1093-1103.
9. Carlson, L.; Schwertmann, U. *Geochim. Cosmochim. Acta* **1981**, *45*, 421-425.
10. Schwertmann, U.; Fechter, H. *Clay Min.* **1982**, *17*, 471-476.
11. Cornell, R. M.; Giovanoli, R. *J. Chem. Soc. Chem. Comm.* **1987**, *6*, 413-414.
12. Cornell, R. M.; Giovanoli, R.; Schindler, P. W. *Clays Clay Min.* **1987**, *35*, 21-28.
13. Kwon, S.-K.; Shinoda, K.; Suzuki, S.; Waseda, Y. *Corrosion Sci.* **2007**, *49*, 1513-1526.
14. Davis, C. C.; Knocke, W. R.; Edwards, M. *Env. Sci. Tech.* **2001**, *35*, 3158-3162.
15. Corrias, A.; Ennas, G.; Mountjoy, G.; Paschina, G. *Phys. Chem. Chem. Phys.* **2000**, *2*, 1045-1050.
16. Kwon, S. K.; Kimijima, K.; Kanie, K.; Suzuki, S.; Muramatsu, A.; Saito, M.; Shinoda, K.; Waseda, Y. *Corrosion Sci.* **2007**, *49*, 2946-2961.
17. Davis, C. C.; Chen, H.-W.; Edwards, M. *Env. Sci. Tech.* **2002**, *36*, 582-587.
18. Swedlund, P. J.; Sivaloganathan, S.; Miskelly, G. M.; Waterhouse, G. I. N. *Chem. Geol.* **2011**, *285*, 62-69.
19. Knight, C. T. G.; Balec, R. J.; Kinrade, S. D. *Angew. Chem. Int.* **2007**, *46*, 8148-8152.
20. Alvarez, R.; Sparks, D. L. *Nature* **1985**, *318*, 649-651.
21. Svensson, I. L.; Sjoberg, S.; Ohman, L. O. *J. Chem. Soc. Faraday Trans.* **1986**, *82*, 3635-3646.
22. Felmy, A. R.; Cho, H.; Rustad, J. R.; Mason, M. J. *J. Solution Chem.* **2001**, *30*, 509-525.
23. Peak, D.; Ford, R. G.; Sparks, D. L. *J. Colloid Interface Sci.* **1999**, *218*, 289-299.
24. Farmer, V. C. In *Infrared Spectra of Minerals*; Farmer, V. C. Ed.; Mineralogical Society, London, Monograph No. 4, 1974.
25. Lazarev, A. N. *Vibrational spectra and structure of silicates*; Consultants Bureau: New York, 1972.
26. McIntosh, G. J.; Swedlund, P. J.; Sohnel, T. *Phys. Chem. Chem. Phys.* **2011**, *13*, 2314-2322.
27. Pokrovski, G. S.; Schott, J.; Garges, F.; Hazemann, J. L. *Geochim. Cosmochim. Acta* **2003**, *67*, 3559-3573.
28. Lippmaa, E.; Maegi, M.; Samoson, A.; Engelhardt, G.; Grimmer, A. R. *J. Am. Chem. Soc.* **1980**, *102*, 4889-4893.
29. Zhang, H. Z.; Chen, B.; Banfield, J. F.; Waychunas, G. A. *Phys. Rev. B* **2008**, *78*, 214106.
30. Stebbins, J. F. In *Mineral physics & crystallography: a handbook of physical constants*; Ahrens, T. J. Ed.; American Geophysical Union: Washington DC, 1995.
31. Stebbins, J. F. *MRS Bulletin* **1992**, *17*, 45-52.
32. Dirken, P. J.; Smith, M. E.; Whitfield, H. J. *J. Phys. Chem.* **1995**, *99*, 395-401.
33. Tada, H.; Akazawa, M.; Kubo, Y.; Ito, S. *J. Phys. Chem. B* **1998**, *102*, 6360-6366.
34. Liebau, F. *Structural chemistry of silicates: structure, bonding, and classification*; Springer-Verlag: Berlin, 1985.

## A chemical perspective on the recreational use of geothermal waters

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**Keywords:** geothermal waters, recreation, visitor ratings, temperature, acidity, turbidity

The potential of geothermal waters for tourism and recreation in New Zealand dates back at least to colonial times, with visits by Prince Albert to the Pink and White Terraces in 1870 and by a subsequent visit of the Duke and Duchess of Cornwall to Rotorua in 1901. Public bathing at the Te Aroha baths overtook their private therapeutic use after 1907,<sup>1</sup> and in Rotorua, the opening of the Blue Baths in the 1930s for recreational swimming was incorporated into a Government strategy of the time to encourage tourism (Fig.1).<sup>2,3</sup>

Nearly a hundred natural hot springs and commercial pools using warm waters from geothermal resources that are currently used for recreation in New Zealand have been described,<sup>6</sup> and this compilation is reflected in the NZ Hot Springs website.<sup>7</sup> This website also provides the opportunity for visitors to the 107 springs and pools listed to provide comment and give a rating. Although there is no indication given as to how these ratings are determined, it would be expected that the characteristics of the waters, the ambience of the setting, and the quality of the service at commercial pools, are factors that would influence the rating. As is shown for the currently closed Waingaro Hot Springs, the quality of service can vary considerably over time (Fig. 2), but more usually the ratings tend to show little variation over time (Fig. 3).

A research project by the University of Waikato and GNS Science is undertaking a survey of hot springs and hot pools aimed at determining the microbial and physicochemical diversity of geothermal features, an archive of microbial diversity and genetic potential, and an 'environmental indicator' ('uniqueness' metric prediction), in which the microbial and physicochemical diversity data will be assessed via a set of criteria that ranks ecosystem 'uniqueness'.<sup>8</sup> As a prelude to the microbiological work, the researchers are measuring selected physicochemical properties of the springs and pools they are sampling, viz., temperature, pH, redox potential (Eh), conductivity, dissolved oxygen, and turbidity.<sup>9</sup> The variations between Eh and pH, conductivity and pH, and Eh with temperature are shown for the hot springs and pools in Rotorua's Kuirau Park in Fig. 4, Fig. 5 and Fig. 6, respectively.

There is no obvious correlation between either dissolved oxygen or turbidity with the other physicochemical parameters.

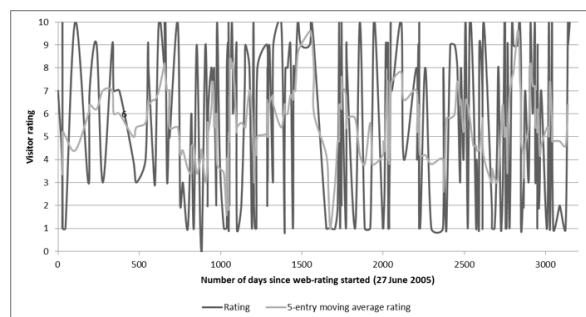
At the time of writing this article about 800 samples had been gathered, although the hot springs and pools for which information is displayed on the 1000 Springs database include comparatively few of those on the Hot Springs website. These are shown in Table 1.

The trends of the physicochemical properties with ratings are given in Table 2. The low correlation coefficients mean that the trends are indicative rather than definitive; but it appears that visitors to the springs and pools prefer waters that are warmer, more basic (higher pH, see Fig. 7), and with low turbidity (see Fig. 8). These trends may be confirmed or countered when additional physicochemical data for fur-

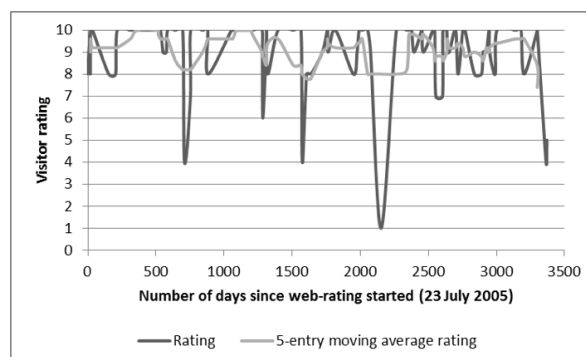
ther hot springs and pools become available from the 1000 Springs Project.



**Fig. 1.** The changing face of tourism: from spectacle to recreation. *Left:* Eruption of the Wairoa Geyser on a booklet cover in 1912 [Photo: Alexander Turnbull Library, Eph-A-TOURISM-Rotorua-1912-02-front].<sup>4</sup> *Right:* Rotorua's newly opened Blue Baths featured on the cover of a 1930s Tourist and Publicity Department booklet [Photo: Alexander Turnbull Library, Eph-A-TOURISM-1930s-02-cover].<sup>5</sup>



**Fig. 2.** Variation in the 348 visitor ratings of Waingaro Springs (near Hamilton) from 27 June 2005 to 8 February 2014



**Fig. 3.** Variation of the 97 visitor ratings for Kerosene Creek (a stream associated with the Waiotapu Geothermal Field, near Rotorua) from 23 July 2005 to 13 October 2014

Table 1. Comparison of hot pool physicochemistry and visitor ratings

System	Location	Feature	Thousand Springs Database						Hot Pools Database		
			Temp, T/°C	Acidity pH	Redox potential Eh/mV	Conductivity $\sigma$ /mS cm <sup>-1</sup>	Dissolved oxygen O <sub>2</sub> /mgL <sup>-1</sup>	Turbidity r/FNU	Average Visitor rating* V	Number of ratings	Spring/Pool
Tikitere	Hells Gate	Tikitere #76 (Hurutini)	42.1	2.24	69.2	3048	40	99.1	6.8	4	Hell's Gate
Rotorua	Kuirau Park	Features 26, 30, 31, 33 <sup>†</sup>	42.8	7.18	-74.4	1702	2.12	13.1	7	6	Kuirau Park Footbaths
Waikite	Waikite Thermal Valley Pools	Feature #5	74.8	8.46	-0.6	1083	2.01	0.9	9.5	63	Waikite Valley Thermal Pools
Waiotapu	Waiotapu	Champagne Pool	74.5	5.29	-222.2	7464	4.93	5.9	8.1	10	Waterfall Spout Bath, Waio-tapu
Wairakei-Tauhara	Spa Park	Otumuheke Stream Feature #1	37.5	7.68	79.2	758	3.13	1.5	7.9	16	Otumuheke Stream, Spa Park
	Wairakei Terraces	Bathing Pool 1-4	39.2	8.44	165.7	7015	4.05	1.5	9.4	5	Wairakei Terraces
	Wairakei Thermal Valley	Feature #2	46.7	6.95	62.1	749	3.84	1.4	9	2	Honeymoon Pool (Te Kiri o Hine Kai Stream)
Turangī	Tokaanu	Features #31, #34 <sup>‡</sup>	33.5	6.05	146.3	4782	2.92	7.4	8.7	47	Tokaanu Thermal Pools

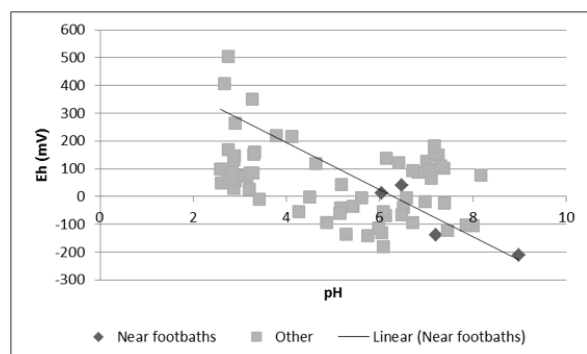
\*Visitor ratings are on a scale of 1 through 10 (10 is best).

<sup>†</sup>The values of the physicochemical properties are averaged over the four features closest to the footbaths<sup>‡</sup>Rating averaged over three pools in streams draining Waiotapu thermal area, considered to be similar to Champagne Pool<sup>¶</sup>Physicochemical properties are averaged over the two pools stated to be in Tokaanu

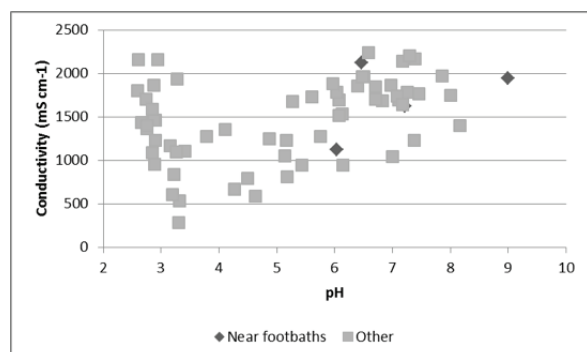
**Table 2.** Linear regression between visitor ratings and physiochemical parameters

Linear regression parameters*	P						
	Temperature (T)	Acidity (pH)	Redox potential (Eh)	Conductivity ( $\sigma$ )	Dissolved oxygen ( $O_2$ )	Turbidity (r)	Log Turbidity (log r)
Slope: M	+5.777	+1.243	+21.993	+662.6	+0.0679	-22.58	-0.533
Intercept: C	0.581	-3.861	-155.8	-2216	2.8071	202.5	5.113
Correlation coefficient	0.133	0.391†	0.0321	0.0608	0.0048	0.465	0.657‡

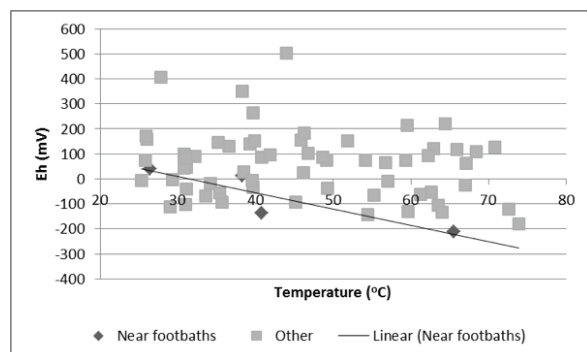
\*For equation  $P = M.V + C$ , where  $V$  is visitor rating; † See Fig. 7; ‡ See Fig. 8



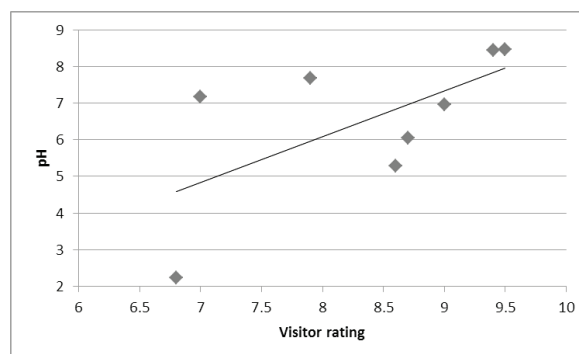
**Fig. 4.** Variation of redox potential, Eh, with pH for hot springs and pools in Kuirau Park, Rotorua. Although a number of reactions contribute to Eh, the data accord with theoretical expectations that Eh decreases with increasing pH



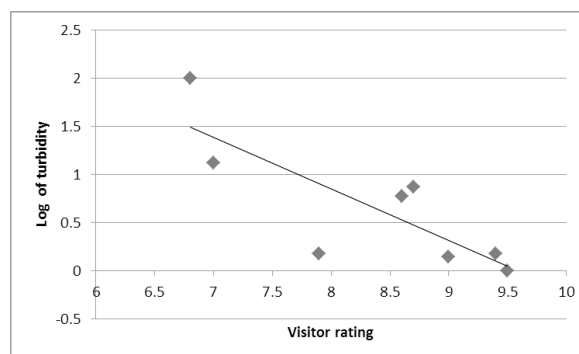
**Fig. 5.** Variation of conductivity with pH for hot springs and pools in Kuirau Park, Rotorua. The V-shaped distribution results from high conductivity at high pH attributed to chlorides in geothermal water rising from some depth, and high conductivity at low pH resulting from near-surface oxidation processes.



**Fig. 6.** Variation of redox potential (Eh) with temperature for hot springs and pools in Kuirau Park, Rotorua. Although the data are 'noisy' there is a trend of decreasing Eh as the temperature increases. This is consistent with the higher temperature water being derived from a deep source.



**Fig. 7.** Variation of pH of hot springs/pools with visitor rating.



**Fig. 8.** Variation of turbidity (as log  $r$ ) of hot springs/pools with visitor rating.

## Notes and References

- Annual attendance at Te Aroha spa, 1904-1909. Data from *Appendices to the Journal of the House of Representatives*; see Hodder, P. *Chemistry in New Zealand* **2014**, 78(4), 158-163.
- Rockel, I. *Taking the Waters: Early Spas in New Zealand*. Government Printer: Wellington, 1986.
- Alsop, P.; Stewart, G.; Bamford, D. *Selling the Dream: The Art of Early New Zealand Tourism*. Craig Potton: Nelson, 2012
- Credit: "Tours and scenes of interest in Geyserland, Rotorua, New Zealand. Wairoa Geyser. Wellington, N.Z., Printed at the Government Printing Office, Wellington, N.Z. [1912]. Tours and scenes of interest in Geyserland, Rotorua, New Zealand. Wellington, N.Z., Printed by John Mackay, Government Printer, 20,000/8/12 - 12111 [1912]. Ref: Eph-A-TOURISM-Rotorua-1912-02-front. Alexander Turnbull Library, Wellington, New Zealand. <http://natlib.govt.nz/records/23248108>"
- Credit: "Mitchell, Leonard Cornwall, 1901-1971. Mitchell, Leonard Cornwall 1901-1971 :Rotorua and New Zealand's thermal wonderland. [1930s].Ref: Eph-A-TOURISM-1930s-02-cover. Alexander Turnbull Library, Wellington, New Zealand.<http://natlib.govt.nz/records/23063926>"
- Jackson, S. *Hot Springs of New Zealand*. Reed: Auckland, 2<sup>nd</sup> edition, 2003.
- Your Guide to all of New Zealand's Thermal Hot Pools and Hot Springs*: <http://www.nzhotpools.co.nz/hot-pools/> (accessed 22 October 2014).
- The 1000 Springs Project*: <http://www.gns.cri.nz/Home/Our-Science/Environment-and-Materials/Extremophiles/Research-activities/The-1000-Springs-Project> (accessed 22 October 2014).
- One Thousand Springs: The microbiology of geothermal hot springs in New Zealand*: <http://www.1000springs.org.nz/> (accessed 22 October 2014).

## Dubious use of fine particle mass-based standards for regulating urban air quality in a hypothermic environment

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### Abstract

In New Zealand, outdoor levels of the common air pollutants show considerable regional and seasonal variability; judged by world standards, average values are low. In winter, supplemental space heating using relatively inexpensive solid fuels is often employed domestically. Arbitrarily chosen National Environmental Air Quality Standards (NESAQ), based on a  $PM_{10}$  24 hour average limit of  $50 \mu\text{g}/\text{m}^3$ , severely restrict such heating. On a mass/mass basis, the gaseous-volatile/semi-volatile fraction is presumably more injurious, or potentially so, than that comprising the inhalable, essentially non-volatile, particulate matter. Also, in New Zealand regulations controlling urban air pollution define air exclusively as that existing outdoors, ignoring the health consequences of indoor air and/or indoor lifestyles. For these and other reasons, estimates concerning lives that can allegedly be potentially saved by reducing air pollution focused solely on compliance with  $PM_{10}$ -based standards are both quantitatively and qualitatively suspect.

### Global health

Firstly, what is meant by the term global health? Logically, global health means the collective health of individual human beings amounting, ultimately, to family, community, country and populations worldwide. Consequently, responsible governance pertaining to public health involves encouraging people to, as much as possible, take good care of themselves and each other independently of government, ensuring key natural resources and environments are appropriately managed or controlled and adopting an inherently conservative approach, bearing in mind the steadily evolving nature of scientific knowledge, economics and population dynamics generally.

### Air pollution versus air quality

Insofar as the relationship between air quality and public health is concerned, this undoubtedly is a very controversial topic particularly in the context of enforceable policy related to the control and/or regulation of urban air pollution. Typically, suspended fine particulate matter mass of less than 10 and  $2.5 \mu\text{m}$  ( $PM_{10}$  and  $PM_{2.5}$ , respectively) determined outdoors is employed as a surrogate for all the harmful consequences allegedly observed or expected pertaining to likely exposures. Since, compared to the recognised causes of death or ill health, the substantive effect of specific instances of ordinary urban air pollution normally are indeterminate, distinguishing between the *actual* consequences and associated hypothetical mortality/morbidity *estimates* related to reduction of the pollution usually is left unresolved to the detriment of affordable, ethical, public health-implicated policy.

Thus according to the WHO<sup>1</sup>

*"...for 2008, the number of premature deaths attributable to urban outdoor air pollution is estimated to amount to 1.34 million worldwide. Of these, 1.09 million deaths could be avoided if the mean annual Air Quality Guideline values of  $PM_{10}=20 \mu\text{g}/\text{m}^3$  and  $PM_{2.5}=10 \mu\text{g}/\text{m}^3$  were implemented."*

Clearly this statement is ambiguous and can be taken as meaning either *could*, in the sense of following directly (i.e. would/will) or *could* in the sense of being possible but by no means certain, with no way of knowing which of these is correct or intended by the author of the report in question.

### Real, attributable, deaths?

Given that numbers of deaths cited typically are de-

rived from very small relative risk factors i.e.  $RR \approx 1.00$  (where  $RR = 1.00$  means zero effect and  $RRs > 2-3$  generally are required if implications of causation are to be taken seriously<sup>2</sup>), not much confidence can be placed in such claims. This is particularly so where, as is usually the case, the crucial exposures are ill-defined, the individuals allegedly affected cannot be identified nor can the substantive causal factors be established with any certainty. In effect, ordinary citizens are being asked to accept the reality of and to fund something they, personally, may never relate to, understand well or benefit from in any substantive way.

Clearly there is a lot at stake here professionally – careers, reputations, industries, economies, statute law, embedded legislation, etc. Whatever the precise explanation, science as a discipline currently is coming a poor second to political expediency employed extensively in the context of urban air quality regulation.<sup>3-5</sup> Thus, for costing purposes, the methodology employed involves i) calculating statistically – from daily mortality data - the number of deaths allegedly attributable to variable (elevated) levels of air pollution and, hence, the number of lives potentially salvable/deaths avoidable in the absence of such pollution and ii) multiplying together such estimates and the monetary value (e.g. NZ\$3.56 million; value of a statistical life)<sup>6</sup> ascribed to the average person dying as a result of a road accident. Typically, very large sums of money as potential net positive benefits are estimated thereby leading to calculation of favourable cost/benefit ratios.

Unfortunately, whereas such traffic-related deaths on average involve people aged around 40 years of age, air pollution is most likely to manifestly affect or harm frail, elderly, people.<sup>7</sup> Hence, rather than attempting to justify control of urban air pollution in terms of ‘saving’ valuable lives, small extensions to (or in some cases detractions from) the lives of already elderly people (cf. population ageing) ought to be accepted instead as a more realistic end result.<sup>8</sup> Also it needs to be acknowledged that such changes are likely to merge more or less seamlessly with the common scenario of steadily increasing life expectancies (2-3 years/decade currently in New Zealand; average life expectancy of approximately 80 years) having little to do with air pollution.

Meanwhile, the situation in Christchurch, New Zealand (population approximately 350,000), exemplifies what can happen when well funded, stridently promoted, authoritatively-couched environmental policies are, nonetheless, ill-conceived and/or mismanaged.<sup>9</sup> Unfortunately, because the topic is complicated, what follows here necessarily deals with only a cross section of the more important aspects.

## Confounding issues

### *Climate*

Typically, mortality is highest during the winter virtually everywhere.<sup>7,10</sup> Comparing the North Island of New Zealand (e.g. Auckland averaging 7-15 °C in winter, 15-24 °C in summer) to the South Island (e.g. Christchurch averaging 2-11 °C in winter, 12-23 °C in summer) reveals

large variations in climate. Yet identical standards (i.e. NESAQ<sup>11</sup>) for permitted air pollution apply everywhere in NZ irrespective of the different domestic heating options available or other local and regional environmental, economic, and demographic distinctions. Sometimes the prevalence of frost or snow and other circumstances favouring low temperatures or otherwise inclement conditions outdoors ensures that provision of adequate warmth indoors is by no means a simple or assured matter.

It follows, therefore, that excessive environmental or other zeal may be a recipe for genuine personal hardship or worse, particularly in the case of elderly or similarly susceptible people of limited means cf. fuel poverty. Having conceded this point, simple logic in the interests of good governance dictates that:

a) standards for air pollution measured outdoors ought to reflect the fact that many interconnected properties of the local environment are capable of influencing public health both positively and negatively and

b) policy-makers/governments desirous of controlling ordinary urban air pollution need, before taking any major, far-reaching, steps, to as much as possible i) take the wider picture into account ii) provide *full* justification, readily understood by ordinary people, for their actions iii) ensure that if mistakes are made these are able to be rectified quickly and with as little collateral damage as possible.

In recent times, assisted by the implementation of various Regional Natural Resource Management Plans formulated by local and regional government, the need for such commonsensical measures have been ignored or overruled possibly in the interests of promoting, ahead of everything else, a “clean, green, 100% pure” image for New Zealand.

### *Indoor versus outdoor air*

As already indicated, for regulatory purposes Ministry for the Environment (MfE)<sup>12</sup> and Regional Councils such as Environment Canterbury (ECan)<sup>13</sup> define air solely in terms of that found outdoors (i.e. where the measurements are made).

However, because *exposures* of interest often occur elsewhere these may not be reflected well by measurements made on air sampled outdoors. Aware of this, the US Environmental Protection Agency (USEPA)’s definitions of ambient<sup>13</sup> are both self consistent and scientifically robust. Thus:

**Ambient Medium (USEPA):** *Material surrounding or contacting an organism (e.g. outdoor air, indoor air, water, soil, through which chemicals or pollutants can reach the organism).*

Whereas, according to Environment Canterbury:

**Ambient air quality** *is the air quality in a general area, outside buildings and structures. It includes air over a wider area and air subject to localized discharges, e.g. street level discharges. It does not include indoor air,*

*air in the workplace, or contaminated air as it is discharged from a source.*

How did this difference and confused picture come about? Clearly, air as a natural resource is mostly located outdoors. Hence it would appear influential New Zealand government officials thought that this explained everything and were unaware of, or attached insufficient importance to, the seemingly benign or neutral indoor environment. Also they clearly did *not* have as a primary concern the public health and safety implications of New Zealand's Resource Management Act,<sup>14</sup> the purpose of which is described as follows:

#### 5 Purpose

*(1) The purpose of this Act is to promote the sustainable management of natural and physical resources.*

*(2) In this Act, **sustainable management** means managing the use, development, and protection of natural and physical resources in a way, or at a rate, which enables people and communities to provide for their social, economic, and cultural well-being and for their health and safety while—*

*a) sustaining the potential of natural and physical resources (excluding minerals) to meet the reasonably foreseeable needs of future generations; and*

*b) safeguarding the life-supporting capacity of air, water, soil, and ecosystems; and*

*c) avoiding, remedying, or mitigating any adverse effects of activities on the environment.*

Either way these same bureaucrats have succeeded in creating fear amongst the population at large that ordinary urban air pollution kills – directly, unambiguously – as many as 182 people each year (approximately 7% of the total deaths)<sup>15</sup> in Christchurch alone even though substantive (i.e. clinical/autopsy) evidence to this effect is completely lacking. But, evidently, not sufficiently “deadly” as to discourage Environment Canterbury from declaring an NESAQ amnesty following a major emergency in Christchurch:

*“...The priority for Environment Canterbury over the last two winters has been to ensure people in damaged properties stayed warm and this priority will continue for winter 2013.*

*....the replacement of older heating sources should reduce particulate air pollution over time. In the short term, however, the need for emergency repairs to heating systems has meant that legislation to prosecute those using polluting older wood burners and open fires has been temporarily relaxed for earthquake damaged homes for the winter of 2011.”<sup>16</sup>*

But, given that laws embodying the NESAQ still prevail and cannot (legally) be challenged even though the science employed thereto appears to be seriously flawed, not indefinitely!

#### Origin of acute effects

Regulatory policy focused on PM<sub>10</sub> 24 hour average (as in New Zealand) assumes that associated *acute* effects are

prevalent. Such effects, presumably, are attributable less to elemental carbon, ammonium nitrate, crustal dust, sea salt and similar comparatively inert, non-volatile, material (conveniently determined by weighing) and more to the gaseous (e.g. NO<sub>2</sub>, CO, SO<sub>2</sub>, O<sub>3</sub>) and organic volatile/semi-volatile co-pollutants present.<sup>17</sup>

“Collectable” naturally-occurring substances possessing irritant/allergenic/infectious properties (e.g. pollen, bacteria, viruses, mould etc.) are an exception here. Similarly, various mineral-based dusts, metals, tobacco smoke, etc., potentially contribute to serious illnesses and disorders such as cancer, usually following many years exposure. Typically, however, much uncertainty exists regarding actual causality in such cases, mainly because of the large number of extraneous confounding factors involved. It is simpler, in a regulatory context, to focus on acute exposure-type monitoring assuming this can be done accurately and that the results are relevant to the actual health effects.

#### Problems related to sampling and monitoring

Being particularly susceptible geographically, Christchurch regularly exhibits “temperature inversion” phenomena during the winter under calm conditions which serves to concentrate the pollutants. Also, because a few, relatively low-lying and hence poorly drained, predominantly residential (St Albans) and/or “industrial” (Woolston) suburbs are especially prone to air pollution attributable to solid-fuelled stoves, boilers and similar equipment, this is where sampling for “worst case scenario” air quality measurement has traditionally been carried out.

Nowadays, such sampling is assumed to reflect maximum (peak) concentrations relevant to NESAQ (PM<sub>10</sub>) compliance. Generally speaking, other sites of interest (e.g. traffic-related) give little cause for concern ordinarily regarding emissions of CO, SO<sub>2</sub> and NO<sub>2</sub> at any time of the year. Meanwhile it seems fair to conclude that, considering all the suburbs and great diversity of living and working conditions that go to make up the whole city, if the Christchurch “airshed” is to be sampled representatively insofar as personal exposures are concerned, many more sampling sites are needed than just the two or three “outdoor” sampling arrangements currently provided for. Nonetheless, a steady decrease in PM<sub>10</sub> levels has been observed over the years with peak levels roughly halved compared to 50 years ago.

Taking such things into account, the inhabitants of Christchurch almost certainly are exposed to relatively low levels of potentially harmful air pollution although few would think so considering the admonishments regularly delivered by MfE and ECan, mostly pertaining to NESAQ (PM<sub>10</sub>) non-compliance.

#### Basis of regulations - credible or not?

Meanwhile, although compliance with a PM<sub>10</sub> 24 hour average-based standard is demanded, cost/benefit justification allied to alleged health risks is ultimately based

on PM<sub>10</sub> annual average-type epidemiological studies mainly conducted overseas such as in the USA.<sup>6</sup> Also, the relevant calculations involve a particularly complex mix of assumptions and approximations in any case.<sup>7</sup> All in all, for the various New Zealand Government departments, public bodies and other authorities involved to continue maintaining that the relevant air quality legislation (NESAQ-based) is scientifically valid is to reveal a distinct unwillingness to come to terms with, if not a profound ignorance of, the subject as a whole.

Beginning around 2002, mortality Relative Risk values of around 1.01 based on short-term/acute exposure (i.e. PM<sub>10</sub> 24 hour average-type epidemiology) were cited as being relevant to Christchurch leading to estimates of 40-70 'premature' deaths each year attributable to PM<sub>10</sub> air pollution.<sup>18</sup> Subsequently, substantially larger RR values of approximately 1.043<sup>15</sup> and, latterly, approximately 1.07<sup>6</sup> emerged related to long-term or chronic exposure-type epidemiology yielding estimates ranging from 158-182 "premature" deaths annually in those aged 30 years and over. Meanwhile, the method for measuring PM<sub>10</sub> has also changed resulting in significantly higher results for this pollutant index related to inclusion of and correction for loss of semi-volatiles.

Taking such matters into account, the topic - air quality - clearly has become something amenable to subjective interpretation (i.e. an art rather than a manifestation of good, sound, applied science as normally understood).

### Air pollution compliance targets

Christchurch as represented by the St Albans and Woolston monitoring stations currently is unlikely to achieve the present NESAQ requirement of a maximum of 3 exceedances per year of 50µg/m<sup>3</sup> PM<sub>10</sub> 24 hour average by 2016 let alone the ultimate target of 1 exceedance by 2020.<sup>19</sup> However, it appears to meet the WHO PM<sub>10</sub> annual average guideline of 20µg/m<sup>3</sup> and seems likely to continue to do so for the foreseeable future.<sup>12</sup> Consequently, considered from the point of view of the city as a whole, the typical exposures to PM<sub>10</sub> (and to PM<sub>2.5</sub> with this comprising on average about 60 % of the PM<sub>10</sub>) would appear to be of little concern judged alongside the standards and guidelines applicable overseas (Table 1).

Furthermore, given that there appears to be little or no connection between measured PM<sub>10</sub> air pollution and both overall and specific types of respiratory health as recorded in New Zealand<sup>20,21</sup> the wisdom and effectiveness of policies aimed at replacing in short order large numbers of relatively modern (enclosed-type) domestic cord wood-fuelled burners with alternative (mostly electrically-operated) sources of heat has to be seriously questioned.

### Nature of the polluting effect

Concerning episodic air pollution as normally experienced in New Zealand, entrapment of "fine" relatively (chemically) inert, essentially non-volatile, material leading to gradual physico-chemical interference of normal respiratory functions (cf. silicosis) would appear to have been the "default" mechanism originally. However, considered in the light of the barely detectable acute effects

observed, such modus operandi would appear to be obsolete in a modern context. According to the authors of the latest version of the oft-cited Health and Air Pollution in New Zealand (HAPINZ) reports:<sup>6</sup>

*Particles of different sizes typically have different sources and different chemical and biological composition. The mechanisms of particle toxicity are complex and still not fully understood. For example, it is not yet certain which of the several classes of toxic effects observed in laboratory experiments are responsible for specific human health effects (Brook et al. 2010).*

Meanwhile, the main pollutant gases NO<sub>2</sub>, CO, SO<sub>2</sub>, and O<sub>3</sub>, despite being routinely monitored, typically are ignored by epidemiologists and planners. Based on the evidence available, a mechanism reliant upon such "reactive" substances and the (mainly) organic gases/volatiles and semi-volatiles would appear to be entirely feasible in the ordinary urban environment. Indicative, however, of the subtleties involved are the results obtained for one Christchurch sampling site shown in Fig. 1.

In practice, determination of PM<sub>10</sub> via FDMS involves the following:

- i) sampling the air under the prevailing (outdoor) ambient conditions
- ii) obtaining, simultaneously, a sub-sample representative of the "fine" particle fraction ≤ 10 µm e.g. via a "50 % efficiency/cut" cyclone
- iii) collecting the suspended, moisture free, particulate matter on a filter while weighing it at a temperature of 30° C
- iv) repeating the weighing step under conditions facilitating calculation/compensation for concomitant loss of attendant "volatiles" whence
- v) the permanent (largely inorganic) gases are not taken into account/recorded as PM
- vi) the more volatile of the volatiles/semi-volatiles (possibly mainly organic) fraction are not taken into account/recorded as such
- vii) the less volatile of the volatiles/semi-volatiles (largely organic) fraction presumably are partly taken into account/recorded
- viii) significant amounts of relatively inert "fine" particulate matter are taken into account/recorded as potentially harmful material simply from a mass perspective
- ix) potential toxicity associated with the "coarse" particle fraction is disregarded/downplayed.

Hence, considering all of the above it seems fair to conclude that monitoring of urban air quality in the interests of public health, as presently carried out, leaves a lot to be desired.

### Precautionary Principle

The Precautionary Principle<sup>22</sup> states that:

*"...if an action or policy has a suspected risk of caus-*

ing harm to the public or to the environment, in the absence of scientific consensus that the action or policy is not harmful, the burden of proof that it is not harmful falls on those taking an action...”

Application of the principle appears to have led, in New Zealand at least, to overly stringent standards for PM (Table 1).

Comparing the shown data above, New Zealand’s PM<sub>10</sub>-based standard is seen to be much more stringent than the equivalent standards favoured by USEPA and the EU. Also, considering that the individual limits, etc. are largely arbitrary, use of the term “standard” in a regional context is contentious. Consistent with this viewpoint, WHO prefers to promulgate limits described as guidelines rather

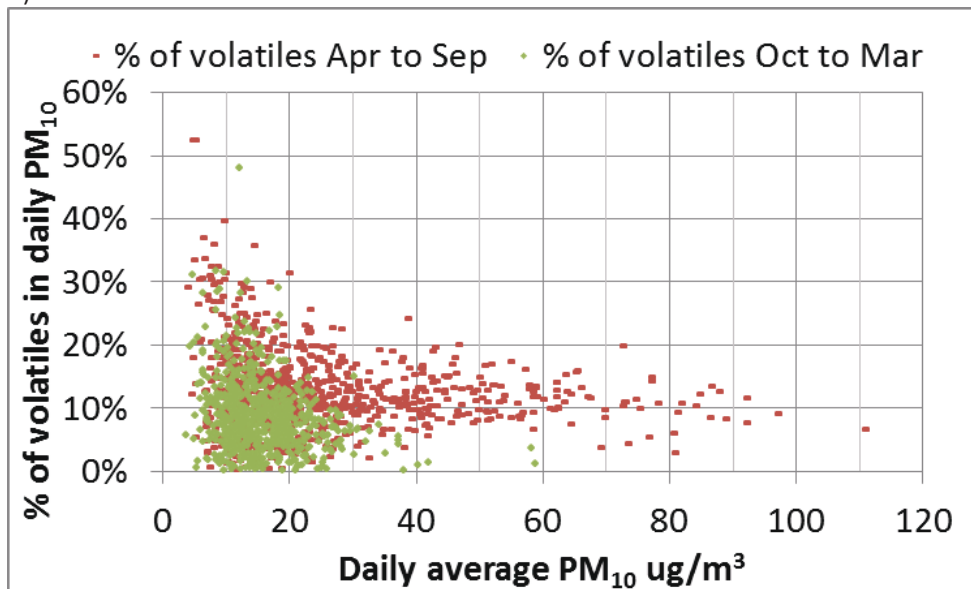
**Table 1** Ambient air quality standards: comparison of allowable air pollution (PM) limits and exceedances (reproduced with permission: Hoare, J.L. *New Directions: Questions surrounding suspended particle mass used as a surrogate for air quality and for regulatory control of ambient urban air pollution, Atmospheric Environment, 2014, 91, 175-177*).

Pollution index	Averaging period	Country		
		United States of America <sup>23</sup>	European Union <sup>24</sup>	New Zealand <sup>11</sup>
PM <sub>10</sub>	24 hours	150*; 1/yr as a 3 yr average	50*; 35/yr	50*; 1/yr (aiming for full compliance by 2020**)
PM <sub>10</sub>	Annual	N/A	40*	N/A (WHO guideline of 20* currently met virtually everywhere)
PM <sub>2.5</sub>	24 hours	35*; 98 <sup>th</sup> percentile averaged over 3 yr.		
PM <sub>2.5</sub>	Annual	12*; (averaged over 3 yr.) Primary 15*; (averaged over 3 yr.) Secondary	25* 20*; (exposure, averaged over 3 yr.) by 2015 18*; (exposure, averaged over 3 yr.) by 2020	N/A (currently ≤15* assuming PM <sub>10</sub> annual avg. is ≤ 20* and 70% of PM <sub>10</sub> is PM <sub>2.5</sub> )

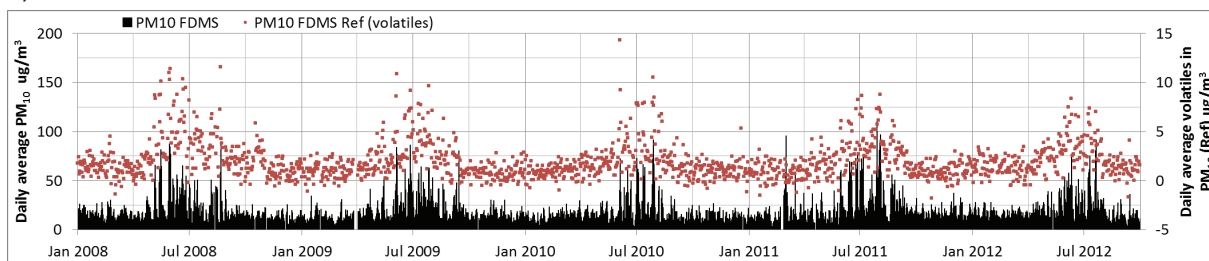
\* Measured in µg/cubic metre

\*\* In some towns and cities in NZ, especially those situated in regions experiencing relatively cold winters, exceedances/yr currently exceed the standard by a considerable margin

A)



B)



**Fig. 1.** 24 hour average PM<sub>10</sub> via Filter Dynamics Measurement System (FDMS), showing seasonal variations in PM and % “volatiles” for Christchurch. (A) Average PM<sub>10</sub> % volatiles: cooler months (red), 12%; warmer months (green), 8%. (B) Variations in daily average PM<sub>10</sub> (black) and daily average volatiles in PM<sub>10</sub> (red). Average volatiles for 2008 - 2012, 11%; “Exceedances”/yr = 20 approximately (graph B reproduced with permission: Hoare, J.L. *New Directions: Questions surrounding suspended particle mass used as a surrogate for air quality and for regulatory control of ambient urban air pollution, Atmospheric Environment, 2014, 91, 175-177*).

than legally enforceable standards stated as follows:

“...governments should consider their own local circumstances carefully before using the guidelines directly as legal standards.”<sup>25</sup>

## Conclusions

Pursuit in an urban context of perfectly clean and/or pure air is unrealistic and impractical. Instead, a reasonable compromise corresponding as much as possible to the likely actual exposures and confirmed risks related to achievable air quality in all its guises is preferable.

Where local supplies of solid fuels are assured, relatively inexpensive and sustainable compared to alternative sources of available energy (e.g. electricity and/or gas) it makes good sense to allow and encourage effective and efficient use of such methods of heating domestically (e.g. as a back-up and/or during very cold or otherwise inclement weather).

Arbitrarily-chosen limits (guidelines) for the individual gaseous inorganic and volatile/semi-volatile organic pollutant categories possibly would be more suitable for regulatory purposes than the epidemiologically-arrived at, PM-based, “standards” currently employed.

Particle-related toxicity probably resides principally in an adsorbed volatile/semi-volatile sub-component; tolerably stable therein provided the ambient temperature is low enough.

Such material probably is capable, at least partly, of being volatilised/desorbed at temperatures approaching “blood heat” (approximately 37 °C) thereby assisting the transfer of otherwise relatively harmless, occluded, material deeper into the lungs.

Probably all airborne particles (i.e. particles  $\leq$  approximately 100 nm in diameter) should be regarded as potentially significant contributors to the acute effects, the latter being mainly attributable to the “permanent” gases and volatiles with additional contributions from the adsorbed semi-volatile and volatile material.

In practice, global health is a composite of that enjoyed by individuals and as such is best tackled from a local/regional perspective.

Compared to the US and EU standards for PM<sub>10</sub> 24 hour average, the equivalent New Zealand standard (NESAQ) permitting no more than 1 exceedance of 50 mg/m<sup>3</sup> per year is particularly stringent with accrued benefits likely to be small or unclear relative to the substantive overall costs incurred. Given that the Government appears unwilling to modify its stance enabling a more realistic/straightforward/honest approach to the science involved, a sense of injustice prevails.

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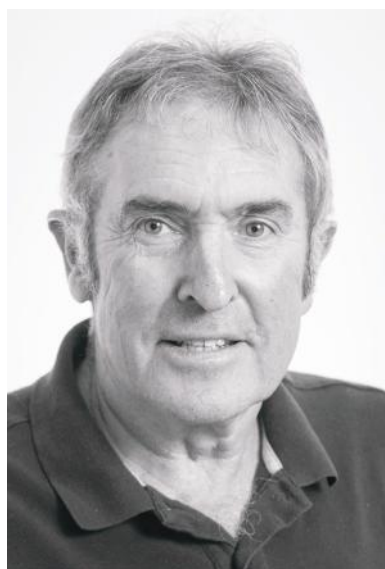
Quality Analyst, Environment Canterbury, Christchurch, New Zealand.

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## References and notes

- World Health Organization. *Burden of disease associated with urban outdoor air pollution for 2008*. Available at: [http://www.who.int/phe/health\\_topics/outdoorair/databases/burden\\_disease/en/](http://www.who.int/phe/health_topics/outdoorair/databases/burden_disease/en/)
- RR (Relative Risk). Available at: <http://www.numberwatch.co.uk/rr.htm>
- <http://junkscience.com.files.wordpress.com/2014/07/epa-health-claims-for-its-coal-plant-co2-rules-are-false1.pdf>
- <http://www.washingtontimes.com/news/2013/jan/22/chinas-bad-air-puts-the-lie-to-epa-scare-tactics/?page=all>
- Phalen, R.F. *Toxicol. Lett.* **1998**, 96-97, 263-267.
- HAPINZ Updated Health and Air Pollution in New Zealand Study (2012). Available at: <http://www.hapinz.org.nz/>
- Hoare, J.L. *NZ Med. J.* **2011**, 124(1330), 66-73. Available at: <http://www.nzma.org.nz/journal/124-1330/4558/>
- Hoare, J.L. *NZ Med. J.* **2011**, 124(1347), 132. Available at: <http://journal.nzma.org.nz/journal/124-1347/5014>
- Moller, P. *NZ Med. J.* **2011**, 124(1345), 103-105. Available at: <http://journal.nzma.org.nz/journal/124-1345/4952/>
- Moller, P. *NZ Med. J.* **2011**, 124(1330), 58-65. Available at: <http://www.nzma.org.nz/journal/124-1330/4562/content.pdf>
- Ministry for the Environment. *2011 amendment to the National Environmental Standards for Air Quality*. December 2013. Available at: <http://www.mfe.govt.nz/laws/standards/air-quality/review/>
- Ministry for the Environment. *2014 Air domain report*. May 2014. Available at: <http://www.mfe.govt.nz/environmental-reporting/air/air-domain-report-2014/index.html>
- Hoare, J.L. *Clean Air Env. Qual.* **2005**, 39(2), 32.
- New Zealand Legislation, Resource Management Act 1991. Available at: <http://www.legislation.govt.nz/act/public/1991/0069/latest/DLM231905.html>
- Fisher, G.W.; Rolfe, K.A.; Kjellstrom T. *et al. Health effects due to motor vehicle air pollution in New Zealand*. Report to the Ministry of Transport, 20 January 2002. Available at: <http://www.transport.govt.nz/research/Documents/health-effects-of-vehicle-emissions.pdf>
- Prepared by Environment Canterbury and Community and Public Health. December 2012. Available at: <http://www.healthychristchurch.org.nz/media/34867/airquality.pdf>
- Hoare, J.L. *Atmos. Environ.* **2014**, 91, 175–177. Abstract available at: <http://dx.doi.org/10.1016/j.atmosenv.2014.04.004>
- Environment Canterbury. *Proposed Canterbury Natural Resources Regional Plan*. 1 June 2002). Chapter 3: Air Quality.
- Mallett, T. *Is Christchurch likely to meet the NESAQ targets for PM<sub>10</sub>?* Environment Canterbury Report No. R12/40, May 2012. Available at: <http://ecan.govt.nz/publications/Reports/air-report-is-christchurch-likely-meet-nesaq-targets-pm10-000512.pdf>
- Palmer, P. *How toxic is your PM10? A review of some aspects of the HAPINZ reports*. 20<sup>th</sup> Conference of the Clean Air Society of Australia and New Zealand, Auckland, New Zealand, July 30 - Aug 2, 2011.
- Kessaram, T.; Stanley, J.; Baker, M.G. *Influenza Other Respir. Viruses* **2015**, 9(1), 14-19.
- Precautionary Principle. See: [http://en.wikipedia.org/wiki/Precautionary\\_principle](http://en.wikipedia.org/wiki/Precautionary_principle)
- National Ambient Air Quality Standards (NAAQS). Available at: <http://www.epa.gov/air/criteria.htm>
- European Commission (Environment) Air Quality Standards. Available at: <http://ec.europa.eu/environment/air/quality/standards.htm>
- WHO (Europe). *Air Quality Guidelines Global Update 2005*. (See Introduction p6). Available at: [http://www.euro.who.int/\\_\\_data/assets/pdf\\_file/0005/78638/E90038.pdf?ua=1](http://www.euro.who.int/__data/assets/pdf_file/0005/78638/E90038.pdf?ua=1)

## Anthony (Tony) David Woolhouse, FNZIC (1946-2014)



Tony gained his 1<sup>st</sup> Class MSc with J.T. Craig in the Chemistry Department at Victoria University of Wellington (VUW) in 1969. He was contracted to the Wellington Hospital Board (Metabolic Unit) where he worked in 1968 and then through the 1970 year, winning the Institute of Medical Laboratory Technology Hilder Memorial prize for his 1971 publication

in their journal. However, he was drawn to a higher degree and he returned to VUW in February 1971 to undertake study as Brian Halton's first PhD student. Tony produced 10 papers from the VUW research, involving the chemistry of ring expanded naphthalenes, triazolines and cyclopropabenzene. The significant number of publications from these years attests to Tony's superb pair of hands in the laboratory and the passionate dedication that enabled him to be such a productive chemist. After PhD graduation in September 1973, some 30 months after starting, Tony went to work with Professor Charles Rees at the University of Liverpool. He gained two publications concerning cycloaddition reactions from his year there.

After returning to NZ for the spring of 1974, Tony joined Chemistry Division of DSIR at Gracefield in March 1975. There, his research at began with the chemistry of lignin, a phenolic component of wood, that was of interest in the discolouration of paper from residues in paper pulp. Tony contributed to three papers in this area and he also managed to keep up with an early interest in photochemistry, publishing four papers over 1978-80. Being then highly regarded by DSIR Chemistry Director, Dr Gordon Leary, Tony was awarded study leave to work with Al Padwa at Emory University in Atlanta, Georgia, USA. In Atlanta, Tony's high work output generated three more papers and a chapter in *Comprehensive Heterocyclic Chemistry* co-authored with Padwa. Such was the admiration he gained for Padwa, Tony attended Padwa's graduate lecture course and kept the notes he took alongside his desk in his office, where they remain today.

The NZ 'think big' era in the early 1980's saw oil exploration expanded in Taranaki and elsewhere. Tony became involved when DSIR Geology asked for help from chemists. Organic biomarkers in oils are the chemical residues of the algae and plant materials from which gas and oil deposits are formed. Tony's work on NZ biomarkers produced 13 papers about the geochemistry of sediments

where the biomarkers are used to aid the source identification and assist in drilling operations.

Tony also worked with the pheromones of NZ insect and animal pests - native leaf roller moths were a kiwifruit orchard pest and ferrets and stoats were predators of native birds. Research with insects, ferrets, opossums and wild cats generated a further 20 papers as he worked on new methods for luring pests into traps.

After 1992 and the formation of Industrial Research Limited (IRL), Tony's career took him back to light activated molecules that were intended for use as laser dyes and the active components in *all optical* switching devices and rewritable holograms. Tony co-authored 32 papers in this area and the optoelectronics and photonics groups now operating at Callaghan Innovation resulted from this initiative.

Tony took the opportunity in 2002 to transfer to the IRL subsidiary, Biopharm - a large scale producer of pharmaceutical products from the fermentation of microorganisms that serviced major overseas pharmaceutical company clients. Tony revelled in scale-up work, being comfortable with the 1,000 litre vessels and kilograms of material. He developed processes that purified crude fermentation material into drug precursors - the warheads required for targeted anti-cancer agents.

Tony returned to laboratory chemistry with the IRL Carbohydrate Chemistry Team in 2006, where he made inhibitors of nucleoside processing enzymes that resulted in 2 papers and a patent. A related study of other possible enzyme inhibitors produced 2 papers detailing novel chiral chemicals.

In 2013, yet another new challenge saw Tony involved with a project for New Zealand Pharmaceuticals Ltd (NZP), for whom he synthesised a large number of novel molecules. Tony became a very popular member of the NZP laboratory team during a four month secondment to Palmerston North with them.

Tony transferred with his colleagues to be a foundation member of the Ferrier Research Institute at Victoria University at the start of 2014, completing the journey back to his *Alma Mater*. His final project was to successfully make a compound required by a VUW colleague for a new technology aimed at measuring very low levels of the male hormone testosterone in water.

There are very few organic chemists who have made such significant contributions to as wide a range of fields as Tony, nor many who were as uniformly liked and admired. In the view of Professor Halton, Tony had one of the best pair of hands in laboratory chemistry in NZ. He will be sadly missed by all.

**Contributed by his VUW colleagues, Doug Crump, Richard Furneaux and Brian Halton**

## Dates of Note

### April

- 21 **Aleksandr Oparin**, the Russian biochemist who is noted for his studies on the origin of life from chemical matter, died on this day in 1980.
- 22 **Fritz Strassmann**, the German physical chemist who, with Hahn and Mietner, discovered neutron-induced nuclear fission in uranium, died in 1980.
- 23 **Max von Laue**, the German physicist and 1914 Nobel Laureate in Physics in 1914 who discovered the diffraction of X-rays in crystals, died this day in 1960.
- 25 **Wolfgang Pauli**, the Austrian-born American who won the 1945 Nobel Prize for his discovery of the Pauli Exclusion Principle, was born in 1900.
- 26 This day in 1900 was the birth date of Dr **Charles Francis Richter**, the seismologist and inventor of the Richter scale.
- 27 **Carl Bosch**, the German industrial chemist who directed development of the industrial scale process for production of ammonia from atmospheric nitrogen at BASF, died in 1940.
- 30 The existence of element 101, *mendelevium*, was announced on this day 1955.

### May

- 1 **Johann Jakob Balmer**, the Swiss mathematician and physicist who discovered a formula basic to the development of atomic theory and is best known for the spectroscopic Balmer lines, was born in 1825.
- 2 This day in 1800 saw the first chemical reaction by electricity – the electrolysis of water by **William Nicholson**.
- 4 The first US national arts and science society was incorporated this day in 1780.
- 5 Sir **Lionel Alexander Bethune Pilkington**, the British industrialist and inventor of the float glass process, died in 1995.
- 6 **Johann Joachim Becher**, the German chemist, physician and adventurer who gave an early theory of combustion in which all flammable objects were supposed to contain a substance which was released when the object burned, was born in 1635. It was the day in 1840 that the adhesive postage stamp was first sold in Great Britain.
- 7 **William Lever** (1<sup>st</sup> Viscount Leverhulme), the British soap manufacturer and philanthropist whose company Lever Brothers morphed into Unilever, died this day in 1925.
- 8 In 1790, 225 years ago, acting on a motion by bishop Charles Maurice de Talleyrand, the French National Assembly created the metric system, initially defining the metre, then revising it to be 1/10,000,000 of the distance between the north pole and the equator and adopting the new system as the Republican Measures of France on April 7, 1795; they adopted Greek prefixes for multiples and Latin for decimal fractions.
- 9 **Joseph Louis Gay-Lussac**, the French chemist best known for his work on gases showing that hydrogen and oxygen combined to form water in ratio of 2:1 by volume and for his law of combining volumes, died in 1850.
- 10 **François-Marie Raoult**, the French chemist who formulated a law on solutions (Raoult's Law) that made it possible to determine the molecular weights of dissolved substances, was born on in 1830. **John Wesley Hyatt**, the US inventor and plastics pioneer who discovered the process for making celluloid, died on this day in 1920. This is the same date that **Stanisloao Cannizzaro**, the Italian chemist, teacher, and legislator who recognised the distinction between atomic and molecular weights and has a reaction named after him, died 10 years earlier in 1910. May 10, 1860 was the day that the German chemists **Bunsen** and **Kirchhoff** announced to the Berlin Academy of Scientists that they had discovered two new elements, *caesium* and *rubidium*.
- 11 This day in 1995 saw scientists confirm that Ebola had broken out in Zaire.
- 12 **Dorothy Mary Crowfoot Hodgkin** (née Crowfoot), the English crystallographer of distinction awarded the 1964 Nobel Prize for Chemistry for her discoveries, using X-ray techniques, of the structure of biologically important molecules, including penicillin (1946), vitamin B-12 (1956), and later, the protein hormone insulin (1969), was born in Cairo, Egypt in 1910. The same day in 1895 saw the birth of **William Francis Giauque**, the Canadian-born American physical chemist who won the 1949 Nobel Prize for Chemistry for his work in the field of chemical thermodynamics, especially his work on the behaviour of matter at very low temperatures and his closely allied studies of entropy. 13 **Marguerite Catherine Perey**, the French chemist who identified element 87 (francium, Fr), the last naturally occurring element to be discovered, died in 1975.
- 14 **George Fownes**, the English chemist who prepared furfural and benzoline, the first examples of vegeto-alkali, or organic salt-bases as they were known then, was born on this day in 1815. **Christian Boehmer Anfinsen**, the American biochemist who (with Moore and Stein) received the 1972 Nobel Prize for Chemistry for studies on the shape and primary structure of ribonuclease, died in 1995.
- 18 **Kasimir Fajans**, the Polish-American physical chemist who discovered the radioactive displacement law (that when a radioactive atom decays by emitting an alpha particle, the atomic number of the resulting atom is two fewer than that of the parent atom) simultaneously with Frederick Soddy, died on on this day in 1975.
- 20 **Eduard Buchner**, the German biochemist awarded the 1907 Nobel Prize for Chemistry for demonstrating that the fermentation of carbohydrates results from the action of different enzymes contained in yeast and not the yeast cell itself, was born in 1860.
- 21 **William Nicholson**, the English chemist who performed the first electrochemical reaction (see May 2 above), died 200 years ago in 1815.
- 23 **Georges Claude**, the French engineer, chemist, and inventor of the neon light, died in 1960.
- 24 On this day in 1935, the *first spectrophotometer* was sold by General Electric Co. and was capable of distinguishing and charting two million different shades of

colour. **John Mayow**, the English chemist and physiologist who identified oxygen as a distinct atmospheric entity about a hundred years before Joseph Priestley and Antoine-Laurent Lavoisier, was born in 1640.

- 27 **John Davy**, the English chemist, doctor and younger brother of Humphry Davy, who first prepared, named and characterised the gas phosgene, was born 1790. **Lars Fredrik Nilson**, the Swedish chemist who discovered scandium and its oxide in the rare-earth minerals gadolinite and euxenite, was born in 1840.
- 31 Sir **Arthur Herbert Church**, the English chemist and mineralogist who was a leading authority in the chemistry of painting, and discovered turacin (an animal pigment containing copper) and several minerals, including the only British cerium mineral, died 100 years ago.

## June

- 2 **Jesse Boot** (1<sup>st</sup> Baron Trent), the English chemist who founded Boots Company Ltd., was born on in 1850.
- 5 **Johan Gadolin**, the Finnish chemist who discovered the element yttrium in 1794, the first of the lanthanides, and has gadolinium named for him, was born in 1760.
- 8 **Tim Berners-Lee**, the English computer scientist who invented the World Wide Web, has his 60<sup>th</sup> birthday this year on this day. It is also the day in 1940 that the discovery of *neptunium* (Np, element 93) was announced by McMillan and Abelson working at the University of California at Berkeley.
- 9 On this day in 1905, Einstein published his analysis of Max Planck's quantum theory and its application to light, the insights of which earned him his Nobel Prize. In addition, in 1795, a provisional metre bar was constructed in brass by Lenoir under the direction of Jean Borda; it was a platinum bar about 25×4 mm, the distance between the ends, in the manner of a guage block, was the accepted unit of length.
- 10 **Wilhelm Friedrich (Willy) Kühne**, the German physiologist whose research focussed on the chemical changes occurring in the retina under the influence of light, died in 1900.
- 15 Comte de **Antoine Francois Fourcroy**, the French chemist who worked with Lavoisier and was one of the first to study foodstuffs and fats, was born in 1775.
- 16 **Max Delbrück**, the German chemist who developed the fermentation industry, established a school for distillation workers, and a glass factory for the manufacture of reliable apparatus and instruments, was born in 1850.
- 17 Sir **Arthur Harden**, the English biochemist who shared the 1929 Nobel Prize for Chemistry (with von Euler-Chelpin) for work on the fermentation of sugar and the enzyme action involved, died in 1940 (see: *This Journal*, 2014, 78, 169-174).
- 18 **Per Teodor Cleve**, the Swedish chemist and geologist who discovered the elements holmium and thulium, died in 1905. It is the day in 1865, 150 years ago that **Edmund Ruffin**, the father of soil chemistry in the US, died.
- 19 **Paul J. Flory**, the American physical chemist, recipient of the Nobel Prize for Chemistry in 1974 for his investigations of synthetic and natural macromolecules, was born this day in 1910. **James Bertram Collip**, the Canadi-

an biochemist who co-discovered insulin, died 50 years ago in 1965. **Nicolas Lémeury**, the French chemist and pharmacist who prepared a comprehensive dictionary of pharmaceuticals in the *Pharmacopée universelle* and the *Traité des drogues simples*, died in 1715 – 200 years ago.

- 24 **Johannes Wislicenus**, the German chemist whose pioneering work led to the recognition of the importance of the spatial arrangement of atoms within a molecule, and studied geometrical isomerism, was born in 1835.
- 26 **Roy J. Plunkett**, the American chemist and inventor of Teflon (polytetrafluoroethylene, PTFE), was born in 1910.
- 28 This day in 1960 was the day *chlorophyll a* was first synthesised by **R.B. Woodward**. **Richard August Carl Emil Erlenmeyer**, the German chemist known for contributing to the early development of the theory of structure, and designing the flask named after him, was born in 1825, the day in 1935 that the first virus was reported in crystalline form by Professor **Wendell Stanley** (1904-1971) who subsequently received the 1946 Nobel Prize in Chemistry for his work on the tobacco mosaic virus.
- 29 **Alexander Parkes**, the British industrial electroplating expert, died in 1890.

## July

- 1 **Franz-Joseph Müller Freiherr von Reichenstein**, the Austrian mineralogist and mining engineer who discovered tellurium, in 1740, 275 years ago.
- 5 **François-Pierre Ami Argand**, the Swiss chemist who invented an oil burner with a tubular wick inside a glass chimney (the Argand lamp), was born in 1750. **Antonio de Ulloa**, the Spanish scientist and naval officer who discovered the element platinum, died in 1795.
- 6 **Nathaniel Wyet**, the American chemist who created the PET plastic beverage bottle, died in 1990.
- 8 **Joseph Loschmidt**, the Austrian chemist and physicist who proposed some kind of cyclic structure for benzene in 1861, died in 1895.
- 12 **Richard Buckminster Fuller**, the American inventor, educator, author, philosopher, engineer and architect who developed the geodesic dome, was born this day in 1895.
- 14 **Jean Baptiste André Dumas**, the French chemist who was a pioneer in organic chemistry and established quantitative organic analysis, was born in 1800. **Maurice de Broglie**, the French physicist who made many contributions to the study of X-rays, died this same day in 1960.
- 18 **Johann Gustav Christoffer Kjeldahl**, the Danish chemist who originated a method for determining the amount of nitrogen in certain organic compounds using a laboratory technique named after him, died in 1800.
- 19 **Georges Friedel**, the French crystallographer who formulated basic laws concerning the external morphology and internal structure of crystals, was born in 1865, 150 years ago.