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$^{14}CO_2$ in the Southern Hemisphere Atmosphere – the Rise and the Fall

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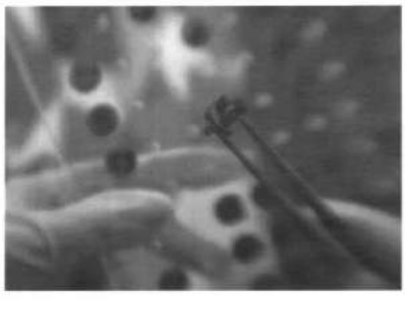
On the cover



Preparation of samples for analysis by Scanning Electron Microscopy.

Photo taken by Matt Walters, School of Biological Sciences, University of Canterbury.

Message from the President

The past few years have seen quite dramatic changes in the Institute. For me, one of the most striking things has been the change in the magazine. CiNZ really has been revitalised under the stewardship of Brian Halton with each issue, broadly speaking, Branch-based. I think this has been a great success and has offered us all the opportunity to learn about what our colleagues are up to across the country.

Last year the Education Specialist Group (ESG), led by Suzanne Boniface in Wellington, started to become more active. I think this offers an opportunity for Chemistry to reach a broader audience with a more inclusive approach towards the school teaching community. One of the perennial criticisms of NZIC has been that its membership, and thus its interests, weight heavily towards academia; hopefully Suzanne and the ESG will be able to offer value to a new sector of people interested in Chemistry.

One innovation under trial this year is that of Corporate Membership. The idea is that Crown Research Institutes and Companies may bulk pay for their staff in order to reduce their, and our, administrative costs. I hope this is a success because it offers the possibility of creating a culture in which large organisations support the NZIC and, more importantly, support their staff in being members of the Institute.

There have also been important operational changes with the secretariat and publishing aspects of the Institute's operation now run from Christchurch. The administrative procedures are in much better shape now than even a year ago. As part of these changes Branches have been asked to have a member looking after the students in the Branch and keeping NZIC informed as to where each student has gone after completing their studies. I think it is important to keep track of our student members to encourage them to stay involved and assist them where we can.

At the start of 2006 we have an Institute that has weathered some administrative difficulties and perhaps now we have an opportunity to take time to think about our role in NZ science. I sense that some within the Institute would like to see a more proactive role for us by, for example, advising government about science policy as it pertains to the chemical sciences. On the other hand, there are those that feel that this is not our role and that we should be more of a networking organisation supporting our members by, for example, providing students with grants to go to overseas conferences. I look forward to getting a feel for the mood among members when I tour the Branches this year.

Finally, 2006 marks a year in which NZIC holds its National Conference. This year it is in Rotorua in December. The conference, ***Back to the Basics: From Small Molecules to Materials and Surfaces***, is being organised by Prof. Peter Schwerdtfeger and his team who have already put together a fantastic programme with a number of noted international plenary speakers. It is going to be a brilliant conference and I urge you to attend.

Associate Professor Keith Gordon (FNZIC)
University of Otago

About the 2006 NZIC President

Keith Gordon received his BSc (1st class Hons.) in 1986 and PhD in 1989 in Chemistry from Queens University (Belfast). His PhD research (directed by Prof. J. J. McGarvey) focused on laser spectroscopy of solar energy compounds. He was awarded a Director's Fellowship at Los Alamos National Laboratories (USA) and worked with Prof. W. H. Woodruff (1990–92) on ultrafast laser spectroscopy of biological systems and solar energy materials. In 1993 Keith took up a lectureship in Chemistry at Otago University where he is currently an Assoc. Prof. Keith's research interests focus on design and synthesis of materials for use in organic light emitting diodes and plastic solar cells, and on understanding the properties of conducting polymers, nanostructured electromaterials, dairy products, and pharmaceuticals using spectroscopy and computational chemistry.

The Use and Limits of Compositional Analysis for Discrimination and Classification of Samples

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The recent decision by the FBI to cease using compositional analysis of bullet lead (CABL)[1] has prompted those of us interested in compositional fingerprinting of materials to re-evaluate our practices, and ensure that we are aware of the boundaries of such techniques. Our group has been particularly interested in whether it is possible to identify the region of origin of wines, or the source of a diesel spill, while others in Australasia have been interested in identifying the origin of items as diverse as cannabis[2] and gold.[3]

The FBI used compositional analysis of bullet lead (CABL) in cases where bullets associated with a crime scene were so badly damaged that they could not be compared by the more traditional method of *e.g.* comparison microscopy. The FBI estimated that it had used CABL for ca. 2500 cases, with ~20% of these proceeding to trial[1] whereas CABL analysis has been used only once in New Zealand, during the third trial[4] of John Barlow in 1995. In CABL, replicate bullet fragments are digested, and then the resulting solutions analysed for Ag, As, Bi, Cd, Cu, Sb, and Sn using inductively-coupled plasma-optical emission spectroscopy (ICP-OES). The data are then compared with analyses from bullets associated with a suspect, with similar results being used to support the hypothesis that the bullets came from the same source.[5,6] Following criticism of CABL,[7] the FBI requested that the US National Research Council evaluate the CABL technique, including the sampling, chemical analysis, statistical analysis, interpretation, and presentation of results. This *Forensic Analysis: Weighing Bullet Lead Evidence* evaluation[5] noted that the sampling, digestion procedure, and ICP-OES compositional analysis of bullets were appropriate. However, concern was expressed over the methods used for the comparison of compositional data from different bullets, and also the interpretation to be placed on such comparisons. The first involved consideration of the appropriate statistical methods to be used in comparisons of multivariate data, and included a recommendation to use a multivariate comparison such as Hotelling's T^2. The second involved the lack of knowledge about the source populations: how bullet lead varied in composition, and how likely it was that two random bullets might be compositionally indistinguishable, together with how conclusions were presented in court.

Compositional Fingerprinting

Compositional fingerprinting aims to characterize a sample in such a way that its similarity or dissimilarity to other samples can be evaluated. The characterization typically includes elemental or GC analysis of organic content, but could also include measurements such as isotope ratios. Commonly, many components are analyzed, with the naïve expectation[8] that discrimination will improve with an increased number of variables. The elemental analyses are frequently performed with instruments such as ICP-OES or inductively-coupled plasma–mass spectrometry (ICP-MS) that are capable of analyzing many elements in a single sample within a short timeframe. Alternatively, organic compounds can be characterized by an information-rich technique such as GC-MS. It is also possible to include measures other than chemical composition into a classification scheme, *e.g.* the patterns of minor peaks in the GC trace of an organic substance can be used for discrimination or classification of oil samples, even if it is not known what compound causes a given peak.[9] It is also possible to have categorical variables, colour, presence or absence of a component, *etc.*, included in a classification or discrimination scheme.

Questions addressed by compositional analysis

It is important to identify the question being asked of a given analysis. A common aim is to determine whether two samples are sufficiently similar that they could have come from the same source - the alternatives being that they are sufficiently dissimilar that they are unlikely to come from the same source, or that there is insufficient information available to make a decision. Such a decision can be referred to as *discrimination*. The identification of similar samples could be expanded to consideration of whether several samples should be placed in a given class – a *classification* decision. An alternative classification decision is to determine whether a sample came from a given source - region/manufacturer/lot, *etc.* There are two key questions that are implicit in such considerations: *What do we mean by source?* and *How do any classes identified within the samples relate to what one might regard as sources?* It is also important to consider the type and strength of conclusions that should be drawn from the observation that two samples are compositionally indistinguishable. Each of these issues is addressed below.

Measurement of elemental (or other) composition

Since fingerprinting analysis usually uses at least five elements, and may involve elements with widely differing concentrations, the most common techniques are ICP-OES and ICP-MS, with neutron activation analysis also having been used. For ICP-OES and quantitative ICP-MS this requires standards for all elements of interest, but if lower accuracy is acceptable ICP-MS in semi-quantitative mode (which does not require standards for all elements)

can be used. With many elements being analyzed over a wide range of concentrations, contamination and interferences are of serious concern, especially when the samples have complex matrices. For example, in a recent ICP-MS analysis of wine, we found that scandium ($^{45}Sc^{+}$) had false high readings due to interference from $^{12}C^{16}O_2{}^{1}H^{-}$ (from the high organic content of the samples),[10] while ^{153}Eu showed interference from $^{137}Ba^{16}O$ when the rare earth element concentrations were very low. Interference can also be caused by multiply charged ions, thus $^{86}Sr^{2+}$ can interfere with $^{43}Ca^{+}$ (^{86}Sr: 9.86%; Ca: 0.135% natural abundance), the latter ion having been used in ICP-MS because of the interference by $^{40}Ar^{+}$ and $^{12}C^{16}O_2{}^{+}$ on the more plentiful isotopes of Ca. In typical samples, the Sr concentration is so much lower than that of Ca that interference is not of concern. However, if a commercial standard with equal concentrations of these elements (provided so that users do not have to purchase many elemental standards separately) is used to standardize the ICP-MS it can lead to a systematic underreporting of the Ca concentrations. These particular interferences are problems in using the most common, and lowest-cost, ICP-MS instruments with quadrupole mass spectrometers – high-resolution ICP-MS instruments, such as that recently acquired at Otago University, are not subject to these interferences at high resolution. ICP-OES also has interferences, including spectral interferences, and this technique is also less sensitive than ICP-MS. However, it is suitable for analyses such as the composition of elements in lead bullets, where the high lead content makes routine ICP-MS analysis difficult.

Obtaining appropriate standards can be difficult also for other compositional analyses, *e.g.* standards for all biomarkers in crude oils can be very expensive. In such cases, it is sometimes possible to rely on GC-MS libraries, or to use a sample of crude oil for which peak identities are known to identify the peaks of interest, as is done for the Eurocrude crude oil identification protocol.[11]

Data Analysis

As noted earlier, component concentrations can vary widely. In general, it is important to ensure that those components with low concentrations receive adequate weight in the analysis (without unduly biasing the results) since trace components may be discriminating. This may require some form of data transformation prior to statistical analysis. It is also important to clearly define the limits of detection, and to decide how to deal with elements that lie below the limit of detection. This is especially true if a logarithmic transformation is to be used on the data prior to statistical analysis (a common practice for natural samples where concentrations are often log-normally distributed).[12]

There are two potential errors that can be made during comparisons: falsely matching samples (*false match*), or incorrectly stating that samples are different when, in fact, they are not (*false exclusion*). Any analysis needs to protect against both of these errors but, depending on the purpose of the analysis, one or other of these errors may be weighted more heavily.

A final consideration is whether or not the composition of the sample could have been altered prior to analysis. This is well known for oil-spill samples, where weathering processes such as evaporation, dissolution, and biodegradation can alter the composition observed by gas chromatography.[13] Compositional changes can also occur due to processing of a foodstuff, *e.g.* the fining of wine[14] or the recovery or smelting of metals.[15] Such changes may require the analyst to place less weight on (or even disregard) some elements or components.

The source

One of the key considerations in classification for provenance is to define and characterize the *source*. It may be a single, invariant material or there may be many sources, or the output from any source may vary with time. An obvious example of multiple invariant sources is human DNA, and a second is the biomarker profiles of crude oils that remain sufficiently constant so that spilled oil can be traced to the field of origin by use of a systematic comparison to a comprehensive database, *e.g.* that in Eurocrude.[11] Temporal variation is shown in examples such as biomarkers in diesel from the NZ refinery at Marsden Point (which uses many different crude oils as feedstock) and gold ore from South African mines (where the exact composition can vary within the veins being extracted from a given mine[15]).

Some source variation is useful since it provides increased potential for discrimination between different samples. However, it can make classification more difficult if the variability is not adequately characterized (through samples of known provenance) or modeled. The Marsden Point example can be expanded to show an additional problem of source identification. Thus it is possible to define many *sources* within the diesel supply chain: the oil fields, the tankers, the refinery, the batch, the service station, and the vehicle. Some of these sources may be useful in a given context whereas others may not. Once a source(s) and any temporal changes have been identified, the mean and spread of each variable characterizing that source (and time period) need to be obtained. Such studies also need to take into account other sources of variability (such as analytical measurement) and confounding factors such as alteration of the profile of the sample, *e.g. weathering* in oil spill analysis, and contamination. It is also necessary to know the overall spread of all populations, and how likely it is for two populations have similar compositions.

The NRC evaluation of the FBI CABL method used the term *compositionally indistinguishable volume* to denote the origin of a population of lead samples that would be analytically indistinguishable using the CABL technique.[5] It also noted that it would be possible for two *different* compositionally indistinguishable volumes to be indistinguishable using CABL, although there were insufficient data to estimate the frequency of this occurrence. The NRC committee estimated that the compositionally indistinguishable volumes of lead could range from ca. 30 to 100,000 kg in size, resulting in estimates of ca 12,000 to 35 million bullets coming from a given compositionally indistinguishable volume (for comparison, the report also

estimated that about 10 billion bullets are produced annually in the US).[5] Based on this high variability, combined with variability in the bullet manufacturing and packaging process, and lack of knowledge of the geographic distribution of bullets produced, the NRC stated that it was not possible to model the bullet manufacturing process in such a way as to predict the compositional distribution of bullets, and that it was not possible to make statements about either the specific times of manufacture of a given bullet or the probability of finding a bullet in a particular geographical area.[5]

The NRC evaluation would not have greatly impacted on decisions that bullets were dissimilar. However, it has been established that it is possible for a single box of bullets to contain samples from two or more different compositionally indistinguishable volumes,[5] so that the CABL analysis could not in general be used as exclusionary evidence between a crime scene and a suspect. In contrast, in many other applications of forensic compositional fingerprinting dissimilarity can result in a decision to exclude a match.

Linking observed classes to specific sources

Often it is only necessary to determine whether or not it is likely that two samples originated from the same *source*. However, sometimes it is desirable to go further than this and link samples to a particular *source*. The observation of clustering in a statistical evaluation of multivariate composition data does not necessarily allow identification with a meaningful source. The apparent clustering could be due to random variation, or it could reflect an effect associated with the processing of the item examined – examples include wines that have been treated with bentonite and show elevated rare earth element content[14] and the effects of metal recovery processes[15] on trace metal profiles in gold. Alternatively, clustering could be due to contamination or other environmental alteration of a subset of samples. If the observed clustering of samples correlates with known origins it may be possible to develop a rule linking samples to a particular origin. Alternatively, supervised classification methods could be used where an adequate number of samples with known provenance are used to develop the classification rules. In this latter case it is important that sufficient samples are used to derive the rules, and that they are representative of all the possible sources from which samples could originate. This requires setting up large inclusive databases that can involve large amounts of time and resources.

Conclusion

The manufacturing, processing, and distribution of bullets in the US are sufficiently variable that it is very difficult to assign a particular meaningful *source*, and differentiate that source from other potential sources. Without detailed distribution knowledge it is also difficult to know the significance of finding two bullets that come from the same source. Our reading of the recent decision to stop CABL analysis is that the FBI felt that it would take too many resources to gain adequate bullet population information compared to the number of times that the information would be used in court. Presumably the FBI decided that the NRC recommendation to present the comparison of two or more bullet analyses in court with the expert witness only stating that the result *is consistent with having come from the same compositionally indistinguishable volume* did not provide sufficient weight to the evidence to justify use of the technique. It should be noted that there are parallel limitations to data interpretations in other fields, *e.g.* the ASTM standard D5739-00 for comparison of spills of petroleum-based products states the oil samples should be interpreted as *similar*, *dissimilar* or *inconclusive*.[16] Such apparently limited statements can still be useful, *e.g.* if there are four ships in port, spilled oil may be described as similar to that obtained from one but dissimilar to the other three. This limitation in data interpretation contrasts to the situation for crude oils, where there is a limited, known population so that comparison of GC profiles with a comprehensive database can identify a match with greater certainty.

In general, discrimination or classification based on compositional analysis requires a large database that is representative of the complete population from which a sample may be drawn. Such a database needs to show the variability within samples, the variability between samples with the same source, and variability between samples with different sources. Such databases can represent substantial investments of time, effort, and money. Many research papers on provenance are based on relatively small databases that can allow discrimination between a few significantly different classes but are not suited for more subtle discrimination; they may not provide true estimates of classification error rates, if the samples are not truly representative of the source populations.

Many of these issues are also being addressed in forensic multi-element glass analysis, traditionally performed using refractive index comparisons. There is a reasonable body of knowledge of glass fragment occurrences, including surveys to estimate the extent and variability of glass fragments being found in the environment or on people, and there is good knowledge of the variability of refractive index across glass populations. However, glass manufacture is now so standardized that the refractive indices of samples of a given type of glass are very similar. Multi-element analysis has shown an improved ability to discriminate between different pieces of glass, and the development of analysis techniques and methods for data interpretation are active areas of research. Given its recent implementation, the databases for multi-element analysis of glass are smaller than those for refractive index, which again places limits on the strength of any conclusion based on glass fragment similarity. The statistical methods to be used in multielement glass discrimination are still under discussion, with recommendations by some authors that Hotelling's T^2 or similar be used,[17] or that a Bayesian approach is more appropriate.[18,19]

Multi-element analysis of gold from mining operations has shown similar needs for extensive databases. Differentiation of gold from significantly different ore deposits is possible with a relatively low number of reference samples.[3] However, differentiation of gold from mines within the relatively homogeneous ores of South Africa

has been much more difficult, and required precise measurements of many elements, combined with selected isotope ratios.[15,20] These latter studies also noted that processing of the gold ore could change selected elemental compositions, and that gold obtained from a given mine could change in profile as different veins were accessed or if a given vein had a composition gradient.

In conclusion, while many of the criticisms of the CABL analysis can be applied to other compositional analyses, there are also some unique features. The issues of statistical data analysis certainly apply to all multivariate comparisons, and there is still controversy over the best approaches to use. The issue that posed greatest difficulty for CABL, however, was the variability inherent in the source bullet populations, and lack of knowledge of the geographical variability of bullet distributions. Clearly, for any provenance study based on compositional analysis, it is imperative that an adequate number of samples be collected to define all possible sources. If the aim is to test for discrimination between samples then it is sufficient to collect enough samples to make good estimates of between-sample variability and within-sample variability. Finally, it is necessary to take note of the comments made in the NRC report on the language used in reporting results, so that the strength of any association is not overstated.

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2007 ROYAL SOCIETY OF CHEMISTRY AUSTRALASIAN LECTURESHIP

Applications are called for the 2007 Royal Society of Chemistry Australasian Lectureship.

This lectureship, financed by an annual grant from the RSC to Australia and New Zealand, is held by a New Zealand resident every fourth year. The 2003 lecturer was Professor Geoffrey Jameson (Massey University). The 2004-2005 lecturers were Professors Bob Gilbert (University of Sydney) and Allan Canty (University of Tasmania).

The lectureship involves lecture tours in Australia and New Zealand, coordinated by the respective RSC Local Representatives: Prof. Graham Bowmaker (University of Auckland) and Prof. Alan Bond (Monash University).

The selection panel for the 2007 Lectureship will be Prof. Graham Bowmaker (Auckland), Prof. John Spencer (Wellington), Prof. Leon Phillips (Christchurch) and A/Prof. Keith Gordon (President of the NZIC).

Applications should include a CV, and an account of the work to be covered in the lectures. The major part of the work should have been carried out in New Zealand.

Applications should be sent to:

Prof. G.A. Bowmaker
Department of Chemistry
University of Auckland
Private Bag 92019
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Email: ga.bowmaker@auckland.ac.nz

by the closing date of 31 August 2006.

Transfer RNA as a Potential Building Block for Nanotechnology

Harold S. Bernhardt and Warren P. Tate

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Nucleic acid nanotechnology

Introduction

It is almost 25 years ago since Nadrian Seeman first proposed the synthesis of X-shaped nucleic acid structures based on the Holliday junction, the cross-over structure of two strands of DNA that occurs as an intermediate in genetic recombination.[1] Since then, Seeman and other groups have produced these and other DNA structures, including a cube and an octahedron.[2-4] One of the main goals of nucleic acid nanotechnology has been the synthesis of components which will form self-assembling 3D arrays with such possible uses as molecular sieves, or scaffolds on which to fix macromolecules for X-ray crystallography, or for use in molecular electronics.

RNA as a potential building block

Over the last five years RNA has been used increasingly in this work. Although more labile than DNA, it has the advantage of being able to form a more diverse range of structures due to its more complex chemistry. It still retains the key attribute of nucleic acids: the specificity of Watson-Crick base-pairing interactions that allows the precise positioning of component parts to form a larger, predictable structure. Systems based upon base-pairing interactions have included the dimerization initiation site of HIV RNA and the right angle (RA) motif found in ribosomal RNA,[5,6] while those based on non-base-pairing tertiary structural associations have included the GAAA tetraloop-receptor interaction which occurs widely in biological systems.[7] A necessary requirement of such systems is inducibility - the ability to be able to control the rate of association; the addition of Mg^{2+} is commonly used to promote aggregation.

Is transfer RNA a candidate building block?

Perhaps surprisingly, as yet there have been no published reports using transfer RNA (tRNA) as a building block for the construction of nanomaterials (tRNA transfers individual amino acids to the growing polypeptide chain during ribosomal protein synthesis). This is surprising, since tRNA possesses a number of attributes that make it particularly suitable. It has a small size and high stability, a well-characterized cloverleaf (2D) and L-shape (3D) structure, and, most importantly, it has the ability to form dimers and higher aggregates through interactions between its single-stranded loops (anticodon loop, D-loop and T-loop). One of the best known examples is yeast $tRNA^{Asp(GUC)}$ (which transfers aspartic acid to the growing polypeptide chain), which forms dimers in solution and in the crystal through its quasi-self-complementary GUC anticodon (Fig. 1).[8] Another example is the A14G mutant human mitochondrial $tRNA^{Leu(UAA)}$ (which transfers leucine to the growing polypeptide chain in the synthesis of mitochondrial proteins), where the mutation creates a self-complementary six base sequence that allows formation of a dimer (Fig. 2).[9] This mutation is associated with two disease conditions, maternally-inherited diabetes and deafness (MIDD), and mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS).[9]

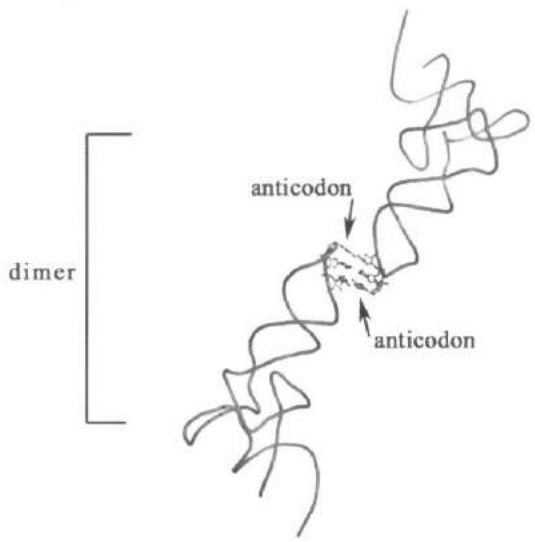

Fig. 1. A dimer from Yeast $tRNA^{Asp(GUC)}$. The graphic (taken from PDB file (see ref. 8) has only anticodons represented fully and shows 3D L-shape of tRNA.

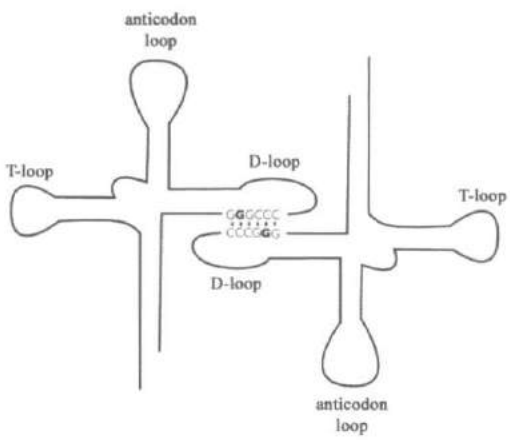

Fig. 2. Dimer from unmodified A14G mutant human mitochondrial $tRNA^{Leu(UAA)}$. The 2D schematic has only complementary sequences represented fully and shows cloverleaf secondary structure including D-loop, anticodon loop and T-loop positions (see ref. 9).

Experimental strategy

$tRNA^{Gly(GCC)}$ from E. coli

One tRNA that has been shown to aggregate is $tRNA^{Gly(GCC)}$ (which transfers glycine to the ribosome) from *E. coli*, yeast and *Bombyx mori* (silkworm).[10-12] Romby *et al.*[13] have provided experimental evidence that *E. coli* $tRNA^{Gly(GCC)}$

forms dimers through its GCC anticodon in a similar manner to yeast $tRNA^{Asp(GUC)}$, but unlike $tRNA^{Asp(GUC)}$, only at pH 4-5. They have suggested this is due to the formation of a stabilizing hemi-protonated C-C(+) base-pair between the two middle bases of the anticodons, whereas, at neutral pH, the C-C interaction is destabilizing.[13] Despite this evidence that it only forms dimers, we chose *E. coli* $tRNA^{Gly(GCC)}$ for our initial studies, partly because it appeared to have the most stable structure of the three $tRNA^{Gly(GCC)}$s, and for the reasons discussed below. As our laboratory already held samples of *E. coli* genomic DNA, our initial approach was to amplify the $tRNA^{Gly(GCC)}$ gene using PCR, and follow this with *in vitro* transcription to produce unmodified $tRNA^{Gly(GCC)}$. Although tRNAs *in vivo* are always modified at a number of positions, unmodified tRNA transcripts produced *in vitro* have been shown to have a similar, if somewhat looser structure. This difference was thought not to be a disadvantage for our studies - and perhaps even preferred, as suggested by the study of the A14G mutant human mitochondrial $tRNA^{Leu(UAA)}$ dimer that used unmodified *in vitro* transcripts.[9]

Multiple gene transcripts

E. coli possesses four identical copies of the $tRNA^{Gly(GCC)}$ gene that occur at two separate loci on opposite strands as well as opposite sides of the *E. coli* genome (Fig. 3).[14] *glyW* occurs as a single gene, while *glyV-glyX-glyY* occur as a triple gene cluster, with the three genes separated by short sequences of 36 and 35 base pairs. Since we wanted principally to amplify the single gene, we used internal primers complementary to the 5'- and 3'- ends of the tRNA, with the forward primer also containing the 17 base T7 RNA polymerase promoter sequence to enable subsequent *in vitro* transcription. We realized however, that the primers had the potential to amplify the double genes (*glyV-glyX* and *glyX-glyY*) and even the triple gene (*glyV-glyX-glyY*) (Fig. 4). The RNA transcripts of these products might possess interesting features of their own for nanotechnological building blocks. They should possess correct tRNA structures as the primary triple gene transcript is cleaved into individual tRNAs *in vivo* by RNase P, an enzyme that recognizes the tertiary structure of the tRNA.[14] The double and triple gene transcripts containing two or three GCC anticodons should also be capable of aggregating (at least at low pH) and the triple gene transcript that contains three GCC anticodons, might possibly be capable of forming 3D structures.

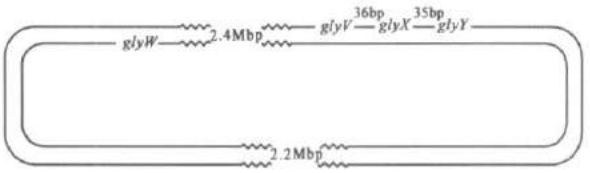

Fig. 3. *In vivo* transcribed $tRNAGly^{(GCC)}$ is a single gene product from *glyW* and a triple gene product from *glyV/glyX/glyY* (see ref. 14); (bp = base pair, Mbp = 10^6 base pairs).

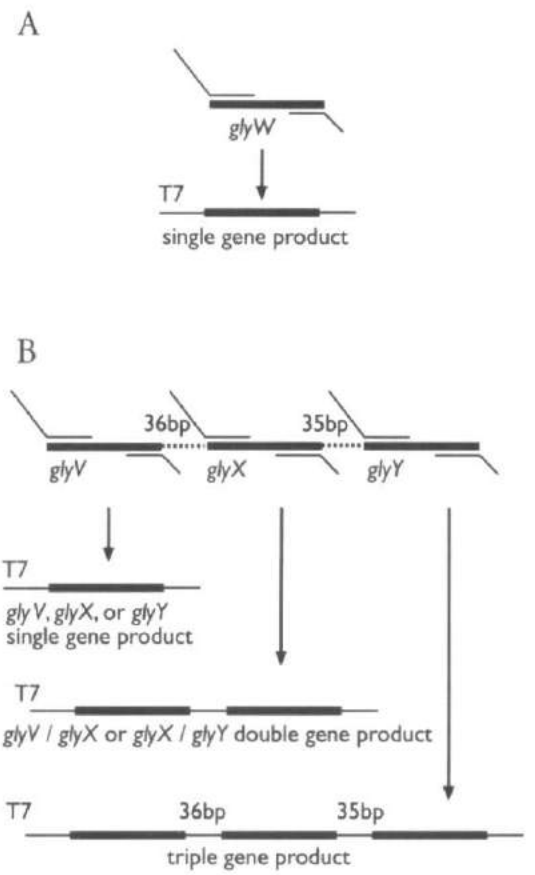

Fig. 4. (a) The predicted amplification of the single $tRNAGly^{(GCC)}$ gene product from the *glyW* locus, and (b) that of the single, double and triple $tRNAGly^{(GCC)}$ gene products from the *glyV/glyX/glyY* locus.

Experimental results

PCR of single, double, and triple gene products

Our first PCR experiment gave bands corresponding to the single, double and triple gene products, but the two double genes were not separated, as they differ in length by only a single base pair (Fig. 5). A higher molecular weight band running at >500 base pairs was unexpected and its origin has yet to be defined. Subsequent attempts to tweak the parameters and increase the yield of the double and (especially) triple gene product have so far been unsuccessful; no triple gene product was detected but consistent quantities of single and double gene products were obtained. This difficulty might be resolved by increasing the concentration of *E. coli* genomic DNA used, and/or by using a different DNA polymerase; strategies for the future.

In vitro *transcription*

In vitro transcription of particular tRNA genes can be notoriously difficult. Sometimes it has necessitated the use of tricks such as incorporating a copy of the hammerhead ribozyme gene in the tRNA gene-containing construct in order to increase the yield of tRNA product.[15] However, in our case, the DNA products generated by PCR (containing a mixture of single, double and triple $tRNA^{Gly(GCC)}$ genes) were converted successfully into RNA, utilizing direct *in vitro* transcription by T7 RNA polymerase; three bands corresponding to the single, double and triple $tRNA^{Gly(GCC)}$ gene transcripts were observed on a denaturing RNA gel (Fig. 6).

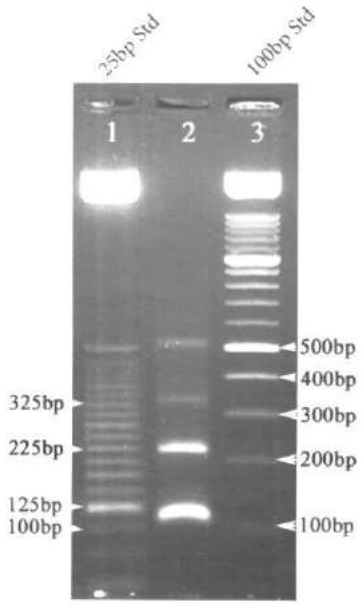

Fig. 5. PCR amplification of single, double and triple $tRNAGly^{(GCC)}$ genes from *E. coli* genomic DNA gave bands equivalent to those expected: 111, 222/223 and 334bp (with the two double gene products running as a single band) on a 2% agarose gel. The origin of an additional >500bp band shown here is unresolved. Lane 1, 25bp DNA standard (25bp band not visible); lane 2, PCR products (as described above); lane 3, 100bp DNA standard (bp = base pairs).

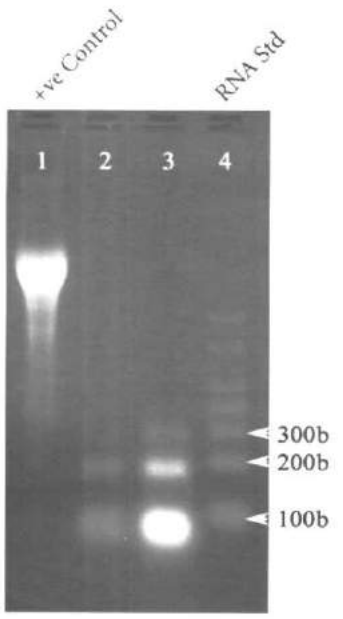

Fig. 6. Products ca. 85, 196/197 and 308b in size from *in vitro* transcription of single, double and triple $tRNA^{Gly(GCC)}$ gene using T7 RNA polymerase; lane 1 is a luciferase mRNA control and b = bases (see text).

In order to achieve these levels of transcription it was necessary to develop the *in vitro* transcription protocol by (*a*) quadrupling the initial T7 RNA polymerase concentration and reducing the concentration of rNTPs, template DNA, and RNase-inhibitor accordingly, and increasing reaction time to 16-24 h (Fig. 6, lane 2); and (*b*), by also adding an extra aliquot of T7 RNA polymerase 3 h into the reaction as described by Roy *et al.*[9] (Fig. 6, lane 3). As Fig. 6 shows, the addition of an extra aliquot of enzyme dramatically increases the level of transcription.

In vitro *transcripts of wild-type and A14G mutant human mitochondrial* $tRNA^{Leu(UAA)}$

Early in this work gifts of two pUC18 plasmids with constructs containing the wild-type and A14G mutant human mitochondrial $tRNA^{Leu(UAA)}$ genes, respectively, downstream of a T7 RNA polymerase promoter were received from Kelley's group (Department of Chemistry, Boston College). We were keen to use these as positive and negative controls for dimerization as Kelley's group had demonstrated that the A14G mutant (but not the wild-type) forms dimers under normal conditions.[9]

The plasmids were linearized using *MvaI* restriction enzyme. The plasmid fragments were separated on a 2% agarose gel, and the 155 base pair fragment that contained either the wild-type or A14G mutant $tRNA^{Leu(UAA)}$ gene with the T7 promoter was then purified by use of a gel-extraction kit. Finally, the wild-type and A14G mutant plasmid DNA fragments were incubated in an *in vitro* transcription reaction modified as previously described. Even then, and using similar amounts of template DNA for each of the three tRNAs, the two $tRNA^{Leu(UAA)}$ plasmid fragments produced a much lower level of transcription than the single $tRNA^{Gly(GCC)}$ gene PCR product as shown in Fig. 7. The single $tRNA^{Gly(GCC)}$ gene was obtained by gel extraction of the single gene band on a preparative 2% agarose gel of a PCR reaction similar to that shown in Fig. 5.

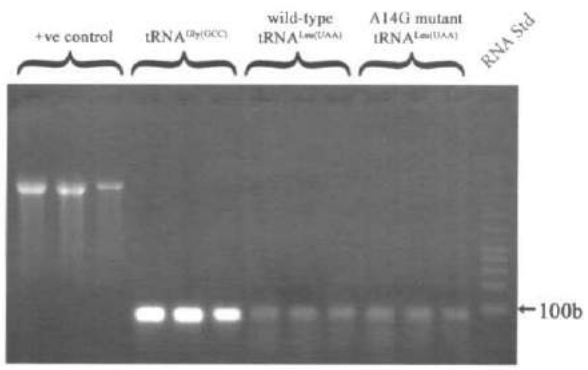

Fig. 7. *In vitro* transcription of single $tRNA^{Gly(GCC)}$ gene and wild-type and A14G mutant human mitochondrial $tRNA^{Leu(UAA)}$ genes; luciferase mRNA positive control; 1st lane of each set of 3: *in vitro* transcription; 2nd lane: DNase cleavage of the template DNA; 3rd lane: RNA extraction.

Reportedly, *in vitro* transcription from a plasmid fragment (as is the case with wild-type/A14G mutant $tRNA^{Leu(UAA)}$) is three to four times more efficient than that directly from a PCR product (as is the case with $tRNA^{Gly(GCC)}$). This makes the difference in level of transcription even more striking and probably results from the different internal promoter sequences of *E. coli* $tRNA^{Gly(GCC)}$ and human mitochondrial $tRNA^{Leu(UAA)}$. As already noted, each of the constructs has a 17 base T7 RNA polymerase promoter sequence upstream of the tRNA. However, the first six bases of the tRNA itself also play a part in the binding of T7 RNA polymerase by functioning as an internal promoter. The internal promoter sequence of *E. coli* $tRNA^{Gly(GCC)}$ is much closer to the ideal T7 sequence than that of human mitochondrial $tRNA^{Leu(UAA)}$ thus allowing more efficient transcription.

Aggregation of building block tRNAs analyzed by native gel electrophoresis

Using single $tRNA^{Gly(GCC)}$ transcript and wild-type and A14G mutant human mitochondrial $tRNA^{Leu(UAA)}$, preliminary experiments to effect aggregation consisted of heating the tRNAs to 70°C for 5 min, rapid cooling on wet ice, and addition of Mg^{2+} to give a 10 mM final concentration as described by Roy *et al.*[9] Aliquots of these reactions have been electrophoresed using native PAGE at either 4°C or RT for 1-4 h. The results provide preliminary evidence of multiple bands with the A14G mutant human mitochondrial $tRNA^{Leu(UAA)}$, *i.e.* aggregation. In order to observe aggregation of the $tRNA^{Gly(GCC)}$ single transcript the gel should be at pH 4-5; the use of a size separation column to isolate the dimer is yet to be investigated.

In search of the first tRNA

An RNA world

In addition to possible applications in nanotechnology, we are also interested in the possible relationship between tRNA aggregation and the origin and evolution of tRNA function, the genetic code and protein synthesis. It appears likely that life was based on RNA prior to the advent of protein synthesis as it can function both as an enzyme and a carrier of genetic information; this is known as the *RNA world*. In light of this hypothesis, and the central role of tRNA in protein synthesis, it seems almost certain that tRNA is an extremely ancient molecule, with its primary, secondary and tertiary structure highly conserved over all three kingdoms of life.

Hairpin loops

Di Giulio has proposed that tRNA arose by the duplication of a shorter hairpin loop.[16] Paul Schimmel's group has demonstrated that hairpin loops combining a single-stranded 3'-terminal CCA sequence with stem sequences related to those of a number of tRNAs are specifically aminoacylated by contemporary aminoacyl-tRNA synthetases, and so could have participated in an early version of non-templated protein synthesis in the absence of the equivalent of an mRNA message.[17] The same group has also demonstrated lateral (side-by-side) H-bonding interactions between these loops that could have brought the two 3'-CCA ends into proximity, enabling the polymerization of amino acids into proteins prior to the advent of the ribosome.[18] Due to the symmetry of base-pairing interactions, it seems possible that these single hairpin loops may have been in equilibrium with hairpin loop-duplexes having a 2D structure similar to tRNA (Fig. 8).

Split tRNAs

Recently, it has been discovered that the archaeal species *Nanoarchaeum equitans* has four split tRNA genes with the split occurring between bases 37 and 38 in the anticodon loop.[19] This is also the most common insertion position for tRNA introns. The anticodon loop sequence of the overwhelming majority of glycine tRNAs that participate in protein synthesis is 5'- YUNCCA↓N - 3', where 'Y' denotes U or C, 'N' any base, and the NCC anticodon is underlined.[20] If one were to cleave glycine tRNA between bases 37 and 38, the position of the arrow in the above, one would be left with two *half* tRNAs of almost identical size, each with a 3'-terminal -CCA, and so conceivably both descended from a single hairpin loop that was aminoacylated by glycine and participated in non-templated protein synthesis.

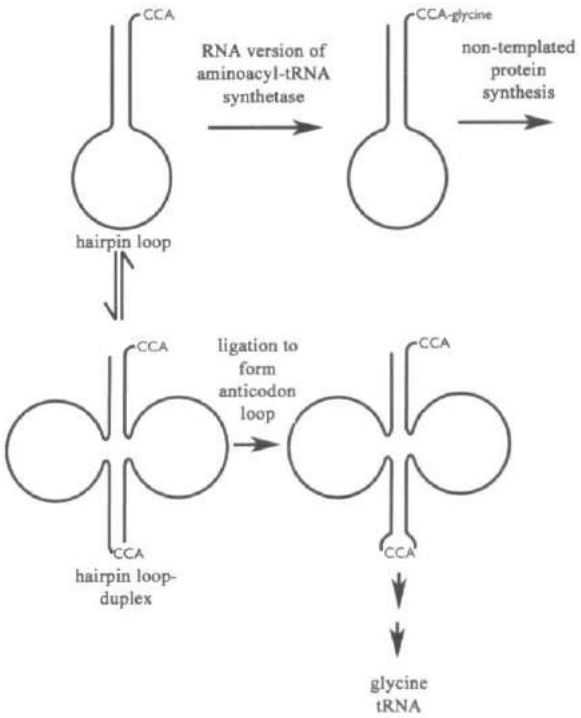

Fig. 8. Proposed evolution of glycine tRNA from hairpin loop.

Origin of the genetic code

Thus it seems possible that tRNA arose by the ligation of two identical hairpin loops, with CCA becoming part of the anticodon loop, (N)CC the first anticodon/GG(N) the first codon (coding for glycine), and glycine tRNA the first tRNA (Fig. 8). That glycine tRNA might have been the first tRNA is not inconsistent with the argument that glycine was possibly the first amino acid. After all it is the smallest, simplest, and only achiral amino acid, and it is almost always produced in experiments that seek to simulate earth's proposed early atmosphere. One of the most highly conserved sequences among cytoplasmic tRNAs is the D-loop -GG- sequence, which in $tRNA^{Gly(GCC)}$ forms part of a seven base sequence complementary to the anticodon loop. It is possible that this highly conserved -GG- sequence is a descendent of the ancestor of the first codon, and that the first anticodon-codon interaction originally had a role in tRNA aggregation.

Summary

We hope that this article gives a taste of the potential for this field of study. Not only may it lead to the creation of 3D arrays for use in nanotechnological applications, but also it may be of critical importance to our understanding of the origin and evolution of tRNA, the genetic code and protein synthesis.

Acknowledgements

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Article

Are Nanoparticles Safe?

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Introduction

Despite the wide application of nanomaterials there is a serious lack of information on their impact on human health and the environment. Engineered nanomaterials are currently found in diverse products: personal care items, sunscreens, abrasion-resistant materials, environmental catalysts, anti-fouling and anti-microbial coatings, in wood preservation, fuel cells, UV-attenuation, scratch resistant and charge dissipating coatings, and even food products.[1] Production ranges from the multi-tonnages of carbon black and fumed silica, for plastic fillers and car tyres, to microgram quantities of quantum dots as biological markers. Nanoparticles are small enough to penetrate small capillaries and pass through biological membranes such that nano-encapsulated therapeutic agents are being proposed or in clinical trials for a wide variety of treatments because of selective targeting and minimisation of side effects.[2]

But are nanoparticles safe to use? Their small size and unique properties may cause adverse effects not found in their larger analogues. Scientific evidence on possible effects on the human body and the environment is just beginning to emerge but there is minimal information on dominant exposure routes, exposure levels, and material toxicity. Consequently, advocates and opponents (who often erroneously equate asbestos to a nanoparticle) of nanotechnology have little information to support or refute their respective position. There is no doubt that toxological effects will vary with the structure, redox activity and preparative history of the nanoparticles in question. This, coupled with the diverse exposure routes, makes for a complex risk analysis.

Herein we look at the possible entry points into the human body and give an assessment of the known risk for some nanoparticles.

Portals for Nanoparticles

As already noted, the size of nanoparticles makes them highly mobile in both humans and the environment. Therefore, they can enter the body through several ports. Translocation can then occur via the blood stream leading to an accumulation in many tissues including the brain and testes.[3] It is still not known whether cells internalise nanoparticles, but at the cellular level nanoparticles can act as a gene vector.[4] Carbon black particles are thought to interfere with cell signalling,[5] an observation that has seen DNA used for the size separation of nanotubes (the DNA wraps itself around the nanotube if the tube diameter is right[6]) but this observation equally raises concerns over the effect of carbon nanotubes on the human body.

Skin

Human skin (ca. 1.5 m^2 in area in an adult human) normally functions as a strict barrier. Despite use in cosmetic and sunscreen products, there is only limited literature on, for example, the penetration of fine-size TiO_2, and none on nano-TiO_2. Nanoparticles may reach the epidermis, and occasionally the dermis, through mechanical agitation but penetration is limited by the hair follicle. There is no hard evidence to suggest they can enter the systemic circulation by this route.

Lung

Many nanomaterials and devices are formed from, or use, aerosols and colloidal suspensions so that exposure is most likely to happen through lung inhalation. While the airways are a relatively robust barrier, in the gas exchange area (the 300 x 10^6 alveoli) the barrier between the alveolar wall and the capillaries is very thin - merely 0.5 μ away from the blood flow. Spherical solid material can be inhaled when its aerodynamic diameter is <10 μm and so nanoparticles travel deeper into the lungs and will deposit in the alveoli via Brownian motion. If inhaled concentrations are low, then the retention time is about 70 days. Since the alveolar macrophages are the defence cells of the lung, nanoparticle accumulation could result in inflammation. Larger particles transmigrate from the alveolar regions to outside the lungs more rapidly, resulting in far greater particle clearance and less risk of inflammation.

Intestine

The intestinal tract is a more complex barrier, and while there are many similarities in the entry of nanomaterials here to that of the lungs, there are also important differences. Non-specific interaction often reduces the toxicity of ingested nanoparticles and consequently they may be less cytotoxic. The transit through the intestinal tract is relatively fast and, as nanomaterials do not remain long in the intestinal tract, their presence will not automatically induce an inflammatory response. In the intestinal tract, the ingested materials move from acidic (stomach) to basic conditions and this markedly changes solubility and the surface characteristics of the particle.

Translocation

Nanoparticles are most likely to enter the body via ingestion and inhalation. Enzymes and the physiological environment could change the properties of nanoparticles (particularly surface activity) and the question *What is the structure of* in vivo *nanoparticles?* has not been answered. This is a particular issue for redox-active metals because cationic nanoparticles would have an immediate toxic effect on the blood-brain barrier.

Once in the body there is no doubt that nanoparticles can translocate to organs and tissues and bioaccumulate. Systemic distribution to other organs, across the blood-brain barrier, and penetration of the blood–testis barrier has been demonstrated.[3] The passage of solid material from the pulmonary epithelium to the circulation system appears to be restricted to nanoparticles. Recent inhalation experiments with rats showed that nanoparticles (25 nm) had reached several organs after 24 h of exposure and (amazingly) the central nervous system. Transportation via the nerves was at a speed of 2.5 mm per hour! Nanoparticles that enter the liver have been found to induce local oxidative stress and, because of the production of radicals, modify the hepatocyte antioxidant systems, but there is no definitive evidence to implicate nanoparticles in liver damage in other than rats.

Translocation from the intestine to lymphatic tissue and capillaries undoubtedly is possible and immune responses may be triggered (such as implicated in Crohn's disease), but to date there are no data to suggest that humans may be affected by transport of the nanoparticle via this portal.

Specific Nanoparticles

Each type of nanoparticle will exhibit its own unique biological or ecological response that will also differ with shape and dosage. It is important to realise that a wide range of nanoparticles have been shown to create reactive oxygen species both *in vivo* and *in vitro* and hence have the potential to induce cell damage. We now provide specific data for nano-sized anatase (*p-TiO*$_2$), the archetypal industrial nanoparticle, and give an overview of the toxic response for some others.

Titanium dioxide

Fine-sized (<2.5 μm) TiO_2, consisting of agglomerates of needle-like particles ca. 20 nm x 100 nm in size (Fig. 1-upper), still is produced mainly by the classical batch sulfate or continuous chloride processes with an annual production of ca. 3.5 million tonne p.a. Nano-sized TiO_2 can be produced from the coarser material by aerosol or gel techniques but a variety of direct methods have been developed, especially starting from $Ti(OPr\text{-}i)_4$. These methods include mesoporous film formation from reverse micelles, supercritical fluid drying of gels, direct particle synthesis under supercritical conditions, and templated approaches.[7] The latter approach can also produce nanotubes (Fig. 1-lower). Gel methods are particularly important as they limit particle nucleation, growth and agglomeration[8] - a significant characteristic of nanoparticles. A recent survey of p-TiO_2 material sold in the US found that it had a size range of 20-50 nm. These are the materials now found in, *e.g.* sunscreens, cosmetics, bulk sprays, powders, coatings, scratch resistant sunglasses, stain repellent fabrics, and anti-graffiti coatings for walls.

TiO_2 is one of several dusts that are grouped into the category of poorly soluble particulates (PSP) by virtue of their low solubility in water and their toxicity. Pulmonary (lung) inflammation is a common response to the inhalation of PSP and has been closely associated with, *e.g.* fibrosis and cancer. In rats, it was found that p-TiO_2 cleared more slowly than fine-sized TiO_2 and translocated more efficiently to lymph nodes. Furthermore, the biological effects correlate better with surface area rather than mass.[9] One study suggested[10] that low exposures (10 mg/m^3) resulted in greater tumour incidence than high exposures (250 mg/m^3). Recent research using p-TiO_2 dots and rods indicated that the surface chemistry of p-TiO_2 may have a role.[11] With silica, cytotoxicity can be correlated with surface area which, in turn, influences the appearance of surface radicals and reactive oxygen species. The surface of TiO_2 is known to be activated to radical formation and analogous toxological responses to silica cannot be ruled out. Furthermore, size specific deposition of nanoparticles, when inhaled as single particles rather than aggregates, appear to contribute to their surface properties and free radical generation. Recent studies on a number of commercial formulations of pigment-grade TiO_2 particles indicate that different surface coatings and surface treatments can also influence the pulmonary toxicity.

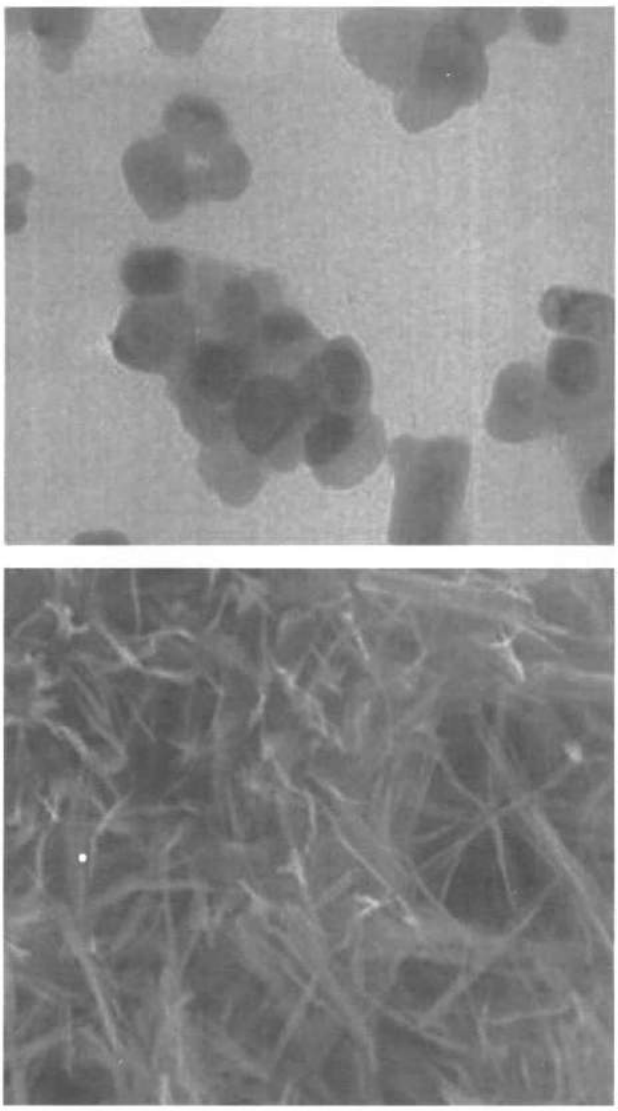

Fig. 1. (upper) Aggregated p-TiO_2; (lower) TiO_2 nanotubes (unpublished work, Otago.

Notwithstanding the experimental evidence for enhanced inflammatory response with p-TiO_2 in rats, we must be cautious in extrapolating this to human responses. Firstly,

nanoparticles have a tendency to clump together and may reach the body as aggregates rather than free entities; all laboratory studies have used artificial nanoparticle aerosols. Secondly, there is evidence to suggest that PSP-induced effects may be unique to the rat as they process inhaled particles in a very different manner to larger mammals and humans. Pulmonary TiO_2 overload leads to the development of pulmonary tumours only in rats[12] and they have a more severe and persistent pulmonary inflammatory response than either mice or hamsters to aerosol p-TiO_2. As yet there is no information on the effects on human health from p-TiO_2 inhalation. Studies involving coal miners exposed to coal-mine dust over a long period of time suggest that humans do not develop overload related tumours. Such evidence, combined with findings from the few studies conducted on particulate exposed primates, indicates that the lungs of larger mammals are less reactive to dust burden insults than rats.

Silver and other metal or metal oxide nanoparticles

Silver nanoparticles (Fig. 2), as antimicrobial agents, have been proposed as constituents of bone cement and other implantable devices.[13] There are clear toxological risks from such use as the nano-Ag could penetrate the dermis and then translocate. Both sperm-stem and liver cells have shown sensitivity to 15 nm Ag, in contrast to ultrafine $AgCO_3$ which had no effect. The cytotoxicity of Ag is related to oxidative stress. Nanoparticles of other metals, and many metal oxides are likely to generate reactive oxygen species but the limited evidence available suggests that nano-Ag is very toxic relative to most other metals and metal oxides. Studies using liver cells classed nano-Ag as highly toxic, nano-MoO_3 moderately toxic, and nano-Fe_3O_4, MnO_2, Al and W non-toxic at low dosage (10- 50 μg/ml); the toxicity increased at higher concentrations (>100 μg/ml).[14,15] Fortunately, some work suggests that metallic nanoparticles are less likely to translocate from the lung to extrapulmonary organs.[16]

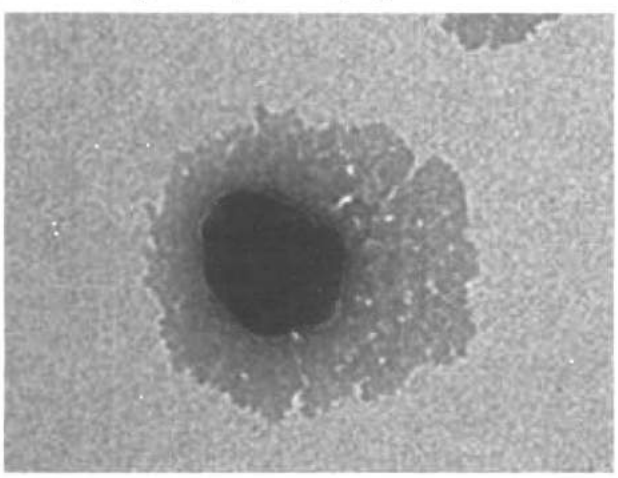

Fig. 2. Ag nanoparticles in chitosan matrix (unpublished work, Otago)

Quantum Dots

Quantum dots (QDs), such as CdSe, have been introduced as new fluorophores for use in bioimaging but to date there is little toxological information on them in the literature. While bulk CdSe is cytotoxic, it has been suggested that CdSe quantum dots are cytocompatible and safe for use in whole animal studies. This postulate is based in part on the use of protective coatings around the CdSe core of the quantum dot. Recent studies found that the cytotoxicity of CdSe QDs towards the liver correlated with the liberation of Cd^{2+} ions due to deterioration of the CdSe lattice.[15] These data suggest that while CdSe QDs may be nontoxic initially for *in vivo* use when appropriately coated, further work is needed on their long-term stability, both *in vivo* and when exposed to environmental conditions.

Nanocarbon

Humans have been exposed to carbon nanoparticles for millennia and carbon nanoforms have been a component of the natural atmosphere since combustion was discovered. As noted already, oxidative stress as a common mechanism for cell damage induced by nanoparticles and free radical cell damage has been demonstrated for C_{60} fullerenes and carbon nanotubes.[17]

Studies on single-walled carbon nanotubes have tended to use uncharacterised materials; it is unclear whether they are unaggregated, aggregated fibrils, nanoropes, carbon black, or mixtures. Therefore, results indicating that single-walled carbon nanotubes inhibit the proliferation of kidney cells in cell culture and cause lung inflammation must be treated with caution. Multi-walled carbon nanotubes (MWCNT) persist in the deep lung after inhalation and, once there, induce both inflammatory and fibrotic reactions in rats. They behave similarly to the notorious nanotube, *chrysotile asbestos*, suggesting that the health risks from exposure to carbon nanoforms may be severe, with an increased risk of carcinogenesis. Occupational exposure limits for the asbestos are ca. 10^6–10^7 fibres/m^3 over an 8 h period. Particle concentrations of nanocarbon aggregates from cooking are ~10^4–10^5/m^3, but as each aggregate may contain 10^3 MWCNT, the asbestos limit could be exceeded if around 10% of the MWCNT made their way to the lung during a 30 min. session at the stove or barbeque!

Charge properties, and the ability of carbon nanoparticles to affect the integrity of the blood-brain barrier as well exhibit chemical effects within the brain, have also been studied. It appears that neutral and low concentration anionic nanocarbon can serve as carrier molecules giving chemicals direct access to the brain; cationic nanoparticles have an immediate toxic effect at the blood-brain barrier.[18] Tests with uncoated, water soluble, colloidal C_{60} fullerenes have shown that redox-active, lipophilic carbon nanoparticles are capable of producing oxidative damage in the brains of aquatic species as well as humans.[19]

Conclusion

Whether or not the use of nanoparticles poses a significant health risk remains unknown. Careful scientific scrutiny of the sparse data, rather than journalistic hype, is required to give an answer to the question posed at the beginning of this article. There is no doubt that nanoparticles can enter the human body via the lungs and the intestines. Once in the body their translocation is a strong function of the surface characteristics of the particles. Nano-sized particles

are more likely to result in a higher lung burden, possibly amplifying any possible chronic effects. Recent studies on carbon nanotubes indicate that they can induce a rather general non-specific pulmonary response. But there is no universal nanoparticle and not all nanoparticles will be either benign or toxic. The presence of contaminants, such as metal catalysts in nanotubes, poses an added risk for evaluation. It is a challenge to devise high throughput and low cost toxological tests for nanoparticles without hindering the advancement of nanotechnology.

Our current understanding on their toxicity centres largely around a limited number of studies conducted (mainly) on laboratory animals, or cell cultures, where the response may not mirror that in a human, nor do they include any *in vivo* interactions.[20] Risk assessment must include the actual toxicity plus the exposure time, and the exposure component is largely unknown. There is also an assumption that because a fine-sized particle, *e.g.* TiO_2, has been approved for use and has no known carcinogenic properties, the nano-equivalent is also safe – a rather large assumption! Particle size is critical. Gold, usually considered inert, is a case in point. Nanogold particles are highly reactive and, for example, can move across the placenta from mother to fetus. The translocation of nanoparticles is certainly an issue the importance of which will only become evident over time.

Chemists in NZ should adopt a precautionary stance and assume that nanoparticles are a potential hazard until shown otherwise, eliminate their presence in the environment, and minimise their unintentional release. Many overseas universities, *e.g.* Cornell, and industrial laboratories have adopted this stance and developed protocols for safe working conditions.

Acknowledgements

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Article

Overtone Spectroscopy: A Sensitive Probe of Hydrogen Bonding

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Hydrogen bonded complexes in the Earth's atmosphere recently have been shown to be of potential importance to climate change.[1,2] These complexes are generally held together by a weak hydrogen bond as shown in Fig. 1 for the water dimer,[3] and are difficult to study under conditions relevant to the atmosphere. So far only the water dimer and the van der Waals complexes, the molecular oxygen dimer (O_2•O_2) and the dimer between molecular oxygen and molecular nitrogen (O_2•N_2), have been observed in the atmosphere or under atmospheric conditions.[4-7] One of the key questions regarding hydrogen bonded complexes is how their spectroscopy affects the absorption of solar radiation in the near infrared (NIR) and visible wavelength regions. The near infrared region is dominated by low-lying electronic transitions and vibrational overtones of XH-stretching transitions (X = any heavy atom). Although there are no distinct boundaries for the near infrared region, typically it is considered to lie within 4000-14,000 cm^{-1}. The calculated changes in absorbance of hydrated complexes H_2O•M, (M is a molecule present in the atmosphere) in the NIR and visible regions have supported the suggestion[8-10] that the complexes are part of the explanation for the so called water vapour continuum, an empirical continuum used in radiative transfer modelling.[11-13]

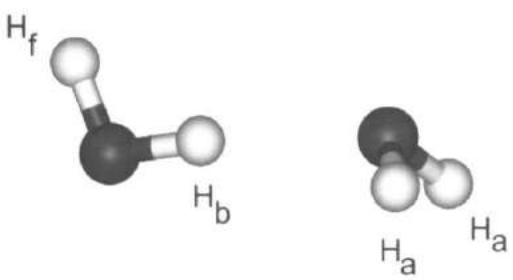

Fig. 1. The structure of the hydrogen bonded water dimer; the OH_b bond length is elongated due to the presence of the other water molecule.

The equilibrium constants for hydrogen bonded complexes in the vapour phase are typically small, which makes their study difficult. Consequently, our attention has been directed to the spectroscopy of molecules with internal hydrogen bonds. There are some infrared studies of hydrogen bonded systems in the vapour phase, however studies in the overtone regions are very limited.[14] One reason for the lack of study is that overtone transitions are inherently weak. Their intensity typically drops by an order of magnitude with each successive quantum of vibrational excitation. Another reason is that many species that undergo hydrogen bonding have relatively low vapour pressures, so low sample concentration is an issue. To overcome these hurdles, one or a combination of conditions such as long sample path lengths, high temperatures, or sensitive spectroscopic techniques are required. One sensitive technique is intracavity laser photoacoustic spectroscopy.

Intracavity photoacoustic spectroscopy

The photoacoustic effect was serendipitously discovered by Alexander Graham Bell in 1880 when he observed that an audible sound is produced when modulated sunlight is incident on an optically absorbing material.[15] Photoacoustic spectroscopy can be categorized as a photocalorimetric or photothermal technique, since it measures the internal heating of a sample due to the absorption of radiation. When a sample is illuminated with modulated monochromatic light, and if some of that light is absorbed by the sample, internal energy levels within the sample are excited. Subsequent de-excitation (relaxation) of the excited states results in all or part of the absorbed energy being transformed into heat energy through nonradiative decay processes. Since the incident light is intensity modulated, the internal heating of the sample is also modulated. This modulated heating creates pressure waves that are detected as sound by a microphone. Modern microphones and related electronic devices are capable of detecting pressure waves caused by temperature rises as low as 10^{-6} °C in gaseous samples.[16] We use microphones that were originally designed for hearing aids.

There are several advantages of photoacoustic spectroscopy over conventional spectroscopy. Since absorption of light by a sample is required before a photoacoustic signal is produced, light that is elastically scattered or transmitted is not detected, and consequently it does not interfere with the intrinsically absorptive photoacoustic measurement. Thus photoacoustic spectroscopy can be considered as a zero noise background technique, and this sensitivity allows for the study of trace amounts of species or species with weak absorptivities. The use of high-powered tunable lasers, such as a titanium:sapphire laser and a dye laser as in our photoacoustic spectrometer, makes this technique very sensitive. The dye and titanium:sapphire lasers of our photoacoustic spectrometer are shown in Figs. 2 and 3.

As mentioned, the vapour pressure of a hydrogen-bonded species is typically low. The vapour pressure of the sample can be increased with heating. We have used a 250 watt heat lamp suspended over the photoacoustic cell to conduct variable temperature photoacoustic experiments. A photograph of this setup is shown in Fig. 3. The temperature can be simply varied by altering the distance of the lamp to the cell. The location around the cavity of the titanium:sapphire laser where the photoacoustic cell is placed is lined with aluminium foil to reflect heat. The foil has an extra role as a dust cover for the laser as the normal

one could not be used during a heating experiment. This heating method has proved quite effective, for temperatures up to 60 °C, with the temperature remaining within ±1 °C during a typical 2 h scan. The vapour pressure increases exponentially with temperature so even a small temperature increase has a significant effect on the vapour pressure. Recently, using our photoacoustic spectrometer, we have recorded vapour phase overtone spectra of compounds that are solids at room temperature.

Fig. 2. The dye laser of a photoacoustic spectrometer in operation; the cavity of the laser extends from the bright spot (front) along the visible beam and the sample cell is housed in it.

Fig. 3. Heating lamp suspended over a titanium:sapphire laser; the photoacoustic cell is in the laser cavity with its glass tip pointing towards the heat lamp.

Intramolecular hydrogen bonding in overtone spectra

There are only a few cases of vapour phase overtone spectra of molecules that are solids at room temperature and these include phenol[17] and naphthalene[18,19] with room temperature pressures of about 0.35 Torr and 50 mTorr, respectively. Herein we present our recent vapour phase overtone spectra of catechol (1,2-dihydroxybenzene) and ethylene glycol (1,2-ethanediol), both molecules with room temperature vapour pressures of a few mTorr.[20,21] The NIR/vis spectra of molecules containing OH bonds are dominated by transitions that involve OH-stretching overtones. These overtone transitions are described well by the local mode model of molecular vibration,[22,23] where each of the non-equivalent OH bonds is described by an isolated anharmonic oscillator, typically the Morse oscillator. We have developed a theoretical model that allows calculation the vibrational overtone spectra from first principles.[21,24,25]

Catechol contains an intramolecular hydrogen bond between the two OH bonds as shown in Fig. 4. The hydrogen bond angle (O-H_b•••O) in catechol is that of a quasi-five-membered ring, far from an optimal linear conformation.[26] Thus the intramolecular hydrogen bond in catechol is expected to be weak. The second OH-stretching overtone spectrum of catechol vapour is shown in Fig. 5. Two distinct peaks are observed in the room temperature intracavity laser photoacoustic spectrum of catechol corresponding to the *free* and *bonded* OH bonds (labelled OH_f and OH_b). The weak intramolecular hydrogen bonding stretches the OH_b bond by about 0.4 pm.[20] It is clear from Fig. 5 that this small change in the OH bond length leads to a large and easily observable frequency shift of the associated vibrational overtone transition. The shift in frequency increases with vibrational excitation making it desirable to record higher overtones. However, as mentioned above, the intensity of these overtone transition decreases with vibrational excitation. This overtone spectrum of catechol can also be recorded at very cold temperatures (1 K) under jet conditions with a technique called *non-resonant ionization detection*.[27] This has the advantage of producing narrow band widths of a few wave numbers and makes it possible to observe even smaller changes in the OH bonds. By combining overtone spectroscopy and the jet cooled technique we have been able to resolve conformational isomers in *m*-aminophenol, where the calculated bond length difference is less than 0.002 pm.[28]

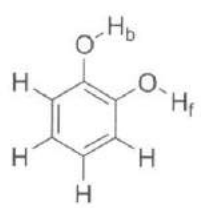

Fig. 4. Catechol is planar with two distinct OH bonds.

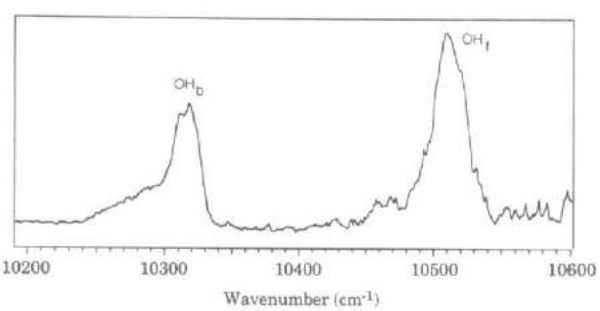

Fig. 5. Intracavity laser photoacoustic spectrum of vapour phase catechol in the 3 quanta of OH-stretch (second overtone) region; the OH_b stretching second overtone is at 10320 cm^{-1} and that for the OH_f stretching is at 10500 cm^{-1}.

Ethylene glycol is one of the simplest molecules with two vicinal OH groups, and it can serve as a simple model for biological molecules such as sugars. Similar to catechol, the conformation of the intramolecular hydrogen bond in ethylene glycol comprises of a five-membered ring. It is a triple rotor molecule that can exist in one of 27 (3^3) conformations. Some of the structures are degenerate due to symmetry and the number of unique conformations is reduced to 10. Of these 10, the 2 most stable contain an intramolecular hydrogen bond (Fig. 6) with conformer 1 favoured over conformer 2 by approximately 2 kJ mol^{-1}. The remaining 8 structures are predicted to have significantly lower populations.[21,29]

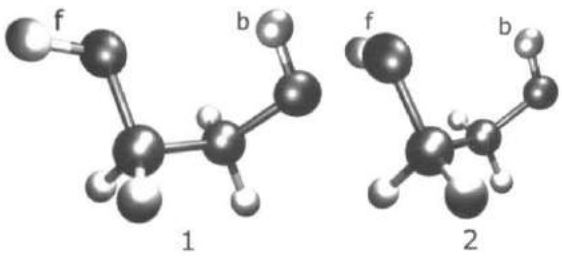

Fig. 6. The two most stable structures of ethylene glycol.

The vapour phase OH-stretching spectra of ethylene glycol in the 2nd to 4th overtone regions are presented in Fig. 7. The abscissa of each overtone region is 550 cm^{-1} wide to illustrate the typical spreading apart of the transitions with increasing vibrational excitation. In these higher overtone spectra, it is possible to identify the transitions from the two lowest energy structures. We have labelled the OH_b and OH_f stretching transitions of structures 1 and 2 as 1b/2b and 1f/2f, respectively. The bonded transitions are observed to undergo a red shift relative to the free transitions, indicative of the presence of hydrogen bonding.

Our experimental work as illustrated by the spectra of catechol and ethylene glycol is complemented with theoretical studies. We use the local mode model of molecular vibration mentioned before to model the OH-stretching overtone transitions using high level ab initio quantum calculations to solve the electronic Schrödinger equation. Potential energy and dipole moment curves are obtained and these make it possible to calculate the frequencies and intensities of the vibrational overtone transitions. The calculated accuracy is highly dependent on the level of the ab initio method applied to the molecule. Recently, we found that with *high level coupled cluster* methods it is possible to obtain accurate predictions of the frequency (ω) and anharmonicity (ωx) of the anharmonic local mode oscillator. The experimental and calculated frequencies and anharmonicities of the lowest energy conformer of ethylene glycol are given in Table 1 (The data are obtained from a potential energy curve calculated with the coupled cluster theory including singles doubles and pertubative triples (CCSD(T)) combined with Dunning's augmented triple zeta (aug-cc-pVTZ) basis set[21]). The agreement between calculated and observed local mode parameters is excellent. This makes it possible to calculate accurate spectra of molecules that have not previously been observed.

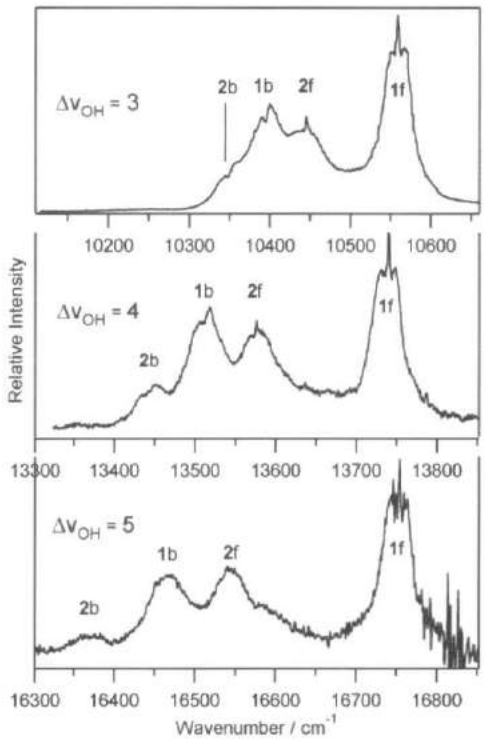

Fig. 7. Photoacoustic spectra of ethylene glycol vapour in the 3 to 5 quanta of OH-stretch regions; the *bonded* and *free* transitions of the two most stable structures are labelled.

Table 1. Observed and CCSD(T) calculated OH-stretching local mode parameters (in cm^{-1}) for the lowest energy conformer of ethylene glycol.

	1b		1f	
	ω	ωx	ω	ωx
Calc.	3806	82.9	3856	84.1
Expt.	3803	85.1	3856	84.3

The level of accuracy possible with the CCSD(T) method alleviates the need for empirical scaling often used in connection with the calculation of vibrational frequencies. The downside of the method is that it is computationally demanding, easily requiring months of computer time even with today's fastest computers!

Contrary to the usual red shift of vibrational frequencies observed for hydrogen bonds, there has been evidence found for a blue-shifting intramolecular hydrogen bond in the overtone spectrum of 1H-nonafluorobutane.[30] The molecule is shown in Fig. 8. In this case, a weak intramolecular hydrogen bond is formed between the hydrogen and a fluorine atom.

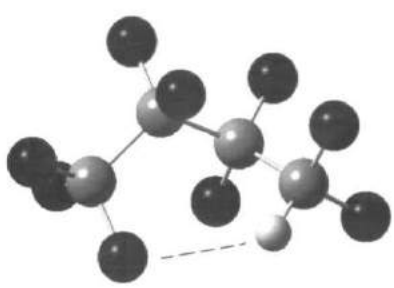

Fig. 8. The structure of 1H-nonafluorobutane with the proposed hydrogen bond.

Efforts are underway at Otago University to record overtone spectra of species undergoing even stronger hydrogen bonding and to observe effects of the aromatic ring π cloud on hydrogen bonding.

Acknowledgments

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Article

$^{14}CO_2$ in the Southern Hemisphere Atmosphere – the Rise and the Fall

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Introduction

In the 1950s a group of NZ scientists had the foresight to establish an atmospheric monitoring programme for $^{14}CO_2$ which has continued to the present day, and has involved many scientists and several institutions. The resulting 50-year record has provided valuable insight into the global carbon and radiocarbon cycles.

The radioactive isotope of carbon, ^{14}C, is formed in the upper atmosphere (stratosphere) when incoming cosmic flux neutrons react with ^{14}N. The ^{14}C is then rapidly oxidised to $^{14}CO_2$, and is distributed throughout the natural carbon reservoirs via the carbon cycle, in particular the atmospheric, terrestrial, and oceanic reservoirs. ^{14}C radioactively decays with a half-life of 5730 years. Before the industrial revolution in the mid-18th century the upper atmosphere production of ^{14}C balanced the radioactive decay over the long term and the system was in an approximately steady state situation.

With the onset of industrialisation and changing land use in the mid-18th century the natural carbon cycle was perturbed. The burning of fossil fuels, such as coal, oil and gas, released carbon to the atmosphere that was previously locked up in relatively inaccessible reservoirs. Atmospheric levels of carbon dioxide have increased by more than 30% in the last 250 years due to human activities, and the anthropogenic CO_2 has also entered the terrestrial and ocean reservoirs. Carbon dioxide released by fossil fuel combustion is devoid of ^{14}C, so-called dead carbon, therefore the atmospheric $^{14}CO_2$ is diluted and the ratio ^{14}C:^{12}C decreases. This consequence is termed the *Suess effect* after Hans Eduard Suess who first described it.[1]

Fossil fuel burning, although not directly producing $^{14}CO_2$, decreases the proportion of $^{14}CO_2$ in the atmosphere, therefore affecting the fluxes with the other reservoirs. During photosynthesis plant uptake reflects the atmospheric composition, so the fraction of ^{14}C in the biomass decreases as the atmospheric ratio decreases. The increasing carbon dioxide in the atmosphere resulting from anthropogenic forcing has increased the CO_2 flux into the oceans, leading to an increase in the total oceanic CO_2. This has only a minor effect on the $^{14}CO_2$ flux.[2]

Atmospheric testing of thermonuclear bombs in the late 1950s produced neutrons around the fireballs of the explosions. Most of the detonations occurred in the northern hemisphere, at the surface or in the lower atmosphere (troposphere), but the fireballs were lifted into the stratosphere. The neutrons then reacted with ^{14}N in the same way as the neutrons of cosmic ray origin. The resulting $^{14}CO_2$ is termed *bomb carbon* and produced a large spike in the atmospheric $^{14}CO_2$ record and, subsequently, in that of other reservoirs as it exchanged with them. The majority of the atmospheric nuclear bomb testings ceased with the signing of the Test Ban Treaty of 1963. However France and China did not sign the treaty immediately but continued atmospheric nuclear bomb testing until 1968 and 1980, respectively. The global atmospheric $^{14}CO_2$ concentration peaked in 1963, and then decreased with a half-life of 12.9 years as the bomb carbon was taken up by the terrestrial and oceanic reservoirs. Enhanced levels of $^{14}CO_2$ are now evident in many other carbon pools, including the oceans,[3] tree rings,[4,5] corals,[6] and ice cores.[7] Over time ocean circulation processes have removed some of the bomb carbon away from the surface ocean. In areas of the ocean where downwelling occurs, such as the North Atlantic Ocean, bomb carbon has been found as deep as 3000 m. When these waters return to the surface, the recycled ^{14}C leads to an increase in surface ^{14}C, and a change in the atmosphere-ocean flux. The distribution of the bomb carbon concentration spike has proved a useful tracer for determining residence times and fluxes between carbon reservoirs, and for teasing out the various underlying processes. However, the large bomb spike has masked the effects of fossil fuel burning and changing land use on the $^{14}CO_2$ distribution making it difficult to directly assess their impact.

Nuclear power stations produce ^{14}C in the reactor, some of which can escape to the environment. Enhanced $^{14}CO_2$ concentrations have been measured close to some reactors,[8] but the effect on the global inventory is small.

A test of global models of CO_2 cycling in the oceans and biosphere is that they are able to reproduce the record of the rate at which the bomb carbon has been removed from the atmosphere.

Methods

Regular measurements of atmospheric $^{14}CO_2$ have been made at Wellington since 1954 (Fig. 1), and comprise the longest such time-series in the world. Initially the samples were collected at Makara, on the west coast near Wellington (41.25 °S, 174.69 °E, 280 m asl; 0), but in 1987 the sampling site was moved to Baring Head, at the entrance to Wellington Harbour, and now the site of a clean air atmospheric monitoring station operated by NIWA (41.41 °S, 174.87 °E, 80 m asl; 0). Samples are collected using static absorption of the CO_2 into a solution of NaOH - a

vessel containing the NaOH solution is simply exposed to the overlying atmosphere for periods of one to two weeks. Back in the laboratory, an aliquot of the solution is acidified, and the released gas is collected and purified by cryogenic distillation. From 1954 until 1995 the ^{14}C in the extracted carbon dioxide was measured by gas proportional counting, but since then accelerator mass spectrometry (AMS) has been used.

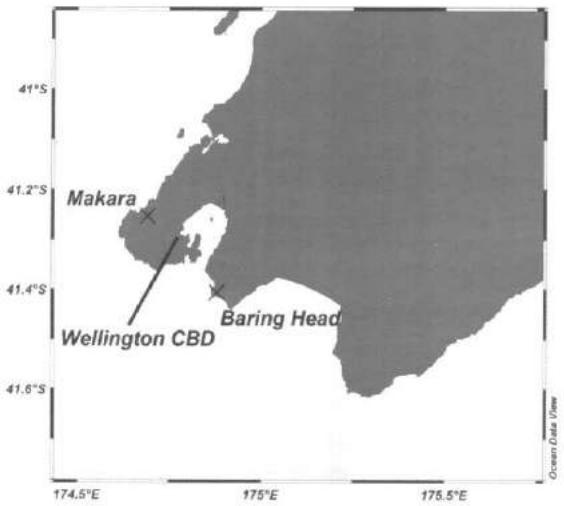

Fig. 1. Map of the southern North Island of NZ showing the location of Makara and Baring Head $^{14}CO_2$ sampling sites.

The abundance of $^{14}CO_2$ is generally expressed as a ratio of ^{14}C to total carbon, and compared to a standard ratio of 1.176×10^{-12}, with corrections applied for fractionation and decay. The standard ratio is that which wood growing in 1950 would have been in the absence of the Suess Effect. The ^{14}C level is reported as Δ14C, parts per thousand greater or less than the standard ratio.

Results

The time series measurements are shown graphically in Fig. 2. $\Delta^{14}CO_2$ increased from a background level of -$10^0/_{00}$ in 1955 to a peak of $690^0/_{00}$ in 1965 due to the input of bomb-derived ^{14}C. The southern hemisphere peak occurred slightly later than that in the northern hemisphere[9] because the majority of the tests were conducted in the northern hemisphere atmosphere and it takes about a year for the atmosphere to mix. The concentration then decreased exponentially with a half-life of 12.9 years to the present day (2005) level of $76^0/_{00}$. The decrease is due to the cessation of the majority of atmospheric nuclear bomb tests, oceanic and terrestrial uptake, fossil fuel dilution and atmospheric mixing processes.

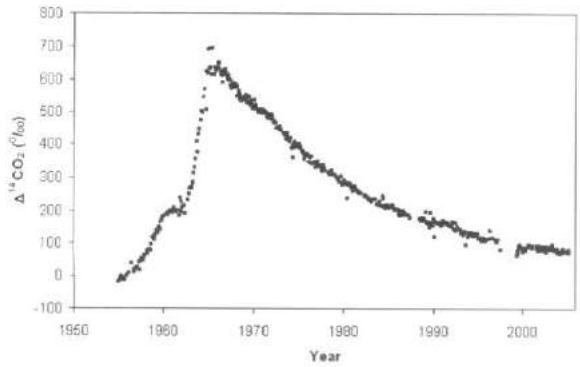

Fig. 2. Time series record of $\Delta^{14}CO_2$ at Wellington, NZ.

The inventory of $^{14}CO_2$ in the global troposphere (Fig. 3) was determined from $\Delta^{14}CO_2$ measured at Wellington, and from the CO_2 mixing ratio measured at Mauna Loa (Hawaii) for the period 1958 to 1970, and Wellington for the period 1970 until 2005. A troposphere:stratosphere ratio of 85:15, and an atmospheric CO_2 burden of 2.1276 PgC/ppmv was used[10] to scale from the point measurements to the global tropospheric inventory. The calculated inventory is shown in Fig. 3. The maximum value of 580 RCU (1 RCU = 1 radiocarbon unit = 10^{26} x ^{14}C atoms) occured in 1965, the values then decrease to 420 RCU in 1998. Fig. 3. also illustrates how the concentration of total CO_2, *i.e.* all carbon isotopes, expressed as a mixing ratio has increased from 316 ppmv in 1958 to 375 ppmv in 2005 due mostly to fossil fuel burning.

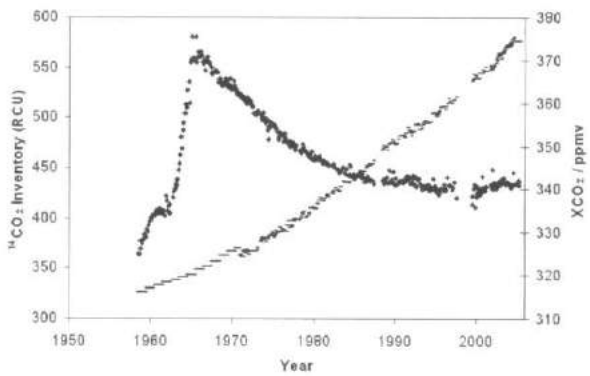

Fig. 3. Global tropospheric inventory of $^{14}CO_2$ (♦ symbol, left-hand axis), and XCO2 (— symbol, right-hand axis).

The changes in the carbon inventories of the rapidly-changing reservoirs have been examined using the Enting-Lassey model.[10,11] In brief, this model considers the three carbon isotopes, including natural, fossil fuel, and bomb-derived carbon; and six reservoirs – the troposphere, stratosphere, short-lived biosphere, long-lived biosphere, surface ocean, and deep ocean. The model simultaneously determines fluxes and reservoir inventories, using various usage/emission histories and predictions, and was calibrated using spot observations. The model is described in detail in the cited references.[10,11]

Several model runs have been performed to examine the effects of varying cosmogenic ^{14}C production, deep ocean diffusivity, fraction of photosynthesis fertilized by CO_2, the size of the long-lived biosphere, and the atmospheric CO_2 turnover time. The E-L model output for the run in which the cosmogenic ^{14}C production is tuned is shown in Fig. 4 where the outputs are given as changes in the inventory of the various reservoirs since 1750, and not absolute inventory size. This is because determination of the absolute inventory is almost impossible, and in large reservoirs such as the ocean the changes are small compared with the absolute value. All of the model runs produce similar outputs, varying only slightly in the magnitude and timing of the various events.

The ^{14}C in the troposphere, stratosphere, and oceans slightly increase from 1900 to 1950 as ^{14}C transfers from the biosphere as a result of deforestation and changing land use. In the 1950s the injection of bomb-carbon initially increases the stratospheric inventory, then is mixed into

the troposphere, and then exchanged into the oceans and biosphere. The tropospheric inventory change decreases exponentially from about 286 RCU in 1966 to 123 RCU in 1997. A broad minimum in the modeled tropospheric inventory is predicted to occur between 1999 and 2007, and this is to be followed by a gradual increase as the ^{14}C is exchanges from the oceans back into the troposphere. The biosphere inventory continues to decline.

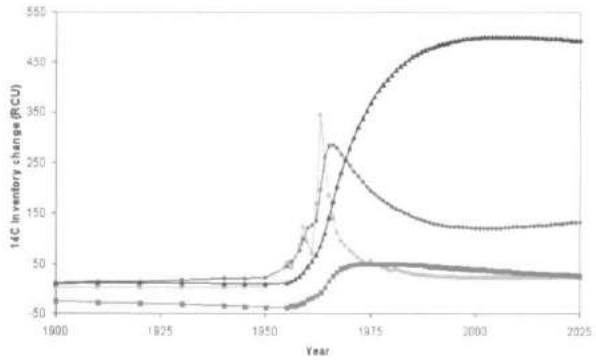

Fig. 4. The modeled change since pre-industrial times in the tropospheric, stratospheric, oceanic, and biospheric ^{14}C inventories for the period 1750-2050.

We are now at the point in time when the observations will soon be able to tell us if the model predictions of an increase in atmospheric $^{14}CO_2$-content is indeed occurring. The 21st century then, could be the time when the oceans change from being a sink for atmospheric ^{14}C to being a source, the first time this is known to have happened.

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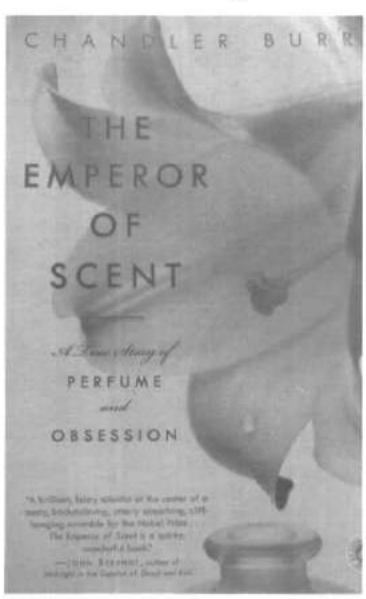
The Emperor of Scent



By Chandler Burr
Published by Random House
ISBN 0 375 75981 6

This book is a must read for anyone with an interest in science and scientific research. That does not mean you need to be a research scientist to enjoy it. The book is written in a very easy going style with plenty of quirky humour and clever analogies.

It is the story of Luca Turin and his struggle to have his theory on how we smell recognised, let alone accepted or even debated. Turin is a biophysicist, a perfumer with an uncanny sense of smell and an even more uncanny way of describing each odour.

The generally accepted way in which we smell is that the molecules entering our nose interact with receptor sites on the basis of the molecular shape fitting the receptor site in some way. The brain recognises the smell of a substance by the shape of its molecules.

Turin reasons for challenging this are:

Firstly the mighty monetary and computer power of the giant perfume manufacturing corporations (referred to as "the big boys" in the book) have yet to come up with a way of predicting the smell of a substance or being able to make to order a substance with a particular smell.

Secondly he compares some other body processes that rely on molecular shape with smell.

• Digestion. Although new foods are being produced they are still based around the same fat, carbohydrate and protein molecules that have been around since humans first evolved. We have the necessary enzymes of the correct shape to catalyse the breakdown of these substances. Digestion is rapid. As additional support of this he makes the point that dogs didn't evolve as chocolate eaters and if

you feed them chocolate they get sick implying that dogs do not have the necessary enzymes to digest chocolate.

• Immune system. Fighting disease also relies on molecular shape. We need antibodies of the right shape to lock onto and neutralise or destroy the invading pathogens. There are pathogens around that were not there when humans evolved. The immune system has to make the appropriate antibodies of the right shape going through maybe thousands of possibilities before a suitable one is found. This takes days which is why, when we get sick, it takes a few days for us to recover.

• Smell. There are substances around that were not present when we evolved. The molecules of many of these substances have entirely different shapes to those of more traditional smelly substances. Therefore, suggests Turin, the receptors of the right shape won't have developed in our nose. Yet we can smell any new odour instantly.

For Turin, shape may have some contribution to the sense of smell but it is by no means the whole story. As the account of his ideas and struggles to have them acknowledged develops it is clear that Turin reads scientific literature very widely and has an almost encyclopaedic memory of what he has read. He also has the ability to make connections that would by-pass many of us. From all of this he develops a previously discarded proposal that smell is due to vibrations of bonds within molecules.

First problem: if this has any credence how are the vibrations detected? On a laboratory scale bond vibrations are detected and measured by a spectroscope. Turin masters the necessary Physics to understand spectroscopy and then goes in search of molecules and molecular phenomena that could mimic a spectroscope.

Second problem; assembling evidence to support his proposals. The search is on for molecules with different shapes but with bonds of similar vibrational wave numbers that are small enough to be able to be smelled.

The main weapon Shapists have against the vibration theory is enantiomers. Right and left handed molecules have different shapes and smell different but as they have the same atoms bonded to each other their vibrational characteristics will be the same. Therefore the answer to smell is shape not vibration.

Isotope replacement. If you replace hydrogen with deuterium in a bond its vibrational characteristics will change and therefore so should its smell.

Turin tackles these issues with an amazing ability to think outside the square. An interesting aspect is the mixed emotions that can be generated when testing ideas. There is the anticipated excitement that what is predicted will happen and provide evidence to support the theory. There is also the apprehension that the results could be other than predicted and torpedo the theory. He finally assembles a paper which is submitted to Nature. It is rejected by the peer reviewers. Turin answers all their criticisms and it is rejected again.

The story highlights a number of aspects of scientific research:

• The scientific community now expects progress to be incremental and is highly suspicious of any radical change of view.

• Most researchers are so defined in their knowledge that it is difficult for them to appreciate work outside their field. One of Turin's problems was that he had brought together some fairly sophisticated ideas from chemistry, physics and biology and there was no one to critique his work who was familiar with all three of these disciplines.

• Big corporations have millions of dollars invested in research in established ideas like shape and smell and are reluctant to see the boat rocked.

• Individuals have reputations earned from research into the shape and smell relationship and will fight hard to protect these reputations.

Through the story Chandler Burr weaves Turin's biographical details; his upbringing, education and his relationships. There are many descriptions of perfumes which I found a bit distracting because I was impatient to get on with the story.

There are a few occasions when the main story is suspended while Burr follows up some other work of Turin's. One that I found particularly interesting was his involvement in a case of a woman who found that everything smelled vile. I read this book shortly after there was a similar well publicised and successfully treated case in New Zealand. After many conversations with the woman Turin finally decides that the cause is epilepsy in the olefactory bulb of the brain. Epilepsy is described in the book as a situation where the sensations detected by the brain are not attenuated as should happen but remain and even build up on themselves to distort and overload the brain's messages and responses. Turin recommends to the hospital specialists that they try epilepsy drugs and effects a cure. As I recall the New Zealand case was cured by surgery.

Two-thirds of the way through the book Burr as the author takes the unusual step of becoming part of the story. He explains how he set out to write an objective account of the vibration theory and intended to give a balanced view of both vibration and shape. However the shapists refused to enter into discussion; none, that he could find, had read the Turin paper (subsequently published in a journal other than Nature) and claimed they didn't need to. All flatly rejected the vibration theory without giving substantive evidence to support this stance.

This book is a fascinating read, somewhat shakes your faith in the objectivity of the scientific community and peer reviewing. The ending emphasises that we are dealing with real life here and not a well crafted novel with all loose ends neatly tied up.

In 1995 the BBC Horizon team produced a TV documentary on Turin's work called *A code in the nose*. If anyone has access to this I'd love to see it.

If you can't find the book in a library or book shop, I bought mine second hand through amazon.com for US$3.95 plus handling and postage.

Reviewed by
Richard Rendle
rendle@xtra.co.nz

New Zealand Institute of Chemistry
supporting chemical sciences

April News

Executive News

Keith Gordon was inaugurated as 2006 President at the February meeting of Council.

Keith Gordon receiving the Chain-of-Office from immediate Past-President Graham Bowmaker.

2006 Contacts

President: A/Prof. K. Gordon (Otago): nzic.president@nzic.co.nz

1st VP: Jan Wikaira (Canterbury): nzic.1-VP@nzic.co.nz

2nd VP: Prof. B. Nicholson (Waikato): nzic.2-VP@nzic.co.nz

Hon. Gen. Secretary: Richard Rendle (Canterbury): nzic.secretary@nzic.co.nz

New Fellows

Congratulations to **Roger Hill** (Hill Laboratories, Hamilton) and **Prof. Alistair Wilkins** (Waikato University) who were elected to the Fellowship in February.

Easterfield Lecture Tour

Dr. Kate McGrath has undertaken the UK lecture tour component of her 2003 Easterfield Award giving lectures *Calcium carbonate crystallisation: the roles of chemical functionality, kinetics and soft templation* and *Emulsion microstructure and stability: understanding and manipulating interfacial control* at the universities of Manchester, Hull, Edinburgh, Bristol, Nottingham on Trent, and Imperial College. The Medal was presented at the University of Manchester during a half-day symposium *Crystallisation - Big Business for Nature and for Mankind* organised by the RSC.

Dr. Kate McGrath receiving the Easterfield medal from Prof. Michael Anderson

Closing date for NZIC Prizes is 30 June 2006

Full details for the HortResearch and Nufarm Prizes, and the Chemical Education and A.C. Kennett Memorial Awards can be found at www.nzic.org.nz – check the *prizes* link on the left-hand panel.

Chemistry in New Zealand

At its February meeting Council elected to make the *Journal* available on-line 6-months after publication by placing it on the web site.

Branch News

AUCKLAND

Following the Branch AGM on November 23, at which **Brent Copp** was re-elected Chairman, **Prof. Jim Metson** spoke of *Big Science in a Big Country* and outlined opportunities available as Australia moves to complete its synchrotron source and research reactor. Jim, promoted to Professor in 2005, started his 3-year term as the Head of Chemistry from February 1.

Chemistry Department UA

Recent promotions are **Brent Copp** to Associate Professor, **Vittorio Caprio** and **Tilo Sohnel** to Senior Lecturer, and **Paul Kilmartin** to Senior Lecturer (extended scale). Three new academics have taken up their appointments. **Dr. Andrew Dingley** (Senior Lecturer Chemistry/Biological Sciences & Director of the NMR Facility; Andrew (PhD Sydney) held postdoctoral positions in Australia and Germany, and a lectureship at UC London prior to his appointment. **Prof. David Williams**, an Auckland

National Branches

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Manawatu	M. Waterland	J. Bendall	D. Shillington	M. Waterland	B. Mulchin
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Waikato	M. Mucalo	K. Lee	Chairman	B. Nicholson	M. Princep
Wellington	K. MacKenzie	K.McGrath	A.Turner	B. Halton	B. Halton

graduate, has the PBRF funded Chair in Electrochemistry. He developed his career in electrochemistry and chemical sensors at the Atomic Energy Research Establishment (Harwell, UK) in the 1980s. He became Thomas Graham Professor of Chemistry at University College London in 1991, co-founded Capteur Sensors Ltd., and was Head of Chemistry (1999-2002) prior to appointment as Chief Scientist at Inverness Medical Innovations, (Unipath Ltd., Bedford, UK). **Dr. Vijayalekshmi (Viji) Sarojini** has been appointed Senior Tutor in Organic and Medicinal Chemistry. She has a PhD from the Indian Institute of Science, Bangalore with 5 years of postdoctoral study in Sweden, England, and USA; previously, she worked as a scientist at Hort Research, Mount Albert.

Margaret Brimble's 2005 HortResearch Prize prize was for her research on the development of flexible synthetic approaches to complex natural products that exhibit important biological activity. Major achievements of the past 5 years include the synthesis of the complex shellfish toxins, *the spirolides*, which are important pharmacological probes that activate calcium channels, and the elegant synthesis of the spiroacetal moiety of the anticancer agent, pectenotoxin-2 (another shellfish toxin). Margaret's research group was also the first to synthesize the novel anti-*Helicobacter Pylori* agent spirolaxine. The latter synthesis is topical given that the 2005 Nobel prize for medicine was awarded to two Australian researchers for the discovery that *H. pylori* causes ulcers. The prize was awarded to Margaret at the November RSNZ Honours Dinner in Wellington.

Margaret Brimble receiving the 2005 HortResearch prize from Andrew Brodie

Peter Swedlund has been awarded a FRST NZST postdoctoral fellowship to work with **Gordon Miskelly** on *Spectroscopic studies of silicate interactions with metal oxyhydroxides relevant to geothermal energy use*. **Dr. Andrej Maroz** has started a Marsden Postdoctoral Fellow in **Bob Anderson's** group. Andrej, from Belorus, completed his first degree at Minsk State University and his doctorate in radiation chemistry at Leipzig. **Gary Fleming** (PhD student with **Hicham Idriss**) gained second prize for his presentation *DL-Proline on TiO_2(110) single crystal surface: a study by high resolution photoelectron and temperature programmed desorption* at the 30th Condensed Matter and Materials Meeting, NSW. The first prize went to **Aloysius Soon**, a former Auckland chemistry student now at Sydney University.

A large contingent from the Auckland region attended Pacifichem 2005 in December, with 12 from the University of Auckland and 2 from Massey Albany.

CANTERBURY

Recent events include a talk *The Hunt for Red Tide Toxins: New Toxins, New Technologies* by **Michael Quilliam** (NRC Institute for Marine Biosciences, Canada). **Dr. Claire Vallance** (Oxford) gave a presentation after the Branch AGM entitled *Molecular photography - velocity map imaging of chemical events* describing the laser-based technique of velocity-map ion imaging. The power of this technique lies primarily in its ability to provide a visual snapshot of the complete product scattering distribution in a single experiment. *Reverse-engineering* of this distribution allows the intimate details of the chemical rearrangement to be unraveled. The Branch 2005 NZIC Prize for the best performance in 200-level Chemistry went to **Daniel Packwood**.

Chemistry Department UC

Greg Russell and *Richard Hartshorn* have been appointed Associate Professors and **Peter Steel** is the recipient of a James Cook Research Fellowship for his work on new metallosupramolecular building blocks. NZIC 2nd VP, **Jan Wikaira** received her 2005 Teaching Medal at the December graduation ceremony. Congratulations to **Jono Hill**, and **Andrea Vernall** who have each gained their PhD degree. **Greg Smith** was awarded the Treloar Prize (for best poster by a young scientist under 30) at the Rotorua joint APS/ASB Conference (see Waikato below) for his theoretical studes of free-radical polymerization. **Alan Downward** is a UC Research Awardee and **Joanna Duncan**, **Reuben Jane**, and **Josh Lehr** are 2006 UC Doctorial Scholars. **Victoria Peddie** is the recipient of a TEC Top Achiever Doctoral Scholarship while **James Bull** and **David Garrett** have are 2006 Senior Scholars.

Peter Slade, has returned to Canada having spent the last few months working in **Jim Coxon's** molecular modelling group; Peter and his wife left the Department a native North American Indian print in recognition of their stay. **Andre Pinkert**, an overseas student who worked with the marine group, has departed for Germany. **Kim van Berkel** has gone to UC-Santa Barbara to undertake a FoRST postdoctoral fellowship on SERS investigations related to biological assays using gold nanoparticles. **Till Cremer** is about to return to Germany to finish his degree following a year with **Owen Curnow** and **Richard Hartshorn**. **Dr. Gerhard Lang** has returned to a position at Kiel (Germany) after a highly productive two years in the Marine Chemistry Group. **Prof. Dilip de Silva**, a return visitor, is to spend the next eight months working with the Marine Group on samples from Sri Lanka, as part of a collaborative project between the Universitise of Canterbury and Colombo.

Summer student **David Garrett** (**Robinson**, **Wikaira** and **Hartshorn**) was in the X-ray lab.

Daniel Packwood, (**Brooksby** and **Downard**) worked on a newly assembled contact-angle measurement system, and **Bryce Jackson** (**Russell**) studied the way by which the spontaneous generation of radicals occurs in emulsion polymerization at temperatures of, for example, 50°C.

CPIT

Michael Edmonds and **Juliette Hamilton** participated in an Australian Chemistry Enhanced Laboratory Learning (ACELL) workshop that encouraged lecturers to test-run experiments they have developed for use in their institution; **Andy Pratt** and **Alan Downward** (UC) also attended this meeting.

CANESIS

Researchers from Canesis Network Ltd. collected three awards at the 11th International Wool Research Conference (Leeds, UK) last September. Canesis also received two of four annual awards for Excellence in Wool Science granted by Australian Wool Innovation (AWI) Ltd. and the German Wool Research Institute (DWI); they are made to encourage creative scientific work in wool science around the world that show practical application. In addition, the paper *Covalent Attachment of Novel Surface Modifications to Wool Fabric via Removal of Surface Bound Lipids* (**Meade, Dyer, Caldwell**, and **Bryson**) won the AWI-sponsored prize for best fundamental science paper presentation at the conference. The methodology developed ultimately will be used to produce smart textiles.

Drs. Jolon Dyer and **Scott Bringans** were joint recipients of a personal award for research into the factors responsible for the photo-yellowing of wool fabric. By utilising quadrupole TOF mass spectrometry, Jolon and Scott have identified 14 yellow compounds derived from tryptophan and tyrosine in 25 locations within the wool proteins, so far. The project is part of a wider AWI-funded collaboration to improve the value of Merino wool and the colour stability of its derived fabrics. **Dr. Simon Causer** received the Project Award for his work on the control of house dust mites and the management of allergens in carpet and wool bedding.

MANAWATU

Selwyn York and **Trevor Kitson** received their NZIC Prizes late in 2005 from Branch Chairperson **Mark Westmorland**.

Selwyn Yorke receiving the NuPharm Prize

Trevor Kitson receiving the NZIC Chemical Education Prize

Massey University

Shane Telfer has taken the position of Lecturer in Chemistry. He gained his BSc(Hons) and PhD degrees (1999; supervisor **Richard Hartshorn**; Canterbury University) and spent postdoctoral time at the Universities of Geneva, Tokyo, and Montreal in the area of supramolecular chemistry. He has developed a diverse range of research interests including stereochemistry and chirality, CD spectroscopy, X-ray crystallography, and photochemistry. He is married to Maiko and his interests include mountain biking and snowboarding.

Dr. Shane Telfer

Len Blackwell, and **Delwyn Cooke**, in partnership with Manawatu Bio-Tech Investment Ltd. are commercialising a take-home fertility-testing kit. The technology aims to pinpoint the most fertile period of a woman's menstrual cycle by measuring the metabolites of the hormones oestrogen and progesterone; the kit is expected to be available within the next two years.

Barbara Gunn has resigned from her position from October 31. She began work as a Technical Assistant in the (then) Massey Chemistry Department in April 1996 and provided back up for the Chemistry teaching technicians. Since inception of the Institute, Barbara also has taken additional duties in the Chemical Services Section. RSNZ Science, Mathematics, and Technology Teaching Fellow **Carol Walkly** has left **Mark Waterland's** group to continue secondary school teaching where he hopes to tie new and exciting research areas into the current high school curriculum.

Jim Salvador (a Marsden-funded post-doctoral with **Geoff Jameson**) has returned home to a position at Michigan State. In his 18-month tenure he mastered structural biology, from site-directed mutagenesis to protein expression, and purification and crystallization to data collection to structure solution, refinement and analysis. He has gained a number of very significant results. Farewell also to **Roger Lins** who has accepted the position of Research Advisor in the College of Sciences.

It seems hard to believe that the recent Massey-Victoria Chemistry Postgraduate Student Seminar Day (14 November 2005) was the 14th in the series. Organised by willing postgraduates from the host university, **Steve Kirk** and **Yvoone Ting** did a great job. The talks were excellent , the science well presented and with humour. A strong contingent of around 25 students and staff from VUW was matched by Massey. It was good to have two Albany students (**Brian Vest** and **Behnam Assadollahzadeh**) join the symposium for the first time. Topics covered a wide range of chemistry including carbon nanotubes, total synthesis, computer modelling and physical chemistry, and metal ligand coordination.

OTAGO

The Branch began 2006 with several new faces; **Prof. Sally Brooker**, **A/Prof Lyall Hanton**, and **Dr. Kim Currie** are joined on the committee by no fewer than 8 members. Congratulations go to **Sally** who has been promoted to **Professor**. In late June she will present an invited lecture at the (joint) Macrocyclic and Supramolecular Symposium (Victoria, Canada). Other members of Sally's group are involved in newsworthy events. **Dr. Jason Price** is to give an invited lecturer at a Supramolecular Chemistry symposium in Sydney to mark **Prof. Len Lindoy's** retirement. **Ryan Hellyer** and **Jonathan Kitchen** have commenced PhD study and **Dr. Andy Noble** (from Ireland) has joined the *Brooker Bunch*.

Prof. Karl Wieghardt (Max Planck Institute -Bioinorganic Chemistry) visited for a month from late February and **Dr. Grace Morgan** (UC, Dublin) is with us for 2 months until late April.

A/Prof Henrik Kjaergaard and students **Joseph Lane**, **Daryl Howard**, and **Daniel Schofield** attended the Pacifichem 2005. **Henrik** organized the *Vibrational overtones - spectroscopy, dynamics and environmental implications* symposium and presented a talk entitled *Accurate calculation of vibrational overtone spectra*. **Daryl Howard** presented *Intra-molecular Hydrogen Bonding in Ethylene Glycol* in this symposium while **Henrik** gave a second address in the *Computational Quantum Chemistry: Methodology and Application* symposium. **Joseph Lane** and **Daniel Schofield** presented *Electronic Spectroscopy in Atmospheric Sulfur Molecules* and *Electronic Spectroscopy of the H_2O-HO Couple*, respectively. Daniel has now completed his PhD degree and has accepted a postdoctoral with **Prof. Ken Jordan** (Pittsburg).

Otago's Biochemistry Department recently hosted two meetings with many NZIC members in attendance. The annual NZSB and Molecular Biology (NZSBMB) conference in conjunction with the NZ Microbiological Society provided three parallel sessions which catered for the interests of almost 400 registrants. A timely and riveting keynote address was provided by **Robert Webster** (Otago graduate and researcher at St. Jude's Children's Research Hospital, Memphis). As the world's leading expert on how influenza virus makes the leap from birds to humans, he spoke with authority and urgency on the H5N1 strain of bird flu. His message is simple: this strain is more lethal to wild birds and model animals (such as ferrets) than any influenza strain he has studied in five decades in the field. We must plan now for the likelihood that H5N1 will acquire the ability to be transmitted from person to person, and thus the potential for a pandemic.

The NZSBMB top award, sponsored by Applied Biosystems, went to **Barry Scott** (Massey University) for his work on the biosynthetic pathways of alkaloids crucial to the symbiotic relationship of fungi with important forage plants such as ryegrass. In particular, the award recognised cloning and analysis of the genes for peramine, a novel cyclic dipeptide and lolitrem, an indole-diterpene.

Over two days preceding the main meeting, the NZSBMB and Australasian Proteomics Society held a satellite meeting gathering NZ scientists using proteomic techniques – including two-dimensional electrophoresis, multidimensional chromatography and mass spectrometry – to identify the broad range of proteins participating in complex processes. Reports of NZ research (from elephant sex pheromones to analytical standards for assessing EU wheat production) were interspersed with advice and examples from half a dozen overseas experts. **Brian Chait** (Rockefeller University) presented an elegant dissection of the nuclear pore, and **John Bergeron** (Int. President - Human Proteome Organization) tracked the path of maturing proteins through a labyrinth of intracellular compartments for us. The meeting made it obvious that over the last five years proteomic techniques have been well-established here and are an increasingly important tool for NZ biochemists.

WAIKATO

The new Fellows **Alistair Wilkins** (Waikato University) and **Roger Hill** (Hill Laboratories) have each made a very significant contribution to chemistry in the Waikato. Roger established Hill Laboratories, the largest privately owned chemistry laboratory in NZ that now employs over 250, and Alistair has developed excellent teaching and research programmes in the University over the last 30 years. **Bill Henderson** is now the NZIC 2nd VP.

ChemQuest™ 2005 was held late last October with a total of 51 teams from 15 schools entered. Students come from the greater Waikato region to compete for the James and Wells trophy, medals, and cash prizes. Questions were categorised under: *Periodic Puzzlers, Sensing the Senses, The Wide World of Chemistry*, and *Demon Demos*. At the conclusion of each round it was Teachers' Turn to compete for a small personal prize and a textbook for their school. It was a most enjoyable night for contestants, presenters and spectators, and the following prizes were awarded:

1st: **Scott Gilbert**, **Hayden Johns**, **Nikhil Ullal** (St Paul's Collegiate School).

2nd: **James Bridgewater**, **Michael Hoy**, **Brent Jackson** (Tauranga Boys' College A).

3rd: **Sophie-Ann Chin**, **James Fisher**, **William Rattray** (St Paul's Collegiate - Team Ramrod).

4th: **Reuben Davis**, **Reginald Nand**, **Paul Randall** (Fairfield College. The Chemistry Chics)

5th: **Chantal Hickey**, **Euna Hwang**, **Lei Ying** (Hillcrest High - Hillcrest Hydrogenators).

Teachers' Turn:

Round 1. **Duncan Smith** (St Paul's Collegiate).

Round 2. **Nigel Roberts** (Trident High)

Round 3. **Mary Thomas** (Trident High)

Round 4. **Kevin Kannan** (John Paul College)

The quiz was presented by **Bill Hen-**

derson and **Brian Nicholson** with **Lyndsay Main** presiding as chief judge. Numerous people contributed to the success of the occasion thatwas sponsored by Waikato School of Science and Engineering, Hill Laboratories, and James and Wells. **Sandra Wilcocks** and **Julie Crisford** from James and Wells attended and presented the prizes to the winning teams.

University of Waikato

The School of Science has taken delivery of a new ICP/MS instrument to enhance trace element analyses. This very powerful tool is able to analyse many elements to the parts per trillion level (and routinely ppb) and it will pair-up with the existing ICP/OES instrument. The first laser ablation equipment in the country is also part of the setup and enables dry, solid samples to be viewed and analysed directly without acid digestion.

Brian Nicholson, **Nick Lloyd**, **Stephen Gardyne**, and **Kelly Kilpin** attended the January Australian and New Zealand Symposium on Organometallic Chemistry (OZOM3) held at the Monash University Gippsland campus. The meeting marked the 70th birthday of **Prof. Glenn Deacon** and gave an interesting emphasis on lanthanide chemistry.

Michael Mucalo and PhD sudent **Dougal Laird** attended the February combined APS/ASB conference in Rotorua. Michael presented a talk on chemically modified substrates for enhancing uptake of bone morphogenetic proteins. It was the first time that the ASB (Australasian Society of Biomaterials; of which Michael is the NZ committee representative) has held a conference outside Australia. Recently, the ASB underwent a name change from Australian to Australasian to reflect its growing NZ membership and held its conference in NZ in conjunction with the Australasian Polymer Society to reflect this. The conference was valuable for the talks, current views, and personal contacts it provided in the biomaterials field. Although this area is truly multidisciplinary, and one in which research is increasingly focussing on cell biological considerations, the essential role of chemistry was made evident in many of the ASB pressentations.

Michèle Prinsep attended the inaugural February AustraLarwood bryozoan symposium organised by **Dennis Gordon** and held at NIWA in Wellington. She gave a talk entitled *Studying the Scum of the Sea: Chemical Investigations of New Zealand Marine Bryozoans*. **Brian Nicholson** survived a very lively **Cafe Scientifique** meeting on the fluoridation of drinking water as part of an effort to educate the Hamilton community, prior to the binding referendum.

MSc degrees have been awarded to **Karen Love** (*Silica Modification of Unbleached Kraft Pulp with Alkoxysilanes*) who now works at Scion, Rotorua,. **Sarah Devoy** (*Reactions of* $[Pt_2(\mu\text{-}S)_2(PPh_3)_4]$), **Kelly Kilpin** (*Synthesis and Anticancer Activity of Novel Gold(III) Complexes*), **Brendan Waugh** (*Studies on CIDRs*), and **Jessica Zhu** (*New Types of Phosphorus-Based Anticorrosive Pigments for Paints*). **Gordon Rajendram** and **Sally Gaw** have submitted their PhD theses (*Evaluation of Near-Infrared Spectroscopy for Analysis of Soil and Plant Analysis in Agriculture* and *Persistence and Availability of Agrichemical Residues in New Zealand Horticultural Soils*); Sally is about to start work as an environmental health scientist at ESR in Christchurch.

NIWA

In early December **Craig Depree** hosted a short visit by **Prof. Mark Hamann** (University of Mississippi) who gave a seminar on his pharmaceutical discovery research *From the Sea to the Clinic: Marine Natural Products with Potential Applications in the Control of Cancer, Infectious Diseases and Neurological Disorders*. **Dr. Michael Stewart** joined the NIWA Aquatic Chemistry group in March from the Centre for Molecular Biodiversity, Queensland, and will work on natural products chemistry of marine organisms.

WELLINGTON

The November AGM saw **A/Prof. Ken MacKenzie** re-elected as Chairperson. The meeting was followed by an enlightening address *Beyond the Horizon: Experiences of a Research Technician on Oceanographic Voyages around New Zealand* by **Lisa Northcote** (NIWA). Conducting oceanographic research at sea was described as often difficult but also rewarding. With the variable work involving extended periods away using heavy equipment, often in adverse weather conditions. Lisa works in the Marine Geology group at NIWA conducting amongst other sediment analyses modern foraminiferal taxonomy and assemblage characterisation from Hawke Bay, Chatham Rise, and Campbell Plateau. The establishment of seasonal to inter-annual variability in ocean temperatures and productivity from living foraminiferal assemblages will allow the derivation of modern analogues for past environmental changes in subtropical and subantarctic waters.

In early November 2005, **Dr. Ian Millar** addressed the Wellington Astronomical Society on planetary formation and chemistry. Effectively this was the first public presentation of a new theory on this matter - and a new chemical theory is not all that common an occurrence!

The first of the 2006 meetings was an address by **Prof. Tom Ziegler** (University of Calgary) on *Atomic scale modeling of polymerization catalysts*. Tom, born in Copenhagen, took Master's degree there, gained his PhD in theoretical inorganic chemistry from Calgary University, and has been one of its Professors of Chemistry since 1993. His research is directed towards the development of new computational methods that are applied to a wide range of chemical problems and processes, including the modeling of polymerization catalysts. He presented an extremely interesting

talk on this topic that was enlivened by superb time-lapse computer simulations; the audience was much appreciative. He is one of the two most cited chemists in Canada and 38th most cited chemist in the world!

Victoria University

Mina Razzak has accepted a Bright Futures postgraduate scholarship to undertake PhD study at Cambridge University from September. **Dr. Rob Keizers** visited en route from one postdoctoral in South Africa to another inVancouver.

ESR

Complex stoichiometric modelling in the 1980s of 2,3,7,8-TCDD airborne releases from the New Plymouth Dow Chemical Plant could not predict actual exposures to local residents, recently confirmed in serum sampling conducted by ESR for the Ministry of Health. While it is assumed that exposures came primarily from fugitive emissions from the plant, this was not considered a viable pathway according to chemists two decades ago.

IRL

Recent visitors have included **Prof. Tom Ziegler**, the major ISAT collaborator with **Dr. Graeme Gainsford** and the Chiral Catalyst project team. The collaboration between the CHIRANZ and Ziegler's group has raised awareness of theoretical chemistry and ab initio modeling of chemical reactions throughout NZ from the seven seminars he gave.

Technical and strategic problems concerning the (critical) computed location of transition state energies for the IRL asymmetric hydrogenation reactions (Rh(CandyPhos)+-based catalysts), and hence the predicted purity of chiral synthesis, have provided for continuing the path-finding study.

Biocryst Inc., the licensee of PNP inhibitors prepared as a result of the ongoing collaboration between the IRL Carbohydrate Chemistry Team and the Albert Einstein College of Medicine (AECOM), Yeshiva University, have sub-licensed two molecules, *Fodosine* and *BCX4208*, that came from this collaboration. The licencees are Roche and Mundipharma in a deal worth $NZ 1 billion. IRL and AECOM will continue to receive, a 1:1 share of many millions of dollars, contingent on event payments laid down in the sublicenses. Additionally, in the event that either Fodosine or BCX4208 progress to market the two parties, will share in royalty payments as a percentage of gross sales.

The New Zealand Science Scene

UC Biomass Gasifier

Research in New Zealand conditions for biomass gasification is now possible. In February a lab-scale biomass gasifier was officially opened at the University of Canterbury.

Biomass gasification is the conversion of solid organic material, including woody residues, sewage sludge and hybrid crop species into a gaseous fuel suitable for combustion to produce heat and electricity.

The gasifier, at the University's Wood Technology Research Centre, is the first step in a programme funded by the Foundation for Research, Science and Technology. The programme's ultimate goal is to develop a system that industry partners can use for a bio-energy demonstration plant.

The Forestry Minister Hon Jim Anderton said at the launch, "New Zealand has a renewable and sustainable plantation forestry resource. It leaves us well placed to take advantage of the technology that can make valuable bio-energy from wood product that would otherwise go to waste."

Associate Professor Shusheng Pang said "while the most promising application of the technology was in the wood processing industry, it could also be used to convert the city's biosolid waste into energy". Professor Shusheng Pang is leading the biomass energy research programme and heads the Wood Technology Research Centre.

The advantages of biomass gasification are reduction in carbon emissions, elimination of waste disposal costs, reduced dependence on fossil fuels and generation of employment. The main disadvantage is that it is currently more expensive than conventional power generation.

After the opening of the gasifier a forum on the role of biofuels in a sustainable energy society was held. Green Party leader Jeanettet Fitzsimons gave the opening address looking at the country's energy demand in the next 10 to 20 years.

Biomass gasification is not a new idea, during World War II there were over a million small gasifiers running cars, trucks, boats and buses.

From left: Jock Brown, a Masters student in the Department of Chemical and Process Engineering, UoC, Dr Robin Mann, Chancellor of UoC, Ms Jeanette Fitzsimons, Green Party Leader and MP, Mr Ian Gilmour, Senior Lecturer in the Department of Chemical and Process Engineering, UoC.

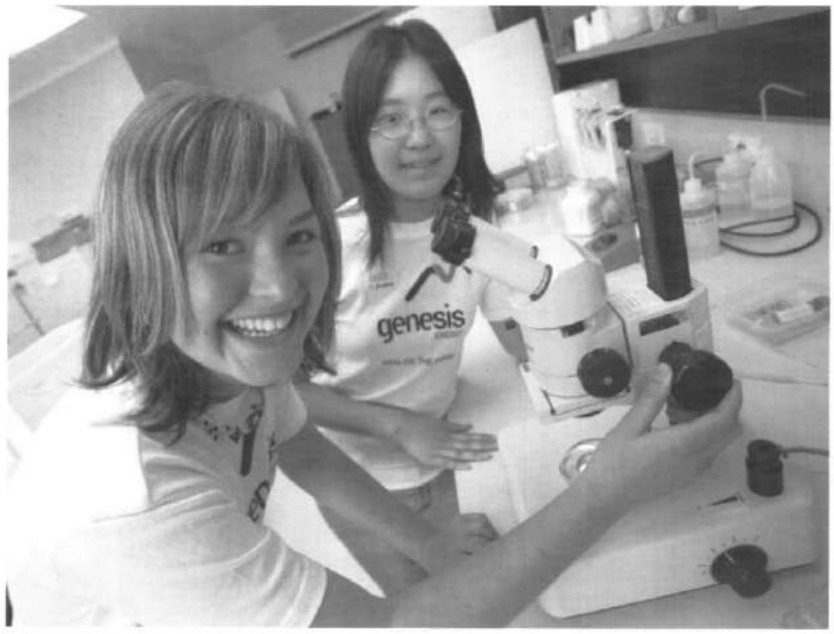
A Window into the Real World of Science for School Students

New Zealand's future generation scientists mixed with those of today in December. AgResearch hosted thirty-six students on its Wallaceville campus as part of the Realise the Dream programme.

AgResearch got involved as a proactive effort to attract students into science careers.

The Realise the Dream programme is a five day national forum for school students who have had winning scientific investigations and inventions at events like regional science fairs. It is run by the Royal Society of New Zealand.

The students had lunch with chief Executive Dr Andy West, viewed the possum breeding unit as well as interacting with scientific staff in labs.



Students enjoy hands-on experience in AgResearch laboratories.

Herbal Medicines

The general lack of good quality information on herbal medicines is being addressed in Auckland.

At the end of 2005 Dr Joanne Barnes was appointed as Associate Professor in Herbal Medicines at The University of Auckland's School of Pharmacy in the Faculty of Medical and Health Sciences. This appointment will mean the start of research activities in this area at the school.

"At present there is very little known about how widely these medicines are used in New Zealand, and the benefits and risks of using them." Dr Barnes said.

"There is reasonable evidence that certain herbal medicines can be very effective but, as with all medicines there are also safety issues surrounding their use. Many people think that because they are natural there won't be any adverse reactions but this isn't necessarily so."

Dr Barnes brings international expertise to the new position. She has written on the topic for both professional and consumer publications and is an honorary consultant to the World Health Organisation's international drug safety monitoring programme.

Fruity Thiols Better with Screw Caps on Wine Bottles

Two thiols with aromas of passion fruit and boxtree as well as those with a grapefruit element were part of a two year study by wine researchers at The University of Auckland led by Dr Laura Nicolau.

The aromas of sauvignon blanc bottles with corks and screw caps were compared using a GCMS machine. Dr Nicolau said the analysis showed the two fruity thiols were up to 23% higher in the bottles using the screw caps.

The tropical smells lose their power when they come into contact with oxygen which explains why sauvignon blanc can lose its fruity character after one to two years.

Are you reaching your full potential at work?

If your organisation is letting you reach your full potential you are fortunate. If not, here is a paper to pass onto your HR department.

A recent report by researchers, Dr Fiona Edgar and Professor Alan Geare from the University of Otago's School of Business, began to fill the gap in the literature about human resource management practices from the employees' perspective. Empirical research in the past has mainly evaluated these practices from the employers' point of view.

Around 600 employees in Wellington and Christchurch were surveyed about human resource management practices in their organisations.

It was found employees wanted quality training and development that was useful while most organisations were focusing on more traditional areas of human resource management such as employee performance and appraisal. The findings suggest employer investment in training and development may have the greatest potential to benefit organisational performance.

Dr Edgar said "It is the quality of HRM practices that is particularly important in influencing employee attitudes. Quality over quantity".

The findings from the research were published in the Asia Pacific Journal of Human Resources in December.

The Squeeze on US Applicants

By Blair Hesp and Helen Palmer

Over the past ten years the policies and practices adopted by the United States Patent and Trade Marks Office (USPTO) have changed markedly for pharmaceutical and biotechnology related patent applications. These changes have proved to be somewhat frustrating for patent applicants.

A patent application can be filed first in New Zealand and then in the course of time may come to be filed at the USPTO. The policies and procedures that applied in the US at the time of filing the New Zealand application are often markedly different from those that apply when the US application is examined. Generally, the timeframe between the original New Zealand filing and examination of the US application is around 5 years.

The changes adopted by the USPTO have had the effect of narrowing the type of protection available for a particular invention. In the case of pharmaceutical inventions, often protection will now only be achievable for a reasonably narrow and tightly defined set of compounds that have been well described and exemplified. Gone are the days of applicants being able to have claims to a reasonably broad generic class of compounds granted in a single patent with minimal supporting experimental data.

We have also noticed in recent times that it is becoming much more difficult to make a general claim as to the suitable indications for the pharmaceutical compounds. It is best practice to show some in vitro and preferably in vivo utility of the compounds or compositions, but the actual types of trials and supporting experimental details can also be important. It would now be difficult to argue before the USPTO that a class of compounds shows utility for treating cancer in general if in vitro trials have been carried out using only one cancer cell line. The approach taken by the USPTO now is that protection is likely to be limited to the particular cancer exhibited by the cell line, possibly extended to some very closely related cancers. Another thing that makes this more frustrating is that 12 months outside of the original New Zealand filing there is little opportunity to add experimental material which could support more general patent claims, unless the applicant is prepared to look at other (costly) options such as filing continuation-in-part applications.

Another factor that is being raised more often by US examiners is the issue of restriction requirements. A restriction requirement identifies different aspects of an invention and requires an applicant to limit its application to one of the identified aspects. It is then necessary to file a divisional application if the applicant wishes to pursue patent protection for the other aspects of the invention. The aspects of the invention are often closely related. In the case of a pharmaceutical invention, these may even be groups of closely related compounds that show similar utility and have the same general structural features. Such closely related classes of compounds are often, in our view, quite fairly incorporated into the same specification. Where a number of different aspects are identified in the specification and an applicant is required to file one or more divisional applications, this adds significantly to the cost of achieving patent protection covering each of the identified aspects.

To add to the overall climate, the US courts are also issuing decisions that have made it more difficult for US patent holders to assert a broad interpretation of their patent claims.

With the goal posts being moved in this way by the USPTO and the US courts - and we can see no likelihood of let up on the positions being taken - it is vitally important that patent applications are not filed on the skinniest of experimental data. We recommend that substantial exemplification of compounds/compositions, along with a multitude of in vitro and in vivo tests, is the best position to take. Of course applicants need to balance the importance of filing an application to obtain an early priority date and perhaps allow publication of the invention, against the need to include strong supporting data in a patent application.

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

Patent Proze
Baldwins
PO Box 852, Wellington.
Email: *email@baldwins.com*

Helen Palmer and Blair Hesp of Baldwins specialise in chemistry and biotechnology patents. Helen joined Baldwins in 2000. She has a PhD in chemistry from The University of Auckland. Helen is a registered patent attorney in New Zealand and Australia. Blair joined Baldwins in 2006. He has a PhD in pharmacology from the University of Otago as well as a NZDipBus with a management focus. Blair is currently studying towards a law degree and registration as a patent attorney.

Pacifichem 2005

The 5th fifth Pacifichem Congress took place in Honolulu in December 2005. Chaired by the CSJ with vice-chairs from the ACS and CSC, the organizing committee (comprising representatives from CSJ, ACS, CSC, Korean CS, RACI and NZIC), provided a programme that attracted over 11,000 participants and an even greater number of oral and poster research presentations.

At the congress opening ceremony

The size of this conference now makes it the most significant event in the chemistry world. New Zealand was represented by approximately 70 participants that included the NZIC President. With a congress of this magnitude it is not possible to organize many conference–wide activities and the technical programme, subdivided into the 11 sections advised previously here, attracted groups of varying sizes with the large groups, *e.g.* organic, running as many as 20 parallel sessions from 07.30 am until 22.00 daily. Obviously, it was impossible to attend all sessions of interest but the website conference planner allowed one to create a workable personal schedule by reviewing the complex programme structure electronically. Full presentation abstracts were supplied on CD and once attendees adapted to this and used the support systems in place then most were satisfied that they maximized their opportunities to attend presentations of interest.

Oral presentations were in PowerPoint™ and this worked very well with virtually no technical delays in speaker changeover. Most sessions were well attended with audiences ranging from 8-10 for very detailed topics, to 500-800 for major figures in the high profile areas. Poster sessions, occupying 2 hour slots, were highly focused and taken quite seriously by the presenters and the attendees. There was little distraction in the form of refreshments and related social activities to deflect the technical discussion over the relatively short time. A student poster competition, based on a prejudging of all 2000 eligible poster abstracts, then 200 selected posters - including 4 from 4 New Zealand - were presented at a separate time and the final 40 winners were congratulated and awarded prizes of Merck Index.

The NZIC profile was promoted by a poster which formed part of the RACI/CSIRO publication display booth in the Congress Exposition and copies of the recent editions of *Chemistry in New Zealand* were available for information and gifting to interested parties. Success can be measured from the enquiries received about NZ, NZIC, and NZ chemistry – mostly from the viewpoint of potential for visits etc.

NZIC president speaks

Pacifichem is a unique, infrequent, gathering which serves to highlight the wide diversity, energy and exciting future of our science. New Zealand must continue to participate in this Congress through the NZIC whereby additional links and profile enhancement with sister organizations in Pacific rim countries can be anticipated.

Robin A J Smith (NZIC Representative)

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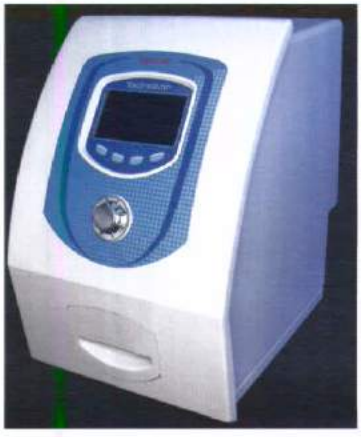

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Secretary: Vesna Davidovic-Alexander

Session Chairs:

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Margaret Brimble, Auckland (Organic and Medicinal Chemistry)
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