

Biotechnology and chemistry: new challenges for metrology

Dr Martin Milton
NPL

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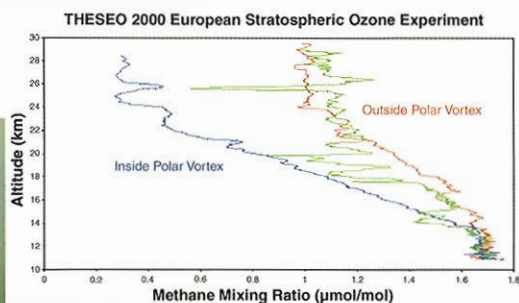
I have been asked to describe some of the challenges that can be foreseen for NPL, and for metrology as a whole, in areas that might be considered to be beyond our historic mission in providing metrology for physical and engineering measurements. I would like to illustrate this by making the point that NPL has never been separated from chemistry, and I have some examples of how we have made measurements of chemical quantities for the last twenty years. I would like to finish with some comments and even speculation about how NPL might be able to contribute to the emerging requirement for measurements in the biotechnologies.

We are celebrating a hundred years of the National Physical Laboratory. It is interesting to see that in that time, as a standards laboratory, work has extended into matters of chemistry. The earliest records of NPL's involvement in chemistry come from a government committee set up specifically to investigate whether NPL should be carrying out chemical testing at all. In an extract from their report in 1907, they concluded that NPL's remit should include *'the standardisation and verification of instruments, but also, under proper restrictions, the testing of materials'*. Of course, the proper restrictions they had in mind was that NPL would not do routine testing, a similar distinction to the one we make today. This little incident illustrates a point, I think, which is that the techniques of physics and chemistry aren't usefully separated.

I would like to give some examples of work we have done measuring chemical quantities over the last twenty years. The first of these centres around the theme of measuring ozone. Ozone first became an issue of national and global concern in the 1970s with the discovery of the depletion of the ozone layer, the so-called Antarctic ozone hole. Even before that time, NPL had a reputation for the development of instrumentation for measuring gases in the stratosphere from high altitude research balloons. A successor to that work now concentrates on the measurement of methane using a high accuracy tunable laser spectrometer. This diagram shows one of the stratospheric balloons that we now use. This rather green scenery is a rather luxurious test flight; the actual measurements in the Arctic and Antarctic, which are the regions of greatest interest around the globe, present a rather more hostile environment than that.

Measurements of Ozone

Traceability: to spectral data
measured at NPL
Uncertainty: $\pm 0.004 \mu\text{mol/mol}$



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The diagram also gives an example of a measurement and shows what can be done with this type of instrumentation. I should say that methane is a tracer gas; it is inert in the upper atmosphere. So it tells us about the motion of the atmosphere itself, from which we can understand the influence of dynamics on the chemistry. So we have here a series of balloon flights. The blue curve shows results made inside the region known as the polar vortex, which characteristically has very low concentrations of methane. Subsequent flights, shown in red, took place outside the polar vortex, with characteristically much higher levels of methane. The interesting flight is the one in green which took place right on the fringe of the polar vortex. We can see here these fingers of air containing very low methane that have been transported out of the vortex to middle latitudes. It is parcels of air like this that are low in ozone that reduce the ozone cover above middle latitudes, certainly down to Scotland and even down to central Europe.

Of course, ozone in the stratosphere is in most ways good for us; ozone at lower atmospheres is not such a great benefit. In the 1980s and early 1990s we pioneered the use of the laser radar method to measure ozone in the lower atmosphere up to altitudes of one kilometre. This is a picture of our mobile laser radar system. In this case it is in the environment of an oil refinery, where we have carried out a large number of measurements of hydrocarbon gases.

This is a natural type of measurement to be made by laser radar because the capability of the system to identify the sources and the quantity of leaks at a distance can actually enable cost savings of up to a million pounds per year to be identified at a large oil refinery.

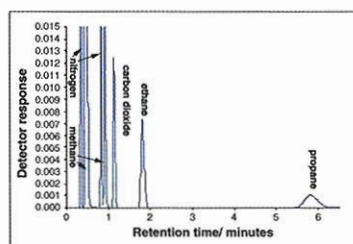
Measurements of Ozone

Traceability: to spectral data
measured at NPL
Uncertainty: $\pm 10\%$ over 100 m

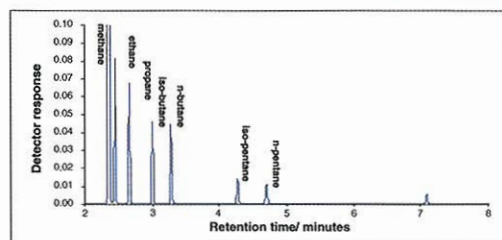


My third example of measuring a chemical quantity comes from measurements of natural gas. The chemical composition of natural gas is very significant to the economy because a lot hinges on the value when we buy and sell natural gas, and that value depends on its energy content or the calorific value, as it's known. Traditionally the calorific value is measured by combusting the gas, a method that has some limitations. More recently, the way we do it at NPL now, is to calculate the calorific value by measuring the components of the gas using high accuracy gas chromatography and standard values of calorific value. This chart shows a typical composition of a natural gas, measured relatively recently. This approach enables us to determine the calorific value without ever carrying out a combustion, to an uncertainty of around a tenth of a percent. It is also an example of another feature of metrology which I think is important and that is what I call the '*coherence of metrology*'. We can do this because we have confidence that the results of a calorimetry measurement will be the same as the results of a gas chromatography measurement multiplied by standard data. That's something that we come to expect from successful metrology.

Natural Gas - by Gas Chromatography



Traceability: to standards prepared at NPL gravimetrically.
Uncertainty: +/- 0.1% (rel)



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The measurement unit of greatest importance to these measurements is the mole, the unit introduced most recently to the SI system. The definition of the mole is quite well known. It emphasises the importance of carbon in chemistry and it also emphasises the importance of mass spectrometry, making measurements relative to carbon. Behind the mole stands the Avogadro constant which is the number of entities within a mole.

“The mole is the elementary amount of substance which contains as many elementary entities as there are atoms in 0.012 kg of carbon 12”

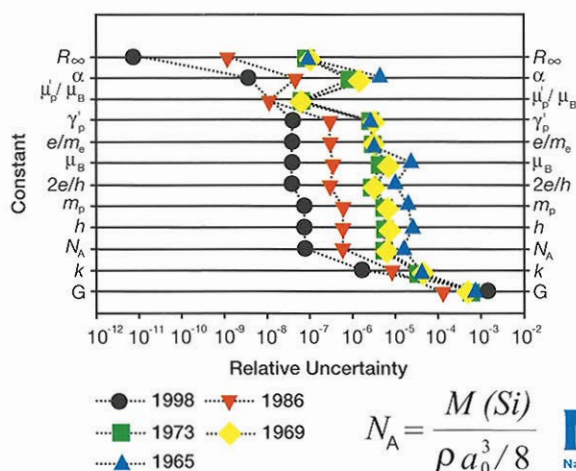
14th General Conference of Weights and Measures 1971

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The diagram shows a chart of progress in determining the fundamental constants over the last thirty-five years and the Avogadro constant, N_A , is shown here - known to a few parts in 10^7 . The most recent direct measurements of the Avogadro constant are made using an experiment which measures the molar mass, the

density and the lattice constant of a pure silicon artefact. An interesting feature of the fundamental constants is the way they are evaluated which takes advantage of knowledge from the physics by which fundamental constants are related. So we see that certain fundamental constants move forward, like an army, together. You can see that the Avogadro constant is a member of a small army of three which indicates fundamental links between the way the Avogadro is defined in chemistry and some aspects of physics. I think it's a further challenge for us to show how this can be used to advance the state of fundamental chemical measurements.

The Avogadro Constant



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I said that the definition of the mole didn't talk about any measurement method - but it is certain that mass spectrometry is very fundamental to chemistry. At NPL, we are carrying out research into the use of mass spectrometry to measure gas concentrations with fractional accuracies beyond the presently attainable limit. The method we use involves taking an unknown sample, adding a known mass of an enriched sample and measuring the isotope ratio of the consequent blend. The diagram shows some recently obtained results of a series of sixty runs. The horizontal red line is the region in which we expect the true answer to lie because we have access to gravimetric reference data. The points indicated in purple and blue are our measurements made by mass spectrometry. We can see that they lie very close indeed to the central point of our reference data.

There is a great deal more work to be done in this area. As we can see, there is a lot of structure that hasn't been sorted out, but nevertheless there is a lot of promise that this method might lead to one of the small holy grails of chemical measurement, which would be to make chemical analysis measurements only by reference to pure chemical substances.

High Accuracy Chemical Measurements by Mass Spectrometry

Enriched spike

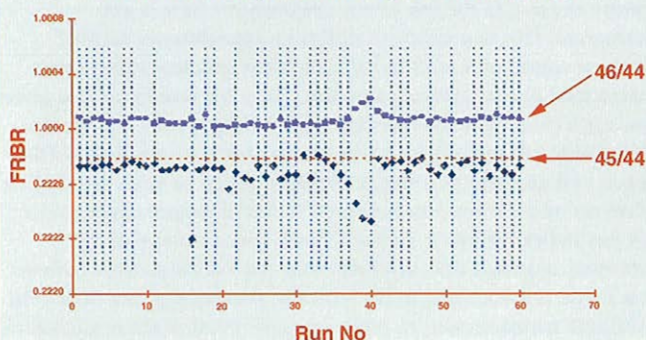
Unknown sample

Blend

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High Accuracy Chemical Measurements by Mass Spectrometry

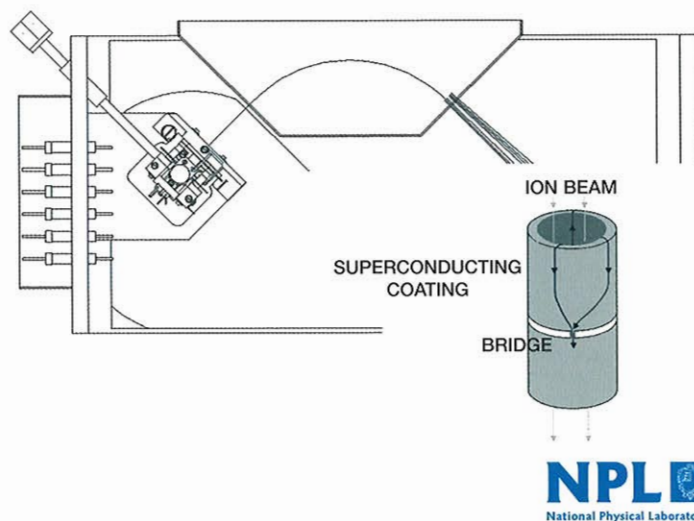
Gravimetric (Reference) versus IDMS



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A further challenge, of course, would be to develop a mass spectrometer that actually worked with such intrinsic accuracy that we never needed to consider standardisation at all. This is a schematic of the mass spectrometer we use at NPL: the gas enters, it's ionised, it flies through a magnetic field and it is dispersed towards the detectors. Now, many of the uncertainties associated with this technique lie in the imperfections in the detectors. A piece of research we are doing at the present looks at the potential to replace those detectors with a superconducting squid magnetometer. Already we've been able to demonstrate that a squid magnetometer can detect ion currents of a few microamps and we expect future progress to get us to the level where such a device could make measurements in a reference mass spectrometer.

Mass Spectrometry with Cryogenic Current Sensing



Measurement Methods Applied to Biotechnology

Bulk/aggregate properties

Biochemical behaviour

Location and identification of molecules

STRUCTURE

FUNCTION/REACTION

STRUCTURE/INTERACTION

Physico-chemical Methods	Biological Methods	Single Molecule Methods
• circular dichroism • scanning calorimetry • FTIR • flow cytometry • mass spectroscopy	Analysis • ELISA • PCR/eletrophoresis • DNA chips Detection • Fluorescent tagging • Spectroradiometry • Radioisotope labels • Electrochemical	Location • FRET • Fluorescence correlation Identification • SERRS • STM / IETS

I said I'd make some comments, some speculations even, about biotechnology. When we look at biotechnology, what type of measurements do we see being made? I would like to talk about three types. The first type of measurement is measurements of what we might call the bulk or aggregate properties of materials or the structure. A particular example might be the temperature or the point in a process at which a protein changes its folding pattern. These measurements are already made by what might be called *physico-chemical methods*; techniques that depend on light, techniques that depend on temperature and so on.

The second type of measurement that we see in the biotechnologies are what we might call biological methods. These access the function and reaction of molecules - for example, to identify the presence of genetically modified material. Biological methods typically consist of some type of biologically sensitive methods such as an immunoassay or a polymerase chain reaction or DNA chips, increasingly followed by detection, using a relatively traditional physical technique.

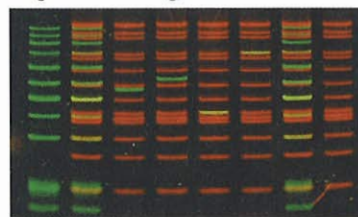
The third type of measurement that we see in biotechnology refers observations on a single molecule to the structure and interaction of individual molecules. These might be used to evaluate

the biocompatibility of materials or the suitability of particular mechanisms for bonding molecules on to DNA chips. These are made using so-called single molecule methods.

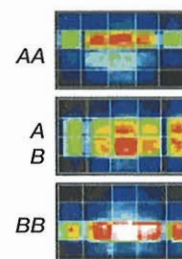
This is a very broad range of methods and it seems likely that they are going to revolutionise research in many areas, particularly the production of new drugs, the development of new crops and even the production of criminal evidence. So it is quite right to ask: do they meet the users requirements and can they be relied upon?

"Detecting" the results in biotechnology

Fluorescence image of gel chromatogram



Traceability: ?



Spectro-radiometric image of an array

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Let me show a specific example. These are two particular examples of measurements. On the left of the diagram we have a gel chromatogram. This is a series of different experiments each of which has resulted in a mixture of biological species which have been separated in the vertical direction. They are visible on this plate because each one has been labelled with a fluorescent tag. On the right hand side are magnified images from above of a so-called DNA chip. Each cell consists of a single stranded piece of DNA bonded to the substrate and we see fluorescence where a tagged single piece of DNA has hybridised to it. In both cases, the accuracy of the measurement depends on our ability and our confidence to believe that this stripe is something to do with the presence of the molecule. It is a difficult measurement to make because in all of these examples, the fluorescent intensity measured is crucially dependent on other things that we don't want to measure, such as the temperature (is there a temperature gradient across here?), such as the presence of other interfering chemicals and, of course, such as non-idealities in the instrument itself.

Conclusions - new challenges for metrology?

Metrology is a single discipline - that provides measurements that are:

- *Comparable between laboratories*
- *Stable over time, and*
- *Coherent between different methods*

Different areas present different challenges

- *At present, many chemical and biochemical measurements are carried out using methods that are not fully characterised*

New applications of metrology require new partnerships

- *Between NPL's metrology missions and organisations with specific expertise.*

How can I summarise such a brief race through these challenges for NPL? I hope I have illustrated that metrology is a single discipline and it has fundamental principles that are applicable to all areas of science and technology including chemistry and biotechnology.

The objectives of metrology include providing results that are comparable between laboratories, stable over time and consistent. For one hundred years we have done that by providing measurements that are traceable, most recently to the SI system. The demand for that type of traceability is now as great as it ever has been. Secondly, having said that metrology is a single discipline, of course different areas present quite different challenges. For example, at present, the majority of measurements supporting the biotechnologies are using methods that have not been fully characterised. Consequently, in some cases, they don't meet the requirements of regulators, consumers and industry. Future work needs to be concentrated on those applications which most need comparability, long-term stability and coherence. Finally, new challenges such as the ones I have talked about can be met through partnership between NPL, with its mission as a metrology institute, and other organisations with very sector specific experience. This will ensure that NPL's skills are effectively exploited to meet new challenges. «

Sir John Rowlinson (chairman)

Thank you Dr Milton. We now return to the partnership of the NPL with industry, and David Richardson, the Director of Marketing and Knowledge Transfer, will tell us more about that.