SAC 92

The following are summaries of fourteen of the papers presented at the tenth SAC Conference held on September 20–26, 1992, in the University of Reading. The conference incorporated the third Spectroscopy Across the Spectrum Conference and the 150th anniversary celebrations of the Laboratory of the Government Chemist.

Bimolecular Photoabsorption Spectroscopy

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The development in recent years of new analytical methods based on non-linear optics has already been rewarded with a remarkable range of applications. In principle, any chemical sample can display optical non-linearity, and non-linear response to laser light in particular underlies the operation of a wide range of modern techniques such as CARS (coherent anti-Stokes Raman scattering), laser mass spectrometry and surface harmonic generation.[1–3] Most types of optical non-linearity owe their origin to the essentially simultaneous interaction of sample molecules, atoms or ions with two or more laser photons: this is precisely why highly intense light is required. However, it has recently become evident that under such high intensity conditions, individual photons can simultaneously interact with two or more sample molecules in a type of role-reversal of conventional optical non-linearity.

Bimolecular photoabsorption generally entails the co-operative excitation of pairs of molecules in close proximity to each other, usually at nearest neighbour distances, through a mechanism similar to Förster energy transfer. In the simplest situation a single photon provides the energy for the excitation of two chemically different molecules A and B

$$A + B + h\nu \rightarrow A^* + B^*$$

Given that neither the spectrum of the pure component A nor that of pure B displays absorption at the frequency ν, then the above mechanism necessarily generates spectral features that can be unambiguously identified as originating from species A in interaction with B.[4] The essential energetics can be understood from Fig. 1.

Two different excitation schemes can be entertained for two-photon bimolecular absorption,[5] as shown in Fig. 2. In Fig. 2(*a*), both photons are absorbed from a single high-intensity laser beam operating at a frequency that is precisely the average of the absorption frequencies of A and B. In Fig. 2(*b*) two photons of different frequencies are absorbed (for example, one from a pump and the other from a dye laser) and two chemically equivalent molecules are simultaneously excited. Both can be regarded as instances of mean-frequency absorption. The scheme shown in Fig. 2(*a*) is of more analytical potential as it identifies the proximity of two components: the main virtue of the scheme shown in Fig. 2(*b*) is that source tunability offers a means of exploiting one of several resonance enhancement mechanisms.[5]

Bimolecular photoabsorption affords a unique *in situ* method for detecting the physical proximity of two chemically different species. As such it offers considerable scope for the study of any inhomogeneous fluid in which two chemically different constituents are both present only in certain areas, as, for example, at micellar boundaries or other types of liquid–liquid interface. Another obvious area of application lies in the microanalysis of surfaces and solid interfaces. In this respect bimolecular excitation is a great deal more surface-specific and also offers considerably more scope for chemical identification than harmonic generation. The method is also applicable to the analysis of samples in which a given analyte comprises either

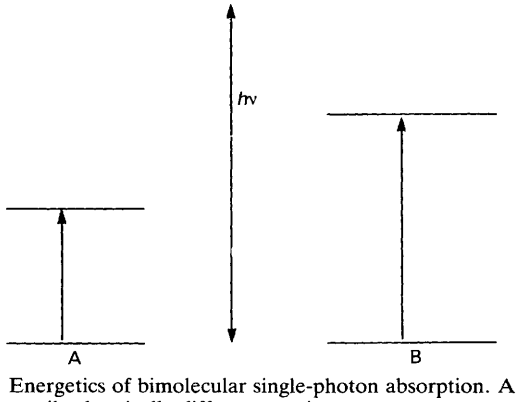

Fig. 1 Energetics of bimolecular single-photon absorption. A and B are necessarily chemically different species

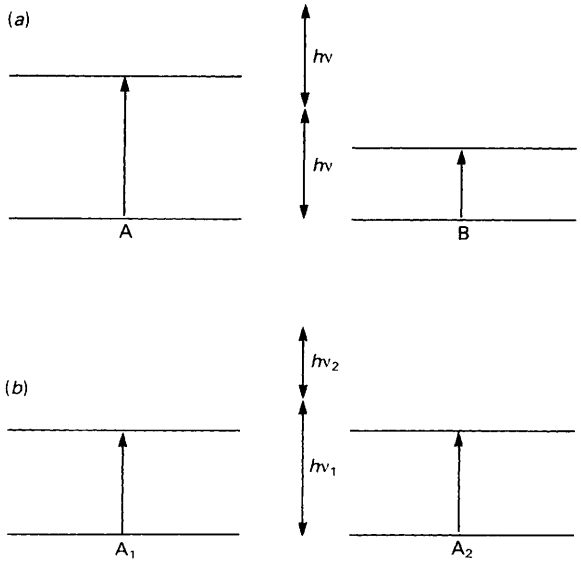

Fig. 2 Energetics of bimolecular two-photon absorption: (*a*) single-frequency excitation involving two chemically different species; (*b*) two-frequency excitation involving two molecules of the same species

one or both of the partner species: here it is possible to improve detection limits for species that are normally considered optically transparent.

Experimental studies have already demonstrated the occurrence of bimolecular photoabsorption in a wide range of media. Most studies have been performed in the gas phase or in crystals, but the effect has also been exhibited in solutions and inert gas matrices (extensive citations are given in refs. 4 and 5). A familiar example is the dimol absorption of liquid oxygen, which, through bimolecular absorption at 634 nm, is responsible for the blue colour of the liquid. Detection is typically based either on absorption or fluorescence measurement: the latter benefits from a much better signal-to-noise ratio, and generally requires monitoring of the fluorescence from only one of the analytes. In certain instances, where the product A^* or B^* spontaneously undergoes chemical reaction or dissociation, chemical or mass spectrometric detection methods can be employed: a simple example is provided by the mixture of H_2CO and D_2CO.[6]

As further studies continue to increase the familiarity of bimolecular excitation, it is to be hoped that experimental developments will enable the full analytical potential of the technique to be realized, so that it can begin to take its rightful place alongside other methods of laser analytical spectroscopy.[7]

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Polarographic Behaviour of *N*-Oxides of Oxazolidines and Their Analytical Application

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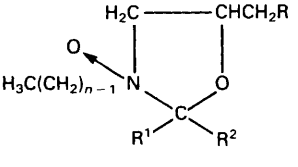
Electroanalytical determinations of *N*-oxides, which complete the classical redox potentiometric and chromatographic determinations, have been reviewed by Devinsky.[1] Rapid, reproducible and precise determination of the *N*-oxides of substituted 3-alkyl-5-chloromethyloxazolidines has been achieved by exploiting the readily accessible technique of d.c. polarography,[2] which has also been used for controlling the synthesis of *N*-oxides by oxidation of the original oxazolidine derivatives with H_2O_2.[3] This technique enables the mixture of reaction products to be determined, including adducts of *N*-oxides and oxazolidines, which do not interfere. It employs the polarographic currents controlled by diffusion of the active compound to the electrode.

The diffusion current for a two-electron reduction limits the sensitivity of the technique to concentration levels of approximately 5×10^{-5} mol l^{-1}. As an increase in sensitivity and the ability to determine a larger number of *N*-oxides are preconditions for the wider application of polarographic methods, it was decided to investigate the use of currents based on adsorption and catalytic effects. These are determined by adsorption of the active compound on the surface of the mercury drop electrode or by its influence on the reduction of the hydrogen overvoltage at this electrode.

The polarographic behaviour of 23 derivatives of *N*-oxides of oxazolidines, and for comparison that of *N,N*-dimethyldodecylamine *N*-oxide, and their adducts with hydrogen peroxide, was investigated. The different substituents, as indicated in the general formula below, are in positions 2, 3 (alkyl; n = number of methylene units) and 5 (R = H, Cl).



The changes of substituent were always performed at one position only, while the substituents at the other positions remained unchanged. The strong electron accepting substituent Cl in position 5 increases the strength of the oxazolidine ring. Consequently, the *N*-oxides are less easily protonated, polarographic currents are controlled by diffusion, there are fewer changes in the current and the half-wave potential, $E_{\frac{1}{2}}$, shifts with the pH. The temperature coefficient does not exceed 3% in each interval. The correlation coefficient (r) for the dependence of i_D on $h^{\frac{1}{2}}$ (h is the height of mercury above the mercury drop electrode) is 0.99 [Fig. 1, curve 1; Fig. 2, current $i_{2(D)}$]. Stabilization of the oxazolidine ring is due to intramolecular interactions between the chlorine atom and the strongly polar *N*-oxide group in the half chair conformation, which agrees with the findings of Pihlaja and Alajoki,[4] based on NMR data.

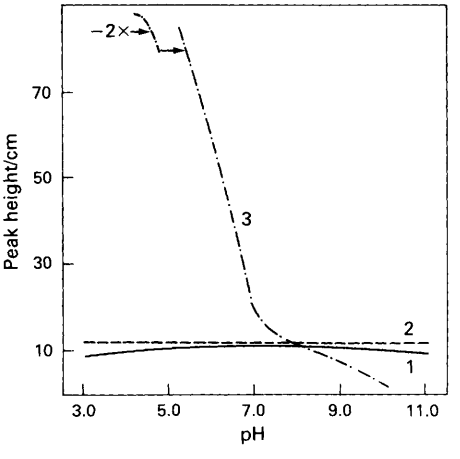


Fig. 1 Dependence of polarographic currents of oxazolidine *N*-oxides on pH. 1, Diffusion current of *N*-oxides; 2, H_2O_2; and 3, mixed (catalytic and adsorption) currents of *N*-oxides; 2×, twice decreased

When R = H, the 2-substituted 3-alkyl-5-methyloxazolidine *N*-oxide becomes less stabilized and the catalytic and adsorption effects are more important and the mixed currents play a larger role [Fig. 1, curve 3; Fig. 2, current $i_{4(K+A)}$]. Changes in $E_{\frac{1}{2}}$ are observed and the current i corresponds to protonation.

An increase in the length of the alkyl chain in position 3 (n increases from 0 to 18) increases the adsorption properties of the *N*-oxide. Derivatives of 2,2-disubstituted 3-dodecyl-5-methyloxazolidine *N*-oxides (Fig. 1, curve 3) are typical representatives of this group. The 3-octyl-5-methyl derivative also belongs to this group.

Substituents in position 2 do not have such a strong influence when they are strongly electronegative, *e.g.*, dinitrophenyl, *p*-nitrophenyl, *p*-bromophenyl or trichloromethyl substituents, and they have an opposite effect on the oxazolidine ring to that of a chloro substituent in position 5. This effect itself influences the character of the polarographic current $i_{3(D+A)}$ or $i_{4(K+A)}$ (see Fig. 2).

When $E_{\frac{1}{2}}$ and the current are not dependent on pH, the curve for H_2O_2 limited by diffusion [Fig. 1, curve 2, $i_{1(D)}$] can be compared with the curve for the acyclic *N*,*N*-dimethyldodecylamine *N*-oxide [Fig. 2, $i_{3(D+A)}$], which is strongly protonated. In the pH range 2–10 the value of $E_{\frac{1}{2}}$ changes by about 500 mV, whereas in the pH range 2–8 the current does not change at all. The current decreases with increasing temperature; this behaviour is typical of an adsorption current. Similarly, there is a slight increase in the current with an increase in the height of the mercury reservoir (non-linearly with the square root), *viz.*, an 8% increase in the range 25–81 cm.

On the other hand, the catalytic current of 3-dodecyl-5-methyloxazolidine *N*-oxide grows rapidly with increasing temperature (the temperature coefficient in the range 20–70 °C is 5.6% per gradient), but decreases slightly with an increase in the height of the mercury above the electrode (about 9% in the range 25–81 cm). Catalytic and adsorption currents are easier to observe at lower concentrations of polarographically active compounds and when the lifetime of the mercury drop is short.

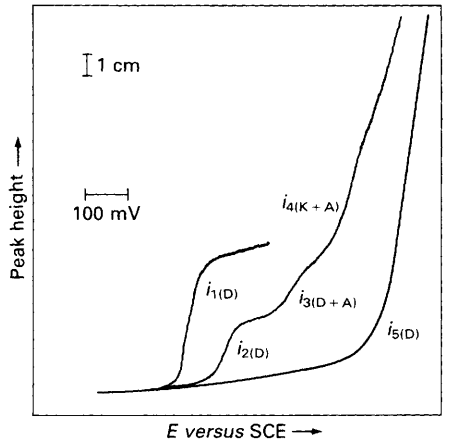


Fig. 2 Typical polarogram of polarographic currents [$i_{(\ )}$]. D, diffusion; K, catalytic; and A, adsorption. 1, H_2O_2 and addition products; 2, 5-chloromethyloxazolidine *N*-oxide; 3, dodecylamine *N*-oxide; 4, *N*,*N*-dimethyl-5-methyloxazolidine *N*-oxide; and 5, supporting electrolyte: Britton–Robinson buffer; pH 8, from −0.8 V *versus* SCE; sensitivity, 1.6×10^{-7} A; temperature, 20 °C

The values of $E_{\frac{1}{2}}$ for H_2O_2 and the addition products are: $i_{1(D)} = -1.0$ V; for the *N*-oxides: $i_{2(D)} = -1.15$ V, $i_{3(D+A)} = -1.35$ V, $i_{4(K+A)} = -1.5$ V; decomposition of the supporting electrolyte occurs at −1.7 V. The precise polarographic data for individual *N*-oxides are given elsewhere.[2,3,5]

Statistical evaluation of the concentration dependencies, employing the limited experimental data available, showed that both groups of compounds are readily determined in the concentration range from 5×10^{-5} to 1×10^{-3} mol l^{-1} using the diffusion-limited current of the addition products ($r = 0.999$; $a = 1.82 \pm 0.2$ mm) (a = intercept) and that of the *N*-oxides ($r = 0.99$; $a = 2.0 \pm 0.6$ mm).[6]

By using the mixed polarographic current the sensitivity can be increased (concentration range from 1×10^{-6} to 1×10^{-4} mol l^{-1}), but the statistical parameters deteriorate (*e.g.*, $r = 0.9$). There is a strong dependence of i on the concentration of the *N*-oxide,[7] but the accuracy of the determinations decreases, thus limiting the sensitivity. Polarographic waves are better developed and determined when the height of the mercury reservoir above the mercury drop electrode is large and the lifetime of the mercury drop is short. The influence of pH and temperature indicates that the analytical application of mixed currents of *N*-oxides requires strict control of the conditions, particularly pH and temperature. It might be possible to optimize the determination so that several *N*-oxides and their addition products with H_2O_2 could be determined in their mixtures, although, in general, the sensitivity would be rather limited. In addition, it might also be possible to differentiate *N*-oxides with similar properties.

The significance of the polarographic technique described here is that it is suitable for controlling the purity and stability of *N*-oxides and for monitoring their synthesis.

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Lasers and Ion Trap Mass Spectrometry

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The combination of lasers and mass spectrometry provides a powerful method for extending the range of the mass spectrometric technique.[1,2] Quadrupole ion trap mass spectrometers are particularly well suited to laser techniques because of the long irradiation times, which can be used for ion photodissociation (PD) studies, and the compatibility of these devices with pulsed ionisation techniques such as laser

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desorption (LD). Various laser interfaces and applications have been reported for ion trap mass spectrometry using either direct laser introduction or fibre optics.[3–6] Fibre optic interfaces are easy to install, require little modification of the ion trap and allow both PD and LD analytical experiments to be performed.

Fig. 1 shows two fibre optic interface configurations implemented on a research ion trap mass spectrometer (ITMS).[7] In one configuration a 1 mm fibre optic is threaded through a vacuum seal mounted in the upper flange of the vacuum housing and enters the trap through a hole in the ring electrode, whilst the other uses a flexible 0.6 mm fibre fitted into a probe, which can be introduced into the spectrometer *via* the solids probe lock without breaking the vacuum. In both instances, the laser light is coupled to the fibre by using a three-axis delivery system outside the vacuum housing.

Photodissociation

Tandem mass spectrometry (MS–MS) has been used widely for structural studies and for the quantitative determination of analytes in the presence of a complex matrix. The dissociation method predominantly used for MS–MS is collisionally activated dissociation (CAD),[8] but it is also possible to use photodissociation for ions with suitable absorption characteristics.[9] PD has the advantage over CAD that, in principle, the energy deposition may be controlled, since it is a function of the photon wavelength. This is illustrated by the PD/MS–MS product ion spectra of the isolated molecular ion of butylbenzene, *m/z* 134, acquired using photons of 488 nm (2.6 eV) and 350 nm (3.7 eV). The branching ratio of the *m/z* 91 ($C_7H_7^+$) and *m/z* 92 ($C_7H_8^{+\cdot}$) ion intensities in the product ion spectrum reflects the energy deposition. An enhanced ratio is observed at the lower wavelength since the high energy dissociation process leading to the *m/z* 91 fragment is preferred at this wavelength, whereas this ion is absent when the ion is irradiated at 488 nm. It is difficult to control the energy in this way by using CAD activation.

CAD and PD/MS–MS spectra may provide complimentary structural information for selected ions because differences in these activation processes allow access to alternative fragmentation pathways. This is demonstrated by the MS–MS product ion spectra of the isolated molecular ion of naringenin, *m/z* 272, [2-(4-hydroxyphenyl)-5,7-dihydroxychroman-4-one] acquired by CAD and PD at 350 nm (Fig. 2). The product ions formed from the two activation methods are quite different. The CAD spectrum shows ions at *m/z* 254, $[M - CO]^{+\cdot}$ and *m/z* 166, $[M - CHC_6H_4OH]^{+\cdot}$, both of which are absent from the PD spectrum, which shows simple cleavage products at *m/z* 179 $[M - C_6H_4OH]^+$, *m/z* 153 $[(HO)_3C_6H_2CO]^+$ and *m/z* 137 $[(HO)_2C_6H_3CO]^+$.

A further advantage of using PD with an ion trap spectrometer is the high dissociation efficiencies (10–100%) which may be obtained compared with those for linear or beam spectrometers (<1%). The sensitivity of PD with the ion trap has been shown to be compatible with chromatographic introduction of samples at the low nanogram level; an analysis which is not possible with the low efficiencies of a beam experiment.[7]

Laser Desorption

The fibre optic interface can also be used for the analysis of non-volatile, thermally labile or ionic compounds by laser desorption. Here the compatibility of the ion trap with pulsed ionization techniques is important.[2] Those samples which are ionic, for example the dye rhodamine 6G, need only be desorbed to yield gas-phase ions and the positive ion spectrum [Fig. 3(*a*)] shows the cationic part of the dye at *m/z* 443. The spectrum was obtained simply by placing approximately 1 μl of a solution of the dye in glycerol on the tip of the probe-mounted fibre optic, which was inserted *via* the probe lock. The sample was subjected to laser pulses (532 nm) from a Nd–YAG laser. At this wavelength no fragments are formed but tandem mass spectrometry on the cation (*m/z* 443), following laser desorption, yields characteristic product ions. Fig. 3(*b*) shows the LD/MS–MS analysis of rhodamine 6G using CAD activation.

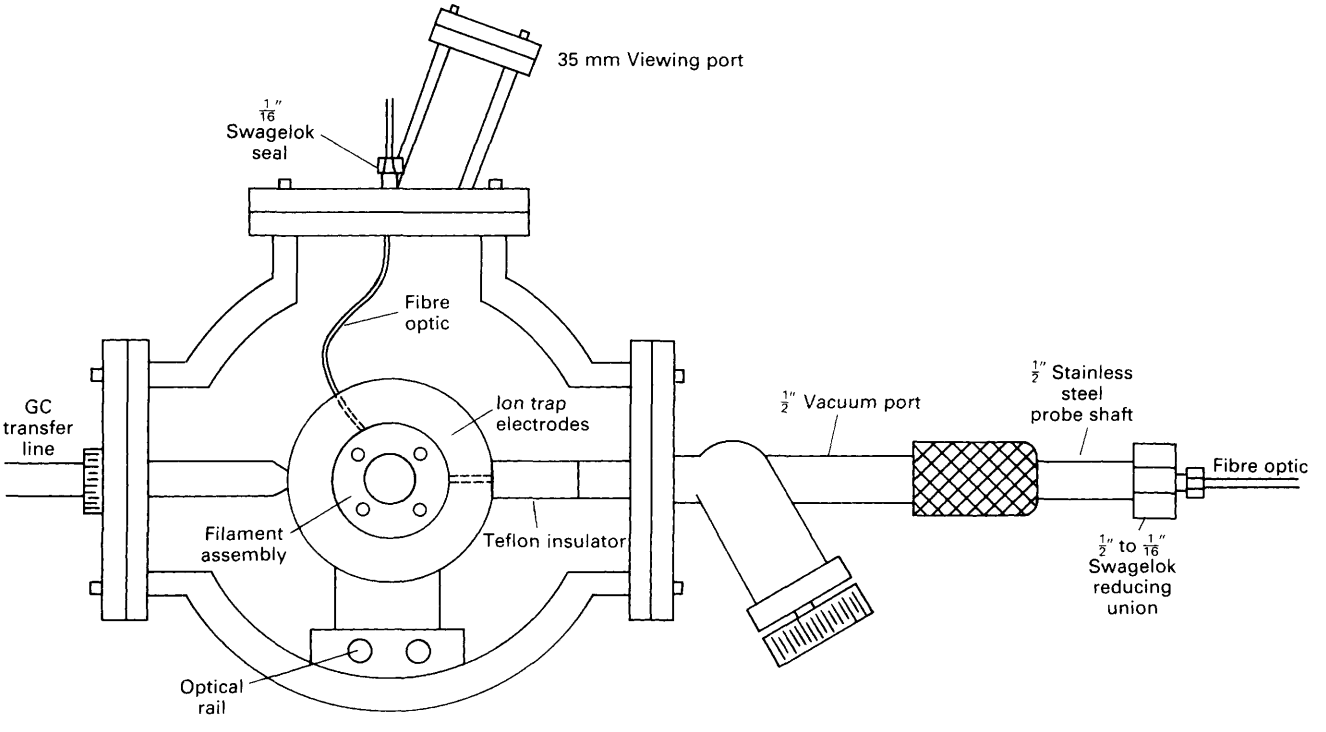

Fig. 1 Fibre optic interfaces for ion trap laser desorption and photodissociation

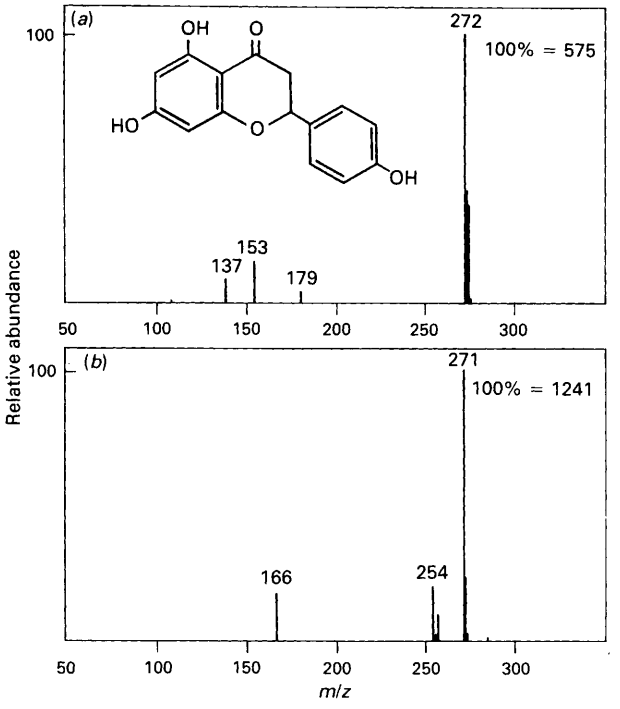

Fig. 2 MS–MS product ion spectra of the *m/z* 272 ion of 4′,5,7-trihydroxyflavanone using: (*a*), PD (350 nm); and (*b*), CAD

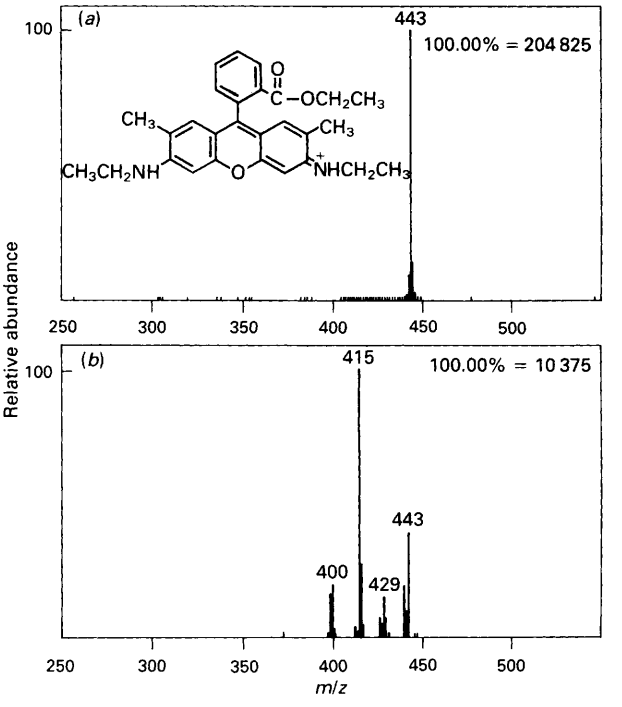

Fig. 3 Laser desorption ion trap mass spectra of rhodamine 6G: (*a*), LD–MS; and (*b*), LD/MS–MS of *m/z* 443

Non-ionic analytes subjected to LD must undergo the dual functions of desorption and ionization. Cationized products such as $[M + Na]^+$ or $[M + K]^+$ are commonly formed by LD in the presence of alkali metals. However, some molecules can be ionized directly by a multi-photon ionization (MPI) mechanism, if there is sufficient energy in the laser beam. For example, the laser desorption/ionization spectrum of naringin, the glucosylrhamnose derivative of naringenin, using a single 355 nm pulse from a Nd–YAG laser at a power of 360 μJ, shows the molecular ion (*m/z* 580) and a fragment ion at *m/z* 271 resulting from the loss of the glycoside group. The simplicity of interfacing continuous wave and pulsed lasers with the ion trap using fibre optics therefore provides a convenient system for a range of laser based analytical applications.

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Measurement Uncertainty in Chemical Analysis

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One of the first problems faced by analysts is whether they have the methodology to provide a result of the required 'accuracy'. The word accuracy is in inverted commas to denote the colloquial use rather than that given in the standard definition of metrological terms. However, after carrying out an analysis, it is very unusual for an analyst to give any indication of the 'accuracy' of the result. This means that the user of the result is unable to make any judgement on the confidence to be placed in it, nor is it possible to compare in a rational way the results of an independent analysis of the same sample.

It is now becoming recognized that a statement of a result is not complete without including in it information about the 'accuracy' or the 'uncertainty' of the result. Indeed many customers now insist that the 'uncertainty' be given and this is a requirement of accreditation standards.

Before going into the concept of uncertainty and its

evaluation, it is essential to be clear about the meaning of the terminology used, since terms such as 'accuracy', 'error', 'trueness' and 'uncertainty' have colloquial meanings that are not necessarily the same as those of the formal definition.

Definitions

The following definitions are based on those given by the International Organization for Standardization (ISO) but have been simplified for the purpose of this paper. Error is the result of a measurement minus the true value; accuracy is the closeness of agreement between the result of a measurement and the true value; uncertainty is an estimate of the range of values within which, at a certain level of confidence, the true value is asserted to lie. These terms will be used without inverted commas to indicate that their meaning is as given in these definitions.

From these definitions it is clear that there is a significant difference between the meanings of error and uncertainty. Firstly, error is the difference of two values whereas uncertainty is a range. Secondly, error requires a knowledge of the true value, or at least a nominal true value, and hence only has meaning when this value is known, *e.g.*, comparison of the result of a measurement of a standard with the value quoted for the standard. Thus, the error cannot be evaluated for measurement of a sample when the value is not known and therefore is not very useful for stating the 'accuracy' of a result of an analysis of a real sample. Uncertainty, however, is an estimate of the range of values which is expected to include the true value, and obviously can be used when the true value is not known. In order to understand how the uncertainty can be evaluated it is necessary to appreciate these differences.

Evaluation of Uncertainty

Detailed evaluation of uncertainty is carried out as common practice, by national standards laboratories on the realization of base and derived units. The techniques they use, sometimes called the 'genealogical' approach, rely on assessing possible causes of error then, by means of subsidiary experiments and/or theoretical analysis, determining the correction for each cause and building up an uncertainty budget by evaluating the uncertainty on the correction, even if the best estimate of the correction is zero. This can be a tedious and time-consuming operation, although often the components of the uncertainty budget associated with just a few corrections dominate.

Fortunately this 'genealogical' approach can be considerably simplified for more routine measurements by calibration of the measurement system with 'traceable' measurement standards, since the calibration can reduce considerably the number of uncertainty components that have to be evaluated.

Traceability

For routine measurements the correct use of 'traceable' standards is essential to ensure reliable results and to enable the uncertainty to be evaluated.

Traceability means that results are traceable to national or international measurement standards through an unbroken chain of comparisons with the uncertainty being stated at each stage.

An essential element of this traceability chain is that the uncertainty is given at each stage; thus the uncertainty on the working standard used for calibration is known whichever level of the chain is used to provide the working standard.

The Measurement System

It is essential that the measurement system, shown schematically in Fig. 1, is in what is known as a state of 'statistical control', *i.e.*, repeated measurements over a period of time of standard samples processed right through the system from point A are consistent with the measured variance (relative

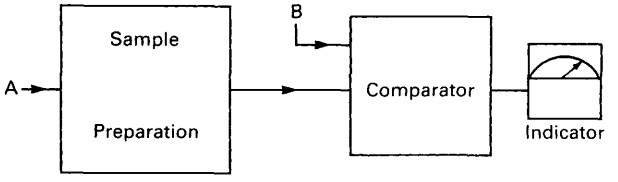

Fig. 1 Schematic diagram of measurement system

standard deviation s_R) of the system. For the sake of simplicity sampling has not been included in the measurement system but in many instances it will need to be.

This measured variance of the measurement system identifies one component of the uncertainty budget, since the over-all uncertainty must be at least as large as the uncertainty on repeated measurements of a standard sample.

The system can be calibrated in a number of ways, each requiring a different number of steps in evaluating the uncertainty. Calibration can be carried out by: (*1*) measurement of a certified reference material (CRM), processed through the complete measurement system (traceability provided by producer of CRM); (*2*) by addition of known amount of analyte to unknown sample (traceability *via* calibrated weights and compounds of known composition); (*3*) addition of a tracer (spiking), *e.g.*, isotopically or radiolabelled compound (traceability from known amount of tracer); (*4*) measurement of a 'pure' standard of the analyte, used to calibrate just the 'comparator' with the calibration standard introduced at point B (traceability *via* calibrated weights and compounds of known composition).

The second component of the uncertainty budget is the uncertainty on the value of the calibration standard itself, which it is assumed can be stated as a relative standard deviation s_{CS}. This should certainly be so if the calibration standard is a CRM, in other instances the uncertainty on the calibration standard may have to be evaluated using the 'genealogical' approach.

The third component of the uncertainty budget arises from the reproducibility of the calibration measurements, relative standard deviation s_{CM}. If the same calibration procedure has been used in calibrating the measurement system when determining the long-term reproducibility then it will be also necessary to consider whether or not s_{CM} has been included in s_R.

The fourth component of the uncertainty budget, and the one most difficult to evaluate, arises from the suitability of the method of calibration for calibrating the whole system. Unfortunately, this component has many sub-components that have to be evaluated using the genealogical approach. If the calibration was carried out using a CRM based on a real sample it would only be necessary to consider the effect of differences in the level of contaminants and any difference in the level of the analyte in the CRM and in the sample. If the composition of the CRM was different from that of the sample then the effect of this would have to be evaluated. Also, if there was a significant difference between the calibration factor using methods (*1*), (*2*) or (*3*) and method (*4*) this would have to be investigated and the associated uncertainty evaluated.

Calibration by method (*4*) alone is clearly unsatisfactory, since it is necessary to apply the genealogical approach to all of the measurement system between points A and B, a formidable task.

Thus, using the genealogical approach it is possible to assess the contributions to the uncertainty budget arising from any lack of suitability of the method used for calibration to calibrate the complete system. If this is assessed as s_G (again as relative standard deviation) then the over-all uncertainty is given by:

$$k[(s_R)^2 + (s_{CS})^2 + (s_{CM})^2 + (s_G)^2]^{\frac{1}{2}} \quad (1)$$

where k is a factor agreed with the user of the result and in most instances a value of two is used.

Example

A detailed evaluation of uncertainty has been carried out by the Laboratory of the Government Chemist (LGC) on the reference material used to calibrate breathalysers. The reference material is a solution of ethanol in water and the ethanol concentration is measured by quantitative oxidation of the ethanol with potassium dichromate, using a CRM for the preparation of the dichromate solutions. The procedure is shown schematically in Fig. 2; dichromate in slight excess is added and this excess is determined by titration with ammonium iron(II) sulfate which is itself calibrated against the standard potassium dichromate.

First the uncertainties arising from the reproducibility of the calibration procedures are evaluated; these are shown in Fig. 3, the largest arising from the reproducibility of the titration.

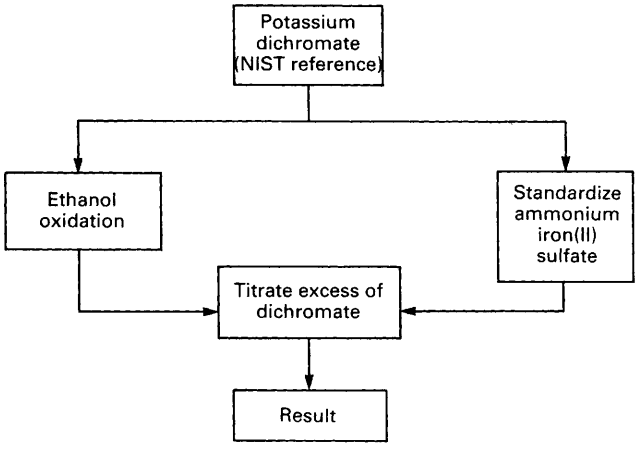

Fig. 2 Procedure for ethanol determination

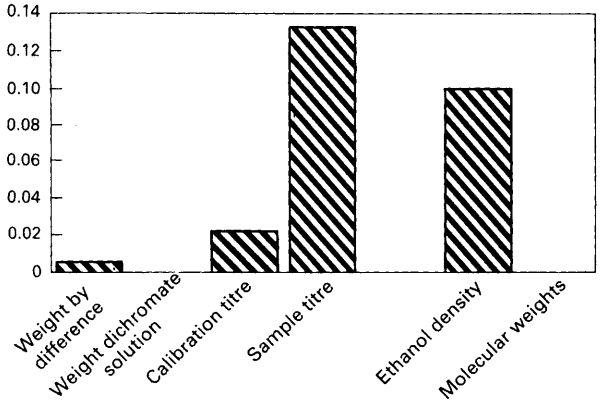

Fig. 3 Physical uncertainty components (%) for ethanol determination

Then considering those arising from the suitability of the calibration procedure to calibrate the complete system, there is a component arising from the conversion of mass to volume since the ethanol reference material value is required in mg ml^{-1}, and there are three further components arising from the 'chemistry' of the method used: (*1*) the purity of the dichromate standard; (*2*) the extent of oxidation which may not be complete or which may proceed further with the oxidation of the product of the initial oxidation; and (*3*) the uncertainty arising from the determination of the end-point of the titration. These are shown in Fig. 4; combining these components gives an over-all uncertainty of 0.5%, using a value of $k = 2$.

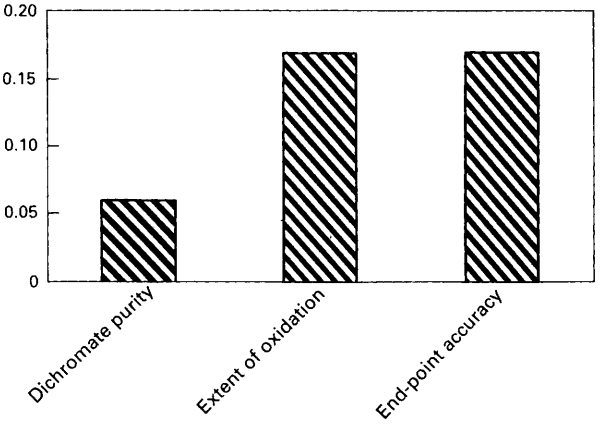

Fig. 4 Chemical uncertainty components (%) for ethanol determination

Proficiency Testing

A very good way of checking the derived uncertainty is by participation in proficiency testing, providing the organizers of the scheme give the uncertainty on the assigned value. Participation is particularly valuable for this purpose when the proficiency scheme utilizes real or natural samples.

Summary

It is now accepted that the statement of an analytical result should include a statement on the uncertainty of the quoted value, evaluated from: reproducibility of measurement system, s_R; uncertainty on calibration standard, s_{CS}; reproducibility of calibration measurement s_{CM}; uncertainty derived from 'genealogical' approach, s_G; all of the above giving an over-all uncertainty using expression (1).

Although calibration of the measurement system using traceable standards considerably simplifies the assessment of the uncertainty, it still relies on the skill of the analyst in assessing the suitability of the calibrated measurement system for carrying out the analysis required, but after all this is what the analyst is paid to do!

The author acknowledges the contribution of Dr. S. Ellison and Mr. T. Farrant in calculating the uncertainties on the data which were kindly supplied by Mr. S. Evans of the Forensic and Customs Division, LGC.

Smuggling and Separation

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Many different types of materials are smuggled across international boundaries, usually with a view to financial gain. By far the most publicity is given to the smuggling of drugs of abuse. The process of separation, at least for drugs from natural sources, begins at the point of harvesting. Separation occurs again at the point of seizure of the drugs by the customs or police officer. Internally concealed drugs (stuffed or swallowed) may need the assistance of a surgeon to effect a separation from the smuggler. Once the suspect drug reaches the laboratory, separation science plays a vital role. Identification of drugs of abuse is most frequently based on chromatographic retention data in conjunction with infrared spectroscopy or mass spectrometry.

A laboratory should be able to provide much more than confirmation of the identity of drugs if it is to support enforcement activities fully. The enforcement officer and scientist should work together as a partnership. The services which a laboratory should provide include analysis, advice, training, intelligence data, and research and development. Laboratory staff should also be competent expert witnesses. The particular services required will depend very much on the nature of the investigation.

Identification of substances is usually straightforward and will determine which (if any) controlled drugs are present. There are many instances where at least some of a consignment is found not to be a drug substance. Purity is relevant for powdered drugs and is often an indication of the position of the suspect in the distribution chain. Although any charges will be brought on the basis of the drug itself, the identification of impurities is valuable in assigning origins of consignments. Heroin and cannabis have characteristics which are indicative of particular geographical origins. These characteristics are partly the result of climate, but also of processing differences in different countries. The Laboratory has an extensive database of chromatographic and other data on major drugs of abuse of known origin. It is thus possible to give opinions on the likely origin of seized drugs. Drugs are processed in batches and the Laboratory can compare seizures made at different times or in different locations to identify any similarities which may indicate the same batch.

Items other than drugs are also examined and the Laboratory can often provide vital evidence of association on, for example, paraphernalia, vehicles, premises, wrapping material and documents. Examination of paraphernalia, vehicles and premises may reveal traces of drugs, which could relate the suspect to colleagues or locations. Such evidence may be of value in relation to the seizure of assets. The materials in which drugs are packaged may also give valuable information. Immediate packaging is often characteristic of a particular source. Other wrappings and particularly textiles, adhesive tapes and polythene bags often have unique compositions or other characteristics which may enable a link to be established between different seizures and suspects. Documents examination can also provide vital evidence. For example, the Laboratory examines air tickets and passports for unauthorized alterations, relates typewritten documents to particular machines, reads typewriter ribbons and compares handwriting with that of known authorship.

Scientific support is not restricted to laboratory based operations and staff are on-call 24 h per day. In large or complex cases, the scientist working at the point of seizure can undertake preliminary tests, identify items which may be of evidential value, and advise on the handling of exhibits which may pose a safety hazard or require special treatment. The advice of the scientist at an early stage in an investigation often enables the enforcement officer to pursue particular avenues of enquiry more rapidly. It also enables the chemist or documents examiner to proceed with his work much more quickly. It minimizes the security problems of transporting bulk quantities of drugs to the Laboratory, and enables representative samples to be taken by the scientist.

The Laboratory supports intelligence on drug trafficking, for example, by studying the 'trade marks' or other features on drugs or their wrappings which are incorporated at the manufacturing or distribution stage. Sometimes trade marks are of immediate evidential value: for example, there have been several cases in the UK where there was a common trade mark on cloth bags containing cannabis resin. Bags with identical marks containing resin of the same chemical composition were seized at various points within the UK over a period of several weeks. This indicated a connection between the suspects undertaking the smuggling. Further scientific investigation, in which an identical cloth bag, but containing some resin shown to be of different composition, was examined, led to the identification of other parts of the distribution chain.

Adulterants and impurities can also be used as markers to correlate different seizures, and can be used to follow drugs from a given illicit operation in the source country to destinations in the user country.

Much of the scientific examination of drugs-related exhibits involves chromatography. Chromatography thus makes a vital contribution to ensuring justice in drug smuggling (and many other) cases.

International Guide for Laboratory Accreditation

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It is increasingly accepted that accreditation, *i.e.*, formal recognition that a testing laboratory is competent to carry out specific tests or types of tests, makes a valuable contribution to the quality of results produced by a laboratory and is one of the key ingredients for facilitating the mutual acceptance of data. Accreditation is usually assessed against the requirements of a particular standard, by an independent, expert body. Laboratories involved in chemical testing, seeking accreditation, have a choice of three main accreditation standards.

Firstly, they may work to ISO 9000 (BS 5750 in the UK, EN 29000 in Europe). This standard, defining quality system requirements, is widely applicable to organizations in production, design and servicing. For chemical testing its main application is in laboratories providing a quality control facility to the rest of the organization. Secondly, laboratories carrying out chemical testing in support of toxicology studies, for food or drug development, may have a legal requirement to comply with the requirements of Good Laboratory Practice (GLP) based on guidelines published by the Organization for Economic Co-operation and Development (OECD) in 1982 and complying with EC Directives. Lastly, and the most appropriate option for laboratories providing a testing or calibration service to others, is to seek accreditation against standards conforming to the guidelines set out in ISO/IEC Guide 25. The most important such standard in Europe is EN 45001 with related national standards, such as NAMAS M10 in the UK.

In order to be applicable to a wide range of sectors of testing or calibration EN 45001 is written in very general terms, and it is sometimes difficult to apply EN 45001 to some types of measurement without additional technical guidance; guidance which interprets the general requirements of the standard without introducing new requirements.

Almost all of the 18 EC and EFTA countries have or are developing accreditation schemes based on EN 45001. The accreditation bodies meet to discuss common points of interest in WELAC, the Western European Laboratory Accreditation Co-operation. Useful achievements include the signing of a multilateral agreement to recognize each other's schemes as equivalent. Five members have signed so far with a further six having applied. WELAC activities cover accreditation in all areas of measurement.

In Europe, the specific interests of chemical testing are represented by EURACHEM, which is a forum of senior decision makers representing government, industry, academics and professionals from the EC and EFTA countries. Both WELAC and EURACHEM recognized the urgent need to produce technical guidance to help chemical testing laboratories interpret EN 45001. They agreed to collaborate to produce suitable guidance notes and formed a joint working group early in 1991. EURACHEM and WELAC have since identified other areas within chemical testing which can best be addressed by mutual co-operation and are working together on further projects.

Production of the Guide

The first draft of the Guide was based on an existing UK document. As it developed, additional material on computers, sampling, measurement uncertainty and validation was added. A full list of subject areas covered is given in Table 1. Inevitably in such a technical exercise, with many contributors, there were often widely differing views on what guidance should be given. Throughout, the group tried to produce advice that would be acceptable to the majority, written in simple English that would be unambiguous in translation.

Table 1 Subject areas within the Guide

Introduction	Reference materials
Scope of accreditation	Computers
Staff	Audit and review
Environment	Sampling, sample handling, sample preparation
Equipment	Quality control
Reagents	Measurement uncertainty
Methods and procedures	Validation
Calibration	Bibliography

In the latter stages of drafting, the working group collaborated with an ISO group producing a similar guide on ISO Guide 25. Because the EURACHEM/WELAC Guide was recognized as being closer to completion, and so as not to duplicate effort, work on the ISO Guide has been suspended for the moment.

Specific Details and Problems

Definitions were taken from the International Standards Organization (ISO) wherever possible. Unfortunately, many were found to be dependent on the ISO source used. The acknowledged definitive source 'VIM', the International Metrology Vocabulary, dates back to 1984 and is currently under revision. A general rule was made to use the most recent source. Other sources of definitions used were IUPAC for sampling and IBM for computing.

Guidance for defining a laboratory's scope of accreditation proved to be a contentious issue. Some favour the documentation of clear rules; others were keen that the matter should be left entirely open. This polarization probably arises from a number of factors. Accreditation may or may not be tied into legal requirements. EN 45001, GLP and ISO 9000 may be the responsibility of one organization or several, depending on the country. Consequently, some wish to see these three standards amalgamated into a single standard. The strength of particular lobbies, such as industry, may influence the way the schemes are run. These issues are yet to be resolved.

The reagents section is a good example of an area fairly specific to chemical testing but not covered directly in EN 45001. EN 45001 makes no specific reference to 'reagents' and yet it is easy to appreciate the importance of reagents to a chemical testing laboratory's quality system. Reagents are very broadly part of the equipment used in performing chemical tests; the requirements for equipment must also be applied to, and interpreted for, reagents. Thus, the Guide provides practical advice on requirements such as choice, grade, use, methods of preparation, storage, labelling and disposal.

Up to now, little guidance has been available in Europe on the validation of the use of computers for chemical testing, but it is an area of increasing importance. The Guide adopts a pragmatic approach, suggesting that within certain guidelines it is possible to assume that a computer is working properly if it produces expected answers when fed with known data. Hence, the validation of computers can be achieved as part of the whole measurement process using standards, reference materials or quality control samples.

EN 45001 makes only the barest mention of the important subject of audit and review. The Guide complements WELAC's recently published document with specific guidance

on the areas which should be examined during audit of a chemical testing laboratory.

The sampling section gives brief general guidance on best practice in sampling, sample handling and sample preparation, and stresses the importance of appropriate sampling in the over-all context of analytical problem solving. It provides suitable pointers for more detailed advice, particularly in specific sectors of measurement, and stresses the importance of correct nomenclature in the sampling process.

In writing the section on measurement uncertainty the aim was to produce statistically correct advice which was meaningful to the non-expert and yet sufficiently detailed to be useful. Production of this section was beset by problems due to inconsistent ISO definitions and variations in what experts considered to be best practice. The philosophy in this area is still subject to debate and future developments may require this section to be updated.

The section on validation gives advice on an important subject for which there is little other advice available, particularly for laboratories working in isolation. The important elements of method validation are discussed with indications of where more detail can be found. The emphasis these days is not to accredit the use of standard methods in preference to non-standard methods, but rather to accredit the use of properly validated methods in preference to those which are poorly validated.

Publication and Use

Complete endorsement of the Guide by EURACHEM and WELAC is expected soon, after which it will probably be published as a working draft, available through EURACHEM, WELAC and national accreditation bodies. The latter may well publish it with a national supplement listing particularly national requirements not covered in the main text. It is expected to be in circulation in this form for about two years, during which time users will be invited to comment on its content. At the end of that time, it will be revised taking into account comments and developments of the accreditation standards themselves. It is hoped that the final text can be formally adopted and published by the European Committee for Standardization (CEN) or ISO.

Conclusion

The joint working group has made rapid progress to produce a guide aimed at meeting the needs of chemical testing laboratories interpreting EN 45001 or ISO Guide 25. It will also assist laboratories working to GLP or ISO 9000. The problems encountered during the drafting were typical of such an exercise but none proved insurmountable. Publication is expected soon and it is hoped that the guidance will prove useful.

Selective Particle Counting by Means of Affinity Ligands Linked to Microscopically Visible Labels

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Microscopically sized particles supporting affinity ligands (antibodies) have found analytical use as reagents in selective separation,[1] cell labelling,[1,2] and homogeneous immunoassays.[3,4] For labelling purposes, particle-based systems have the particular advantage that selective detection is still possible when only small numbers (a few tens) of antigen–antibody conjugates are formed.[5] If this property can be exploited to its extreme limit, it should prove possible to detect small molecules which possess as few as two antigenic sites.

Liposomes, which are spherical particles consisting of an aqueous lumen enclosed by a lipid bilayer membrane,[6] are particularly attractive options for antibody labelling applications, in that (*a*) they are easy to produce in relatively homogeneous populations, (*b*) they can be detected as single entities, or as small clusters, by the encapsulation of an enzyme or a fluorescent dye,[7] and (*c*) phospholipids which contain chemical functionalities suitable for attachment onto proteins can be conveniently incorporated as membrane components. This paper describes the application of liposomes containing a fluorophore as antibody labels. The system is demonstrated by the selective flow cytometric[8] detection of *Legionella pneumophila*, while the possible extension of this work to allow the detection of single molecules is discussed.

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Experimental

The liposomes used in the flow cytometry work contained 15 mmol l^{-1} of carboxyfluorescein (CF) and 1 mmol l^{-1} of ethylenediaminetetraacetic acid (EDTA) dissolved in 10 mmol l^{-1} tris(hydroxymethyl)methylamine (Tris) buffer (pH 7.4). These were prepared by a freeze–thaw sonication procedure,[6] followed by passage ten times under pressures of up to 700 psi‡ through two polycarbonate membranes (Nucleopore; pore diameter approximately 0.4 μm) in an extruder (Lipex, Vancouver, Canada). Non-encapsulated fluorophore was removed by gel filtration on a Sephadex G-25M column (Pharmacia; PD-10). The proportions of the various forms of phospholipids constituting the liposome membranes were egg lecithin : cholesterol : DL-α-phosphatidyl-L-serine : 1,2-distearoyl-*sn*-glycero-3-phosphorylethanolamine (PE), 13 + 4 + 6 + 1 by mass. 3-(2-Pyridoxyldithio)propanoic acid, *N*-hydroxysuccinimide ester (SPDP) was covalently attached to the PE[6] before liposome preparation.

Formaldehyde fixed (1% m/v) suspensions of *L. Pneumophila* sero group 1 (w74/81) and rabbit antiserum to *L. Pneumophila* lipopolysaccharide were kindly provided by Dr. R. Fitzgeorge, Centre for Applied Microbiology and Research (CAMR). *Escherichia coli* K12 was also fixed with buffered formaldehyde. Antiserum was purified on immobilized protein A at CAMR. Fragments of these antibodies were prepared by pepsin digestion [ImmunoPure $F(ab')_2$ Preparation Kit;

‡ 1 psi = 6.894 76 × 10^3 Pa.

Pierce], with subsequent conversion to Fab′ achieved by reduction with dithiothreitol. The Fab′ fragments were cross-linked with the liposomes *via* SPDP-derivatized PE by mixing the two overnight under a nitrogen atmosphere.[6] The molar ratio of Fab′ fragments : liposomes was approximately 30 000 : 1.

Macroscopic fluorescence measurements on liposome populations were performed on a Baird Nova-3 spectrofluorimeter, with excitation at 494 nm and emission at 515 nm. The flow cytometer used was an Argus 100 (Skatron Ltd.), with a mercury arc lamp excitation beam of wavelength 470–495 nm, and fluorescence detection at 520–550 nm.

Results and Discussion

Optimization of Liposome Populations

Spectrofluorimetric investigations were performed on populations of liposomes prepared from egg lecithin and cholesterol in equimolar proportions and constant concentrations, in the presence of a range of concentrations of CF. Fig. 1(*a*) shows a plot of the relative fluorescence intensity against the concentration of entrapped dye, which, as previously reported[2] gives a maximum fluorescence at 15 mmol l^{-1} CF. Concentrations of CF greater than 15 mmol l^{-1} lead to a self-quenching,[2] with weak fluorescence signals observed when the concentration of the entrapped dye is greater than 50 mmol l^{-1}.

Liposome stability was determined by monitoring the rate of CF leakage for a population prepared from a solution containing 50 mmol l^{-1} CF. The initial fluorescence of the liposome suspension was low, owing to self-quenching. However, the fluorescence increased steadily over a period of 4 months as the CF leaching from the liposomes became diluted and thus unquenched. The proportion of fluorophore lost to the external medium was calculated as the ratio of the rise in fluorescence of the liposome solution to that which occurred when the membranes were deliberately disrupted (by the addition of Triton X-100). Fig. 1(*b*) shows that liposomes composed of egg lecithin leach less than 5% of their entrapped fluorophore per month when stored refrigerated under air and in buffer having the same ionic strength as the liposomally encapsulated solution. Stability is further improved by the inclusion of cholesterol in the liposome membrane. The data in Fig. 1(*b*) were obtained with an external medium of pH 7.4; when stability studies were performed at pH 6.4, the leakage rates were considerably higher. This was attributable to the higher solubility in lipids of CF with undissociated carboxylic acid groups at pH 6.4, whilst lipid bilayer properties might also be pH-dependent.

Selective Detection of *L. pneumophila*

Light scattering signals [Fig. 2(*a*)] are observed when *L. pneumophila* cells flow through the beam of a flow cytometer;[8] however, under normal circumstances these signals do not correlate with any rise in the detected fluorescence above background. Thus, since other bacteria also scatter light [as illustrated in Fig. 2(*a*) with *E. coli*], it is not possible to identify the presence of *L. pneumophila* definitively in complex solutions containing many bacterial forms by flow cytometry unless the cells are first selectively labelled with fluorophore.[8]

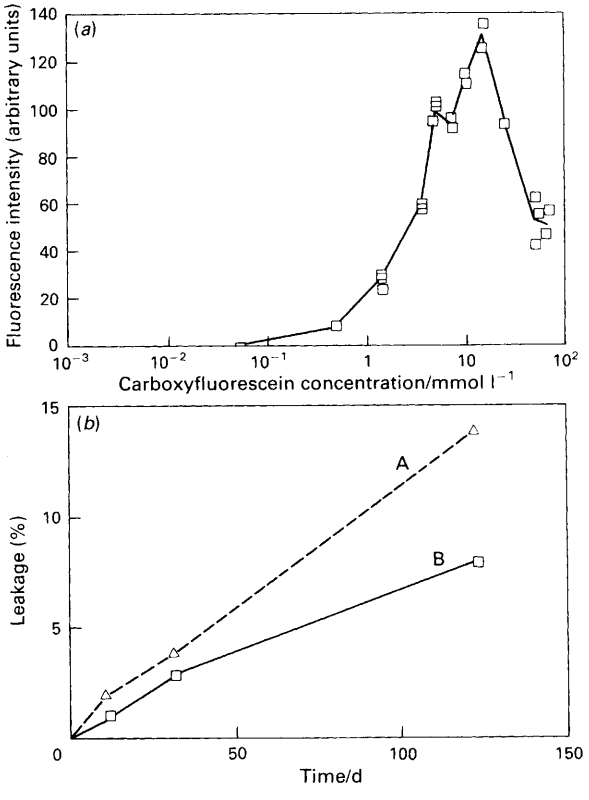

Fig. 1 Fluorescence studies of liposomes containing carboxyfluorescein (CF). (*a*) Relative fluorescence *versus* concentration of entrapped CF; (*b*) % leakage (as defined in text) *versus* time; A, 100% egg lecithin; B, 50% mol/mol egg lecithin : cholesterol. Unless otherwise stated the liposomal membranes consisted of a 1 + 1 (by mass) mixture of egg lecithin and cholesterol. The liposomes were prepared by the hydration of thin films of the lipid mixture with CF solutions, followed by five cycles of freeze–thawing (using liquid nitrogen), and finally by transmembrane extrusion (Nuclepore; pore diameter 100 nm)

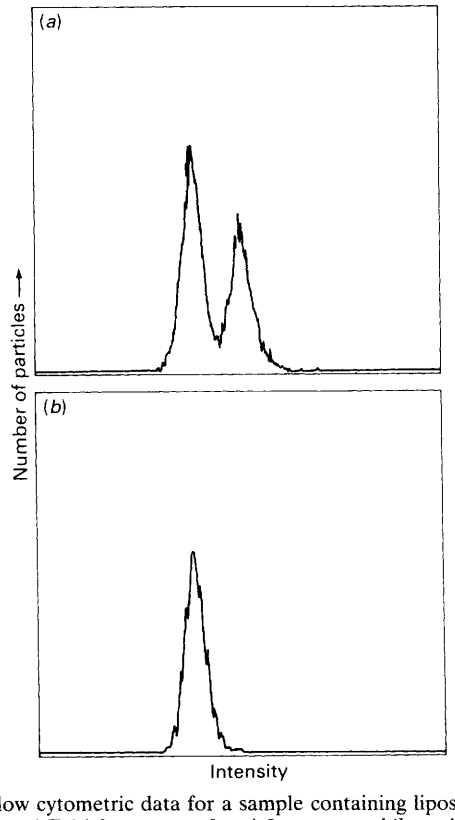

Fig. 2 Flow cytometric data for a sample containing liposomes with surface-bound Fab′ fragments of anti-*L. pneumophila* antibodies, *L. pneumophila*, and *E. Coli*: (*a*) forward angle light scattering signal; (*b*) forward angle light scattering coincident with fluorescence. The *x*-axis gives the relative intensity of the light scattering signal, with the *y*-axis indicating the numbers of particles producing that response. Liposomes were manufactured in CF (15 mmol l^{-1}). The gain voltage on the forward angle light scattering photomultiplier detector was 550 V. Specimens were introduced into the laminar stream at a rate of 0.5 μl min^{-1}

Liposomes containing CF are weaker light scatterers than bacteria, but they do produce fluorescent signals which are discernible above background in a flow cytometer. When a population of these lipsomes carrying Fab′ fragments specific for *L. pneumophila* was mixed with a solution containing *L. pneumophila*, conjugates were formed which could be selectively measured by configuring the flow cytometer to detect only particles which produced simultaneous response at both the fluorescent and light scattering detectors [Fig. 2(*b*)]. Thus, liposome conjugation provides a possible route to the selective measurement of bacterial cells, in an approach which is analogous to that of conventional fluorescein isothiocyanate (FITC) labelling,[8] but has the potential advantage that it may be applied where there are very few antigenic sites present on the surface of the bacteria.[6] In order to measure *L. pneumophila* quantitatively by liposome labelling it is essential that all the bacteria in the sample are present as conjugates, and this inevitably requires that the liposomes are present in excess. In our experience, the required ratio varies from a few tens to a few hundreds between different batches of liposomes, and therefore some care is required in selecting reagent strengths appropriate for a *Legionella* assay. Nevertheless, we have quantitatively measured *L. pneumophila* at concentrations as low as 10^3 ml^{-1} in specimens which contains a 10 000-fold excess of *E. coli*.

Detection of Single Molecules

We feel that it should be possible to detect and count individual molecules by flow cytometry, using microscopically visible fluorescent labels coupled to antibodies. The principle of such a measurement is that the antigenic sites on the molecule act as a 'glue' for the formation of small clusters of labels, which can be distinguished by the magnitude of their fluorescence signal. A necessary prerequisite for this approach to be successful is that the population of the labels are monodisperse with respect to the level of their fluorescence under illumination. Unfortunately, however, the liposome populations described above did not fulfil this criterion, as indicated by Fig. 3, which shows that a wide spectrum of fluorescence intensities was observed in flow cytometric investigations of liposome samples. The reason for this broad range of fluorescence signals is unclear. However, since we have found by electron and fluorescence microscopy and by photon correlation spectroscopy that liposome populations can be prepared with a relatively monodisperse size distribution, it seems likely that the CF is unevenly distributed between liposomes.

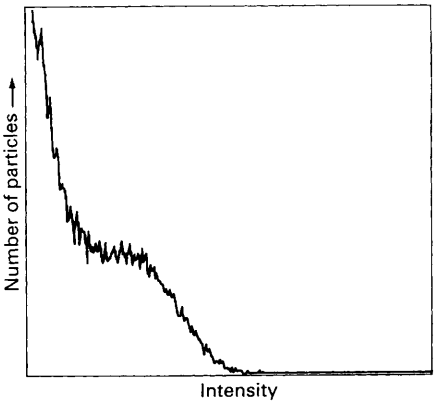

Fig. 3 Flow cytometric data for a sample of liposomes which contained 15 mmol l^{-1} CF. The *x*-axis gives the relative intensity of the fluorescence signal, with the *y*-axis indicating the numbers of particles producing that response. The gain voltage on the fluorescence photomultiplier detector was 650 V. Specimen flow rates and sheath fluid were as in Fig. 2

Conclusions

Liposomes containing high concentrations of fluorescent dyes, after they are coupled with an appropriate antibody, can serve as alternative labels for the selective measurement of bacteria by flow cytometry. They are relatively stable with respect to long-term storage, and their high fluorescence makes them suitable for measuring cells which possess relatively few antigenic sites. The application of such labels to the detection of molecules might yet be possible. However, this will require some improvements to the existing preparatory methods to provide greater uniformity in the population.

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European Community Measurement and Testing Programme

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The scientific activities carried out by the European Community (EC) go back to the mid-1950s when nuclear energy research was first carried out under the EurAtom treaty. By the early 1980s, a much broader range of activities, collectively known as the EC R & D Framework Programme, had been laid down which attempts to provide even coverage of those scientific areas judged to be important to the Community as a whole. For example, energy, the environment, industry, food and agriculture and raw materials. Each Framework programme runs for five years and, in 1990, the European Council adopted the 3rd Framework Programme which runs until 1994.

The main objectives of the 3rd Framework Programme are essentially to: (*a*) improve industrial competitiveness (the emphasis being on pre-competitive research rather than fundamental blue skies research or near to the market development); (*b*) direct the attitude of the industrial sector towards further pan-European initiatives; (*c*) ensure scientists and engineers are firmly aware of the Community dimension and spirit during their training; (*d*) increase economic and social cohesion within the Community whilst maintaining the quality of R & D projects; (*e*) take into account the protection of the environment and quality of life; (*f*) face the challenges relating to the completion of the Single Market particularly with respect to Directives and standards.

In practical terms, EC R & D largely boils down to international collaborative projects jointly funded by the

European Commission and by the participants—industry, academia, government departments and other organizations with a vested interest in R & D across the Community. The current programme has an over-all budget of 5.7 billion ECU, or about £4 billion, and contains 15 specific research and technical development activities (Table 1). Perhaps the most important activity for analytical chemists is the measurement and testing programme which is run by the Community Bureau of Reference (BCR).

The Community Bureau of Reference

The principal objective of the BCR programme is to improve the reliability of chemical analysis and physical metrology in order to achieve harmonization of measurement results across the Community. Much of the BCR work programme is therefore geared towards solving measurement problems which could lead to trade disputes or hinder the operation of the Single Market.

Based on this premise, the type of projects which tend to be looked on favourably by the BCR include those where: the accuracy of measurements is not sufficient for the industrial requirements related to manufacture and quality control; measurement discrepancies between laboratories are greater than commercial specifications allow; measurement inaccuracies may induce heavy losses in the trade of goods of high value or in large volumes (food, raw materials); inaccuracies related to pollution or health care may lead to wrong conclusions with considerable damage to industry, the public or the environment.

In some instances, the strategic importance of measurements for European industry is also taken into account, for example, measurements in the field of microelectronics. In the context of these criteria, the BCR has for the past five years or so concentrated essentially on the following priority areas: agriculture and food analysis; environmental analysis; applied metrology measurements; testing and measurements of industrial products; biomedical analysis.

There are essentially two ways in which the BCR programme is funded. Firstly, concerted action, which is used where the aim of the project is to co-ordinate the work of several laboratories in order to improve a measurement or test, for example, improvements in the accuracy of calibrations between national metrology laboratories. In this instance, the BCR will pay up to 100% of the costs of meetings, the scientific and administrative co-ordination of the project and the processing and publication of the data produced.

The second, and most common, means of funding is known as shared-cost action which is used to fund collaborative studies between Community laboratories studying improvements in methodology or when certifying a reference material. In this instance, projects are funded on a 50:50 basis between BCR and the participants, although for certain non-industrial participants, for example universities, it is possible to secure 100% funding.

The usual approach adopted by the BCR is to invite laboratories from as many Member States as possible, with perhaps an upper limit of 20–30, to carry out a number of collaborative studies such that any measurement discrepancies are reduced to what is considered to be an acceptable level of uncertainty. As regards chemical measurements, the laboratories will then often participate in a certification exercise whereby they rigorously characterize a candidate reference material. In this way, participating laboratories gain considerable expertise which is then disseminated to other European laboratories who use the certified reference materials (CRMs) produced.

It should be noted that the certification exercise should always be undertaken by a number of laboratories using different independent methods of analysis. In this way, any measurement bias is reduced to a minimum. Of course, this approach to certification may occasionally lead to the elimination of the data from one or more of the methods being used if their inaccuracies are shown to be unacceptable and, at the limit, data from only one method used by relatively few laboratories may be all that is suitable for certification.

Table 1 EC R & D Framework Programme

1	Information technologies
2	Communications technologies
3	Development of telematics systems
4	Industrial and materials technologies
5	Measurement and testing
6	Environment
7	Marine science and technology
8	Biotechnology
9	Agricultural and agro-industrial research
10	Biomedical and health research
11	Life sciences and technologies for developing countries
12	Non-nuclear energies
13	Nuclear fission safety
14	Controlled thermonuclear fusion
15	Human capital and mobility

The New (1992–94) BCR Programme

Since its formation in 1973, the BCR programme has carried out over 600 measurement projects and, as a result, has produced over 400 reference materials.

Recently, the BCR has had a major call for proposals in advance of carrying out its 1992–94 work programme. Essentially, the new programme breaks down into four main areas: Area 1, Support to Directives and Regulations (12 MECU); Area 2, Support to Standards (11.5 MECU); Area 3, Common Means of Calibration (12 MECU); Area 4, New Methods of Measurement (12 MECU). The distribution of the budget is shown in parentheses (1 MECU = 1 million ECU) and includes the cost of personnel and management for running the programme. In other words, a total of 47 MECU or less than 1% of the total Framework Programme budget.

Area 1 will cover the work required to improve those methods of measurement and testing which are necessary for the implementation of Directives. This is vital for the Single Market because although European Directives provide the legal basis for the harmonization of national regulations, their implementation sometimes requires measurements and analyses which are beyond the capabilities of many laboratories at present. Typical topics of interest will include the analysis of food products (*e.g.*, pesticides in cereals, vegetables and fruit; nitrate in baby food, cheese and milk powder; and heavy metals in olive oil), agricultural products (*e.g.*, drug residues, vitamins and toxic impurities in animal feeds; and cow's milk in goat's cheese), environmental analyses [*e.g.*, pollution in the sea; leachable heavy metals from building and demolition waste; and polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and dioxins in contaminated soils], air in the workplace (*e.g.*, aldehydes, isocyanates, nitrosamines, asbestos and heavy metals), noise measurements (*e.g.*, the evaluation of hearing protectors and the transmission of noise in buildings), biomedical analysis (*e.g.*, toxic substances in blood and urine) and quality assurance (*e.g.*, supporting collaboration between the national quality assurance systems).

Area 2 will focus on the development of standardized methods for the testing of industrial products as specified by European standards. Topics so far identified include the development of test methods for determining the fire behaviour of upholstered furniture, constructive products, hearing protectors against impulse noise and cold spots in microwave ovens.

Area 3 has been the area where the BCR has concentrated

most of its effort over the years, essentially producing CRMs and improving calibrations between the National Physical Laboratories. In the new programme this area will produce CRMs mainly for Area 1 and possibly Area 2, for example, certified nitrate content of baby food.

Finally, Area 4 will concentrate on developing instrumental measurement methods either as a result of work carried out under Areas 1–3, or for the on-line or *in situ* determination of physical, chemical or biological parameters where either no suitable direct methods currently exist or where there are severe operational conditions.

Summary

In conclusion, European scientific collaboration in areas such as the Framework Programme is becoming increasingly important. For example, depending on the type of projects undertaken, it is often possible to realize substantial benefits such as: (*a*) sharing the cost and risks of R & D by making use of complementary skills and common facilities, thus enabling participation in projects where the scale of investment would otherwise be beyond an individual organization; (*b*) gaining a commercial advantage from tapping into the scientific expertise of European laboratories/organizations; (*c*) achieving a more significant role in the development of international scientific standards; (*d*) establishing business contacts with overseas counterparts in order to be well placed to take advantage of the Single Market.

It should be noted, however, that EC programmes are often oversubscribed and the preparation of a suitable proposal can be extremely time consuming and therefore expensive. But, that said, the benefits of participation are often great and in most instances easily outweigh the costs of participation.