PAGES 1–116

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ROYAL SOCIETY OF CHEMISTRY

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Edited by: K. E. Jarvis, A. L. Gray, and J. G. Williams, *Royal Holloway and Bedford New College, London* and I. Jarvis, *Kingston Polytechnic*
Special Publication No. 85

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November 1990

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Series: Methodological Surveys in Biochemistry and Analysis
Volume: 20

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Analysis for Drugs and Metabolites, including Anti-infective Agents is divided into three sections covering: policies for method validation and ensuring sound results acceptable to the authorities; the bioanalysis of anti-infective drugs, ranging from ß-lactams to anti-parasitics and anti-virals, with procedural details and informative accounts of method-development rationale and problems; and other drug types, plus recent assay approaches such as SFC and laser induced fluorescence.

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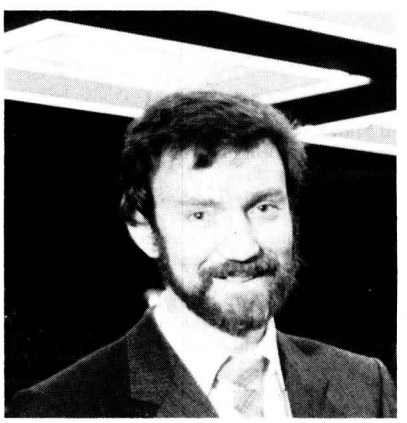
Editorial

This has been a challenging year in the Analytical Journals office with various staff, procedural (for North American authors) and editorial changes being made, in order to keep *The Analyst* abreast of current developments in both the style and terminology used in analytical chemistry. Some of these changes may become evident as you read through this first issue of 1991; however, most are minor, hence there is no over-all change in format. These changes are detailed in the revised 'Instructions to Authors' given on page 105. One of the most obvious points of note is that *The Analyst* will no longer publish 'short papers', mainly because the criteria for these were exactly the same as those for full papers and the larger page size of recent years has meant that the distinction on the basis of length has become blurred. The main reason for the editorial and style changes is to achieve consistency between all primary journals of the RSC.

A major step forward in 1990 was the establishment of a North American connection for *The Analyst*, with Professor J. F. Tyson being appointed US Associate Editor (see photograph). Hopefully his appointment will give *The Analyst* the foothold necessary to make further progress and keep a high profile in the North American market in order to encourage authors and to recruit referees. Thus, North American authors may now send their papers directly to Professor Tyson. Papers will usually be refereed within North America, thereby saving valuable time in the publication process.



Professor Julian F. Tyson, US Associate Editor for The Analyst

Professor Tyson and Professor J. D. R. Thomas (outgoing Chairman of the Analytical Editorial Board) both contributed Editorials for the July issue of *The Analyst* about the history and future of *The Analyst*, respectively. Professor Thomas is now the President of the Analytical Division and has been succeeded as Chairman by Dr. A. G. Fogg (Loughborough University).

The Editorial staff would like to take this opportunity to express their appreciation for all the hard work Professor Thomas has put in over the last seven years and for the assistance and advice he has given throughout his term of office. We are certain he will continue to take an active interest in all the Analytical Journals and we wish him success in his new role.

L to R: Harpal Minhas, Paul Delaney, Monique Warner, Claire Harris, Judith Egan (front), Brenda Holliday, Sheryl Whitewood, Paula O'Riordan and Roger Young

There have also been several staff changes within the office (see photograph). Judith Egan remains Editorial Manager, Analytical, and Editor of the *Journal of Analytical Atomic Spectrometry* (*JAAS*) and Roger Young remains the Editor of *Analytical Proceedings*. However, Harpal Minhas (formerly Senior Assistant Editor) was appointed Editor of *The Analyst* in May. The former Editor, Janet Dean, has been promoted to the post of Editorial Manager, *Dalton Transactions*. Paul Delaney is now Senior Assistant Editor, along with Assistant Editors Paula O'Riordan, Sheryl Whitewood and Brenda Holliday. Claire Harris the Editorial Secretary for *The Analyst* and *Analytical Proceedings* and Monique Warner the Editorial Secretary for *JAAS* complete the Editorial team.

All these changes inevitably disrupted production. However, everybody, including many of our referees, worked extremely hard and managed to keep *The Analyst* on schedule for most of the year. Our high standards were maintained with a rejection rate of 48% and average times to publication falling to their lowest since we relocated to Cambridge (in 1988/9) by September.

Readers, authors and referees should note that there have also been several changes to the Advisory Board and Regional Advisory Editors, hence, you should consult the inside front cover of *The Analyst* to ensure that you are aware of your local Advisory Board member/editor. Of course, if you would like further information or to discuss any aspects of the publication procedures, please do not hesitate to contact either myself or Professor Tyson.

Finally, The Royal Society of Chemistry celebrates its 150th Anniversary in 1991. To commemorate this event the Analytical Divison has organised two symposia (one jointly with the Faraday Division) to coincide with RSC celebrations during the Annual Chemical Congress, at Imperial College from 8th to 12th April, and we hope to see you there.

Harpal Minhas, *Editor*

Basic Statistical Methods for Analytical Chemistry Part 2. Calibration and Regression Methods* A Review

James N. Miller
Department of Chemistry, Loughborough University of Technology, Loughborough, Leicestershire LE11 3TU, UK

Summary of Contents

Keywords: *Analytical calibration method; statistics and rectilinear graph; curve fitting method; robust and non-parametric method; review*

Introduction

Most methods in modern analytical science involve the use of optical, electrical, thermal or other instruments in addition to the manipulative 'wet chemistry' skills which are an essential part of the analyst's training. Instrumental methods bring chemical benefits, such as the ability to study a wide range of concentrations, achieve very low limits of detection and perhaps study two or more analytes simultaneously. They also bring the practical benefits of lower unit costs and increased speed of analysis, perhaps through partial or complete automation. The results of instrumental analyses are evaluated by using calibration methods that bring about and reflect these advantages and are, to some extent, distinct from the statistical approaches discussed in Part 1 of this review.[1] Nonetheless many of the concepts summarized in Part 1 are also applied in the statistics of calibration methods, and familiarity with these concepts is assumed here.

A typical calibration experiment (a single analyte–multivariate calibration has recently been surveyed[2]) is performed by making up a series of standard solutions containing known amounts of the analyte and taking each solution separately through an instrumental analysis procedure with a well defined protocol. For each solution, the instrument generates a signal, and these signals are plotted on the y-axis of a calibration graph, with the standard concentrations on the x-axis. A straight line or curve is drawn through the calibration points and may then be used for the determination of a test ('unknown') sample. The unknown is taken through exactly the same analysis protocol as the standards, the instrument signal is recorded and the test concentration estimated from the calibration graph by interpolation—and not, with one special exception described below, by extrapolation. It is apparent that one calibration graph can be used in the determination of many test samples, provided that instrument conditions and the experimental protocol do not change. This approach thus offers the desired feature of being able to analyse many samples rapidly over a range of concentrations; less obvious advantages include the ability to estimate limits of detection (see below) and eliminate the effects of some types of systematic error. For example, if the monochromator in a spectrophotometer has an error in its wavelength scale, errors in calculated concentrations using this instrument should cancel out between the standards and the samples.

This approach to the determination of concentrations poses several problems. What type of line—straight, curved, or part-straight, part-curved—should be drawn through the calibration points? Given that the instrument signals obtained from the standards will be subject to random errors, what is the best straight line or curve through those points? What are the errors in test concentrations determined by interpolation? What is the limit of detection of the analysis? These and other statistical questions posed by calibration experiments still generate new methods and excite considerable controversy. Not surprisingly, it is in the area of curve-fitting that most new procedures are being introduced, but linear regression methods also generate their own original literature, as will become apparent.

Linear Calibration

Correlation Coefficient

Many analytical procedures are carefully designed to give a linear calibration graph over the concentration range of interest, and analysts who use such methods routinely may assume linearity with only occasional checks. In the develop-

* For Part 1 of this series see reference 1.

ment of new methods, and in any other case where there is the least uncertainty, the assumption of linearity must be carefully investigated. It is always valuable to inspect the calibration graph visually on graph paper or on a computer monitor, as gentle curvature that might otherwise go unnoticed is often detected in this way (see below). Here, and in many other aspects of calibration statistics, the low-cost computer programs available for most personal computers are very valuable. As will be seen, it is important to plot the graph with the instrument response on the y-axis and the concentrations of the standards on the x-axis. One of the calibration points should normally be a 'blank', *i.e.*, a sample containing all the reagents, solvents, *etc.*, present in the other standards, but no analyte. It is poor practice to subtract the blank signal from those of the other standards before plotting the graph. The blank point is subject to errors as are all the other points and should be treated in the same way. As shown in Part 1 of this review,[1] if two results, x_1 and x_2, have random errors e_1 and e_2, then the random error in $x_1 - x_2$ is not $e_1 - e_2$. Thus, subtraction of the blank seriously complicates the proper estimation of the random errors of the calibration graph. Moreover, even if the blank signal is subtracted from the other measurements, the resulting graph may not pass exactly through the origin.

Linearity is often tested using the correlation coefficient, r. This quantity, whose full title is the 'product-moment correlation coefficient', is given by

$$r = \frac{\sum_i [(x_i - \bar{x})(y_i - \bar{y})]}{\{[\sum_i (x_i - \bar{x})^2][\sum_i (y_i - \bar{y})^2]\}^{\frac{1}{2}}} \quad (1)$$

where the points on the graph are $(x_1, y_1), (x_2, y_2), \ldots (x_i, y_i), \ldots (x_n, y_n)$, and $\bar{x}$ and $\bar{y}$ are, as usual, the mean values of x_i and y_i respectively. It may be shown that $-1 \leq r \leq +1$. In the hypothetical situation when $r = -1$, all the points on the graph would lie on a perfect straight line of negative slope; if $r = +1$, all the points would lie exactly on a line of positive slope; and $r = 0$ indicates no linear correlation between x and y. Even rather 'poor' calibration graphs, *i.e.*, with significant y-direction errors, will have r values close to 1 (or -1), values of $|r| <$ about 0.98 being unusual. Worse, points that clearly lie on a gentle curve can easily give high values of $|r|$. So the magnitude of r, considered alone, is a poor guide to linearity. A study of the 'y-residuals' (see below) is a simple and instructive test of whether a linear plot is appropriate. A recent report of the Analytical Methods Committee[3] provides a useful critique of the uses of r, and suggests an alternative method of testing linearity, based on the weighted least squares method (see below).

'Least Squares' Line

If a linear plot is valid, the analyst must plot the 'best' straight line through the points generated by the standard solutions. The common approach to this problem (not necessarily the best!) is to use the unweighted linear least squares method, which utilizes three assumptions. These are (*i*) that all the errors occur in the y-direction, *i.e.*, that errors in making up the standards are negligible compared with the errors in measuring instrument signals, (*ii*) that the y-direction errors are normally distributed, and (*iii*) that the variation in the y-direction errors is the same at all values of x. Assumption (*ii*) is probably justified in most experiments (although robust and non-parametric calibration methods which minimize its significance are available, see below), but the other two assumptions merit closer examination.

The assumption that errors only occur in the y-direction is effectively valid in many experiments; errors in instrument signals are often at least 2–3% [relative standard deviation (RSD)], whereas the errors in making up the standards should be not more than one-tenth of this. However, modern automatic techniques are dramatically improving the precision of many instrumental methods; flow injection analysis, for example, shows many examples of RSDs of 0.5% or less.[4] In such cases, it may be necessary either to abandon assumption (*i*) (again, suitable statistical methods are available—see below), or to maintain the validity of the assumption by making up the standards gravimetrically rather than volumetrically, *i.e.*, with an even greater accuracy than usual. If the assumption is valid, the line calculated as shown below, is called the line of regression of y on x, and has the general formula $y = bx + a$, where b and a are, respectively, its slope and intercept. This line is calculated by minimizing the sums of the squares of the distances between the standard points and the line in the y-direction. (Hence the term 'least squares' for this method.) It is important to note that the line of regression of x on y would seek to minimize the squares of x-direction errors, and therefore would be entirely inappropriate when the signal is plotted on the y-axis. (The two lines are not the same except in the hypothetical situation when all the points lie exactly on a straight line.) The y-direction distances between each calibration point and the point on the calculated line at the same value of x are known as the y-residuals and are of great importance in several calculations, as will be shown later in this paper.

Assumption (*iii*), that the y-direction errors are equal, is also open to comment. In statistical terms it means that all the points on the graph are of equal weight, *i.e.*, equal importance in the calculation of the best line—hence the term 'unweighted' least squares. In recent years this assumption has been tested for several different types of instrumental analysis, and in many cases it is found that the y-direction errors tend to increase as x increases, though not necessarily in linear proportion. Such findings should encourage the use of weighted least squares methods, in which greater weight is given to those points with the smallest experimental errors. These points are discussed further in a later section.

If assumptions (*i*)–(*iii*) are accepted then the slope, b, and intercept, a, of the unweighted least squares line are found from

$$b = \frac{\sum_i [(x_i - \bar{x})(y_i - \bar{y})]}{\sum_i (x_i - \bar{x})^2} \quad (2)$$

$$a = \bar{y} - b\bar{x} \quad (3)$$

The equations show that, when b has been determined, a can be calculated by using the fact that the fitted line passes through the centroid, $(\bar{x}, \bar{y})$. These results are proved in reference 5, a classic text on the mathematics of regression methods. The values of a and b can be simply applied to the determination of the concentration of a test sample from the corresponding instrument output.

Errors and Confidence Limits

The concentration value for a test sample calculated by interpolation from the least squares line is of little value unless it is accompanied by an estimate of its random variation. To understand how such error estimates are made, it is first important to appreciate that analytical scientists use the line of regression of y on x in an unusual and complex way. This is best appreciated by considering a conventional application of the line in a non-chemical field. Suppose that the weights of a series of infants are plotted against their ages. In this case the weights would be subject to measurement errors and to inter-individual variations (*e.g.*, all 3 month old infants would not weigh the same), so would be correctly plotted on the y-axis: the infants' ages, which would presumably be known exactly, would be plotted on the x-axis. The resulting plot would be used to predict the average weight (y) of a child of given age (x). That is, the graph would be used to estimate a y-value from an input x-value. The y-value obtained would of course be subject to error, because the least squares line itself

is subject to uncertainty. The graph would not normally be used to estimate the age of a child from its weight!

In analytical work, however, the calibration graph is used in the inverse way—an experimental value of y (y_0, the instrument signal for a test sample) is input, and the corresponding value of x (x_0, the concentration of the test sample) is determined by interpolation. The important difference is that x_0 is subject to error for two reasons, (1) the errors in the calibration line, as in the weight *versus* age example, and (2) the random error in the input y_0 value. Error calculations involving this 'inverse regression' method[5] are thus far from simple and indeed involve approximations (see below).

First, we must estimate the random errors of the slope and intercept of the regression line itself. These involve the preliminary calculation of the important statistic $s_{y/x}$, which is given by

$$s_{y/x} = \left[\frac{\sum_i (y_i - \hat{y}_i)^2}{n-2}\right]^{\frac{1}{2}} \quad (4)$$

In this equation, each y_i value is a measured signal value from the analytical instrument, while the corresponding $\hat{y}_i$ is the value of y on the fitted straight line at the same value of x. Each $(y_i - \hat{y}_i)$ value is thus a y-residual (see above). It is clear that equation (4) is similar to the equation for the standard deviation of a series of replicate results, except that the term $(n - 2)$ appears in the denominator as the number of degrees of freedom of the data, rather than $n - 1$. This difference is explained below in the discussion of analysis of variance applied to regression calculations. After $s_{y/x}$ has been determined, the standard deviation of the slope, s_b, and the standard deviation of the intercept, s_a can be determined from

$$s_b = \frac{s_{y/x}}{[\sum_i (x_i - \bar{x})^2]^{\frac{1}{2}}} \quad (5)$$

$$s_a = s_{y/x}\left[\frac{\sum_i x_i^2}{n\sum_i (x_i - \bar{x})^2}\right]^{\frac{1}{2}} \quad (6)$$

These standard deviations can then be used to estimate the confidence limits for the true slope and intercept values. The confidence limits for the slope are given by $b \pm ts_b$, where the value of t is chosen at the desired confidence level (two-tailed values) and with $n - 2$ degrees of freedom. Similarly, the confidence limits for the intercept are given by $a \pm ts_a$. These confidence limits are often of practical value in determining whether the slope or intercept of a line differs significantly from a particular or predicted value. For example, to test whether the intercept of a line differs significantly from 0 at the 95% confidence level, we need only see whether or not the 95% confidence interval for a includes zero.

The statistic $s_{y/x}$ is also used to provide equations for the confidence interval of the mean value of y_0 at a particular x_0 value, and for the (wider) confidence interval for a new and single value of y_0 measured at $x = x_0$. These equations are of limited value in analytical work, as already noted, and examples are given in standard texts.[5–7] Estimating the confidence limits for the entire line is more complex, as a combined confidence region for a and b is required. This problem was apparently first addressed by Working and Hotelling[8] in 1929, and there is a useful summary of their method and of related studies in the often-cited paper by Hunter.[9] The general form of the confidence limits is shown in Fig. 1(*a*), from which it is clear that the confidence limits are at their narrowest (best) in the region of $(\bar{x}, \bar{y})$, as the regression line must pass through this point.

We can now reconsider the principal analytical problem, that of estimating the standard deviation, s_{x_0}, and the confidence interval of a single concentration value x_0 derived from an instrument signal y_0. As shown diagrammatically in Fig. 1, the confidence interval for this x_0 value results from the uncertainty in the measurement of y_0, combined with the confidence interval for the regression line at that y_0 value. The standard deviation s_{x_0} is given by

$$s_{x_0} = \frac{S_{y/x}}{b}\left[1 + \frac{1}{n} + \frac{(y_0 - \bar{y})^2}{b^2\sum_i (x_i - \bar{x})^2}\right]^{\frac{1}{2}} \quad (7)$$

It can be shown[5] that this equation is an approximation that is only valid when the function

$$g = \frac{t^2}{\left\{b \Big/ \left[\frac{S_{y/x}^2}{\sum_i (x_i - \bar{x})^2}\right]^{\frac{1}{2}}\right\}^2} \quad (8)$$

has a value less than about 0.05. For g to have low values it is clearly necessary for b and $\sum_i (x_i - \bar{x})^2$ to be relatively large and $s_{y/x}$ to be small. In an analytical experiment with reasonable precision and a good calibration plot these results are indeed obtained; for example the data given in reference 9 yield a g value of 0.002.

In a typical analysis, the value of y_0 might be obtained as the mean of m observations of a test sample, rather than as a single observation. In which case, the (approximate) equation for s_{x_0} becomes

$$s_{x_0} = \frac{S_{y/x}}{b}\left[\frac{1}{m} + \frac{1}{n} + \frac{(y_0 - \bar{y})^2}{b^2\sum_i (x_i - \bar{x})^2}\right]^{\frac{1}{2}} \quad (9)$$

After s_{x_0} has been calculated, the confidence limits for x_0 can be determined as $x_0 \pm ts_{x_0}$, with t again chosen at a desired confidence level and $n - 2$ degrees of freedom. Inspection of equations (7) and (9) provides important guidance on the performance of a calibration experiment, presuming that we wish to minimize s_{x_0}. In cases where $m = 1$, the first of the three terms within the bracket in these equations is generally the largest. Thus, making only a small number of replicate determinations of y_0 can dramatically improve the precision of x_0. Similarly, increasing the number of calibration points, n, is beneficial. If considerations of time, material availability, *etc.* limit the total number of experiments $(m + n)$ that can be

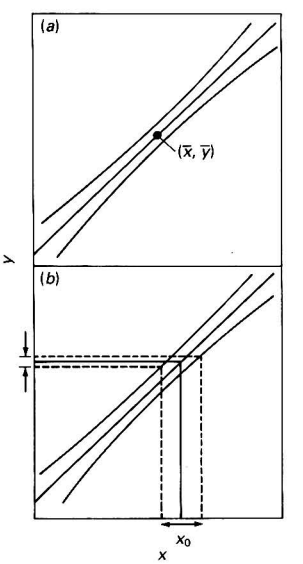


Fig. 1 Confidence limits in linear regression: (*a*) shows the hyperbolic form of the confidence limits for a predicted y-value; and (*b*) shows how these confidence limits combine with the uncertainty in y_0 to yield a confidence interval for a predicted x-value, x_0

performed, the sum of the first two components of the bracketed term in (7) and (9) is minimized by setting $m = n$. However, small values of n are to be avoided for a separate reason, *viz.*, that the use of $n - 2$ degrees of freedom then leads to very large values of t and correspondingly wide confidence intervals. Calculation shows that, in the simple case where $y_0 = \bar{y}$, then for any given values of $s_{y/x}$ and b, the priority (at the 95% confidence level) is to avoid values of $n <$ 5 because of the high values of t associated with <3 degrees of freedom. When $n \geqslant 5$, maximum precision from a fixed number of measurements is obtained when $m = n$.

The last bracketed term in equations (7) and (9) shows that precision (for fixed m and n) is maximized when y_0 is as close as possible to $\bar{y}$ (this is expected in view of the confidence interval variation shown in Fig. 1), and when $\sum_i (x_i - \bar{x})^2$ is as large as possible. The latter finding suggests that calibration graphs might best be plotted with a cluster of points near the origin, and another cluster at the upper limit of the linear range of interest [Fig. 2(*a*)]. If n calibration points are determined in two clusters of $n/2$ points at the extremes of a straight line, the value of the term $\sum_i (x_i - \bar{x})^2$ is increased by a factor $[3(n - 1)/(n + 1)]$ compared with the case in which the n points are equally spaced along the same line [Fig. 2(*b*)]. In practice it is usual to use a calibration graph with points roughly equally distributed over the concentration range of interest. The use of two clusters of points gives no assurance of the linearity of the plot between the two extreme x values; moreover, the term $[(y_0 - \bar{y})^2/b^2 \sum_i (x_i - \bar{x})^2]$ is often the smallest of the three bracketed terms in equations (7) and (9), so reducing its value further may have only a marginal over-all effect on the precision of x_0.

Method of Standard Additions

In several analytical methods (*e.g.*, potentiometry, atomic and molecular spectroscopy) matrix effects on the measured signal demand the use of the method of standard additions. Known amounts of analyte are added (with allowance for any dilution effects) to aliquots of the test sample itself, and the calibration graph (Fig. 3) shows the variation of the measured signal with the amount of analyte added. In this way some matrix effects are equalized between the sample and the standards. The concentration of the test sample, x_c, is given by the intercept on the x-axis, which is clearly the ratio of the y-axis intercept and the slope of the calibration line, calculated using equations (2) and (3), *i.e.*,

$$x_c = a/b \tag{10}$$

The standard deviation of x_c, s_{x_c}, is given by a modified form of equation (7):

$$s_{x_c} = \frac{S_{y/x}}{b}\left[\frac{1}{n} + \frac{\bar{y}^2}{b^2 \sum_i (x_i - \bar{x})^2}\right]^{\frac{1}{2}} \tag{11}$$

This standard deviation can as always be converted into a confidence interval using the appropriate t value. It might be expected that such confidence intervals would be wider for this extrapolation method than for a conventional interpolation method. In reality, however, this is not so, as the uncertainty in the value of x_c derives only from the random errors of the regression line itself, the corresponding value of y being fixed at zero in this case. The real disadvantages of the method of standard additions are that each calibration line is valid for only a single test sample, larger amounts of the test sample may be needed and automation is difficult.

The slope of a standard additions plot is normally different from that of the conventional calibration plot for the same sample. The slope ratio is a measure of the proportional systematic error produced by the matrix effect, a principle used in many 'recovery' experiments.[10] The use of the conventional standard additions method has been discussed at length by Cardone.[11,12] The generalized standard additions method (GSAM)[13] is applicable to multicomponent analysis problems, but belongs to the realm of chemometrics.[14]

Limit of Detection and Sensitivity

The ability to detect minute amounts of analyte is a feature of many instrumental techniques and is often the major reason for their use. Moreover, the concept of a limit of detection (LOD) seems obvious: it is the least amount of material the analyst can detect because it yields an instrument response significantly greater than a blank. Nonetheless, the definition and measurement of LODs has caused great controversy in recent years, with additional and considerable confusion over nomenclature, and there have been many publications by statutory bodies and official committees in efforts to clarify the situation. Ironically, the significance of LODs, at least in the strict quantitative sense, is probably overestimated. There is clearly a need for a means of expressing that (for example) spectrofluorimetry at its best is capable of determining lower amounts of analytes than absorptiometry, and the principal use of LODs in the literature appears to be to show that a newly discovered method is indeed 'better' than its predecessors. But there are many reasons why the LOD of a particular method will be different in different laboratories, when

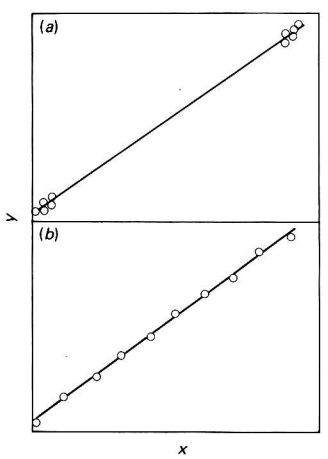


Fig. 2 Calibration graphs with: (*a*) clusters of standards at high and low concentrations; and (*b*) equally spaced standards

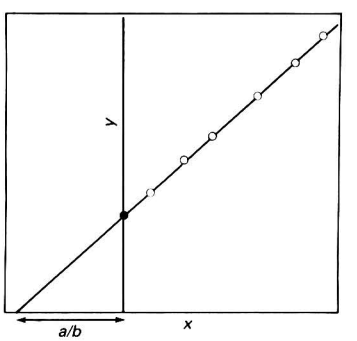


Fig. 3 Calibration graph for the method of standard additions. The point ● is due to the original sample; for details see text

applied to different samples or used by different workers. Not least among the problems is, as always, the occurrence of hidden and possibly large systematic errors, a point rightly emphasized by a recent report of the Analytical Methods Committee of the Analytical Division of the Royal Society of Chemistry.[15] It can thus be very misleading to read too much into the absolute value of a detection limit.

One principle on which all authorities are agreed is that the sensitivity of a method is not the same as its LOD. The sensitivity is simply the slope of the calibration plot. As calibration plots are often curved (see below) the concentration range over which the sensitivity applies should be quoted. In practice the concept of sensitivity is of limited value in comparing methods, as it depends so much on experimental conditions (*e.g.*, the sensitivity of a spectrophotometric determination can simply be increased by increasing the optical path length). Comparisons of closely related methods —for example, of spectrophotometric methods for iron(III) using three organic chelating reagents with different molar absorptivities in 10 mm cuvettes[16]—may be of value.

The most common definitions of the LOD take the form in which the lowest detectable instrument signal, y_L, is given by

$$y_L = y_B + ks_B \quad (12)$$

where y_B and s_B are, respectively, the blank signal and its standard deviation. Any sample yielding a signal greater than y_L is held to contain some analyte, while samples yielding signals $<y_L$ are reported to contain no detectable analyte. The constant, k, is at the discretion of the analyst, and it cannot be emphasized too strongly that there is no single, 'correct', definition of the LOD. It is thus essential that, whenever an LOD is quoted, its definition should also be given. After y_L has been established, it can readily be converted into a mass or concentration LOD, c_L, by using the equation

$$c_L = ks_B/b \quad (13)$$

This equation shows the relationship between the LOD and sensitivity, the latter being given by the slope of the calibration graph, b, if the graph is linear throughout.

Kaiser[17] suggested that k should have a value of 3 (although other workers have, at least until recently, used $k = 2$, $k = 2^{3/2}$, *etc.*). This recommendation has been reinforced by the International Union of Pure and Applied Chemistry (IUPAC)[18] and others, and is now very common. It is important to clarify the significance of this definition. Fig. 4(*a*) illustrates the distribution of the random errors of y_B, the standard deviation σ_B being estimated by s_B, as always. The probability that a blank sample will yield a signal greater than $y_B + 3s_B$ is given by the shaded area, readily shown, using tables of the standard normal distribution, to be 0.00135, *i.e.*, 0.135%. This is the probability that a false positive result will occur, *i.e.*, that analyte will be said to be present when it is in fact absent. This is analogous to a type I error in conventional statistical tests.[1] However, the second type of error (type II error), that of obtaining a false negative result, *i.e.*, deducing that analyte is absent when it is in fact present, can also occur. If numerous measurements are made on a solution that contains analyte at the LOD level, c_L, the instrument responses will be normally distributed about y_L, with the same standard deviation (estimated by s_B) as the blank. (This assumption of equal standard deviations was noted earlier in this review and has thus far been used throughout.) Half the measurements on a sample with concentration c_L will thus yield instrument responses below y_L. If any sample, yielding a signal less than y_L, is reported as containing no detectable analyte, the probability of a type II error is clearly 50%; this would always be true, irrespective of the separation of y_B and y_L.

Many workers, therefore, separately define a 'limit of decision', a point between y_B and y_L, to establish more sensible levels of type I and type II errors. This procedure is

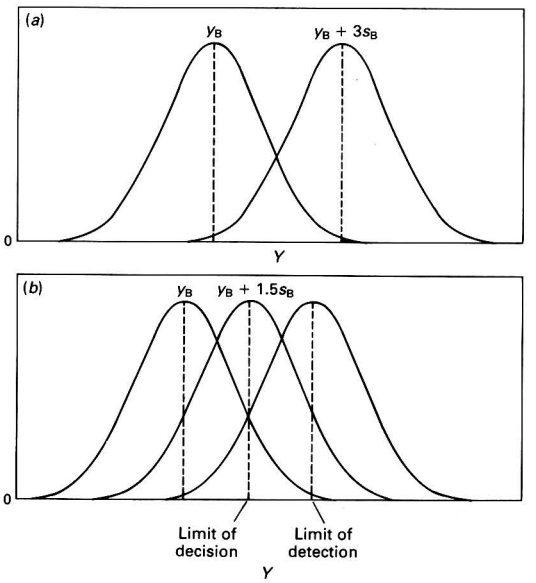


Fig. 4 Limits of detection; for details see text

again analogous to simple statistical tests; in this case the null hypothesis is that there is no analyte present, and the alternative hypothesis is that an analyte concentration c_L is present. The critical value for testing these hypotheses is set by the establishment of the limit of decision. In most cases the assumption is made that type I and type II errors are equally to be minimized (although it is easy to imagine practical instances where this is not appropriate), so the limit of decision would be at $y_B + 1.5s_B$ [Fig. 4(*b*)]. If analyte is reported present or absent at y-values, respectively, above and below this limit, there is a probability of 6.7% for each type of error. Many analysts feel this to be a reasonable criterion; it is not much different from the 95% confidence level routinely used in most statistical tests. Clearly, if the probability of both types of error is to be reduced to 0.135%, the limit of decision must be at $y_B + 3s_B$, and the LOD at $y_B + 6s_B$.[16]

Some workers further define a 'limit of determination', *i.e.*, a concentration which can be determined with a particular RSD. Using the IUPAC definition of LOD, it is clear that the RSD at the limit of detection is 33.33%. A common definition of the limit of determination is $y_B + 10s_B$, indicating an RSD of 10%. It is to be noted that this result again assumes that the standard deviation s_B applies to measurements at all levels of y. The effects on LODs of departures from a uniform standard deviation have been considered by Liteanu and Rica[19] and by the Analytical Methods Committee.[15]

If the LOD definition of $y_B + 3s_B$ is accepted, it remains to discuss the estimation of y_B and s_B themselves. The blank signal, y_B might be obtained either as the average of several readings of a 'field blank' (*i.e.*, a sample containing solvent, reagents and sample matrix but no analyte, and examined by the same protocol as all other samples), or by utilizing the intercept value, a, from the least squares calculation. If all the assumptions involved in the latter calculation are valid, these two methods should yield values for y_B that do not differ significantly. Repeated measurements on a field blank will also provide the value of s_B. Only if a field blank is unobtainable (a not uncommon situation) should the intercept, a, be used as the measure of y_B, in this situation $s_{y/x}$ will provide an estimate of s_B.

Intersection of Two Straight Lines

Analytical scientists frequently use methods requiring the determination of the point of intersection of two straight lines. This approach is used in Job's method[20] and in other studies of molecular interactions such as drug–protein binding. The usual requirement is to determine the x-value (often a concentration ratio rather than a single concentration in this case) of the point of intersection. If the two lines, each determined by the methods described above, are given by $y = b_1x + a_1$ and $y = b_2x + a_2$, the intersection point, x_x, is easily shown to be given by

$$x_x = (a_2 - a_1)/(b_1 - b_2) \quad (14)$$

The confidence interval for x_x has been calculated in several ways (reviewed in reference 19), and continues to excite interest;[21] it is clearly related to the hyperbolic curves representing the confidence intervals for each line (Fig. 5). For the line $y = b_1x + a_1$ these curves are given by

$$y = (b_1x + a_1) \pm ts_{(y/x)_1}\left[1 + \frac{1}{n_1} + \frac{(x - \bar{x}_1)^2}{\Sigma(x - \bar{x}_1)^2}\right]^{\frac{1}{2}} \quad (15)$$

This equation yields the confidence limits for the true mean value of y at any given value of x. The t value is taken at the desired confidence level (usually 95%) and $n_1 - 2$ degrees of freedom. A similar equation applies to the line $y = b_2x + a_2$. One reasonable definition for the lower confidence limit for x_x, (x_L), is the abscissa value of the point of intersection of the upper confidence limit of line 1 and the lower confidence limit of line 2 (Fig. 5). At this point

$$b_1x_L + a_1 + ts_{(y/x)_1}\left[1 + \frac{1}{n_1} + \frac{(x_L - \bar{x}_1)^2}{\Sigma(x - \bar{x}_1)^2}\right]^{\frac{1}{2}} = b_2x_L + a_2 - ts_{(y/x)_2}\left[1 + \frac{1}{n_2} + \frac{(x_L - \bar{x}_2)^2}{\Sigma(x - \bar{x}_2)^2}\right]^{\frac{1}{2}} \quad (16)$$

which can be solved for x_L. An analogous equation can be written for x_u, which is similarly defined by the intersection of the lower confidence limit for line 1 and the upper confidence limit for line 2

$$b_1x_u + a_1 - ts_{(y/x)_1}\left[1 + \frac{1}{n_1} + \frac{(x_u - \bar{x}_1)^2}{\Sigma(x - \bar{x}_1)^2}\right]^{\frac{1}{2}} = b_2x_u + a_2 + ts_{(y/x)_2}\left[1 + \frac{1}{n_2} + \frac{(x_u - \bar{x}_2)^2}{\Sigma(x - \bar{x}_2)^2}\right]^{\frac{1}{2}} \quad (17)$$

As the confidence intervals for lines 1 and 2 may be of different width, and as the two lines may interesect at any angle, the confidence limits for x_x may not be symmetrical about x_x itself. It should also be noted that the confidence limits for x_x derived from (for example) the 95% confidence limits for the two separate lines are not necessarily the 95% confidence limits for x_x. As the estimation method used above assumes the worst case in combining the random errors of the two lines, the derived confidence limits are on the pessimistic (*i.e.*, realistic!) side. Finally it is important to note that the practical applications of this method utilize extrapolations of the two straight lines to the intersection point. These extrapolations are generally short, and care is usually taken to perform the experiments in conditions where the extrapolations are believed to be valid. However, if this belief is erroneous (*e.g.*, in studies of drug–protein binding where there is more than one class of binding site instead of the single class often assumed), even the best statistical methods cannot produce chemically valid results.

Residuals in Regression Statistics

Previous sections of this review have shown that the unweighted regression methods in common use in analytical chemistry are based on several assumptions which merit critical examination and that it is not a straightforward matter to decide whether a straight line or a curve should be drawn through a set of calibration points. Important additional information on both these topics can be obtained from the y-residuals, the $(y - \hat{y})$ values which represent the differences between the experimental y-values and the fitted y-values. The residuals thus represent the random experimental errors in the measurements of y, if the statistical model used (the unweighted regression line of y on x) is correct. Many statistical tests can be applied to these residuals (a comprehensive survey is given in reference 5) but for routine work it is often sufficient to plot the individual residuals against $\hat{y}$ or against x. Many regression programs for personal computers offer this facility and some provide additional refinements, *e.g.*, the inclusion of lines showing the standard deviations of the residuals.

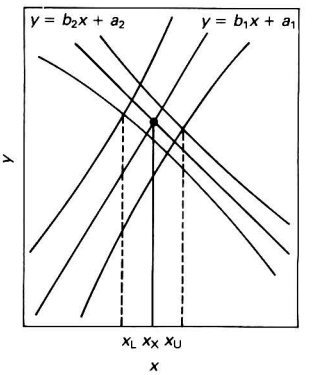


Fig. 5 Confidence limits for the point of intersection of two straight lines

It can be shown that, if the calibration line is calculated from the equation $y = bx + a$ (but not if it is forced through the origin by using the form $y = bx$), the residuals always total zero, allowing for rounding errors. As already noted, the residuals are assumed to be normally distributed. Fig. 6(*a*) shows the form that the residuals should thus take if the unweighted regression line is a good model for the experimental data. Fig. 6(*b*) and (*c*) indicates possible results if the unweighted regression line is inappropriate. If the residuals tend to become larger as y (or x) increases, the use of a weighted regression line (see below) is indicated, and if the residuals tend to fall on a curve, the use of a curved calibration graph rather than a linear one is desirable. In the latter case the signs (+ or −) of the residuals, which should be in random order if an appropriate statistical model has been used, will tend to occur in sequence ('runs'); in the example given, there is clearly a sequence of positive residuals, followed by a sequence of negative ones followed by a second positive sequence. The number of 'runs' (three in the example given) is thus significantly less than if the signs of the residuals had been + and − in random order. The Wald–Wolfowitz method tests for the significance of the number of runs in a set of data[5,22] by comparing the observed number of runs with tabulated data,[23] but it cannot be used if there are fewer than nine points in the calibration graph. Like the other residual diagnostic methods described here, the test is thus of restricted value in instrumental analysis, where the number of calibration points is frequently less than this. Tests on residuals are not, however, limited to linear regression plots: they can also be applied to non-linear plots, and indeed to any situation in which experimental data are fitted to a statistical model and some unexplained variations occur.

Examination of the residuals may shed light on a further problem, that of outliers among the data. The first part of this

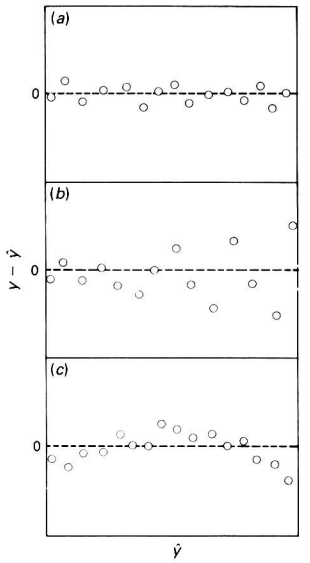


Fig. 6 Residuals in regression; for details see text

review[1] emphasized the importance of examining possible outliers carefully before rejecting them, if only because an observation that appears to be an outlier if one statistical model (*e.g.*, linear regression with normally distributed errors) is used, might not be an outlier if an alternative model (*e.g.*, a weighted or a polynomial regression equation) is fitted. After the residuals of a calibration graph have been calculated, it is usually easy to identify any that are exceptionally large. Again, many personal computer programs 'flag' such data points automatically. Unfortunately it is not legitimate simply to examine the residuals by the Q-test[1] or related outlier tests, as the residuals are not independent measurements (they must total zero). However, several methods have been developed for studying potential outliers in regression.[5,7] These methods transform the residuals before examination, and will not be treated in detail here. Perhaps the best-known approach involves the estimation of 'Cook's Distance'[24] for the suspect point. This distance is a measure of the influence of an observation, *i.e.*, of how much the regression line would be altered by omission of the observation from the usual calculations of a and b. A discussion of this method, along with a BASIC computer program which implements it, has recently been published.[25] The problem of outliers can alternatively be by-passed by the use of the robust and non-parametric methods described below.

Regression Techniques in the Comparison of Analytical Methods

When a novel analytical method is developed there is a natural desire to validate it by comparing it with well established methods. This is normally achieved by applying both new and established methods to the analysis of the same group of test samples. As calibration methods are designed for use over wide concentration ranges, these samples will properly contain widely differing amounts of the analyte under study. The question then arises, how are the paired results (*i.e.*, each sample examined by each of the two methods) evaluated for systematic errors? The paired t-test[1] cannot be used, as it ascribes the same weight to any given difference between a pair of results, irrespective of the absolute value measured. The approach most commonly used is to plot the results of the two methods on the two axes of a regression graph; each point on the graph thus represents a single sample measured by the two techniques being compared. It is clear that, if both methods give identical results for all samples, the resulting graph will be a straight line of unit slope and zero intercept, with the correlation coefficient $r = +1$. Some departure from these idealized results is inevitable in practice, and the usual requirements if the new method is to be regarded as satisfactory are that r is close to $+1$, that the confidence interval for the intercept, a, includes zero, and that the confidence interval for the slope, b, includes 1. The new method is tested most rigorously if the comparison is made with a considerable number of samples covering in a roughly uniform way the concentration range of interest.

There are several ways in which the plotted line can deviate from the ideal characteristics summarized above. Sometimes one method will give results which are higher or lower than those of the other method by a constant amount (*i.e.*, $b = 1$, $a >$ or <0). In other cases there is a constant relative difference between the two methods ($a = 0$, $b >$ or < 1). These two types of error can occur simultaneously ($a >$ or < 0, $b >$ or < 1), and there are instances in which there is excellent agreement between the two methods over part of the range of interest, but disagreement at, *e.g.*, very high or very low concentrations. Finally, there are experiments where some of the points lie close to the ideal line ($b = 1$, $a = 0$), but another group of samples give widely divergent points; speciation problems are the most probable cause of this result.

These possibilities have been summarized by Thompson,[26] whose paper also studies an important problem in the use of conventional regression lines in method comparisons. The line of regression of y on x assumes that the random errors of x are zero. This is clearly not the case when two experimental methods are being compared, so despite its almost universal use in this context the conventional regression line is not a proper statistical tool for such comparisons. (The line of regression of x on y would be equally unsuitable.) It has, however, been shown that by using Monte Carlo simulation methods,[26] the consequences of this unsoundness are not serious provided that at least ten samples covering the concentration range of interest fairly uniformly are used in the comparison, and the results from the method with the smaller random errors are plotted on the x-axis. A rigorous solution of the method comparison problem would be a calculation of the best straight line through a series of points with both x and y values subject to random errors. Over a century ago, Adcock[27] offered a solution which assumed that the x- and y-direction errors were equal. A complete solution, based on maximum likelihood methods, has been proposed by Ripley and Thompson.[28] Their technique utilizes the statistical weight of each point (thus requiring more information—see below) and as it is a computer-based iterative approach, it may not command ready acceptance for routine use.

Robust and Non-parametric Regression Methods

Previous sections of this review have shown that the unweighted least-squares regression line of y on x may not be appropriate if the y-direction errors are not normally distributed, or if both x- and y-direction errors occur (as in method comparisons). Moreover, the calculation and interpretation of this line are complicated by the presence of possible outliers. Statisticians use the term robustness to describe the property of insensitivity to departures from an assumed statistical model; a number of robust regression methods have been developed in recent years. One obvious approach is to use non-parametric methods, which do not assume any particular distribution for the population of which the experimental data are a sample. (Note that robust methods are not necessarily non-parametric, but non-parametric methods are generally robust.)

Perhaps the best-known non-parametric regression method is that developed by Theil.[29] He suggested that the slope, b, of the regression line should be estimated by determining the median of the slopes of all the lines joining pairs of points. (The median of a set of measurements is the middle value when an odd number of measurements is arranged in numerical order, or the average of the two middle values when there is an even number of measurements.) A graph with n points will thus have $[n(n + 1)/2]$ independent estimates of the slope. After b has been determined, n estimates of the intercept, a, can be obtained from the equation $a_i = y_i - bx_i$. The median of these n values of a is taken as the value of the intercept estimate. Theil's procedure is open to two objections. Firstly, it seems that the slope estimates of points with well separated x_i values should carry more weight than those from neighbouring pairs of points; Jaeckel[30] has proposed a modified method that achieves this. Secondly, computation of the median slope value becomes tedious even for fairly moderate values of n; a graph with ten points will yield 55 separate estimates of the slope to be determined and sorted into numerical order. This problem is overcome by the use of a shorter technique (Theil's abbreviated or incomplete method) in which slope estimates are obtained from x_i and the first point above the median value of x, from x_2 and the second point above the median, and so on. (If n is odd, the middle x_i value is not used at all in the slope calculation.) After the slope of the line has been estimated in this way, the intercept is estimated as in the 'complete' method. An example of this approach, which also illustrates its robustness towards outliers, is given in reference 22. The Theil methods make no assumptions about the directions of the errors, so are suitable for method comparisons (see above). Hussain and Sprent[31] have shown that the Theil (complete) method is almost as efficient as the least-squares method when the errors are normally distributed, and much more efficient, especially when n is small, when the errors are not normally distributed. (Efficiency in statistics is a relative concept: it allows the comparison of two statistical tests in their ability to detect alternative hypotheses which are close to the null hypothesis, H_o.[32]) Maritz[33] has reviewed Theil's method and Sprent[32] and Maritz[34] have surveyed other robust and non-parametric regression methods, including those which handle curved graphs. It should be noted that the spline techniques of curve-fitting discussed below are non-parametric methods.

When a line is to be drawn through a large number of points (*e.g.*, in method comparisons), a relatively rapid and preliminary method of plotting it may be of value. The points are divided as nearly as possible into three equal groups of x-values, and the median x-value for each of the three groups is identified. These three data points are known as the summary points. The slope of the resistant line (*i.e.*, resistant to outliers) through all the points is then estimated by the slope of the line joining the two outermost summary points. The intercept of the line is calculated as the average of the three intercepts obtained from the determined slope and the three summary points (*cf.* Theil's method above). The values of the slope and intercept may be polished by iterative minimization of the y-residuals. This method (and many other 'quick and dirty' methods used in exploratory data analysis) is discussed in reference 35.

In some experiments the results cannot be expressed in quantitative terms, but only in terms of a rank order.[1] Examples—perhaps infrequent in analytical work—include the preferences of laboratory workers for different pieces of equipment, the state of health of laboratory animals or the taste quality of a food or drink sample. Relationships in such cases are studied using rank correlation methods. Spearman's rank correlation coefficient, ρ,[36] is famous as being the first statistical method to use ranks, and is readily shown[33] to be the product-moment correlation coefficient, r, converted for use when both x and y variables are expressed as ranks. Spearman's ρ is given by

$$\rho = 1 - \frac{6\Sigma d_i^2}{n(n^2 - 1)} \qquad (18)$$

and, like r, lies in the range $-1 \leqslant \rho \leqslant +1$. In equation (18) d_i is the difference between the x and y rankings for the ith measurement. If the calculated value of ρ exceeds the critical value (taken from tables) at the appropriate confidence level and value of n, a significant correlation between x and y is established (although not necessarily a causal relationship). As in other ranking methods, tied ranks (*i.e.*, observations of equal rank in x or y) are given mid-rank values. Thus, if several food samples were ranked, with the two best samples judged equal in quality, the ranking would be 1.5, 1.5, 3, 4, . . . instead of 1, 2, 3, 4, . . .

The Kendall rank correlation coefficient, τ,[37] is based on a different idea. If, in a ranking experiment, high x-values are generally associated with high y-values, we expect that $y_j > y_i$ if $x_j > x_i$. Pairs of observations having this property are said to be concordant, while observations where the x and y values are in opposite order are said to be discordant (if $x_i = x_j$ or $y_i = y_j$ a tie has occurred). Kendall's method involves examining each of the $[n(n - 1)/2]$ pairs of data and evaluating n_c, the number of concordances, and n_d, the number of discordances. The rank correlation coefficient is then given by

$$\tau = \frac{n_c - n_d}{n(n - 1)/2} \qquad (19)$$

Again, it is evident that $-1 \leqslant \tau \leqslant +1$, the value -1 corresponding to all the pairs of data points giving discordances, and $+1$ corresponding to complete concordance. Intermediate values are again compared with tabulated values. Kendall's method has the advantage that the data do not have to be converted into ranks for n_c and n_d to be calculated. Moreover, the computation can be further simplified[38] if the term $[n(n - 1)/2]$ is omitted, the test statistic being simply calculated as $T = n_c - n_d$. It is rare, however, for the results of the Kendall and Spearman methods to disagree, and both have been used successfully as tests for trend, *i.e.*, to examine whether there is a correlation when one of the variables is time. The concept of concordance introduced by Kendall can be extended to problems with more than two variables; examples are given in standard texts.[38]

Analysis of Variance in Linear Regression

Analysis of variance (ANOVA) is a powerful and very general method which separates the contributions to the over-all variation in a set of experimental data and tests their significance. The sources of variation (one of which is invariably the random measurement error) are each characterized by a sum of squares (SS), *i.e.*, the sum of a number of squared terms representing the variation in question, a number of degrees of freedom (DF) (as defined in reference 1), and a mean square, which is the former divided by the latter and can be used to test the significance of the variation contribution by means of the F-test.[1] The mean square and the number of degrees of freedom for the over-all variation are, respectively, the sums of the mean squares and degrees of freedom of the several contributing sources of variation: this additive property greatly simplifies the calculations, which are now widely available on personal computer software.

In analytical calibration experiments, variation in the y-direction only is considered. This variation is expressed as the sum of the squares of the distances of each calibration point from the mean y value, $\bar{y}$, *i.e.*, by $\Sigma(y_i - \bar{y})^2$. This is the total SS about $\bar{y}$. There are two contributions to this over-all variation. One is the SS due to regression, *i.e.*, that part of the variation due to the relationship between y and x; each term in this SS is clearly of the form $(\hat{y}_i - \bar{y})^2$. This SS has just one DF,

as just one function of the y_i values, *i.e.*, the slope, b, will calculate $\Sigma(\hat{y}_i - \bar{y})^2$ from $b^2\,\Sigma(x_i - \bar{x})^2$. The second source of variation is the SS about regression, *i.e.*, the variation due to deviations from the calibration line, each term in the SS being of the form $(y_i - \hat{y}_i)^2$. This SS has $(n - 2)$ DF, reflecting the fact that the residuals come from a model requiring the estimation of two parameters, a and b. In accordance with the additivity principle described above, it is possible to show[5] that

Total SS about $\bar{y}$ = SS due to regression + SS about regression (20)

Moreover, the number of DF for the total SS is $(n - 2) + 1 = (n - 1)$. This result is expected, as only $(n - 1)$ y_i values are needed to determine the total SS about y, as $\Sigma(y_i - \bar{y}) = 0$ by definition. A typical ANOVA table for a linear regression plot is shown in Table 1. This is a one-way ANOVA calculation, there being only one source of variation in addition to the inevitable experimental error. The significance of the correlation can be tested by using the F-test, *i.e.*, by calculating

$$F_{1,\,(n-2)} = \mathrm{MS_{reg}}/\mathrm{MS_{res}} \qquad (21)$$

In practice this is rarely necessary (though readily available in software packages), as the F-values are generally vastly greater than the critical values. A more common estimate of the goodness of fit is given by the statistic R^2, sometimes known as the (multiple) coefficient of determination or the (multiple) correlation coefficient. The prefix 'multiple' occurs because R^2 can also be used in curvilinear regression (see below). If the regression line (straight or curved) is to be a good fit to the experimental points, the SS due to regression should be a high proportion of the total SS about $\bar{y}$. This is expressed quantitatively using the equation

$$R^2 = \text{SS due to regression/total SS about } \bar{y} \qquad (22)$$

R^2 clearly lies between 0 and 1 (although it can never reach 1 if there are multiple determinations of y_i at given x_i values[5]), however, it is often alternatively expressed as a percentage—the percentage of goodness of fit provided by a regression equation. It can be shown[5] that, for a straight line plot, $R^2 = r^2$, the square of the product-moment correlation coefficient. The application of R^2 to non-linear regression methods is considered further below.

Weighted Linear Regression Methods

The preceding discussion of regression methods has assumed that all the points on the regression line have equal weight (*i.e.*, equal importance) when the regression line is plotted. This is a reflection of the assumption that the y-direction errors are equal at all the values of x used in the calibration graph. In practice this is often an unrealistic assumption, as it is very common for the standard deviations of the measurements to alter with x. As already noted, RSDs will be high at analyte levels just above the LOD. However, there may be other variations at much higher concentrations. In some cases the standard deviation is expected to rise in proportion to the concentration, *i.e.*, the RSD is approximately constant,[39] while in other cases the standard deviation rises, though less rapidly than the concentration.[40] Many attempts have been made to formulate rules and equations for this concentration related behaviour of the standard deviation for different methods.[41,42] In practice, however, it will frequently be better to rely on the analyst's experience of a particular method, instrument, *etc.* in this respect.

If experience suggests that the standard deviation of replicate measurements does indeed vary significantly with x (heteroscedastic data), a weighted regression line should be plotted. The equations for this line differ from equations (2)–(7) because a weighting factor, w_i, must be associated with each calibration point x_i, y_i. This factor is inversely proportional to the variance of y_i, s_i^2, and must either be estimated from a suitable model (see above), or determined directly from replicate measurements of y_i:

$$w_i = s_i^{-2}/(\textstyle\sum_i s_i^{-2}/n) \qquad (23)$$

Equation (23) conveniently scales the weighting factors so that their sum is equal to n, the number of x_i values. The slope and intercept of the weighted regression line are then given, respectively, by

$$b = \frac{\sum_i w_i x_i y_i - n\bar{x}_w\bar{y}_w}{\sum_i w_i x_i^2 - n(\bar{x}_w)^2} \qquad (24)$$

$$a = \bar{y}_w - b\bar{x}_w \qquad (25)$$

Both these equations use the coordinates of the weighted centroid, $(\bar{x}_w, \bar{y}_w)$, given by $\bar{x}_w = \sum_i w_i x_i/n$ and $\bar{y}_w = \sum_i w_i y_i/n$, respectively; the weighted regression line must pass through this point. The standard deviation, $s_{x_{0w}}$, and hence a confidence interval of a concentration estimated from a weighted regression line is given by

$$s_{x_{0w}} = \frac{s_{(y/x)_w}}{b}\left\{\frac{1}{w_0} + \frac{1}{n} + \frac{(y_0 - \bar{y}_w)^2}{b^2[\sum_i w_i y_i^2 - n(\bar{x}_w)^2]}\right\}^{\frac{1}{2}} \qquad (26)$$

where w_0 is an interpolated weight appropriate to the experimental y_0 value, and $s_{(y/x)_w}$ is given by

$$s_{(y/x)_w}\left\{\frac{[\sum_i w_i y_i^2 - n(\bar{y}_w)^2] - b^2[\sum_i w_i x_i^2 - n(\bar{x}_w)^2]}{n-2}\right\}^{\frac{1}{2}} \qquad (27)$$

The confidence limits for weighted regression lines have the general form shown in Fig. 7, with the weighted centroid closer to the origin than its unweighted counterpart.

Calculations of weighted regression lines are evidently more complex than unweighted regression computations, and only the more advanced computer software packages provide suitable programs. The slope and intercept of a weighted regression line are often very similar to those obtained when

Table 1 Anova table for linear regression

Source of variation	Degrees of freedom	Sum of squares	Mean square (MS)
Regression	1	$\sum_{i=1}^{n}(\hat{y}_i - \bar{y})^2$	
About regression (*i.e.*, residual)	$n - 2$	$\sum_{i=1}^{n}(y_i - \hat{y}_i)^2$	$s_{y/x}^2 = \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n-2}$
Total	$n - 1$	$\sum_{i=1}^{n}(y_i - \bar{y})^2$	

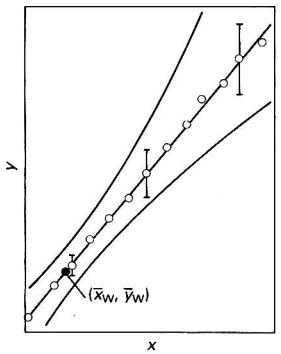


Fig. 7 Confidence limits in weighted regression. The point ● is the weighted centroid ($\bar{x}_w$, $\bar{y}_w$)

unweighted calculations are applied to the same data, but only the weighted line provides proper estimates of standard deviations and confidence limits when the weights vary significantly with x_i. Weighted regression calculations must also be used when curvilinear data are converted into rectilinear data by a suitable algebraic transformation (see below).

The Analytical Methods Committee has recently suggested[3] the use of the weighted regression equations to test for the linearity of a calibration graph. The weighted residual sum of squares (*i.e.*, the squares of the *y*-residuals, multiplied by their weights and then summed) should, if the plot is linear, have a chi-squared distribution with $(n - 2)$ DF. Significantly high values of chi squared thus suggest non-linearity. The same principle can be extended to test the fit of non-linear plots (see below).

Partly Straight, Partly Curved Calibration Plots

In many analytical methods a typical calibration plot is linear at low concentrations, but shows curvature towards the *x*-axis at higher analyte levels. This is often because an intrinsically non-linear relationship between instrument signal and concentration approximates to a linear function near the origin (*e.g.*, fluorescence spectrometry). In such cases it would be logical to fit all the data, including the low-concentration points, to a curve. In practice, however, linear regression equations have been regarded as so much simpler to calculate than non-linear ones that the former are usually used over as wide a concentration range as possible. This gives rise to the question—to what upper concentration limit is the linear approximation satisfactory?

A simple exploratory approach to this problem is exemplified in reference 22. It involves calculating with equations (1)–(3) the correlation coefficient, slope, intercept and SS of the residuals, first for all the calibration points, and then for the data sets with the highest, next-highest, *etc.* points being omitted successively. If the highest point(s) lie on a significantly non-linear portion of the plot, omitting them from the calculations will produce large reductions in the SS of residuals, significant increases of r towards 1 (note again that absolute values are of little significance) and changes of a and b towards the values suggested by the calibration points near the origin. When the omission of further points produces only minor changes in r and the other parameters mentioned, then the linear portion of the graph has been successfully identified. During this stepwise removal of calibration points, a situation may arise where the omission of a point produces only slight improvements in the value of r *etc.*, at the expense of a further restriction in the accepted linear range of the experiment. Judgement must then be exercised to balance the advantages of an increased linear range against the possible loss of accuracy and precision.

Treatment of Non-linear Data by Transformations

A large number of analytical methods are known to produce non-linear calibration graphs. In some cases these curvilinear relationships are only important at higher analyte concentrations (see above), but in other cases (*e.g.*, immunoassays) the entire plot is non-linear. Methods for fitting such data to curves are well established (see below) but the simplicity of linear methods has encouraged numerous workers to try to transform their data so that a rectilinear graph can be plotted. The most common transformations involve the use of logarithms (*i.e.*, plotting y against $\ln x$, or $\ln y$ against $\ln x$) and exponentials. Less commonly used transformations include reciprocals, square roots and trigonometric functions. Two or more of these functions are sometimes used in combination, especially in calibration programs supplied with commercial analytical instruments. This topic has been surveyed by Bysouth and Tyson.[43] Draper and Smith[5] have reviewed such procedures at some length, and logical approaches to the best transformations have been surveyed by Box and Cox[44] and by Carroll and Ruppert.[45]

It is important to note that all such transformations may affect the relative magnitudes of the errors at different points on the plot. Thus a non-linear plot with approximately equal errors at all values of x (homoscedastic data) may be transformed into a linear plot with heteroscedastic errors. It can be shown[5,46] that if a function $y = f(x)$ with homoscedastic errors is transformed into the function $Y = BX + A$, then the weighting factors, w_i used in equations (24)–(27) above are calculated from

$$w_i = \left[1/\left(\frac{dY_i}{dy_i}\right)\right]^2 \qquad (28)$$

In some cases transformations make the use of a weighted regression plot less necessary. Thus a line of the form $y = bx$ with *y*-direction errors dependent on x may be subjected to a log–log transformation. The errors in $\log y$ then depend less markedly on $\log x$ and, therefore, a homoscedastic approach may be reasonable. Similarly, Kurtz *et al.*[47] have applied a series of power functions to transform chromatographic data to constant variance.

In some analytical methods data transformations are so common that specialist software may be available to perform the calculations and present the results graphically. This is particularly true in the field of competitive binding immunoassays, where several different (but related) transformations are in common use, including those involving the logit function $\{\text{logit}\, x = \ln[x/(1 - x)]\}$ and logistic functions such as $y = A/(B + Ce^{-Dx})$, with the A–D values to be found by iteration. (Immunoassay practitioners also use spline functions—see below.) These methods have been reviewed.[48]

Curvilinear Regression

In many analytical methods, non-linear regression plots arise from several experimental factors, making it impossible to predict a model equation for the curve. For example in molecular fluorimetry, theory[49] shows that a plot of fluorescence intensity against concentration should be linear—but only as the result of several assumptions about the optical system used and the sample under study, and with the aid of a mathematical approximation. In practice some or all of these assumptions will fail, but to an unpredictable extent, giving a calibration graph that may approximate to a straight line near the origin (see above) but is, in reality, a curve throughout. In such cases it is sensible to adopt an empirical fit of a curve to

the observed data. This is most commonly attempted by using a polynomial equation of the form

$$y = a + bx + cx^2 + dx^3 + \ldots \quad (29)$$

The advantage of this type of equation is that, after the number of terms has been established, matrix manipulation allows an exact solution for *a*, *b*, *c*, *etc.* if the least-squares fitting criterion (see above) is used. Most computer packages offer such polynomial curve-fitting programs, so, in practice the major problem for the experimental scientist is to decide on the appropriate number of terms to be used in equation (29). The number of terms must clearly be $<n$ for the equation to have any physical meaning and common sense suggests that the least number of terms providing a satisfactory fit to the data should always be used (quadratic or cubic fits are frequently excellent).

Several approaches to this problem are available, the simplest (though probably not the best) being the use of the coefficient of determination, R^2. As described above, this coefficient expresses the extent to which the total SS about $\bar{y}$ can be explained by the regression equation under scrutiny. Values of R^2 close to 1 (or 100%—computer packages often present the result in this form) are thus apparently indicative of a good fit between the chosen equation and the experimental data. In practice we would thus examine in turn the values of R^2 obtained for the quadratic, cubic, quartic, *etc.* fits, and then make a judgement on the most appropriate polynomial.

This method is open to two objections. The first is that, like the related correlation coefficient, r (see above), R^2 can take very high values ($\gg 0.9$) even when visual inspection shows that the fit is obviously indifferent. More seriously still, it may be shown that R^2 always increases as successive terms are added to the polynomial equation, even if the latter are of no real value. (Draper and Smith[5] point out that this presents particular dangers if the data are grouped, *i.e.*, if we have several y-values at each of only a few x-values. The number of terms in the polynomial must then be less than the number of x-values.) Thus, if this method is to be used, it is essential to attach little importance to absolute R^2 values and to continue adding terms to the polynomial only if this leads to substantial increases in R^2.

An alternative and probably more satisfactory curve-fitting criterion is the use of the 'adjusted R^2' statistic, given by[50]

$$R^2 \text{ (adjusted)} = 1 - (\text{residual MS/total MS}) \quad (30)$$

The use of the mean square (MS) instead of SS terms, allows the number of degrees of freedom $(n - p)$, and hence the number of fitted parameters (p), to be taken into account. For any given data set and polynomial function, adjusted R^2 always has a lower value than R^2 itself; many computer packages provide both calculations.

Among several other methods for establishing the best polynomial fit[5,7] the simple use of the F-test[1] has much to commend it. In this application (often referred to as a partial F-test), F is used to test the null hypothesis that the addition of an extra polynomial term to equation (29) does not significantly improve the goodness of fit of the curve, when compared with the curve obtained without the extra term. Thus

$$F = \frac{(\text{extra SS due to adding } x^n \text{ term})/1}{\text{residual MS for } n\text{-order model}} \quad (31)$$

The calculated F value is compared with the tabulated value of $F_{1,n-p}$ at the desired probability level; p is again the number of parameters to be determined, *e.g.*, 3 (a, b, c), in a test to see whether a quadratic term is desirable. This test is simplified if the ANOVA calculation breaks down the SS due to regression into its component parts, *i.e.*, the SS due to the term in x, the additional SS due to the term in x^2 *etc.*

It is worth noting that, if the form of a curvilinear plot is known, the calculation of the parameter values can be regarded as an optimization problem and can be tackled by methods such as simplex optimization.[51] This approach offers no particular benefit in cases where exact solutions are also available by matrix manipulation, such as the polynomial equation (29), but may be very advantageous in other cases where exact solutions are not accessible. Again commercially available software is plentiful. A recent paper[52] describes the calculation of confidence limits for calibration lines calculated using the simplex method.

Whichever model and calculation method is chosen to plot a calibration graph, it is desirable to examine the residuals generated and use them to study the validity of the chosen model. If the latter is suitable the residuals should show no marked trend in sign, spread or value when plotted against corresponding x or y values. As for linear regression, outlying points can also be studied.

Spline Functions and Other Robust Non-linear Regression Methods

As non-linear calibration plots often arise from a combination of physico-chemical effects (see above) and failure of mathematical approximations *etc.*, it is perhaps unrealistic to expect that any single curve will adequately describe such data. It may thus be better to plot a curve which is a continuous series of shorter curved portions. The most popular approach of this type is the cubic spline method, which seeks to fit the experimental data with a series of curved portions each of cubic form, $y = a + bx + cx^2 + dx^3$. These portions are connected at points called 'knots', at each of which the two linked cubic functions and their first two derivatives must be continuous. In practice, the knots may coincide with experimental calibration points, but this is not essential and a variety of approaches to the selection of the number and positions of the knots is available. Spline function calculations are provided by several software packages, and their application to analytical problems has been reviewed by Wold[53] and by Wegscheider.[54]

A group of additional regression methods now attracting considerable attention relies on the use of fitting criteria other than or in addition to the least squares approach. In particular the 'reweighted least squares' method described in detail by Rousseeuw and Leroy[55] utilizes the least median of squares criterion (*i.e.*, minimization of the median of the squared residuals) to identify large residuals. The least squares curve is then fitted to the remaining points. These modern robust methods can of course be applied to straight line graphs as well as curves, and despite their requirement for more advanced computer programs they are already attracting the attention of analytical scientists (*e.g.*, reference 56). Such developments provide timely reminders that the apparently simple task of fitting a straight line or a curve to a set of analytical data is still provoking much original research.

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Liquid–Solid Extraction of Tributyltin From Marine Samples*

Otis Evans, Betty J. Jacobs and Arnold L. Cohen
Environmental Monitoring Systems Laboratory, US Environmental Protection Agency, 26 West Martin Luther King Drive, Cincinnati, OH 45268, USA

An analytical method based on a liquid–solid extraction (LSE) procedure followed by gas chromatography (splitless 'hot' injection mode) with electron-capture detection (GC-ECD) has been developed and evaluated for tributyltin (TBT) chloride in fresh and marine waters. Splitless 'hot' injection GC requires the rigorous exclusion of water from the eluting solvent and the sample extract solutions. Tributyltin chloride is thermally sensitive and tends to degrade in the injection liner. This thermal degradation, possibly a debutylation reaction, is enhanced in the presence of trace amounts of water. Electron capture detection, although offering adequate sensitivity, has insufficient specificity for TBT chloride at trace level concentrations. Also, the solvent, ethyl acetate, can extract compounds from the LSE column or cartridge housings. These compounds, phthalates, cresols, adipates, *etc.*, may interfere with the measurement and detection of the analyte. Chromatograms of extracts of LSE discs and procedural blanks have 'clean' backgrounds in contrast to many of the LSE columns and cartridges. Extraction discs mounted in a tandem arrangement show no breakthrough of analyte from the front to the rear disc for sample volumes which ranged from 100 to 500 ml. Liquid–solid extraction appears to meet the need for sample collection (on-site), preservation/storage (column or disc) and convenient and inexpensive sample shipment. The TBT chloride can be preserved on column(s) or disc(s) for at least 1 month. Recoveries of the tin analyte at the 0.1 ng ml^{-1} level range between 91 and 104%.

Keywords: *Liquid–solid extraction; tributyltin chloride; gas chromatography with electron-capture detection; splitless 'hot' injection; speciation*

Butyltin compounds, because of their versatility, have permeated many aspects of human society, exerting a profound effect on the environment and the economy. These compounds have found extensive use in industry, agriculture, aquatic areas (fresh water and marine) and medicine.[1]

The principal butyltin compounds are the tributyltins (TBTs). The TBTs are used as insecticides, fungicides, acaricides and preservatives for many different types of materials. However, their use in antifouling paints (as biocides) on ships, boats and docks and as slimicides in cooling towers has raised many issues and concerns.[2–5] The organotin-based paints release the TBTs directly into the aquatic environment. Tributyltins are effective biocides against marine fouling organisms; however, they are non-specific and extremely toxic to non-target animal and plant species. These compounds have also caused major pollution problems in areas with restricted water circulation and significant recreational boating activity.

As a result of the adverse manner in which these organotin compounds affect the environment, an awareness has developed of the need for reliable analytical methodology to identify and quantify them. The analytical research community has responded with a plethora of methods and techniques which address the speciation of organotins in biological tissues,[6–10] sediments[7,9–15] and various aqueous matrices.[14,16–28] The chemical literature is growing rapidly with the introduction of new and more sophisticated instrumentation[29–32] which has the capability to detect and identify organotins at extremely low levels.

This research was initiated by the US Environmental Protection Agency in order to meet the mandates of its monitoring programme and provide methods for the community to use. The work involved a review and evaluation of the technical literature, and in-house development of standardized methodology for organotin speciation. The general method criteria are rapid and inexpensive procedures which are selective for specific organotin compound(s).

The current analytical needs of the Agency are: firstly, standardized methodology for TBT in marine and fresh waters; secondly, a method detection limit (MDL) of 2–5 ng l^{-1}; thirdly, the identification and determination of TBT decomposition products and mixed butyltin compounds; and fourthly, methods for TBT and other organotins in sediments and biotissues.

The liquid–solid extraction (LSE) procedure described here, initially reported by Junk and Richard,[17,33] appears to meet the criteria of the Agency and current analytical needs with respect to TBT. However, the measurement–detection [splitless injection gas chromatography (GC) with electron-capture detection (ECD)] portion of the procedure presents certain problems that make consistent and accurate quantitative measurement difficult.[34] We are also, to our knowledge, presenting the first scientific report, in the chemical literature, on the application of LSE discs [chemically bonded silica particles enmeshed in a polytetrafluorethylene (PTFE) matrix] for organometallic compounds in environmental matrices, *i.e.*, the extraction of TBT.[34,35] Recent publications[36,37] report the use of LSE discs for the extraction of organic analytes from aqueous matrices.

Experimental†

Materials and Reagents

The solvents used were ethyl acetate and methanol (Fisher Scientific, Fairlawn, NJ, USA), Ultrex hydrochloric acid and ammonia solution (J. T. Baker, Phillipsburg, NJ, USA) and distilled, de-ionized water, prepared by passing distilled water through mixed bed cation- and anion-exchange resins.

Liquid–solid extraction columns, Bakerbond [octadecyl (C_{18}) 40 μm, 1 ml, 100 mg (J. T. Baker)] and discs, Empore [extraction discs, octadecyl 25 mm (Analytichem International, Harbor City, CA, USA)] were used in the tributyltin chloride extractions.

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† Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the US Environmental Protection Agency.

Standards

The tributyltin chloride stock standard solution (Alfa Products, Danvers, MA, USA) was prepared in methanol at a concentration of 100 mg l^{-1} and stored in PTFE at 4 °C. Subsequent dilutions were made with methanol and the solutions stored in glass at the same temperature. Individual working external standard solutions were prepared in ethyl acetate containing 15 μl of 20% hydrochloric acid per 50 ml of solvent.

Standards taken through the LSE procedure were prepared in distilled, de-ionized water adjusted to pH 4.5. Artificial sea-water (Forty Fathoms, Marine Enterprises, Towson, MD, USA) was used to simulate waters from the natural ocean environment.

Instrumentation

A Model GC-5880A gas chromatograph equipped with an electron-capture detector, and a 7672A automatic sampler (Hewlett-Packard, Avondale, PA, USA) were used. The 5880A was equipped with a split–splitless injection port operated in the splitless mode. A DB-1 fused silica capillary column (30 m × 0.32 mm i.d., 0.25 μm thickness) (J and W Scientific, Rancho Cordova, CA, USA) was used with helium as the carrier gas at a flow-rate of 3.9 ml min^{-1}. The linear velocity was 20.8 cm s^{-1} at 160 °C (measured isothermally). The argon–methane (5% methane in argon) make-up gas flow-rate was 30 ml min^{-1}. The injector and detector port temperatures were 200 and 260 °C, respectively. After injection the column temperature was held isothermally at 80 °C for 1.0 min, then the temperature was increased at a rate of 15.0 °C min^{-1} to 180 °C and held for 10.0 min, and finally the temperature was increased again at a rate of 20.0 °C min^{-1} to 230 °C and held there for 8.0 min in order to reduce column bleed interferences in ensuing runs.

Samples were extracted with a Baker LSE column processor vacuum manifold (J. T. Baker) (stainless-steel basin), together with a vacuum hose fitting, a cover with Luer fittings and gasket, a vacuum gauge controller and manifold Luer plugs. A 304 stainless-steel syringe (pressure filter) holder, 25 mm, was used to support the LSE discs. Extraction column reservoirs (75 ml) and adapters (1, 3 and 6 ml) were used when applying the sample to the LSE columns. A glass syringe (50 ml capacity) with a stainless-steel Luer tip was used as a reservoir when applying the sample to the extraction discs.

A digital pipette (Brinkman Instruments, Westbury, NY, USA) was used to dispense specified volumes during standard and sample solution preparations.

Extraction Procedure

Adjust a 100 ml sample containing 1–2% v/v methanol to pH 4.5 (add 1–2 ml of methanol per 100 ml of sample). Take a 1 ml, 100 mg silica (C_{18}) column or 25 mm PTFE enmeshed extraction disc (C_{18}) and add three column volumes (about 3 ml) of non-acidified ethyl acetate using a squeeze bottle. (After pre-conditioning the PTFE filter disc with ethyl acetate, create a vacuum in order to pull air through it for 5 min. The disc must not be allowed to become dry with subsequent conditioning and sample application.) Add four column volumes (about 4 ml) of methanol, 2 column volumes (2 ml) of de-ionized water and 2 column volumes of pH 4.5 de-ionized water. (Do not allow the column to become dry during additions of column conditioners and before the sample is added.)

Next attach the sample reservoir to the column. (If the disc is used, the reservoir is attached prior to conditioning.) Add the sample solution and adjust the flow-rate to about 5 ml min^{-1}. Following sample application, dry the LSE column and/or the disc by drawing room air in through the device, using the vacuum manifold for at least 30 min, then place the LSE disc and/or column in a desiccator (charged with calcium sulphate) overnight to effect complete removal of all residual water. (It is essential that all residual water be removed from LSE discs and columns prior to elution of TBT with the eluting solvent.) The analyte (TBT) is eluted with two 250 μl portions of ethyl acetate (acidified with 15 μL of HCl per 50 ml of ethyl acetate) into a calibrated GC glass sample vial. [Each portion of ethyl acetate (HCl acidified[34]) remains in contact with the column for at least 30 s.] The final volume of eluate (column/disc extract) is adjusted to 0.5 ml with a few drops of ethyl acetate (HCl acidified).

Finally, the sample vial is refrigerated overnight (4 °C) to allow the extract solution to equilibrate. It was found that allowing the extract to stand overnight (refrigerated) resulted in more stable and consistent responses being achieved upon sample analysis.

Caution. Tributyltin chloride is an extremely toxic and combustible substance. Pure standard material (liquid) and stock standard solutions of this compound should be handled with suitable protection for skin, eyes, *etc.*

Results and Discussion

The purpose of this research was to develop an analytical procedure for the detection and quantitative determination of tributyltin chloride in marine waters. Splitless injection capillary column gas chromatography with electron-capture detection was chosen as the determinate step because such equipment is common to most environmental laboratories.[39] On-column injection GC and element-selective detectors have been shown to improve the quality of the data and enhance sensitivity and selectivity and should be used whenever possible.

Details concerning the many factors, *i.e.*, interferences, moisture, thermal decomposition, analyte adsorption, and special conditioning requirements, affecting and influencing the LSE procedure and subsequent GC separation and detection, have been presented elsewhere.[34] Fig. 1 shows a chromatogram for a tributylin chloride external standard prepared in pure solvent. The analyte peak has a retention time of approximately 14.6 min. However, samples (and standards) taken through the extraction procedure can be affected by interferences which can originate from commer-

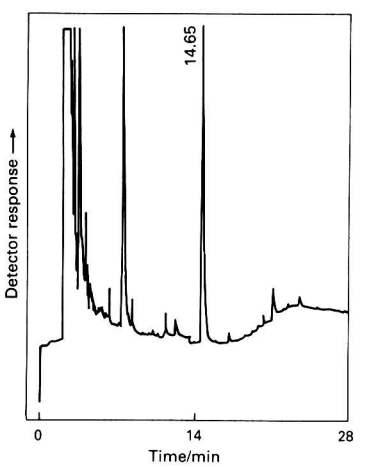

Fig. 1 GC-ECD trace of a 100 ppb solution of tributyltin chloride (retention time, 14.65 min)

cially available LSE columns and cartridges.[40] The eluting solvent may extract these potentially interfering compounds from the polypropylene housing, polyethylene frit and C_{18} bonded porous silica during the critical elution step (Fig. 2). The number of compounds extracted, and the number of potential interferences varies between manufacturers and also between and within a particular batch of columns. A representative number of columns should be analysed from each batch before use in order to determine background variability. Extraneous peaks, if present, can be deleterious to trace level measurements of tributyltin chloride when using an electron-capture detector. The exceptional sensitivity of the detector can be compromised by its insufficient specificity at the parts per trillion (ppt) concentration level.

Table 1 presents the results that can be obtained using this procedure, providing certain criteria are met.[34] The mean recoveries are greater than 95% at the 25 ppt level.

The recent introduction of LSE discs provides an opportunity for a comparison to be made between the two extraction technologies, and for the procedure to be evaluated further. Fig. 3 presents a series of chromatograms which show the apparent advantage of the LSE discs. Fewer extraneous peaks are observed, resulting in fewer potential interferences in the area of analyte elution (identified by the arrow in Fig. 3). However, a disadvantage of the discs is that they cannot be air dried (by vacuum) easily and as a consequence the extraction process is lengthened. Moisture (water) must be completely eliminated, because its presence is detrimental to the splitless injection GC of a thermally sensitive compound, such as tributyltin chloride. Accuracy and precision data for analyte measurements at the 50 ppt level, using the discs, are shown in Table 2. The results are comparable to those obtained by use of the extraction columns.

Further studies were performed to determine the potential breakthrough volume of this particular extraction device. The typical sample volume used was 100 ml; however, to increase the sensitivity and lower the detection limit it was necessary to extract larger amounts of solution. Table 3 presents the results for discs mounted in a tandem arrangement for measuring breakthrough. There was no measurable breakthrough of the analyte from the front to the rear disc for all of the volumes attempted. As the final extract volume is 0.5 ml, enhancement factors of up to 1000 are achievable with 500 ml sample solutions.

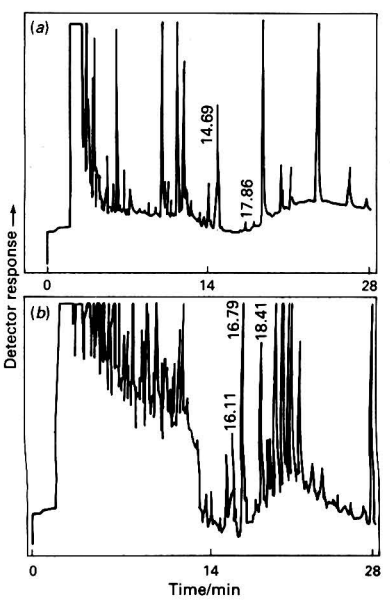

Fig. 2 GC-ECD traces of extracts from commercial C_{18} bonded porous silica columns

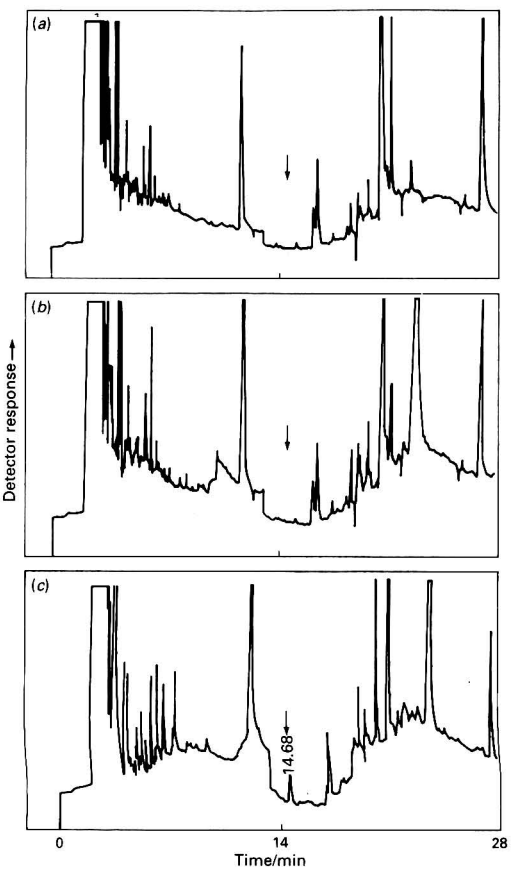

Fig. 3 GC-ECD traces of LSE solutions from C_{18}, 25 mm discs. (*a*) Extract from pre-conditioned disc; (*b*) extract from laboratory reagent blank; and (*c*) extract from sea-water containing 0.05 μg l^{-1} tributyltin chloride

Table 1 Accuracy and precision data for 18 determinations of the analyte (tributyltin chloride) at 0.025 μg l^{-1} with LSE and GC-ECD, using a C_{18} silica-based column (100 mg)

Technique	Volume of sample/ml	Concentration after extraction: Expected/ μg l^{-1}	Concentration after extraction: Mean observed/ μg l^{-1}	Standard deviation/ μg l^{-1}	Relative standard deviation (%)	Mean accuracy (% true concentration)
LSE	200	10	10.3	0.8	7.7	102.9
GC-ECD	200	12.5	12.0	1.4	11.7	96.1

Table 2 Accuracy and precision data for eight determinations of the analyte (tributyltin chloride) using a 25 mm C_{18} PTFE enmeshed extraction (filter) disc*

True concentration ng l^{-1}	Mean observed/ ng l^{-1}	Standard deviation/ ng l^{-1}	Relative standard deviation (%)	Mean accuracy (% true concentration
50	52.7	3.79	7.2	105

* 200 ml sample volume, standards taken through the LSE procedure.

Table 3 Recovery from tandem mounted discs.* Expected recovery, 20 μg l^{-1}; final volume (V_f) (extract), 0.5 ml; enhancement factors (EF) (theoretical), 200, 400 and 1000, respectively; 6–7 determinations per volume of sample; and ND = not detected

Volume of sample/ ml	Disc position	Mean observed concentration μg l^{-1}	Standard deviation/ μg l^{-1}	Relative standard deviation (%)	Mean accuracy (% true concentration)
100†	Front	18.7	0.97	5.2	93.5
	Rear (back)	ND			
200‡	Front	22.3	1.52	6.8	111.5
	Rear (back)	ND			
500§	Front	18.8	0.92	4.9	94
	Rear (back)	ND			

* 25 mm, C_{18}.
† 0.1 μg l^{-1} tributyltin chloride.
‡ 0.05 μg l^{-1} tributyltin chloride.
§ 0.02 μg l^{-1} tributyltin chloride.

The results indicate that the LSE of TBT can avoid the possible complications associated with a comparable liquid–liquid extraction (LLE). Liquid–liquid extractions tend to be time consuming and laborious. They are also expensive as large volumes of solvent may be required and many of these solvents are potential health hazards. The volume of eluting solvent in this procedure was less than 1 ml. Of course, slightly larger amounts were used during column conditioning.

Other potential advantages that LSEs appeared to offer were further explored. Table 4 shows data for the preservation and storage of TBT on the extraction columns for approximately 1 month. Comparable results have been achieved for analogous experiments using the discs. The space occupied by the latter is minimal and avoids the storage of bulky containers and the manpower required to handle them. The implication is that these devices would be convenient and inexpensive for the shipment of samples. Several research groups have investigated the preservation of TBT *via* freezing to determine applicability for storage and exchange of samples.[28] However, an acknowledged disadvantage is the possible loss of analyte should the samples tend to thaw during transport. In our experiments, the extraction procedure was followed to the desiccation step, then the columns were refrigerated. For the evaluation phase the procedure was continued at the stage of elution into the GC vial. Subsequent experiments, of a shorter duration, indicated that TBT contained on an LSE disc or column could be stored at room temperature (in a desiccator). No further research is planned in this area.

Conclusion

The LSE of TBT from aqueous solution offers simplicity in extracting, concentrating, preserving and storing an important environmental pollutant of great concern. The discs were found to have a low background, good collection efficiency and no observable breakthrough. An electron capture detector does not have the specificity to allow measurement of TBT at trace concentrations in environmental samples. Even

Table 4 Tributyltin preservation on LSE columns. Initial concentration 0.1 ng ml^{-1}, 100 ml sample volume, and expected recovery 20 ng ml^{-1}

Week	No. of determinations	Concentration after LSE, mean observed/ ng ml^{-1}	Standard deviation/ ng ml^{-1}	Relative standard deviation (%)	Recovery (% true concentration)
1	4	19.8	1.1	5.5	99
2	4	19.2	0.8	4.0	96
3	3	19.5	1.1	5.8	97.5
4	3	18.6	0.4	2.3	93

though the results presented here are good, determinations of TBT by using splitless injection GC are tedious and require a considerable amount of time. Therefore, its use is not recommended for routine analytical measurements of this analyte. Element-selective detectors, atomic absorption, flame photometric, *etc.*, should be strongly considered for complex aqueous matrices.

The authors thank Craig Markell (3M Company) for the liquid–solid extraction discs, and Gregor Junk (Ames Laboratory–US Department of the Energy) for his helpful discussions and comments during the course of this work. We gratefully acknowledge the assistance and advice of Thomas Bellar, Thomas Behymer, William Middleton, Jerry Bashe, Jeffrey Collins and other members of the Chemistry Research Division of the Environmental Monitoring Systems Laboratory and Technology Applications, Inc., Cincinnati, Ohio.

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Application of Tryptamine as a Derivatizing Agent for Airborne Isocyanate Determination

Part 4.* Evaluation of Major High-performance Liquid Chromatographic Methods Regarding Airborne Isocyanate Determination With Specific Investigation of the Competitive Rate of Derivatization

Weh S. Wu, Robert E. Stoyanoff† and Virindar S. Gaind
Occupational Health Laboratory, Ontario Ministry of Labour, 101 Resources Road, Weston, Ontario M9P 3T1, Canada

Several amines were investigated to determine their competitive rates in the derivatization of isocyanates. The amines studied included *N*-(*p*-nitrobenzyl)-*N*-propylamine, 1-(2-pyridyl)piperazine, 1-(2-methoxyphenyl)piperazine, *N*-ω-methyl tryptamine and tryptamine. Phenyl isocyanate, which might find application in future studies on sampling isocyanate vapour on solid absorbents, because it possesses a much higher vapour pressure than any of the industrially used isocyanates, was employed as the reference isocyanate. This approach was adopted because only the relative, rather than actual, rates of derivatization were of interest. By comparing the significant features of the methods for the determination of isocyanates using high-performance liquid chromatographic techniques, it was concluded that the proposed method, which uses tryptamine (and possibly *N*-ω-tryptamine), was the most promising for practical application. The theoretical background of the proposed method was based on the isolation of a selected π-system in a derivative for specific detection. This approach should be applicable to other areas involving analysis with chromatographic techniques. The competitive rate study has also provided a better assessment in selecting a particular amine for further research in personal sampling of isocyanates on amine-coated solid absorbents.

Keywords: *Isocyanate derivatization; competitive derivatization rate; isolation of selected π-system*

High-performance liquid chromatography (HPLC) techniques for determining airborne isocyanates have been routinely practiced in occupational health laboratories. The reason for this is that high-performance liquid chromatography is capable of handling larger molecules or compounds that are unstable to heat such as the industrially used isocyanates or their common derivatives. This has become even more important in recent years because the monitoring of airborne polymeric isocyanates in workplaces has been in demand. In spite of the many published isocyanate monitoring methods using HPLC techniques, the representative methods that have involved major developments are few and are discussed below in order to draw some distinct comparisons to the attention of the reader.

Dunlap *et al.*[1] developed the earliest significant method using *N*-(*p*-nitrobenzyl)-*N*-propylamine (NNNP) to derivatize isocyanates. Quantification of the derivatives is carried out by reversed-phase high-performance liquid chromatography with ultraviolet (UV) detection. This method has been applied almost exclusively to the determination of monomeric isocyanates. The NNNP reagent, however, can only be purchased in the form of a salt owing to the instability of the amine itself. An additional disadvantage of this method is that it lacks the sensitivity of detection often required for the determination of extremely low levels of airborne isocyanates. Despite the drawbacks of this method, viewed by present day standards, many laboratories are still accustomed to applying it as the ultimate regulatory method for monitoring isocyanates in the workplace.

Subsequently, several HPLC methods have been published, with the emphasis on improving the sensitivity of detection, but not for determining polymeric isocyanates. The most important methods are those of Levin *et al.*,[2] which uses the fluorescent reagent 1-naphthalenemethylamine to derivatize isocyanates, and Goldberg *et al.*,[3] which uses 1-(2-pyridyl)-piperazine (PP) as the derivatizing agent to enhance the UV chromophore for detection. Interestingly, the latter workers have also used PP to derivatize a number of polymeric isocyanates.[4] However, procedures for quantifying the derivatized polymeric components have not been addressed.

In order to satisfy the increasing demand for monitoring both monomeric and polymeric isocyanates, Bagon *et al.*[5] have devised a dual-detection HPLC method using 1-(2-methoxyphenyl)piperazine (MPP) as the derivatizing agent. Polymeric components of a particular isocyanate are identified by the ratio of the responses from both the amperometric oxidation and UV detectors. Each of these components is quantified to the corresponding amount of –NCO by calibration with the parent monomer. However, this method has some major drawbacks; hence the reliability of the analysis is often open to question. Establishing a reliable response ratio from amperometric oxidation and UV detection is, in general, not an easy task. The reason for this is that the former is regarded as a detector with 'low stability' and the latter lacks both sensitivity and selectivity. This method, regardless of its drawbacks, has, nevertheless, provided an unprecedented approach to the determination of various polymeric isocyanates together with their monomers. In fact, in the UK, this method has been designated as the official regulatory method for monitoring total isocyanates exposure in workplaces apparently because no other methods are available.

Recently, Wu *et al.*[6] developed a method for determining total isocyanates with considerable reliability. This method uses tryptamine to react with all the isocyanates and the derivatives are quantified by a dual-detection system consisting of a fluorescence and an amperometric oxidation detector. As both detectors are very sensitive and selective, very low

* For Part 3 of this series see reference 6.

† Present address: Concord Scientific Corporation, 2 Tippett Road, Toronto, Ontario M3H 2V2, Canada.

detection levels can be attained with minimum detection interferences. The uniqueness of this method is that all the tryptamine-derivatized isocyanates can be quantified by calibration against a single, pure standard such as tryptamine-derivatized toluene diisocyanate. The amount of isocyanato groups (–NCO) for individual components of the sample can be quantified without necessarily identifying the appropriate type of isocyanate. The theoretical basis of this method is that the π-orbitals of the indolyl moiety of tryptamine, responsible for the fluorescence and amperometric oxidation activities, are unperturbed before and after derivatization.[7,8] In the present study this approach has been generalized to analysis with chromatographic techniques as the isolation of a selected π-system in a derivative for specific detection.

The evaluation of analytical methods for determining isocyanates would be more conclusive if the derivatization reaction rates could be compared. When collecting airborne isocyanates in solutions of various derivatizing agents, the derivatization reaction rates would affect the over-all efficiency of the methods. This is particularly important for sampling airborne isocyanates using a solid absorbent coated with a derivatizing agent, which would become indispensable for personal sampling in the workplace. A relatively slow reaction involving a reagent coated on a solid absorbent would be even slower than the same reaction occurring in solution. Unfortunately, information regarding competitive derivatization rates has not appeared in any of the published methods, which clearly indicates that some research in this area is necessary.

Derivatization of an isocyanate (RNCO) with an amine (Am) is a second-order chemical reaction and the reaction rate can be written as follows:

$$-\mathrm{d[RNCO]}/\mathrm{d}t = k[\mathrm{RNCO}]\,[\mathrm{Am}] \quad (1)$$

where k is the rate constant. Assuming the initial concentrations of amine and isocyanate are a and i, respectively, and the concentration of the derivative formed after a given time, t, is x, the reaction rate can be expressed as

$$\mathrm{d}x/\mathrm{d}t = k(a - x)(i - x) \quad (2)$$

However, if a second amine is also involved in the derivatization to compete with the first amine, the rate kinetics for both reactions are given by equations (3) and (4), respectively

$$\mathrm{d}x/\mathrm{d}t = k(a - x)\,(i - x - y) \quad (3)$$

$$\mathrm{d}y/\mathrm{d}t = k'(b - y)\,(i - x - y) \quad (4)$$

where b is the initial concentration of the second amine, y the concentration of the corresponding amine-derivatized isocyanate and k' the corresponding rate constant.

As both reactions proceed concurrently at different rates, the combined rate kinetics can be expressed by equation (5) with the time variable, t, being considered as a constant

$$\left(\frac{\mathrm{d}x}{\mathrm{d}y}\right) = (k/k')\frac{(a-x)}{(b-y)} \quad (5)$$

By appropriately integrating equation (5) as shown in equation (6), the relative rate (k/k') for the competitive derivatization reaction[9,10] is finally obtained [equation (7)]

$$\int \frac{\mathrm{d}x}{(a-x)} = (k/k') \int \frac{\mathrm{d}y}{(b-y)} \quad (6)$$

$$k/k' = \log[(a - x)/a]/\log[(b - y)/b] \quad (7)$$

Experimentally, it is unnecessary to conduct the rate study on industrially used diisocyanates because multiple derivatives would be produced due to random attack on the isocyanato groups by the amines thus complicating the investigation. As only the relative derivatization rate with the isocyanato functional group was of interest, a relatively pure and stable mono-isocyanate, *i.e.*, phenyl isocyanate, was preferred for this work.

Experimental and Results

Chemicals and Apparatus

Tryptamine and *N*-ω-methyl tryptamine (NMTP) were purchased from Sigma (St. Louis, MO, USA). Phenyl isocyanate, 1-(2-pyridyl)piperazine (PP) and 1-(2-methoxyphenyl)piperazine (MPP) were from Aldrich (Milwaukee, WI, USA). *N*-4-Nitrobenzyl-*N*-propylamine (NNNP) was a Regis product, obtained through Caledon Laboratories (Georgetown, Ontario, Canada) as the hydrochloride salt. Amine solutions of NNNP were freshly prepared immediately before use. Final dilutions of these solutions were made in acetonitrile. All solvents were of glass-distilled quality and were obtained from Caledon Laboratories. Water was doubly distilled after treatment with $KMnO_4$.

The HPLC system consisted of a Beckman 112 solvent delivery module, a Scientific System LP-21 pulse damper and a Shoeffel 970 fluorescence detector. The excitation wavelength was set at 275 nm and the emission wavelength was filtered at 320 nm. A 5 μm Hypersil-ODS column (25 cm × 4.6 mm i.d.) from Chromatography Science was used. The mobile phase was acetonitrile–water (50 + 50). The flow-rate was set at 0.8 ml min^{-1}. The infrared (IR) spectrometer was a Beckman Model 4240 instrument and the spectrum of tryptamine-derivatized phenyl isocyanate (KBr disc) was obtained at a scan rate of 600 cm^{-1} min^{-1}.

Preparation of the Tryptamine Derivative of Phenyl Isocyanate

A solution of phenyl isocyanate (1 g) in 10 ml of acetonitrile was added dropwise, with stirring, to 100 ml of an acetonitrile solution containing 0.5 g of tryptamine. The solution was allowed to stand for 1 h and the derivative was recrystallized from acetonitrile. The urea derivative [m.p. 196 °C (decomp.)] was also identified by the IR band at about 1650 cm^{-1}.

Study of Competitive Reaction Rates by Derivatizing Phenyl Isocyanate With Amines

There were several options for conducting the experiments for this study. The competitive reaction rate is a relative rate between two competing amine reagents reacting concurrently with the isocyanate. Therefore, only two derivatizing agents were used for each specific rate study. To a set of 50 ml calibrated flasks, each containing a mixture of amines, 5 ml aliquots of acetonitrile solutions of phenyl isocyanate were added individually. For the sake of simplicity, the amount of the individual amines in each flask was kept constant while the amount of phenyl isocyanate added was varied. The contents of the flasks were allowed to react for 1 h before diluting to volume with acetonitrile. All solutions were diluted with acetonitrile to the appropriate concentrations for HPLC evaluation. A small amount of acetic anhydride (in acetonitrile), sufficient to remove the excess of amine, was also added to each flask before HPLC analysis. The data for the competitive derivatization rates for tryptamine and MPP, tryptamine and PP, tryptamine and NNNP, and NMTP and MPP are listed in Tables 1–4. A typical HPLC trace of a solution of phenyl isocyanate containing tryptamine and MPP is shown in Fig. 1.

Study of Competitive Reaction Rates by Derivatizing Phenyl Isocyanate With Tryptamine and Water

As water is known to react with isocyanates and exists in the atmosphere, it is important to have some knowledge about its relative reaction rate in comparison with those of the amines. It is also known that isocyanates react much more slowly with water than with amines. Therefore, the amount of water used for the experiments has to be fairly large in order to

Table 1 Relative rate for derivatizing phenyl isocyanate with tryptamine and MPP

Tryptamine (a)/μmol	MPP (b)/μmol	Phenyl isocyanate (i)/μmol	PI-TP* (x)/μmol	PI-MPP† (y)/μmol	Log[$(a-x)/a$]	Log[$(b-y)/b$]
1.563	1.115	1.050	0.599	0.451	−0.21006	−0.22485
1.563	1.115	0.840	0.470	0.370	−0.15534	−0.17512
1.563	1.115	0.630	0.349	0.281	−0.10974	−0.12611
1.563	1.115	0.420	0.227	0.193	−0.06825	−0.08240
1.563	1.115	0.210	0.105	0.105	−0.03029	−0.04282

$k/k' = 0.979$‡

* Tryptamine derivative of phenyl isocyanate.
† MPP derivative of phenyl isocyanate, obtained with the assumption that $i = x + y$.
‡ Obtained from the slope of the log[$(a-x)/a$] *versus* log[$(b-y)/b$] plot.

Table 2 Relative rate for derivatizing phenyl isocyanate with tryptamine and PP

Tryptamine (a)/μmol	PP (b)/μmol	Phenyl isocyanate (i)/μmol	PI-TP* (x)/μmol	PI-PP† (y)/μmol	Log[$(a-x)/a$]	Log[$(b-y)/b$]
1.563	1.154	1.050	0.724	0.326	−0.27020	−0.14418
1.563	1.154	0.840	0.569	0.271	−0.19657	−0.11625
1.563	1.154	0.630	0.407	0.223	−0.13100	−0.09326
1.563	1.154	0.420	0.280	0.140	−0.08573	−0.05617
1.563	1.154	0.210	0.128	0.082	−0.03711	−0.03201

$k/k' = 2.011$

* Tryptamine derivative of phenyl isocyanate.
† PP derivative of phenyl isocyanate, obtained with the assumption that $i = x + y$.

Table 3 Relative rate for derivatizing phenyl isocyanate with tryptamine and NNNP

Tryptamine (a)/μmol	NNNP (b)/μmol	Phenyl isocyanate (i)/μmol	PI-TP* (x)/μmol	PI-NNNP† (y)/μmol	Log[$(a-x)/a$]	Log[$(b-y)/b$]
1.563	15.03	1.050	0.716	0.334	−0.26608	−0.00976
1.563	15.03	0.840	0.560	0.280	−0.19266	−0.00817
1.563	15.03	0.630	0.449	0.181	−0.14707	−0.00526
1.563	15.03	0.420	0.320	0.100	−0.09949	−0.00290
1.563	15.03	0.210	0.163	0.047	−0.04783	−0.00136

$k/k' = 23.63$

* Tryptamine derivative of phenyl isocyanate.
† NNNP derivative of phenyl isocyanate, obtained with the assumption that $i = x + y$.

Table 4 Relative rate for derivatizing phenyl isocyanate with NMTP and MPP

NMTP (a)/μmol	MPP (b)/μmol	Phenyl isocyanate (i)/μmol	PI-NMTP* (y)/μmol	PI-MPP† (x)/μmol	Log[$(a-x)/a$]	Log[$(b-y)/b$]
1.437	0‡	1.050‡	1.050	0	—	—
1.437	2.800	1.050	0.640	0.410	−0.25600	−0.06876
1.437	2.800	0.840	0.512	0.328	−0.19132	−0.05411
1.437	2.800	0.630	0.396	0.234	−0.14001	−0.03790
1.437	2.800	0.420	0.259	0.161	−0.08631	−0.02572
1.437	2.800	0.210	0.134	0.076	−0.04251	−0.01195

$k/k = 3.744$

* NMTP derivative of phenyl isocyanate.
† MPP derivative of phenyl isocyanate.
‡ Derivatization without addition of MPP to the reaction mixture, used for calibrating the amount of PI-NMTP produced in the set.

differentiate the rate from that of the amine. The competitive reaction rate for tryptamine and water is shown in Table 5.

Summary of Competitive Derivatization Rates and Over-all Comparison of the Methods

The competitive derivatization rates obtained above would be more meaningful if relative rates could be assigned to all the amines investigated. Table 6 shows the relative rate constants listed in descending order; the rate for MPP was arbitrarily assigned a value of 100. An over-all comparison of the various HPLC methods is presented in Table 7. Experimental data reflecting the relative rate constant of k/k' are plotted in Fig. 2.

Discussion

Although many HPLC methods for determining airborne isocyanates have been published, no study of the competitive derivatization rate of amines has been reported. This work has shown that the relative rates for the derivatization of phenyl isocyanate by MPP and tryptamine are almost identical and

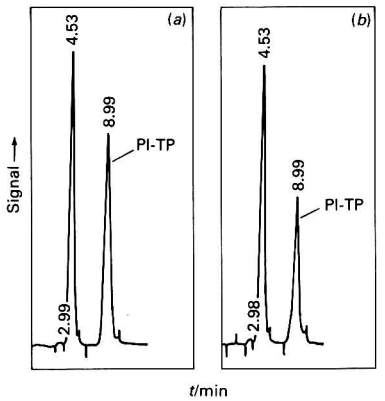

Fig. 1 Chromatogram of competitive derivatization of phenylisocyanate with tryptamine and MPP. (*a*) Solution containing 1.05, 1.56 and 1.12 μmol of phenyl isocyanate, tryptamine and MPP, respectively, before dilution; and (*b*) solution containing 0.63, 1.56 and 1.12 μmol of phenyl isocyanate, tryptamine and MPP, respectively, before dilution

Table 5 Relative rate for derivatizing phenyl isocyanate with tryptamine and water

Tryptamine (*a*)/μmol	H_2O (*b*)/μmol	Phenyl isocyanate (*i*)/μmol	PI-TP* (*x*)/μmol	PI-H_2O† (*y*)/μmol
1.563	1.39×10^4	1.050	A‡	B§
1.563	1.39×10^4	0.840	A	B
1.563	1.39×10^4	0.630	A	B
1.563	1.39×10^4	0.420	A	B
1.563	1.39×10^4	0.210	A	B

$\text{Log}[(a - x)/a] \geqslant -0.4$¶ $\log[(b - y)/b] \geqslant -3 \times 10^{-6}$¶; $k/k' \geqslant 1 \times 10^5$

* Tryptamine derivative of phenyl isocyanate.
† Derivative of phenyl isocyanate with water.
‡ Approximately 100% yield.
§ Approximately zero.
¶ Assuming the HPLC technique fails to differentiate up to a 5% yield of PI-H_2O.

Table 6 Relative reaction rates for derivatizing phenyl isocyanate with various amines

Derivatizing agent	Relative rate constant, *k*
NMTP	374
MPP	100
Tryptamine	98
PP	49
NNNP	4
Water	$\leqslant 1 \times 10^{-5}$

are two and 25 times faster than those for PP and NNNP, respectively. Experiments conducted earlier[6] by using a Test Atmosphere Generation System indicated that the recoveries of airborne toluene diisocyanate in the respective impinger solutions of MPP and tryptamine were indeed very competitive. As can be seen from Table 6, NMTP is the most reactive of the amines investigated, being about four times more reactive than MPP. However, a detailed study of the application of NMTP to the determination of isocyanates has not been conducted because of the high cost of this amine. Further, the amines used for derivatization are always present in a large excess, which is unlikely to affect the over-all yield of the derivatives caused by reactions with slightly slower rates.

On the other hand, a very much slower derivatization rate was obtained with NNNP. The main concern is that the NNNP method is still being used widely for quantifying airborne isocyanates in workplaces and may not be able to reflect the true exposure. The results indicate that a much smaller amount of tryptamine or MPP is required than of NNNP to efficiently derivatize an equivalent amount of isocyanate. This would also be of benefit in the HPLC system, because less material would need to be loaded on to the column if tryptamine were to be used for derivatization.

It has been observed in previous experimental work[6] that the derivatization of isocyanates with NNNP is less efficient than with tryptamine or MPP, as reflected by the consistently lower results. It was suspected that the solvated water might have been partly responsible as NNNP has to be extracted from water before use. However, the relative derivatization rate study indicates that this is not the situation as it would have affected the rate of the other competing amines

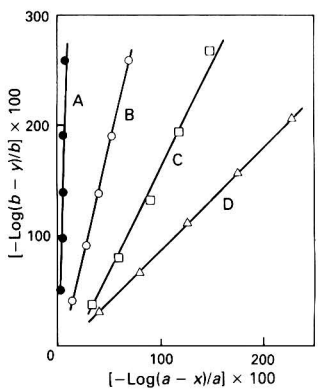

Fig. 2 Plot of second-order reaction kinetics for competitive rates for derivatizing phenyl isocyanate with amines. A, Tryptamine and NNNP; B, NMTP and MPP; C, tryptamine and PP; and D, tryptamine and MPP

Table 7 Comparison of representative HPLC methods for determining isocyanate

Derivatizing agent	Type of detection	No. of specific detection modes	Availability for determining polyisocyanate	Availability for quantifying NCO* of unidentified isocyanate
NNNP	Ultraviolet	0†	No	No
NAMA‡	Fluorescence	1	No	No
PP	Ultraviolet	0	No	No
MPP	Ultraviolet and amperometric	1	Yes	No
Tryptamine§	Fluorescence and amperometric	2	Yes	Yes

* Reactive isocyanato functional group.
† UV absorption common for organic chemicals, regarded as a non-specific detection mode.
‡ 1-Naphthalenemethylamine.
§ Employed in the proposed method.

simultaneously if the derivatization rate for water were competitive. In fact, the relative rate for water to compete with amine for the derivatization of isocyanate is negligible. For instance, our work shows that the derivatization of phenyl isocyanate by tryptamine is at least 1×10^5 times faster than by water.

A slower derivatization rate for NNNP could also be caused by the instability of the amine form of the reagent. However, as the NNNP solutions for derivatization were always prepared freshly before use, the instability of the amine form of the reagent does not appear to be the reason for the slower rate. Therefore, it is more probable that the nature of the reaction kinetics is responsible, but the exact cause is still unknown.

It should be noted that in all the experiments conducted on competitive derivatization rates the actual yield of tryptamine-derivatized phenyl isocyanate was used as the basis for all the necessary calculations. This was because of the highly fluorescent nature of this derivative. The analytical interferences on high-performance liquid chromatography would be minimized by fluorescence detection.

One of the most important reasons for performing the relative rate study was to provide the best possible assessment in developing personal sampling techniques for isocyanate exposure in the workplace. The preferred air collection media for personal sampling at work sites are solid absorbents instead of impinger solutions. For a reagent-coated solid absorbent, this implies that the derivatization would occur in the unfavourable solid phase of the reagent. Amines with faster derivatization rates would therefore be more suitable for coating the solid absorbents. Considering all the factors such as the sensitivity and selectivity of detection of the methods discussed for isocyanates, the use of tryptamine is recommended for future studies on sampling airborne isocyanates using solid absorbents. By comparing all the features of the HPLC methods shown in Table 7, the proposed method is the most suitable for practical applications. Moreover, the proposed concept of the isolation of a selected π-system in a derivative for specific detection has promoted a new area for exploration in analysis with chromatographic techniques.

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NOTE—References 6, 7 and 8 are to Parts 3, 1 and 2 of this series, respectively.

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Determination of Titanium(IV) in River Water by Ion-pair Reversed-phase High-performance Liquid Chromatography With 4,4′-Diantipyrylmethane

Nobuo Uehara, Kazuhiro Morimoto and Yoshio Shijo
Department of Applied Chemistry, Faculty of Engineering, Utsunomiya University, Ishii-cho, Utsunomiya 321, Japan

An ion-pair reversed-phase high-performance liquid chromatographic method for the selective determination of Ti^{IV} with 4,4′-diantipyrylmethane (DAM) is described. The Ti^{IV}–DAM complex was separated on an ODS column using acetonitrile–water (30 + 70) containing 1×10^{-4} mol dm^{-3} DAM, 0.01 mol dm^{-3} ammonium iodide and 0.02 mol dm^{-3} chloroacetate (pH 2.25). The detection limit for Ti^{IV} with the proposed method is 1.8 μg l^{-1}. Titanium(IV) in river water can be determined without the interference of foreign ions after pre-concentration.

Keywords: *Titanium(IV); 4,4′-diantipyrylmethane; ion-pair reversed-phase high-performance liquid chromatography; river water; pre-concentration*

4,4′-Diantipyrylmethane (DAM) is the most popular reagent for the spectrophotometric determination of Ti^{IV} because of its selectivity.[1,2] The reagent has been applied to the determination of Ti^{IV} in cements,[3] ferro-niobium,[4] silicate rocks[5] and plant materials.[6] However, it is difficult to use DAM for the determination of low levels of Ti^{IV} because the molar absorptivity of the Ti^{IV}–DAM complex is not very large.

Recently, more sensitive spectrophotometric reagents for Ti^{IV} have been reported. Inoue *et al.*[7] described an extraction spectrophotometric method for Ti^{IV} with *N*-*p*-octyloxybenzoyl-*N*-phenylhydroxylamine and phenylfluorone. Marini *et al.*[8] used 2-(5-chloro-2-pyridylazo)-5-dimethylaminophenol and hydrogen peroxide for the determination of Ti^{IV}. Gregorowicz *et al.*[9] determined Ti^{IV} in steel with Eriochrome Azurol G. However, these reagents are not as selective as DAM.

Most of the work combining high-performance liquid chromatography (HPLC) with spectrophotometric detection has been aimed at the development of sensitive and selective analytical methods for determining metal ions.[10–14] However, there are relatively few reports on the application of HPLC to Ti^{IV}. Main and Fritz[15] determined Ti^{IV} by HPLC with bis(quaternary ammonium hydrazones) of 2,6-diacetylpyridine. The detection limit of Ti^{IV} was 8×10^{-8} mol dm^{-3} when 2,6-diacetylpyridinebis(*N*-methylenepyridinohydrazone) was used as the chelating agent. However, the molar absorptivities of these Ti^{IV}–bishydrazone complexes are smaller than that of the Ti^{IV}–DAM complex. Therefore, DAM appears to be more sensitive than these bishydrazones for the determination of Ti^{IV} by HPLC.

This paper describes the use of DAM as a pre-column chelating agent for the sensitive determination of Ti^{IV} by ion-pair reversed-phase HPLC. The proposed method was applied to the determination of Ti^{IV} in river and rain water after pre-concentration using a simple and rapid evaporation of the sample solution.

Experimental

Apparatus

The liquid chromatographic system consisted of a Nihon Seimitsu Kagaku (Tokyo, Japan) NSP-800-3U pump, a Japan Spectroscopic (Tokyo, Japan) hexane dumper, a Japan Spectroscopic 870-UV spectrophotometric detector, a Rheodyne 7125 loop injector with a 20 μl sample loop and a Shimadzu (Kyoto, Japan) U-125 MU recorder. A Yamamura Kagaku (Tokyo, Japan) YMC R-ODS-5 column (250 × 4.6 mm i.d.) was used for all experiments. The simple laboratory-built evaporator shown in Fig. 1 was used for pre-concentration; it was of the same type as that described in a previous paper.[16]

Reagents

Distilled, de-ionized water was purified further by means of a Millipore Milli-Q system. Analytical-reagent grade acetonitrile and methanol were filtered through a Millipore filter (0.45 μm) after distillation. The DAM was obtained from Dojindo (Kumamoto, Japan). The Ti^{IV} standard solution (1000 mg l^{-1}) for atomic absorption spectrometry was obtained from Wako Pure Chemicals (Osaka, Japan). All other chemicals were of guaranteed-reagent grade.

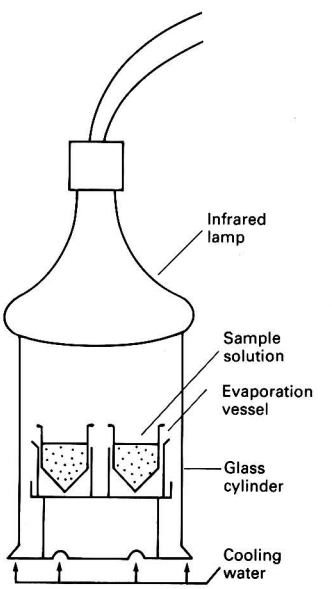

Fig. 1 Schematic diagram of the laboratory-built evaporator

Eluent and Chromatographic Conditions

The eluent used was acetonitrile–water (30 + 70) containing 1 × 10^{-4} mol dm^{-3} DAM, 0.01 mol dm^{-3} ammonium iodide and 0.02 mol dm^{-3} chloroacetate buffer (pH 2.25). The pH of the eluent was adjusted before addition of acetonitrile. The flow-rate of the eluent was 1.0 ml min^{-1}. The eluate was monitored at 390 nm.

Procedure

Ten millilitres of 60% nitric acid were added to 1.0 l of sampled river water and the solution was filtered through a Millipore filter (0.45 μm). To 10 ml of river water sample, 1.0 ml of 60% nitric acid and 0.1 ml of 3 mol dm^{-3} sulphuric acid were added. The solution was evaporated to dryness with the laboratory-built evaporator. The residue was dissolved in 0.4 ml of 2% DAM solution (0.5 mol dm^{-3} HCl) by shaking for 2 min and the solution was allowed to stand for 20 min. To a 0.3 ml aliquot of the solution, 0.1 ml of 4 mol dm^{-3} sodium chloroacetate solution and 0.1 ml of methanol were added. An aliquot of the solution (20 μl) was injected on to the HPLC column.

Results and Discussion

Derivatization Studies

The Ti^{IV}–DAM complex was formed within 15 min in an acidic medium of 0.5 mol dm^{-3} HCl. However, direct injection of 0.5 mol dm^{-3} HCl solution into the chromatograph is inadequate. Therefore, the pH of the solution was adjusted to 2.25, which is a suitable pH for the ODS column. The solution must be injected into the chromatograph immediately after adjustment of the pH because the complex decomposes gradually at pH 2.25.

HPLC Studies

Fig. 2 shows a typical chromatogram of the Ti^{IV}–DAM complex. All the other metal ions studied, *viz.*, Al^{III}, Cr^{VI}, Cu^{II}, Fe^{III}, In^{III}, Ni^{II}, Mn^{II}, V^{V} and Zr^{IV}, gave no peaks on the chromatogram under the conditions used.

Iodide ion, which has been used for the ion-pair extraction of Ti^{IV} with DAM was examined as a counter anion for HPLC of the Ti^{IV}–DAM complex, as the complex has a positive charge.[2] Fig. 3 shows the effect of ammonium iodide concentration on the mass distribution ratio of the Ti^{IV}–DAM complex. As the ammonium iodide concentration increased the mass distribution ratio of the complex also increased, which indicated that ammonium iodide acted as an ion-pair reagent. The peak of the complex was well resolved from the solvent front above an ammonium iodide concentration of 1 × 10^{-2} mol dm^{-3}.

Acetonitrile was a good organic modifier for the retention of the Ti^{IV}–DAM complex. The mass distribution ratio of the complex decreased as the content of acetonitrile in the mobile phase increased, as shown in Fig. 4. An acetonitrile content of 30% v/v was selected in order to obtain a suitable chromatogram for the detection of Ti^{IV}.

The retention behaviour of the Ti^{IV}–DAM complex was investigated for various pH values of the eluent. As the pH of the eluent increased, the mass distribution ratio of the complex also increased and the peak broadened, as shown in Fig. 5 Such behaviour appears to be due to hydrolysis of the complex and interaction between the residual silanol groups of the ODS column and the complex.

The DAM concentration of the eluent also affected the retention of the Ti^{IV}–DAM complex. The peak height of the complex increased and the mass distribution ratio decreased with an increase in the DAM concentration. The addition of DAM to the eluent prevented the decomposition of the complex and allowed its rapid elution.

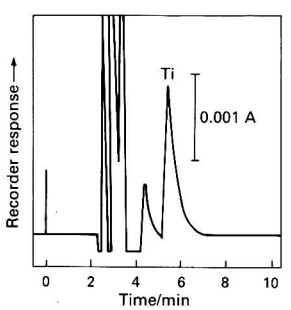

Fig. 2 Chromatogram of the Ti^{IV}–DAM complex. Column, YMC R-ODS-5 (250 × 4.6 mm i.d.); eluent, acetonitrile–water (30 + 70) containing 1 × 10^{-4} mol dm^{-3} DAM, 0.01 mol dm^{-3} ammonium iodide and 0.02 mol dm^{-3} chloroacetate (pH 2.25). Ti^{IV}, 0.1 mg l^{-1}; flow-rate, 1.0 ml min^{-1}; detection wavelength, 390 nm; detector sensitivity, 0.01 a.u.f.s.; and injection volume, 20 μl

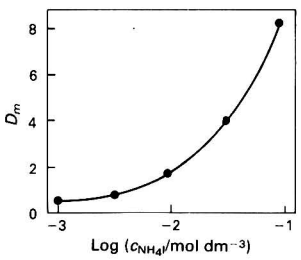

Fig. 3 Effect of concentration of ammonium iodide on the mass distribution ratio. For chromatographic conditions see Fig. 2

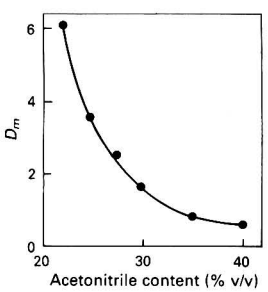

Fig. 4 Effect of acetonitrile content on the mass distribution ratio. For chromatographic conditions see Fig. 2

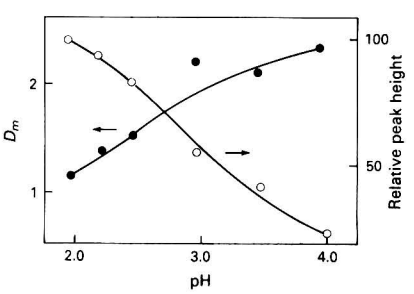

Fig. 5 Effect of pH of the eluent on the mass distribution ratio and peak height. For chromatographic conditions see Fig. 2

Table 1 Effect of foreign ions on the determination of Ti^{IV}. Concentration of Ti^{IV} added, 0.1 mg l^{-1}

Foreign ion	Concentration added/ mg l^{-1}	Error* (%)
Al^{III}	100	+1.3
Cu^{II}	100	+2.5
Ni^{II}	100	+2.2
V^{V}	100	−3.1
Fe^{III}	50	+2.9
Fe^{II}†	50	−0.9
Co^{II}	20	+1.3
Cr^{III}	20	+0.2
In^{III}	20	+0.9
Mn^{II}	20	+2.9
Mo^{VI}	20	−3.3
Pb^{II}	20	−3.6
Sn^{II}	20	−0.9
U^{VI}	20	−3.5
Zn^{II}	20	−1.2
Zr^{IV}	20	+1.8
Bi^{III}	0.5	−4.9

* A plus sign indicates a positive error, a minus sign indicates a negative error.

† Ascorbic acid was added as a masking agent.

Calibration Graph and Detection Limit

The calibration graph of peak height *versus* Ti^{IV} concentration was a straight line in the concentration range 0.01–0.3 mg l^{-1} Ti^{IV} with 20 μl injections. The detection limit for Ti^{IV} was 1.8 μg l^{-1} at a signal to noise ratio of 3. The reproducibility of the method is 3.9%, expressed as the relative standard deviation for ten replicate analyses of solutions containing 0.1 mg l^{-1} Ti^{IV}.

Interferences

The effect of foreign ions on the determination of Ti^{IV} with HPLC was studied. Table 1 shows the results. None of the metal ions tested interfered with the determination of Ti^{IV} at concentrations commonly found in river water.[17] Only Bi^{III} interfered seriously with the determination of Ti^{IV}. However, the interference from Bi^{III} would not be a problem in practice because the concentration of Bi^{III} in river water is very low. The presence of a large amount of Fe^{III} gave a broad peak which interfered with the determination of Ti^{IV}. However, the Fe^{III} peak disappeared completely on adding ascorbic acid to the sample solution, because Fe^{II} does not react with DAM.

Applications

The simple evaporation system used in a previous paper[16] was employed for pre-concentration because the HPLC method was not sufficiently sensitive to determine Ti^{IV} in river water directly. The 15-fold enrichment achieved by pre-concentration enabled Ti^{IV} to be determined in river water by HPLC.

Table 2 Results for the determination of Ti^{IV} in river and rain water

Sample	Concentration of Ti^{IV}*/ μg l^{-1}
Watarase river	1.85 ± 0.10 (n = 4)
Kinu river	0.76 ± 0.09 (n = 3)
Rain water†	2.66 ± 0.27 (n = 3)

* Mean ± standard deviation.

† Sampled at Utsunomiya University.

The water sampled at the Watarase and Kinu rivers (both in Tochigi, Japan) and also rain water were analysed by the standard additions method. To 10 ml of the water sample, 0–80 μl of 1 mg l^{-1} Ti^{IV} standard solution were added. The results are summarized in Table 2. The determination of Ti^{IV} with the proposed method is sensitive and free from interferences. The technique can be used for the determination of μg l^{-1} levels of Ti^{IV} in the presence of large amounts of foreign ions after pre-concentration.

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Simultaneous Determination of Silver and Copper by Flame Atomic Absorption Spectrometry With Alternate Irradiation by Two Hollow Cathode Lamps

Osamu Sakurada, Shunitz Tanaka and Mitsuhiko Taga*
Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060, Japan
Teiji Kakizaki
Laboratory of Chemistry, Hokkaido University of Education at Iwamizawa, Iwamizawa 068, Japan

The simultaneous flame atomic absorption spectrometric determination of silver and copper is described. The resonance lines of both silver (328.07 nm) and copper (327.40 nm) were introduced within the same bandpass of the monochromator. Therefore, it was possible to measure the absorption signals of silver and copper simultaneously, when the silver and copper hollow cathode lamps were alternately irradiated. Acquisition of the absorption data was synchronized with the irradiations using a computer. For the introduction of the sample into the flame, a discrete nebulization method was investigated in order to minimize the sample volume required and the analysis time.

Keywords: *Simultaneous atomic absorption spectrometry; flame atomization; discrete nebulization; silver; copper*

Atomic absorption spectrometry (AAS) is an excellent analytical method in terms of selectivity and sensitivity. The selectivity of AAS is attributed to the use of an analyte-specific resonance line being emitted from the radiation source, a hollow cathode lamp (HCL). However, this indicates that for simultaneous multi-element determination by AAS, it is necessary to prepare multi-channel radiation sources and optical systems for each element. Simultaneous multi-element determination by AAS has been attempted by many workers[1,2] and the instrumentation is now commercially available.[3] Harnly *et al.*[4] proposed a multi-element AAS system consisting of a continuum light source such as an Xe arc lamp, a high-resolution échelle polychromator and a computerized high-speed data system. Nakamura and Kubota[5] also reported an instrument for multi-element AAS consisting of a specific multi-element HCL, a single detection channel with one photomultiplier tube (PMT) and a time-divided high-speed data acquisition system. However, these instruments are very complicated and expensive to use for routine analyses.

On the other hand, the spectral interferences that arise in AAS from the overlap of the absorption lines of the analytes consequently lead to large experimental errors.[6] Therefore, a graphite furnace AAS method has been developed for the simple simultaneous determination of copper and silver by the use of their neighbouring resonance lines.[7] This method is based on the difference in the appearance temperatures of the analytes in the graphite furnace. Silver is atomized at a lower temperature than copper. The mixed radiation of the resonance lines of silver (328.07 nm) and copper (327.40 nm) is introduced into the graphite furnace atomizer simultaneously. By measurement of the peak heights of silver and copper on a chromatographic absorption–time profile, the simultaneous determination of silver and copper is achieved.

In this work, the simultaneous determination of silver and copper by flame AAS was investigated using the neighbouring resonance lines. In flame AAS, the difference in the appearance temperature, used in the graphite furnace method, cannot be expected. Therefore, alternate irradiation of the sample with the silver and copper HCLs was attempted. By utilizing a computerized high-speed data acquisition system to collect the absorption signals alternately, the absorption signal for each element can be obtained with one measurement. A discrete nebulization method[8] for the introduction of the sample into the flame was also investigated in order to minimize the sample volume required and the analysis time. The proposed simultaneous method is simpler and less expensive than other methods. The method was applied to the determination of silver and copper in commercially available silver brazing filler metals used for welding.

Experimental

Apparatus

A Hitachi Model 170-50 atomic absorption spectrometer was used, with a pre-mix burner for the air–acetylene flame. In order to introduce the mixed radiation of the resonance lines of silver and copper into the flame, the optical system for deuterium background correction was used as the secondary radiation source. As shown in Fig. 1, the silver and copper HCLs were placed in the positions of the HCL and the deuterium lamp (in the normal mode), respectively. The light beams from the silver and copper HCLs were pulsed at 50 Hz alternately, as shown in Fig. 2. The light beams were made spatially coincident using the half-mirror and subsequently passed through the flame atomizer into the spectrometer. The

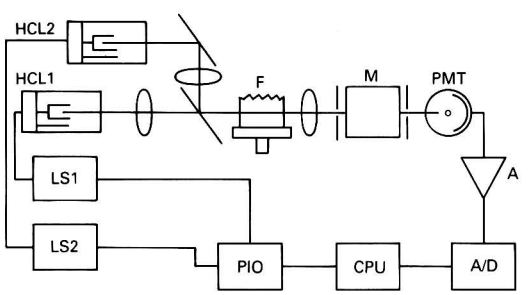

Fig. 1 Schematic diagram of the proposed system. HCL1 and HCL2, Hollow-cathode lamps for silver and copper; LS1 and LS2, lamp power supply; F, flame; M, monochromator; PMT, photomultiplier tube; A, logarithmic amplifier; A/D, analogue to digital converter; CPU, computer; and PIO, parallel input–output module

* To whom correspondence should be addressed.

spectral bandpass of the monochromator was set at 1.1 nm to detect both the silver and copper resonance lines. The voltage analogues of the logarithmic converted circuit of the PMT (Hamamatsu R456) were converted into digital data by a 12 bit analogue to digital converter (A/D) [Contec AD12-16A(98)]. Timing of the A/D cycle was obtained using software-controlled radiation pulses from the HCLs, with a parallel input–output module [Contec (PIO-48W(98)]. The vertical scale in Figs. 3–6 is shown in volts (V) and is comparable to absorbance. The peak area integrated the peak height with the time (s). A Model PC-9801UV2 personal computer (Nippon Electric) was used for data acquisition. Software to perform the data acquisition and analysis was written in BASIC and incorporated a Machine Code subroutine for rapid data acquisition.[9]

The discrete nebulization method was used to introduce the sample solution into the flame. The device for this was assembled from a miniature polytetrafluoroethylene funnel connected to the nebulizer capillary. The sample solution was injected into the funnel and nebulized in the flame. The computer-controlled data acquisition was triggered by the detection of the sample passing through the nebulizer capillary by means of a photocoupler (Omron Model EE-SV3) registering the change in the light transmission. The absorbance–time signals were digitally stored in the memory of the computer. The stored time, namely, the time required for the sample volume, was pre-set, normally 4 s for a 100 μl injection. The system for the detection of the injection was a modification of the system proposed by Goto *et al.*[10] Micropipettes (Eppendorf 4700 and 4710) were used for sample injection.

Reagents

Standard solutions of silver and copper were prepared by dissolving silver nitrate (analytical-reagent grade, Wako Pure Chemicals) in 1 mol dm^{-3} HNO_3 and by dissolving copper metal (99.999% pure, Mitsuwa Pure Chemicals) in concentrated nitric acid and diluting with water to a final concentration of 1 mol dm^{-3} HNO_3. Other reagents were of analytical-reagent grade. Doubly distilled water was used throughout.

Results and Discussion

Flame Conditions

For the simultaneous atomic absorption spectrometric determination of silver and copper, it is important to determine the optimum flame conditions for the two elements. In order to do this, response surface graphs[11,12] were constructed from the results of the atomic absorption spectrometric determination of silver and copper plotted as burner height *versus* acetylene pressure. The burner height values were indicated by the scale reading on the AAS apparatus. In the distribution pattern of silver and copper shown in Fig. 3, the maximum absorbance peaks are located at a flame height of approximately 2.5 and an acetylene pressure of 0.25 kg cm^{-2}. In subsequent experiments, the following experimental conditions were chosen as the optimum flame conditions; air flow pressure, 1.6 kg cm^{-2}; acetylene flow pressure, 0.25 kg cm^{-2}; and burner height, 2.5 (arbitrary units).

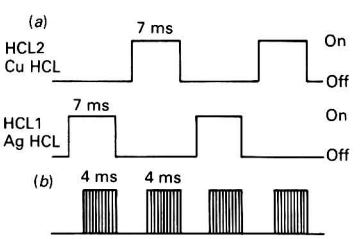

Fig. 2 (*a*) Timing chart of emission signals from the silver and copper HCLs and (*b*) signal sampling

Absorbance Signals of Copper and Silver Obtained by Alternate Irradiation of the Sample

Silver and copper are simultaneously atomized and hence there is usually no time difference in the appearance of the signal, in conventional AAS. Therefore, the different absorption signals cannot be distinguished when the mixed resonance lines of silver and copper pass through the flame at the same

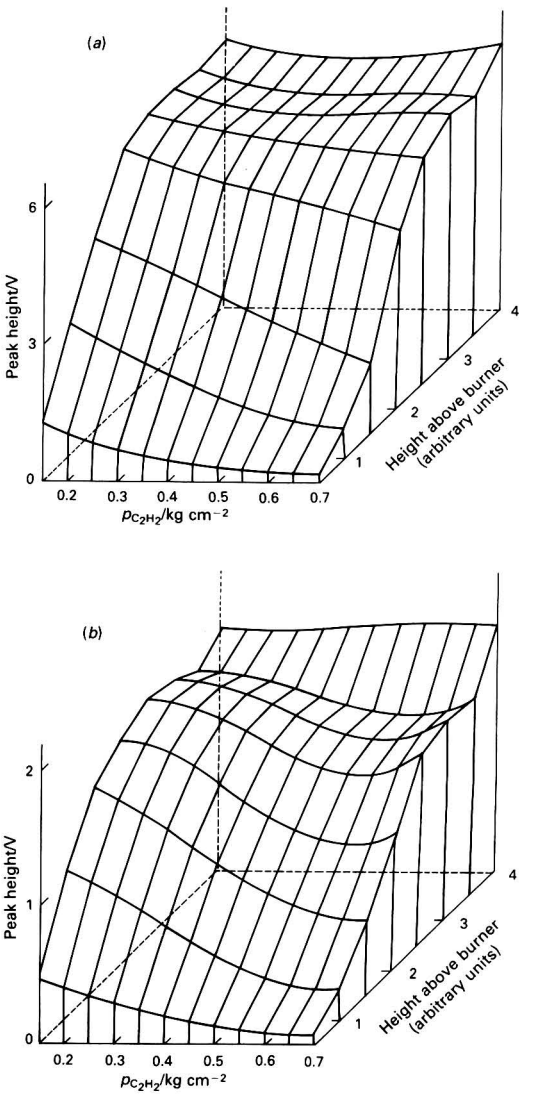

Fig. 3 Response surfaces of silver and copper obtained by continuous nebulization. Sample solution of (*a*) silver (5 ppm); and (*b*) copper (5 ppm). Burner height value was indicated by the scale reading on the AAS apparatus

time. However, if the silver and copper HCLs alternately irradiate the sample and acquisition of the absorption data is synchronized with the irradiation, it is possible to distinguish between the absorption signals of silver and copper. The two HCLs are pulsed at 50 Hz alternately as shown in Fig. 2 and both radiations pass through the flame. By utilizing a computerized high-speed data acquisition system to collect both of the absorption signals alternately, each element could be determined with a single measurement. The absorbance–time profiles for silver and copper, obtained simultaneously using the instrument previously mentioned, are shown in Fig. 4(*a*). A sample solution of silver (5 ppm) and copper (5 ppm)

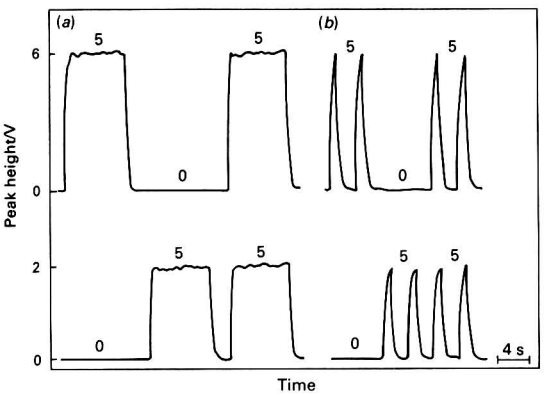

Fig. 4 Absorption profiles of silver (top) and copper (bottom). (*a*) Continuous-flow nebulization method. (*b*) Discrete nebulization method. Sample injection volume was 100 μl. The numbers on the profiles refer to sample composition and correspond to the concentration of the metal in ppm

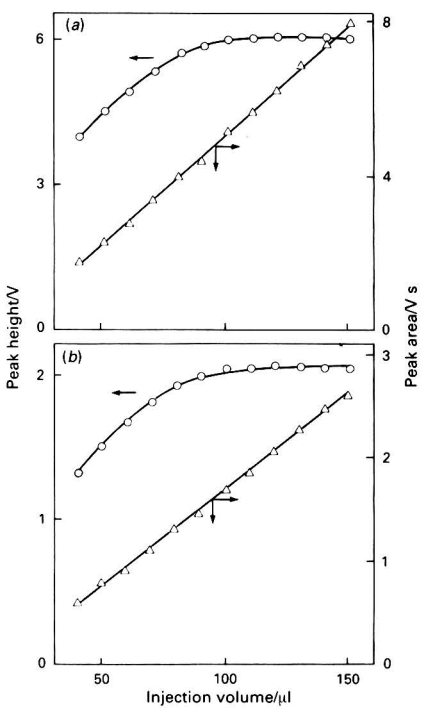

Fig. 5 Effect of injection volume on peak height and peak area of (*a*) silver and (*b*) copper obtained with 5 ppm silver and 5 ppm copper mixed solutions

in 0.1 mol dm^{-3} HNO_3 was injected by using a conventional method. The absorbance–time profiles obtained in the mixed solution were compatible with the signals obtained in each single element solution. Mutual interference due to the coexistence of silver and copper was not observed. Consequently, it is clear that the simultaneous determination of silver and copper can be performed with the alternate irradiation method.

In conventional flame AAS, the continuous flow injection method is popular for sample injection. However, it has the disadvantage that a large sample volume is required. In order to minimize the sample volume required and the analysis time, a discrete nebulization method was investigated. The signal peak shape obtained with this method is shown in Fig. 4(*b*).

Effect of Injection Volume

The dependence of the peak height and the peak area on various injection volumes of the mixed solution, containing 5 ppm of Ag and 5 ppm of Cu, is shown in Fig. 5. The peak height increases with the volume of sample solution injected up to about 100 μl. Thereafter, the limit of the peak height is the same as the height obtained with continuous aspiration.

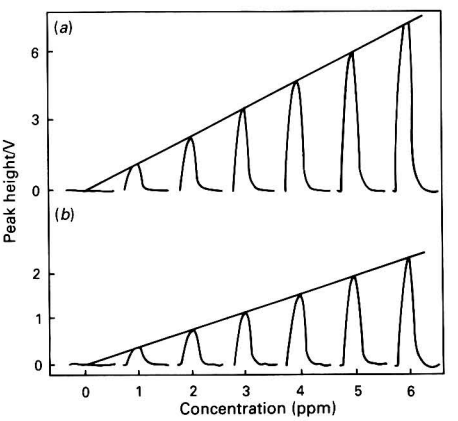

Fig. 6 Calibration graphs for (*a*) silver and (*b*) copper obtained with a constant volume (100 μl) of mixed solutions of silver and copper

Table 1 Results for the determination of silver and copper in silver brazing filler metals. Figures in parentheses represent the relative standard deviations (%) of five analyses. The proposed method of simultaneous flame AAS used a 100 μl injection volume; flame AAS was the conventional single-element flame method using continuous-flow nebulization. Reference values are as reported by the manufacturer

		Content (% m/m)			
		Proposed method			
Sample	Element	Peak height	Peak area	Flame AAS	Reference value (% m/m)
Low melt*	Ag	43.9 (0.5)	44.1 (2.4)	45.1 (1.1)	44–46
	Cu	15.6 (2.7)	15.2 (1.8)	15.3 (2.5)	14–16
Sample No. 318†	Ag	25.7 (2.1)	25.2 (2.1)	25.7 (1.4)	≈25
	Cu	34.4 (2.3)	33.7 (1.3)	34.8 (2.5)	≈35

* Obtained from Nippon Yushi.
† Obtained from Kinzokuyouzai.

The reproducibility of the signal is improved by increasing the injection volume. With an injection volume of greater than 100 μl, the relative standard deviation for ten determinations of peak height is less than 1%. The peak-area value shows a linear relationship to the injection volume.

Calibration Graph

The calibration graphs for silver and copper, as the absorbance–time profiles, are shown in Fig. 6. The peak height and peak area gave straight lines for silver and copper. As a constant injection volume was used, there was a linear relationship between the peak-area value and the concentration.

Application

The proposed simultaneous AAS method using discrete nebulization was applied to the determination of silver and copper in two types of silver brazing filler metal alloys that consisted of silver, copper, zinc and cadmium. Samples were dissolved in nitric acid. The results shown in Table 1 were obtained by measuring the peak height and peak area and are in agreement with the reference values reported by the manufacturers. In addition, the proposed method was validated by conventional single-element flame AAS using continuous-flow nebulization. In applying the Student's *t*-test to the two methods, there was no significant statistical difference in the results obtained using the two methods.

The simultaneous determination of silver and copper by flame AAS was also performed using the neighbouring resonance line. The method requires only a simple modification to the normal AAS system. The proposed method can be employed in order to minimize both the sample volume and the analysis time.

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Two-step Coprecipitation Method for Differentiating Chromium Species in Water Followed by Determination of Chromium by Neutron Activation Analysis

Chi-Ren Lan, Chia-Liang Tseng and Mo-Hsiung Yang*
Institute of Nuclear Science, National Tsing Hua University, 30043 Hsinchu, Taiwan
Zeer B. Alfassi
Department of Nuclear Engineering, Ben Gurion University, 84102 Beer Sheva, Israel

A two-step method for the determination of Cr^{VI} and Cr^{III} in natural waters was developed. The method is based on the variation of the coprecipitation yields with $Pb(PDC)_2$ (PDC = pyrrolidine dithiocarbamate) as a function of pH. By using two different pH values both species can be determined separately. Firstly, Cr^{VI} was coprecipitated at pH 4.0 and then Cr^{III} was separated at pH 9. Total chromium was determined by reduction of Cr^{VI} followed by coprecipitation at pH 9. The validity of the procedure was checked with the National Institute of Standards and Technology Standard Reference Material 1643b Trace Elements in Water and the result was found to be in good agreement with the certified value.

Keywords: *Water; chromium species analysis; ammonium pyrrolidine dithiocarbamate; coprecipitation; neutron activation analysis*

Chromium is present in natural waters in two different oxidation states, Cr^{III} and Cr^{VI}. The former is considered an essential element in mammals, whereas the latter is considered to be a toxic material.[1–6] Thermodynamic calculations indicated that in natural waters Cr should exist almost exclusively as Cr^{VI}.[7] However, it was found experimentally that the actual ratio of Cr^{III} to Cr^{VI} in natural waters varied from 0.02 to 0.99.[8] Arrhenius and Bonatti[9] pointed out that this variation and contradiction with theory might be due to the *in situ* coprecipitation of chromate only, with strontium or barium sulphate. This selective coprecipitation can also be used as an analytical tool for the separation of the two species followed by their individual determination. Chuecas and Riley[10] studied the coprecipitation of Cr from water using ^{51}Cr as a radiotracer. They found that both aluminium and iron(III) hydroxides (hydrated oxides) will coprecipitate Cr^{III} efficiently. The pH range for 99% coprecipitation is considerably larger for iron(III) hydroxide (pH 7.0–9.0) than for aluminium hydroxide (pH 7.5–8.0). When coprecipitating Cr^{VI} spiked with $^{51}Cr^{VI}$, about 1.2% of the Cr was precipitated. This might be due to partial coprecipitation caused by a small amount of $^{51}Cr^{III}$ in the radiotracer.

Fukai[11] measured both Cr^{III} and Cr^{VI} in sea-water by coprecipitation with iron(III) hydroxide first from the untreated water and then after reduction of the sample with sodium sulphite in acidic medium. Several studies have been carried out on the extraction of Cr species by means of ammonium pyrrolidine dithiocarbamate (APDC)–ethyl methyl ketone (EMK) or diethyl dithiocarbamate(DDTC) with EMK. However, the percentage extraction varied considerably (50–100%).[12,13]

De Jong and Brinkman[14] selectively determined Cr^{VI} and Cr^{III} in sea-water using solvent extraction,. They found that Cr^{VI} was extracted with high efficiency (>99%) from various acidic solutions with tertiary amines. These workers used a pH of 2 (0.01 mol dm^{-3} HCl) and Aliquat 336 as the extractant; Aliquat 336 is a mixture of methyl trialkylammonium chlorides with alkyl groups that are mainly C_8–C_{10}. The extracting organic solvent was toluene. Chromium(III) was not extracted at all in this medium. However, by using the same extractant at pH 6–8 and in the presence of at least 1 mol dm^{-3} thiocyanate (in CCl_4 rather than toluene, in order to dissolve the KSCN), Cr^{III} was quantitatively extracted while none of the Cr^{VI} was extracted in this pH range. One of the methods of Cr speciation recommended by the US Environmental Protection Agency (EPA)[15] involves the extraction of Cr^{VI} with APDC into isobutyl methyl ketone (IBMK); however, several problems are associated with this method.[16]

Isozoki *et al.*[17] studied the different ionic species of Cr in natural water using ion-exchange chromatography; the work was similar to that of Naranjit *et al.*[18] and Leyden *et al.*[19] Isozoki *et al.* used a chelating column of Chelex 100 for the quantitative adsorption of Cr^{III} while Cr^{VI} was not absorbed and passed through the column. Chromium(VI) was determined after reduction to Cr^{III}. Miyazaki and Barnes[20] used a poly(dithiocarbamate) chelating resin. Only Cr^{VI} was retained on the column, the Cr^{III} passed through. Total Cr was obtained after oxidizing Cr^{III} to Cr^{VI} with $KMnO_4$ in acidic media. Wai *et al.*[21] differentiated between Cr^{III} and Cr^{VI} by extracting Cr^{VI} from natural waters into chloroform with DDTC followed by back-extraction into aqueous Hg^{II} solution for determination by graphite furnace atomic absorption spectrometry. The Cr^{III} remaining in the extracted solution can be oxidized to Cr^{VI} with $KMnO_4$ and then extracted with DDTC.

Subramanian[22] developed procedures using the APDC–IBMK extraction system for the selective determination of Cr^{III}, and the simultaneous determination of Cr^{III} plus Cr^{VI}, without the need to convert Cr^{III} into Cr^{VI}. He used phthalate buffer and found that at about 0.02–0.1% of phthalate, both species were extracted efficiently, whereas above 0.8% phthalate, only Cr^{VI} was quantitatively extracted (all in the pH range 2.5–4.0).

For both atomic absorption spectrometry and inductively coupled plasma atomic emission spectrometry and also for spectrophotometric determination it is preferable to obtain the concentrated sample in a liquid phase; however, for neutron activation analysis (NAA) and also for X-ray fluorescence spectrometry it is preferable to have the sample in the form of a solid.

This is particularly true for Cr as thermal neutron activation leads to two radionuclides of which the short-lived radionuclide ^{55}Cr has a low abundance of the parent isotope (2.36%), a relatively small cross-section for formation (0.36 barn) and, most significantly, it is almost a pure β-emitter and emits very few γ-rays (0.043%). The lower limit of detection using this radionuclide is very high and measurement of Cr by

* To whom correspondence should be addressed.

NAA is carried out using the long-lived radionuclide ^{51}Cr (half-life, 27.71 d). In order to obtain high sensitivity the sample should be irradiated at high fluxes for long periods of time (several days or at least 10–20 h). With these long irradiation times liquid samples will suffer considerable radiolysis, leading to the formation of large amounts of gases, which are likely to lead to explosion of the irradiation ampoule. Hence a solid sample should be used and, rather than drying the liquid sample, it is preferable to pre-concentrate the trace elements by coprecipitation. The best coprecipitants for NAA will be compounds that have small thermal neutron absorption cross-sections and which do not form radioisotopes on neutron absorption or where the radioisotopes formed are very short-lived and/or are only β-emitters. Materials that fulfil these criteria are organic compounds of lead and bismuth. In earlier works,[23–27] the precipitation of several trace elements from natural waters and biological fluids with $Pb(PDC)_2$ and $Bi(PDC)_3$ (PDC = pyrrolidine dithiocarbamate) have been described; the present work is concerned with the two species of Cr, *viz.*, Cr^{III} and Cr^{VI}.

Nakayama *et al.*[28] found that in the pH range 4–10 both Cr^{III} and Cr^{VI} were coprecipitated with bismuth oxide. Hence they first coprecipitated Cr^{III} with iron(III) hydroxide at about pH 8, and then Cr^{VI} was collected at the same pH with bismuth oxide. Pik *et al.*[29] first coprecipitated Cr^{III} with iron(III) hydroxide at pH 8.5 and then coprecipitated Cr^{VI} from the remaining solution with $Co(PDC)_2$ at pH 4.

Experimental

All chemicals used were of analytical-reagent grade and the solutions were prepared using doubly distilled, de-ionized water.

Two methods were used to determine the coprecipitation yields of Cr^{III} and Cr^{VI} with $Pb(PDC)_2$. In the first, the radiotracer ^{51}Cr was used. The coprecipitation yield was measured from the radioactivity counts [with an NaI(Tl) detector] of the original radiotracer solution and the precipitate. Solutions of $^{51}Cr^{VI}$ and $^{51}Cr^{III}$ were prepared by the method of Collins *et al.*[30] About 50 mg of K_2CrO_4 or $K_2Cr_2O_7$ were irradiated in the reactor for 2–3 d. The irradiated salt was dissolved in 5 ml of a solution containing 15 mg of $Zn(NO_3)_2$ and 50 mg of CrO_3. After complete dissolution, 3 ml of 1 mol dm^{-3} KOH were added and the solution was heated at 90–95 °C for about 30 min. While the reaction mixture was still hot, 1 ml of a solution containing 3 mg of $Zn(NO_3)_2$ was added, with stirring. The resulting suspension was filtered through a 10 × 6 mm i.d. column of alumina or celite washed with 5 ml of 1 mol dm^{-3} KOH and 5 ml of 1×10^{-4} mol dm^{-3} KOH, and kept wet with a KOH solution of pH 10. The filtered solution was used as a radiotracer for Cr^{VI}. Some of the ^{51}Cr changed its valency due to the Szilard–Chalmers process and was retained on the column as Cr^{III}. The $^{51}Cr^{III}$ was eluted from the column with 5 ml of 1 mol dm^{-3} HCl and was used as a radiotracer for Cr^{III}.

In the second method, standard solutions of unlabelled Cr^{III} and Cr^{VI} were used and the standard solution and the precipitate were analysed simultaneously by NAA.

The coprecipitation studies were carried out by the addition of 1 ml of a standard solution containing 1 mg ml^{-1} of either Cr^{III} or Cr^{VI} to 250–500 ml of tap water (for the radiotracer experiments) or distilled water (for the NAA experiments) followed by the addition of 2 mg of $Pb(NO_3)_2$, 5 ml of 1 mol dm^{-3} acetate buffer and adjustment of the pH with HNO_3. Then, 100 mg of APDC were added to the solution which was stirred for 30 min. The precipitates were filtered through a 0.45 μm Millipore filter (Gelman) and dried in a desiccator containing silica gel until completely dry. In the NAA experiments the dried samples were introduced into polyethylene vials which were heat-sealed. A standard was prepared by introducing 1 ml of the standard solution into a polyethylene vial and heating to dryness under an infrared lamp. The precipitates and the standard were placed in an irradiation capsule and irradiated for 30 h at a flux of 5×10^{12} n cm^{-2} s^{-1}. The radioactivity induced in the samples was measured by a Ge(Li) detector connected to a multi-channel analyser, after 1 week of cooling. The coprecipitation yields were calculated as the radioactivity ratio of the samples to the standard.

Results and Discussion

The results for the recovery of Cr^{III} and Cr^{VI} by coprecipitation with $Pb(PDC)_2$ as a function of pH for 250 ml of distilled water, tap water and sea-water are given in Table 1. As can be seen from these results, it is possible to precipitate Cr^{VI} almost exclusively in the pH range 2.5–4.5. Chromium(III) cannot be coprecipitated alone, as at pH 9 about 16–44% of Cr^{VI} is also precipitated. However, a two-step coprecipitation on the same sample can give information about the concentrations of both Cr^{VI} and Cr^{III}. The sample is adjusted to pH 4 and $Pb(NO_3)_2$ (2 mg) and APDC (100 mg) are added to the solution. The solution is stirred for 30 min and then filtered on a 47 mm Millipore filter (0.45 μm). The precipitate on the filter-paper is used to determine Cr^{VI}. The filtrate is adjusted to pH 9 with 25% ammonia solutions (about 3 ml). Then, 100 mg of APDC and 2 mg of $Pb(NO_3)_2$ are added, the solution is stirred (for 30 min) and filtered on a 47 mm diameter 0.45 μm Millipore filter and the precipitate is used for the determination of Cr^{III}.

Table 2 gives the results for the recovery of Cr^{III} and Cr^{VI} from 2 l of sea-water or tap water spiked with Cr^{III} or Cr^{VI} at a total concentration of 20 ng ml^{-1}. As can be seen from this table even at this low concentration there is nearly a 100% recovery (about 95–105%).

Total Cr Determination

Total Cr can be determined either by oxidation of Cr^{III} to Cr^{VI} and coprecipitation at pH 4 or by reduction of Cr^{VI} to Cr^{III} and coprecipitation at pH 9.0. It is easier to reduce Cr^{VI} than to oxidize Cr^{III} and consequently the latter option was preferred. Chromium(VI) was reduced with $NaHSO_3$.

Table 1 Recovery of Cr^{III} and Cr^{VI} by coprecipitation with $Pb(PDC)_2$ as a function of pH. All values in per cent. Conditions: water sample, 250 ml; Cr^{III} or Cr^{VI}, 100 μg; APDC, 100 mg; and $Pb(NO_3)_2$, 2 mg

	Distilled water		Tap water		Sea-water	
pH	Cr^{III}	Cr^{VI}	Cr^{III}	Cr^{VI}	Cr^{III}	Cr^{VI}
3	0.8	92	0.8	92	0.5	95
4	1.8	98	1.8	95	0.5	95
5	3.6	95	3.6	95	0.5	95
6	13.2	95	13	95	4	34
7	88	58	66	58	67	14
8	93	52	93	52	96	14
9	97	44	97	44	95	16

Table 2 Recovery tests carried out by spiking sea-water and tap water samples with Cr^{III} or Cr^{VI}. Water sample, 2 l; Cr, 20 ng ml^{-1}

		Recovery (%)	
Sample		Cr^{VI}	Cr^{III}
Sea-water		101.6	98.5
		95.7	99.2
		105.3	102.1
	Average:	100.9	99.9
Tap water		102.9	98.6
		101.0	95.9
		100.7	103.1
	Average:	101.5	99.2

Table 3 Recovery of Cr^{VI} by reduction and precipitation at pH 9 as a function of the amount of $NaHSO_3$ added (Cr^{VI}, 104 μg)

Amount of $NaHSO_3$/mg	1.7	6.7	16.7	33.3	66.6	83.5	167	500
Recovery (%)	25.4	28.7	41.5	56.3	100	99.3	97.6	99.9

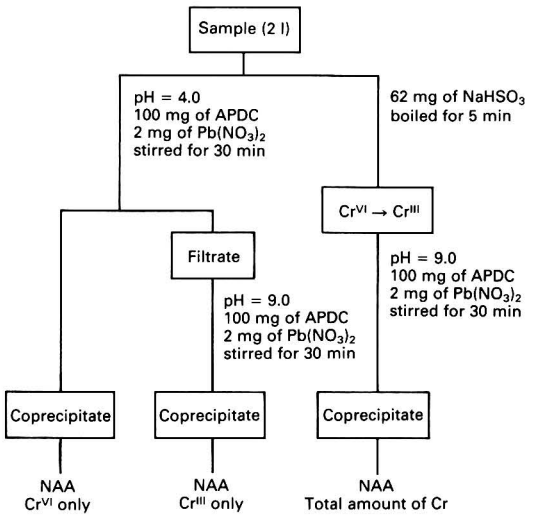

Fig 1 Scheme for the speciation of Cr^{III} and Cr^{VI} and for the determination of total Cr

Different amounts of $NaHSO_3$ were added to a series of solutions containing 104 μg of Cr^{VI}. The solutions were stirred and then placed in a microwave oven for heating (2 min at 100% power followed by 3 min at 60% power). After boiling for 5 min the solutions were rapidly cooled in an ice-bath. The pH was adjusted to 9 with 1 ml of 25% ammonia solution and the Cr^{III} in the solution was then determined as described above. Table 3 shows the percentage recovery of Cr^{VI} obtained with this method as a function of the amount of $NaHSO_3$ added. It can be seen that 66.6 mg of $NaHSO_3$ are sufficient for complete reduction and recovery. Consequently in later experiments, about 70 mg of $NaHSO_3$ were used for the reduction of Cr^{VI} in the procedure for the determination of total Cr. Fig. 1 illustrates schematically the determination of the two species of Cr and of total Cr. This method of separate determination of the two species of Cr has advantages over most of the previous methods in that it does not require the oxidation of Cr^{III} to Cr^{VI} or the reduction of Cr^{VI} to Cr^{III} which is usually carried out with an excess of the reagents. The proposed method does, however, suffer from the disadvantage that even if one is interested only in Cr^{III}, one must first coprecipitate Cr^{VI}. If only Cr^{III} is required, then coprecipitation with hydrated iron(III) hydroxide might be a better procedure.

Real Samples

Three samples of sea-water, well water and tap water were analysed according to the scheme illustrated in Fig. 1. The results are given in Table 4. It can be seen that the agreement between the values for Cr^{VI} plus Cr^{III} and total Cr is good. A certified reference water, *viz.*, National Institute of Standards and Technology Standard Reference Material 1643b Trace Elements in Water, was also analysed as a quality control material during the measurement of the total amount of Cr.

Table 4 Results for the determination of Cr^{III}/Cr^{VI} in natural waters. Results given are mean ± standard deviation (n = 3)

	Found/ng ml^{-1}		
Sample	Cr^{VI}	Cr^{III}	Total Cr
Sea-water	0.10 ± 0.01	0.49 ± 0.04	0.54 ± 0.03
Well water	0.13 ± 0.05	0.11 ± 0.02	0.25 ± 0.02
Tap water	0.14 ± 0.04	0.20 ± 0.05	0.33 ± 0.05

The resulting value (18.1 ± 1.5 ng ml^{-1}, mean of three sample analyses ± standard deviation) was in good agreement with the certified value (18.6 ng ml^{-1}).

Limit of Detection

Blank values were measured by using tap water as the metal-free solution after coprecipitation of total Cr. Analysis of five samples of the blank gave an average value of 0.261 ± 0.006 ng ml^{-1}. Employing the usual convention, that the detection limit is 4.7 times the square root of the background, or 4.7 times the standard deviation, gave a limit of detection of 0.03 ng ml^{-1}.

Mechanism

The variation of the amount of Cr^{VI} with pH might be associated with the equilibrium between CrO_4^{2-} and $Cr_2O_7^{2-}$. At acidic pH, CrO_4^{2-} is the major species and it is this species that is coprecipitated, while $Cr_2O_7^{2-}$ is not precipitated to any great extent. The precipitation of Cr^{III} at pH 9 cannot be in the form of $Cr(PDC)_3$. It is possible that the insoluble hydrated Cr^{VI} oxide is coprecipitated with $Pb(PDC)_2$. It is known[31] that Cr^{III} exists at acidic pH as the hexaaqua ion $[Cr(H_2O)_6]^{3+}$, which has a pK of 4. At higher pH the hydroxide ion $[Cr(H_2O)_5(OH)]^{2+}$ is formed, which can give soluble dimers and polymers. At even higher pH values, dark green gels are formed which are coprecipitated with $Pb(PDC)_2$.

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Ion-selective Electrodes in Organic Analysis: Determination of Alkyl Halides *via In Situ* Generation of *S*-Alkylisothiouronium Salts

Wing Hong Chan,* Albert Wai Ming Lee* and Yu Man Cheung
Department of Chemistry, Hong Kong Baptist College, 224 Waterloo Road, Kowloon, Hong Kong

Low relative molecular mass alkyl halides, after *in situ* derivatization to the corresponding *S*-alkylisothiouronium salts in the presence of an excess of thiourea, were determined with a poly(vinyl chloride) membrane *S*-alkylisothiouronium-selective electrode based on *S*-butylisothiouronium tetraphenylborate. This membrane electrode exhibited Nernstian response in the range 1.0×10^{-1}–1.6×10^{-4} mol dm^{-3} with an average cationic (positive) slope of 58.8 mV per concentration decade at 25 °C. The electrode had a reasonably wide working pH range (6.5–8.5), fast dynamic response time (30 s–2 min), stable response for at least 2 months and high selectivity for the *S*-alkylisothiouronium ion in the presence of many inorganic and organic ions. The electrode functioned satisfactorily for the determination of primary and secondary alkyl halides, excluding alkyl fluorides.

Keywords: *Alkyl halide determination; ion-selective electrode; organic analysis;* in situ *S-alkylisothiouronium salt generation*

Organohalides are essential industrial chemicals. They are important intermediates in many chemical reactions and are used extensively as solvents. Other significant uses of this class of compound are as anaesthetics, refrigerants, and grain and fruit fumigants.[1] There is a wide range of published methods for the determination of organohalides. The basic approach involves the decomposition of an organohalide sample to halide ions. After decomposition, the liberated halide ions are determined by using a halide ion-selective electrode,[2] gravimetry or visual titrimetry.[3] For compounds containing a tightly bound halogen atom such as alkyl halides, the oxygen flask combustion technique is used.[3] All of these methods require either a fairly tedious sample preparation procedure or a large sample size.

In order to increase the use of ion-selective electrodes (ISEs) in organic analysis, a new strategy has been proposed for converting a covalent organic compound into a water-soluble ionic derivative which is amenable to potential measurement. Many important organic functional groups such as esters, alcohols, aldehydes and amines, after derivatization to ionic species, can be subjected to selective electrode measurement.[4] However, a general method for the determination of alkyl halides using an ISE has not yet been developed. The aim of this investigation was to develop a poly(vinyl chloride) (PVC) membrane *S*-alkylisothiouronium-selective electrode for the determination of alkyl halides *via in situ* generation of the corresponding *S*-alkylisothiouronium salts in 95% ethanol by means of a bimolecular nucleophilic substitution reaction,[5] *viz.*,

$$RX + H_2N\text{–}\overset{\overset{S}{\|}}{C}\text{–}NH_2 \xrightarrow[\text{ethanol}]{95\%} R\text{–}S\text{–}\overset{\overset{+}{N}H_2\,X^-}{\overset{\|}{C}}\text{–}NH_2$$

where X is Br or I.

For the less reactive alkyl halides, such as primary alkyl chlorides and secondary alkyl bromides, it was first necessary to convert these compounds into the corresponding alkyl iodides by refluxing overnight with an excess of sodium iodide in 95% ethanol.[6] The crude iodo compound, without purification, was then converted into the *S*-alkylisothiouronium salt by treatment with an excess of thiourea.

* To whom correspondence should be addressed.

Experimental

Apparatus

Potentiometric measurements were made at a constant temperature in the range 20–25 °C with an Orion digital pH/ion meter (Model SA720). A platinized platinum electrode (Model 3401) from Yellow Springs Instruments was used as an internal reference electrode. A saturated calomel electrode (SCE) from Orion (Model 9006) was used as an external reference electrode. For pH measurements, a Sensonex combined pH electrode (Model 5200C) was used. The nuclear magnetic resonance (NMR) spectra were recorded with a Jeol NMR spectrometer (60 MHz) (Model PMX 60SI).

Reagents

All solutions were prepared with analytical-reagent grade reagents in distilled, de-ionized water unless stated otherwise. The organic solvents and reagents were also of analytical-reagent grade. Tetrahydrofuran (THF) was distilled over sodium under nitrogen before being used. High relative molecular mass PVC was obtained from Aldrich. Sodium tetraphenylborate and bis(2-ethylhexyl) phthalate (DEHP) were obtained from Fluka. The Britton–Robinson buffer of pH 7.5 consisted of 57.5 ml of 0.2 mol dm^{-3} sodium hydroxide and 100 ml of a stock solution containing 0.04 mol dm^{-3} acetic acid, 0.04 mol dm^{-3} orthophosphoric acid and 0.04 mol dm^{-3} boric acid.

S-Butylisothiouronium Tetraphenylborate–PVC Matrix Membrane Electrode

Preparation of the sensing material

S-Butylisothiouronium bromide (0.01 mol) was dissolved in 20 ml of distilled, de-ionized water. An equimolar amount of sodium tetraphenylborate was dissolved in another 20 ml of distilled, de-ionized water. The solutions were mixed and stirred for 10 min. The white precipitate of *S*-butylisothiouronium tetraphenylborate was filtered off by suction filtration, washed with distilled, de-ionized water, air-dried for 24 h and ground to a fine powder.

Preparation of the sensing membrane

A mixture of *S*-butylisothiouronium tetraphenylborate (0.04 g), PVC powder (0.26 g) and plasticizer (DEHP) (0.10 g) was dissolved in 20 ml of distilled THF. The solution was poured into a 75 mm i.d. Petri dish and covered with a piece of

filter-paper. After all the THF had evaporated, an *S*-butylisothiouronium tetraphenylborate–PVC matrix membrane sheet was obtained.

Assembly of the electrode

A portion of the sensor membrane (diameter, 1 cm) was cut and fitted into a screw-cap adaptor with an O-ring placed above the membrane as the electrode body.[7] A solution of 1.00×10^{-2} mol dm^{-3} *S*-butylisothiouronium bromide at pH 7.5 was used as the internal reference solution and a platinized platinum electrode was used as an internal reference electrode.

Conditioning and storage procedure

The assembled electrode was conditioned by soaking it in 1×10^{-2} mol dm^{-3} *S*-butylisothiouronium bromide solution at pH 7.5 for 1 d before use; the electrode was also stored in the same solution when not in use.

Construction of the ISE system

The assembled electrode was immersed in an *S*-butylisothiouronium bromide (SBB) solution and acted as a half-cell. The other half of the cell was formed by inserting an SCE into a saturated KCl solution. The ISE system was completed by connecting the two half-cells by a KCl salt-bridge. The electrochemical cell was constructed as follows:

Internal reference electrode (Pt–Pt)	Internal reference solution, 0.01 mol dm^{-3} SBB at pH 7.5	PVC matrix membrane with *S*-butylisothiouronium tetraphenylborate (ion pair)	Standard solution at pH 7.5	Saturated KCl solution	External reference electrode (SCE)

In Situ Derivatization of Alkyl Halides

In the bimolecular nucleophilic substitution reaction, the order of reactivity follows the sequence RI > RBr > RCl (R = alkyl). For a given halide, the reactivity decreases in the order primary > secondary > tertiary. Based on the difference in reactivity, alkyl halides can be grouped into two classes in the *in situ* derivatization reaction.

Class 1. *Halides with higher reactivity*, i.e., *alkyl iodides and primary alkyl bromides*

The alkyl halide (about 0.5 g) was weighed accurately in a 100 ml round-bottomed flask and 1.2 equiv. of thiourea were added. The mixture was dissolved in 30 ml of 95% ethanol and the solution was refluxed for a certain period of time (90–150 min). After refluxing, the solvent was removed under reduced pressure to leave a colourless oily liquid, which was dissolved in a pH 7.5 buffer solution which consisted of boric acid, acetic acid, orthophosphoric acid and sodium hydroxide. The resulting solution was diluted to the mark with the buffer in a 100 ml calibrated flask and then potential measurements were performed.

Class 2. *Halides with lower reactivity*, i.e., *alkyl chlorides and secondary alkyl bromides*

The alkyl halide (about 0.5 g) was accurately weighed into a 100 ml round-bottomed flask and 3 equiv. of sodium iodide were added. The mixture was dissolved in 40 ml of 95% ethanol and the solution was refluxed overnight. Thiourea (1.5 equiv.) was then added to the cool solution and the mixture was refluxed for 150 min. After removal of the solvent *in vacuo*, the oily residue was dissolved in pH 7.5 buffer and diluted to the mark with the same buffer in a 100 ml calibrated flask. The solution was then ready for potential measurement.

Calibration

The butyl bromide sample, after the aforementioned *in situ* derivatization, was used to prepare a series of standard solutions in the concentration range 1×10^{-1}–1×10^{-5} mol dm^{-3}. Aliquots (80 ml) of the standard solutions were used for the ISE measurements. The potentials of the stirred solutions were recorded when they became stable and were plotted as a function of the logarithm of the butyl bromide concentration. The graph was used for subsequent determination of alkyl halides.

Results and Discussion

Nature and Composition of the Membrane

S-Butylisothiouronium bromide reacts readily with sodium tetraphenylborate to form a stable, crystalline 1 : 1 ion-pair complex whose composition was unambiguously verified by proton NMR spectroscopy. The integration ratio and the multiplicity of the various proton signals of the NMR spectrum agree well with the proposed structure (Fig. 1). The response characteristics of the PVC membrane doped with various amounts of *S*-butylisothiouronium tetraphenylborate were systematically investigated. The suitability and sensitivity of the membrane, based on both the slope of the calibration graph and the limit of detection, were studied. The calibrations were performed in Britton–Robinson buffer (pH 7.5), in which all of the *S*-butylisothiouronium ions are in the univalent form. As shown in Table 1, the response was dependent on the proportion of active compound contained in the membrane formulation. For optimum performance, 9–12% m/m of the sensor in the membrane formulation was required. When less carrier was used, the response deviated considerably from the Nernst equation. In the fabrication of the PVC sensing membrane, about 25% m/m of plasticizer (DEHP) was added to improve the plasticity of the membrane.

In Situ Derivatization Reaction

In the presence of an excess of thiourea, the water-soluble ionic *S*-alkylisothiouronium salts can be prepared quantitatively from alkyl halides by heating under reflux. For compounds containing a tightly bound halogen atom, the alkyl halides are first converted into the corresponding alkyl iodides by refluxing overnight with 3 equiv. of sodium iodide in 95% ethanol. For quantitative work, it is essential that the extent of *S*-alkylisothiouronium salt formation is reproducible under a given set of conditions. In order to ensure this and to determine the time required for quantitative conversion of the alkyl halide into the *S*-alkylisothiouronium salt, the reaction was monitored by potentiometric measurement using the selective electrode. The measured potential readings were plotted as a function of reaction time (Fig. 2). It was found that quantitative (or constant) conversion of butyl bromide into *S*-butylisothiouronium bromide was obtained when the

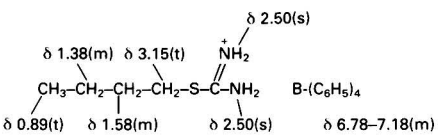

Fig. 1 Assignment of the proton NMR data for the sensor (in $CDCl_3$)

Table 1 Response characteristics of a PVC membrane doped with various amounts of *S*-butylisothiouronium tetraphenylborate (sensor)

Sensor (% m/m)	Total mass (PVC + DEHP + sensor)/g	Sample	Slope/mV per concentration decade	Detection limit/ 10^{-4} mol dm^{-3}	Correlation coefficient
2.5	0.4091	1	38.4	3.2	0.9989
		2	37.2	3.5	0.9985
6.6	0.4112	1	51.5	2.3	0.9990
		2	52.7	2.0	0.9985
9.5	0.4075	1	58.0	2.2	0.9986
		2	57.7	2.0	0.9983
12.3	0.4105	1	57.4	2.0	0.9990
		2	57.6	1.6	0.9988
24.8	0.4048	1	53.7	1.3	0.9931
		2	52.4	1.6	0.9970

Table 2 Conditions for the *in situ* derivatization of different alkyl halide samples

Primary alkyl iodide	1-Iodobutane	Reflux with excess of thiourea in 30 ml of 95% ethanol for 90 min
Primary alkyl bromide	1-Bromobutane	Reflux with excess of thiourea in 30 ml of 95% ethanol for 150 min
	1-Bromopropane	
Primary alkyl chloride	1-Chlorobutane	Reflux with 3 equiv. of NaI in 40 ml of 95% ethanol for 24 h, then reflux with excess of thiourea for 150 min
Secondary alkyl bromide	2-Bromobutane	

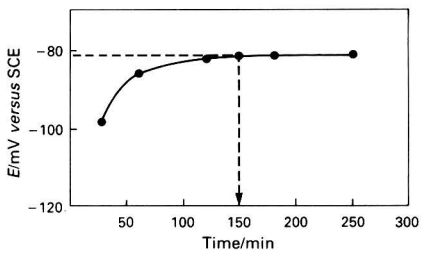

Fig. 2 Time required for the quantitative conversion of butyl bromide into the *S*-butylisothiouronium salt (monitored with the ISE)

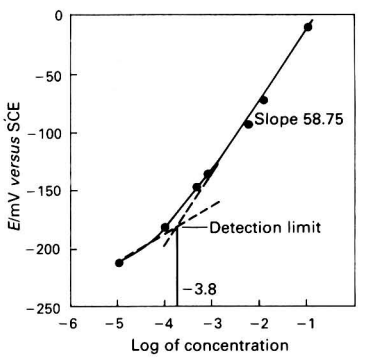

Fig. 3 Response of the electrode to the concentration of butyl bromide in Britton–Robinson buffer of pH 7.5

former was refluxed with an excess of thiourea in 95% ethanol for 150 min. Similarly, the reaction conditions required for quantitative conversion of other alkyl halides into the corresponding *S*-alkylisothiouronium salts were determined and these are given in Table 2. The results obtained are consistent with the relative reactivities of alkyl halides in nucleophilic substitution reactions.

Characteristics of the Electrodes

As shown in Fig. 3, the PVC membrane electrode exhibited an average Nernstian slope of 58.8 mV per concentration decade

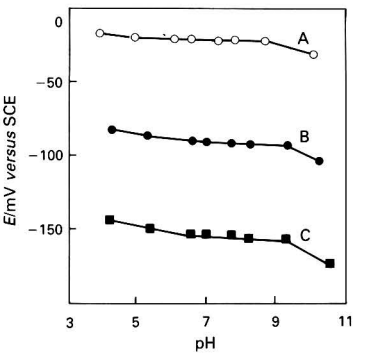

Fig. 4 Effect of pH on the potential of the *S*-butylisothiouronium tetraphenylborate–PVC matrix membrane electrode. A, 5×10^{-2}; B, 5×10^{-3}; and C, 5×10^{-4} mol dm^{-3} butyl bromide

over five determinations (standard deviation = 1.17 mV) with good linearity (correlation coefficient = 0.9991) from 1.0×10^{-1} to 1.6×10^{-4} mol dm^{-3} butyl bromide. Both the slope of the calibration graph and the correlation coefficient demonstrate the suitability and sensitivity of this membrane for the determination of alkyl halides.

Effect of pH

The influence of pH on the response of the *S*-butylisothiouronium tetraphenylborate–PVC matrix membrane electrode was evaluated by performing the potential measurements on the derivatization product of butyl bromide at different pH values. The electrode showed stable and constant readings (±1 mV) in the pH range 6.5–8.5 for various concentrations of butyl bromide (Fig. 4). The *S*-butylisothiouronium ion can be protonated in a strongly acidic medium to give a divalent cation, whereas it is readily hydrolysed to butanethiol in a strongly alkaline medium

$$C_4H_9S{-}\overset{+NH_2}{\overset{\|}{C}}{-}NH_3^+ \overset{H^+}{\rightleftharpoons} C_4H_9S{-}\overset{+NH_2}{\overset{\|}{C}}{-}NH_2 \xrightarrow[pH]{high} C_4H_9SH + NH_2{-}\overset{O}{\overset{\|}{C}}{-}NH_2$$

Therefore, subsequent potential measurements were made at pH 7.5, at which nearly all of the *S*-butylisothiouronium ions are in the univalent form.

Response Time and Stability of the Electrode

The *S*-butylisothiouronium tetraphenylborane–PVC matrix membrane electrode has a rapid response time. The response time of the standard solutions was recorded in increased order of their concentration. The results indicated that the average dynamic response time was 30 s and 2 min for a concentrated ($>1 \times 10^{-3}$ mol dm^{-3}) and dilute ($<1 \times 10^{-3}$ mol dm^{-3}) solution, respectively (Fig. 5). On the other hand, ageing of the membrane was not a serious problem. The performance of the electrode in terms of linearity and Nernstian response was reproducible over a period of 2 months, after repeated measurements.

Effect of Interfering Ions

The response of the electrode to *S*-alkylisothiouronium ion in the presence of various foreign ions was examined. The potential given by solutions each containing 1×10^{-2} mol dm^{-3} of the foreign compound and various *S*-butylisothiouronium concentrations in the range 1×10^{-1}–1×10^{-5} mol dm^{-3} was measured. The selectivity coefficients were calculated by using the fixed interference method.[8] The results obtained (Table 3) demonstrated that no significant effect was caused by organic compounds such as thiourea and urea, and by inorganic ions such as ammonium and chloride. As an excess of thiourea was used in the derivatization of the alkyl halides, it was fortuitous that thiourea did not interfere with the determination. The tetrabutylammonium ion interfered only when present at concentration levels at least several times greater than that of the *S*-butylisothiouronium ion. In addition, the calibrations were carried out in Britton–Robinson buffer solution which consisted of boric acid, acetic acid, orthophosphoric acid and sodium hydroxide; none of these inorganic components of the buffer solution caused any interference.

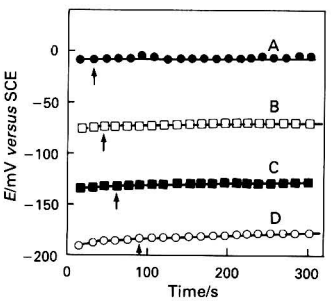

Fig. 5 Response time of the membrane electrode for different concentrations of butyl bromide in Britton–Robinson buffer of pH 7.5 (stable reading indicated by the arrow). A, 1×10^{-1}; B, 1×10^{-2}; C, 1×10^{-3}; and D, 1×10^{-4} mol dm^{-3} butyl bromide

Table 3 Selectivity coefficients ($k^{pot}_{S,B}$) for the *S*-butylisothiouronium tetraphenylborate–PVC matrix membrane electrode. Concentration of each foreign ion, 1.0×10^{-2} mol dm^{-3}

Interfering compound (B)	$k^{pot}_{S,B}$
Thiourea	0*
Urea	0*
Tetrabutylammonium hydrogen sulphate	0.5
Ammonium chloride	0*

* Identical calibration graphs were obtained both in the presence and absence of the foreign ion.

Determination of Alkyl Halides

At the onset of this investigation, it was expected that the sensing material of the electrode could be used to detect both primary and secondary aliphatic alkyl halides. *S*-Alkylisothiouronium solutions in the concentration range 1×10^{-1}–5×10^{-4} mol dm^{-3} were prepared from the corresponding alkyl halides and determined by using the *S*-butylisothiouronium tetraphenylborate–PVC matrix membrane electrode. The potentials given by these solutions were compared with the calibration graph prepared from the butyl bromide derivatization product in order to assess the accuracy and reproducibility of the method. The results obtained for five samples (Table 4), each analysed in triplicate, showed that 1-iodobutane and 1-bromobutane could be quantitatively converted into the *S*-butylisothiouronium salts and determined with the membrane electrode. The average recovery was 98.1% with a mean standard deviation of 0.88%. The lower recovery of 1-bromopropane (Table 4), which is a reactive alkyl halide, may be due to its greater volatility. For the less reactive alkyl halides, 1-chlorobutane, and 2-bromobutane, the two-step derivatization reaction is more prone to side-reactions, such as an elimination reaction. The recovery of these two halides was 86.6 and 61.3%, respectively. However, the excellent precision observed for this electrode method rendered the determination of these less reactive halides equally feasible. The determination of primary chloroalkanes and secondary bromoalkanes, using the same calibration graph, adjusting the amount of the less reactive halides found with the electrode by a factor of 0.87 and 0.61, respectively, will give the equivalent amount of the halide in the sample. Moreover, the absolute potentials recorded by the electrode for various alkyl halides at the same concentration level were very similar. Therefore, the electrode can be used

Table 4 Determination of alkyl halides using the *S*-butylisothiouronium tetraphenylborate–PVC matrix membrane electrode

Sample	Trial	Mass used/g	Mass measured by ISE/g	Recovery (%)	Mean (%)	Standard deviation (%)
1-Iodobutane	1	0.9228	0.8933	96.8		
	2	0.3809	0.3790	99.5	98.2	1.35
	3	0.3123	0.3067	98.2		
1-Bromobutane	1	0.6906	0.6726	97.4		
	2	0.5571	0.5460	98.0	98.1	0.70
	3	0.2503	0.2473	98.8		
1-Bromopropane	1	0.1682	0.1600	95.1		
	2	0.5369	0.5235	97.5	95.8	1.45
	3	0.5893	0.5592	94.9		
1-Chlorobutane	1	0.4317	0.3708	85.9		
	2	0.1079	0.0940	87.1	86.6	0.61
	3	0.4662	0.4042	86.7		
2-Bromobutane	1	0.6063	0.3771	62.2		
	2	0.6790	0.4094	60.3	61.3	0.97
	3	0.1516	0.0934	61.6		

either to determine the concentration of an individual alkyl halide or to measure the total concentration of a mixture of alkyl halides.

Conclusion

An indirect ISE system for the determination of alkyl halides has been described. The *in situ* generation of the ionic *S*-alkylisothiouronium salt from the corresponding covalent alkyl halide in the presence of an excess of thiourea is the key factor in the viability of the method. This highly selective electrochemical method has been shown to be applicable to the determination of low relative molecular mass primary and secondary alkyl halides, excluding alkyl fluorides.

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Masking of Zirconium(IV) in the Determination of Fluoride With an Ion-selective Electrode: Application to Zirconium(IV) Fluoride-based Glasses

Akio Yuchi, Jun-ichi Baba, Hiroko Wada and Genkichi Nakagawa
Department of Applied Chemistry, Nagoya Institute of Technology, Gokiso, Showa, Nagoya 466, Japan

The performance of six chelating reagents [ethylenediamine-*N,N,N′,N′*-tetraacetic acid (EDTA); *trans*-1,2-cyclohexanediamine-*N,N,N′,N′*-tetraacetic acid (CDTA); *N′*-(2-hydroxyethyl)ethylenediamine-*N,N,N′*-triacetic acid (HEDTA); triethylenetetraamine-*N,N,N′,N″,N‴,N‴*-hexaacetic acid (TTHA); diethylenetriamine-*N,N,N′,N″,N″*-pentaacetic acid (DTPA); and citrate] has been studied for masking zirconium(IV) in the determination of fluoride with an ion-selective electrode. Citrate was not suitable because it produced a prolonged electrode response. Of the aminopolycarboxylates, DTPA has a much greater masking ability than the others. Using DTPA at pH 5–6, fluoride was successfully determined at a concentration of 1×10^{-5} mol dm^{-3} in the presence of up to 4×10^{-6} mol dm^{-3} zirconium(IV). The proposed method was applied to the analysis of a number of zirconium(IV) fluoride compounds and ZrF_4-based glasses after fusion with sodium carbonate.

Keywords: *Fluoride determination; ion-selective electrode; fluoride glass; zirconium masking; diethylenetriamine-*N,N,N′,N″,N″*-pentaacetic acid*

In the last decade, heavy metal fluoride glasses, which generally contain zirconium(IV), hafnium(IV) or thorium(IV), have attracted much attention in relation to their potential use in long-distance fibre optics.[1,2] In accordance with progress in this area, the determination of fluoride in the presence of these metal ions has increasingly been required; *e.g.*, chemical durability testing is essential to assess the utility of each fluoride glass, as the transparency of the glass in the middle infrared region (0.2–8 μm) easily deteriorates as a result of attack from environmental water. To monitor the dissolution rate of a fluoride glass in an aqueous solution, a recent review[3] recommends following the appearance of the dissolution products over a period of time, by analysis of the soaking solution, instead of following the loss in the mass of the glass. Metal components have been determined by various spectroscopic methods, and fluoride by potentiometry with a fluoride ion-selective electrode.[4–9] Zirconium(IV), hafnium(IV) or thorium(IV) contained in these glasses has an extremely high affinity for fluoride[10–15] and thus is expected to interfere seriously with the determination of fluoride. To eliminate the interference, commercially available TISAB (total ionic strength adjustment buffer solution), which contains citrate or *trans*-1,2-cyclohexanediamine-*N,N,N′,N′*-tetraacetic acid (CDTA) as a masking reagent for metal ions,[16,17] has been used. These reagents are effective for masking common metal ions such as Al^{3+}, Fe^{3+} and Ca^{2+} but are not always suitable for metal ions in higher oxidation states. For example, Simmons and Simmons[8] have observed that all the fluoride ions were not in the free form when CDTA was used as a masking reagent for zirconium(IV). No further work appears to have been undertaken since that report.

In previous papers,[18–20] Yuchi and co-workers have studied the reaction of the fluoro complexes of trivalent metal ions with various masking reagents and found that mixed ligand complexes were generally present with a masking reagent and fluoride. Reagents forming less stable mixed ligand complexes are more efficient for the masking of a metal ion in the determination of fluoride by potentiometry using a fluoride ion-selective electrode. It has been found that ethylenediamine-*N,N,N′,N′*-tetraacetic acid (EDTA) complexes of tetravalent metal ions also form stable mixed ligand complexes with fluoride.[21]

In the present paper, six chelating reagents [EDTA, CDTA, *N′*-(2-hydroxyethyl)ethylenediamine-*N,N,N′*-triacetic acid (HEDTA), diethylenetriamine-*N,N,N′,N″,N″*-pentaacetic acid (DTPA), triethylenetetraamine-*N,N,N′,N″,N‴,N‴*-hexaacetic acid (TTHA) and citrate] have been examined for the determination of fluoride in the presence of zirconium(IV). Using DTPA as a masking reagent, fluoride was successfully determined in some zirconium fluoride compounds and ZrF_4-based glasses. As fluoride in these materials is prone to be replaced by oxygen-containing species such as OH^- and O^{2-} ions, these data will be complementary to the results for the determination of oxygen in fluoride glasses by charged particle activation analysis.[22]

Experimental

Reagents

All the reagents used were of analytical-reagent grade. Potassium nitrate was recrystallized twice. Carbonate-free potassium hydroxide solution was prepared as described elsewhere.[23] Potassium fluoride was dried in a platinum crucible for 24 h at 110 °C. Fluoride solutions were stored in polyethylene containers. Zirconium(IV) stock solution was prepared by dissolving zirconium(IV) oxide nitrate, $ZrO(NO_3)_2$, in a 4 mol dm^{-3} nitric acid solution, which prevents the formation of polymeric hydrolysed species.[24] The concentration of zirconium(IV) was determined by titration with EDTA in 1 mol dm^{-3} HNO_3 at 90 °C using Xylenol Orange.

The zirconium tetrafluoride (Morita Kagaku Kogyo) and potassium hexafluorozirconate (Kanto Chemicals) used as samples were of technical grade.

Measurement

The equipment used was the same as that described previously.[18–21] All the potentiometric measurements were performed at 25 °C and at an ionic strength of 0.1 mol dm^{-3} KNO_3.

The effects of pH and the concentrations of fluoride and zirconium(IV) on the electrode response were studied by utilizing various masking reagents; a series of solutions containing 2.5×10^{-6}–2.5×10^{-4} mol dm^{-3} zirconium(IV), 1.25×10^{-5}–1.25×10^{-3} mol dm^{-3} fluoride ion and 5×10^{-6}–1×10^{-1} mol dm^{-3} masking reagent were titrated with 0.1 mol dm^{-3} potassium hydroxide. After each addition the

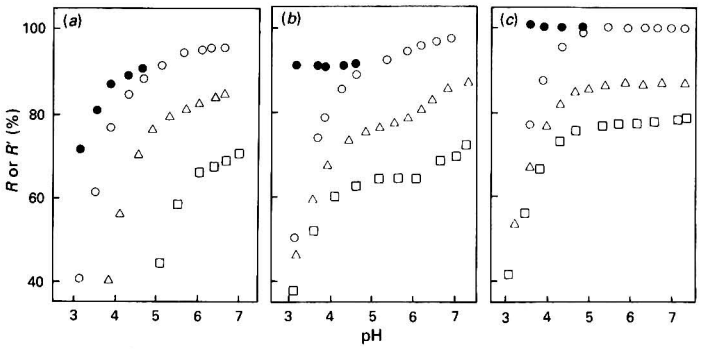

Fig. 1 R or R'(%) *versus* pH. Masking reagent: (*a*) none; (*b*) EDTA; and (*c*) DTPA. c_F/mol dm^{-3}: □, 1.25×10^{-3}; △, 1.25×10^{-4}; and ○, ●, 1.25×10^{-5}. R: □, △ and ○; and R': ●. $c_F : c_{Zr} = 5$. $c_L : c_{Zr} = 2$

pF and pH were measured with a fluoride ion-selective electrode and a fluoride-resistant glass electrode. The recovery of fluoride, $R = [F^-]/c_F$ (c_F = total fluoride concentration), was calculated from the pF values. The recovery, taking the protonation of fluoride into account, R', was also calculated by using the relevant constants and both pF and pH values[18] (K is the formation constant in each instance).

$$R' = ([F^-] + [HF] + 2[HF_2^-])/c_F$$
$$= ([F^-] + K_{HF}[H^+][F^-] + 2K_{HF_2}K_{HF}[H^+][F^-]^2)/c_F$$

Recommended Procedure

A 0.05 g portion of the sample to be analysed is placed in a platinum crucible and covered with 1 g of Na_2CO_3. The crucible is heated at 900 °C for 15 min. The cooled melt is digested with 60–70 cm^3 of 1×10^{-2} mol dm^{-3} DTPA. After dissolution, 20 cm^3 of 1 mol dm^{-3} HNO_3 are added, and the solution is diluted to 250 cm^3. After a further 500-fold dilution the solution is analysed for fluoride.

Results and Discussion

Zirconium(IV) seriously interferes with the determination of fluoride as shown in Fig. 1(*a*). Although R increases with an increase in pH or with dilution of the sample, it does not reach 100% in the pH range suited to the use of the fluoride ion-selective electrodes. Addition of a masking reagent generally improves the recovery. The effects of pH and the concentrations of fluoride, zirconium(IV) and masking reagent were studied for each system (Figs. 1–3).

Effects of pH and Fluoride Concentration

In the presence of a masking reagent, R also increases with pH, steeply in an acidic medium and gradually in a neutral medium [Fig. 1(*b*) and (*c*)]. The increase in R at pH <5 is due to the deprotonation of HF. As the formation of HF is negligible above pH 5, a plateau or an inflection point appears in the graph of R *versus* pH depending on the concentrations of fluoride and zirconium(IV).

At higher concentrations of fluoride and zirconium(IV), the formation of ZrF_n is mainly responsible for the interference in a slightly acidic medium, whereas at lower concentrations of fluoride and zirconium(IV) the interference is caused by ZrLF. [As the fully deprotonated ligands (L) used in this study have different electric charges, the net charges on the zirconium complexes are different to each other and therefore have been omitted for simplicity.]

$$ZrF_n + L' \rightleftharpoons ZrLF + (n-1)F^- \quad (1)$$

$$ZrLF \rightleftharpoons ZrL + F^- \quad (2)$$

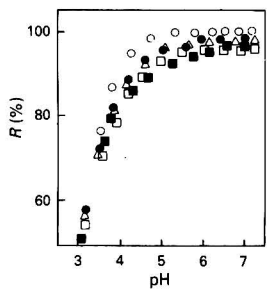

Fig. 2 Comparison of aminopolycarboxylates as masking reagents for Zr^{IV}. Masking reagent: ○, DTPA; ●, TTHA; △, HEDTA; □, CDTA; and ■, EDTA. $c_F = 1.25 \times 10^{-5}$ mol dm^{-3}. $c_{Zr} = 2.5 \times 10^{-6}$ mol dm^{-3}. $c_L = 5 \times 10^{-6}$ mol dm^{-3}

Both equilibria, particularly that given by equation (1), shift to the right by simple dilution of the sample. Hence, the sample should be diluted as much as possible within the dynamic range of the fluoride ion-selective electrodes. Such an effect has also been utilized to eliminate interference from aluminium.[20,25]

The increase in R with pH found for the EDTA system [Fig. 1(*b*)] at pH >6 is ascribed to the replacement of fluoride in the mixed ligand complexes by hydroxyl ions to form ZrL(OH) or $Zr_2L_2(OH)_2$.

$$ZrLF + OH^- \rightleftharpoons ZrL(OH) + F^- \quad (3)$$
$$\downarrow\uparrow$$
$$1/2[Zr_2L_2(OH)_2]$$

The R *versus* pH curves obtained agree well with those calculated using the relevant stability constants.[21,26–28]

Comparison of Masking Reagents

Fig. 2 shows the masking abilities of aminopolycarboxylates for 0.25×10^{-5} mol dm^{-3} zirconium(IV) at a total fluoride concentration of 1.25×10^{-5} mol dm^{-3}. Satisfactory recovery was obtained only with DTPA at pH >5.

As R exceeds 80% for a sample with $c_F : c_{Zr} = 5$ in the neutral pH region (Fig. 2), the average number of fluoride ions bound to zirconium is less than 1. A higher concentration of an aminopolycarboxylate did not give a higher recovery. Hence, the equilibrium [equation (1)] is completely shifted to the right. In such solutions, the following relationships hold:

$$c_F = [ZrLF] + [F^-] \quad (4)$$

$$c_{Zr} = [ZrL] + [ZrLF] \quad (5)$$

$$K^F_{ZrLF} = [ZrLF]/[ZrL][F^-] \quad (6)$$

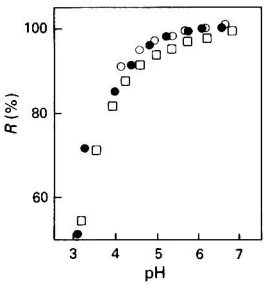

Fig. 3 Effect of citrate concentration on the recovery of fluoride. $c_F = 1.25 \times 10^{-5}$ mol dm^{-3}. $c_{Zr} = 2.5 \times 10^{-6}$ mol dm^{-3}. c_L/mol dm^{-3}: ○, 0.1; ●, 0.01; and □, 0.001

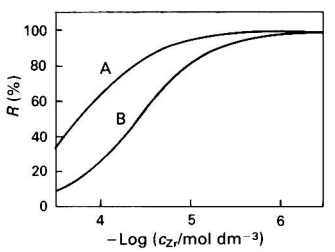

Fig. 4 Calculated recovery of fluoride *versus* $-\log c_{Zr}$ when masking zirconium with A, DTPA and B, EDTA. $c_F = 1.25 \times 10^{-5}$ mol dm^{-3}

For a solution containing known concentrations of fluoride and zirconium(IV), the recovery of fluoride can be calculated from these equations. The formation constant of the mixed ligand complex, K^{E}_{ZrLF}, for DTPA was found to be $10^{3.80}$, whereas that for EDTA has been previously reported[21] to be $10^{4.52}$. Recovery of 1.25×10^{-5} mol dm^{-3} fluoride in the presence of various concentrations of zirconium(IV) was calculated and is shown in Fig. 4. When DTPA was used as a masking reagent, the tolerable amounts of zirconium(IV) for 99 and 98% recovery of fluoride are 2×10^{-6} and 4×10^{-6} mol dm^{-3}, respectively. These values correspond to 16 and 32% as the molar ratio of zirconium(IV) to total fluoride and are sufficient for the analysis of fluoride glasses, because the molar ratios of these glasses are generally lower than 25%. Using EDTA, on the other hand, the tolerable amounts of zirconium(IV) are 4×10^{-7} and 8×10^{-7} mol dm^{-3} corresponding to only 3 and 6%, respectively. The potentially octadentate ligand, DTPA, may be the correct size to form a stable and coordination-saturated complex with zirconium(IV) similar to bis(nitrilotriacetate)zirconium,[29] and the resultant complex has a much lower affinity for fluoride ion.

For citrate (Fig. 3), a slight increase in recovery with an increase in the concentration of citrate from 0.001 to 0.1 mol dm^{-3} indicates a different reaction scheme. Even 0.1 mol dm^{-3} citrate solution, however, has a masking ability inferior to DTPA. Moreover, a higher concentration of citrate results in a prolonged response time of the fluoride ion-selective electrodes, which has been pointed out in relation to the masking of aluminium.[30,31]

Pre-treatment

Dissolution of zirconium fluoride compounds is not easy; *e.g.*, 0.004 g of finely powdered ZrF_4 suspended in 100 cm^3 of a 0.5×10^{-3} mol dm^{-3} DTPA solution stirred continuously required 7 h at pH 6–7 and 3 h at pH 3 to dissolve. Fusion with sodium carbonate was examined as a general method of pre-treatment for the dissolution of zirconium fluoride compounds. After fusion, even a fluoride glass sample could be dissolved in a DTPA solution. As fusion for 15, 30 or 60 min produced the same results, 15 min proved to be sufficient. It was necessary to treat the cooled melt with a DTPA solution before neutralization with nitric acid, in order to avoid prolonging the dissolution time. For samples containing relatively large amounts of zirconium, small amounts of a white precipitate, zirconium hydroxide or hydrated zirconium oxide, were formed during the neutralization of the DTPA solution with nitric acid. As potassium hexafluorozirconate was soluble in water, fluoride could be determined without fusion. When the same sample was pre-treated as described above, a white precipitate was formed but the analytical results were in good agreement with each other; thus the amount of fluoride in the precipitate is negligible. The addition of nitric acid results in a pH of about 6, which gradually increases with time owing to the evolution of CO_2. Neither the precipitation of zirconium compounds nor the increase in pH interferes with the subsequent determination of fluoride. The solutions thus obtained can be kept for at least 1 week without any deterioration.

Table 1 Determination of fluoride in samples containing zirconium

Sample	Fluoride (%) Measured	Calculated
ZrF_4	43.5, 43.6, 44.0	45.5
K_2ZrF_6	37.6, 37.9, 38.3	40.2
ZB glass*	35.1, 35.1, 35.2	37.3
ZBLAN glass†	40.7, 41.2, 41.7	39.1

* $ZrF_4 : BaF_2 = 2 : 1$.
† $ZrF_4 : BaF_2 : LaF_3 : AlF_3 : NaF = 53 : 20 : 4 : 3 : 20$.

Determination of Fluoride in Samples Containing Zirconium

Fluoride in commercially available zirconium fluoride compounds of technical grade and in fluoride glasses was determined using the proposed procedure. Diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid was effective for masking Ba^{2+}, La^{3+} and relatively small amounts of Al^{3+}. The results in Table 1 show a satisfactory reproducibility. Purities of commercial ZrF_4 and K_2ZrF_6 were 96.2 and 94.3%, respectively. The infrared absorption band at 1640 cm^{-1} suggests the presence of strongly adsorbed water molecules.[22]

Prior separation of fluoride by conventional steam distillation was virtually impossible, although a recent paper[32] describes a modified method, which is effective in the presence of zirconium(IV). As demonstrated above, potentiometry with a fluoride ion-selective electrode by using DTPA as a masking reagent is a more convenient and less time consuming method for the determination of fluoride in samples containing zirconium.

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Indirect Determination of Chloride by Gas-diffusion Flow Injection With Amperometric Detection

Snežana D. Nikolić and Emil B. Milosavljević
Faculty of Chemistry, University of Belgrade, P.O. Box 550, 1101 Belgrade, Yugoslavia
James L. Hendrix and John H. Nelson
Departments of Chemistry and Chemical and Metallurgical Engineering, Mackay School of Mines, University of Nevada, Reno, NV 89557, USA

A rapid, indirect gas-diffusion flow injection (FI) method with amperometric detection has been developed for the selective and sensitive determination of Cl^-. The method is based on permanganate oxidation of Cl^- to chlorine. The chlorine diffuses through the micro-porous membrane and is quantified amperometrically at a platinum working electrode. Calibration graphs were linear up to the maximum concentration of Cl^- investigated (10 mmol dm^{-3}). The precision of the technique was better than a relative standard deviation of 1% at 2 mmol dm^{-3} levels and better than 2% at 10 μmol dm^{-3}, with a throughput of 30 samples h^{-1}. At elevated temperatures (50 °C) and higher acidities (5 mol dm^{-3} H_2SO_4), the detection limit was 0.1 μmol dm^{-3} (0.7 ng of Cl^-). The effects of temperature, sample acidity, working potential and interferents on the FI signals were studied. The method was successfully applied to the determination of Cl^- in natural and tap waters.

Keywords: *Gas-diffusion flow injection method; amperometric detection; indirect chloride determination*

The ubiquitous nature of the chloride ion, and its importance, makes the determination of Cl^- one of the most frequently required analyses. It is not surprising, therefore, that a number of flow injection (FI) methods have been developed for this analyte. One of the first FI publications[1] describes the spectrophotometric determination of Cl^- in brackish waters. Other papers utilizing spectrophotometric detection followed.[2–11] An interesting approach to the determination of Cl^- is the combination of FI dialysis with spectrophotometric detection.[2,12] Martínez-Jimenez *et al.*[13,14] determined Cl^- and mixtures of Cl^- and I^-, with use of FI in conjunction with indirect atomic absorption spectrometric detection. The combination of FI and potentiometry for the determination of Cl^- has also been extensively studied. Various ion-selective electrodes[15–26] and a copper-wire indicator electrode[27] were used for this purpose. Zaitsu *et al.*[28] combined FI with turbidimetry in order to develop a method for the determination of Cl^-. The same analyte was determined by FI methods, with use of chemiluminescence[29] and conductivity[30] detection.

A literature search revealed that there are only two FI amperometric methods designed for the determination of Cl^-. Polta and Johnson[31] utilized pulsed amperometric detection to determine Cl^-, which alters the rate of surface oxide formation at the platinum working electrode. A triple-step potential waveform had to be used, hence the method required an apparatus more sophisticated than that required for single-potential amperometry. Also, the method suffers from various interferents and, as these workers pointed out, it is more suitable for detection in liquid chromatographic analysis where sufficient resolution of species is provided. The FI method, with direct amperometric detection of Cl^- at a silver working electrode, developed by Frenzel *et al.*,[32] is also non-selective as any species that forms either insoluble silver salts or stable complexes with Ag^+ would necessarily interfere. Hence, special calibration methods were required.

The present paper describes an approach to the use of amperometric detection for the determination of Cl^-. In the FI manifold developed, the injected analyte is converted on-line into chlorine, which diffuses from the donor stream through the micro-porous membrane into the acceptor solution. The latter carries chlorine to the flow-through amperometric detector, where it is reduced at a platinum working electrode. The cathodic current measured is proportional to the concentration of Cl^- in the original sample or standard. To the best of our knowledge, there are only two FI publications[33,34] that combine gas diffusion with amperometric detection. This is surprising, as the inherent sensitivity of amperometry and selectivity of the gas-diffusion processes render this combination a powerful analytical tool.

Experimental

Reagents and Materials

All the chemicals used were of analytical-reagent grade. The aqueous reagent and standard solutions were stored in polyethylene bottles. De-ionized water was used throughout. A saturated solution of $KMnO_4$ served as the oxidizing agent. It was prepared by boiling the saturated solution with subsequent filtering in order to remove MnO_2 and any excess of $KMnO_4$ that might be present. A stock solution of 0.1 mol dm^{-3} NaCl was prepared from BDH (Poole, Dorset, UK) concentrated volumetric standards, which are certified to have an accuracy within the factor limits of 0.999 and 1.001. Standard Cl^- solutions, which in most of the experiments were made in 3 mol dm^{-3} H_2SO_4, were prepared by diluting aliquots of the stock solution to the appropriate volumes.

Instrumentation and Apparatus

The FI manifold is illustrated in Fig. 1. Two peristaltic pumps were used. One was a Model Mini S-840 (Ismatec, Zurich, Switzerland) and the other was a Model HPB 5400 (Iskra, Kranj, Yugoslavia). The injection valve was a Model 5020 (Rheodyne, Cotati, CA, USA) equipped with a 200 μl sample loop. The gas-diffusion unit, which was obtained from Shenyang Film-Projector Reflector Factory (Shenyang, China), is similar in construction to the Tecator (Högänas, Sweden) Chemifold V gas-diffusion cell. The membrane used, which was of Teflon, was supplied with the unit. All connections were made with 0.5 mm i.d. Teflon tubing except for the long mixing coil (MC_1), which was made from a 0.8 mm i.d. Teflon tube.

The flow-through amperometric cell (Dionex, Sunnyvale, CA, USA), described earlier,[35] consisted of platinum working and counter electrodes. The reference electrode was an Ag–AgCl (1 mol dm^{-3} NaCl) electrode and it was separated from the flowing stream by an ion-exchange Nafion mem-

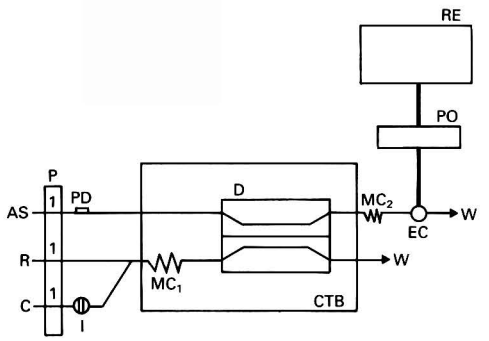

Fig. 1 FI manifold used for the indirect determination of chloride: C, carrier (3 mol dm^{-3} H_2SO_4); R, reagent (saturated $KMnO_4$); AS, acceptor solution (0.01 mol dm^{-3} H_2SO_4); P, peristaltic pump; PD, pulse damper; I, injection valve; MC_1, long mixing coil (2 m × 0.8 mm i.d.); D, diffusion cell; CTB, constant-temperature bath; MC_2, short mixing coil (0.3 m × 0.5 mm i.d.); EC, electrochemical flow-through cell; PO, potentiostat; RE, recorder; and W, waste. Flow-rates are given in ml min^{-1}

brane (all electrode potentials are reported *versus* this reference electrode). The platinum working electrode was polished occasionally with a small amount of toothpaste and a paper tissue. The potential to the flow-through amperometric cell was applied and currents were measured with a Model MA5450 polarograph (Iskra, Kranj, Yugoslavia); the resulting FI signals were recorded on a Model 61 Servograph (Radiometer, Copenhagen, Denmark) strip-chart recorder. The measurements were made with both donor and acceptor streams continuously flowing.

Temperature regulation was achieved with a constant-temperature bath, type NBE (VEB Prüfgerate–Werl, Medingen, Germany).

Results and Discussion

The rate of oxidation of Cl^- to chlorine by permanganate is slow. Hence, in order to apply this reaction in FI, steps must be taken to increase the reaction rate. The logical step is to use the saturated $KMnO_4$ solution as the oxidant, as has been carried out recently in the non-FI method developed for the determination of Cl^- by flame infrared emission.[36]

The effects of several parameters on the performance of the FI system, illustrated in Fig. 1, designed for the indirect determination of Cl^-, were studied.

In order to find a suitable acceptor solution, several potential candidates were tested (H_2O, NaOH, Na_2CO_3, KNO_3 and H_2SO_4). It was found that the optimum signal to noise ratio was obtained with 0.01 mol dm^{-3} H_2SO_4, and in all the subsequent experiments this medium was used as the acceptor solution.

The effect of the applied potential at the working platinum electrode was investigated in the range +0.10 to +0.70 V *versus* an Ag–AgCl reference electrode. The hydrodynamic voltammogram for a 2.50 mmol dm^{-3} sodium chloride standard in 3 mol dm^{-3} H_2SO_4 is shown in Fig. 2. As can be seen, the optimum potential is +0.30 V. However, if selectivity concerns dictate otherwise, slight variations of the applied potential are possible, bearing in mind that at potentials lower than about +0.15 V, the background current becomes too high, probably as a result of the onset of oxonium ion reduction.

The effect of H_2SO_4 concentration on the peak currents was studied by injecting the same Cl^- standard (2.50 mmol dm^{-3}) while varying the concentration of the acid from 1.0 to 5.0 mol dm^{-3}. The data obtained are shown in Fig. 3. As can be

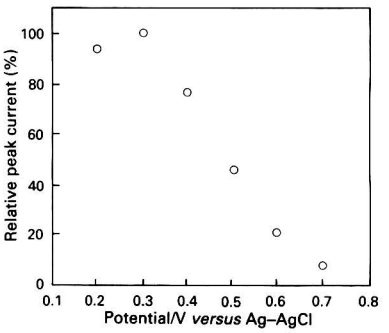

Fig. 2 Hydrodynamic voltammogram for a 200 μl injection of a 2.50 mmol dm^{-3} sodium chloride standard

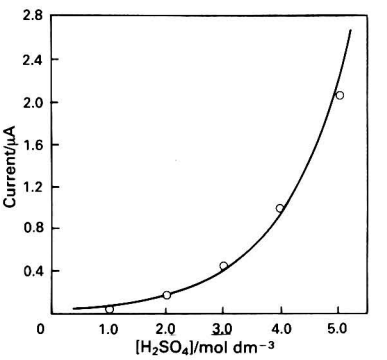

Fig. 3 Variation of peak current as a function of sulphuric acid concentration. ○, Experimental data points; and —, calculated according to the equation $i = \exp(-3.50 + 0.86c)$. For details see text

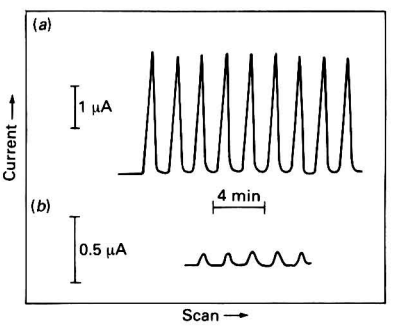

Fig. 4 Response of the amperometric detector to: (*a*) nine repetitive injections of a 2.00 mmol dm^{-3} chloride standard; and (*b*) five repetitive injections of a 10.0 μmol dm^{-3} chloride standard

seen, the current increases exponentially with an increase in H_2SO_4 concentration. The simple equation of the type: $i = \exp(-3.50 + 0.86c)$ (where i is the current in μA, and c is the concentration of H_2SO_4 in mol dm^{-3}) fits the data very well. The corresponding correlation coefficient was found to be 0.9989.

The temperature effects were studied by injecting a 2.50 mmol dm^{-3} sodium chloride standard made in 3 mol dm^{-3} H_2SO_4, while varying the temperature in the interval 30–60 °C. A linear relationship was obtained with an increase in sensitivity of (0.187 ± 0.009) μA $°C^{-1}$, with a correlation coefficient of 0.9975. This finding is interesting, as the change in temperature not only affects the oxidation rate, but also the solubility of the chlorine formed, the diffusion process and the

Table 1 Solutions tested for their possible interference*

Compound	NH_4SCN	CH_3COONa	Na_2HPO_4	Na_2EDTA†	NaF	NH_4NO_3
Concentration/mol dm^{-3}	0.01	0.1	0.1	0.01	0.1	0.1

* The response of the amperometric detector to 200 μl injections of the solutions tested could not be distinguished from the background noise.
† Na_2EDTA = Disodium ethylenediaminetetraacetate.

Table 2 Comparison of FI results for the determination of Cl^- in the presence of Br^- and I^- [all samples contained 2.50 mmol dm^{-3} (88.6 μg ml^{-1}) Cl^-]

Sample	FI/μg ml^{-1}	Difference (%)*
Cl^-	88.6 ± 0.3	0
Cl^-/Br^-†	96.5 ± 0.9	+8.9
Cl^-/I^-‡	88.2 ± 0.7	−0.45
Cl^-/Br^-§	84.3 ± 0.3	−4.8

* Compared to a pure Cl^- (88.6 μg ml^{-1}) standard.
† Cl^- + 1 μg ml^{-1} of Br^-.
‡ Cl^- + 1 μg ml^{-1} of I^-.
§ Cl^- + 1 μg ml^{-1} of Br^- + 0.5 mmol dm^{-3} IO_3^- (bromine expelled by boiling the solution for 10 min).

Table 3 Comparison of Cl^- determination by FI and argentimetric titration in three water samples

	Concentration/μg ml^{-1}		
Sample	Argentimetric	FI	Label
Belgrade tap water	22.0 ± 0.1	21.2 ± 0.2	
Prolom*	4.60 ± 0.08	4.50 ± 0.1	32.0
Knjaz Miloš*	14.0 ± 0.2	12.9 ± 0.2	12.0

* Commercial mineral waters.

reduction of chlorine at a platinum working electrode. The last process is by itself a complicated one. Temperature changes affect the working potential (they change the potential of the reference electrode), and also the diffusive and migratory properties of the analyte, *etc.* As satisfactory sensitivity is achieved even at the lowest temperature studied (30 °C), most of the subsequent experiments were carried out at this temperature.

The linearity studies were conducted by injecting in triplicate a total of eight standards between 0.10 and 10 mmol dm^{-3} made in 3 mol dm^{-3} H_2SO_4. The linear regression equation for a typical calibration run was: $i = (-5.05 \pm 0.04) \times 10^{-3} + (0.164 \pm 0.007) \times c$ (i is the peak current in μA, and c is the concentration of Cl^- in mmol dm^{-3}), with a correlation coefficient of 0.9992 (all the statistics were calculated for a 95% confidence level). The repeatability of the analytical system is illustrated in Fig. 4. For example, the relative standard deviation for a 2.00 mmol dm^{-3} standard was found to be 0.8% (n = 9). The detection limit under these experimental conditions (30 °C; 3 mol dm^{-3} H_2SO_4), calculated according to the recommended procedure,[37] was 5 μmol dm^{-3} of Cl^-. At elevated temperatures (50 °C) and higher acidities (standards were made in 5 mol dm^{-3} H_2SO_4) the detection limit was 0.1 μmol dm^{-3}, which corresponded to 0.71 ng of Cl^- (the sample loop volume was 200 μl).

It has been established previously that PTFE membranes used in the FI gas-diffusion studies are effective barriers for ionic species.[38,39] Nevertheless, a number of anions were tested. The concentrations of these species given in Table 1 are the maximum concentrations at which they were tested. As expected, in all these examples the response of the amperometric detector could not be distinguished from the baseline.

It has been established previously[34] that anions such as NO_2^-, SO_3^{2-}, CO_3^{2-}, $S_2O_3^{2-}$, CN^- and S^{2-}, which, when acidified, form acidic gases, could potentially interfere when a particular amperometric flow-through cell is used. These anions at sufficiently high concentrations, even if they are not electroactive at the working potential, could interfere in an indirect manner. The Ag–AgCl reference electrode in the configuration used is separated from the flowing stream by the ion-exchange Nafion membrane. If the buffer capacity of the acceptor solution is too low, there will be a significant change in pH when the acidic gases, formed by on-line acidification in the FI manifold, diffuse through the Teflon membrane and are trapped in the acceptor solution. This pH change alters the potential of the reference electrode assembly, which is probably induced by a shift in the ion-exchange equilibria at the Nafion membrane. However, in the method developed in this work, the standards and samples are acidified off-line as they are made in 3 mol dm^{-3} H_2SO_4, so that the aforementioned anions would not interfere in the determination of Cl^-, as they are evolved prior to injection. (**Danger!** If some of the aforementioned anions are present, the acidification of the samples should be performed with all due precautions as in some instances poisonous gases are formed.)

Other potential interferents are the anions that can be oxidized on-line by permanganate to form molecular species. If these species diffuse through the membrane and are reducible at the platinum electrode at the applied potential, they would cause a positive error in the determination of Cl^-. Likely candidates are Br^- and I^-. It has been established that Br^- interferes, whereas I^- does not. Under the experimental conditions used for the determination of Cl^-, it is probable that Br^- is mainly oxidized to bromine, while I^- yields higher oxidation states, which form ionic species. Bromide and I^- can be present in natural waters up to levels of 1 and 0.1 μg ml^{-1}, respectively. As can be seen from Table 2, a 2.50 mmol dm^{-3} Cl^- standard spiked with Br^- at 1 μg ml^{-1} levels increases the signal by 8.9%. Kubala *et al.*,[36] in their flame infrared emission method for the determination of Cl^-, utilized the iodate pre-treatment method, which is usually applied to the determination of Cl^- by classical argentimetric procedures:

$$IO_3^- + 6Br^- + 6H^+ \rightarrow I^- + 3Br_2 + 3H_2O$$

This pre-treatment produces bromine, which can be boiled out of the solution, and I^-, which was shown not to interfere with the FI method for the determination of Cl^-. The applicability of this pre-treatment method is also illustrated in Table 2, from which it can be seen that iodate treatment decreases the absolute percentage difference from the pure Cl^- standard by about 50% (see Table 2).

In order to illustrate the potential of the indirect FI/gas-diffusion/amperometric method for the determination of Cl^-, three water samples have been analysed. Table 3 compares the values obtained with those given by argentimetric titrations. It is interesting to note that FI results always gave slightly lower values than those of argentimetric titrations. This could be explained by the fact that argentimetric titrations are influenced by the presence of anions such as phosphate, which might be present in the water samples analysed. Therefore, considering that the titration method is not error free, the agreement between the results obtained by the two methods is very good.

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Determination of Manganese(II) by a Photoactivated, Catalytic Method

Juergen Mattusch and Gerhard Werner
Department of Chemistry, Karl-Marx-University Leipzig, Talstr. 35, Leipzig 7010, Germany

Helmut Mueller
Department of Chemistry, Technical University "Carl Schorlemmer," Leuna-Merseburg, Otto-Nuschke-Str., Merseburg 4200, Germany

A method is described for the determination of Mn^{II} based on the photoactivated oxidation of sulphite using the specific photosensitizer Rose Bengal. The reactivity of Mn^{II} is approximately ten times higher for the photosensitized, acetylacetone-activated reaction than for the system that is only chemically activated. The determination of Mn^{II} is not interfered with by a large excess of Co^{II}, Cu^{II}, Ni^{II}, Cr^{III}, Cd^{II} or Fe^{III}. The detection limit of the proposed method is 0.3 ppb with a relative standard deviation of about 5.5%. A proposal for a possible reaction mechanism is made on the basis of spectrophotometric measurements. The catalytic method described was used for the determination of Mn^{II} in drinking water.

Keywords: *Kinetic catalytic method; photosensitization; chemical activation; trace analysis; manganese(II) determination*

Kinetic or reaction-rate methods of chemical analysis utilize the dynamic properties of reaction systems. Catalytic determinations are the most widely used of the kinetic methods. In the last 10 years many such methods have been developed.[1-5] Using a catalytic reaction one can determine extremely small concentrations of the catalyst through an increase in the reaction rate because a catalytic species may participate in a large number of cycles of the catalytic reaction. The best way to increase the rate of a catalysed reaction (and thereby the sensitivity while decreasing the limit of detection) is through the use of activators.[4,5] Activation is understood from a catalytic point of view as the increase in the rate of a catalysed reaction resulting from the action of a chemical species (the activator) that takes part in a step for which the activation energy is lower than that involving the catalyst only (classical chemical activation). The effect of irradiation on catalysed reactions has been studied and the influence of light on their rate and selectivity (photoactivation) has been shown.[6]

Such photoactivation applied to the photosensitization of catalysed reactions can be used as an alternative to classical activation. Photoactivation can be subdivided into: direct photoactivation of a reactant; indirect photosensitization; and catalyst photoactivation. In this work, the analytical utility of the activation of the catalyst Mn^{II} by photosensitization has been examined. Veprék-Šiška and co-workers[7,8] have demonstrated that the auto-oxidation of sulphite is catalysed by metal ions. Both the thermal and the photochemical or photo-initiated oxidation of sulphite proceed *via* the same mechanism.[7-10] The reactive intermediates of the thermal reaction are probably ternary complexes of the metal ion, *e.g.*, Cu^{II}, with sulphite and oxygen. Also, in the photo-initiated auto-oxidation of sulphite the catalytic action of Fe^{III} is connected with the existence of sulphitoiron(III) complexes.[6,11-13] In the present paper the photosensitized auto-oxidation of sulphite, catalysed by Mn^{II} ions, was examined in detail.

Experimental

Materials

All chemicals and reagents were of analytical-reagent grade. Acetylacetone (acac) (Merck) was distilled before use. The other chemical activators, *i.e.*, 1,10-phenanthroline (phen), ethylenediamine (en), nitrilotriacetic acid (nta), 2,2'-bipyridine (bpy), histidine (his) and oxalate (ox) were used as received. A 0.3 mol dm^{-3} stock solution of Na_2SO_3 was prepared fresh daily by dissolving 3.78 g of Na_2SO_3 in 100 ml of doubly distilled water. The 0.1 mol dm^{-3} buffer solution was obtained by dissolution of sodium tetraborate decahydrate (38.14 g l^{-1}). The Mn^{II} stock solution was prepared by dissolution of $Mn(NO_3)_2$. The exact amount of Mn^{II} was determined by complexometric titration.[14]

Apparatus

Continuous photolysis was performed with a 500 W superpressure mercury lamp (HBO 500). Excitation was at 546 nm with a 10 nm bandpass. The reaction solution was contained within a 50 ml glass vessel and was stirred magnetically. The continuous oxygen consumption during the reaction time was measured with an oxygen-selective electrode of the Clark-type (Meinsberg, Germany). The reaction rate is expressed as oxygen consumption during a defined time ($v = -\Delta[O_2]/\Delta t$) in mg O_2 l^{-1} s^{-1} in all instances. Detailed information about the absorbance of the photosensitizer and the reaction intermediates was obtained with a Specord spectrophotometer (Jena, Germany).

Procedure

A 2 ml volume of 0.1 mol dm^{-3} buffer solution, 6 ml of a 0.01 mol dm^{-3} solution of acac, 0.02 ml of 4×10^{-4} mol dm^{-3} Rose Bengal (RB) and appropriate amounts of Mn^{II} were mixed with doubly distilled water to obtain a volume of 50 ml. The injection of 0.2 ml of sulphite stock solution was carried out at the beginning of the irradiation of the reaction solution in order to initiate the reaction. The oxygen consumption was measured for a fixed time interval.

Results and Discussion

Photosensitized Activation

Preliminary experiments were performed to determine the general influence of the photosensitizer on the catalysed and uncatalysed auto-oxidation of sulphite. The use of Methylene Blue (MB) as a photosensitizer at a wavelength of 575 nm, which approximately agrees with the absorption band of the dye, *i.e.*, 665 nm, has shown that non-optimum excitation conditions existed leading to a fast uncatalysed reaction and a

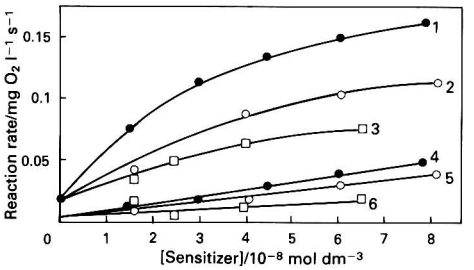

Fig. 1 Dependence of the reaction rate on the concentration of the sensitizer with 1–3, the catalysed and 4–6, the uncatalysed reaction. 1 and 4, RB; 2 and 5, MB; and 3 and 6, Rh. 1.2 mmol dm^{-3} SO_3^{2-}; 15.4 μmol dm^{-3} Mn^{II}; and HBO 500, λ = 546 nm

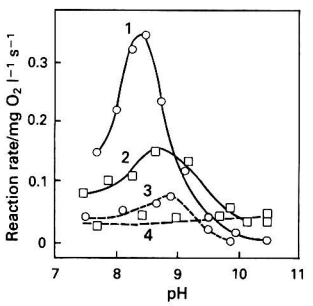

Fig. 2 pH-dependence of the reaction rate with different buffer systems: solid line, borate buffer; and broken line, non-complexing buffer. 1 and 3, catalysed reaction; 2 and 4 uncatalysed reaction. Experimental conditions: 1.2 mmol dm^{-3} SO_3^{2-}; 4 × 10^{-8} mol dm^{-3} RB; HBO 500, λ = 546 nm; and 15.4 μmol dm^{-3} Mn^{II}

low catalytic activity of Mn^{II}. Unfortunately, the catalysed reaction was not accelerated by increasing the concentration of MB. Therefore, it was necessary to find the optimum combination of photosensitizer, irradiation wavelength and catalyst. Hence, a number of relevant triplet sensitizers and irradiation with the intensive emission band of the lamp at 546 nm were tested. The reaction-rate dependence on dye concentrations for the catalysed and uncatalysed reactions is shown in Fig. 1. Of the dyes investigated only Rhodamine B (Rh), MB and RB sensitized the catalytic reaction. The most effective photosensitizer was RB, firstly, because its absorption band corresponds to the irradiation wavelength at 546 nm, and secondly, because RB is highly effective for intersystem crossing (ISC) (quantum yield $\phi = 0.8$ for ISC) and other irradiationless de-activation processes.[15] It can be concluded from Fig. 1 that the activating influence of the photosensitizer attains a maximum at a concentration of about 4 × 10^{-8} mol dm^{-3}. The calibration graphs over the range 0–1 × 10^{-8} mol dm^{-3} Mn^{II} in unbuffered solution for different RB concentrations were not suitable for analysis because of the non-linearity of the graphs. Another disadvantage was the fast uncatalysed reaction compared with the catalysed reaction. The pH dependence of the photosensitized auto-oxidation of sulphite catalysed by Mn^{II} was determined in the pH range 7–10. The results for two buffer systems are shown in Fig. 2, which indicates that the catalytic activity of Mn^{II} attains a maximum at pH 8.4 and drops sharply for pH values different from 8.4 using the borate buffer system. No catalysed dark reaction could be observed in the pH range investigated. Finally, with optimized reaction conditions for the Mn^{II}-catalysed reaction [1.2 × 10^{-2} mol dm^{-3} sulphite, 4 × 10^{-8} mol dm^{-3} RB, pH 8.4 (borate), T = 20 °C and HBO 500, λ = 546 nm] a linear calibration graph was obtained from 8 to 50 ppb of Mn^{II}. The relative standard deviations (RSDs) for 8 and 42 ppb of Mn^{II} are typically 10 and 6.4%, respectively.

Table 1 Comparison of the action of chemical activators and photosensitizers

Sensitizer/activator	Concentration/ mol dm^{-3}	Reaction rate*/ mg O_2 l^{-1} s^{-1}
Rose Bengal, *hν*	4 × 10^{-8}	0.069
Rose Bengal, *hν*	4 × 10^{-7}	0.240
1, 10-Phenanthroline	8 × 10^{-4}	0.031
Histidine	1 × 10^{-3}	0.018
Acetylacetone	1.2 × 10^{-3}	0.015
Nitrilotriacetic acid	8 × 10^{-7}	0.108

* Reaction rate = $v_{catalysed} - v_{uncatalysed}$. Reaction conditions: 1.2 mmol dm^{-3} SO_3^{2-}, pH = 8.4, 0.62 μmol dm^{-3} Mn^{II}.

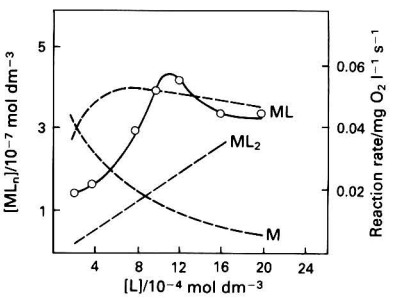

Fig. 3 Comparison of the reaction rate on complex formation of the different forms of the Mn^{II}–acac complex: solid line, reaction rate; and broken line, concentration of the different complex forms. Experimental conditions: 0.62 μmol dm^{-3} Mn^{II}; pH = 8.4; and 1.2 mmol dm^{-3} SO_3^{2-}

Chemical Activation

The detection limit of the catalytic method should be improved further by combining the photoactivated system with the chemical activation of Mn^{II}. At the same time the chemical activator should be capable of decreasing the rate of the uncatalysed photosensitized reaction. The influence of different ligands which are known to increase the catalytic activity of Mn^{II} was compared with the action of light and RB.

A comparison of the reaction rates presented in Table 1 indicates that only the activation by nta is comparable to the activation by photosensitization. The activation properties of the ligands can be expressed in order of decreasing efficiency as follows: nta > phen > his > acac >> ox≈en≈bpy. The dependence of the reaction rate on the concentration of nta shows that the most catalytically active form is the 1 : 1 complex. This complex is able to coordinate oxygen or sulphite or both. Nitrilotriacetic acid also stabilizes the +3 state of the catalyst [log β (Mn^{III}, nta) = 20.35],[16] which probably initiates the radical chain reaction of sulphite. The use of nta as an activator for Mn^{II} using the indicator reaction of Malachite Green and periodate has been described by Mottola and Heath.[17] Similar results to those for nta have been obtained with acac, where again the 1 : 1 complex was the most effective form of the catalyst (Fig. 3). On the basis of the following equilibrium constants, log β (Mn^{II}, acac) = 4.24 and log β_2 (Mn^{II}, acac) = 7.35,[16] plots of simulated complex equilibria (COMICS[18]) of the Mn^{II}–acac complexes indicate that the 1 : 1 complex is the most effective form. The slight difference between the maxima of reaction rate and complex formation of the 1 : 1 complex can probably be attributed to a kinetic hindrance. The activation of the catalytic reaction by en, bpy or ox is negligible. With the activation of the auto-oxidation of sulphite by ligands such as phen, his and acac, the determination of Mn^{II} is possible with a detection limit of greater than 2 ppb. The ligand nta is apparently the most effective activator for Mn^{II} with a detection limit of 1.1

Table 2 Influence of interfering ions on the photosensitized, acac-activated and thermal, acac-activated methods

Interfering ion	Ratio of [Mn^{II}] to [interfering ion] Photosensitized*	Thermal†	Ratio of photosensitized to thermal method
Co^{II}	1:100	1:0.08	1250
Cu^{II}	1:500	1:2.5	200
Ni^{II}	1:650	1:23	28
Cr^{III}	1:585	1:20	29
Cd^{II}	1:1000	1:1000	1
Fe^{III}	1:2000	1:13	160
Sulphate	$1:3 \times 10^6$	$1:3 \times 10^6$	1
Nitrate	$1:1.3 \times 10^5$	1:6500	20
Phosphate	—	1:1300	—

* 3.1×10^{-8} mol dm^{-3} Mn^{II}. Conditions for the photosensitized, acac-activated method: 1.2 mmol dm^{-3} SO_3^{2-}; 1.2 mmol dm^{-3} acac; 1.6×10^{-7} mol dm^{-3} RB; pH = 8.4; and HBO 500, λ = 546 nm.

† 6.2×10^{-7} mol dm^{-3} Mn^{II}. Conditions for the thermal, acac-activated method: 0.6 mmol dm^{-3} SO_3^{2-}; 1.2 mmol dm^{-3} acac; and pH = 8.4.

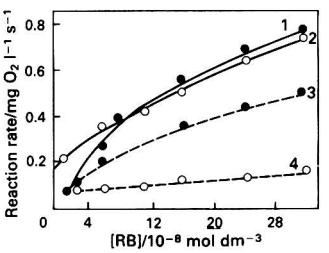

Fig. 4 Dependence of the reaction rate on the RB concentration in the presence (1 and 3) and absence (2 and 4) of acac: solid line, catalysed reaction; and broken line, uncatalysed reaction. Experimental conditions: 1.2 mmol dm^{-3} SO_3^{2-}; 0.62 μmol dm^{-3} Mn^{II}; and pH = 8.4

ppb, but the linear range of the calibration graph depends strongly on the Mn : nta ratio. It is also possible to determine the nta concentration by this method. The interference levels for several metal ions and anions are summarized in Table 2. Data in the second column of Table 2 indicate that Co^{II} catalysed the indicator reaction more effectively than did Mn^{II}. The interference levels of Cu^{II}, Ni^{II}, Cr^{III} and Fe^{III} are fairly close to the Mn^{II} concentration. A comparison of the method proposed above with the Mn^{II}-catalysed reaction between iodide and periodate[19] shows only a small improvement in the selectivity of the proposed method in most instances.

Combination of Photosensitized and Chemical Activation

The first two parts of this paper have shown the improvement of the sensitivity of catalytic methods by chemical activation of Mn^{II} and also by photosensitization. Therefore, it was of interest to investigate the simultaneous influence of irradiation with light and chemical activation by the ligand. Preliminary experiments for the characterization of the catalytically most active form of the catalyst in the photoactivated system provided the same results as for chemical activation alone. The combination of photoactivation and chemical activation led to a change in the efficiency of the ligands used as follows: acac > nta > his > phen >> bpy > ox ≈ en.

The chemical activator acac has two significant attributes, shown in Fig. 4. Firstly, the formation of a complex between Mn^{II} and this ligand improves the catalytic action of the catalyst and secondly, the uncomplexed ligand decreases the

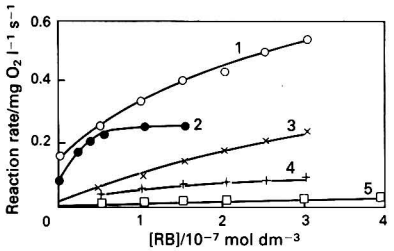

Fig. 5 Dependence of the reaction rate on the concentration of the sensitizer for different chemical activators. Reaction rate = $v_{catalysed} - v_{uncatalysed}$ (reaction rate in mg O_2 l^{-1} s^{-1}). 1, 1.2 mmol dm^{-3} acac; 2, 2 μmol dm^{-3} nta; 3, 0.8 mmol dm^{-3} phen; 4, 0.4 mmol dm^{-3} his; and 5, en, ox, bpy. Experimental conditions: 1.2 mmol dm^{-3} SO_3^{2-}; 0.62 μmol dm^{-3} Mn^{II}; and pH = 8.4

Table 3 Comparison of the analytical parameters of the thermal, photosensitized and combined method with acac as chemical activator and RB as photosensitizer

Method	Parameter: Detection limit (ppb)	RSD (%)
Thermal	1.8	3.3
Photosensitized	8.0	9.5
Combined	0.3	5.5

rate of the uncatalysed reaction. Therefore, it is possible to increase the RB concentration in order to enhance the detection limit further (Fig. 5). The influence of the light intensity on the acceleration of the catalysed reaction was investigated with a continuous argon laser. The excitation occurred at 514.5 nm. The experiments have shown that the reaction rate depends more strongly on the light intensity than on the concentration of the photosensitizer. Unfortunately, because the use of powerful light sources in analytical chemistry is limited, an increase in the photosensitizer concentration was necessary in order to improve the sensitivity. With the optimized reaction conditions [1.2 mmol dm^{-3} SO_3^{2-}, pH 8.4 (4 mmol dm^{-3} borate), T = 20 °C, HBO 500, λ = 546 nm] a linear correlation between the reaction rate and the concentration of Mn^{II} exists from the detection limit up to approximately 9 ppb of Mn^{II}. The RSDs for 0 and 8.5 ppb of Mn^{II} are 5.5 and 1.3%, respectively. The average and RSD values were obtained from five parallel measurements. With the RSD of the uncatalysed reaction the detection limit for Mn^{II} using the photosensitized, chemically activated reaction was calculated to be 0.32 ppb of Mn^{II}. A comparison of the analytical parameters of the three proposed methods is presented in Table 3. It can be seen from the results that the combination of photoactivation with chemical activation yields the maximum sensitivity. The first column of Table 2 lists the ratio of Mn^{II} to interfering ion, when the interference began (average ± 2 RSD). A comparison of the first and second columns shows the further improvement of selectivity in most instances by the choice of a selective excitation of the catalyst by photosensitization. The reason for the differences in selectivity appears to be due to the different photochemical reaction pathways used to oxidize the sulphite radicals. As described in the introduction the photo-reduction process, *e.g.*, for the Fe^{III} ion, occurs *via* complex formation of Fe^{II} with sulphite, absorption of light by this complex at approximately 300–350 nm and electron transfer in the excited state. The irradiation of the reaction solution with light which cannot be absorbed by this complex leads to the same behaviour of the Fe^{III} ions as in the non-photoactivated, acac-activated reaction. The enhanced reactivity of Mn^{II} in contrast to Fe^{III}

or Co^{II} arises from the possibility of excitation of the Mn^{III}–acac complex by photosensitization with RB and the acceleration of the Mn^{III} reduction process by sulphite. The chain reaction of the sulphite radicals formed in the previous reaction increases the catalytic reactivity of Mn^{II}. It should be noted that the reaction mechanism was not investigated in detail. However, the formation of Mn^{II} during the catalytic reaction was detected by complex formation with pyrophosphate and by optical absorption spectrometry of the Mn^{III}–acac complex. Fig. 6 shows that the conversion of Mn^{III}–acac into $[Mn(H_2P_2O_7)_3]^{3-}$ led to a decrease in the catalytic activity of Mn^{2+} at $[P_2O_7^{4-}] > 1 \times 10^{-5}$ mol dm^{-3}. Pyrophosphate is known to stabilize manganese in the +3 state.[20] Further support for Mn^{III} is provided by spectrophotometric observation. The slow oxidation of Mn^{II} by oxygen can be accelerated by using acac and a ligand.[21] In aqueous solution (pH 8.4) the Mn^{II}–acac complex shows an absorption band at 294 nm. In the presence of oxygen (0.2 mmol dm^{-3}) and perchlorate (1.5 mmol dm^{-3}) the absorption of the Mn^{II}–acac complex was changed. The 294 nm band disappeared and was replaced by a band at 222 nm. This band originates from Mn^{III}(aq) which is stable in the presence of perchlorate. This value shows good agreement with that previously measured by Wells and Davies[22] and Bielski and Chan.[23] Without oxygen no Mn^{III} was observed.

The photosensitized oxidation of Mn^{II} to Mn^{III} was five times faster than the corresponding dark reaction. Other photochemical experiments suggested that the uncatalysed reaction occurs *via* singlet oxygen ($^1O_2$). The reaction can be inhibited by a powerful quencher of $^1O_2$ such as his or acac. Another fact is that the triplet state of chlorophyll or benzophenone is quenched by Mn^{II}.[24,25] The triplet energies (E^t) of chlorophyll and RB of 13 200 and 13 800 cm^{-1}, respectively, are similar, which means that a similar energy transfer from the photosensitizer to the catalyst should be possible. With the experimental data obtained the catalysed reaction mechanism of the photosensitized auto-oxidation of sulphite seems to be a combination of two amplification processes; firstly, the catalytic cycle of Mn^{II}–Mn^{III} and secondly, the chain reaction of sulphite to sulphate initiated by Mn^{III}.[26] The uncatalysed reaction occurs *via* sulphite oxidation by singlet oxygen. The reaction mechanism is shown schematically below (L represents the ligand and the asterisk indicates the activated complex).

Catalysed reaction scheme:

$$\mathrm{RB} \xrightarrow{h\nu} {}^3\mathrm{RB}$$
$${}^3\mathrm{RB} + \mathrm{Mn^{II}L} \longrightarrow {}^3\mathrm{RB}\ldots\mathrm{Mn^{II}L}$$
$${}^3\mathrm{RB}\ldots\mathrm{Mn^{II}L} \longrightarrow \mathrm{RB} + {}^*\mathrm{Mn^{II}L}$$
$${}^*\mathrm{Mn^{II}L} + \mathrm{O_2} \longrightarrow \mathrm{Mn^{III}(aq)} + \mathrm{O_2^-} + \mathrm{L}$$
$$\mathrm{Mn^{III}(aq)} + \mathrm{SO_3^{2-}} \longrightarrow \mathrm{Mn^{II}(aq)} + \mathrm{SO_3^{\cdot -}}$$
$$\mathrm{SO_3^{\cdot -}} + \mathrm{O_2} \longrightarrow \mathrm{SO_5^{\cdot -}}$$
$$\mathrm{SO_5^{\cdot -}}\ (\text{chain reaction}) \xrightarrow{\mathrm{O_2}} \mathrm{SO_4^{2-}}$$
$$\mathrm{Mn^{II}(aq)} + \mathrm{L} \longrightarrow \mathrm{Mn^{II}L}$$

Uncatalysed reaction scheme:

$${}^3\mathrm{RB} + {}^3\mathrm{O_2} \longrightarrow {}^1\mathrm{RB} + {}^1\mathrm{O_2}$$
$${}^1\mathrm{O_2} + \mathrm{SO_3^{2-}} \longrightarrow \mathrm{SO_3^{\cdot -}} + \mathrm{O_2^-}$$
$$\mathrm{SO_3^{2-}} + {}^3\mathrm{O_2} \longrightarrow \mathrm{SO_5^{\cdot -}}$$
$$\mathrm{SO_5^{\cdot -}}\ (\text{chain reaction}) \xrightarrow{\mathrm{O_2}} \mathrm{SO_4^{2-}}$$

The applicability and accuracy of the proposed photosensitized, acac-activated method for the determination of Mn^{II} were tested on simulated and natural drinking water. The simulated drinking water (SDW) was prepared by mixing the individual components. The composition of the water based on World Health Organization (WHO) standard values is summarized in Table 4. The average values of five parallel measurements are given in the first column of Table 5. For comparison the data of the acac-activated (thermal) reaction are given in the second column. Only the photosensitized, acac-activated method makes it possible to determine Mn^{II} in this complex matrix with sufficient accuracy. A 0.1 ml volume of natural drinking water was directly introduced into the reaction solution. The concentration of Mn^{II} was in the range 0.09–0.13 mg l^{-1} depending on the source of the water and the day on which the sample was taken.

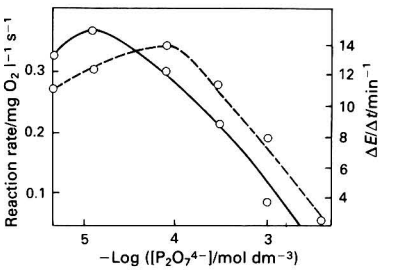

Fig. 6 Dependence of the reaction rate on the pyrophosphate concentration; reaction was recorded by: solid line, oxygen consumption and broken line, changes of the absorption of sulphite at 231 nm. Experimental conditions: 1.2 mmol dm^{-3} SO_3^{2-}; pH = 8.4; 4×10^{-8} mol dm^{-3} RB; 1.5×10^{-5} mol dm^{-3} Mn^{II}; and HBO 500, λ = 546 nm

Table 4 Composition of the SDW and WHO standard values*

Component	WHO/ mg l^{-1}	SDW/ mg l^{-1}	Component	WHO/ mg l^{-1}	SDW/ mg l^{-1}
Pb	0.05	0.1	Ca	220	80
As	0.05	0.05	Mg	125	75
Cr	0.05	0.05	Ni	—	0.02
Cd	0.005	0.02	Co	—	0.015
Ba	1.0	0.7	F^-	1.7	0.95
Fe	1.0	1.0	NO_3^-	45	50
Mn	0.5	1.1	P_2O_5	5	5
Zn	15	10	Cl^-	600	350
Cu	0.05	0.13	SO_4^{2-}	400	290
Ag	0.1	0.1	BO_3^{3-}	—	0.2

* See reference 27.

Table 5 Comparison of the photosensitized and thermal acac-activated methods

Parameter	Method: Photosensitized	Method: Thermal
Average concentration/mg l^{-1}	1.145	0.850
SD/mg l^{-1}	0.033	0.010
RSD (%)	2.9	1.4
Theoretical concentration/mg l^{-1}	1.100	1.100
Deviation from the theoretical value (%)	+4.1	−27.7

Conclusion

The proposed method for the determination of Mn^{II} is based on a combination of photoactivation with chemical activation of the catalyst by ligands. The reactivity of Mn^{II} is approximately ten times higher for the photosensitized, acac-activated reaction than for the system that is only chemically activated. For monitoring the reaction rate, the oxygen consumption was measured with an oxygen-selective Clark electrode. The determination of Mn^{II} is possible in the presence of a 100–1000-fold excess of Co^{II}, Cu^{II}, Ni^{II}, Cr^{III} and Cd^{II}. Iron(III) interferes at about a 2000-fold excess. The detection limit of the proposed method is 0.3 ppb with an RSD

of about 5.5%. The energy transfer from the triplet RB to the Mn^{II}–acac complex represents the photoactivation. The oxidation of the excited Mn^{II} complex by oxygen is therefore accelerated. The reaction of Mn^{III} with sulphite initiates the chain reaction to form sulphate. The catalytic method described was used for the determination of Mn^{II} in drinking water.

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Determination of Citric Acid Based on Inhibition of the Crystal Growth of Calcium Fluoride

F. Grases, A. Costa-Bauzá and J. G. March
Department of Chemistry, University of Balearic Islands, 07071-Palma de Mallorca, Spain

Inhibition of the growth of calcium fluoride crystals in the presence of citrate was followed using a kinetic–potentiometric technique and a calcium ion-selective electrode, and as a consequence, a method for the determination of citrate in the range 0.5–2.4 μg ml^{-1} has been developed. The method was successfully applied to the determination of citrate contained in pharmaceutical products and urine. Urine analysis requires prior separation of phosphate, sulphate and magnesium(II). Elimination of these interferences was studied and accomplished using precipitation processes. Magnesium and phosphate were jointly eliminated in basic media by the addition of ammonium ions. Phosphate and sulphate were eliminated with barium(II). Phosphate was also eliminated as a lithium salt.

Keywords: *Citric acid determination; calcium fluoride; crystal growth; urine analysis*

The determination of citric acid in various biological and non-biological samples has aroused increasing interest in recent years. A range of determinations in food[1–11] and pharmaceutical preparations[12,13] have been described. The determination of citrate in urine is also very important clinically because of its effective inhibitory action on calcium oxalate urolithiasis.[14–16]

Common methods for the determination of citric acid can be classified into three main groups: gravimetric and volumetric methods;[12,17–19] enzymic analyses;[20–23] and chromatographic determinations, mainly high-performance liquid chromatography (HPLC).[1–11] Enzymic and HPLC procedures present notable advantages when the determination of citric acid is performed in a complex matrix. Owing to the specificity and selectivity of these techniques, sample preparation is minimal. A very simple spectrophotometric procedure has been proposed for the determination of citric acid in urine through the formation of a yellow complex with Fe^{III} after the prior separation of phosphate through its precipitation using magnesium and ammonium ions before the complexation.[24]

In the present work, the inhibition of the growth of calcium fluoride seed crystals by citrate was investigated. As a consequence, a kinetic method for the determination of citrate is proposed; this method was applied to several samples. The use of inhibition processes in crystal growth has also been demonstrated by determining phytic acid using seed crystals of calcium oxalate monohydrate[25] and by determining phosphate ions using calcite (calcium carbonate) seed crystals.[26]

Experimental

Reagents and Apparatus

Sodium fluoride, calcium chloride, acetic acid and ammonia were purchased from Panreac (Barcelona, Spain), and sodium citrate (disodium salt) and calcium fluoride from Probus (Barcelona, Spain). All reagents used were of analytical-reagent grade. A suspension of calcium fluoride seed crystals was prepared by mixing 5 g of calcium fluoride and 25 g of water, then stirring magnetically for 48 h or more (aged seed). Enzymic citric acid analyses were performed using a Test-combination supplied by Boehringer Mannheim (Catalogue No. 139076).

Potentiometric measurements were obtained using a Crison (Barcelona, Spain) 2002 micropotentiometer equipped with a calcium ion-selective electrode [Ingold (Urdorf, Switzerland)] coupled with a silver–silver chloride reference electrode separated from solution with an intermediate junction containing potassium chloride. The seed crystals were characterized by using a Hitachi S-530 scanning electron microscope.

Procedure for the Determination of Citric Acid

To a 250 ml glass beaker, the following solutions were added: 8 ml of acetic acid–ammonium acetate buffer solution (total concentration 5 mol dm^{-3}, pH 6.0); 2 ml of 0.10 mol dm^{-3} calcium chloride; and sufficient citrate solution to give a final concentration in the final volume (200 ml made up with water) of 0.5–2.4 μg ml^{-1}. This solution was stirred magnetically at 500 rev min^{-1} with a synchronous motor stirrer (controlled agitation is always essential in order to keep the slurry in suspension), and 1 ml of aged calcium fluoride suspension was added. The electrodes were immersed in the resulting suspension and, when the potentiometer gave a constant reading (after a few seconds), 0.70 ml of a 0.80 mol dm^{-3} sodium fluoride solution was added, which initiated crystal growth, and consequently a decreasing electrical response which was measured continuously on a chart recorder. The calibration graph was obtained from the difference, in millivolts, between the suspension that contained citrate and the suspension that did not (blank), 5 min after the addition of fluoride. All experiments were carried out at room temperature (25 °C).

Procedure for Urine Analysis

A 2 ml aliquot of urine and 1 ml of 5 mol dm^{-3} ammonia solution were placed in a test-tube, the resulting turbid solution was warmed at 90 °C for 30 min and after cooling, filtered through a disposable 0.22 μm filter membrane coupled to a syringe. The filtering system was washed out with 1 ml of water, then 1 ml of 0.35 mol dm^{-3} barium chloride was added and the warming and filtering procedure repeated. An aliquot of the resultant solution was used for the determination of citrate using the procedure described earlier.

Results and Discussion

Effects of Polycarboxylic Acids on the Crystal Growth of Calcium Fluoride

The growth of calcium fluoride seed crystals was studied in the presence of several di- and tricarboxylic acids. As can be seen in Fig. 1, the growth of calcium fluoride crystals was notably delayed by the presence of some carboxylic acids, such that citric acid and oxalic acid delayed crystal formation for the longest and shortest periods, respectively. However, succinic, glycocholic and malonic acids had no significant effect under the experimental conditions used. These results demonstrate that the presence of two or more adjacent carboxylic acid groups permits very effective adsorption of the organic acids

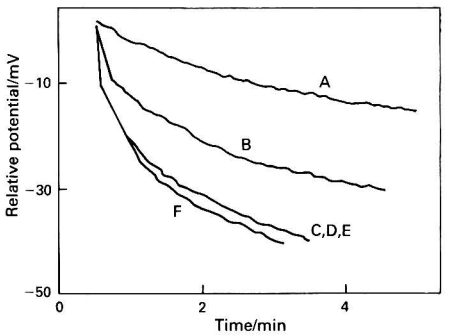


Fig. 1 Calcium fluoride crystallization in the presence of various carboxylic acids: A, citric acid; B, oxalic acid; C, D and E, succinic, glycocholic and malic acids; and F, crystallization in the absence of a foreign carboxylic acid. The initial concentrations of these carboxylic acids were, in all instances, 2×10^{-5} mol dm^{-3}. All other experimental conditions are given under Procedure for the Determination of Citric Acid

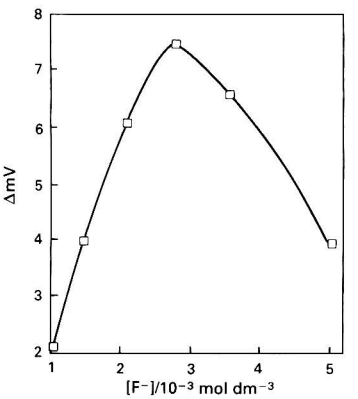


Fig. 2 Effect of initial fluoride concentration on ΔmV, after 5 min, between the blank and a sample containing citrate at a concentration of 5×10^{-6} mol dm^{-3}. All other experimental conditions are given under Procedure for the Determination of Citric Acid

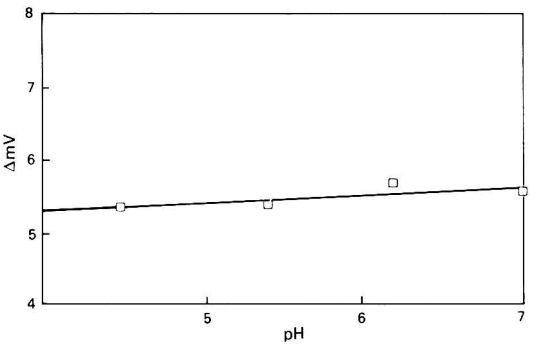


Fig. 3 Effect of pH on ΔmV, after 5 min, between the blank and a sample containing citrate at a concentration of 5×10^{-6} mol dm^{-3}. The different pH values were obtained by adding distinct buffer solutions prepared by mixing suitable volumes of 5 mol dm^{-3} acetic acid and 5 mol dm^{-3} ammonia. The final pH of the suspension was checked with a pH-meter. No significant pH change was detected during crystal growth. All other experimental conditions are given under Procedure for the Determination of Citric Acid

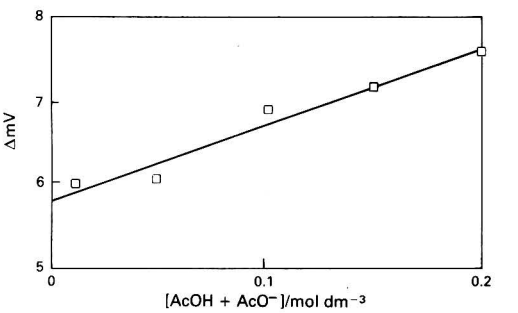


Fig. 4 Effect of total concentration of buffer solution (acetic acid + acetate) on ΔmV, after 5 min, between the blank and a sample containing citrate at a concentration of 5×10^{-6} mol dm^{-3}. All other experimental conditions are given under Procedure for the Determination of Citric Acid

on the crystal surface. The adsorption of the polycarboxylic acids onto the active growth sites is likely to be responsible for the reduction in the crystal growth rates.

In order to find the optimum conditions for the development of a kinetic procedure for the determination of citric acid, based on its inhibition of calcium fluoride crystal growth, the influence of supersaturation (initial concentrations of fluoride and calcium), pH and ionic strength (total concentration of buffer solution) on the rate of reaction was studied.

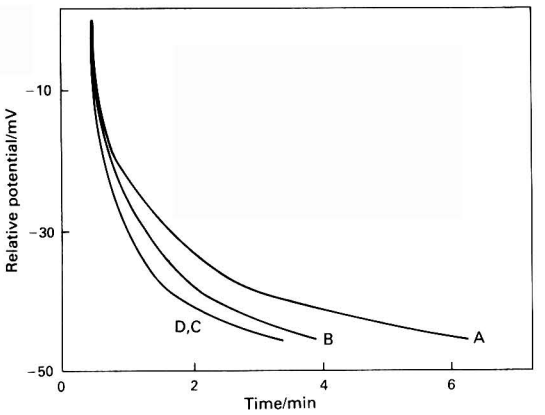
Fig. 2 shows the voltage difference in millivolts, after 5 min, between sample and blank corresponding to several fluoride concentrations. As can be seen for a total initial fluoride concentration of 2.8×10^{-3} mol dm^{-3} the inhibitory effect of citrate was at a maximum. Consequently this concentration was chosen to establish the calibration graph.

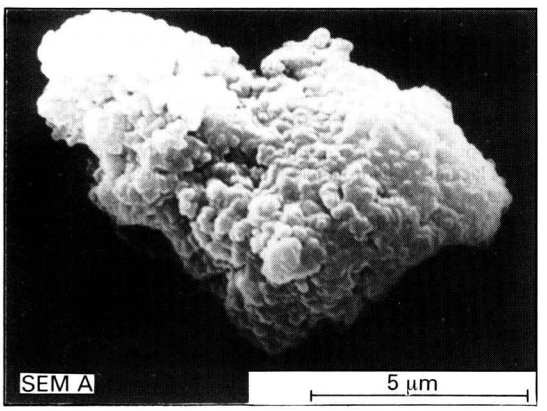
The results of the study of the influence of pH are summarized in Fig. 3. As is shown, the difference in electrical potential is only slightly affected for pH values between 4.5 and 7.0, thus indicating that some protonation of the citrate molecule did not affect its inhibitory capacity. When the influence of the ionic strength is considered it can be seen (Fig. 4) that again citrate inhibition is only slightly affected. The weak influence of the pH and ionic strength on the inhibitory effects of citrate can be explained by the observation that the interactions between citrate oxygen atoms and calcium ions are not exclusively of an electrostatic nature.

It is known that the morphology and surface characteristics of seed crystals can, in some instances, have considerable effects on the rate of crystal growth[27] and consequently can modify the influence of adsorbing inhibitor molecules in crystal growth reactions. Therefore, to evaluate the effect of size and morphology of the calcium fluoride seed on crystallization runs, experiments were carried out using seeds with two different surface characteristics: with and without ageing treatment. As can be seen in Fig. 5, noticeable variations were observed depending on the seed, *i.e.*, recently prepared or aged seed, indicating that citrate was more effective in reducing the rate of growth of the most perfect crystals. This can be explained as a consequence of a relatively small number of active growth sites that can be blocked by the inhibitor while increasing the perfection of the crystals. With the aim of attaining maximum sensitivity values (lower detection limit), an aged seed was chosen for the preparation of the calibration graph.

Characteristics of the Analytical Methods

The fixed-time method (5 min) was applied to the potential (mV) *versus* time curves, recorded in the presence of different amounts of citrate under selected conditions in order to obtain





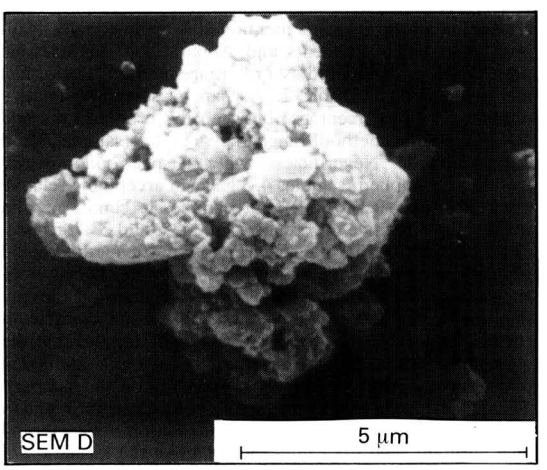


Fig. 5 Scanning electron micrographs (SEM) and crystallization graphs of calcium fluoride obtained from different aged seed crystals. Stirring time: A, 30 min; B, 24 h; C, 48 h; and D, 5 d

the calibration graph, which was linear in the range of citrate concentrations between 0.5 and 2.4 μg ml^{-1}. The relative standard deviation was 2.0% (n = 11, α = 0.05). The selectivity was tested by obtaining the rate curves in the presence of several species that in some instances accompany citrate in biological samples such as urine. The results are summarized in Table 1. Most of the interfering species caused a decrease in the difference of electrical potential. As can be seen, the main interferences were caused by phosphate, sulphate and magnesium ions. Elimination of these interferents was extensively studied and accomplished through precipitation processes as described below. It is interesting to compare the selectivity of the proposed procedure with that described in previous papers[25,26] using seed crystals of a different nature. Thus it can be observed that, in general terms, the selectivity obtained when using calcium fluoride seed crystals is considerably inferior to that obtained when using calcium oxalate monohydrate or calcium carbonate (calcite) seed crystals. These observations can be explained by considering that owing to the simpler crystal structure of calcium fluoride seed crystals, the number of molecules or ions that can cause some blockage of the active growth sites is noticeably increased. When working with more complex crystalline structures the number of species that can adapt to the morphology of the active growth sites is restricted.

Table 1 Effect of foreign ions on the determination of 6 × 10^{-6} mol dm^{-3} citrate

Ion	Concentration tested/mol dm^{-3}	Error* (%)
Na^{I}, Ba^{II}, Li^{I}, Cl^{-}, urea	(1–2) × 10^{-3}	0.1–1.5
Succinate	1 × 10^{-3}	50
	1 × 10^{-4}	1.7
Sulphate	1 × 10^{-3}	134
	1 × 10^{-4}	56
	1 × 10^{-5}	1.7
Phosphate	1 × 10^{-4}	300
	1 × 10^{-5}	60
	1 × 10^{-6}	1.9
Ethylenediaminetetraacetic acid	1 × 10^{-3}	456
	1 × 10^{-5}	220
	1 × 10^{-6}	1.1
Oxalate	1 × 10^{-5}	34
	4 × 10^{-6}	10
	1 × 10^{-6}	1.9
Tartrate	1 × 10^{-3}	268
	1 × 10^{-4}	156
	1 × 10^{-6}	1.7
Malate	1 × 10^{-4}	207
	1 × 10^{-5}	34
	1 × 10^{-6}	1.8
Mg^{II}	1 × 10^{-3}	414
	1 × 10^{-4}	230
	1 × 10^{-5}	44
	1 × 10^{-6}	4
	5 × 10^{-7}	1.6

* The error was always positive.

Elimination of Interferences

The elimination of the interference from phosphate, sulphate and magnesium(II) was studied in order to establish the reliability of the determination of citrate in human urine. The separation processes were carried out on solutions prepared in the laboratory; the composition of these solutions was considered to be representative of human urine.[28] Ordinarily a solution containing SO_4^{2-} (10.5 mmol dm^{-3}), $H_2PO_4^-$ (22 mmol dm^{-3}), HPO_4^{2-} (3.0 mmol dm^{-3}), Cl^- (240.2 mmol dm^{-3}), $C_6H_5O_7^{3-}$ (4.0 mmol dm^{-3}), Na^+ (170.7 mmol dm^{-3}), NH_4^+ (43.4 mmol dm^{-3}), K^+ (81.3 mmol dm^{-3}) and Mg^{2+} (3.0 mmol dm^{-3}) was used as synthetic urine.

The elimination of Mg^{II} to a concentration level that did not interfere in the determination of citrate in urine was accomplished by adding 1 ml of 5 mol dm^{-3} ammonia to 2 ml of synthetic urine. Under such conditions, ammonium magnesium phosphate precipitated. After the precipitate had been digested at 90 °C over a period of 30 min, the solid phase was

Table 2 Comparison of results obtained for the determination of citric acid in commercial formulations and urine. Results, the average of three separate determinations, are expressed as mg l^{-1} with standard deviation (%) given in parentheses

Sample	Proposed method	Spectrophotometric method	Enzymic (reference) method
Pharmaceutical product—			
Benadryl (Parke-Davis)*	8800 (2.2)	8450 (1.9)	8700 (6.2)
Benylin (Parke-Davis)†	4300 (3.2)	4300 (2.0)	4400 (6.5)
Urine—			
Synthetic‡	700 (3.7)	725 (2.6)	720 (6.1)
Human 1	600 (4.0)	819 (3.1)	561 (5.9)
2	500 (4.2)	679 (3.5)	474 (6.2)
3	940 (4.0)	1162 (3.2)	910 (6.3)
4	1110 (3.9)	1148 (3.7)	1140 (7.0)
5	550 (3.5)	600 (2.9)	530 (5.7)
6	730 (3.2)	809 (3.1)	715 (5.4)
7	640 (3.0)	700 (2.8)	650 (6.7)
8	700 (3.1)	715 (2.5)	716 (5.0)

* Value claimed by the manufacturer, 8350 mg l^{-1}.
† Value claimed by the manufacturer, 5183 mg l^{-1}.
‡ Amount added, 750 mg l^{-1}.

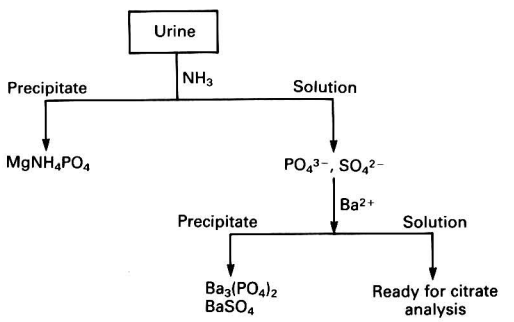


Fig. 6 Schematic diagram for the elimination of interferent species normally present in human urine

separated from the solution by filtration through a disposable 0.22 μm filter. Phosphate and sulphate were then separated jointly from the solution, which did not at this stage contain Mg^{II}, by adding 1 ml of 0.35 mol dm^{-3} barium chloride. The new solid phase formed (barium sulphate and barium phosphate) was removed from the solution in a manner similar to that used for the ammonium magnesium phosphate, *i.e.*, digestion and filtration. After such treatment, the determination of citrate by applying the proposed procedure was reliable with an error of 6.7%, as can be seen in Table 2, confirming that the elimination of interferents was satisfactory. Further experimental details are given under Procedure for Urine Analysis. Fig. 6 shows a schematic diagram of the separation processes. It is obvious that if the sample did not contain phosphate, the elimination of magnesium would not take place by the addition of ammonia only; under such circumstances the addition of phosphate would be necessary. If phosphate was the only interferent present in the sample, its elimination could be carried out, either by adding barium(II), as described, or by adding lithium chloride and sodium hydroxide (1 ml of 3 mol dm^{-3} lithium chloride and 2 ml of 2 mol dm^{-3} sodium hydroxide were added to 2 ml of synthetic urine without magnesium and sulphate). The remaining lithium(I) in solution did not affect the subsequent determination of citrate. Finally, if sulphate was the only species to be eliminated, its quantitative precipitation as a barium salt from a synthetic urine which contained neither phosphate nor magnesium would take place without the addition of ammonia. However, when phosphate was the species being separated, the satisfactory precipitation of barium phosphate took place only in basic media and the addition of ammonia, before the addition of barium(II), was necessary.

Application

The proposed method was applied to the determination of citrate in pharmaceutical products, synthetic urine and several samples of human urine. It should be noted that the determination of citrate in pharmaceutical products was carried out without any previous treatment of the sample (except appropriate dilution). Nevertheless, application to urine samples required previous separation of phosphate, sulphate and magnesium(II), as mentioned earlier. In order to confirm the reliability of the proposed procedure the results were compared with those obtained by spectrophotometry based on the formation of the yellow Fe^{III}–citrate complex,[24] and with those obtained with the enzymic procedure based on the transformation of citrate to oxaloacetate and acetate catalysed by the enzyme citrate lyase (CL):

$$\text{Citrate} \xrightarrow{\text{CL}} \text{oxaloacetate} + \text{acetate}$$

In the presence of the enzymes malate dehydrogenese (MDH) and lactate dehydrogenase (LDH), oxaloacetate and its decarboxylation product, pyruvate, are reduced to L-malate and L-lactate, respectively, by reduced nicotinamide adenine dinucleotide (NADH):

$$\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{L-malate} + \text{NAD}^+$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{L-lactate} + \text{NAD}^+$$

Then, the amount of NADH oxidized is stoichiometric with the amount of citrate. The diminution in the concentration of NADH is evaluated spectrophotometrically at 340 nm.[22] A kit containing all the reagents mentioned is commercially available. (See under Reagents and Apparatus.) This enzymic procedure was considered as a reference method. The results are summarized in Table 2. As can be seen, the relative difference (as a percentage) between the proposed method and the enzymic method, ranged between 1.5 (sample 7) and 7.0 (sample 1). Nevertheless, when a spectrophotometric method was applied, samples 4–8 gave an acceptable relative difference (from 0.1 to 13.2), but, samples 1, 2 and 3 gave an unacceptable relative difference (46.0, 43.2 and 27.7, respectively), indicating that the spectrophotometric method could give erroneous results depending on the sample. The discrepancy can be explained by taking into consideration the fact that the formation of the yellow Fe^{III}–citrate complex is strongly dependent on the pH of the medium, and that the amounts of substances with acid–base behaviour contained in real urine samples vary widely (depending on the sample). Thus, the addition of the same amount of hydrochloric acid to different samples (after the elimination of interferences) could give different final pH values, which affect the formation of the Fe^{III}–citrate complex and the absorbance value corresponding to the Fe^{III} and urine blanks.

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Determination of Trace Amounts of Fluorine, Boron and Chlorine From a Single Sodium Carbonate Fusion of Small Geological Sample Masses

Alfons Hofstetter and Georg Troll
Institut für Mineralogie und Petrographie der Universität München, Theresienstr. 41, 8000 München 2, Germany
Dietmar Matthies
Lehrstuhl für Forstliche Arbeitswissenschaft und Verfahrenstechnik, Hohenlindenerstr. 5, 8000 München 80, Germany

Trace amounts of fluorine, chlorine and boron (all below 100 ppm) in a single sodium carbonate fusion of 150 mg of geological material were determined potentiometrically (F) and spectrophotometrically (Cl and B). The accuracy and precision of this method were tested on six international reference samples.

Keywords: *Fluorine, chlorine and boron determination; geological material; potentiometry; spectrophotometry*

Despite the importance of fluorine and boron in geochemical processes (for example in lowering of the solidus temperature for crystal growth during magma fractionation or as complexing agents in ore deposit forming events), the database for these elements is still small compared with other major and trace elements. This is particularly true for extra-terrestrial material or related matter, such as tektites or impact rocks. There are several reasons for this: (*i*) concentrations of fluorine, boron and chlorine in tektites or impact rocks are usually a factor of 10–100 lower than in igneous, metamorphic or sedimentary rocks (common values for tektites are 12–40 ppm of fluorine, 6–50 ppm of boron and 6–15 ppm of chlorine according to Moore *et al.*,[1] Matthies and Köberl[2] and Mills[3]), (*ii*) very small amounts of these materials are available (usually substantially less than 1 g) and (*iii*) analytical techniques are generally complex and time consuming. Before a systematic investigation of the fluorine, boron and chlorine distribution in tektites and meteorites was carried out, the analytical method described in this paper was developed.

Modern analytical techniques, such as prompt gamma neutron activation analysis (PGNAA), inductively coupled plasma atomic emission spectrometry (ICP-AES), X-ray fluorescence (XRF), atomic absorption spectrometry (AAS), ion chromatography, and others, were unable to determine all three elements at the level of sensitivity required for the current application. Fluorine and chlorine cannot be determined by PGNAA, nor can boron be measured by this technique to the accuracy and precision required, as the sample mass of 1–2 g that is routinely required for analysis (Shaw *et al.*[4]) was not available in this application. X-ray methods such as electron microprobe and XRF do not meet the required detection limits (Schäfer and Meduna[5]); in addition, insufficient amounts of the rock or sample powder were available for routine XRF analysis (Hahn-Weinheimer *et al.*[6]). Interferences from iron and silica, and the mass of sample required for analysis precluded the determination of boron by ICP-AES (Owens *et al.*[7] and Din[8]). The AAS technique was considered unsuitable as it had a low sensitivity for boron and there is a strong tendency for boron to form carbides.

Several papers have been published, dealing with trace analytical procedures for single-element determinations, *e.g.*, Langer and Baumann[9] for fluorine and Aruscavage and Campbell[10] for chlorine. In most of these instances however, a reagent containing boron or fluorine is used for sample decomposition (H_3BO_3 or HF, respectively), which invalidates the use of these techniques for the proposed method. Therefore, it was necessary to develop and apply an alternative analytical technique, in order to fulfil completely the requirements of the proposed procedure.

The analytical procedure described here offers the possibility of high accuracy and precision for the determination of all three elements at low concentration levels (all below 50 ppm) and with a minimum of sample consumption (<200 mg). Furthermore, uncertainties due to possible inhomogeneity in the distribution of elements are avoided as all the determinations are made on a single sample split.

Experimental

Apparatus

For the sodium carbonate fusion an electric muffle furnace, suitable for continuous operation at 950 °C, is required. Polyethylene beakers (100 ml) and polytetrafluoroethylene separating funnels from Nalgene are used for boron extraction. Fluorine is measured potentiometrically with an ion-selective fluoride combination electrode (Orion 96-09-00) with a digital pH/mV-meter (Orion, Model 701A). Boron and chlorine are determined spectrophotometrically using a double-beam spectrophotometer (Perkin-Elmer, Model 552).

Reagents

The reagents used for the determination of each element are listed below the appropriate heading:

Fluorine

Buffer solution. Tris(hydroxymethyl)aminomethane (242 g) (No. 93349, Fluka) and 230 g of sodium tartrate (No. 6663, Merck) are dissolved in 800 ml of H_2O and adjusted to pH 5.25 by the addition of approximately 125 ml of concentrated HCl (36–38%).

Standard sodium fluoride solution. Standard solutions of 1×10^{-3}, 1×10^{-4}, 1×10^{-5}, 5×10^{-5} and 1×10^{-6} mol dm^{-3} sodium fluoride are prepared by dilution of 0.1 mol dm^{-3} sodium fluoride solution (Orion 96-09-06).

Chlorine

Ammonium iron(III) sulphate solution. Ammonium iron(III) sulphate (12.055 g) $[FeNH_4(SO_4)_2 \cdot 12H_2O]$ (No. 3776, Merck) is dissolved in 100 ml of 9 mol dm^{-3} HNO_3.

Mercury(II) thiocyanate solution. Mercury(II) thiocyanate (0.35 g) $[Hg(SCN)_2]$ (No. 4484, Merck) is dissolved in 100 ml of methanol (95%) for approximately 12 h.

Standard sodium chloride solution. Sodium chloride (0.8242 g) (dry) is dissolved in 1 l of doubly distilled water. A 10 ml aliquot is diluted to 1 l (Cl^- concentration = 10 μg ml^{-1}).

Boron

2-Ethylhexane-1,3-diol (EHD) solution. 2-Ethylhexane-1, 3-diol (200 ml) (No. 820032, Merck) is mixed with 800 ml of chloroform.

Carmine reagent. Carminic acid (100 mg) (No. 211, Merck) is dissolved in 200 ml of concentrated H_2SO_4 (95–97%).

Boron standard solution. Boric acid (10.5716 g) (Suprapur, No. 765, Merck) is dissolved in 1 l of distilled H_2O. A 5 ml aliquot is diluted to 500 ml with distilled H_2O (boron concentration = 1 μg ml^{-1}).

Procedures

Sodium carbonate fusion

Sodium carbonate (0.9 g) and zinc oxide (0.1 g) are added to 150 mg of the powdered sample and transferred into a 25 ml Pt crucible, which is covered with a lid. The crucible is placed in a muffle furnace at 600 °C and the temperature increased to 950 °C. After 30 min at 950 °C, the crucible is removed and cooled to room temperature. The fusion cake is then dissolved and washed out of the crucible with 28 ml of dilute nitric acid (1 + 30) into a 100 ml polyethylene beaker. Three drops of ethanol are added to reduce any Mn that may be present. The dissolution should last about 12 h at 40 °C after which the pH is adjusted to between 5.5 and 6.0 before the solution is filtered into a 250 ml polyethylene bottle; the solid residue is rinsed with doubly distilled water. The volume of the solution should not exceed 100 ml. Blank solutions are treated in the same manner. Plastic gloves should be worn during the entire procedure in order to minimize contamination (particularly for the determination of chlorine).

Fluorine determination

Fluorine is determined potentiometrically. A buffer solution (20 ml) is added to 20 ml of the sample solution and stirred continuously, while the electrochemical potential is measured with an ion-selective fluoride electrode. Using the standard additions technique the concentration of fluorine in the sample can be calculated using the Nernst equation.

Chlorine determination

Chlorine is measured spectrophotometrically using the method described by Huang and Johns.[11] A 20 ml sample solution, 2 ml of ammonium iron(III) sulphate solution and 2 ml of mercury(II) thiocyanate solution are mixed and diluted to 25 ml with doubly distilled water in a flask. The time for complete development of the iron(III)–thiocyanate complex is 10 min. Blank and calibration solutions are treated similarly. The absorbance is measured against water at a wavelength of

Table 1 Fluorine, chlorine and boron concentrations in six international reference samples and corresponding data from Govindaraju[13]

	Reference sample	Data from this work* (ppm)	Mean (ppm)	Standard deviation (ppm)	Relative standard deviation (%)	Compiled data from reference 13
Fluorine—						
	DTS-1	16, 16, 18, 18, 21	18	2.0	11.5	13†
	BIR-1	43, 44, 47, 50, 50	47	3.3	7.0	44†
	BHVO-1	365, 372, 373, 381, 400	378	13.3	3.5	385‡
	AGV-1	431, 440, 443, 459, 460	447	12.3	2.8	425‡
	MAG-1	756, 775	766	—	—	770‡
	NIM-L	3898, 3992, 4057, 4356	4076	198.0	4.9	4400‡
	R1/IV	289, 295, 307, 312, 315, 315, 323, 327	310	13.0	4.2	—
Chlorine—						
	DTS-1	25, 28, 31, 33, 35	30	4.0	13.1	10.5†
	BIR-1	24, 27, 27, 31, 36	29	4.6	16.0	26†
	BHVO-1	96, 99, 107, 108, 114	105	7.3	6.9	92‡
	AGV-1	107, 110, 115, 117, 117	113	4.5	4.0	119‡
	MAG-1	Not determined				
	NIM-L	1157, 1190, 1211, 1289	1212	56.1	4.6	1200‡
	R1/IV	200, 207, 216, 220, 223, 225, 231, 240	220	12.7	5.8	—
Boron—						
	DTS-1	10, 11, 11, 13, 14	12	1.6	14.0	0.50§
	BIR-1	<Detection limit				0.33§
	BHVO-1	2, 3, 5, 5, 6	4	1.6	39.1	2.5†
	AGV-1	8, 10, 10, 11, 12	10	1.5	14.5	7.8†
	MAG-1	126, 134	130	—	—	136‡
	NIM-L	18, 18, 19, 21	19	1.4	7.4	—
	R1/IV	10, 11, 11, 11, 12, 12, 13, 15	12	1.6	13.0	—

* = Data in ascending order.
† = Information values.
‡ = Proposed values.
§ = Recommended values.

460 nm. The chlorine concentration is calculated from a calibration graph.

Boron determination

The remaining sample solution (between 50 and 60 ml) is used for the spectrophotometric determination of boron following the method of Troll and Sauerer.[12] Five drops of concentrated HCl and 20 ml of EHD are added to the sample solution. After intensive shaking of the mixture for 45 min, the EHD solution containing boron is separated from the aqueous sample solution. The solvent extraction is repeated, this time using only 10 ml of EHD solution. The re-extraction is carried out with 10 ml of 0.5 mol dm^{-3} NaOH. Blank and calibration solutions are treated similarly. For the determination of boron, 1 ml of the sample (NaOH), blank and standard solutions, respectively, is transferred into quartz beakers and two drops of concentrated HCl are added to each. While cooling in a water-bath, 5 ml of concentrated H_2SO_4 and 5 ml of carmine reagent are added to the solutions (caution, violent reaction!). After 1 h the carminic complex is completely developed, and the absorbance is measured at a wavelength of 610 nm. The boron concentration in the sample is calculated from a calibration graph.

Results and Discussion

The accuracy and precision of the proposed method were tested by the analysis of six international reference samples of silicate rocks: USGS (United States Geological Survey) DTS-1 (Dunite), BIR-1 (Basalt), AGV-1 (Andesite), BHVO-1 (Basalt), MAG-1 (Marine Mud) and NIM-L (Lujavrite) (obtained from the National Institute of Metallurgy, South Africa) and an internal reference sample (R1/IV, Volcanic Glass, Whalers Bay, Deception Island, Antarctica). The results are given in Table 1 and are in good agreement with the recommended values.

Obviously the precisions (expressed as the relative standard deviations) decrease as concentrations fall towards detection limit levels. However, at higher concentrations, the following limiting precisions were measured: fluorine, 2% at >800 ppm of fluorine; chlorine, 6% at >100 ppm of chlorine; and boron, 4% at >100 ppm of boron. Typical total blank values were measured as 2 ppm for fluorine, 27 ppm for chlorine and 3 ppm for boron. The detection limits, calculated as 3σ confidence levels, following the International Union of Pure and Applied Chemistry (IUPAC)[14] recommendations, were <1 ppm fluorine, 4 ppm chlorine and 1 ppm boron. These data refer to an analysis of 150 mg of sample powder.

In order to achieve reliable results at low concentration levels, several conditions must be fulfilled. For fluorine, the readings of the electrochemical potential must be left to attain constant values for a time which depends on the fluoride concentration as follows: 30 min $<1 \times 10^{-5}$ mol dm^{-3} NaF, 10 min between 1×10^{-5} and 1×10^{-4} mol dm^{-3} NaF and 5 min $>1 \times 10^{-4}$ mol dm^{-3} NaF. (For further information on electrode analysis refer to Nicholson.[15]) The determination of chlorine is the most problematic. To avoid contamination, the analytical equipment must not be touched without gloves. In addition, the time span between mixing of the solutions and spectrophotometric measurements should not exceed 30 min; after this time, the colour of the iron(III)–thiocyanate complex begins to fade. This fading is due to the oxidation of thiocyanate by iron(III) in daylight (Huang and Johns[11]). The limiting factor in the determination of boron is the impurity of the sodium carbonate used for the fusion (Troll and Sauerer[12]). It is, therefore, necessary to analyse a blank with each batch of samples.

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Enhancement of Precision and Accuracy in Derivative Spectrophotometry of Highly Absorbing Samples

Isabel Dol, Moisés Knochen and Carmen Altesor
Universidad de la República, Facultad de Química, Cátedra de Análisis Instrumental, Av. Gral. Flores 2124, Casilla 1157, 11800 Montevideo, Uruguay

The extension of relative absorptiometric methods to derivative spectrophotometry is proposed as a way of improving precision and accuracy for extremely high absorbance values. The concept was developed by applying the transmittance-ratio method to second-derivative measurements of two model systems where the total absorbance is kept very high, leading to a significant enhancement of precision and accuracy. The variation of the signal to noise ratio and of the total error as a function of absolute and relative absorbance was studied. Some of the factors affecting the analytical behaviour of this method are discussed.

Keywords: *Derivative spectrophotometry; precision; noise; transmittance-ratio method; high absorbances*

Derivative spectrophotometry[1–6] is often used as a means of overcoming various types of additive interference in absorption spectrophotometry and other spectrometric techniques. The number of analytical applications in different areas has increased considerably in the last few years, and many reports have appeared in the literature reflecting the widespread interest.[7–17]

The basis for its application is simple. Let $A(\lambda)$ be the mathematical expression of the absorbance of a given analyte as a function of wavelength, and let $B(\lambda)$ be the corresponding expression for an overlapped spectrum. Assuming the additive property of absorbances, the following expression for the total absorbance, A_T, can be written:

$$A_T(\lambda) = A(\lambda) + B(\lambda)$$

As the derivative is an additive operator it follows that:

$$\frac{d^n A_T}{d\lambda^n} = \frac{d^n A}{d\lambda^n} + \frac{d^n B}{d\lambda^n} \quad (1)$$

Hence, if a convenient order, n, of derivation can be found so that the second term of the right-hand side of equation (1) becomes negligible, the contribution of the interference to the total analytical signal will be cancelled out. The effective cancellation of the interference depends on the relative amplitudes of the two terms in the right-hand side of equation (1).

Despite its inherent simplicity, this technique is not trouble-free, and its application is not as straightforward as it might seem. The influence of such factors as height- and width-ratios of interfering to analyte bands should be taken into account when predicting the systematic and random errors to be expected in a given determination. This point was studied by O'Haver and Green[18] by means of a model based on Gaussian bands.

One of the aspects to take into consideration is the influence of noise. The so-called 'analytical noise,' *i.e.*, the random variations generated in the various steps of sample processing, concerns all types of analytical determinations and will not be considered here. Instead we will focus on instrumental noise, the type of random variability originating in the spectrophotometer itself. As it depends on the operating conditions, it is important to have some knowledge of the factors that affect it.

Instrumental noise originates mainly in the photoelectric detector and in the electronic amplification.[19,20] Detector noise is dominated by shot, or quantum, noise, while electronic noise has a high proportion of Johnson noise. Detector noise depends to a large extent on the amount of light striking the photodetector surface. This in turn depends on such factors as the intensity of the light emitted from the lamp, slit-width and the total absorbance of the cell plus analyte plus solvent and matrix. In digital instruments, where an analogue to digital (A/D) converter is employed, this converter is also a source of variability.

Raw data [transmittance (T), absorbance (A), *etc.*] must first be obtained before they can be processed, hence it is important to ensure the best possible signal to noise (S/N) ratio during data acquisition in order to generate usable derivatives. It is well known from the theory of classical spectrophotometry that there are optimum ranges of transmittance, and hence of absorbance, where measurements with the lowest possible spectrophotometric error can be made, leading to the lowest analytical error.[21,22]

In those situations where the absorbance is either very high or very low, differential, or relative, methods[23–26] can be used to improve the S/N ratio and therefore to lower the analytical error. These methods were developed mostly during the 1950s as a means of improving the precision of the measurement under conditions of high spectrophotometric error, *i.e.*, extremely high or low absorbances. However, with the advent of highly sophisticated spectrophotometers, usable spectrophotometric ranges have widened, and absorbances in excess of 2 can now be measured with a reasonable error, while the precision of low-absorbance measurements has also been considerably improved. Hence, precision methods have fallen into general disuse, and a few words should be said about them at this point.

Three precision methods are described in the literature: transmittance-ratio, trace-analysis and ultimate-precision methods.[26]

When the absorbance is too high, the transmittance-ratio (or high-absorbances) method is employed. In this method, a solution with an absorbance lower than that of the sample, but higher than that of the blank, is used instead of the blank for making the 100% T (*i.e.*, zero absorbance) adjustment, thus obtaining a 'relative absorbance' which is lower than the true absorbance. Usually, a dilute solution of the analyte is used for this purpose. This provides an easy way of improving the S/N ratio in those situations where a dilution cannot be carried out, such as the measurement of the absorbance of solid samples.

Similarly, the trace-analysis (or low-absorbances) method consists of using a solution with a transmittance different from zero for the 'dark-current' (0% T) adjustment. The 100% T adjustment is made as usual. The relative absorbance thus measured is higher than the actual absorbance. As suggested by its name, this method should be employed when the absorbance is very low, a situation often found in trace analysis.

Finally, the ultimate-precision method involves a combina-

tion of both of the above-mentioned procedures, and is supposed to achieve the best possible precision.

Using any of these methods, a convenient expansion of the scale and a gain in precision of the measurement are attained. Further details of these methods are given in the Appendix at the end of this paper.

Unfortunately, in most digital spectrophotometers the 0% T adjustment is performed automatically and is not accessible to the user, hence it is not possible to carry out the low-absorbances and ultimate-precision methods with this type of instrument.

In derivative spectrophotometry on the other hand, one is often faced with the situation of trying to determine a given analyte in the presence of a highly absorbing matrix or extreme turbidity. The over-all shape of the spectrum of the matrix or turbid solution may be such that its interference on the analyte spectrum could be overcome by a derivative of a certain order, but, as the total absorbance becomes higher, the noise increases, and may become intolerable, thus precluding the use of the derivative method, which otherwise would be useful. In addition, one cannot resort simply to diluting the sample as this would lead to a loss of analytical signal (derivative amplitude), a situation not desirable when the amplitude obtained is already small.

In this paper, the use of an adaptation of the transmittance-ratio method in derivative absorption spectrophotometry is explored. Only this method was investigated, because the other two precision methods cannot be carried out with the instrument used for the reasons mentioned above.

Measurement of High Absorbances in Derivative Spectrophotometry

Adaptation of the transmittance-ratio method to derivative spectrophotometry warrants some comment. If the detector response is assumed to be linear with light intensity, the general equation for the relative transmittance in differential methods[26] can be written

$$T_R = \frac{T_x - T_o}{T_{ref} - T_o} \quad (2)$$

The subscripts R, x and ref refer to relative, sample and reference, respectively, whereas the subscript o refers to the object used for the 0% T adjustment. 'Reference' is the equivalent of 'blank,' *i.e.*, the solution or object used for the 100% T adjustment.

For the transmittance-ratio method, $T_o = 0$, hence equation (2) becomes

$$T_R = \frac{T_x}{T_{ref}}$$

and hence

$$A_R = A_x - A_{ref} \quad (3)$$

where A_R, A_x and A_{ref} are the corresponding absorbances.

Differentiating n times in the wavelength domain

$$\frac{d^n A_R}{d\lambda^n} = \frac{d^n A_x}{d\lambda^n} - \frac{d^n A_{ref}}{d\lambda^n} \quad (4)$$

In the original version of this method, a less concentrated solution of the same analyte was used as the reference to obtain the desired scale expansion. This procedure cannot be followed when working with derivative spectrophotometry which, as opposed to a simple absorbance measurement, is performed over a wavelength range instead of at a single wavelength. In classical transmittance-ratio measurements, only the transmittance (or absorbance) value of the reference solution is important, whereas in derivative spectrophotometry its spectral shapes become of major concern. It is clear from equation (4) that the use of a dilute solution of the same analyte would result in a loss of analytical sensitivity. Hence, the absorption spectrum of the object used for compensation purposes in the reference beam should be such that its derivative equals zero at the wavelengths to be used for measurement. If the absorption spectrum is flat (*i.e.*, $A_{ref}(\lambda) = k$), this condition is met for all orders of derivation. In fact, this approach applies also to conventional zero-order relative measurements, a fact not often mentioned in the literature.

Experimental

Instrumentation

A Shimadzu UV-240 double-beam, microprocessor-controlled spectrophotometer with an OPI-2 spectral processing unit (Shimadzu) was used. This instrument is fitted with an R-928 photomultiplier, and is capable of performing digital derivation of spectra either in real time or from data previously stored in random-access memory (RAM). In this work the real-time mode was used in order to save time. Other instrumental conditions were as follows: mode, 721 (second derivative, 1 nm wavelength increment); scan speed, fast; slit, 1 nm; wavelength scale, 3; wavelength range, 510–530 nm (didymium filter) or 240–280 nm (homatropine methylbromide); and T/A range, −0.25 to +0.25 (didymium filter) or −0.5 to +0.5 (homatropine methylbromide). Second-derivative negative-peak values were printed out by means of the 'peak pick' function.

A Shimadzu UV-160 spectrophotometer was also used for some complementary measurements. Solutions were measured in 1 cm silica cells. In order to simulate the analyte, a didymium-glass filter (Shimadzu No. 202-30242) was used.

A variable-beam attenuator (Beckman No. 104 186) was employed. Other similar laboratory-built attenuators were also used. The spectral flatness of the attenuators was checked. These attenuators were installed in the path of the reference beam of the spectrophotometer.

In order to be able to achieve the desired levels of total absorbance in one of the experiments, a slide filter consisting of a variable number of layers of wire mesh (17 wires per centimetre; diameter, 0.4 mm) was constructed. The spectral behaviour of the mesh was checked and it was found to have a virtually flat spectrum within the wavelength range of interest, the absorbance of a single layer being about 0.4. Its second-derivative spectrum followed the zero line. Henceforth, this device will be referred to as the neutral filter.

Reagents

Homatropine methylbromide (HMB) of pharmaceutical grade, and analytical-reagent grade barium sulphate and glycerine were used.

Methods

Two different types of experiment were carried out. In the first, the spectrum of a didymium filter (Fig. 1) was scanned in the visible range (510–530 nm) and the second derivative was obtained under a number of different conditions of total and relative absorbance (Fig. 2). The variable neutral filter was used to simulate the 'additive interference.'

When the didymium filter was placed in the sample-cell holder, the neutral filter was affixed next to it in the light path, and the number of layers of mesh was varied from 0 to 7 in order to obtain values of total absorbance in the range 0.4–3.7. The variable-beam attenuator was set in each instance to obtain a given relative absorbance. One hundred and seven different combinations of total and relative absorbance were investigated. The amplitude of the second-derivative spectrum of the didymium filter was measured at the minimum at about 525 nm, corresponding to a shoulder in the zero-order spectrum.

In order to simulate more closely an actual analytical application, in a different experiment, the second-derivative spectrum of a solution of HMB in water–glycerine (1 + 1) (625 mg l^{-1}) was scanned in the ultraviolet (UV) range (240–280 nm) in the presence of a turbidity obtained by suspending various amounts of barium sulphate (1.2–6.0 g l^{-1}). The glycerine was added to stabilize the suspension. Forty different combinations of total and relative absorbance were investigated, the total absorbance level being in the range 1.8–3.7.

Measurements were made at the minimum at about 258 nm, which corresponds to the main maximum of the HMB absorption band (Figs. 3 and 4).

This model system can be considered to be representative of many real-world situations, because of the spectral region where the benzenoid absorption bands of HMB occur, and because of the presence of an additive interference which does not present a flat spectrum.

In both experiments, the spectral baseline was memorized previously using air as the reference. Measurements were made with various degrees of optical compensation by placing the variable-beam attenuator in the reference-cell holder. Ten second-derivative scans were then made and the average, standard deviation, S/N ratio, relative bias and total error[27] were calculated as follows:

$$\text{S/N ratio} = \frac{\text{average}}{\text{standard deviation}}$$

$$\text{Bias} = \text{average} - \text{true value}$$

$$\text{Relative bias (\%)} = \frac{\text{bias}}{\text{true value}} \times 100$$

$$\text{Total error (\%)} = \frac{|\text{bias}| + 2\,(\text{standard deviation})}{\text{true value}} \times 100$$

where 'true value' refers to the corresponding measurement performed without interference.

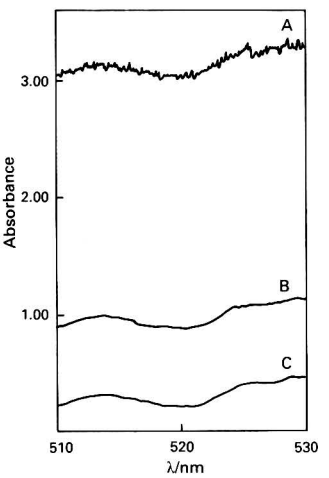

Fig. 1 Absorption spectra of A, the didymium glass + neutral filter; B, didymium glass + neutral filter, with reference-beam attenuation; and C, didymium glass. The didymium glass was used as a simulated analyte, while the additive spectral interference was simulated by means of a neutral filter consisting of seven layers of wire mesh (see text)

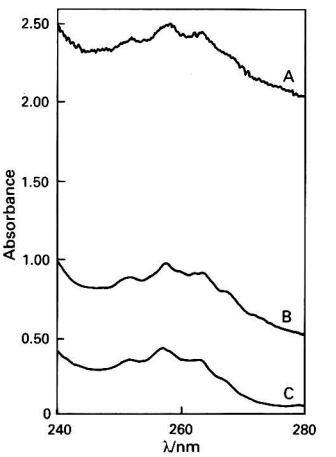

Fig. 3 Absorption spectra of A, 625 mg l^{-1} aqueous HMB solution + $BaSO_4$ suspension, uncompensated; B, HMB + $BaSO_4$ suspension, with optical compensation; and C, HMB without interference

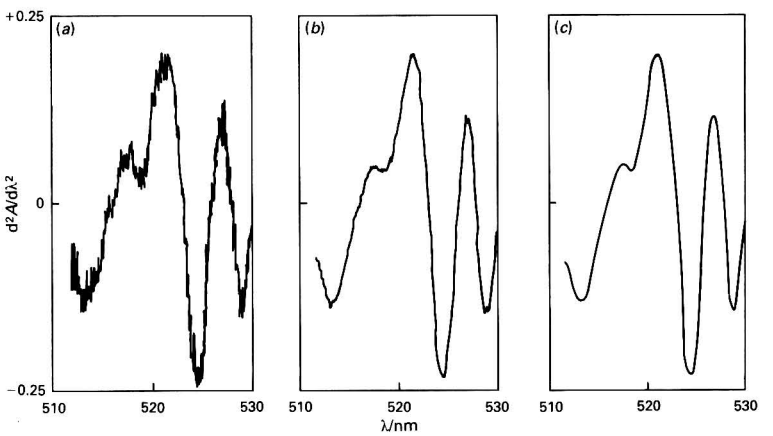

Fig. 2 Second-derivative spectra of (*a*) didymium glass + neutral filter; (*b*) didymium glass + neutral filter, with reference-beam attenuation; and (*c*) didymium glass. The didymium glass was used as a simulated analyte, while the additive spectral interference was simulated by means of a neutral filter consisting of seven layers of wire mesh (see text)

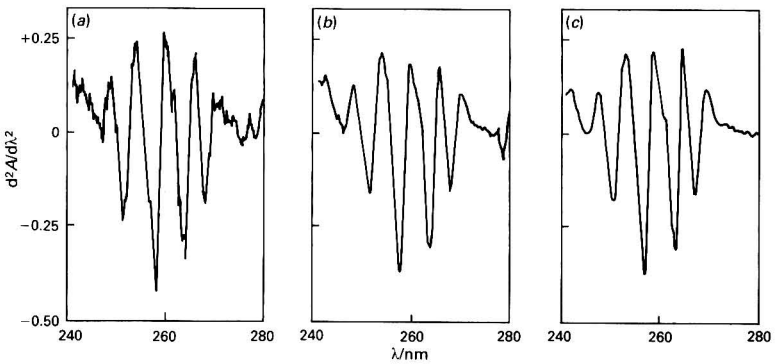

Fig. 4 Second-derivative spectra of (*a*) aqueous HMB solution (625 mg l^{-1}) + $BaSO_4$ suspension, uncompensated; (*b*) HMB + $BaSO_4$ suspension, with optical compensation; and (*c*) HMB solution without interference

Table 1 Variation of relative bias, S/N ratio and coefficient of variation (CV) with relative absorbance (A_R) for the didymium-glass spectrum (amplitude of the second derivative at 525 nm), at a total absorbance level of 3.7

A_R*	Bias (%)	S/N ratio	CV (%)
0.2	2.2	16.9	5.91
0.8	1.3	18.3	5.47
1.1	2.9	23.0	4.35
1.5	3.5	41.4	2.42
1.8	1.2	30.1	3.32
2.2	4.9	18.3	5.45
2.4	8.2	12.0	8.35
2.7	16.0	7.1	14.10
3.1	29.2	6.6	15.23

* Measured at 525 nm.

Table 2 Variation of relative bias, S/N ratio and coefficient of variation (CV) with relative absorbance (A_R) for the HMB spectrum (amplitude of the second derivative at 258 nm), at a total absorbance level of 3.7

A_R*	Bias (%)	S/N ratio	CV (%)
0.4	−1.39	30.5	3.3
0.6	2.59	12.6	7.9
1.1	3.02	9.9	10.1
1.6	−0.88	18.4	5.4
2.1	2.94	12.8	7.8
2.5	6.74	8.8	11.3
2.9	2.85	5.5	18.1

* Measured at 258 nm.

Results and Discussion

The evaluation of the experimental data demonstrates that a significant reduction in noise and analytical bias in the derivative can be attained by using differential techniques. Tables 1 and 2 exemplify the S/N ratio and bias values found for high total absorbances, at different levels of relative absorbance (measured at the same wavelengths as the second derivative), for the two experiments. As a demonstration of the gain in precision, during the measurements with the didymium glass, at a total absorbance level of 3.7 (at 525 nm), no usable data could be acquired from the conventional derivative method, as the noise in the second derivative was so high that the peak-finding algorithm of the instrument became inoperative. However, through the use of differential measurements, a value for the S/N ratio of 41, equivalent to an instrumental coefficient of variation of 2.4%, could be attained at a relative absorbance level of 1.5.

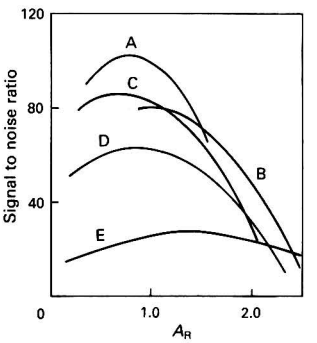

Fig. 5 Variation of S/N ratio as a function of relative absorbance (A_R) for the second derivative at 525 nm (didymium glass), at total absorbance levels of A, 1.6; B, 2.5; C, 2.8; D, 3.0; and E, 3.7

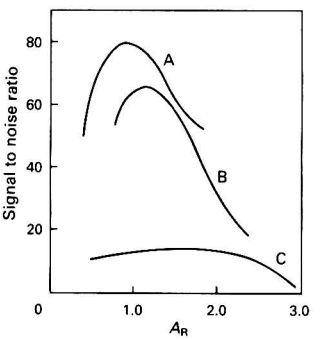

Fig. 6 Variation of S/N ratio as a function of relative absorbance (A_R) for the second derivative at 258 nm (HMB), at total absorbance levels of A, 1.8; B, 2.4; and C, 3.7

Signal to noise ratio enhancements ranging from 2.5 to more than 13-fold were observed in this work. The enhancement to be expected depends on such factors as the wavelength region, slit-width and the intensity of the light reaching the detector. In addition, it will also depend on the spectral shape. At high absorbance levels, the absorbance range spanned by a given absorption band may correspond to a variation of several decades in % T, and hence in light intensity, because of the logarithmic nature of absorbance. Therefore, the S/N ratio at the wavelength of the maximum may be considerably worse

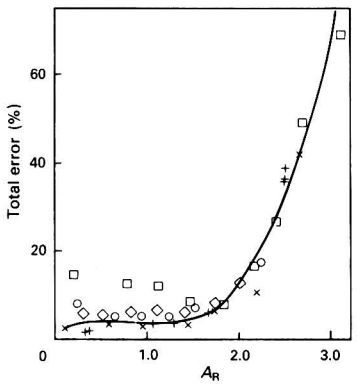

Fig. 7 Variation of total error as a function of relative absorbance (A_R) for the second derivative at 525 nm (didymium glass), at total absorbance levels of +, 2.5; ×, 2.7; ◇, 2.8; ○, 3.0; and □, 3.7

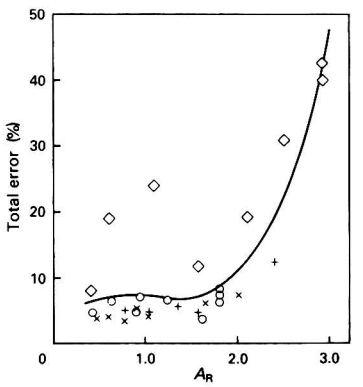

Fig. 8 Variation of total error as a function of relative absorbance (A_R) for the second derivative at 258 nm (HMB), at total absorbance levels of ○, 1.8; ×, 2.0; +, 2.4; and ◇, 3.7

than at other wavelengths. The absorbance values reported here correspond to the wavelengths where the derivative measurements were carried out in each instance.

Figs. 5 and 6 are plots of S/N ratio *versus* relative absorbance at various levels of total absorbance for the didymium glass and HMB, respectively, while Figs. 7 and 8 show similar plots for the total error as defined by McFarren *et al.*[27] From these plots, the existence of optimum ranges of relative absorbance can be observed. It could be stated tentatively that for the present instrument, the optimum S/N ratio is obtained for relative absorbances of about 1. This could be expected as the error pattern of the derivative follows that of the original data.

The total error increases considerably at absorbances above 2, as a consequence of the increase in both noise and bias. The above results show that an increasing positive analytical bias appears as relative absorbances become higher, following the same over-all trend as the noise. At high values of total absorbance, the bias could be diminished by applying the transmittance-ratio method to lower the relative absorbance to values of about 1. This phenomenon, which attracted our attention, could not be explained on the basis of the considerations put forward by O'Haver and Green.[18] As it was not clear whether this behaviour could be general or specific to the particular instrument being used, some complementary measurements were carried out using a different spectrophotometer (Shimadzu UV-160), which is fitted with photodiodes instead of a photomultiplier. Despite the fact that the number of measurements made was not as high as with the UV-240 instrument, a similar trend was found for the total error. This bias could probably be assigned to the influence of stray light, although this requires further investigation.

Digital smoothing is often used to improve the S/N ratio in spectrophotometric data, hence it is valid to compare both methods. Digital smoothing is usually performed on the data previously stored, and depending on the algorithm employed it may involve a compromise between precision and analytical sensitivity. If the noise level is very high, such as with the measurements carried out at absorbances of 3 and above, the degree of smoothing required would be so high that an appreciable loss of spectral resolution and analytical sensitivity would occur. Similar reasoning could be applied to other procedures used for lowering the noise level, such as the use of wider slits, as this would result in a loss of spectral detail and hence of derivative amplitude in most instances. On the other hand, optical compensation using the transmittance-ratio method does not cause any degradation, either in the signal amplitude or in the resolution attained. In fact, the data collected in this work show that under some circumstances it can make the difference between usable information and no information at all. In any event, spectrophotometric compensation and data smoothing can be considered as complementary strategies aiming at the same objective.

Baseline correction was made with air as the reference. As with most digital spectrophotometers, the instrument used in this work memorizes the spectral baseline in the working wavelength range. These data are stored in RAM and later subtracted automatically from the absorbance values measured in the sample position to obtain the true absorbance value, which is displayed subsequently. Therefore, it is not always necessary to use a cell with the solvent or blank in the path of the reference beam. Instead, one can place a cell with solvent (or blank) only in the path of the sample beam during the process of baseline memorizing, which is then made with air as the reference. This procedure was followed in both experiments, because, as the variable-beam attenuator had to be installed in the reference-cell position during measurements, there was no possibility of simultaneously placing a reference cell when measuring the aqueous solutions. The didymium-glass filter is usually measured against air, hence this makes no difference.

It should be pointed out that the procedure explained previously for baseline memorizing may not be adequate in certain instances, when the dynamic range of the instrument may be exceeded.

The choice of the second derivative for this investigation was based on the spectrum of the neutral filter, which was flat, and of the barium sulphate suspension, which displayed a small slope within the wavelength range scanned. The corresponding second-derivative spectra followed the baseline. In addition, the second derivative is perhaps the most widely used derivative in analytical applications. Further, considerations of error propagation allow an extrapolation to derivatives of other orders to be made, because the noise reduction attained in the derivative originates in the gain in precision in the acquisition of the absorption spectra.

Conclusions

The transmittance-ratio method may be used for enhanced precision and accuracy in derivative spectrophotometry in situations of extremely high absorbances. The method is no more complex than the ordinary method, and it provides a significant reduction in noise and bias without loss of spectral resolution or analytical sensitivity.

This technique can be used advantageously in the presence of a highly absorbing interference or intense light scattering, hence the field of application of derivative spectrophotometry is widened considerably.

Although the proposed methodology was applied only to model systems, its utility in actual analytical situations is obvious, and some applications are currently being developed in our laboratory.

The existence of optimum ranges of relative absorbance, which could be expected from theory, was also demonstrated.

As to the limitations, the two most important limitations are detector sensitivity and stray light. When working with absorbances of 3, it should be remembered that this is equivalent to a transmittance of 0.1%. If an attenuator is placed in the reference beam, the amount of light reaching the detector through this path may be very small. For photomultipliers, this means that the maximum voltage available may be applied to the dynodes and still not be sufficient to reach the 100% T (zero absorbance) level. Stray light may cause a severe distortion of the spectra, thus rendering the measurements obtained under these conditions useless. The first of these problems was not found during this work, while stray light could be responsible for the bias effects noted at high absorbances; however, the quality and performance (state of the lamps, photodetector, *etc.*) of a given instrument will determine whether or not these problems will be of concern.

In this work only the method for high absorbances was investigated. Extension of these concepts to the other two precision methods requires further investigation, and this is currently under study.

Appendix

For the benefit of those not familiar with relative absorptiometric methods, a brief explanation of the properties of these methods, with the emphasis on the transmittance-ratio method, is presented below.

Let us assume that the response, R, of a spectrophotometer is a linear function of the power, P, of the light falling on its detector. Then

$$R = kP + k'$$

where k is the sensitivity of the instrument and k' is an additive constant adjustable by means of the 'dark current' control. The zero adjustment is made by interposing an absorbing system (cuvette, shutter, *etc.*) such that the light power reaching the detector is P_o, while the full-scale (usually 100% T) adjustment is made with a cuvette containing blank or solvent, such that the light power falling on the detector is P_B. If this adjustment is made with an absorbing system rather than the blank or solvent, the power reaching the detector will be P_{ref}. When a given sample is measured, the light power reaching the detector will be P_x. Obviously, $P_o < P_x < P_{ref} < P_B$. The respective instrumental responses, R, will then be

$$R_o = 0 = kP_o + k' \Rightarrow k' = -kP_o$$

$$R_{ref} = 1 = kP_{ref} + k' = k(P_{ref} - P_o)$$

$$R_x = kP_x + k' = k(P_x - P_o)$$

The 'relative transmittance' can then be defined as

$$T_R = \frac{R_x}{R_{ref}} = \frac{(P_x - P_o)}{(P_{ref} - P_o)}$$

If we divide all the powers by P_B, the respective transmittances are substituted in place of the powers

$$T_R = \frac{(T_x - T_o)}{(T_{ref} - T_o)} \quad \text{(A1)}$$

and hence

$$T_x = T_R T_{ref} - T_R T_o + T_o \quad \text{(A2)}$$

In the 'ordinary method' the zero adjustment is made with a shutter interposed in the light path, and the full-scale response is adjusted with blank or solvent, hence $T_o = 0$ (0% T) and $T_{ref} = 1$ (100% T); therefore, equation (A1) simplifies to the usual form,

$$T_x = T_R$$

When using the high-absorbances method, $T_o = 0$ and $T_{ref} \neq 1$, hence

$$T_x = T_R T_{ref}$$

In the low-absorbances method, the zero adjustment is not made with a shutter, but rather with a system whose transmittance, T_o, is different from 0, and the full-scale adjustment is made with a blank or solvent

$$T_x = T_R - T_R T_o + T_o$$

In the ultimate-precision method, both the 0 and 100% T points are adjusted with solutions, and equation (A2) fully applies.

On the other hand, according to the Bouguer–Beer law,

$$-\log T_x = A_x = abc$$

where a is the absorptivity, b the length of the light path and c the concentration of the analyte, and, considering equation (A2)

$$-\log T_x = -\log(T_R T_{ref} - T_R T_o + T_o) = A_x = abc \quad \text{(A3)}$$

For the estimation of the error, this equation will be differentiated, taking into account that T_o and T_{ref} are constant

$$-\frac{0.4343(T_{ref} - T_o)}{(T_R T_{ref}) - (T_R T_o) + T_o} dT_R = abdc \quad \text{(A4)}$$

Dividing by equation (A3) and considering the approximation of increments to be sufficiently small,

$$\frac{\Delta c}{c} = \frac{0.4343(T_{ref} - T_o)}{(T_R T_{ref} - T_R T_o + T_o)\log(T_R T_{ref} - T_R T_o + T_o)} \Delta T_R \quad \text{(A5)}$$

or

$$\frac{\Delta c}{c} = \frac{0.4343(T_{ref} - T_o)}{T_x \log T_x} \Delta T_R \quad \text{(A6)}$$

which is the same.

If the relative error in absorbances is sought, the same equation applies, substituting $\Delta A/A$ in place of $\Delta c/c$.

If the S/N ratio is preferred, we have the reciprocal

$$\text{S/N ratio} = \frac{T_x \log T_x}{0.4343(T_{ref} - T_o)} \frac{1}{\Delta T_R} \quad \text{(A7)}$$

From equations (A5) and (A6) the corresponding analytical errors can be calculated. These are the usual forms for the expression of the relative error of precision methods.

In the older literature, the gain in precision attained is assigned to the scale expansion and hence to the lower reading error. Ingle[28] has correctly pointed out that this is only part of the truth, as other sources of uncertainty are present. The enhancement of precision however will depend on which of the noise sources will be significant in a given instrument. If the development of theoretical equations is required, the noise characteristics of the detector should be known, something that is not always available.

Classically, error equations in spectrophotometry have been calculated assuming a given behaviour for the dependence of the spectrophotometric error, ΔT, on T. This in turn depends mainly on the nature of the photodetector. For quantum detectors such as those used in UV–visible spectrophotometry, it has been postulated that ΔT can be considered to be proportional to $T^{\frac{1}{2}}$ or some more complex form such as $(T^2 + T)^{\frac{1}{2}}$.[20]

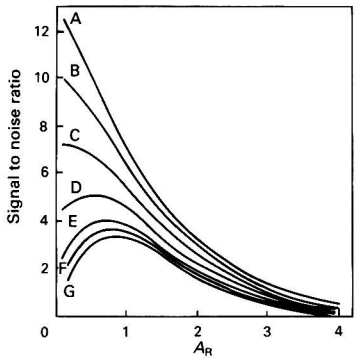

Fig. 9 Theoretical variation of S/N ratio as a function of relative absorbance (A_R) assuming a dependence of the form $KT^{\frac{1}{2}}$ between spectrophotometric noise and transmittance [equation (A10)], for the transmittance-ratio method, at values of A_{ref} of: A, 1.3; B, 1.0; C, 0.7; D, 0.4; E, 0.2; F, 0.1; and G, 0

As an example, the following equation can be derived from equation (A7) for the $T^{\frac{1}{2}}$ dependence:

$$\text{S/N ratio} = \frac{(T_R T_{ref} - T_R T_o + T_o)\log(T_R T_{ref} - T_R T_o + T_o)}{0.4343(T_{ref} - T_o)\,K(T_R)^{\frac{1}{2}}}$$

where K is a proportionality constant.

$$0.4343K\,(\text{S/N ratio}) = \frac{T_x \log T_x}{(T_{ref} - T_o)\,(T_R)^{\frac{1}{2}}} \qquad \text{(A8)}$$

In the high-absorbances method $T_o = 0$, hence equation (A8) becomes

$$0.4343K\,(\text{S/N ratio}) = \frac{T_x \log T_x}{T_{ref}\,(T_R)^{\frac{1}{2}}} = (\text{S/N ratio})' \qquad \text{(A9)}$$

$$(\text{S/N ratio})' = (T_R)^{\frac{1}{2}} \log (T_R T_{ref}) \qquad \text{(A10)}$$

Fig. 9 shows the plot of S/N ratio as a function of A_R for the transmittance-ratio method, for different values of A_{ref}, assuming a simple dependence for the spectrophotometric error of the form $T^{\frac{1}{2}}$. It can be seen that the error can be diminished by application of the precision method, to an extent that depends on the values of A_{ref}.

However, these types of error equation, although useful, are not rigorous, as they depend on certain assumptions about the error that are not necessarily true, as shown by Youmans and Brown,[22] who, for this reason, preferred to develop error equations based only on statistical processing of experimental results. Their considerations were further developed by Bense and Dol[29] for application to the precision methods.

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Sensitive Colorimetric Determination of Ammonium Ion in Water by Laser Photothermal Detection

Eugen Strauss
Department of Physics and Astronomy, University of Georgia, Athens, GA 30605, USA
Jan-Paul Favier, Dane Bićanić* and Kees van Asselt
Department of Agricultural Engineering and Physics, Agricultural University, Duivendaal 1, 6701 AP Wageningen, The Netherlands
Marcel Lubbers
Department of Soil Science and Geology, Agricultural University, Duivendaal 1, 6701 AB Wageningen, The Netherlands

Photothermal beam deflection was combined with the established and proven indophenol colorimetric method for the measurement of trace amounts of ammonium ion in water. A 7 mW 632 nm He–Ne laser and a 4 mW 780 nm Al–Ga–As semiconductor laser provided a limit of detection below 0.25 mmol m^{-3}.

Keywords: *Ammonium determination; trace detection; photothermal method; aluminium–gallium–arsenic diode laser; helium–neon laser*

Ammonia produced in large amounts from organic nitrogen compounds (*e.g.*, proteins, amino acids and urea) by reductive chemical reactions and under anaerobic conditions by microbiological processes plays an active part in the chemistry of soils. A considerable fraction is also released into the air; its characteristic odour does not go unnoticed in areas with a livestock industry under most meteorological conditions. Ammonia contributes significantly to various atmospheric processes and to water pollution.[1] In particular, the ammonium ion dissolved in rain-water is the main base contributing to the neutralization of atmospheric acidity and is eventually returned to the ground with precipitation. Water bodies are also directly polluted by ammonia and nitrate because of the excessive use of nitrogen fertilizers in agriculture and from the discharge of sewage treatment plants. Ammonium is found at increasing concentrations in surface- and ground-water and in drinking water, which is a source of increasing public concern.

As for other pollutants, sensitive, fast and low-cost analytical methods for the determination of both ammonia and ammonium ion are required for the control of pollution. The same is true for the investigation of ammonia deposition, propagation and decomposition in the environment and its consumption by plants and micro-organisms and by chemical processes. Various detection methods and techniques are presently known; of these, the classical colorimetric (both manual and automated) methods for the determination of ammonia and ammonium ion in sewage effluents, and in raw and potable waters have received much attention. After Nessler's reaction,[2] the methods based on the spectrophotometric measurement of the absorbance of the coloured complexes formed (related to Indophenol Blue)[3,4] are the most well established. In particular, the indophenol reaction is the standard method for the examination of water recommended for example by the Environmental Protection Agency (EPA)[5] and Deutsche Industrie Norm (DIN).[6] The indophenol method is recommended for use at concentration levels ranging from 2 to 70 mmol m^{-3}, but the practical detection limit reported is close to 0.6 mmol m^{-3}.[4–6] Alternative methods include titration, ion-selective electrodes and spectrofluorimetric detection. Under controlled conditions a gas-selective ammonium electrode may reach a limit of detection of <10 mmol m^{-3}, if the non-linear part of the response curve is included.[7] Spectrofluorimetric detection involves a reaction with *o*-phthalaldehyde[8] and fluorescence at about 430 nm; it has been demonstrated to be particularly useful with ion chromatography[9] and shown[10] to be sensitive to <1 mmol m^{-3}.

Of the methods for the detection of ammonium, standard indophenol colorimetry appears to be advantageous for most of the routine applications mentioned above. It requires the least sophisticated instrumentation and is relatively cheap and simple in its chemistry. Furthermore, the possible interference of various salts and other substances frequently present in water has been studied and documented.[4,5] Extending the range of reliable detection for indophenol colorimetry to below the 0.25 mmol m^{-3} level using a simple instrument suitable for field work would be useful in soil science studies and in agricultural work, hence the present investigation of the potential of laser-based photothermal detection in indophenol colorimetry. Photothermal laser spectroscopy is considered here because it is particularly suitable for the measurement of small absorptions, mainly because the signal is zero with no absorption and in addition weak turbidity and scattering do not interfere. Recently, the suitability of this technique for the detection of <0.1 mmol m^{-3} of orthophosphate in Molybdenum Blue colorimetry has been shown.[11] Here, some details of photothermal detection are presented and a reliable detection method for ammonium in water at concentration levels below 0.25 mmol m^{-3} is described.

Experimental

Photothermal Absorption Measurement

Laser-based photothermal detection techniques have been employed in recent years for a wide range of applications, in particular they have been shown to be capable of ultrasensitive trace detection.[12] Photothermal techniques are based on detection of the various effects of heat released in a sample as a result of the absorption of intensity-modulated radiation. Photoacoustics, a technical variant that measures, either by a gas microphone or a piezo-transducer, the acoustic wave generated by thermal expansion, is commonly known and various other photothermal effects can be employed for detection. The photothermal effect on the optical index of refraction has proved to be particularly useful.[13] With laser sources it is experimentally advantageous to measure the gradient of the refractive index rather than the refractive index itself. A technical variant called 'collinear' photothermal

* To whom correspondence should be addressed.

beam deflection spectroscopy[14] (BDS) is obviously particularly suitable for the detection of small absorptions in transparent liquid samples at a fixed wavelength because the entire path length in the sample contributes to the signal and residual small air bubbles do not interfere. In BDS two different laser sources are used. The wavelength of one laser (pump laser) must, as closely as possible, match the absorption wavelength of the species to be detected. The energy absorbed from the periodically modulated pump laser beam heats, optically, the excited region of the sample and produces a time varying spatial profile of the refractive index that acts as a thin diverging 'thermal lens.' A second laser (probe laser), if possible emitting at a different wavelength that is negligibly absorbed by the sample, is used to detect the refractive index gradient. The thermal lens deflects the probe beam and the a.c. deflection is observed at the modulation frequency (with no absorption in the absence of the substance to be measured) using a synchronous lock-in detection technique.

Apparatus

With indophenol colorimetry, ammonium is determined as an emerald green complex which is formed by the reaction of ammonia at pH 12.8–13.0 with hypochloride and salicylate in the presence of sodium nitroprusside as a catalyst. The reagents were prepared following standard procedures:[6] standard NH_4^+ solutions for calibration were prepared from $(NH_4)_2SO_4$ [Merck, *pro analysi* (p.a.)]; sodium phosphate buffer (Merck, p.a.) using a nitrogen-free NaOH solution (Baker) and potassium sodium tartrate solution (Merck, p.a.); the sodium salicylate–sodium nitroprusside (Merck, p.a.) solution was kept in the dark and the sodium hypochlorite (BDH) solution was prepared fresh daily.

The indophenol complex has an absorption band between 600 and 700 nm, with a maximum at 650 nm. This band closely matches the red line of the He–Ne laser at 632.8 nm (Fig. 1). A readily available, moderate power (7 mW) laser suffices as the pump laser for the sensitivity required. A cheap, low power Al–Ga–As semiconductor laser (4 mW, 780 nm) equipped with a collimation lens was used as the probe beam source. At 780 nm the absorption of the complex and of the reagents is small and water absorption is negligible. Both beams have Gaussian profiles and are aligned to intersect in the middle of the sample cuvette at a small angle (about 3°) in order to maximize their spatial overlap (Fig. 2). Two suitable lenses focus the beams into the cuvette; the probe beam to a tighter focus (1/e diameter 50 μm; where e is the base of the natural logarithm) than the pump beam (1/e diameter 150 μm). The probe beam is displaced vertically from the pump beam such that it intersects with the thermal lens in the region of the maximum gradient of the refractive index. This relative displacement is easily aligned by adjusting (with micropositioners) the lens which focuses the pump laser. The deflection of the probe beam by the thermal lens was measured by use of a quadrant position sensor made of silicon diodes (Centronix DQ-50). A 780 nm interference filter was used to block the light from the pump beam which was scattered by the sample and cuvette windows. The photothermal effect was measured in the frequency domain. The intensity of the pump laser was mechanically chopped at 14 Hz. The deflection signal was then modulated at that frequency and fed to a standard lock-in amplifier for de-modulation.

Transmission at the He–Ne wavelength through the cuvette for the range of ammonium concentration of interest was ≥0.5, *i.e.*, the sample was 'optically thin.' The deflection signal is proportional to the absorption coefficient, *i.e.*, the indophenol complex concentration, provided the d.c. temperature (only the modulating component of the temperature is being detected) power of both laser powers and the modulation frequency are constant. It was noted that the residual absorption of the probe beam in the sample did not distort the measured deflection signal when it was normalized to the power intensity reaching the position sensor. The reagents had a small residual absorption at the 632 nm pump wavelength (Fig. 1), which contributed to the deflection measured. It is therefore necessary in practice to subtract the signal generated by a blank reference sample prepared with ammonium-free water from the same reagents as the sample.

The relatively simple experimental set-up was assembled from standard mounts (provided with a magnetic base) and optical components available in a spectroscopy laboratory and arranged on a laboratory-built vibration-damped table. The deflection is typically of the order of 1×10^{-3}° at the concentration level of 2–3 mmol m^{-3} and the displacement at the detector is about 1 μm, *i.e.*, the measurement is prone to noise from mechanical vibrations. However, no special vibrational insulation is necessary if a single sturdy bench carrying all the components is used. Standard solutions and samples could be pipetted directly into a 10 mm photometer cuvette without removing the cuvette from the set-up. Fluid turbulences and rising air bubbles disappeared almost immediately. A lock-in amplifier integration time constant of 10 s was sufficient. Several successive amplifier readings were averaged and the statistical deviations calculated.

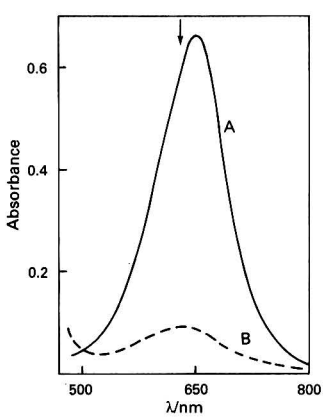

Fig. 1 A, The absorbance of the indophenol complex recorded with a 10 mmol m^{-3} ammonium solution in the 1 cm sample cell; and B, the residual absorption of the blank reagents solution (multiplied by a factor of $\frac{10}{3}$ to enable distinction from the *x*-axis). The arrow indicates the wavelength of the He–Ne pump laser

Results and Discussion

Determination of Ammonium in Standard Solutions

The sensitivity of photothermal indophenol colorimetry was tested with several dilution series prepared from the standard NH_4^+ solution (see above). A low concentration (below 20

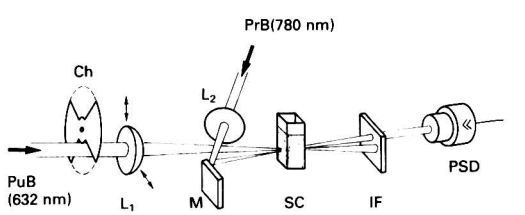

Fig. 2 Experimental set-up for photothermal beam deflection detection of ammonium. C, Sample cuvette; Ch, mechanical chopper; PSD, position-sensitive detector; PrB, probe beam; PuB, pump beam; SC, sample cuvette; L_1 and L_2, lenses; M, plane mirror; and IF, interference filter

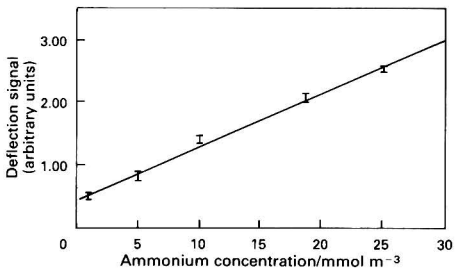

Fig. 3 Variation of the signal as a function of the ammonium concentration in photothermally detected indophenol colorimetry

mmol m^{-3}) in the range of interest was chosen for all further studies. At higher concentrations, a conventional spectrophotometer can be used. However, such samples are also photothermally accessible using dilution or a thinner cuvette to ensure that the sample is optically thin. The result of five replicate runs is depicted in Fig. 3. The reproducibility of the absolute values of deflection was good and limited only by the mechanical stability of the fairly simple experimental set-up. The net deflection signal, *i.e.*, the difference between the signal measured for the sample and the blank reagents solution, is linear in the concentration range 0.25–25 mmol m^{-3}. This detection limit is not restricted by the signal to noise ratio of the deflection signal but rather by the residual absorption near 650 nm observed in the blank solution (the linear absorption coefficient was found to be about 0.02 cm^{-1}), see Fig. 1. A typical value for the absorption of the blank reported in the literature is 0.06 A for a cell 40 mm in length.[4] The indophenol complex has a comparable linear absorption coefficient at a concentration of 0.25 mmol m^{-3}. Hence, the residual absorption of the blank prevents the full utilization of the potential of photothermal detection in the determination of ammonium. The band shape and position of the residual absorption in the red region are about the same as those of the indophenol complex so that the use of a different pump laser wavelength would not alleviate the problem. This similarity may indicate the presence of ammonium impurities in the stock solutions.

Conclusion

The investigation shows that photothermal detection is a very suitable technique for the sensitive colorimetric determination of ammonium in water. Taking into account the interfering residual absorption, the data obtained imply an improvement in the practical detection limit (<0.25 mmol m^{-3}) when compared to that attainable[6] with standard spectrophotometers. An important advantage of this technique is that it is not affected by the slight turbidity present in most 'real' samples. Furthermore, the set-up is basically very simple and constructed from readily available and inexpensive components. A compact instrument[15] suitable for routine laboratory and field-work based on beam deflection detection is feasible. The design could include a 780 nm Al–Ga–As probe laser and a 670 nm semiconductor pump laser instead of an He–Ne laser. A red region semiconductor laser is also absorbed by the indophenol complex and can be expected to become a low-cost item in the near future. The instrument could also be used for phosphate colorimetry by simply interchanging the pump and probe beams of the two lasers.[11]

Colorimetric determination of ammonium in water by means of photothermal detection has been shown to be a sensitive and reliable technique with a practical detection limit of <0.25 mmol m^{-3}. Recent developments in the technology of inexpensive diode lasers[16] indicate their potential for practical and analytical work, in particular for trace detection, in the near future.

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Spectrofluorimetric Flow-through Sensor for the Determination of Beryllium in Alloys

M. de la Torre, F. Fernández-Gámez, F. Lázaro, M. D. Luque de Castro* and M. Valcárcel
Department of Analytical Chemistry, Faculty of Sciences, University of Córdoba, 14004 Córdoba, Spain

A spectrofluorimetric sensor for the determination of beryllium based on the use of morin, immobilized in a resin exchanger located in a flow cell, is proposed. The determinative method developed with this sensor has a linear range between 1 and 40 ppb, with a relative standard deviation of 1.71% and a sampling frequency of 30 h^{-1}. Its selectivity allows the determination of beryllium in simulated alloy samples with excellent results.

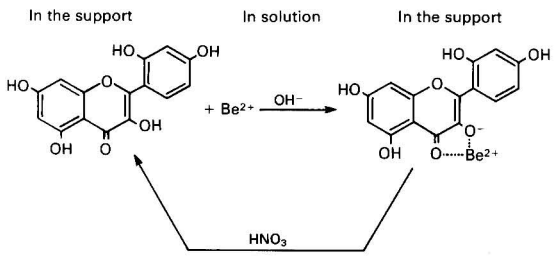
Keywords: *Spectrofluorimetric sensor; beryllium determination; alloy samples; flow injection*

The presence of small concentrations of beryllium in manufactured materials (*e.g.*, in aluminium-based alloys to retard the oxidation of the melt[1] and in nickel alloys to produce high-strength alloys resistant to corrosion and wear[2]) calls for sensitive, accurate analytical methods for the appropriate control of these concentrations. A variety of methods have so far been proposed for this analyte.[3] Because of the lack of specific methods for beryllium, its determination may require a prior separation step depending on the sample matrix. Conventional atomic spectrometric techniques, which are very selective, do not provide sufficiently low determination ranges.[4] On the other hand, spectrofluorimetry is more sensitive and affords lower determination ranges. Morin (2′,4′,3,4,7-pentahydroxyflavone) is one of the spectrofluorimetric reagents for beryllium[5–8] that has been studied most often.

Several flow injection (FI) methods for this analyte have also been proposed, particularly spectrophotometric methods (determination of beryllium in copper-based alloys using Xylenol Orange[9]) and atomic methods (liquid–liquid extraction with acetylacetonate prior to introduction into an inductively coupled plasma atomic emission spectrometer[10]).

The principles behind the proposed sensor have only been used partly, although not successfully, by other workers. Saari and Seitz[11] developed a spectrofluorimetric probe for beryllium based on the immobilization of morin on cellulose. A thin layer of support plus reagent on cellophane was located at the end of an optical fibre, which was then introduced into the solution being measured. The sensor had a response time of 2 min and a restoration period of 3 min. No practical application has been reported. Recently, Capitán *et al.*[12] have reported an ion-exchange batch spectrofluorimetric method for the determination of beryllium with morin. A series of tedious steps makes this method impractical for routine analysis.

The proposed sensor uses morin, previously immobilized on an ion-exchange resin located at the spectrofluorimetric flow cell, which eliminates all manipulation as the sample is injected into a carrier–eluting stream (HNO_3) and, after measurement, the sensor is regenerated, and hence rendered ready for subsequent analyses, according to the following reactions:



* To whom correspondence should be addressed.

Experimental

Reagents

Beryllium (1 g dm^{-3}) and morin (1 g dm^{-3} in a 40% solution of ethanol) were used. More dilute solutions of Be^{II} were prepared in 0.02 mol dm^{-3} NaOH. Dowex 1 X2-100, 1 X4-100, 1 X8-100, 1 X4-50, 1 X4-200, 1 X4-400 and 50 X4-100; Sephadex QAE 50–120 and 25–120 and diethylaminoethyl dextran (DEAE) 50–120 and 25–120 mesh resins, obtained from Sigma, were also used.

Apparatus

Fluorescence measurements were made on a Kontron SFM 25 spectrofluorimeter equipped with a Knauer *x–t* recorder, a Gilson Minipuls-3 peristaltic pump, a Rheodyne 5041 injection valve of variable volume and a fluorescence flow cell with a path length of 1.5 mm packed with the appropriate resin.

Procedure

Solid samples (alloys) must be dissolved in nitric acid and diluted in order to bring the beryllium concentration within the linear range of the calibration graph (1–40 ppb). Ethylenediaminetetraacetic acid (EDTA) was added up to a concentration of 1×10^{-2} mol dm^{-3}. A 1 ml volume of this solution was injected into a single-channel FI manifold through which an eluent stream (2×10^{-2} mol dm^{-3} HNO_3 + 2×10^{-2} mol dm^{-3} $NaNO_3$ + 20 ppb morin) carried the sample plug to the flow cell, where the resin support housed the immobilized ligand (morin). The transient signal (FI peak) thus obtained rapidly reached the baseline as a result of the dissociation of the complex and elution of the analyte. A blank was also used under the same working conditions. The difference between both signals was compared with the data from the calibration graph which had been constructed, under the same conditions, using standards in an acidic medium similar to that used for the samples.

Results and Discussion

Study of Variables

This study was aimed at finding the optimum conditions (sensitivity, stability and regenerability) for the spectrofluorimetric sensor. The variables influencing the system were divided into three groups, namely: those typical of the reaction–detection unit, chemical and FI.

Variables of the reaction–detection unit

The most suitable flow cell was a Hellma Model 176.052 QS with an inner volume of 25 μl and a 1.5 mm path length. This path length was sufficient to ensure adequate sensitivity without the detector capacity being saturated by the fluorescence of the resin plus morin it contained.

The basic variables of the solid support were its chemical nature, mesh size and degree of cross-linking.

Both cationic and anionic resins of different types (Sephadex and Dowex) were studied. The most suitable type of support was Dowex because of its high capacity to retain and elute the anionic complex. This is a result of the electrostatic charges of the complex and exchanger being involved in aromatic interaction with the support. Cationic Dowex supports were unsuitable as they neither immobilized morin nor allowed the formation of the Be–morin complex. The excellent immobilization of morin on the anionic Dowex exchanger was a result of the resonance forms of the reagent present in the basic medium used for immobilization.

The time taken for equilibrium to be reached between the solid phase, on which the ligand is immobilized, and the beryllium solution, increases with decreasing mesh size, hence Dowex 1 X4-200 and 1 X4-400, with smaller mesh sizes, resulted in less effective Be–morin contact than Dowex 1 X4-100.

Increasing degrees of cross-linking (between 2 and 8%) resulted in decreasing fluorescence signals. The most suitable support (Dowex 1 X4-100) was thus used in subsequent experiments.

The level of the resin in the flow cell was a key variable because the maximum formation of the complex took place at the solid/liquid interface. This phenomenon was most intense at the top of the packed support as the flow impinged from above; thus the top of the resin was kept as close as possible to the path length, all of which should be occupied by the resin. The optimum level was 8.0–8.5 mm.

Other variables affecting the reaction–detection unit were those related to the reagent immobilization, *i.e.*, morin concentration in the resin, ethanol content and time required for the immobilization.

The baseline fluorescence signal increased with the concentration of morin retained in the resin up to a constant fluorescence intensity (I_f) of 68% (on a full scale of 100% I_f), which resulted in a narrow range for the occurrence of the analytical signal. On the other hand, high concentrations of morin resulted in uneven formation of the Be^{II} complex possibly because of the difficulty in accessing active sites. This gave rise to an erratic elution equilibrium and, hence, to major irreproducibility of the measurements. A solution of morin at a concentration of 2.0 μg ml^{-1} provided adequate immobilization with good reproducibility and an acceptable range within which to perform the spectrofluorimetric measurements of the analyte.

Increasing the ethanol content of the aqueous solution of morin (between 0 and 100%) resulted in no change in the amount of morin immobilized and produced similar baselines in all instances. Nevertheless, the analyte signal was most clearly defined when the immobilization was carried out with morin in a 40% solution of ethanol. The shape of the transient signal was significant when measurements of the slope of this signal were made.

The influence of the time of contact between the resin and the morin solution was studied for periods between 5 and 420 min. When the period of contact was completed the resin was washed with distilled water and stored in 0.05 mol dm^{-3} HNO_3 for 24 h. The baseline obtained was constant for times equal to or longer than 20 min; 45 min was chosen as the time producing the most reliable results.

Chemical variables

The chemical variables studied included sample pH, type and concentration of eluent, ionic strength and morin replenishment.

The pH of the sample was changed with 1×10^{-3} and 1×10^{-1} mol dm^{-3} NaOH for samples containing 10 ppb of Be^{II}. The maximum signal was obtained for an NaOH concentration of 2×10^{-2} mol dm^{-3}, which resulted in a broadened linear analytical range.

An eluent that could also be used as carrier was the best for this type of sensor as it simplified the FI manifold dramatically. An acid carrier–eluent allowed the Be^{II}–morin complex to be decomposed very rapidly, the Be^{II} being separated and the morin being kept in the resin, ready for the next determination. A 2×10^{-3} mol dm^{-3} solution of HNO_3 provided a lower baseline and higher analytical signal with effective elution.

The ionic strength of the eluent was a key factor because of the degree of hydration, on which the compactness of the resin depends markedly. A decrease in the ionic strength yielded a higher fluorescence intensity; thus, $NaNO_3$ at a concentration of 2×10^{-2} mol dm^{-3} was used in the eluting carrier.

A study of the stability of the immobilized morin was performed by repetitively injecting Be^{II} under the optimum working conditions. There was a slight decrease in the height of the transient peaks with time, which was indicative of a small loss of the reagent caused by the eluting carrier. A morin concentration between 0 and 30 ppb in the carrier was investigated to compensate for this loss. The concentration of ligand in the carrier providing stable signals was 20 ppb.

Flow injection variables

The FI variables studied were the sample volume, which influenced the sensitivity of the method, and the flow-rate, which determines the rate at which the analyte can be eluted and hence the sampling frequency. Another typical FI variable such as the reactor length had no influence on the performance of this method as no chemical reaction was involved during the transport of the sample to the detection system. Its length must be as short as possible in order to minimize dispersion of the sample into the eluting carrier.

The flow-rate was varied between 0.44 and 2.48 ml min^{-1}. These changes indicated that the retention of the Be^{II} (complex formation) was not instantaneous as the transient signal increased as the flow-rate decreased. Nevertheless, very low flow-rates were incompatible with short residence times and rapid baseline restoration; thus, a flow-rate of 1.2 ml min^{-1} was selected as a compromise.

Sample volumes smaller than 100 μl yielded signals that were similar to that of the blank. The signal obtained with sample volumes larger than 100 μl increased linearly up to a sample volume of at least 2.5 ml, which leads to the conclusions that (*i*) the immobilized reagent was in large excess and (*ii*) the rate of formation of the Be^{II}–morin complex was fairly low. As large sample volumes resulted in decreased sampling frequency, an injected volume of 1.0 ml was selected.

Features of the Method

The calibration graph was constructed under the optimum working conditions. The data obtained revealed a linear

Table 1 Tolerated ratios of interferents in the determination of beryllium

Ion added	Tolerated ratio of Be^{II} : foreign ion (m/m)*	
Ca^{II}	1 : 100	—
Cu^{II}	1 : 100	—
Co^{II}	1 : 40	(1 : 400)
Fe^{III}	1 : 40	(1 : 400)
Mg^{II}	1 : 10	(1 : 300)
Al^{III}	1 : 1	(1 : 100)

* Ratios in parentheses correspond to the tolerance in the presence of 1×10^{-2} mol dm^{-3} EDTA.

Table 2 Determination of beryllium in simulated typical alloys

Alloy	Composition (%) Be	Co	Ni	Ag	Uses	Be found (%)*
Copper-based	4.00	—	—	—	Master alloy	4.00 ± 0.04
	1.90	0.24	—	—	Electrical terminals, springs	2.00 ± 0.05
	3.40	0.24	—	—	Welder bar	3.30 ± 0.03
	0.50	2.50	—	—	Good electrical conductor	0.51 ± 0.04
	0.40	1.60	—	0.95	Bearings	0.43 ± 0.07
	0.40	—	1.50	—	Welding equipment	0.40 ± 0.02
	2.10	0.50	—	—	Casting alloy	2.30 ± 0.03
	2.45	—	1.10	—	Casting alloy	2.44 ± 0.06
Aluminium-based	5.00	—	—	—	Master alloy	4.97 ± 0.04
	0.25	—	—	—	Aircraft alloy	0.25 ± 0.02
Nickel-based	1.80	—	—	—	Springs	1.85 ± 0.03
	2.70	—	—	—	Castings	2.73 ± 0.03

* ± standard deviation (n = 3).

analyte concentration range between 1 and 40 ppb. Two equations were obtained according to whether the height of the transient signal or its rising slope was measured between 15 and 30 s. The equations and their regression coefficients are as follows:

$$A_{max}: S_1 = 10.48X + 6.767 \quad (r^2 = 0.9960)$$
$$T_g\ (15\text{–}30\ s): S_2 = 0.50X + 0.440 \quad (r^2 = 0\ 9964)$$

where S_1, S_2 and X are % fluorescence intensity, % fluorescence intensity s^{-1} and ppb of beryllium, respectively; T_g is the slope of the rising portion of the analytical signal, S, and is given by $T_g = (S_{30} - S_{15})/(t_{30} - t_{15})$ (the subscript numbers correspond to time, in seconds).

The reproducibility of the method was studied on 11 samples containing 20 ppb of Be^{II} injected in triplicate. The precision obtained, expressed as per cent. relative standard deviation (RSD), was 1.71. The sampling throughput was 30 samples h^{-1}.

A study of potential interferences in the determination of Be^{II} using the proposed method was performed. The determinations were carried out at a Be^{II} concentration of 20 ppb and the maximum Be^{II} : foreign ion ratio was 1 : 100. Table 1 lists the tolerated ratios of the species determined. A given species was considered not to interfere when the signal obtained lay in the interval $S_s \pm \sigma$ (S_s = fluorescence intensity of the analyte without interferent, σ = RSD). Only one of the ions assayed (Al^{III}) interfered at the same level as the analyte, while most of the others did not interfere in excesses of up to 100-fold over the analyte concentration. Nevertheless, the selectivity of the method was inadequate for application to the determination of beryllium in alloys. Thus, the masking of these species with EDTA at different concentrations was performed in order to increase their tolerated level.[6] The tolerated levels achieved by masking with 1×10^{-2} mol dm^{-3} EDTA are listed in parentheses in Table 1.

Application of the Method to Simulated Alloys

As the most common samples for the determination of beryllium are alloys, and taking into account the lack of standards of this type, simulated alloys,[13] with similar compositions to the most common alloys and with an acidic medium to assimilate the matrix of real samples, were prepared. The acid concentration in the samples was that required for the dissolution of the alloys, taking into account that the mass of the sample would be 2.5 mg. Table 2 lists the composition of these samples and the use of each, and also the percentage of Be^{II} obtained for the different samples that were analysed. The procedure used is described under Experimental.

These results show good agreement between the experimental and actual values for all the alloys studied.

Conclusions

The results obtained by applying the proposed method to synthetic samples similar to alloys lead to the conclusion that the proposed sensor is suitable for this type of analysis as the determination is fast, selective, inexpensive and simple. The proposed method has the following features: (*i*) it uses a very simple single-channel manifold with a conventional spectrofluorimetric detector, (*ii*) requires no sample pre-treatment, (*iii*) has low reagent consumption, (*iv*) provides low determination limits and a good linear determination range, (*v*) allows good sample throughput, and (*vi*) provides excellent selectivity, which allows the determination of the analyte in real samples.

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Thiophosphoryl Compounds as Novel Inducing Agents in the Iodine–Azide Reaction

Witold Ciesielski, Włodzimierz Jędrzejewski and Zbigniew H. Kudzin
Institute of Chemistry, University of Łódź, Narutowicza 68, 90-136 Łódź, Poland
Piotr Kiełbasiński and Marian Mikolajczyk
Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363 Łódź, Poland

The application of organophosphorus compounds as inducing agents in the iodine–azide reaction was investigated. The induction activity was exhibited by thiophosphoryl compounds; their induction coefficients were dependent on the number and nature of sulphur atoms in the $P(S)_n$ function. These relationships can be used for the group differentiation of organophosphorus compounds, for example phosphates, thiophosphates and dithiophosphates. On the basis of their induction activity the microdetermination of thiophosphoryl inductors on a nmol scale was elucidated.

Keywords: *Induced iodine–azide reaction; organophosphorus inductors; microdetermination of thiophosphoryl inductors*

The induced iodine–azide reaction, originally carried out by Raschig,[1] has been explored extensively for decades in analytical chemistry.[2–10] This reaction, represented schematically in equation (1), occurs in the presence of inducing agents (inductors), usually divalent sulphur-containing compounds:

$$I_2 + 2N_3^- \xrightarrow{\text{inductor}} 2I^- + 3N_2 \qquad (1)$$

The greatest induction was exhibited by dithiocarbamates,[8,11] thiolamino acids, *e.g.*, cysteine[4,9,12] or reduced gluthathione,[9,13] thioureas[14] and also compounds bearing thiopurine[15] or thiopyrimidine[16] moieties. However, of the many analytical papers published on the iodine–azide reaction there is only one describing the induction activity of the P–S-containing compounds, and this is limited to thin-layer chromatographic detection of phosphorothiono ester-based pesticides.[17] Taking into account the industrial importance of organic thiophosphoryl compounds (*e.g.*, plant protection agents, extraction and flotation agents, rubber industry),[18–20] the large positive effect caused by thiophosphoryl inductors can constitute the basis for a novel procedure for their determination. In this paper the results of studies on the inductive effect of some thiophosphoryl compounds on the iodine–azide reaction are presented.

The efficiency of thiophosphoryl compounds as inductors has been characterized and compared on the basis of their induction coefficients (F_i), defined by

$$F_i = n_I/n_i \qquad (2)$$

where n_I = millimoles of iodine consumed in the induced reaction and n_i = millimoles of the inductor.

Experimental

The iodine determinations were performed using a VSU-2P spectrophotometer (Carl Zeiss, Jena) equipped with quartz cuvettes of path length (l) 1 or 5 cm.

Reagents and Solutions

All compounds **1–12** (Table 1) were synthesized according to the literature and were of the same purity as reported previously.[21] They were also found to be homogeneous according to ^{31}P NMR data. Organophosphorus compounds **1** and **2** were used as 0.001 mol dm^{-3} aqueous solutions and **3–12** as 0.001 mol dm^{-3} ethanolic solutions. All aqueous solutions were prepared using re-distilled (in glass apparatus) water.

Iodine solution. A 0.01 mol dm^{-3} aqueous stock solution of iodine, containing 4 g l^{-1} of potassium iodide, was prepared and a 0.0015 mol dm^{-3} solution was prepared by dilution of the stock solution.

Potassium iodide solution. A 0.35 mol dm^{-3} aqueous solution was prepared.

Reaction solution. The solution contained 20 g l^{-1} of sodium azide and was buffered to pH 6 with hydrochloric acid.

Determination of the Inductive Effect on the Iodine–Azide Reaction

The consumption of iodine in the induced iodine–azide reaction was determined spectrophotometrically by measuring the absorbance at 350 nm, characteristic of the triiodide complex (I_3^-). The molar absorptivity of the triiodide was dependent on the concentration of the iodide anion owing to the reversibility of the formation reaction

$$I_2 + I^- \rightleftharpoons I_3^- \qquad (3)$$

with a plateau where the potassium iodide concentration is about 3×10^{-2} mol dm^{-3}. At this point the molar absorptivity reaches a maximum value of 2.5×10^4 dm^3 mol^{-1} cm^{-1}.

However, at the same time the induced reaction is adversely affected by the presence of the iodide anion and is highly suppressed when the iodide concentration reaches about 1×10^{-2} mol dm^{-3}. To reconcile these two opposing effects, all experiments were carried out at the lowest possible iodide concentration (about 1.2×10^{-4} mol dm^{-3}), resulting only from the applied solution of potassium triiodide. Subsequently, the concentration of potassium iodide was adjusted to 3×10^{-2} mol dm^{-3}, *i.e.*, the optimum for the subsequent spectrophotometric determination of iodine.

Procedure for Determination of the Induction Coefficient

A 50 μl volume of a solution of iodine was injected into the reaction cell containing 10 ml of a stirred reaction solution and a sample of the organophosphorus inductor (0.5–160 nmol). The reaction mixture was allowed to stand for the time indicated in Table 1, 1 ml of potassium iodide solution was added and the mixture was measured spectrophotometrically at 350 nm, giving the total absorbance of unconsumed iodine (A). A similar measurement on a blank solution without organophosphorus inductor gave the total absorbance (A_0).

The amount of iodine consumed in the induced iodine–azide reaction was calculated from the equation

$$n_I = \frac{(A_0 - A)\,V}{0.5\,\varepsilon_{I_3^-}\,l} \quad (4)$$

where V is the volume of the reaction mixture (11.1 ml), l is the light path of the cuvettes (1 cm) and $\varepsilon_{I_3^-}$ is the molecular absorptivity of the triiodide anion (2.5×10^4 dm^3 mol^{-1} cm^{-1}). The induction coefficients of the organophosphorus inductors (F_i) were calculated from equation (2).

Procedure for Determination of Thiophosphoryl Inductor

The determination of the thiophosphoryl inductor (*e.g.*, disulphide **7**, see Table 2) was performed under conditions identical with those described for the determination of the induction coefficients. For the determination of compound **7** at levels of 0.05–0.25 nmol a 0.0015 mol dm^{-3} iodine solution was used, followed by ultraviolet spectrophotometric measurements in cuvettes with a light path l = 5 cm.

The relationship $\Delta A = A_0 - A$ as the function of the amount of inductor was used in the construction of the calibration graph (linear in the ranges 0.050–0.25 and 0.25–2.00 nmol of compound **7**) and was applied to the spectrophotometric determination of compound **7**.

Results and Discussion

The results obtained for the induction activities of organophosphorus compounds **1–12** in the induced iodine–azide reaction are given in Table 1. They indicate that the induction activity of thiophosphoryl compounds is strongly dependent on the structure, especially on the nature of the P–S bonds. Thus, potassium diethyl phosphorodithioate (**1**) exhibits a high induction activity (F_i = 220), apparently due to the presence of the thiolate function in the PS_2^- anion. For disulphide **7**, which may be formally considered to be a dimer of compound **1**, the induction activity (F_i = 450) is approximately double that of compound **1**, probably owing to the facile cleavage of **7** under the reaction conditions. However, the induction activity of potassium diethyl phosphorothioate (**2**) (F_i = 40) is only about 20% of that of compound **1**.

Conversion of a free thiolate function in compound **1** into a stable thioester function causes a decrease in the induction activity of the resulting compounds. Thus, comparison of the induction activities of triethyl thiophosphates, compounds **3–6**, reveals a lack of activity of the phosphorothiolate **4** (F_i = 0), low activity of triethyl phosphorothionate (**3**) (F_i = 6) and triethyl phosphorotetrathiolate (**6**) (F_i = 8) and a slightly higher activity of triethyl phosphorodithioate (**5**) (F_i = 20). In contrast, phosphine sulphides **10** and **11**, containing the P–S bond, exhibit remarkably high induction effects, comparable to that of potassium diethyl phosphorodithioate (**1**), with induction activity coefficients of 190 and 220, respectively. However, the replacement of the phenyl group in phosphine sulphide **11** by the ethoxy group leads to ethyl diphenylphosphinothionate (**12**) and to a substantial decrease in the induction activity of this compound (F_i = 105). Tetraethyl monothiopyrophosphate (**9**) and tetraethyl dithiopyrophosphate (**8**) also exhibit low induction activities (F_i = 6 and 12, respectively).

The proposed method can also be used for the determination of thiophosphoryl compounds by application of the calibration procedure described for C–S inductors.[9] Thus, the determination range of disulphide (**7**) (F_i = 450) is between 0.25 and 2.0 nmol (or 0.05–0.25 nmol using a cuvette with a light path l = 5 cm) (Table 2). The determination ranges for other thiophosphoryl compounds tested are higher, corresponding to their lower induction activities expressed by their F_i values (Table 1). The analytical evaluations of the induction activities of thiophosphoryl compounds will be published elsewhere.

The determination of the induction coefficients can also be used to distinguish between phosphates, phosphorothioates and phosphorodithioates and also phosphine sulphides from phosphine oxides.

Table 1 Induction coefficients (F_i) of thiophosphoryl compounds*

No.	Structure	Reaction time/min	Induction coefficient (F_i)	Range of determination/ nmol
1	$(EtO)_2PS_2K$	3	220	0.5–4
2	$(EtO)_2PSOK$	5	40	2.5–25
3	$(EtO)_3P{=}S$	15	6	20–160
4	$(EtO)_2P(O)SEt$		0	
5	$(EtO)_2P(S)SEt$	15	20	5–40
6	$(EtS)_3P{=}S$	10	8	15–120
7	$(EtO)_2P(S)SP(S)(OEt)_2$	3	450	0.25–2.0
8	$(EtO)_2P(S)OP(S)(OEt)_2$	10	12	10–80
9	$(EtO)_2P(O)OP(O)(OEt)_2$	15	6	20–160
10	$Bu_3P{=}S$	1	190	0.6–5
11	$Ph_3P{=}S$	10	213	0.5–4
12	$Ph_2(EtO)P{=}S$	12	105	1.0–8

* Phosphoric acid, diethylphosphoric acid and triphenylphosphine oxide did not exhibit the induction.

Table 2 Results for the determination of disulphide **7** (n = 6)

Determination No.	Cuvette path length, l/cm	Taken/ nmol	Found/ nmol	Relative standard deviation (%)
1	1	0.25	0.26	5.2
2	1	0.50	0.52	2.4
3	1	1.00	1.02	3.6
4	1	1.50	1.48	3.0
5	1	2.00	2.01	2.1
6	5	0.050	0.047	7.3
7	5	0.100	0.101	4.6
8	5	0.150	0.153	3.1
9	5	0.200	0.198	3.5
10	5	0.250	0.245	2.4

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Spectrophotometric Determination of Trace Amounts of Copper(I) and Reducing Agents With Neocuproine in the Presence of Copper(II)

Esma Tütem and Reşat Apak*
Department of Chemistry, Faculty of Engineering, University of Istanbul, Vezneciler 34459, Istanbul, Turkey
Fikret Baykut
Department of Chemical Engineering, Faculty of Engineering, University of Istanbul, Vezneciler 34459, Istanbul, Turkey

The existing spectrophotometric method for the determination of total Cu with neocuproine (Nc) does not allow the differentiation of Cu^{I} and Cu^{II}. It is shown here that the use of a dilute (3.0×10^{-3} mol dm^{-3}) Nc solution in weakly acidic or neutral media makes the determination of Cu^{I} feasible at the 1×10^{-5} mol dm^{-3} level in the presence of up to 0.1 mol dm^{-3} Cu^{II}. For the determination of trace amounts of Cu^{I} in the presence of 1 mol dm^{-3} Cu^{II}, the latter can be masked by NH_3–NH_4Cl buffer at pH 10, giving almost the same molar absorptivity for Cu^{I}, *i.e.*, 7.5×10^3 dm^3 mol^{-1} cm^{-1}. The ability to measure the absorption due to Cu^{I} in the presence of an excess of Cu^{II} was exploited as the basis of an indirect method for the determination of trace amounts of reductants. Superior to the existing individual methods of determination, which are usually specific for a given reducing agent, the proposed system consists of treating the reductant with the Cu^{II}–Nc reagent in ammonium acetate buffered media, followed by measurement of the absorbance of the Cu^{I}–Nc chelate at 450 nm. The quantification of a given reductant in the concentration range 1×10^{-6}–1×10^{-4} mol dm^{-3} usually takes 3 min with a mean relative standard deviation of 3%. The molar absorptivity of an *n*-electron reductant which has reacted stoichiometrically is approximately $7.5n \times 10^3$ dm^3 mol^{-1} cm^{-1}, *i.e.*, *n* times that of Cu^{I}–Nc. Hence, hydrogen peroxide, ascorbic acid, cysteine, hydroxylamine, hydrazine, thiosulphate, dithionite, mitoxantrone, glutathione, iron(II) and thiourea were determined with theoretical molar absorptivities; carminic acid, sulphite, tin(II) chloride, 2,4-dinitrophenylhydrazine, sodium tetrahydroborate(III) and 2,3-dimercaptopropan-1-ol were determined with the aid of empirical linear absorbance–concentration plots. The proposed spectrophotometric method is rapid and allows the quantification of biologically important reducing agents.

Keywords: *Copper(I) determination; reducing agent determination; copper(II)–neocuproine reagent; neocuproine; spectrophotometry*

The formation of the charge-transfer complex[1] between Cu^{I} and neocuproine(Nc)(2,9-dimethyl-1,10-phenanthroline) is the basis of the existing spectrophotometric method for the determination of total Cu.[2–5] However, this method cannot differentiate between the mono- and divalent forms of Cu. Copper ion-selective electrodes are also useless for analysing mixtures of Cu^{I} and Cu^{II}.[6]

The Cu^{I}–Cu^{II} redox couple is the focus of much attention in the studies of metalloenzyme modelling[7] and Cu speciation in environmental waters.[8] Therefore, the quantification of trace amounts of Cu^{I} in the presence of an excess of Cu^{II} is important from an analytical standpoint.

Considering the relative stabilities of the Nc and ethylenediaminetetraacetic acid (EDTA) complexes of Cu^{I} and Cu^{II} under specified experimental conditions, Ulibarri *et al.*[9] concluded that the selective determination of Cu^{I}, stabilized by allyl alcohol in the mixture, should be possible in the presence of Cu^{II} by masking the Cu^{II} with EDTA. However, the present study showed that such a high concentration of Nc (0.1% in ethanol), as used in the above work,[9] would inevitably result in a positive interference from Cu^{II}. Moreover, the unexpectedly low absorbances at 450 nm reported by the same workers[9] of mixtures containing Cu^{I}, Cu^{II}, Nc and EDTA, which could have arisen from incomplete conversion of Cu^{I} into the Cu^{I}–Nc complex *via* mixed-ligand complex formation, suggest that there is a need to develop more satisfactory analytical procedures.

Moffett *et al.*[10] were able to measure the absorbance due to Cu^{I}–bathocuproine while preventing the reduction of 1×10^{-5} mol dm^{-3} Cu^{II} by NH_2OH by masking the Cu^{II} with ethylenediamine; however, concentrations of Cu^{II} were not tested.

Hence a simple and reliable analytical method for Cu^{I} has been developed in the present study which does not require the use of masking agents for Cu^{II} up to a concentration of 0.1 mol dm^{-3}; most masking agents lead to negative errors in the quantification of Cu^{I}. The proposed method is also suitable for the determination of reducing agents with high sensitivity.

Various spectrophotometric procedures exist for the determination of biologically important reductants: hence, thiol groups can be determined with sulphanilamide–naphthylethylenediamine,[11] *N*-ethylmaleimide,[12] *p*-chloromercuribenzoate,[13] *N,N*-dimethyl-1,4-phenylenediammonium chloride and chloramine-T,[14] aminophenols and iron(III)[15] or by using 1-chloro-2,4-dinitrobenzene and the Ellman reagent.[16,17] Glutathione can be determined with alloxan;[18] 1,2-dithiols [*e.g.*, 2,3-dimercaptopropan-1-ol (BAL)] with benzidine;[19] thiourea with iron(III) thiocyanate[11] or tetraiodomercurate;[20] ascorbic acid with phosphomolybdate,[11] 2,6-dichlorophenolindophenol[11] or sulphanilamide–naphthylethylenediamine;[11] and hydroxylamine by using the sulphanilic acid–1-naphthylamine azo coupling[21] or aldoxime[22] reaction.

Reducing agents can be determined by reduction of a metal ion exhibiting multiple valencies, followed by treating the lower valency of the metal with a chromogenic reagent. Ascorbic acid was determined by oxidation with Fe^{III}–1,10-phenanthroline[23–25] or by oxidation with Cu^{II} and subsequent colour development with Nc in a mono-[26] or biphasic system.[27] Schilt and Di Tusa[28] used a chromogenic reagent for Fe^{II} such that the reductant to be determined reduced Fe^{III} to a coloured product. However, these redox reactions required elevated temperatures (60–90 °C) and considerable periods of time (30–240 min) and were mostly incomplete,[28] so that the

* To whom correspondence should be addressed.

calibration graphs did not correspond to the theoretical n-electron oxidations of the reductants being determined.

In this work the ability to measure trace amounts of Cu^{I} in the presence of an excess of Cu^{II} was exploited with the aim of indirectly quantifying reducing agents that could reduce the Cu^{II}–Nc reagent at a suitable pH to the coloured Cu^{I}–Nc chelate. Hence, an n-electron reductant that reacted stoichiometrically would exhibit an effective molar absorptivity of approximately $7.5n \times 10^3$ dm^3 mol^{-1} cm^{-1}.

Experimental

Reagents

All chemicals were obtained from Merck and were of analytical-reagent grade with the exception of mitoxantrone, which was synthesized according to the procedure of Murdock *et al.*[29]

All solutions and distilled water were de-aerated with oxygen-free nitrogen before use. Copper(II) solutions of 0.1, 0.01 and 0.001 mol dm^{-3} were prepared by appropriate dilution of a 1 mol dm^{-3} $CuCl_2$ stock solution.

Copper(I) solution, 1×10^{-3} mol dm^{-3}. Prepared from a newly opened pack of CuCl which had been kept in a de-aerated desiccator. A 0.0099 g amount of CuCl was dissolved in 25 ml of 7% NH_4Cl solution and diluted to 100 ml with water, the operation being carried out under a flow of nitrogen. This solution can only be used for four measurements within a period of 15 min after which it must be discarded, otherwise probable oxidation and disproportionation reactions of Cu^{I} would cause absorbance decreases.

When Cu^{I} alone is to be used rather than a synthetic mixture of both Cu^{I} and Cu^{II}, a 1×10^{-3} mol dm^{-3} Cu^{I} solution can be prepared from the corresponding amount of $CuCl_2$ in 1×10^{-2} mol dm^{-3} NH_2OH solution.

Stable Cu^{I} *solution in the form of* $Cu(Nc)_2Cl$, 1×10^{-3} mol dm^{-3}. Copper(II) chloride (0.05 mmol) was dissolved in 50 ml of 1×10^{-2} mol dm^{-3} NH_2OH·HCl solution and 10 ml of ethanolic 0.015 mol dm^{-3} Nc solution were rapidly added. The solution was shaken with consecutive 10 ml portions of $CHCl_3$ until the $CHCl_3$ phase was colourless. The combined organic phases were evaporated under a gentle stream of nitrogen, and 25 ml of ethanol were added before the $CHCl_3$ solution became dry. The addition of ethanol and the evaporation procedure were repeated twice under nitrogen, ensuring that the ethanolic solution did not evaporate to complete dryness. The ethanolic residue was taken up in 50 ml of 96% ethanol and stored in a refrigerator. The resulting solution is stable and has a Cu^{I} concentration of 1×10^{-3} mol dm^{-3}.

Ammonia–ammonium chloride concentrated buffer of pH 10. Prepared from commercial (25% m/m) NH_3 solution and solid NH_4Cl as 7.2 ± 0.1 mol dm^{-3} NH_3 and 1.3 mol dm^{-3} NH_4Cl. The variation in NH_3 concentration was caused by bubbling nitrogen through the buffer occasionally.

Working solutions (5.0×10^{-4} mol dm^{-3}) of the reducing agents (including NH_2OH·HCl) were prepared from the corresponding 1×10^{-2} mol dm^{-3} stock solutions. A 1×10^{-2} mol dm^{-3} hydrogen peroxide solution was prepared from commercial hydrogen peroxide (35%) and standardized by titration with $KMnO_4$ solution. A 5.0×10^{-4} mol dm^{-3} 2,4-dinitrophenylhydrazine solution was prepared in ethanol. A 1×10^{-2} mol dm^{-3} $NaBH_4$ solution was prepared in 1×10^{-2} mol dm^{-3} NaOH immediately before use. The pH 7 buffer was 1 mol dm^{-3} ammonium acetate. The pH 7.4 phosphate buffer was obtained by mixing equimolar (1×10^{-3} mol dm^{-3}) Na_2HPO_4 and NaH_2PO_4 solutions in appropriate proportions. A 0.3 mol dm^{-3} solution of EDTA was prepared from Na_2EDTA. A 3.0×10^{-3} mol dm^{-3} Nc solution was prepared in 96% ethanol.

Apparatus

The absorptions at 450 nm were recorded with a Beckman DB-GT ultraviolet–visible spectrophotometer in silica cuvettes. The pH measurements were made with a Metrohm E-512 pH meter. A Texas TI 58C programmable calculator was used for regression analysis of the absorbance–concentration data.

Spectra

Copper(I) chloride, or Cu^{I} obtained by reduction of Cu^{II} with NH_2OH in Cu^{II} solutions up to 0.1 mol dm^{-3}, absorbs at 450 nm against water in the Nc procedure. As for the NH_3 buffered 1 mol dm^{-3} Cu^{II} solutions, both blank and Cu^{I}-containing samples have a relatively high absorption at 450 nm; however, the Cu^{I} sample exhibits maximum absorbance at 450 nm against the reagent blank.

Procedure for Studying the Cu^{I}–Cu^{II} System

A 1 ml aliquot of a $CuCl_2$ solution of the desired concentration (1.0, 0.1, 0.01 or 0.001 mol dm^{-3}) was placed in a test-tube and a flow of nitrogen through a capillary tube was started. After de-aerating for 1 min, either x ml of 1.0×10^{-3} mol dm^{-3} CuCl ($0.1 \leqslant x \leqslant 1.0$) or x ml of 5.0×10^{-4} mol dm^{-3} NH_2OH·HCl ($0.1 \leqslant x \leqslant 1.0$) was added. In the latter instance, where Cu^{I} is produced in the mixture by the addition of NH_2OH, bubbling of nitrogen was continued for a further 1 min to allow the reduction to go to completion. Then, $(4 - x)$ ml of water was added for the unbuffered medium studies or $(4 - x - y)$ ml of water for the buffered work, followed by y ml of concentrated NH_3 buffer. Finally, 2.5 ml of 3.0×10^{-3} mol dm^{-3} Nc in ethanol were added. Thirty seconds after the Nc had been added, the capillary tube was removed and the contents of the test-tube were transferred into a silica cuvette. The absorbance was measured at 450 nm against water 2 min after the Nc had been added. A reagent blank (containing no CuCl or NH_2OH) was run for all the experiments. Air must be removed from all the solutions and from the distilled water by purging with nitrogen prior to the experiments and all additions of reagents must be carried out under a flow of nitrogen. The total aqueous and ethanolic volumes in the final solution are 5.0 and 2.5 ml, respectively. For studies with pure CuCl (without Cu^{II}) either $(5 - x)$ or $(5 - x - y)$ ml of water was added depending on whether the unbuffered or buffered medium was being employed.

Recommended Procedure for the Determination of Trace Amounts of Cu^{I} in the Presence of Cu^{II}

Procedure A or B should be selected depending on the Cu^{II} concentration in the sample. If the total Cu concentration is not known before analysis, it should be measured by the conventional NH_2OH–Nc method.[2–4]

If the Cu^{II} concentration is $\leqslant$0.1 mol dm^{-3}, procedure A should be used; if the Cu^{II} concentration is of the order of 1 mol dm^{-3}, procedure B should be used.

Procedure A

Place an aliquot of a weakly acidic or neutral sample solution (x ml) containing preferably 0.5×10^{-4}–10×10^{-4} mmol of Cu^{I} in a test-tube. Dilute the sample with 7% NH_4Cl solution before analysis if necessary to bring the Cu^{I} concentration within the desired range. Add $(5 - x)$ ml of water and 2.5 ml of 3.0×10^{-3} mol dm^{-3} Nc in ethanol. Measure the absorbance at 450 nm against water after 2 min and make the necessary correction for the reagent blank. (Carry out the passage of nitrogen throughout the procedure as described above.) The molar absorptivity for Cu^{I}, obtained by reduction of Cu^{II} with suitable amounts of NH_2OH, is 8.0×10^3 dm^3 mol^{-1} cm^{-1}. For the determination of Cu^{I} in the range $1.33 \times 10^{-5} \leqslant c_{Cu^{I}} \leqslant$

1.6×10^{-4} mol dm^{-3} in the final solution (11 different concentrations), Beer's law is obeyed, and the equation of the straight line is: $A = 8.0 \times 10^3 c + 0.044$ (correlation coefficient = 0.9995), where A is the net absorbance and c the Cu^{I} concentration (mol dm^{-3}) in the final solution. The relative standard deviation is 4.2%. The limit of detection and the limit of quantification for Cu^{I} in the final solution are 1.5×10^{-6} and 5.0×10^{-6} mol dm^{-3}, respectively. However, the molar absorptivity for 'unprotected' Cu^{I}, *i.e.*, Cu^{I} used in the form of CuCl rather than produced *in situ* by the addition of NH_2OH, might be lower (4.5×10^3–7.5×10^3 dm^3 mol^{-1} cm^{-1}) depending on the Cu^{II} concentration. Therefore, in such instances the Cu^{I} concentration can be found from a calibration graph obtained with variable amounts of standard CuCl in a standard Cu^{II} solution with a Cu^{2+} concentration identical with that of the solution to be analysed.

Procedure B

Place an aliquot of a weakly acidic or neutral sample solution (x ml) containing preferably 0.5×10^{-4}–5×10^{-4} mmol of Cu^{I} in a test-tube. Dilute the sample with 7% NH_4Cl solution before analysis if necessary to bring the Cu^{I} concentration within the desired range. Add $(4.35 - x)$ ml of water, 0.65 ml of pH 10 buffer and 2.5 ml of 3.0×10^{-3} mol dm^{-3} Nc in ethanol in this order. Measure the absorbance at 450 nm against water after 2 min and correct for the reagent blank. (Carry out the passage of nitrogen throughout the procedure and stop the flow of nitrogen 30 s after the addition of Nc.) When the amount of Cu^{II} is 1 mmol, the reagent blank has an absorbance of 0.34 against water. The effective molar absorptivity for Cu^{I} produced *in situ* by reduction with NH_2OH is 7.7×10^3 dm^3 mol^{-1} cm^{-1}. The equation of the straight line is: $A = 7.7 \times 10^3 c + 0.037$ (correlation coefficient = 0.998), where A is the net absorbance and c the Cu^{I} concentration (mol dm^{-3}) in the final solution. However, the molar absorptivity for 'unprotected' Cu^{I} (used in the form of CuCl) is 4.4×10^3 dm^3 mol^{-1} cm^{-1} under the described experimental conditions. Therefore, in such instances the Cu^{I} concentration can be found from a calibration graph constructed by preparing solutions containing various CuCl concentrations in a standard 1 mol dm^{-3} $CuCl_2$ solution.

If the Cu^{II} concentration of the sample is between 0.1 and 1.0 mol dm^{-3}, the amount of NH_3 (in mol) should be 4.6 times that of Cu^{II} (15% in excess of the stoichiometric amount required for the tetraammine complex) and hence the volume of buffer should be adjusted accordingly.

Recommended Procedure for the Determination of Reducing Agents

A 1 ml aliquot of a 0.1 mol dm^{-3} Cu^{II} solution was placed in a test-tube and the flow of nitrogen through the capillary tube was started. Then, 2.5 ml of Nc, 1 ml of ammonium acetate, $(3 - x)$ ml of water and x ml of reductant (5.0×10^{-4} mol dm^{-3}) were added in this order. The absorbance at 450 nm was recorded against water 2 min after the addition of the reductant. The measured absorbances were corrected for the absorbance of the reagent blank containing no reducing agent. If the n-electron reductant did not yield a molar absorptivity of approximately $7.5n \times 10^3$ dm^3 mol^{-1} cm^{-1} (*i.e.*, n-times that of Cu^{I}–Nc) in the final (7.5 ml) solution, it was concluded that the redox reaction was not stoichiometric and the procedure was repeated by using an initial Cu^{II} concentration of 0.01 mol dm^{-3} and/or a period of 20 min prior to measuring the absorbance. The absorbances for ten different concentrations of each reductant were measured within the concentration range in which Beer's law was obeyed. The linear equation for the quantification of each reductant was found by regression analysis of absorbance–concentration data. The mean molar absorptivities [$\bar{\varepsilon} = (A_{net}/c)$] and the standard deviations (s) were obtained. The value of ε at the 95% confidence level was calculated from the equation: $\varepsilon = \bar{\varepsilon} \pm ts/\sqrt{n}$, where $t_{0.975} = 2.26$ and $n = 10$.

Results and Discussion

By using an excess of the Nc reagent in a weakly acidic or neutral solution, EDTA can prevent Cu^{II} from forming the yellow Cu^{II}–Nc complex only for a limited period of time, whereas the Cu^{I} itself can be masked. Afterwards both Cu^{I} and Cu^{II} tend to exhibit similar absorbances. The effectiveness of EDTA in masking Cu^{II} selectively in preference to Cu^{I} is a function of pH, buffer concentration, EDTA concentration, Nc concentration and time. Ethylenediamine also masks Cu^{II}; however, selective decoloration of Cu^{II}–Nc from equimolar mixtures of Cu^{I} and Cu^{II} was not possible with ethylenediamine at pH 7.2–7.5 (achieved with phosphate buffer) when an excess of Nc was present.

At the working pH used by Ulibarri *et al.*,[9] *i.e.*, pH 6–7, the value of log β_1 for Cu^{II}–EDTA is actually comparable to the value of log β_2 for Cu^{II}–Nc (log $\beta_1 = 11.5$ for Cu^{II}–HY^{3-}; log $\beta_2 = 11.7$ for Cu^{II}–Nc).[30] Hence, EDTA does not hinder the formation of Cu^{II}–Nc at this pH, and this was confirmed experimentally in the present work. The suppression of the absorption due to Cu^{II} was not possible when a concentration of Nc as high as 0.1% in ethanol was maintained.

Standard mixtures of Cu^{I} and Cu^{II} were prepared by adding a measured amount of NH_2OH (a two-electron reductant for Cu^{II})[21] to an excess of Cu^{II}. The molar absorptivity of Cu^{I} produced indirectly by this method is slightly higher than that obtained with pure CuCl (ε = approximately 8.0×10^3 and 7.5×10^3 dm^3 mol^{-1} cm^{-1}, respectively). Similar observations have been reported in the literature, where NH_2OH gave rise to a molar absorptivity which was higher than expected for a two-electron reductant.[28]

The method developed in this work is based on the control of complex formation by the ligand by using 3.0×10^{-3} mol dm^{-3} Nc in ethanol as the ligand. The values of log β_2 for Cu^{I}–Nc and Cu^{II}–Nc are 19.1 and 11.7, respectively.[30] Hence Cu^{II}–Nc cannot be formed in a concentrated Cu^{II} solution within 2 min with such a low ligand concentration, allowing the absorption of Cu^{I}–Nc at 450 nm to be measured. If the Cu^{II} concentration is about 1 mol dm^{-3}, masking with NH_3 buffer at pH 10 is to be preferred. Masking of Cu^{II} with NH_3 buffer is unnecessary for Cu^{II} concentrations $\leq$0.1 mol dm^{-3}, and has

Table 1 Variation of the molar absorptivity of Cu^{I} (as CuCl) with the concentration of Cu^{II}

Cu^{II} concentration/mol dm^{-3}	0	0.001	0.01	0.1
$\varepsilon/10^3$ dm^3 mol^{-1} cm^{-1}	7.5	5.8	4.7	4.5

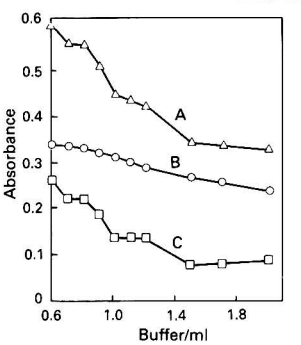

Fig. 1 Variation of absorbance with volume of NH_3 buffer. 1.0×10^{-4} mmol of NH_2OH added to 1 mmol of Cu^{II}. Nc: 2.5 ml of a 3.0×10^{-3} mol dm^{-3} solution in ethanol, total volume = 7.5 ml. A, Sample; B, reagent blank; and C, net absorbance at 450 nm against water

Table 2 Determination of reductants with Cu^{II}–Nc

Reductant	No. of electrons per reductant	Absorbance–concentration line equation*	Correlation coefficient	ε/dm^3 mol^{-1} cm^{-1}	Relative standard deviation (s/ε) (%)	Linear range/ mol dm^{-3}	Comments†
Hydrogen peroxide‡	2	$A = 1.48 \times 10^4 c - 0.010$	0.9999	$(1.44 \pm 0.04) \times 10^4$	3.9	0–1.4×10^{-4}	Quantitative
Ascorbic acid§	2	$A = 1.56 \times 10^4 c - 0.005$	0.9999	$(1.56 \pm 0.02) \times 10^4$	1.2	0–1.3×10^{-4}	Quantitative
Cysteine§	1	$A = 7.48 \times 10^3 c - 0.006$	0.9992	$(7.45 \pm 0.23) \times 10^3$	2.9	0–2.7×10^{-4}	Quantitative
Hydroxylamine hydrochloride§	2	$A = 1.76 \times 10^4 c + 0.004$	0.9990	$(1.75 \pm 0.06) \times 10^4$	2.8	$6.67 \times 10^{-6} \leqslant c \leqslant 1.1 \times 10^{-4}$	Quantitative
Sodium dithionite§	2	$A = 1.63 \times 10^4 c - 0.021$	0.9991	$(1.59 \pm 0.12) \times 10^4$	7.3	0–1.0×10^{-4}	Quantitative
Mitoxantrone dihydrochloride§	2	$A = 1.54 \times 10^4 c + 0.042$	0.9999	$(1.62 \pm 0.06) \times 10^4$	4.0	0–1.1×10^{-4}	Quantitative
Glutathione§	1	$A = 8.51 \times 10^3 c + 0.007$	0.9994	$(8.70 \pm 0.33) \times 10^3$	4.5	$6.0 \times 10^{-6} \leqslant c \leqslant 2.3 \times 10^{-4}$	Quantitative
Hydrazine dihydrochloride§	4	$A = 3.07 \times 10^4 c - 0.003$	0.9992	$(3.05 \pm 0.08) \times 10^4$	2.1	0–6.3×10^{-5}	Quantitative
Fe^{II} (in the form of Mohr's salt)§	1	$A = 7.49 \times 10^3 c + 0.007$	0.9997	$(7.51 \pm 0.22) \times 10^3$	3.1	0–2.6×10^{-4}	Quantitative
Carminic acid‡	4	$A = 2.55 \times 10^4 c + 0.023$	0.9996	$(2.66 \pm 0.09) \times 10^4$	3.9	0–6.7×10^{-5}	Incomplete, but suitable
Thiourea¶	4	$A = 3.19 \times 10^4 c + 0.041$	0.9995	$(3.40 \pm 0.12) \times 10^4$	3.7	0–4.7×10^{-5}	Quantitative
Thiosulphate§	1	$A = 7.41 \times 10^3 c + 0.002$	0.9999	$(7.55 \pm 0.36) \times 10^3$	4.5	0–2.7×10^{-4}	Quantitative
Sulphite§	2	$A = 1.36 \times 10^4 c + 0.029$	0.9999	$(1.43 \pm 0.08) \times 10^4$	4.5	$6.6 \times 10^{-6} \leqslant c \leqslant 1.3 \times 10^{-4}$	Quantitative
Tin(II) chloride§	2	$A = 7.69 \times 10^3 c + 0.045$	0.9998	$(8.29 \pm 0.52) \times 10^3$	5.0	$6.6 \times 10^{-6} \leqslant c \leqslant 2.0 \times 10^{-4}$	Incomplete reaction; can be quantified by an empirical equation
2,4-Dinitrophenylhydrazine§	4(?)	$A = 1.84 \times 10^4 c + 0.074$	0.9990	$(2.05 \pm 0.16) \times 10^4$	6.2	0–9.3×10^{-5}	Incomplete reaction; can be quantified by an empirical equation
Sodium tetrahydroborate(III)§	8(?)	$A = 4.31 \times 10^4 c + 0.035$	0.9995	$(4.54 \pm 0.27) \times 10^4$	5.6	0–4.0×10^{-5}	Incomplete reaction; can be quantified by an empirical equation
2,3-Dimercaptopropan-1-ol§	2	$A = 1.19 \times 10^4 c + 0.032$	0.9995	$(1.31 \pm 0.07) \times 10^4$	6.2	$6.6 \times 10^{-6} \leqslant c \leqslant 1.0 \times 10^{-4}$	Incomplete reaction; can be quantified by an empirical equation

* A = net absorbance; c = concentration of reductant in the final solution (mol dm^{-3}).
† With respect to whether the reaction can be used for quantification of the reductant.
‡ Reaction conditions for colour development: 0.01 mol dm^{-3} Cu^{II} medium; absorbance measured within 2 min.
§ Reaction conditions for colour development: 0.1 mol dm^{-3} Cu^{II} medium; absorbance measured within 2 min.
¶ Reaction conditions for colour development: 0.01 mol dm^{-3} Cu^{II} medium; absorbance measured within 20 min.

an adverse effect on the molar absorptivity. The half-cell potential of the $[Cu(NH_3)_4]^{2+}$–$[Cu(NH_3)_2]^{+}$ couple becomes more negative as the $[Cu(NH_3)_2]^{+}$ or NH_3 concentration increases, favouring the oxidation of Cu^{I} species. Hence, the decrease in the molar absorptivity accompanied by deviations from linearity of the absorbance *versus* concentration graphs observed with increasing Cu^{I} or NH_3 concentration might be due to oxidation before chelation by Nc.

With synthetic mixtures obtained by the addition of trace amounts of CuCl to an excess of $CuCl_2$, the absorbance decreases and deviations from linearity become more marked as NH_3 is added. Moreover, the molar absorptivity for Cu^{I} decreases with increasing Cu^{II} concentration in the absence of NH_3 (Table 1).

For a fixed amount of Cu^{I}, *i.e.*, 2.0×10^{-4} mmol, in excess of Cu^{II} (1 mmol) solution, the variation of the blank, sample and net absorbances with the volume of NH_3 buffer added is illustrated in Fig. 1. The net absorbance decreases significantly when the NH_3 concentration is greater than the stoichiometric concentration required for the formation of $[Cu(NH_3)_4]^{2+}$. A slight excess of NH_3 buffer was found to be optimum for analysis in order to prevent precipitation. (The use of 0.65 ml of NH_3 buffer allows a value for ε of 7.7×10^3 $dm^3\ mol^{-1}\ cm^{-1}$ to be obtained for Cu^{I}.)

Copper(I) alone, as CuCl without Cu^{II}, in 0.65 ml of NH_3 buffer gives an increase in absorbance with concentration that deviates slightly from linearity; however, when Cu^{I} (1.0×10^{-4}–4.0×10^{-4} mmol) is either protected by a 10-fold excess of NH_2OH or used in the form of a $Cu(Nc)_2Cl$ stock solution, the maximum molar absorptivity of 7.7×10^3 $dm^3\ mol^{-1}\ cm^{-1}$ can be attained even in the presence of the same amount of buffer (0.65 ml). This observation shows that Cu^{I} is not masked, but is probably oxidized in NH_3 buffer when NH_2OH is not used.

Of the potential reducing agents, H_2O_2, ascorbic acid, cysteine, NH_2OH, N_2H_4, $S_2O_4^{2-}$, $S_2O_3^{2-}$, mitoxantrone, glutathione, Fe^{II} and thiourea yield molar absorptivities corresponding to stoichiometric *n*-electron oxidations of these substances (Table 2).

Carminic acid, sulphite, tin(II) chloride, 2,4-dinitrophenylhydrazine, $NaBH_4$ and 2,3-dimercaptopropan-1-ol (BAL) are incompletely oxidized, but can be quantified by the aid of empirical equations in the linear absorbance–concentration range.

Citric acid, an interferent in the procedure of Lau *et al.*,[31] which hinders the Cu^{II}-catalysed oxidation of ascorbic acid, does not pose a problem in the Cu^{II}–Nc system. Ascorbic acid was determined successfully in the presence of a 20-fold amount of citrate.

Cysteine undergoes a stoichiometric one-electron oxidation in the Cu^{II}–Nc system, and the rapid oxidation of cysteine by the Cu^{II}–Nc reagent is not affected by the Cu-catalysed auto-oxidation of cysteine.[32]

The ease of oxidation of the potent anticancer drug, mitoxantrone, with the Cu^{II}–Nc reagent might provide support for its oxidative metabolism; the mechanism of its antitumour action is still not fully known. However, mitoxantrone has recently been shown to be oxidized enzymically to the iminoquinone form *via* irreversible and reversible steps, the whole oxidation process involving two electrons.[33]

The slow reaction of Cu^{II}–Nc with thiourea might be due to the formation of a stable complex between this substance and the reduction product of the system, *i.e.*, Cu^{I}, with $\log \beta_3 = 13$ and $\log \beta_4 = 15.4$.[34] Nevertheless, the more stable Cu^{I}–Nc complex predominates given sufficient time (20 min), thus allowing the theoretical molar absorptivity typical of a four-electron reductant to be attained.

Hexacyanoferrate(II) and iodide could not be quantified in the Cu^{II}–Nc system because of their undesirable reactions with Cu^{II} and Cu^{I}, respectively. Glucose (and reducing sugars with the $-(CHOH)_n-CHO$ group), oxalate, citrate, phosphate, arsenite and thiocyanate did not react, demonstrating that the Cu^{II}–Nc system is selective to a limited extent and might be of potential use in the quantification of other reductants in biological fluids with minimal interference from the major constituents.

The potential utility of the proposed system has not been investigated fully as other reducing agents capable of being oxidized by Cu^{II}–Nc might also be quantified.

In principle, the Cu^{II}–Nc system allows the spectrophotometric determination of a reducing agent, A_{red}, provided that the redox reaction

$$n Cu^{2+} + 2n Nc + A_{red} \rightleftharpoons n[Cu(Nc)_2]^{+} + A_{ox} \qquad (1)$$

is complete with the formation of an equivalent amount of $[Cu(Nc)_2]^{+}$ with respect to the *n*-electron reductant, A_{red}. Copper(II) is a strong oxidizing agent only when its reduction product, Cu^{I}, is stabilized by a strong complex-forming ligand, *e.g.*, Nc. The standard potential of the Cu^{2+}–Cu^{+} couple (0.17 V) is shifted to more positive values by preferential complexation of Cu^{I}. Even strong oxidizing agents such as H_2O_2, which are weak reductants, can be oxidized in such a system. For example, when 7.5×10^{-3} mmol of Nc and 5.0×10^{-5} mmol of reductant are used, the cell potential appears to be less favourable and the reduction is incomplete for H_2O_2 in the presence of 0.1 mmol of Cu^{II}. The same reasoning applies to the stronger reductant, NH_2OH, in the presence of 1.0 mmol of Cu^{II}.

The oxidizing power of Cu^{II} in a solution containing Nc is dependent on the ease of formation of $[Cu(Nc)_2]^{+}$. A large excess of Cu^{II} can exhibit an affinity for Nc, thereby preventing the preferential quantitative formation of $[Cu(Nc)_2]^{+}$. The stronger the reductant, the more quantitative will be the reduction of Cu^{II} with the subsequent formation of a stoichiometric amount of this complex. On the other hand, weak reductants should be determined either by masking the excess of Cu^{II} so that it will not compete with Cu^{I} for complex formation or by using a more dilute solution of Cu^{II}.

Most *n*-electron reductants give a molar absorptivity that is approximately *n*-times that of $[Cu(Nc)_2]^{+}$, demonstrating that the reduction is essentially complete within 2 min. Empirical methods of determination were established for those reducing agents whose oxidation was not complete within the prescribed period of time provided that their absorbance–concentration plots were linear. Usually there was a useful range of Cu^{II} concentrations for reductants, possibly as a result of favourable oxidation potentials; hence the theoretical (expected for an *n*-electron reduction of Cu^{II}–Nc) molar absorptivities could be achieved.

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Highly Sensitive Spectrophotometric Determination of Trace Amounts of Uranium(VI) With the Thiocyanate–Basic Triphenylmethane Dyes–Gum Arabic System

Liu Zhongfan and Liu Shaopu
Department of Chemistry, Southwest China Teachers University, Chongqing 630715, People's Republic of China

Two highly sensitive spectrophotometric methods for the determination of trace amounts of uranium(VI) have been developed, based on its colour reactions with thiocyanate and triphenylmethane dyes (TPMD) in aqueous solution in the presence of gum arabic. Uranium(VI) reacts with thiocyanate and TPMD to form ion association complexes of composition $(TPMD)_2[UO_2(SCN)_4]$. The molar absorptivities are between 0.81×10^5 and 5.71×10^5 dm^3 mol^{-1} cm^{-1}, the highest value being found with Crystal Violet. Suitable conditions for the reactions, the effects of foreign ions and a pre-concentration procedure for uranium(VI) were investigated. The methods can be applied to the spectrophotometric determination of trace amounts of uranium(VI) in water and some ores.

Keywords: *Uranium(VI) determination; spectrophotometry; thiocyanate–triphenylmethane dye–gum arabic system*

The spectrophotometric determination of molybdenum(V),[1] zinc,[2,3] cobalt(II),[4] indium(III),[5] selenium(IV),[6] iron(III)[7] and vanadium(V)[8] as the ion association complexes of the metal ion–thiocyanate complex and some basic dyes in aqueous solution in the presence of a surfactant has been reported. However, the possibility of the spectrophotometric determination of uranium(VI) using an ion association complex of this type has not been studied to date.

Experimentally it was found that uranium(VI) forms an anionic $[UO_2(SCN)_4]^{2-}$ complex in the presence of a large excess of thiocyanate. This anionic complex reacts with some basic triphenylmethane dyes (TPMD) such as Crystal Violet (CV), Malachite Green (MG), Brilliant Green (BG), Iodine Green (IG) and Ethyl Violet (EV) to form association complexes of the type $(TPMD)_2[UO_2(SCN)_4]$. These complexes can be kept in solution, avoiding precipitation in the presence of gum arabic. These colour reactions have very high sensitivity, their molar absorptivities are between 0.81×10^5 and 5.71×10^5 dm^3 mol^{-1} cm^{-1} depending on the dyes used and the experimental conditions; the highest sensitivities were obtained with the CV and MG systems.

Suitable conditions for the colour reactions, the effects of foreign ions and the pre-concentration procedure for uranium(VI) were studied and a highly sensitive spectrophotometric method for the determination of trace amounts of uranium(VI) in water and some ore samples is proposed.

Experimental

Reagents

Sulphuric acid, 0.5 and 1.0 mol dm^{-3}.

Sodium thiocyanate solution, 15% m/v.

Gum arabic solution, 1% m/v.

Basic triphenylmethane dye solutions. Crystal Violet (analytical-reagent grade, Beijing Chemical Plant), 0.05%; MG (chemically pure reagent, Beijing Chemical Plant), 0.05%; BG (chemically pure reagent, Shanghai Third Chemical Reagent Plant), 0.05%; IG (BDH), 0.05%; and EV (Fluka), 0.025%.

Potassium aluminium sulphate solution, 8.8% m/v. Dissolve 22 g of $KAl(SO_4)_2.12H_2O$ in water and dilute to 250 ml with water. This solution contains 5 mg ml^{-1} of aluminium.

Standard uranium(VI) solution. Dissolve 0.1782 g of uranyl acetate (guaranteed-reagent grade, BDH) in water. Transfer this solution into a 1000 ml calibrated flask, then dilute to the mark with water. Dilute further to 5 μg ml^{-1} as required.

Apparatus

A 721 spectrophotometer (Third Analytical Instrument Factory, Shanghai), wavelength range 360–800 nm, was employed for absorbance measurements.

Procedures

Place 10 μg of uranium(VI) in a 25 ml calibrated flask. Add suitable amounts of sulphuric acid (1 mol dm^{-3}) and sodium thiocyanate solution and set aside for 10 min. In the following order, add 1 ml of gum arabic solution and 4–7 ml of basic dye solution depending on the dye used. Dilute to the mark with water and mix. Measure the absorbance in a 1 cm cell at the maximum absorbance wavelength against the reagent blank solution.

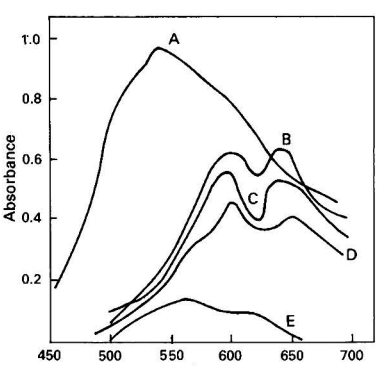

Fig. 1 Absorption spectra of the ion association complexes. A, CV system; B, MG system; C, BG system; D, IG system; and E, EV system. $[U^{VI}]$ = 10 μg per 25 ml. A 1 cm cell was used and measured against the blank

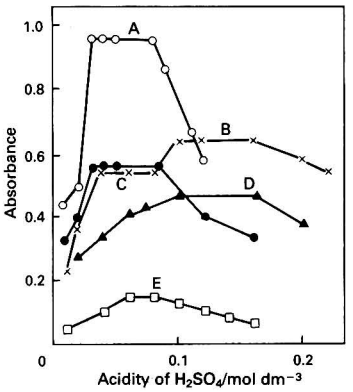

Fig. 2 Effect of solution acidity on absorbance. A, CV system; B, MG system; C, BG system; D, IG system; and E, EV system

Results and Discussion

Spectral Characteristics

Fig. 1 shows the absorption spectra of the ion association complexes of uranium(VI) with thiocyanate and five basic triphenylmethane dyes.

The maximum absorption wavelengths are at 540 and 565 nm for the ion associate systems of CV and EV, respectively. However the MG, BG and IG systems also have two absorption peaks, the maximum absorption wavelengths are at 640 (MG system) and 600 nm (BG and IG systems).

Effect of Solution Acidity

Fig. 2 shows the dependence of the absorbance of the ion association complexes on solution acidity. All the ion association reactions are carried out in acidic media, but the acidities differ according to the system.

The optimum acidity is between 0.10 and 0.16 mol dm^{-3} sulphuric acid for MG and IG systems, 0.03 and 0.08 mol dm^{-3} sulphuric acid for CV and BG systems and 0.06 and 0.08 mol dm^{-3} sulphuric acid for the EV system. Other acids such as hydrochloric, nitric and phosphoric acids were tested, but were found to be unsuitable. The use of nitric acid caused a rapid decrease of the absorbance because of the oxidation of the dyes by this acid. With hydrochloric and phosphoric acids, the chloride and phosphate ions formed complexes with uranium(VI). The highest and most stable absorbance was obtained with sulphuric acid; hence sulphuric acid was used in this work.

The MG and IG systems have the widest and the EV system the narrowest acidity range for the systems mentioned above.

Effects of Reagent Concentration

A higher thiocyanate concentration is required for complete formation of the anionic $[UO_2(SCN)_4]$ complex. The optimum concentration of sodium thiocyanate is between 0.15 and 0.30 mol dm^{-3} for all systems with the exception of the MG system for which the optimum concentration is between 0.45 and 0.60 dm^{-3} mol.

The optimum concentrations of the basic dyes are as follows: (2.0–2.5) $\times$ 10^{-4} mol dm^{-3} of CV; (0.84–1.7) $\times$ 10^{-4} mol dm^{-3} of MG; (2.1–2.9) $\times$ 10^{-4} mol dm^{-3} of BG; (1.8–2.4) $\times$ 10^{-4} mol dm^{-3} of IG; and (4.0–8.4) $\times$ 10^{-5} mol dm^{-3} of EV, separately.

Effects of Surfactants and Stability of the Absorbance

In the absence of a surfactant, the ion-association complexes will precipitate out of the aqueous solution due to their hydrophobicities. When a surfactant is added or a colloid stabilizer such as gum arabic, gelatin, poly(vinyl alcohol), Triton X-100 and Tween 20, the solution remains clear and there is a marked colour change. The effects of surfactants on the absorption spectra characteristics are not marked, but their effects on the sensitivity and stability of the colour reaction are different. Gum arabic is the best in all systems, however, a gum arabic–Triton X-100 solution is used in the EV system.

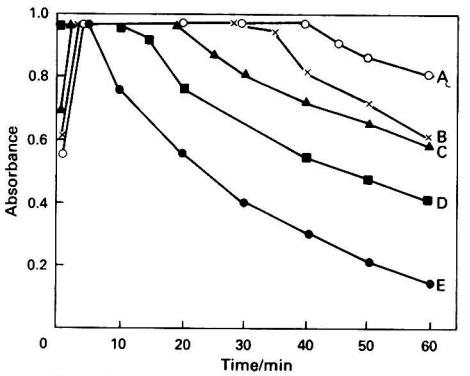

Fig. 3 Effects of temperature on the absorbance of the CV system. A, 15; B, 18; C, 20; D, 25; and E, 30 °C

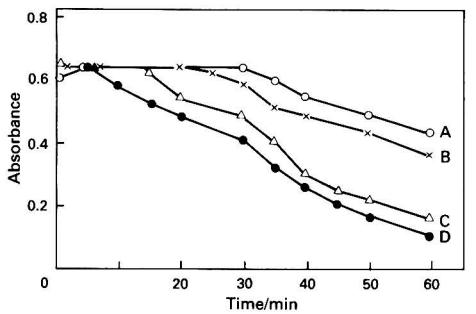

Fig. 4 Effects of temperature on the absorbance of the MG system. A, 15; B, 20; C, 25; and D, 30 °C

Figs. 3 and 4 show the effects of temperature on the speed and stability of colour development for the CV and MG systems in aqueous solution in the presence of gum arabic. With an increase in the temperature, colour development is faster and the stability of the absorbance is reduced. For example, for the colour to develop fully a period of 5 min is necessary; the absorbance is stable for about 40 min when the temperature is at 15 °C. However, colour development is instantaneous, but the absorbance is only stable for 5 min at 30 °C for the CV system. For the MG system, the effect of temperature was similar to that of the CV system (see Fig. 4). Therefore, the measurement of the absorbance should be carried out immediately at higher than ambient temperatures.

Composition of the Ion Associates

The molar ratio of uranium(VI) to SCN was 1 : 4 as established by the equilibrium shift method and uranium(VI) to CV or MG 1 : 2 as established by Job's method of continuous variation and the equilibrium shift method.

Owing to the uranium(VI) ion, the main species in acidic solution is UO_2^{2+}, therefore the composition of the ion association complexes in the CV and MG systems may be inferred to be $CV_2[UO_2(SCN)_4]$ and $MG_2[UO_2(SCN)_4]$.

Table 1 Sensitivity of various methods for the spectrophotometric determination of uranium

Reagent*	pH/acidity	λ_{max}/nm	$\varepsilon/10^4$ dm^3 mol^{-1} cm^{-1}	Reference
Arsenazo(III)	5.0–7.5 mol dm^{-3} HNO_3 or HCl	655	6.0	9
Chlorophosphonazo(III)	0.5–4.0 mol dm^{-3} HCl	670	7.36	9
PAR	6.8–10.7	550	4.25 (Extracted into $CHCl_3$)	10
TAR	7.5–7.8	540–545	3.30	11
Pyrogallol Red	6.0–7.0	650	2.9	12
CAS–CTMAC–PY	4.0–5.5	625	10.0	13
Thorin	3.0–7.0	600	2.12	14
Dibromophenylfluorone–Tween 20	6.5–9.0	565	17	15
5-Br-PADAP–sal–CPB	5.5–5.9	595	8.8	16
Benzoic acid–Rhodamine B	4.5	555	10.3 (Extracted into benzene–diethyl ether–2-methylpentan-1-one	17
Benzoic acid–MG	6.0	635	8.3 (Extracted into cyclohexane)	18
SCN–CV–gum arabic	0.03–0.08 mol dm^{-3} H_2SO_4	545	57	This work
SCN–MG–gum arabic	0.10–0.16 mol dm^{-3} H_2SO_4	640	37.4	This work

* PAR = 4-(2-pyridylazo)resorcinol; TAR = 4-(2′-thiazolylazo)resorcinol; CAS = Chrome Azurol S; CTMAC = cetyltrimethylammonium chloride; PY = pyridine; 5-Br-PADAP = 2-[2-(5-bromopyridyl)azo]-5-diethylaminophenol; sal = salicylic acid; and CPB = cetylpyridinium bromide.

Table 2 Tolerated amounts of foreign ions in the determination of uranium(VI) with the CV system (U^{VI}: 5 μg; relative deviation ±5%)

Foreign ion	Amount tolerated/μg	Foreign ion	Amount tolerated/μg
Mn^{II}	2000	Cd^{II}	20
Al^{III}	1000	Sn^{IV}	20, 100*
Ca^{II}	1000	Ni^{II}	20, 80*
Ba^{II}	200	Nb^{V}	10
Sr^{II}	200	Sc^{IV}	10
As^{III}	200	W^{VI}	10
Ge^{IV}	100	Mo^{V}	10
Cr^{III}	100	Zr^{IV}	10, 100*
Pb^{II}	100	Ti^{IV}	10, 40*
Sb^{III}	100	Bi^{III}	5, 400*
Ga^{III}	50	Tl^{III}	5
Th^{IV}	50	Cu^{II}	— 100*
Re^{VII}	50	Zn^{II}	— 20*
Te^{IV}	50	Fe^{III}	— 1000*
Ce^{IV}	30	In^{III}	— 20*
La^{III}	30	Hg^{II}	— 20*
Be^{II}	20		

* 1 ml of 0.1 mol dm^{-3} EDTA solution and 1 ml of 5% thiourea solution added.

Sensitivities and Selectivities of the Methods

Different amounts of uranium(VI) were used for colour development under optimum conditions, the measurement of absorbance and the construction of calibration graphs. The molar absorptivities of the colour reactions were calculated and the following values found: $\varepsilon = 5.71 \times 10^5$ dm^3 mol^{-1} cm^{-1} for the CV system; $\varepsilon = 3.74 \times 10^5$ dm^3 mol^{-1} cm^{-1} for the MG system; $\varepsilon = 3.27 \times 10^5$ dm^3 mol^{-1} cm^{-1} for the BG system; $\varepsilon = 2.74 \times 10^5$ dm^3 mol^{-1} cm^{-1} for the IG system; and $\varepsilon = 8.03 \times 10^4$ dm^3 mol^{-1} cm^{-1} for the EV system. The concentration ranges of uranium(VI) obeying Beer's law were 0–15 μg per 25 ml for CV, MG, BG and IG systems and 0–20 μg per 25 ml for the EV system.

As can be seen from Table 1 the methods are highly sensitive for the spectrophotometric determination of uranium.

The selectivity of the method using the CV system was investigated in the determination of 5 μg of uranium(VI) in the presence of a series of other ions.

Table 2 shows the tolerance of foreign ions in the determination of uranium(VI). The main interfering ions are iron(III), molybdenum(VI), zinc, cobalt(II), indium(III), vanadium(V), mercury(II). The addition of 1 ml of 0.1 mol dm^{-3} EDTA solution and 1 ml of 5% thiourea solution can improve the selectivity of the method. If the absorbance is measured against a solution containing the same amount of aluminium, then, up to 20 mg of aluminium were found not to interfere. Hence, pre-concentration of uranium(VI) by the $Al(OH)_3$ coprecipitation method greatly increases the selectivity of the method and it can be applied to the spectrophotometric determination of trace amounts of uranium(VI) in the presence of many metal ions.

The selectivity of the MG system was also investigated and found to be very similar to that of the CV system and the selectivity can also be improved by similar procedures.

Spectrophotometric Determination of Trace Amounts of Uranium(VI) in Synthetic Water Samples

Place 100 ml of the synthetic water sample in a 250 ml beaker, add 1 ml of nitric acid (1 + 1) and heat to boiling, then add 3 ml of 8.8% m/v potassium aluminium sulphate solution and 1 drop of Methyl Red solution. Add ammonia solution (1 + 1) dropwise until the solution changes colour from red to yellow and set aside for 3–4 h. Filter the precipitate and wash it several times with hot water.

Crystal Violet method

Dissolve the precipitate in a 25 ml calibrated flask by adding 2.5 ml of 0.5 mol dm^{-3} sulphuric acid and heating, and wash a few times with up to 10 ml of hot water. In the following order add 1 ml of 0.1 mol EDTA solution, 1 ml of 5% thiourea solution, 3 ml of sodium thiocyanate solution, mix and then set aside for 10 min. Then add 1 ml of gum arabic solution, 4 ml of CV solution and dilute to the mark with water and mix.

The absorbance of the solution was measured in a 1 cm cell at 545 nm, against the reagent blank.

Malachite Green method

The procedure is similar to that for the CV system, but differs in that the precipitate is dissolved in 3.0 ml of 1 mol dm^{-3} sulphuric acid. Then 6 ml of sodium thiocyanate solution and 5 ml of MG solution are added. Finally, the absorbance of the solution is measured at 640 nm. The results obtained with both of these methods are summarized in Table 3.

Table 3 Results for the determination of uranium(VI) in synthetic water samples

Sample	Uranium(VI) content/mg l^{-1} Added	Found	Elements other than uranium(VI)/mg l^{-1}
1	0.025	0.023* 0.026†, 0.027† 0.023†	Pb^{II} 0.6, Cd^{II} 0.6, W^{VI} 0.2, Ca^{II} 50 Mg^{II} 9, Bi^{III} 0.08, Ti^{IV} 0.6, Hg^{II} 0.2 Tl^{III} 0.3, Th^{IV} 0.4, Zr^{IV} 0.5, Be 1, Cr^{III} 5, In^{III} 0.2, Te^{IV} 0.3, Ga^{III} 0.4, Cu^{II} 0.1, Mn^{II} 1, As^{III} 0.15, Zn^{II} 0.2, Fe^{III} 2, Ni^{II} 0.5, Mo^{VI} 0.1, Sb^{III} 0.3, Ba^{II} 10
2	0.050	0.051*, 0.050* 0.049* 0.052†, 0.053 0.049†	Fe^{III} 3, Cr^{III} 4, Sn^{IV} 0.4, V^{V} 0.6 Pb^{II} 0.7, Cd^{II} 0.5, W^{VI} 0.7, Ca^{II} 60, Mg^{II} 8, Bi^{III} 0.06. Ti^{IV} 0.1, Hg^{II} 0.1, Tl^{III} 0.4, Th^{IV} 0.5, Zr^{IV} 0.6, Be^{II} 0.2, In^{III} 0.3, Cu^{II} 0.2
3	0.100	0.102*, 0.101* 0.099* 0.099†, 0.101† 0.102†	Cu^{II} 0.3, Fe^{III} 5, Cr^{III} 4, Sn^{IV} 0.5 V^{V} 0.1, Pb^{II} 0.8, Cd^{II} 0.2, W^{VI} 0.3, Ca^{II} Mg^{II} 7, Bi^{III} 0.1, Ti^{IV} 0.2, Hg^{II} 0.3, Tl^{III} 0.5, Th^{IV} 0.6, Zr^{IV} 0.1, Be^{II} 0.2, In^{III} 0.3, Te^{IV} 0.5, Ga^{III} 0.2, Mn^{II} 2.5, As^{III} 0.5, Zn^{II} 0.05, Ni^{II} 0.3, Mo^{VI} 0.03, Sb^{III} 0.2, Ba^{II} 5
4	0.125	0.124*, 0.126* 0.123*	Cu^{II} 0.45, Fe^{III} 4, Cr^{III} 2, Sn^{IV} 0.6 V^{V} 0.4, Pb^{II} 0.4, Cd^{II} 0.5, W^{VI} 0.6, Ca^{II} 1, Mg^{II} 0.4, Bi^{III} 0.25, Ti^{IV} 0.5, Hg^{II} 0.5, Tl^{III} 0.2, Zr^{IV} 0.4, Th^{IV} 0.1, Be^{II} 0.6, In^{III} 0.1, Te^{IV} 0.2, Ga^{III} 0.5, Mn^{II} 2, As^{III} 0.1, Zn^{II} 0.1, Ni^{II} 0.1, Sb^{III} 0.5, Ba^{II} 1
5	0.150	0.149*, 0.148* 0.147* 0.147†, 0.146† 0.151†	Cu^{II} 0.4, Fe^{III} 7, Cr^{III} 4.5, Sn^{IV} 0.2 V^{V} 0.2, Pb^{II} 0.3, Cd^{II} 0.4, W^{VI} 0.4, Ca^{II} 7, Mg^{II} 5, Bi^{III} 0.15, Ti^{IV} 0.3, Hg^{II} 0.4, Tl^{III}, 0.08, Th^{IV} 0.5, Zr^{IV} 0.2, Be^{II} 0.3, In^{III} 0.4, Te^{IV} 0.5, Ga^{III} 0.2, Mn^{II} 2.5, As^{III} 0.5, Zn^{II} 0.15, Ni^{II} 0.2, Mo^{VI} 0.15, Ba^{II} 7

* Results of the CV method.
† Results of the MG method.

Table 4 Results for the determination of uranium in ores

Sample	Uranium content (%) Certified	Found*	Sample	Uranium content (%) Certified	Found*
Granite 85-1-01	0.0005	0.00055	Phosphonate rock 72-12-01	0.0011	0.0010
Granite 85-1-02	0.0009	0.00092	Phosphonate rock 72-12-02	0.0058	0.0054

* Average of three determinations.

Spectrophotometric Determination of Uranium in Ores With the Crystal Violet System

Dissolve 0.1–1 g of sample ore (containing 1–10 μg of uranium) in a platinum crucible with 5–10 ml of nitric acid and 5 ml of hydrochloric acid and heat. Add 2–5 ml of hydrofluoric acid and heat gently to near dryness. Add 1–2 ml of sulphuric acid and heat until sulphuric acid fumes are no longer evolved. Repeat the treatment with hydrofluoric acid and sulphuric acid once or twice. To the residue add 1 ml of nitric acid and 5–10 ml of hot water and pass the solution through a filter into a 250 ml beaker, then wash the residue several times with hot water. Dilute to about 150 ml with water and add 2 ml of 8.8% m/v potassium aluminium sulphate solution and 1 drop of Methyl Red solution. Then heat the solution to boiling, add ammonia solution (1 + 1) dropwise until the solution changes colour from red to yellow and set aside for 3–4 h. Filter the precipitate and wash several times with hot water. The remainder of the procedure is the same as for the water samples. Results are presented in Table 4.

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Fluorescence Reaction of Sodium 7-Phenylazo-8-aminoquinoline-5-sulphonate With Gold and its Analytical Application

Ruan Chuanmin and Xu Qiheng*
Department of Chemistry, University of Yunnan, Kunming 650091, People's Republic of China

Sodium 7-phenylazo-8-aminoquinoline-5-sulphonate has been synthesized and its identity confirmed by infrared spectrometry, thermogravimetry and elemental analysis. This compound reacts with gold(III) in slightly acidic media forming a red complex which has an intense fluorescence at $\lambda_{em}/\lambda_{ex}$ = 380 nm/325 nm. The fluorescence intensity is proportional to the concentration of gold(III) in the range 0–320 ppb with a detection limit of 0.5 ppb. The method has been used for the determination of trace amounts of gold in mines.

Keywords: *Gold determination; sodium 7-phenylazo-8-aminoquinoline-5-sulphonate; spectrofluorimetry*

Both 8-hydroxy- (8HQ) and 8-mercaptoquinoline (8MQ) are known reagents which are widely used as chelating agents in analytical chemistry.[1] However, the 8-amino derivative of quinoline (8AQ) has received little analytical attention, resulting in only a few published papers.[2,3] The 8-amino derivative of quinoline with (*N,N*) as its chelating atom is more selective than 8HQ(*N,O*) and 8MQ(*N,S*). In recent years, a series of 8-aminoquinoline-5-azo derivatives have been synthesized and used in spectrofluorimetric and spectrophotometric analysis.[4,5]

As part of an investigation of the 8AQ chelating system, this paper reports the synthesis of the fluorescent reagent, sodium 7-phenylazo-8-aminoquinoline-5-sulphonate (SPAAQ), its fluorescence reaction with gold(III) and its use for the spectrofluorimetric determination of trace amounts of gold(III).

Experimental

Apparatus

The fluorescence spectra and intensities were measured with a Shimadzu (Japan) RF-540 spectrofluorimeter in 10 × 10 mm quartz cells.

Reagents

8-Aminoquinoline-5-sulphonate (AQS). This was synthesized from 8-hydroxyquinoline.[6]

Sodium 7-phenylazo-8-aminoquinoline-5-sulphonate. This was synthesized by the following procedure. Aniline (0.5 g) was dissolved in 3 ml of ice-cold concentrated hydrochloric acid and 10 ml of ice-cold doubly distilled water and slowly diazotized with a solution of 0.4 g of sodium nitrite in 5 ml of water. The diazotized solution was then added dropwise with stirring to an ice-cold solution of AQS (1.1 g) in 40 ml of 2 mol dm^{-3} acetic acid; 2 mol dm^{-3} sodium hydroxide was added to keep the pH constant and the mixture left for 1 h, with stirring in the ice-bath, then neutralized with sodium hydroxide and filtered. The red precipitate was recrystallized several times from 95% ethanol to give a yield of 20% with a melting point of 239–241 °C. Elemental analysis was as follows, calculated: C, 48.91; H, 3.53; N, 15.21; and S, 8.69. Found: C, 48.34; H, 3.71; N, 14.81; and S, 8.72%. The data obtained from thermogravimetry (TG), infrared (IR) and nuclear magnetic resonance (NMR) spectra confirmed the structure of SPAAQ to be as shown in Fig. 1.

SPAAQ solution, 1 × 10^{-4} mol dm^{-3}. This was prepared by dissolving 0.0368 g of the reagent in 1 l of ethanol. The solution is stable for several months.

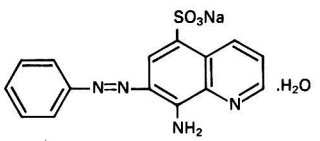

Fig. 1 Structure of SPAAQ confirmed by data obtained from TG, IR and NMR spectra

Gold(III) standard solution, 100 μg ml^{-1}. This was prepared by dissolving gold in *aqua regia*. Working standards were prepared from this solution as required.

Buffer solution (pH about 3.6). A mixture of 50 ml of 0.2 mol dm^{-3} potassium hydrogen phthalate solution and 6 ml of 0.2 mol dm^{-3} hydrochloric acid.

All other chemicals used were of analytical-reagent grade.

Procedure for the Determination of Gold(III)

To a sample solution in a 25 ml calibrated flask, add 0.4 ml of 1 × 10^{-4} mol dm^{-3} SPAAQ and 3 ml of buffer solution (pH 3.6). Dilute to the mark with distilled water and mix well, heat for 20 min in boiling water and cool for 5 min, then measure the relative fluorescence intensity at λ_{em} = 380 nm and λ_{ex} = 325 nm.

Results and Discussion

Fluorescence Spectra

The excitation and emission spectra with maxima at 325 and 380 nm, respectively, of the reagent blank and the complex are shown in Fig. 2.

Effect of pH

The effect of the pH on the relative fluorescence intensity was studied; the optimum pH range was found to be between 2.5 and 4.0, hence the pH was fixed in the optimum interval with a buffer solution of potassium hydrogen phthalate–hydrochloric acid of pH 3.6.

Effect of Amount of SPAAQ

The maximum fluorescence intensity was obtained in the concentration range 1.2 × 10^{-7}–2.0 × 10^{-7} mol dm^{-3} of SPAAQ solution. Higher reagent concentrations caused a decrease in the fluorescence intensity, when 0.4 ml of 1.0 × 10^{-4} mol dm^{-3} of reagent was used. The reason for this effect has not been studied.

* To whom correspondence should be addressed.

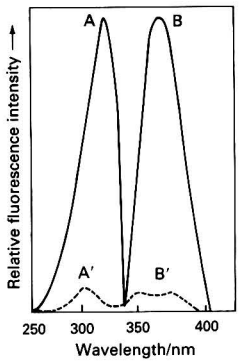

Fig. 2 Excitation and emission spectra of the gold complex (A and B) and the reagent blank (A′ and B′)

Effect of Heating Time

Without heating, the SPAAQ–gold(III) complex has a low fluorescence intensity. When heat is applied for 15–40 min the relative fluorescence intensity attains a constant maximum value. In this experiment a time of 20 min was selected.

Composition of Complex

The composition of the complex was determined by Job's method of continuous variation and by the molar ratio method. The molar ratio of gold to SPAAQ was found to be 1:1. The apparent stability constant of the complex was calculated from the results of the molar ratio and Job's method. An average value of log $K = 8.1 \pm 0.1$ was obtained at 20 °C.

Effect of Foreign Ions

The effect of foreign ions on the determination of 2 μg of gold(III) is summarized in Table 1. The limiting value of the concentration for each ion was taken as that value which caused an error of not more than 5% in the fluorescence intensity. The positive interference can be attributed to the fact that those elements also form complexes with SPAAQ in slightly acidic solutions. Platinum(II), Fe^{II} and Fe^{III} cause a serious positive interference.

Calibration Graphs

If the recommended conditions are used, a linear relationship is found between the emitted fluorescence intensity and gold concentration in the range 0–320 ppb, with a detection limit of 0.5 ppb.

Determination of Gold in Minerals

A 2–5 g amount of sample was transferred into a 200 ml beaker and 30–50 ml of concentrated hydrochloric acid were added. The beaker was covered and heated gently to dissolve the sample. About 50 ml of distilled water were added and the mixture was filtered. The residue was dissolved in *aqua regia* and boiled twice almost to dryness with distilled water to reduce the acidity; the solutions were then transferred into 50 ml calibrated flasks. To an aliquot of this solution was added 0.4 ml of 1×10^{-4} mol dm^{-3} SPAAQ and the pH was adjusted (2.5–4.0) with buffer solution. Iron was masked with 1 ml of 1% NaF. The results obtained are given in Table 2.

Table 1 Interference of other ions in the determination of gold. [The concentration of gold(III) was 2 μg per 25 ml]

Tolerance limit (M^{n+}/Au)	Ion
2	Pt^{2+}, Fe^{2+}, Fe^{3+}
4	Ga^{3+}
50	Mn^{2+}, Cu^{2+}, Cr^{3+}, Zn^{2+}, Ag^{+}
100	Co^{2+}, Pb^{2+}
500	Hg^{2+}, Ni^{2+}, W^{VI}, Mo^{VI}, Al^{3+}
1000	Sn^{4+}, Cr^{VI}, F^{-}, PO_4^{3-}, $C_2O_4^{2-}$

Table 2 Determination of gold in minerals

	Sample 1	Sample 2
Gold content*/g per tonne	4.33	34.20
Gold found/g per tonne	4.37	34.40

* Values obtained by atomic absorption spectrometry.

Conclusion

A number of spectrofluorimetric methods have been reported for the determination of trace amounts of gold, *e.g.*, rhodamines,[7,8] rhodanines,[9] kojic acid,[10] bipyridylglyoxal diphenylhydrazone,[11] and 2-phenylbenzo[8,9]quinolizino-[4,5,6,7-*fed*]phenanthridinylium perchlorate[12] have been used. However, these methods are not always convenient. One of the major problems seems to be the use of organic solvents. The proposed method can be used to determine gold(III) directly in the aqueous phase.

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BOOK REVIEWS

Ion Chromatography

Hamish Small. *Modern Analytical Chemistry.* Pp. xii + 276. Plenum Press. 1990. Price $49.50. ISBN 0 306 4329 0.

Hamish Small is regarded as the father of ion chromatography, a technique first described in 1975. It is surprising that he has not already written a book on the subject. This is a book of theory for those ion chromatographers who do not have a background in high-performance liquid chromatography. All the basic theory of ion exchange and chromatography is discussed and treated mathematically. Similar treatment is given to the other types of separation now classed as ion chromatography such as ion-pair and ion-exclusion chromatography. The nature of the column materials used in ion chromatography is described in some detail and a large section covers detection and detectors. This ranges from a discussion of signal to noise ratios to descriptions of detectors such as conductivity with chemically suppressed background, amperometry, and direct and reverse ultraviolet absorption. Many of the phenomena observed in ion chromatography are explained, *e.g.*, the 'water dip' and the fact that sensitivity increases with concentration when suppressed conductivity is used with carbonate eluents. It is difficult to think of any aspect that is not discussed.

All the chapters are well referenced although some pre-1975 ion chromatography authors are missing, *e.g.*, Dionex workers are given credit for pioneering conductivity detection in ion-exclusion chromatography whereas it had been published in *Talanta* in 1969 by Goodman, Lewis and Taylor and possibly by others. Another small criticism of the book is that the index is not as complete as it could be.

The book concludes with a chapter on applications in which the range of species that can be separated is illustrated. Again, a theoretical approach is taken with detailed descriptions of the processes involved in effecting the separation and detecting the separated species. Although all the main classes of compounds are mentioned it is by no means a full enough guide to decide how to separate *A* from *B* in matrix *C*. Other books exist which are more helpful in the choice of methods for samples. This book provides the theoretical background and explanations missing in the 'cookery books' and some manufacturers' application notes.

D. Mealor

Methods for Analysis of Musts and Wines. Second Edition

C. S. Ough and M. A. Amerine. Pp. 377. Wiley-Interscience. 1988. Price £67.50. ISBN 0 471 62757 7.

The scientific study of wine, oenology, is dominated by Continental European schools and by the University of California. The latter establishment is largely responsible for the modern revitalization of the subject, with the consequent overall improvements in wine quality, and Ough and Amerine are two of the University's leading oenologists.

The first edition of this book has become a standard work of reference for laboratories engaged in the analysis of grape-musts, wines and spirits. This second edition follows the format of its predecessor, consisting of chapters devoted to chemically related analytes or oenologically related properties. Examples of the chapter headings include: 'Soluble Solids'; 'Acidity and Individual Acids'; 'Alcohols'; 'Carbonyl Compounds'; 'Esters'; and a survey of analytical equipment. Each chapter is made up of monographs concerned with individual analytes or properties, each monograph giving a short account of the occurrence, biochemical origin and relative importance of the analyte to the character of a wine.

The heart of the book is its description, in full detail, of the methods for determining the desired analyte or property. Several methods are given for the most important analytes, ranging from traditional chemical procedures to instrumental (often chromatographic) and enzymic where appropriate. The scope, sensitivity and selectivity of each is discussed, so that if confronted with the need to determine an unfamiliar analyte, this book is the place to start. It is the best single source of the methods available and is suitable for most laboratories and circumstances.

The authors identify officially approved methods and quote the current legal maxima for controlled analytes. These should be treated with some caution and used only to indicate that controls exist, because of the rapid change in national and European Community regulations. Indeed, diethyleneglycol makes its appearance in this edition only because of the so-called 'antifreeze' scandal and its regulatory aftermath.

Overall this is a well referenced, well indexed book and a worthy successor to its predecessor which can be recommended to everyone concerned with wine and spirits analysis.

C. P. Richards

Handbook of Chemistry and Physics (70th Edition)

Edited by Robert C. Weast, David R. Lide, Melvin J. Astle and William H. Beyer. CRC Press. 1989. Price £73.00. ISBN 0 8493 0470 9.

The 70th Edition of the CRC Press 'Rubber Handbook' contains all of the features that make this series so useful. These include brief descriptions of the elements, the table of Physical Constants of Inorganic Compounds, the heats of fusion of inorganic compounds, the Table of the Isotopes, the physical constants of organic compounds and the structural formulae of organic compounds, among many, many other items.

The book is well presented in the usual leather binding and will be an invaluable aid to all practising chemists.

Roger Young

Reviews on Immunoassay Technology. Volume 3

Edited by S. B. Pal. Pp. viii + 173. Macmillan. 1989. Price £45.00. ISBN 0 333 49795 3.

This multi-author volume consists of ten chapters contributed by authors from Europe, Japan, the USA and the USSR. The book covers a diverse range of topics on immunological assays and techniques. The first two chapters review methods for isolating antigen-specific B lymphocytes and the second sub-component of the first complement component, respectively. Both chapters give comprehensive and practical information on the procedures involved. There is an interesting contribution by two authors from the USSR describing how antibodies to respiratory pathogens can be evaluated and quantified for epidemiological as well as for more clinically orientated studies. Tests involving haemagglutination inhibition and the more recent ELISA techniques are described.

There are three chapters in the book describing novel approaches to what is conventionally called immunoassays (as opposed to immunological techniques). The preparation and use of liposomes, either conjugated to antigens or antibodies

or as carriers of reporter molecules in diagnostic immunoassays, are described. Several different types of homogeneous enzyme immunoassays which utilize enzyme (β-galactosidase) sub-units prepared by recombinant DNA techniques are described in another chapter. The sub-units are only enzymically active when aggregated together, a process inhibited if antibody binding to an antigen-labelled sub-unit intervenes. The use of enzyme channelling and immunochromatography to produce simple and rapid, competitive and sandwich immunoassays is illustrated in another contribution. The book also contains three useful reviews on the use of immunological reagents for staining antigens and antibodies in tissues and cells. Immunocytochemical techniques, including direct and indirect methods utilizing immunoperoxidase, immunofluorescence, avidin/biotin and ferritin and immunogold labels for light and electron microscopy, are reviewed. The applications described include double labelling of antigens, localization of peroxisomal enzymes and the visualization of viral antigens and antibodies. Finally, there is a chapter (which appears to be a little out of date) describing a procedure and computer program for the interpolation of immunoassay dose response curves.

The volume under review is a well presented and illustrated book. However, it is unlikely that it would be of interest in its entirety to any one individual reader because of the wide range of topics it covers. Each chapter gives a relatively short but clear and concise insight into a subject and it is therefore the type of book that will be consulted, from time to time, by newcomers to a particular field of interest or by students during their course of study. Readers should, however, be aware that recent references to the literature (after 1985–86) were remarkably few and the information given in the various contributions should be supplemented by reference to the more recent scientific literature.

G. Wynne Aherne

Packings and Stationary Phases in Chromatographic Techniques

Edited by Klaus K. Unger. *Chromatographic Science Series Volume 47.* Pp. viii + 836. Marcel Dekker. 1990. Price $150.00 (USA and Canada); $180.00 (Export). ISBN 0 8247 7940 1.

Since the inception of the Marcel Dekker monograph series on Chromatographic Science, with the publication of Calvin Giddings' excellent account of the dynamics of chromatography, over 40 volumes have appeared covering virtually all aspects of chromatography. Hitherto, the stationary phase, which is an essential component of all chromatographic systems, has largely been neglected. Although somewhat belated this omission has now been rectified by the publication of a splendid review, written by internationally recognized experts under the editorial guidance of Klaus Unger, a scientist well known for his innovative work on liquid chromatographic packing materials.

The book provides a comprehensive review and critical analysis of the role of stationary phases and packings in gas, thin-layer and column-liquid chromatography. Valuable information is given on the plethora of phases available for use by the analyst, including details of manufacture, structural properties and chromatographic behaviour, with special emphasis on factors affecting retention and selectivity. In the reviewer's opinion the book provides the essential knowledge to enable analytical chemists to select, handle and evaluate stationary phases for the solution of the full spectrum of chemical and biomedical separation problems.

The monograph consists of 13 chapters, the first of which presents a thorough historical review of the development of chromatography, essential reading for research degree candidates, which includes over 90 references enabling further research into the origins of the technique. This is followed by an overview of packing materials which concentrates upon the characterization of the physical and chemical structural properties of stationary phases and the role of these properties in determining the efficiency and selectivity of systems in both gas and liquid chromatography.

Chapter 3 covers column packings for use in gas–solid and gas–liquid chromatography. This chapter contains a wealth of detail about the full range of commercially available sorbents, liquid phases and supports. Included is information on the manufacture, chemical structure and chromatographic properties along with guidelines for the selection of phases for a wide range of assays that should prove to be useful to the beginner and the established practitioner. There follows a brief account of column materials for liquid–liquid partition chromatography with solvent generated phase systems.

Stationary phases for thin-layer chromatography are reviewed in Chapter 5. Here again a lot of practically useful data are given about the full spectrum of sorbents for normal- and reversed-phase TLC. Details are given about bulk sorbents and pre-coated layers and in each instance mobile phase systems are recommended for a wide range of applications and typical separations illustrated.

A comprehensive account of column packings for liquid chromatography is given in Chapter 6. Here inorganic and organic sorbents are considered along with the full range of chemically bonded phases. Details are given of the preparation, physical and chemical characterization together with typical analytical applications and a discussion of elution mechanisms. As with the gas chromatographic phases, discussed earlier in the text, valuable information is given on commercially available phases.

The next three chapters deal with stationary phases for size exclusion, donor–acceptor complex and ligand-exchange chromatography. These chapters not only contain useful information about the respective column materials but also helpful accounts of the theory and applications of these less familiar techniques.

A substantial account of ion-exchange chromatography is given in Chapter 10. In addition to a thorough treatment of ion exchangers, essential theoretical and practical aspects of the technique are discussed. Details are given of the majority of phases currently available, including polymer- and silica-based materials, and their analytical application is illustrated by typical examples. This chapter is followed by short, yet informative, accounts of phases for ion-pair, affinity and chiral-liquid chromatography.

The book is highly recommended.

M. B. Evans

Standardization Within Analytical Chemistry

P. Kivalo. Pp. 157. Akademiai Kiado. 1989. Price £23.00. ISBN 963 05 5604 9.

Raising the topic of standardization usually guarantees that many analytical chemists will rapidly lose interest! However, there is growing awareness of the necessity for involvement as more practising analytical chemists realize the pit-falls of working across national and continental boundaries.

Professor Kivalo's book is certainly going to help in promoting understanding of the issues and challenges involved in harmonization and standardization of analytical methodology. I found the short historical perspective and the overview of the roles of IUPAC and ISO particularly helpful. Another useful feature of the work is that the appendices contain 5 key documents, brought together and containing much of the detail outlined in Chapter 5 on methods standardization.

These are: (*i*) ISO/IEC Guide 2-1986 (E/F/R). General terms and definitions concerning standardization and related activities; (*ii*) ISO 78/2-1982 (E). Layouts for standards. Part 2. Standards for chemical analysis; (*iii*) ISO 5725-1986 (E). Precision of test methods. Determination of repeatability and reproducibility for a standard test method by interlaboratory trials; (*iv*) AOAC Guidelines for interlaboratory collaborative study procedure to validate characteristics of a method of analysis; and (*v*) ISO/16C Guide 43-1984 (E). Development and operation of laboratories proficiency testing.

I recommend this timely book to those involved not only in the international arena but also to those working within organizations involving disparate laboratory groups.

C. Burgess

Multivariate Calibration

Harald Martens and Tormold Naes. Wiley-Interscience. 1989. Pp. xvii + 419. Price £75.00. ISBN 0 471 90979 3.

In their preface, the authors set out their objective to address a number of topics relating to multivariate calibration, namely: why multivariate calibration?; useful statistical tools; how to do it; how to make sure it really works; how to find extreme outliers or errors in the data set; how to get the right type of input data; and how to tailor your data for optimal calibration. Given such an ambitious target, the authors have done well to keep the size of the book to 8 chapters and just over 400 pages.

Chapter 1 takes the reader reasonably gently through the rationale of multivariate calibration and the problems associated with calibration prediction and interference. The basic mathematical tools for vector and matrix manipulation together with geometric representations in linear algebra are covered in Chapter 2. Concepts of orthogonality and projection provide the introduction to statistical considerations such as covariance and correlations leading to the matrix representations of the least squares principle and calibration models. Both these chapters were well written and digestible to the non-specialist. Indeed it may be difficult for those more used to the formal presentation of linear algebra to be comfortable with the geometrical illustration of matrices and vectors. I found this approach to be most helpful.

Chapter 3 forms the true meat of the book being in essence the mathematical basis for the Unscrambler computer program used throughout the book. The authors have attempted to write this at two levels and provide more statistical and mathematical rigour for the more advanced reader, achieved by the use of material in the form of statistical extensions which may be omitted. Laudable though this objective is, I felt that the chapter is probably the least satisfactory in the book. It is a highly indigestible 163 pages and takes the reader rapidly through univariate calibration and into data compression followed by a discussion of principle component analysis and principle component regression. There then follows detailed algorithmic discussion of PLS1 and PLS2 which was very heavy going in marked contrast to the deft light touch shown in Chapters 1 and 2. Large portions of this would only be of interest to the advanced reader and could be safely removed to an appendix. Newcomers to the field on the first reading would be well advised to skip large sections of this for the sake of their digestion and continued interest in the topic.

Fortunately, matters rapidly improve when contact with the real work is regained in Chapter 4, on assessment, validation and choice of calibration method. Chapter 5 continues the good work by addressing the difficult question of errors and outliers. Chapter 6 tackles the problem, usually ignored, of designing the experiment so that the data are fit for the purpose of the later analysis. I find it strange that the authors left this to the end, rather than putting it at the beginning where it belongs, together with the section on pre-treatment of data and linearization (Chapter 7).

The final chapter gives a worked example using the Unscrambler package. This is not as useful as it could have been, as the data set is omitted and the arguments are almost entirely pictorial, which is a pity because a feel for the numbers generated would have been an advantage.

In conclusion, the authors have made a bold attempt to write a book for analytical scientists, who are not mathematical specialists, to encourage the use of multivariate calibration techniques. Notwithstanding my reservations about the readability of Chapter 3, I'm sure that this book marks a significant milestone in the progress of chemometrics and am happy to recommend it.

C. Burgess

CUMULATIVE AUTHOR INDEX

JANUARY 1991

The Analyst
INSTRUCTIONS TO AUTHORS

The *Analyst* publishes original research papers on all aspects of the theory and practice of analytical chemistry, fundamental and applied, inorganic and organic, including chemical, physical, biochemical, clinical, pharmaceutical, biological, automatic and computer-based methods. Papers on new techniques and instrumentation, detectors and sensors, and new areas of application with due attention to overcoming limitations and to underlying principles are all equally welcome. All contributions are judged on the criteria of (*i*) originality and quality of scientific content and (*ii*) appropriateness of the length to content of new science. Thus, papers reporting results which would be routinely predicted or result from application of standard procedures or techniques are unlikely to prove acceptable in the absence of other attributes which themselves make publication desirable.

Although short articles are acceptable, the Society strongly discourages fragmentation of a substantial body of work into a number of short publications. Unnecessary fragmentation will be a valid reason for rejection of manuscripts. Papers may be submitted for publication by members of The Royal Society of Chemistry or by non-members.

There is no page charge for papers published in *The Analyst*.

The following types of papers will be considered.

Original research papers.

Communications, which must be on an urgent matter and be of obvious scientific importance. Rapidity of publication is enhanced if diagrams are omitted, but tables and formulae can be included. Communications receive priority and are usually published within 5–8 weeks of receipt. They are intended for brief descriptions of work that has progressed to a stage at which it is likely to be valuable to workers faced with similar problems. A fuller paper may be offered subsequently, if justified by later work. Although publication is at the discretion of the Editor, communications will be examined by at least one referee.

Reviews, which must be a critical evaluation of the existing state of knowledge on a particular facet of analytical chemistry. However, original work may be included. Simple literature surveys will not be accepted for publication. It is desirable that potential review writers should contact the Editor before embarking on their work.

Copyright. The whole of the literary matter (including tables, figures, diagrams and photographs) in *The Analyst* is Royal Society of Chemistry copyright and may not be reproduced without permission from the Society or such other owner of the copyright as may be indicated. **Papers that are accepted must not be published elsewhere except by permission.** Submission of a manuscript will be regarded as an undertaking that the same material is not being considered for publication by another journal in any language.

US Associate Editor. Papers from North America can be submitted to Dr. J. F. Tyson, Department of Chemistry, University of Massachusetts, Amherst, MA 01003, USA. To enhance the speed of processing of manuscripts, these papers will usually be refereed in the United States or Canada.

Regional Advisory Editors. For the benefit of potential contributors outside the United Kingdom and North America, a Group of Regional Advisory Editors exists. Requests for help or advice on any matter related to the preparation of papers and their submission for publication in *The Analyst* can be sent to the nearest member of the Group. Currently serving Regional Advisory Editors are listed in each issue of *The Analyst*.

Manuscripts. Papers should be typewritten in double spacing on one side *only* of the paper. Copies of any related, relevant, unpublished material and raw data should be made available on request. Three copies of text and illustrations should be sent to the **Editor, The Analyst, The Royal Society of Chemistry, Thomas Graham House, Science Park, Milton Road, Cambridge CB4 4WF, or directly to the US Associate Editor,** and a further copy retained by the author.

Administration and Publication Procedure. Receipt of a contribution for consideration will be acknowledged immediately by the Editorial Office. The acknowledgement will indicate the paper reference number assigned to the contribution. Authors are particularly asked to quote this number on all subsequent correspondence.

All papers (including conference presentations submitted for special issues) are sent simultaneously to at least two referees, whose names are not disclosed to the authors. On the basis of the referees' reports, the Editor decides whether the paper is suitable for publication, either unchanged or after appropriate revision. This decision and relevant comments of the referees are communicated to the author. Differences of opinion are mediated by the Editor, possibly after consultation with further referees, or, in the last resort, by the Editorial Board.

When rejection of a paper is recommended, the Editor informs the author, and returns the top copy of the manuscript. Authors have the right to appeal to the Editorial Board if they regard a decision to reject as unfair.

Authors will receive formal notification when papers are accepted for publication.

Proofs. The address to which proofs are to be sent should accompany the paper. Proofs should be carefully checked and returned immediately (by Air Mail from outside Europe). Particular attention should be paid to numerical data both in the tables and text.

Reprints. Fifty reprints of each paper are supplied free on request. Additional reprints can be purchased if ordered at the time of publication. Details are sent to authors with the proofs.

Notes on the Writing of Papers for *The Analyst*

Manuscripts should be in accordance with the style and usage shown in recent copies of *The Analyst*. Conciseness of expression is expected: clarity is increased by adopting a logical order of presentation, with suitable paragraph or section headings. Spellings should be in accordance with the Oxford English Dictionary.

To facilitate abstracting and indexing by Chemical Abstracts Service, and other abstracting organisations, it would be helpful if at least one forename could be included with each author's family name. The corresponding author should be clearly indicated.

Descriptions of new methods should be supported by experimental results showing accuracy, precision and selectivity.

The recommended order of presentation is as indicated below:

(*a*) *Title.* This should be as brief as is consistent with an adequate indication of the original features of the work. The title should usually include the analyte being determined or identified, the matrix and the analytical method used.

(*b*) *Summary.* A summary of about 250 words, giving the salient features and drawing attention to the novel aspects, should be provided for all papers. It should be essentially independent of the main text and include relevant quantitative information such as detection limits, precision and accuracy data.

(*c*) *Keywords.* Up to 5 keywords or key phrases, indicating the topics of importance in the work described, should be included after the summary.

(*d*) *Aim of investigation.* A concise introductory statement of the novel features of the work and the object of the investigation with any essential historical background, followed, if necessary, by a *brief* account of preliminary experimental work with relevant references.

(*e*) *Description of the experimental procedures.* Working details must be given concisely. Analytical procedures should preferably be given in the form of instructions; well known operations should not be described in detail. Suppliers of equipment and materials, and their locations, should be mentioned.

(*f*) *Results and Discussion.* Results are best presented in tabular or diagrammatic form (but not both for the same results), followed by an appropriate statistical evaluation, which should be in accordance with accepted practice. For example, a new procedure for multi-element determinations which produced results for which the concentrations of 8 out of 10 of the elements determined in a standard reference material were statistically indistinguishable from the certificate values should be described in those terms and not referred to as 'excellent agreement'. This is particularly important in the summary. Any discussion should comment on the scope of the method and its validity, followed by a statement of any conclusions drawn from the work. A separate conclusions section is not encouraged but, if included, it should not simply duplicate statements in the discussion.

(*g*) *Acknowledgements.* Contributors other than co-authors, companies or sponsors may be acknowledged in a separate paragraph at the end of the paper. Titles may be given but not degrees.

(*h*) *References.* References should be numbered serially in the text by means of superscript figures, *e.g.*, Foote and Delves,[1] Burns *et al.*[2] or Hirozawa,[3] and collected in numerical order under 'References' at the end of the paper. They should be listed, with the authors' initials, in the following form (double-spaced typing):

1 Yerian, T. D., Christian, G. D., and Růžička, J., *Analyst*, 1986, **111**, 865.
2 Sharp, B. L., Barnett, N. W., Burridge, J. C., Littlejohn, D., and Tyson, J. F., *J. Anal. At. Spectrom.*, 1988, **3**, 133R.
3 Committee for Analytical Methods for Residues of Pesticides and Veterinary Products in Foodstuffs and the Working Party on Pesticide Residues of the Ministry of Agriculture, Fisheries and Food, *Analyst*, 1985, **110**, 765.
4 Hara, H., Horvai, G., and Pungor, E., *Analyst*, 1988, **113**, 1817; *Anal. Abstr.*, 1989, **51**, 6H57.
5 Norwitz, G., and Keliher, P. N., *Analyst*, 1987, **112**, 903 (and references cited therin).
6 L'Vov, B. V., Polzik, L. K., Romanova, N. P., and Yuzeforskii, A. I., *J. Anal. At. Spectrom.*, in the press.
7 *Official Methods of Analysis of the Association of Official Analytical Chemists*, ed. Horwitz, W., Association of Official Analytical Chemists, Arlington, VA, 13th edn., 1980, sect. 20. 104.
8 O'Connor, A., Sigma, St. Louis, MO, personal communication, 1989.
9 Appelqvist, R., *PhD Thesis*, University of Lund, Sweden, 1987.
10 Klinger, J. A., and Harrison, W. W., paper presented at the 1990 Winter Conference on Plasma Spectrochemistry, St. Petersburg, FL, USA, January 8th–13th, 1990.
11 Beauchemin, D., and Craig, J. M., in *Plasma Source Mass Spectrometry. The Proceedings of the Third Surrey Conference on Plasma Source Mass Spectrometry, University of Surrey, July 16th–19th*, 1989, ed. Jarvis, K. E., Gray, A. L., Jarvis, I., and Williams, J. G., Royal Society of Chemistry, Cambridge, 1990, pp. 25–42.

Journal titles should be abbreviated according to the *Chemical Abstracts Service Source Index* (*CASSI*).

For books, the edition (if not the first), the publisher and the place and date of publication should be given, followed by the page number.

1 Harrison, W. W., and Donohue, D. L., in *Treatise on Analytical Chemistry*, ed. Kolthoff, I. M., and Winefordner, J. D., Wiley, New York, 2nd edn., 1989, pt. 1, vol. 11, ch. 3, pp. 189–235.
2 Gutscht, C. D., *Calixarenes*, The Royal Society of Chemistry, Cambridge, 1989.
3 *British Pharmacopoeia 1988*, HM Stationery Office, London, 1988, vol. 1, p. 140.
4 Růžička, J., and Hansen, E. H., *Flow Injection Analysis*, 2nd edn., Wiley, New York, 1988, pp. 299–304.
5 Moody, G. J., and Thomas, J. D. R., in *Ion Selective Electrodes in Analytical Chemistry*, ed. Freiser, H., Plenum Press, New York, 1978, ch. 4.

Authors must, in their own interest, check the lists of references against the original papers; second-hand references are a frequent source of error. References to conference abstracts which have not been published in the open literature are not acceptable. The number of references must be kept to a minimum.

Nomenclature. Current internationally recognised (IUPAC) chemical nomenclature should be used. Common trivial names may be used, but should first be defined in terms of IUPAC nomenclature. A listing of all relevant IUPAC nomenclature publications appear in the January issue.

Symbols and units. The SI system of units, as recommended by IUPAC, should be followed. Their basis is the 'Système Internationale d'Unités' (SI). A detailed treatment is given in the so-called Green Book: Quantities, Units and Symbols in Physical Chemistry (Blackwell Scientific Publications, Oxford, 1988 edn.).

The following will be the guidelines used:

(*a*) A metric system will always be used in preference to a non-metric one.

(*b*) SI will be the standard usage.

(*c*) The units used to record the definitive values of 'critical data' or quantitities measured to a high degree of accuracy will be SI. These units are summarised in the Appendix.

The effect on current style of papers for *The Analyst* includes the following:

(*a*) dimensions should preferably be given in metres (m) or in millimetres (mm);

(*b*) temperatures should be expressed in K or °C (not °F);

(*c*) wavelengths should be expressed in nanometres (nm) (not mμ);

(*d*) frequency should be expressed in Hz (or kHz, etc.), not in c/s or c.p.s.; rotational frequency can be denoted by use of s^{-1}; in mass spectrometry, signal intensity should be expressed in counts s^{-1} and not in Hz;

(*e*) radionuclide activity will be expressed in becquerels (Bq) or curies (Ci); 1 Ci = 3.7×10^{10} Bq;

(*f*) the micron (μ) will not be used; 10^{-6} m will be 1 μm.

When non-SI units are used they must be adequately explained unless their definition is obvious (*e.g.*, degree Celsius, mmHg). The derivation of derived non-SI units should be indicated.

Abbreviations. Abbreviational full stops are omitted after the common contractions of metric units (*e.g.*, ml, g, μg, mm) and other units represented by symbols. Abbreviations other than those of recognised units should be avoided in the text after definition. Upper case letters without points should be used for abbreviations for techniques and associated terms, *e.g.*, HPLC, AAS, XRF, UV, NMR, SCE. Other common abbreviations and contractions require full points, *e.g.*, eqn., m.p., Dr., except when sub- or super-script, λ_{max} for example. The abbreviations Me, Et, Pr^n, Bu^n, Bu^i, Bu^s, Bu^t, Ph, Ac, Alk, Ar and Hal can be used; others should be defined. Carboxy groups are written CO_2R, not COOR. Substituents should be indicated by R (one) or by R^1, R^2, R^3 . . . (more than one).

Percentage concentrations of solutions should be stated in internationally recognised terms. Thus the symbols 'm' instead of 'w' for mass and 'v' for volume are to be used. The following show the manner of expressing these percentages together with an acceptable alternative given in parentheses: % m/m (g per 100 g); % m/v (g per 100 ml); % v/v. Further implications of the use of the term 'mass' are that 'relative atomic mass' of an element (A_r) replaces atomic weight, and 'relative molecular mass' of a substance (M_r) replaces molecular weight.

Concentrations of solutions of the common acids are often conveniently given as dilutions of the concentrated acids, such as 'dilute hydrochloric acid (1 + 4),' which signifies 1 volume of the concentrated acid mixed with 4 volumes of water. This avoids the ambiguity of 1 : 4, which might represent either 1 + 4 or 1 + 3. Dilutions of other solutions can be expressed in a similar manner. Molarity is generally expressed as a decimal fraction (*e.g.*, 0.375 mol dm^{-3}).

Tables and diagrams. Table column headings should be brief. Tables consisting of only two columns can often be arranged

horizontally. Tables must be supplied with titles and be so set out as to be understandable without reference to the text.

Either tables or graphs may be used but not both for the same set of results, unless important additional information is given by so doing. The information given by a straight-line calibration graph can usually be conveyed adequately as an equation or statement in the text.

Column headings and graph axis labels should be in accord with SI conventions. Thus, the expression of numerical values of a physical quantity should be dimensionless, *i.e.*, the quotient of the symbol for the physical quantity and the symbol for the unit used, *e.g.*, p/atm, or some mathematical function of a number, *e.g.*, ln (p^m/atm). Further examples are ν/cm^{-1}, λ/cm, mass of substance/g and flow-rate/ml min^{-1}. For units which are already dimensionless, *i.e.*, ratios such as % or ppm, the type of ratio is indicated in parentheses, *e.g.*, c (%) or c (ppm). The diagonal line (solidus) will not be used to represent 'per.' In accordance with the SI system, units such as grams per millilitre are already expressed in the form g ml^{-1}. It should be noted that the 'combined' unit, g ml^{-1}, must not have any 'intrusive' numbers. To express concentration in grams per 100 millilitres, the word 'per' will still be required: Concentration/g per 100 ml. It may be preferable for an author to express concentrations in grams per litre (g l^{-1}) rather than grams per 100 ml.

Most diagrams will be retraced and lettered in order to achieve uniform line thicknesses and lettering size and style. However, all diagrams should be carefully and clearly drawn on good quality paper and should be carefully and clearly lettered. If possible, chromatograms and spectra, complicated flow charts, circuit diagrams, etc., should be supplied as artwork for direct reproduction in order to avoid time-consuming and expensive redrawing. The clearest copy should be without lettering.

Three complete sets of illustrations should be provided, two sets of which may be made by any convenient copying process for transmission to the referees.

All diagrams should be accompanied by a separately typed set of captions. Wherever possible, extensive identifying lettering should be placed in the caption rather than on lines on graphs, etc.

Photographs. Photographs can be submitted if they convey essential information that cannot be shown in any other way. They should be submitted as glossy or matt prints made to give the maximum detail. Colour photographs will be accepted only when a black-and-white photograph fails to show some vital feature and can be supplied either as prints or transparencies.

Appendix

The SI System of Units

In the SI system there are seven base units—

Physical quantity	*Name of unit*	*Symbol for unit*
length	metre	m
mass	kilogram	kg
time	second	s
electric current	ampere	A
thermodynamic temperature	kelvin	K
amount of substance	mole	mol
luminous intensity	candela	cd

There are two supplementary dimensionless units for plane angle (radian, rad) and solid angle (steradian, sr). Some derived SI units that have special names are as follows—

Physical quantity	*Name of unit*	*Symbol for unit*	*Definition of unit*
energy	joule	J	kg m^2 s^{-2}
force	newton	N	kg m s^{-2} = J m^{-1}
power	watt	W	kg m^2 s^{-3} = J s^{-1}
electric charge	coulomb	C	A s
electric conductance	siemens	S	m^{-2} kg^{-1} s^3 A^2 (Ω^{-1})
electric potential difference	volt	V	kg m^2 s^{-3} A^{-1} = J A^{-1} s^{-1}
electric resistance	ohm	Ω	kg m^2 s^{-3} A^{-2} = V A^{-1}
electric capacitance	farad	F	A^2 s^4 kg^{-1} m^{-2} = A s V^{-1}
frequency	hertz	Hz	s^{-1}
magnetic flux density (magnetic induction)	tesla	T	kg s^{-2} A^{-1} = V s m^{-2}
radionuclide activity	becquerel	Bq	s^{-1}
presssure, stress	pascal	Pa	m^{-1} kg s^{-2} (= N m^{-2})
energy, work, heat	joule	J	m^2 kg s^{-2} (= N m = Pa m^3)
inductance	henry	H	m^2 kg s^{-2} A^{-2} (= VA^{-1} s)

Examples of other derived SI units are—

Physical quantity	*SI unit*	*Symbol for unit*
area	square metre	m^2
volume	cubic metre	m^3
density	kilogram per cubic metre	kg m^{-3}
velocity	metre per second	m s^{-1}
angular velocity	radian per second	rad s^{-1}
acceleration	metre per second squared	m s^{-2}
magnetic field strength	ampere per metre	A m^{-1}

Certain units will be allowed in conjunction with the SI system, *e.g.*—

Physical quantity	*Name of unit*	*Symbol for unit*	*Definition of unit*
time	minute	min	60 s
plane angle	degree	°	$(\pi/180)$ rad
volume	litre	l	10^{-3} m^3 = dm^3
magnetic flux density (magnetic induction)	gauss	G	10^{-4} T
temperature, t	degree Celsius	°C	t/°C = T/K − 273.16
radionuclide activity	curie	Ci	3.7×10^{10} Bq
energy	electronvolt	eV	1.6021×10^{-19} J
pressure	bar	bar	10^5 Pa
mass	unified atomic mass unit	u	$1.660\,54 \times 10^{-27}$ kg

The other common units of time (*e.g.*, hour and day) will continue to be used in appropriate contexts.

Decimal multiples and submultiples have the following names and symbols (for use as prefixes)—

10^{-3}	milli	m	10^3	kilo	k
10^{-6}	micro	μ	10^6	mega	M
10^{-9}	nano	n	10^9	giga	G
10^{-12}	pico	p	10^{12}	tera	T
10^{-15}	femto	f	10^{15}	peta	P
10^{-18}	atto	a	10^{18}	exa	E
10^{-21}	zepto	z	10^{21}	zetta	Z
10^{-24}	yocto	y	10^{24}	yotta	Y

Compound prefixes (*e.g.*, mμm) should not be used; 10^{-9} m = 1 nm.

The Royal Society of Chemistry,
Thomas Graham House,
Science Park, Milton Road,
Cambridge CB4 4WF, UK

Telephone 0223 420066; Fax 0223 423623 (Group III); E-mail RSC1 @ UK.AC.RL.GB (JANET)

JOURNALS OF THE ROYAL SOCIETY OF CHEMISTRY

Refereeing Procedure and Policy (1991)

1.0 Contributions to Dalton, Perkin, and Faraday Transactions, J. Mater. Chem., The Analyst, J. Anal. At. Spectrom. and J. Chem. Research

1.1 Introduction

This document summarises the procedure used for assessing papers submitted to the four *Transactions*, *J. Mater. Chem.*, *The Analyst*, *J. Anal. At. Spectrom.*, and *J. Chem. Research*, and provides guidelines for referees engaged in this assessment.

1.2 Subject matter

Papers are submitted to the various journals according to subject matter. If a referee feels that a paper would be published more appropriately in an RSC journal other than the one suggested by the author, he should inform the Editor. The topics covered by the various journals are as follows:

Dalton Transactions (*Inorganic Chemistry*). All aspects of the chemistry of inorganic and organometallic compounds, including bioinorganic chemistry and solid-state inorganic chemistry; the applications of physicochemical techniques to the study of their structures, properties, and reactions, including kinetics and mechanism; new or improved experimental techniques and syntheses.

Faraday Transactions (*Physical Chemistry and Chemical Physics*). Gas-phase kinetics and dynamics; molecular beam kinetics and spectroscopy, photochemistry and photophysics; energy transfer and relaxation processes: laser-induced chemistry; spectroscopies of molecules, molecular and gas-phase complexes: quantum chemistry and molecular structure, statistical mechanics of gaseous molecules and complexes; spectroscopies, statistical mechanics and quantum theory of the condensed phase, computational chemistry and molecular dynamics; colloid and interface science, surface science, physisorption and chromatographic science, chemisorption and heterogeneous catalysis, zeolites and ion-exchange phenomena; electrode processes, liquids and solutions; solid-state chemistry (microstructures and dynamics); reactions in condensed phases; physical chemistry of macromolecules and polymers; materials science; thermodynamics; biophysical chemistry and radiation chemistry.

Perkin Transactions 1 (*Organic Chemistry*). All aspects of organic and bio-organic chemistry. These include synthetic organic chemistry of all types, organometallic chemistry, chemistry and biosynthesis of natural products, the relationship between molecular structure and biological activity, the chemistry of polymers and biological macromolecules, and medicinal and agricultural chemistry where there is originality in the science.

Perkin Transactions 2 (*Physical Organic Chemistry*). Physicochemical aspects of organic, organometallic, and bio-organic chemistry, including kinetic, mechanistic, structural, spectroscopic, and theoretical studies. Such topics include structure–activity relationships and physical aspects of biological processes and of the study of polymers and biological macromolecules.

Journal of Materials Chemistry. The chemistry of materials, particularly those associated with advanced technology; modelling of materials; synthesis and structural characterisation; physicochemical aspects of fabrication; chemical, structural, electrical, magnetic and optical properties; applications.

The Analyst (*Analytical Chemistry*). Theory and practice of all aspects of analytical chemistry, fundamental and applied, inorganic and organic, including chemical, physical, and biological methods.

Journal of Analytical Atomic Spectrometry. The development and analytical application of atomic spectrometric techniques.

Journal of Chemical Research. All areas of chemistry. The format of this journal (one- or two-page printed synopsis in Part S, plus microform version of authors' full text typescript in Part M) makes it particularly suitable for papers containing lengthy experimental sections or extensive data tabulations.

1.3 Procedure

Each manuscript is considered independently by two referees. The referees' reports constitute recommendations to the appropriate Editorial Board, which is empowered to take final action on manuscripts submitted. The Editor, acting for the Editorial Board, is responsible for all administrative and executive actions, and is empowered to accept or reject papers. It is the Editor's duty to see that, as far as possible, agreement is reached between authors and referees; although the referees may need to be consulted again concerning an author's reply to comments, further refereeing will be avoided as far as possible.

1.3.1 *Adjudication of disagreements.* If there is a notable discrepancy between the reports of the two referees, or if the difference between authors and referees cannot be resolved readily, a third referee may be appointed as adjudicator. In extreme cases, differences may be reported to the appropriate Editorial Board for resolution.

When a paper is recommended for rejection by referees, the Editor will inform the authors and return the top copy of the manuscript. Authors have the right to appeal to the Editorial Board if they regard a decision to reject as unfair. The Editor may refer to the Editorial Boards any papers which have been recommended for acceptance by the referees, but about which the Editor is doubtful.

1.3.2 *Anonymity.* The anonymity of referees is strictly preserved, and reports should be couched in terms which do not disclose the identity of the writer. A referee should never communicate directly with an author, unless and until such action has been sanctioned by the Society, through the Editor.

1.3.3 *Confidentiality.* A referee should treat a paper received for assessment as confidential material. Information acquired

by a referee from such a paper is not available for citation until the paper is published.

1.4 Policy

The primary criterion for acceptance of a contribution for publication is that it should advance scientific knowledge significantly. Papers that do **not** contain new experimental results may be considered for publication **only** if they either reinterpret or summarise known facts or results in a manner presenting an advance in chemical knowledge. Papers in interdisciplinary areas are acceptable if the chemical content is considered satisfactory.

Papers reporting results regarded as **routine** or **trivial** are not acceptable in the absence of other, desirable attributes.

Although short papers are acceptable, the Society strongly discourages the **fragmentation** of a substantial body of work into a number of short publications; such fragmentation is likely to be grounds for rejection.

The **length** of an article should be commensurate with its scientific content; however, authors are allowed every latitude (consistent with reasonable brevity) in the **form** in which their work is presented. Figures and flow-charts can often save space as well as clarify complicated arguments, and should not be excised unless they are unhelpful or really extravagant.

If a paper as a whole is judged suitable for the *Journal*, minor criticisms should not be unduly emphasised. It is the responsibility of the Editor to ensure the use of reasonably brief phraseology, and to assist the author to present his work in the most appropriate format.

However, referees should not hesitate to recommend rejection of papers which appear incurably badly composed.

It should be clearly understood that referees' reports are made in confidence to the Editor, at whose discretion comments will be transmitted to the author. To assist the Editor, referees are requested to indicate which comments are designed only for consideration, as distinct from those which, in the referee's view, require specific action or an adequate answer before the paper is accepted.

Referees may ask for sight of **supporting data** not submitted for publication, or for sight of a previous paper which has been submitted but not yet published. Such requests must be made to the Editor, not directly to the author.

1.4.1 *Authentication of new compounds.* Referees are asked to assess, as a whole, the evidence in support of the homogeneity and structure of all new compounds. No hard and fast rules can be laid down to cover all types of compounds, but the Society's policy is that evidence for the unequivocal identification of new compounds should wherever possible include good elemental analytical data; for example, an accurate mass measurement of a molecular ion does not provide evidence of purity of a compound and must be accompanied by independent evidence of homogeneity. Low-resolution mass spectrometry must be treated with even more reserve in the absence of firm evidence to distinguish between alternative molecular formulae. Where elemental analytical data are not available, appropriate evidence which is convincing to an expert in the field may be acceptable.

Spectroscopic information necessary to the assignment of structure should normally be given. Just how complete this information should be must depend upon the circumstances; the structure of a compound obtained from an unusual reaction or isolated from a natural source needs much stronger supporting evidence than one derived by a standard reaction from a precursor of undisputed structure.

Referees are reminded of the need to be exacting in their standards but at the same time flexible in their admission of evidence. It remains the Society's policy to accept work only of high quality and to permit no lowering of standards.

1.5 Titles and summaries

Referees should comment on Titles and Summaries with the following points in mind.

Titles of papers are used out of context by several organisations for current awareness purposes. To enable such systems to serve chemists adequately, titles must be written around a sufficient number of scientific words carefully chosen to cover the important aspects of the paper.

Summaries should preferably be self-contained, so that they can be understood without reference to the main text.

1.6 Speed of Refereeing

The Editorial Boards are anxious to maintain and to reduce further if possible the publication times now being achieved. In this connection, referees should submit their reports with the minimum of delay, or return manuscripts immediately to the Editor if long delay seems inevitable.

1.7 Suggestion of Alternative Referees

The Editor welcomes suggestions of alternative referees competent to deal with particular subject areas. Such suggestions are particularly helpful in cases where referees consider themselves ill-equipped (in terms of specialist knowledge) to deal with a specific paper, and in highly specialized or new areas of research where only a limited number of experts may be available. If, in such a case, the alternative and the original referee work in the same institution, the manuscript may be passed on directly after informing the Editor.

1.8 Notes (Short Papers) and Letters

'Notes' are published in *Dalton Transactions*; the corresponding format in *The Analyst* and *J. Anal. At. Spectrom.* is referred to as a 'Short Paper'. These articles are intended for the description of essentially complete pieces of work which are not of the length to justify a full paper. They are *NOT* preliminary communications, nor in any way an alternative to *Chemical Communications*, for which there are additional criteria of novelty and urgency. The quality of material contained in a Note (Short Paper) should be the same as that in a full paper. Investigations arising out of some larger project but not prosecuted to the same degree are particularly appropriate for this format.

A Note (Short Paper) should not normally exceed in length about 8 pages of typescript, including figures, tables, *etc.* It should comprise a short abstract (except in *The Analyst* and *J. Anal. At. Spectrom.*) and Discussion, but adequate experimental details are required.

In *J. Chem. Research*, a 'Short Paper' is essentially of the same type. As a consequence of its length, it appears in full in Part S with no microform version in Part M.

'Letters', published only in *Dalton Transactions*, are a medium for the expression of scientific opinions and views normally concerning material published in that journal; it is intended that contributions in this format should be published rapidly. The Letters section is for scientific discussion, and is not intended to compete with media for the publication of more general matters such as *Chemistry in Britain*.

Only rarely should a Letter exceed one printed column in length (about 1–2 pages of typescript). Where a Letter is polemical in nature, and if it is accepted, a Reply will be solicited from other parties implicated, for consideration for publication alongside the original Letter.

1.9 Relationship with Communications Journals

In cases where a preliminary report of the work described has appeared (for example in *Chemical Communications*), referees should alert the editor to any excessive and unnecessary repetition of material; this can arise in connection with communications journals whose restrictions on length and the reporting of experimental data are less severe than those of *Chemical Communications*. Furthermore, the acceptability of the full paper must be judged on the basis of the significance of the additional information provided, as well as on the criteria outlined in the foregoing sections.

2.0 Contributions to Chemical Communications

Chemical Communications is intended as a forum for preliminary accounts of original and significant work, in any area of chemistry that is likely to prove of wide general appeal or exceptional specialist interest. Such preliminary reports should be followed up in most cases by full papers in other journals, providing detailed accounts of the work. It is Society policy that only a fraction of research work warrants publication in *Chemical Communications*, and strict refereeing standards should be applied. The benefit to the reader from the rapid publication of a particular piece of work before it appears as a full paper must be balanced against the desirability of avoiding duplicate publication. The needs of the reader, not the author, must be considered, and priority in publication should not be allowed to determine acceptability. Acceptance should be recommended only if, in the opinion of the referee, the content of the paper is of such urgency that rapid publication will be advantageous to the progress of chemical research.

The length of Communications is strictly limited; only in exceptional circumstances should it exceed one printed page (two-and-a-half to three A4 pages of typescript) and referees should be particularly critical of manuscripts longer than this. Communications do not contain extensive spectroscopic or other experimental data, but referees may ask for sight of such data before reaching a decision.

The refereeing procedure for Communications is the same as that for full papers, except that rapidity of reporting is crucial in order to maintain rapid publication. The Journals Committee functions as the Editorial Board of *Chemical Communications* and as such acts as final arbiter in cases of dispute.

3.0 Communications submitted to The Analyst and J. Anal. At. Spectrom.

Criteria for acceptance of Communications submitted to *The Analyst* and *J. Anal. At. Spectrom.* are similar to those for contributions to *Chemical Communications*, except that they should be concerned specifically with analytical chemistry. However Communications to *The Analyst* and *J. Anal. At. Spectrom.* are not subjected to refereeing in the usual way; a decision whether or not to publish rests with the Editor, who may or may not obtain advice from a referee.

4.0 Communications submitted to Perkin or Faraday Transactions or J. Mater. Chem.

Criteria for acceptance of Communications submitted to *Perkin* or *Faraday Transactions or J. Mater. Chem.* are similar to those for contributions to *Chemical Communications*, except that the work will be of more specialist interest. For *Perkin Communications* inclusion of key experimental data is expected. Assessment is carried out by a small nucleus of referees, consisting largely of members of Perkin Editorial Board or of the Faraday or Materials International Advisory Editorial Board, as appropriate.

5.0 Contributions to Mendeleev Communications

Mendeleev Communications, published jointly by the Royal Society of Chemistry and the USSR Academy of Sciences, is a sister publication to *Chemical Communications*, containing preliminary reports of the same type, in any area of chemistry. The majority of contributions are from Soviet authors.

Assessment involves two stages of refereeing. Manuscripts submitted to the Soviet Editorial Office are refereed initially by a Soviet scientist. If found acceptable they are then reviewed by Western scientists chosen by the Royal Society of Chemistry. A favourable recommendation at this stage, from one referee, is sufficient authority for acceptance. If the recommendation is unfavourable, however, a second RSC referee is consulted; two unfavourable reports are required for rejection. Manuscripts submitted to the UK Editorial Office undergo this two-stage refereeing process in reverse.

6.0 X-Ray Crystallographic Work

6.1 Crystallographic papers are of two types:

(A) The majority, which contain definitive data on completely refined determinations.

(B) A minority which include brief accounts of structures containing feature(s) of unusual interest and where the structure solutions are clear but where (for any of a variety of reasons) the full refinement has not been completed. These are then regarded as preliminary publications, at least so far as the X-ray results are concerned.

Both types of publication are appropriate for *Chem. Commun.*; only those of type (A) should normally appear in *Dalton* or *Perkin Transactions*.

6.2 Papers of type (A) in *Dalton* and *Perkin Transactions* should normally contain the information in their *titles* that an X-ray structure determination has been carried out; this is often appropriate in *Chem. Commun.* also, but not obligatory. Papers of type (B) need not do so if the X-ray determination forms only a minor part. *Summaries* should always contain this information unless the paper is of type (B) and the structure determination is not a main point of the communication.

6.3 All papers containing crystallographic determinations will be refereed by two referees, one a structural chemist. If the editor considers it advisable, the paper may also be sent to a crystallographer for comment. Referees will not normally be expected to check values of structural parameters for publication (*e.g.* bond lengths and angles against atomic co-ordinates; this will be done after publication by CCDC or Bonn), but should still pay attention to the quality of the experimental crystallographic work. However their primary concern should be such new chemistry as is involved in the structure.

6.4 On occasions *Chem. Commun.* will publish preliminary accounts [type (B)] of crystal structures of unusual *chemical* interest. By 'preliminary' is meant that the data have not yet been fully refined. Sufficient supplementary data must be provided for the referee to judge whether the 'not-fully-refined' structure does indeed prove the desired point, and care should be taken by the referees to ensure that the authors do not overstate the case they have—for example by reporting bond

lengths to very high degrees of apparent precision when they have poor *R*-factors. Such papers will always be refereed by a professional crystallographer. Authors must indicate in the paper or the supplementary data the justification for publishing without full refinement and referees should comment on whether the case for publication is convincing.

6.5 In many cases the structure referred to in *Chem. Commun.* will be fully refined. The *Chem. Commun.* can then be considered to fulfil the archival function, and the structure determination may not require further detailed refereeing when presented as part of a full paper. In the full paper, the author's purpose will then be served by a simple reference back to the original communication. However, if the crystallography is discussed again at any length in the full paper, the data should be re-presented to the referees in full, and re-published if considered necessary.

6.6 There may be other cases when an author wishes to publish a paper in *Dalton* or *Perkin* in which the result of a crystal structure determination is discussed, but in which details or extensive discussion are considered unnecessary. The crystallographer may even be omitted as a co-author (for example when the determination is carried out by a commercial company). If the author is able to show the referees that this procedure is appropriate, it will be allowed provided that it does not lead to unnecessary fragmentation. However, the author must provide, as supplementary information, sufficient data relating to the crystal structure determination to allow a referee to make sure that the point made is correct, and co-ordinates *etc.* will be deposited with CCDC (or Bonn). The brief published description of the determination should be supplemented by appropriate reference to 'unpublished work'.

IUPAC Publications on Nomenclature and Symbolism

1.0 Compilations

1.1 Nomenclature of Organic Chemistry, a 550-page hardcover volume published in 1979, available from Pergamon, Oxford.

Section A: Hydrocarbons
Section B: Fundamental heterocyclic systems
Section C: Characteristic groups containing carbon, hydrogen, oxygen, nitrogen, halogen, sulphur, selenium, and tellurium
Section D: Organic compounds containing elements not exclusively those referred to in the title of Section C
Section E: Stereochemistry
Section F: General principles for the naming of natural products and related compounds
Section H: Isotopically modified compounds

1.2 Nomenclature of Inorganic Chemistry, a 278-page hardcover volume published in 1990, available from Blackwell Scientific Publications, Oxford.

Chapter 1: General aims, functions and methods
Chapter 2: Grammar
Chapter 3: Elements, atoms, and groups
Chapter 4: Formulae
Chapter 5: Names based on stoichiometry
Chapter 6: Neutral molecular compounds
Chapter 7: Names for ions, substituent groups and radicals, and salts
Chapter 8: Oxoacids and derived anions
Chapter 9: Co-ordination compounds
Chapter 10: Boron hydrides and related compounds

1.3 Biochemical Nomenclature and Related Documents, a 220-page softcover manual published in 1978 by The Biochemical Society for IUB, and available from the Biochemical Society Book Depot, PO Box 32, Commerce Way, Colchester, Essex CO2 8HP. The contents are as follows:

General
Nomenclature of organic chemistry. Section E: Stereochemistry (1974)
Nomenclature of organic chemistry. Section F: Natural products and related compounds (1976)
Nomenclature of organic chemistry. Section H: Isotopically modified compounds (1977)
Isotopically labelled compounds: common biochemical practice
Recommendations for measurement and presentation of biochemical equilibrium data (1976)
Abbreviations and symbols for chemical names of special interest in biological chemistry (1965)
Abbreviations and symbols: a compilation (1976)
Citation of bibliographic references in biochemical journals (1971)
Amino acids, peptides and proteins
Nomenclature of α-amino acids (1974)
Symbols for amino-acid derivatives and peptides (1971)
Rules for naming synthetic modifications of natural peptides (1966)
Abbreviated nomenclature of synthetic polypeptides or polymerized amino acids (1971)
A one-letter notation for amino-acid sequences (1968)
Abbreviations and symbols for the description of the conformation of polypeptide chains (1969)
Nomenclature of peptide hormones (1974)
Recommendations for the nomenclature of human immunoglobulins
Protein data bank. A computer-based archival file for macromolecular structures (1977)
Nomenclature of multiple forms of enzymes (1976)
Nucleotides and nucleic acids
Abbreviations and symbols for nucleic acids, polynucleotides and their constituents (1970)
Lipids
Nomenclature of lipids (1976)

Nomenclature of steroids (1967)
Nomenclature of quinones with isoprenoid side chains (1973)
Tentative rules for the nomenclature of carotenoids (1970). Amendments (1974)
Nomenclature of tocopherols and related compounds (1973)
Carbohydrates, etc.
Tentative rules for carbohydrate nomenclature. Part 1 (1969)
Nomenclature of cyclitols (1973)
Phosphorus-containing compounds
Nomenclature of phosphorus-containing compounds of biochemical importance (1976)
Miscellaneous
Trivial names of miscellaneous compounds of importance in biochemistry (1965)
Nomenclature and symbols for folic acids and related compounds (1965)
Nomenclature for vitamins B-6 and related compounds (1973)
Nomenclature of corrinoids (1973)

1.4 Compendium of Analytical Nomenclature, a 280-page hardcover volume published in 1987, available from Blackwell Scientific Publications, Oxford. The contents are as follows:

Presentation of the Results of Chemical Analysis
Solution Thermodynamics (activity coefficients, equilibria, pH)
Recommendations for Terminology to be used with Precision Balances
Recommendations for Nomenclature of Thermal Analysis
Recommendations for Nomenclature of Titrimetric Analysis
Electrochemical Analysis
Analytical Separation Processes (precipitation, liquid-liquid distribution, zone melting and fractional crystallization, chromatography, ion exchange)
Spectrochemical Analysis (radiation sources, general atomic emission spectroscopy, flame spectroscopy, *X*-ray emission spectroscopy, molecular methods)
Recommendations for Nomenclature of Mass Spectrometry
Recommendations for Nomenclature of Radiochemical Methods
Surface Analysis (including photoelectron spectroscopy)

1.5 Compendium of Chemical Terminology: IUPAC Recommendations, a 456-page volume published in 1987, available in hardcover and softcover from Blackwell Scientific Publications, Oxford.

1.6 Quantities, Units, and Symbols in Physical Chemistry, a 134-page hardcover volume published in 1988, available from Blackwell Scientific Publications, Oxford.

2.0 Documents not included in the compilations

2.1 Nomenclature of Elements and Compounds

2.1.1 *Amino acids and Peptides*

Nomenclature and symbolism for amino acids and peptides (*Pure Appl. Chem.*, 1984, **56**, 595; *Eur. J. Biochem.*, 1984, **138**, 9).

2.1.2 *Analytical Reagents*

Guide to trivial names, trade names, and synonyms for substances used in analytical chemistry (*Pure Appl. Chem.*, 1978, **50**, 339).

2.1.3 *Boron Compounds*

Nomenclature of inorganic boron compounds (*Pure Appl. Chem.*, 1972, **30**, 681).

2.1.4 *Carbohydrates*

Conformational nomenclature for five- and six-membered ring forms of monosaccharides and their derivatives (provisional) (*Pure Appl. Chem.*, 1981, **53**, 1901; *Eur. J. Biochem.*, 1980, **111**, 295).

Abbreviated terminology of oligosaccharide chains (provisional) (*Pure Appl. Chem.*, 1982, **54**, 1517; *J. Biol. Chem.*, 1982, **257**, 2347).

Polysaccharide nomenclature (provisional) (*Pure Appl. Chem.*, 1982, **54**, 1523; *J. Biol. Chem.*, 1982, **257**, 3352).

Nomenclature of unsaturated monosaccharides (provisional) (*Pure Appl. Chem.*, 1982, **54**, 207; *Eur. J. Biochem.*, 1981, **119**, 1; errata *Eur. J. Biochem.*, 1982, **125**, 1).

Nomenclature of branched-chain monosaccharides (provisional) (*Pure Appl. Chem.*, 1982, **54**, 211; *Eur. J. Biochem.*, 1981, **119**, 5; errata *Eur. J. Biochem.*, 1982, **125**, 1).

Symbols for specifying the conformation of polysaccharide chains (provisional) (*Pure Appl. Chem.*, 1983, **55**, 1269; *Eur. J. Biochem.*, 1983, **131**, 5).

2.1.5 *Delta Convention*

Nomenclature for cyclic organic compounds with contiguous formal double bonds (*Pure Appl. Chem.*, 1988, **60**, 1395).

2.1.6 *Elements*

Recommendations for the names of elements of atomic number greater than 100 (*Pure Appl. Chem.*, 1979, **51**, 381).

2.1.7 *Enzymes*

Enzyme Nomenclature (1984), published by Academic Press in hardcover and softcover editions.

2.1.8 *Folic Acid*

Nomenclature and symbols for folic acid and related compounds (*Pure Appl. Chem.*, 1987, **59**, 833; *Eur. J. Biochem.*, 1987, **168**, 251).

2.1.9 *Glycoproteins*

Nomenclature of glycoproteins, glycopeptides, and peptidoglycans (*Pure Appl. Chem.*, 1988, **60**, 1389).

2.1.10 *Heterocyclic Compounds*

Revision of the extended Hantzsch-Widman system of nomenclature for heteromonocycles (*Pure Appl. Chem.*, 1983, **55**, 409).

2.1.11 *Hydrogen*

Names for hydrogen atoms, ions, and groups, and for reactions involving them (*Pure Appl. Chem.*, 1988, **60**, 1115).

2.1.12 *Isotopically Modified Compounds*

Nomenclature of inorganic chemistry. Part II. 1. Isotopically modified compounds (*Pure Appl. Chem.*, 1981, **53**, 1887).

2.1.13 *Lambda Convention*

Treatment of variable valence in organic nomenclature (*Pure Appl. Chem.*, 1984, **56**, 769).

2.1.14 *Nitrogen Hydrides*

Nomenclature of hydrides of nitrogen and derived cations, anions, and ligands (*Pure Appl. Chem.*, 1982, **54**, 2545).

2.1.15 *Nucleotides*

Abbreviations and symbols for the description of conformations of polynucleotide chains (provisional) (*Pure Appl. Chem.*, 1983, **55**, 1279; *Eur. J. Biochem.*, 1983, **131**, 9).

2.1.16 *Numerical Terms*

Extension of Rules A-1.1 and A-2.5 concerning numerical terms used in organic chemical nomenclature (*Pure Appl. Chem.*, 1986, **58**, 1693).

2.1.17 *Polymers*

Nomenclature of regular single-strand organic polymers (*Pure Appl. Chem.*, 1976, **48**, 373).

Nomenclature for regular single-strand and quasi single-strand inorganic and co-ordination polymers (*Pure Appl. Chem.*, 1985, **57**, 149).

Source-based nomenclature for copolymers (*Pure Appl. Chem.*, 1985, **57**, 1427).

Stereochemical definitions and notations relating to polymers (*Pure Appl. Chem.*, 1981, **53**, 733).

Use of abbreviations for names of polymeric substances (*Pure Appl. Chem.*, 1987, **59**, 691).

Basic definitions of terms relating to polymers (*Pure Appl. Chem.*, 1974, **40**, 477).

Definitions of terms relating to individual macromolecules, their assemblies, and dilute polymer solutions (*Pure Appl. Chem.*, 1989, **61**, 211).

A classification of linear single-strand polymers (*Pure Appl. Chem.*, 1989, **61**, 243).

Definition of terms relating to crystalline polymers (*Pure Appl. Chem.*, 1989, **61**, 769).

2.1.18 *Polyanions*

Nomenclature of polyanions (*Pure Appl. Chem.*, 1987, **59**, 1529).

2.1.19 *Prenols*

Nomenclature of prenols (*Pure Appl. Chem.*, 1987, **59**, 683; *Eur. J. Biochem.*, 1987, **167**, 181).

2.1.20 *Retinoids*

Nomenclature of retinoids (provisional) (*Pure Appl. Chem.*, 1983, **55**, 721; *Eur. J. Biochem.*, 1982, **129**, 1).

2.1.21 *Steroids*

Nomenclature of steroids (*Pure Appl. Chem.*, 1989, **61**, 1783).

2.1.22 *Tetrapyrroles*

Nomenclature of tetrapyrroles (*Pure Appl. Chem.*, 1987, **59**, 779).

2.1.23 *Tocopherols*

Nomenclature of tocopherols and related compounds (*Pure Appl. Chem.*, 1982, **54**, 1507; *Eur. J. Biochem.*, 1982, **123**, 473).

2.1.24 *Vitamins*

Nomenclature of Vitamin D (provisional) (*Pure Appl. Chem.*, 1982, **54**, 1511; *Eur. J. Biochem.*, 1982, **124**, 223).

2.1.25 *Zeolites*

Chemical nomenclature and formulation of compositions of synthetic and natural zeolites (*Pure Appl. Chem.*, 1979, **51**, 1091).

2.2 Terminology, Symbols, and Units, and Presentation of Results

2.2.1 *General*

Glossary of terms used in physical organic chemistry (*Pure Appl. Chem.*, 1983, **55**, 1281).

2.2.2 *Analytical*

Nomenclature, symbols, units, and their usage in spectrochemical analysis. Part VII, Molecular absorption spectroscopy, u.v. and visible (*Pure Appl. Chem.*, 1988, **60**, 1449). Part X, Preparation of materials for analytical atomic spectroscopy (*Pure Appl. Chem.*, 1988, **60**, 1461).

Recommendations for publication of papers on a new analytical method based on ion exchange or ion-exchange chromatography (*Pure Appl. Chem.*, 1980, **52**, 2555).

Recommendations for presentation of data on compleximetric indicators. 1. General (*Pure Appl. Chem.*, 1979, **51**, 1357).

Recommendations for publishing manuscripts on ion-selective electrodes (*Pure Appl. Chem.*, 1981, **53**, 1907).

Recommendations on use of the term amplification reactions (*Pure Appl. Chem.*, 1982, **54**, 2553).

Recommendations for the usage of selective, selectivity, and related terms in analytical chemistry (*Pure Appl. Chem.*, 1983, **55**, 553).

Nomenclature for automated and mechanised analysis (*Pure Appl. Chem.*, 1989, **61**, 1657).

Nomenclature for sampling in analytical chemistry (*Pure Appl. Chem.*, 1990, **62**, 1193).

2.2.3 *Clinical*

Physicochemical quantities and units in clinical chemistry with special emphasis on activities and activity coefficients (*Pure Appl. Chem.*, 1984, **56**, 567).

Quantities and units in clinical chemistry (*Pure Appl. Chem.*, 1979, **51**, 2451).

Quantities and units in clinical chemistry: nebulizer and flame properties in flame emission and absorption spectrometry (*Pure Appl. Chem.*, 1986, **58**, 1737).

List of quantities in clinical chemistry (*Pure Appl. Chem.*, 1979, **51**, 2481).

2.2.4 *Colloids and Surface Chemistry*

Definitions, terminology, and symbols in colloid and surface chemistry. I (*Pure Appl. Chem.*, 1972, **31**, 577). II, Heterogeneous catalysis (*Pure Appl. Chem.*, 1976, **46**, 71). Part 1.14: Light scattering (provisional) (*Pure Appl. Chem.*, 1983, **55**, 931).

Reporting experimental pressure-area data with film balances (*Pure Appl. Chem.*, 1985, **57**, 621).

Reporting physisorption data for gas/solid systems with special reference to the determination of surface area and porosity (*Pure Appl. Chem.*, 1985, **57**, 603).

Reporting data on adsorption from solution at the solid/solution interface (*Pure Appl. Chem.*, 1986, **58**, 967).

2.2.5 *Electrochemistry*

Nomenclature for transfer phenomena in electrolytic systems (*Pure Appl. Chem.*, 1981, **53**, 1827).

Electrode reaction orders, transfer coefficients, and rate constants—amplification of definitions and recommendations for publication of parameters (*Pure Appl. Chem.*, 1980, **52**, 233).

Classification and nomenclature of electroanalytical techniques (*Pure Appl. Chem.*, 1976, **45**, 81).

Recommendations for sign conventions and plotting of electrochemical data (*Pure Appl. Chem.*, 1976, **45**, 131).

Electrochemical nomenclature (*Pure Appl. Chem.*, 1974, **37**, 499).

Recommendations on reporting electrode potentials in nonaqueous solvents (*Pure Appl. Chem.*, 1984, **56**, 461).

Definition of pH scales, standard reference values, measurement of pH and related terminology (*Pure Appl. Chem.*, 1985, **57**, 531).

Interphases in systems of conducting phases (*Pure Appl. Chem.*, 1986, **58**, 437).

The absolute electrode potential: an explanatory note (*Pure Appl. Chem.*, 1986, **58**, 955).

Electrochemical corrosion nomenclature (*Pure Appl. Chem.*, 1989, **61**, 19).

2.2.6 *Kinetics*

Symbolism and terminology in chemical kinetics (provisional) (*Pure Appl. Chem.*, 1981, **53**, 753).

2.2.7 *Photochemistry*

Recommended standards for reporting photochemical data (*Pure Appl. Chem.*, 1984, **56**, 939).

Glossary of terms used in photochemistry (*Pure Appl. Chem.*, 1988, **60**, 1055).

2.2.8 *Quantum Chemistry*

Expression of results in quantum chemistry (*Pure Appl. Chem.*, 1978, **50**, 75).

2.2.9 *Reactions*

Nomenclature for organic chemical transformations (*Pure Appl. Chem.*, 1989, **61**, 725).

System for symbolic representation of reaction mechanisms (*Pure Appl. Chem.*, 1989, **61**, 23).

Detailed linear representation of reaction mechanisms (*Pure Appl. Chem.*, 1989, **61**, 57).

2.2.10 *Rheological Properties*

Selected definitions, terminology, and symbols for rheological properties (*Pure Appl. Chem.*, 1979, **51**, 1215).

2.2.11 *Spectroscopy*

Recommendations for publication of papers on methods of molecular absorption spectrophotometry in solution (*Pure Appl. Chem.*, 1978, **50**, 237).

Recommendations for the presentation of infrared absorption spectra in data collections. A, Condensed phases (*Pure Appl. Chem.*, 1978, **50**, 231).

Definition and symbolism of molecular force constants (*Pure Appl. Chem.*, 1978, **50**, 1709).

Nomenclature and conventions for reporting Mössbauer spectroscopic data (*Pure Appl. Chem.*, 1976, **45**, 211).

Recommendations for the presentation of NMR data for publication in chemical journals. A, Proton spectra (*Pure Appl. Chem.*, 1972, **29**, 625). B, Spectra from nuclei other than protons (*Pure Appl. Chem.*, 1976, **45**, 217).

Presentation of Raman spectra in data collections (*Pure Appl. Chem.*, 1981, **53**, 1879).

Names, symbols, definitions and units of quantities in optical spectroscopy (*Pure Appl. Chem.*, 1985, **57**, 105).

A descriptive classification of the electron spectroscopies (*Pure Appl. Chem.*, 1987, **59**, 1343).

Presentation of molecular parameter values for i.r. and Raman intensity (*Pure Appl. Chem.*, 1988, **60**, 1385).

Recommendations for EPR/ESR nomenclature and conventions for presenting experimental data in publications (*Pure Appl. Chem.*, 1989, **61**, 2195).

2.2.12 *Thermodynamics*

A guide to procedures for the publication of thermodynamic data (*Pure Appl. Chem.*, 1972, **39**, 395).

Assignment and presentation of uncertainties of the numerical results of thermodynamic measurements (*Pure Appl. Chem.*, 1981, **53**, 1805).

Notation for states and processes; significance of the word 'standard' in chemical thermodynamics and remarks on commonly tabulated forms of thermodynamic functions (*Pure Appl. Chem.*, 1982, **54**, 1239).

Announcement and Call for Papers

1991 Joint Meeting FACSS/Pacific Conference

October 6-11, 1991
Disneyland Hotel, Anaheim, California, USA

A combined meeting of the Federation of Analytical Chemistry and Spectroscopy Societies (FACSS) and the Pacific Conference on Chemistry and Spectroscopy will be held in 1991. FACSS is considered by many to be the premier annual technical analytical conference. The Pacific Conference is a regional meeting of the American Chemical Society and the Society for Applied Spectroscopy. This joint meeting will provide a program with expanded technical coverage with an emphasis in emerging technologies in analytical, spectroscopic, chemical, and biochemical science.

The deadline for submission of your title and a preliminary 100 word brief is **March 25, 1991**. A title submission form appears on the next page.

For information concerning the scientific program, please contact **James Holcombe, FACSS/Pacific Conference Program Chairman**, Dept. of Chemistry, University of Texas, Austin, TX 78712.

Nominations are requested for the **Tomas Hirschfeld Student Awards**, which will be presented at the conference for the most outstanding papers submitted by graduate students. The student nominees will give their papers at the FACSS/Pacific Conference. To be considered for these awards, students must submit the title of their paper, two letters of nomination and any reprints/preprints and a 250 word abstract to the program chairman by March 25, 1991.

Contributed papers are solicited in all areas of analytical chemistry including atomic and molecular spectroscopy, chromatography, laser spectroscopy, mass spectrometry, nuclear magnetic resonance, process analysis, computers and software, environmental analysis, biotechnology, pharmaceutical and clinical analyses. In addition, papers in the fields of biochemistry, inorganic, organic, physical, atmospheric and environmental chemistry will be presented. The scientific program will also include various Award Symposia.

For general information please contact one of the General Chairmen: **Richard Deming,** Dept. of Chemistry and Biochemistry, California State University at Fullerton, Fullerton, CA 92634 (714)-773-2170 or **Connie Sobel,** 1800 N. Altadena Dr., Passadena, CA 91107 (818)-794-0737.

For other information please contact: **FACSS, P.O. Box 278, Manhattan, KS 66502 or phone (301)-846-4797.**

1991 Joint Meeting FACSS/Pacific Conference
Title Submission Form

Title Deadline: March 25, 1991 ***Please print clearly or type***

Topic Codes _____ (maximum of 3 from Topic Code List)

Title:

Authors:

Corresponding Author Information:

First Name _____ M.I. ___ Last Name _____

Company/University _____

Address _____

_____

City _____ State _____ Zip Code _____ Country _____

Phone ( ) _____

Preferred Format *: Talk ☐ Poster ☐ Either ☐

* Actual format may be determined by space availability and format of similar talks in your topical area.

Affiliations: ACS ☐ Anachem ☐ Coblentz ☐ CFDV ☐ ISA ☐ RSC ☐ SAS ☐ Other ☐

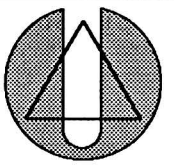
[Please check author(s) affiliation(s). Excess funds may be distributed amongst supporting organizations in relation to participation.]

Topic Codes:

- A Atomic Spectroscopy
- B Biomed/Biological/Pharmacological Analyses
- C Chromatography
- D Electroanalytical
- E Lasers
- F Mass Spectroscopy
- G Molecular Spectroscopy
- H Process Control/Computers/Chemometrics
- J Solid State/Surfaces/Materials
- K Other Analytical
- L Other Chemistry Sessions:
  - L1 Biochemistry/Biotechnology
  - L2 Inorganic Chemistry
  - L3 Organic Chemistry
  - L4 Physical Chemistry
  - L5 Atmospheric Chemistry
  - L6 Environmental Chemistry

Preliminary 100 word brief:

Please send this completed form to: FACSS
P.O. Box 278 (1928 Leavenworth *for overnight express*)
Manhattan, KS 66502



Federation of
Analytical Chemistry and
Spectroscopy Societies

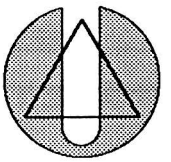
Did you miss the 1990 FACSS Conference?

We're sorry if you were unable to attend FACSS this year. We thought you might be interested in purchasing a copy of the final program which includes all of the abstracts. If you would like a copy please send a check in the amount of $10.00 (U.S. funds) made payable to: FACSS XVII.

Mail to: FACSS, P.O. Box 1405, Frederick, MD 21702-0405

Please be sure to include your name and address. Thanks!

We're looking forward to seeing you in Anaheim !



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1991 FACSS/Pacific Conference
October 6-11, 1991
Disneyland Hotel, Anaheim, CA

Nominations are requested for the Tomas Hirschfeld Student Awards, which will be presented at the Joint Meeting of the FACSS/Pacific Conference. Awards are given for the most outstanding papers submitted by graduate students in the field of analytical chemistry. The student nominees will present 20 minute papers at the 1991 FACSS/Pacific Conference. To be considered for these awards, students must submit the title of their paper, two letters of nomination, any reprints/preprints and a 250 word abstract to : **James Holcombe, FACSS/Pacific Conference Program Chairman**, Dept. of Chemistry, University of Texas, Austin, TX 78712.

The deadline for submission of all materials is **March 25, 1991**.

The awardees' travel expenses will be paid by FACSS.

For further information concerning the Tomas Hirschfeld Student Awards contact either of the Student Award Co-chairs:

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The XXVII Colloquium Spectroscopicum Internationale

XXVII CSI

1991
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will be held in

Grieg Hall, Bergen, Norway
June 9–14 1991

IUPAC

This traditional biennial conference in analytical spectroscopy will once again provide a forum for atomic, nuclear and molecular spectroscopists worldwide to encourage personal contact and the exchange of experience.

Participants are invited to submit papers for presentation at the XXVII CSI, dealing with the following topics:

Basic theory and instrumentation of—

- Atomic spectroscopy (emission, absorption, fluorescence)
- Molecular spectroscopy (UV, VIS and IR)
- X-ray spectroscopy
- Gamma spectroscopy
- Mass spectrometry (inorganic and organic)
- Electron spectroscopy
- Raman spectroscopy
- Mössbauer spectroscopy
- Nuclear magnetic resonance spectrometry
- Methods of surface analysis and depth profiling
- Photoacoustic spectroscopy

Application of spectroscopy in the analysis of—

- Metals and alloys
- Geological materials
- Industrial products
- Biological samples
- Food and agricultural products

Special emphasis will be given to trace analysis, environmental pollutants and standard reference materials.
The scientific programme will consist of both plenary lectures and parallel sessions of oral presentation. Specific times will be reserved for poster sessions.

PRE- AND POST-SYMPOSIA

In connection with the XXVII CSI the following symposia will be organised:

Pre-symposia—

I. GRAPHITE ATOMISER TECHNIQUES IN ANALYTICAL SPECTROSCOPY
June 6–8, 1991, Hotel Ullensvang, Lofthus, Norway.

II. CHARACTERISATION OF OIL COMPONENTS USING SPECTROSCOPIC METHODS
June 6–8, 1991, Hotel Hardangerfjord, Øystese, Norway.

III. MEASUREMENT OF RADIO-NUCLIDES AFTER THE CHERNOBYL ACCIDENT
June 6–8, 1991, Hotel Solstrand, Bergen, Norway.

Post-symposium—

IV. SPECIATION OF ELEMENTS IN ENVIRONMENTAL AND BIOLOGICAL SCIENCES
June 17–19, 1991, Hotel Alexandra, Loen, Norway.

For further information contact:

THE SECRETARIAT
XXVII CSI
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CONTENTS

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