PAGES 321–416

ISSN 0003—2654

The Analyst

A monthly international journal dealing with all branches of analytical chemistry

Vol. 110 No. 4
April
1985

ROYAL SOCIETY OF CHEMISTRY

The Analyst

The Analytical Journal of The Royal Society of Chemistry

Editorial Office: The Royal Society of Chemistry, Burlington House, Piccadilly, London, W1V 0BN. Telephone 01-734 9864. Telex No. 268001

Advertisements: Advertisement Department, The Royal Society of Chemistry, Burlington House, Piccadilly, London, W1V 0BN. Telephone 01-734 9864. Telex No. 268001

The Analyst (ISSN 0003–2654) is published monthly by The Royal Society of Chemistry, Burlington House, London W1V 0BN, England. All orders accompanied with payment should be sent directly to The Royal Society of Chemistry, The Distribution Centre, Blackhorse Road, Letchworth, Herts. SG6 1HN, England. 1985 Annual subscription rate UK £134.50, Rest of World £141.00, USA $260.00. Purchased with *Analytical Abstracts* UK £293.00, Rest of World £308.00, USA $566.00. Purchased with *Analytical Abstracts* plus *Analytical Proceedings* UK £330.00, Rest of World £347.00, USA $638.00. Purchased with *Analytical Proceedings* UK £169.00, Rest of World £178.00, USA $327.00. Air freight and mailing in the USA by Publications Expediting Inc., 200 Meacham Avenue, Elmont, NY 11003.
USA Postmaster: Send address changes to: *The Analyst*, Publications Expediting Inc., 200 Meacham Avenue, Elmont, NY 11003. Second class postage paid at Jamaica, NY 11431. All other despatches outside the UK by Bulk Airmail within Europe, Accelerated Surface Post outside Europe. PRINTED IN THE UK.

Information for Authors

Full details of how to submit material for publication in *The Analyst* are given in the Instructions to Authors in the January issue. Separate copies are available on request.

The Analyst publishes papers on all aspects of the theory and practice of analytical chemistry, fundamental and applied, inorganic and organic, including chemical, physical and biological methods. There is no page charge.

The following types of papers will be considered:

Full papers, describing original work.

Short papers, also describing original work, but shorter and of limited breadth of subject matter; there will be no difference in the quality of the work described in full and short 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 should not be simple claims for priority: this facility for rapid publication is 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.

Reviews, which must be a critical evaluation of the existing state of knowledge on a particular facet of analytical chemistry.

Every paper (except Communications) will be submitted to at least two referees, by whose advice the Editorial Board of *The Analyst* will be guided as to its acceptance or rejection. 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.

Regional Advisory Editors. For the benefit of potential contributors outside the United Kingdom, a Panel 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 Panel. Currently serving Regional Advisory Editors are listed in each issue of *The Analyst*.

Manuscripts (three copies typed in double spacing) should be addressed to:
The Editor, *The Analyst,*
Royal Society of Chemistry,
Burlington House,
Piccadilly,
LONDON W1V 0BN, UK

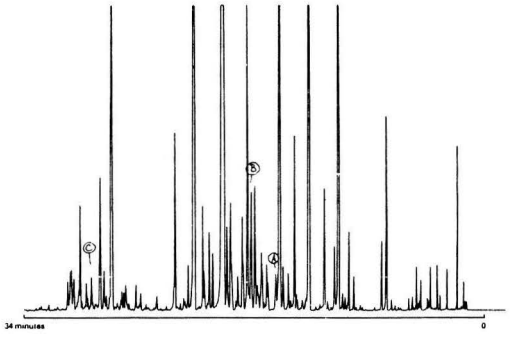
Particular attention should be paid to the use of standard methods of literature citation, including the journal abbreviations defined in Chemical Abstracts Service Source Index. Wherever possible, the nomenclature employed should follow IUPAC recommendations, and units and symbols should be those associated with SI.

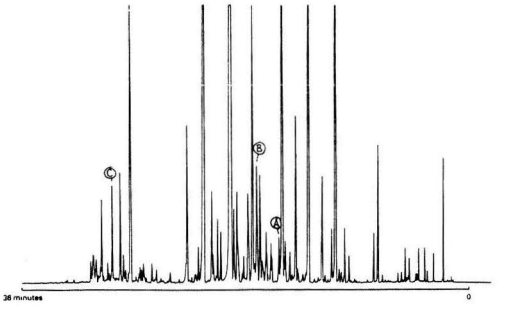
All queries relating to the presentation and submission of papers, and any correspondence regarding accepted papers and proofs, should be directed to the Editor, *The Analyst* (address as above). Members of the Analytical Editorial Board (who may be contacted directly or via the Editorial Office) would welcome comments, suggestions and advice on general policy matters concerning *The Analyst.*

Fifty reprints of each published contribution are supplied free of charge, and further copies can be purchased.

Advertisement Feature

Analysis of Essential Oils by Capillary Gas Chromatography





INTRODUCTION

Essential oils, the distilled extracts of plants, fruits and spices, are used in the food and perfume industries for flavouring and for the production and enhancing of odours. Being derived from natural products they are invariably complex mixtures of relatively volatile compounds, and to obtain a meaningful analysis high resolution capillary columns must be used.

Because the components are frequently polar in nature, Carbowax 20M is the most commonly used stationary phase. A non-polar column may be used also in order to substantiate results.

As an example of the kind of problem that has to be solved, although lemon oils are derived from lemons, the area where those lemons were grown determines the composition, and hence the quality and price of the final product. Condsequently the source of the oil often has to be established and this can usually be judged by small variations in composition. Only a capillary column can provide such detailed information.

In all flavour work it must be recognised that, even when a component is present in large concentrations, it does not necessarily contribute proportionately to the flavour or odour. It may be odourless or its role may be that of a flavour enhancer. Conversely the smallest peak in the chromatogram may arise from a compound which greatly influences the flavour.

For further information please contact: D. F. K. Swan, Pye Unicam Ltd. York Street, Cambridge CB1 2PX (telephone: 0223 358866).

INSTRUMENTATION

Pye Unicam Series 304 Gas Chromatograph
Column: 25 m × 0.25 mm Carbowax 20M WCOT
Column temperature: 2 min at 60 °C then rising at 4 °C min^{-1} to 220° C.
Injector temp.: 250 °C
Detector: FID
Detector temp.: 250 °C
Carrier: hydrogen, 2 ml min^{-1}
Attenuation: 16 × 10
Sample size: 0.05 µl of pure geranium oil
Injector split: 25 : 1

RESULTS AND DISCUSSION

The work reported here was done on two geranium oils which gave virtually identical chromatograms with a packed column, yet whose odours and colours were quite different. Figures 1 and 2 are the corresponding WCOT column chromatograms. Several differences here are immediately obvious. Peaks A and B are higher, and peak C is considerably higher in Figure 1 than in Figure 2.

It is relatively easy to produce good chromatograms for essential oils, but to obtain very high resolution it is necessary carefully to optimise the analysis conditions. Even though the two chromatograms reveal a large number of components there are still a number of peaks with shoulders and peaks not fully resolved. If these are to be separated it is necessary to use a column in good condition and a slower programme rate.

The companies appearing on this page are able to offer scientific support to users of laboratory instrumentation. THE ANALYST will regularly publish specific Application Notes provided by their applications chemists.

EDITORIAL

JOURNAL OF ANALYTICAL ATOMIC SPECTROMETRY—JAAS

A Sister Journal for *The Analyst*

We are delighted to announce that the Publications and Information Board of the Royal Society of Chemistry has authorised the publication of a new primary journal in the field of analytical chemistry, the *Journal of Analytical Atomic Spectrometry* (*JAAS*). Publication is to commence at the beginning of 1986 and will be on a bimonthly basis. This will become the third Primary Journal published by the RSC in the analytical chemistry area and will join *The Analyst* and *Analytical Proceedings* as a responsibility of the Analytical Editorial Board, a sub-committee of the Society's Journals Committee. As with all RSC journals, *JAAS* will be organised by the full-time staff of the Society, and the Editor, Mrs. Judith Brew of the RSC office in London, will be delighted to receive enquiries from potential authors and subscribers.

The publication of a journal in a specific scientific discipline such as atomic spectrometry will be a new venture for the RSC, but the need for the establishment of a journal in this specific subject has received strong support from members of the Analytical Division Council, the Atomic Spectroscopy Group of the Analytical Division and the Executive Committee and Board of *Annual Reports on Analytical Atomic Spectroscopy* (*ARAAS*). A market survey carried out by the Society also revealed strong national and international support for the proposed concept of *JAAS*.

As indicated in the headline, *JAAS* is viewed very much as a sister journal to *The Analyst*, catering much more for the practical user of analytical instrumentation and procedures than the research worker. In addition, we hope it will serve as a vehicle to assist in the transfer of research ideas from the research laboratory into the routine analytical laboratory. Whilst papers will be accepted for publication on all aspects of analytical atomic spectrometry, including more fundamental studies, we anticipate that the emphasis will be on novel instrument developments and practical analytical applications of direct relevance to the working analyst. In addition to atomic-absorption, atomic-emission and atomic-fluorescence spectrometry, papers will be published on atomic mass spectrometry and X-ray fluorescence/emission spectrometry. The growing interest in the development and applications of hybrid techniques involving atomic spectrometry (*e.g.*, GC coupled AAS and HPLC - ICP) will also find a home in the pages of *JAAS*. Manuscripts on other general subjects of direct interest to atomic spectroscopists, including sample preparation, and dissolution and pre-concentration procedures, and also the statistical interpretation and use of atomic spectrometric data, will also be acceptable for publication in *JAAS*. The format of the journal will be similar to that of *The Analyst*, and will include full papers, short papers and letters and will provide a similar facility for the rapid publication of short communications. It is also intended to have a news section.

A special feature of *JAAS* will be created by the inclusion of the *Annual Reports on Analytical Atomic Spectroscopy* (*ARAAS*) now published by the RSC in book form. Volume 14 of *ARAAS*, which is currently in production and due to appear in late 1985, will be the last, and from the beginning of 1986 sections of *ARAAS* will be published in each issue of the new journal. We believe that this will make a particularly exciting and innovative contribution to the journal. This publishing format will allow much more rapid publication of the reviews, which will continue to be written in the distinctive style of *ARAAS*, with critical appraisal of new work presented at conferences in addition to the primary journal literature. Each bimonthly issue of *JAAS* will include approximately one sixth of the review articles now published in *ARAAS*, and a compilation of the abstract references from a two-month period. Each review will be based on the most recent literature consistent with the journal publication times, and will provide what we believe will be a unique current appreciation of developments in analytical atomic spectrometry.

No doubt some of our members and scientific colleagues will wonder if there is really a need for another new scientific journal in this specific topic area, and will be concerned with its possible effect on *The Analyst* and other journals. Judged by recent receipts of papers for *The Analyst*, and the increase in size and consequently the cost of this and many other journals, we believe that this is an entirely appropriate time and field for the launch of a journal of this concept. The greater volume of material to be published presents a choice between higher prices for present journals due to the increased cost of publication of increasing numbers of pages, or the creation of new journal titles. In our opinion, there is more than sufficient primary material to support a new journal in this field, and *JAAS* as conceived will co-exist happily and without undue competition with general analytical journals such as *The Analyst*, and owing to their different emphasis, with other existing specialist atomic spectroscopy journals. We believe, and our market survey supported the idea, that the practical use of atomic spectrometric instrumentation represented a homogeneous area of applied science, and that scientists in many laboratories would welcome a specific journal such as *JAAS*. The Royal Society of Chemistry has not traditionally published journals in specific topic areas, but has accepted the strongly supported case that this would be an important service to scientists working in this area. The price advantage, to member and non-member subscribers to the journal, of publication by a learned society such as the RSC should be appreciated by all. It must be emphasised that *The Analyst* will continue to welcome and carry papers in the field of atomic spectrometry, and the Analytical Editorial Board will ensure that *The Analyst* remains a journal that presents important developments in all branches of analytical science.

The combination of a primary journal with the *ARAAS* reviews, which is possible only within the RSC, provides the opportunity for establishing a unique publication that we anticipate will become essential reading to workers in this field. Our market survey indicated very positive enthusiasm on a world-wide basis for the concept of *JAAS*, and we look forward to the active support and collaboration of our many friends and colleagues in the analytical community to ensure the success of this important new publishing venture.

J. M. Ottaway
Chairman, Analytical Editorial Board

Determination of Strongly Curved Calibration Graphs in Flame Atomic-absorption Spectrometry: Comparison of Manually Drawn and Computer-calculated Graphs

Leo de Galan, Hans P. J. van Dalen and Guy R. Kornblum
Laboratorium voor Analytische Scheikunde, Technische Hogeschool Delft, Jaffalaan 9, 2628 BX Delft, The Netherlands

The non-linear portion of the calibration graphs in flame atomic-absorption spectrometry (AAS) offers more precise analytical results provided that the calibration graph can be determined reliably. Experimental data for three elements (Cu, Ni, Pb) and synthetic data were submitted to nine analysts and to three mathematical expressions (polynomial, cubic spline and rational function). The quality of hand-drawn and computer-calculated graphs was judged for goodness of fit and the accuracy of determining unknowns. It is concluded that results within 1% can be obtained in either instance, provided that 5–8 selected reference solutions are used and weighting factors are applied in the least-squares fit. With a simple criterion to guide the selection of the correct degree, the ordinary polynomial is the preferred expression to fit flame AAS data. With these recommendations it is possible to extend the calibration graph at least to the point where the slope is reduced to 25% of its value at the origin.

Keywords: *Calibration graphs; severe curvature; atomic-absorption spectrometry*

Relative methods of analysis, such as flame atomic-absorption spectrometry, require a calibration graph based on experimental data obtained from reference solutions. The determination of the calibration graph either by hand or by computer is hampered by two uncertainties: the scatter in the data points owing to measurement error (noise) and incorrect concentration values, and the ignorance about the true functional relationship between the signal and the concentration.

The preference for linear calibration graphs may well be explained by the fact that it avoids the second error source. If the calibration graph is known to be linear, it can be determined simply with a ruler or by fitting a first-degree polynomial. The remaining problem of data scatter has been treated in several publications that discuss the choice of the reference concentrations,[1] the repercussion of the confidence range,[2,3] the use of weighting factors[4,5] and the appropriate procedure when both the signal and the concentration are subject to random errors.[6] These discussions generally refer to linear calibration graphs.

Unfortunately, in AAS the initially linear relation according to Beer's law breaks down at higher absorbance owing to instrumental imperfections[7,8] and the influence of atomic spectral line profiles.[9] The onset and the degree of curvature vary from one element to another. In some instances (*e.g.*, Ni) a restriction to the linear part of the calibration graph would unacceptably reduce the dynamic range of the method. In practice, therefore, an extension into the non-linear part is often recommended, although standard methods[10] warn that the slope should not fall below 70% of the value in the origin. This limitation, however, seems rather arbitrary. Indeed, the linear part of the calibration graph may be the most accurately determined, but at least for AAS it does not offer the best precision. Studies by Roos[11–13], Ingle,[14] Bower and Ingle,[15–17] Steglich and Stahlberg[18,19] and ourselves[20] all agree that the region of lowest relative standard deviation in the concentration only starts at about 0.1 absorbance unit and extends well into the non-linear part of the calibration graph. The examples in Fig. 1 show clearly that the upper range of good precision extends to the point where the slope has decreased to 25% (Cu, Pb) or even 10% (Ni) of its value in the origin. However, this advantage can be exploited only if the calibration graph can be determined accurately over this range. Any error in the location of the curve will be translated into systematic errors in the consecutive analytical results.

Some confidence can be derived from the initial and hence somewhat limited studies by Marshall for AAS[21] and by Goode and Northington for flame emission spectrometry.[22] For calibration graphs extended to the point where the slope had fallen to half its original value good agreement was observed between the results from manually drawn and computer-calculated graphs.

Several current, computerised AAS instruments permit the use of non-linear calibration graphs using a variety of mathematical expressions. The variety stems partly from the lack of an exact theoretical description of the calibration graph in AAS. As Tyson[23] remarked, however, the accuracy of these approaches may sometimes be insufficient and cannot be adequately tested with current instrumentation.

This study is concerned with maintaining accuracy when severely non-linear calibration graphs are used. Despite the current emphasis on computerised data handling, manually drawn graphs were included in the study, not only to accommodate the less affluent reader, but also to obtain a frame of reference for the computer-calculated results.[23] Conclusions will be drawn concerning the number of reference solutions, the acceptable curvature, the need for weighting factors and the validity of the mathematical expressions. Although the conclusions apply strictly to flame AAS, from which the experimental data are drawn, they are expected to have a broader significance.

Experimental

Data Sets

Experimental absorbance values were measured for about 20 reference solutions of copper (324 nm), lead (283 nm) and nickel (232 nm) using a Perkin-Elmer Model 2280 atomic-absorption spectrometer, Varian Techtron single-element hollow-cathode lamps and a 10-cm air - acetylene flame. The instrument was operated under standard conditions and met the manufacturer's specifications.

Reference solutions extending from blank to over 10 times the linear range were prepared from 1000 μg ml^{-1} standards by ordinary volumetric dilution. The concentrations of the

reference solutions are all well above the detection limit and refer mostly to the region of highest precision. The final concentrations are taken as fixed, which means that any (small) error in the concentration translates into an absorbance error. Each absorbance was read 10 times with an integration time of 4 s. The average values and standard deviations were used to construct the calibration graphs and the precision plots in Fig. 1.

To obtain a better assessment of the accuracy of determining "unknowns" and the influence of noise, a synthetic data set ("Syn") was generated from a quadratic expression ($A = a_0 + a_1c + a_2c^2$). Noise with a relative standard deviation of up to 2% was added with a random number generator.

Procedure for Manually Drawn Graphs

Manually drawn calibration graphs were solicited from nine analysts in our laboratory (not including the authors) of varying experience. After preliminary tests showed that all used A4 formatted graphs, they were supplied with copies of DIN A4 graph paper on to which the calibration points were already entered. In addition, they received five numerical absorbance values. They were asked to return the graph paper with "their" calibration graph drawn in and concentration data derived for the five absorbances, which thus acted as answers to unknowns."

The number of data points supplied was successively increased according to a scheme exemplified for nickel in Table 1. Always included were the blank and the highest absorbance. The five "unknowns" were also maintained throughout. Results from the previous set were always collected (with no copies retained by the analysts) before the next set with a larger number of data points was handed out.

As a result of this exercise, we received from each analyst for Cu, Ni, Pb, Syn I (quadratic without noise) and Syn II (2% noise added) a total of 20 calibration graphs and 20 answers to each of the five unknowns. The total number of 900 concentrations values was reduced to 100 values for the average and standard deviation among the nine analysts.

Procedure for Computerised Curve Fitting

The same collection of data as handed out to the analysts was used for curve-fitting three mathematical expressions, as follows.

(a) A polynomial of degree one to four:

$$A = \sum_{k=0}^{n} a_k c^k \quad (1 \leqslant n \leqslant 4) \quad . . \quad . . \quad . . \quad (1)$$

Standard least-squares techniques were used and weighting factors of 1 (no weights) and $1/c^2$ were applied. The quality of the fit was judged by the quality coefficient proposed by Knegt and Stork[24]:

$$QC = 100 \left\{ \frac{\sum_{i}^{N} [1-(A_i/\hat{A}_i)]^2}{N-1} \right\}^{\frac{1}{2}} \quad . . \quad . . \quad (2)$$

where A_i is the absorbance entered for reference solution i and $\hat{A}_i$ is the value predicted for this concentration by the fitted curve. From the fitted curve the absorbance values of the "unknowns" were converted into concentrations by an iterative procedure.

Table 1. Experimental data and subsets for nickel

Concentration/ mg l^{-1}		Absorbance	Data used in subsets 5	7	10	19
0		0.000	×	×	×	×
0.5		0.020				×
1.0		0.041			×	×
2.5		0.094		×		×
3.75		0.137			×	×
5		0.182	×			×
7.5		0.255		×	×	×
10		0.338				×
15		0.470			×	×
20		0.587	×	×		×
25		0.683			×	×
30		0.760				×
40		0.868		×	×	×
50		0.951	×			×
60		1.009			×	×
70		1.059		×		×
80		1.108			×	×
90		1.148				×
100		1.183	×	×	×	×
Unknowns	C_1	0.405				
	C_2	0.824				
	C_3	0.963				
	C_4	1.107				
	C_5	1.168				

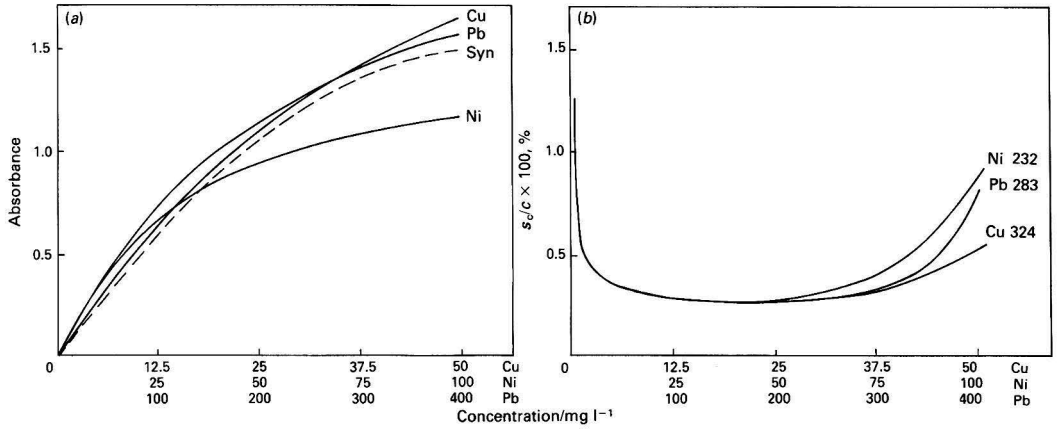


Fig. 1. (*a*) Experimental calibration graphs (Cu, Pb and Ni) and synthetic graph (Syn) showing the severe curvature of the extended concentration on range used. (*b*) Experimental Ringbom plots expressed as a coefficient of variation of the concentration, c, illustrating that optimum precision is obtained in the non-linear part of the calibration graphs

(b) A rational function as used in some commercial AAS instruments:

$$c = K_0 \left(\frac{K_1 A + K_3 A^2}{K_2 A - 1} \right) \quad \ldots \ldots (3)$$

where K_0 is only a reslope factor.[25]

Again least-squares fitting is used when the number of data points exceeds the number of parameters, as is always the case in this study. Strictly, fitting the error-free, independent parameter (the concentration) to the error-sensitive, dependent variable (the absorbance) is not permitted statistically. Equation (3) has the advantage, however, that unknown concentrations can be calculated directly from the measured absorbance (these calculations were performed for us by Dr. W. B. Barnett, Perkin-Elmer, Ridgefield, CT, USA).

(c) A cubic spline,[26] whereby the 5–20 reference data are subdivided into adjacent subsets that are separately covered by a third-degree polynomial. A smooth transition between consecutive polynomials is realised by imposing constraints on the first and second derivatives. Through an interactive procedure the subdivision of the reference data and the quality of the final graph are judged by the human observer on a video screen (these calculations were performed for us by Drs. J. H. H. G. van Willigen, Technische Hogeschool Twente, The Netherlands).

Results and Discussion

Manually Drawn Graphs

A preliminary inspection of the raw data received from the analysts revealed two phenomena. Firstly, and expectedly, the hand-drawn graphs become smoother and more reliable with increasing number of data points. However, some analysts, even with several years' experience, drew the graphs systematically above or below the centrally located data points. As a result, the data points did not scatter randomly around the graph as would be expected for a well drawn graph. These results were not excluded from further analysis.

Secondly, despite the fact that the conversion of the numerical absorbance values to "unknown" concentrations was generally carried out with great care, a few per cent. of the reported concentrations were subject to gross errors in scale reading. For example, a value of 48.1 p.p.m. was reported, whereas the pencil mark on the graph paper clearly pointed to a value of 38.1 p.p.m. Such gross errors were corrected before the data were subjected to statistical analysis.

To check the reproducibility of individual analysts, the synthetic data sets based on a pure quadratic (Syn I without noise and Syn II with 2% noise added) were handed out twice at an interval of several weeks. Because in this instance the "unknown" concentrations are known exactly, the returns could be checked for accuracy (systematic error) and precision (from the 10 duplicate values received from each analyst).

Contrary to our expectation, the precision and accuracy varied little with the experience of the analyst or with the number of data points used in drawing the graph (five being the minimum). However, addition of noise increased the difference between duplicates from less than 3% in the absence of noise to up to 6% in the presence of 2% noise.

At first sight we were surprised to learn that the lowest unknown concentration in the nearly linear part of the graph was subject to a *larger* random and systematic error (up to 7%) than the high concentrations in the strongly curved portion of the graph (1% for Syn I). Because the absorbance of the unknowns is fixed, this difference must reflect a poorer representation of the initial part of the calibration graph. On closer analysis, the most likely cause is the analysts' habit of drawing a single graph for the entire concentration range. As a result, reading the scale becomes inaccurate at low concentrations and the errors are enhanced, at least when they are reported on a relative scale. As will be seen below, a similar problem, although with a different origin, arises with computer-calculated graphs.

Because the above results revealed no significant differences between the nine analysts, their results could be pooled to yield average values and standard deviations for all 100 unknown concentrations. The standard deviation was used as a measure of the precision. The accuracy was judged by comparing the average with the true concentration (for Syn I and Syn II) or with the average derived from the calibration graph with about 20 data points (for Cu, Ni and Pb).

Fig. 2 shows the precision and the accuracy of the reported concentrations (increasing from C_1 to C_5) as a function of the number of reference solutions for two representative examples (Pb and Syn I). From this figure and similar data for Cu, Ni and Syn II the following conclusions can be drawn. As explained above, the lowest concentration (C_1) is frequently, though not always, less precise and accurate than the other four concentrations. Even for Ni the highest concentration can be determined within 1%, although the slope of the calibration graph has diminished to 10% of its value at the origin. The addition of 2% noise to the synthetic data set did not markedly affect the precision between analysts, but it did lower the accuracy of their average results. Obviously, a noise level of 2% is more typical for furnace AAS than for flame AAS.

The number of reference solutions should not be taken too small, but a very large number is also unnecessary. As is clear from Fig. 2, precision and accuracy of typically about 1% are obtained with 7–10 reference solutions. For Cu and Pb, where the final slope of the calibration graph is about 25% of the slope at the origin, 7 data points are sufficient. For Ni, where the slope decreases to 10% of its original value, 10 points may be recommended.

Based on these results, the following procedure is recommended for manually drawn calibration graphs.

1. Prepare eight reference solutions (preferably by weighing to avoid concentration errors) containing 0, 5, 10, 20, 40, 60, 80 and 100% of the expected useful concentration range.
2. Measure first the two highest reference solutions (80 and 100%) several times and use the standard deviations to calculate s_c/c and to verify the range of acceptable precision [compare Fig. 1(*b*)].
3. As long as the absorbance difference between the two highest concentrations (80 and 100%) is larger than that between the two lowest concentrations (0 and 5%), the final slope of the calibration graph is more than 25% of the initial slope, as recommended.
4. If the above tests are satisfactory, measure all eight solutions a few times against the pure solvent. Enter the average absorbance values on A4 graph paper and plot a five-times enlarged entry for the bottom 10% of the graph.
5. Draw the calibration graph carefully and check the result for random scatter of the data points around the graph. A quick impression of the smoothness can be obtained by looking at the graph at grazing incidence.
6. To avoid gross errors, double-check any concentration read for an unknown sample.

In a final test the analysts were supplied with eight reference data for Cu, Ni and Pb and eight new "unknowns" ranging from very low to maximum absorbance. They were requested to follow the above recommendations. In the results, gross reading errors could no longer be detected and the precision and accuracy of the reported concentrations roughly improved two-fold. Except for very low and very high concentrations, where errors up to 2% could be observed, all data were reliable to within 0.5–1% (precision and accuracy), which agrees with the Ringbom plot in Fig. 1(*b*).

Computer-calculated Graphs

The flexibility and computational ease offered by polynomials make them ideally suited to curve-fitting data with an

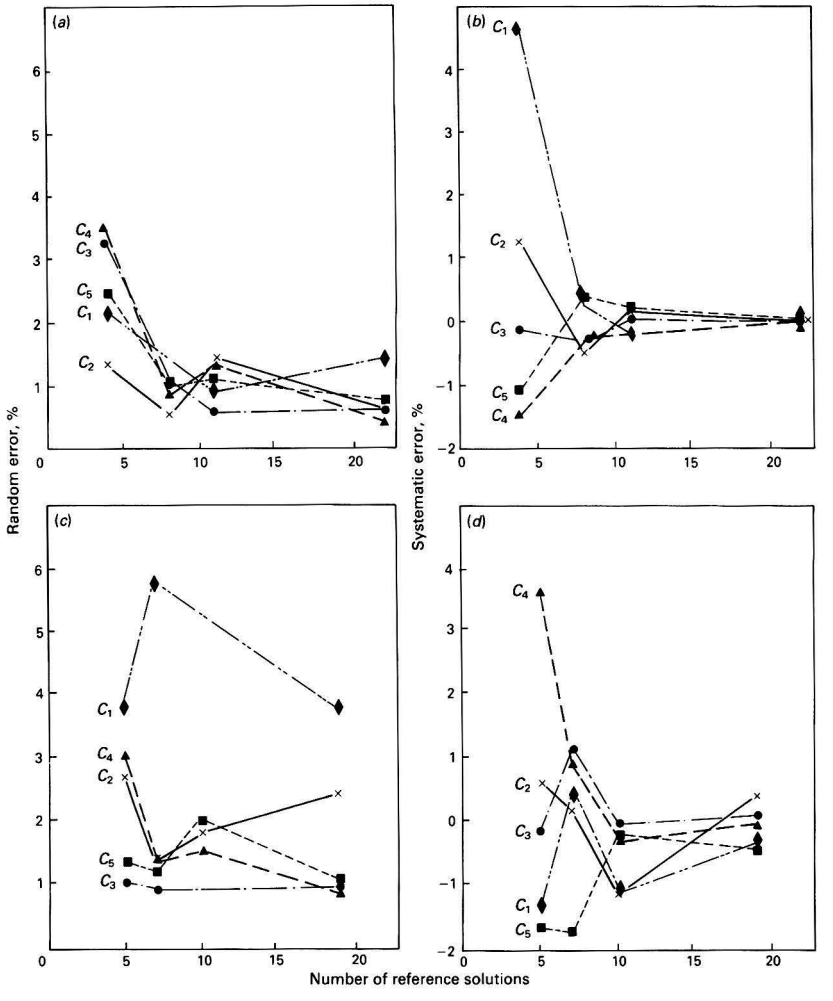


Fig. 2. Precision and accuracy of manually drawn calibration graphs for (*a*) and (*b*) Pb and (*c*) and (*d*) Syn I. The random error between analysts (*a*) and (*c*) and the systematic error (*b*) and (*d*) in reporting "unknown" concentrations are presented as a function of the number of reference solutions used in constructing the calibration graphs. Concentration values increasing from C_1 to C_5 correspond to about 80, 170, 260, 325 and 385 mg l^{-1} for Pb and 8, 23, 45, 77 and 103 mg l^{-1} for Syn I

unknown functional relationship as is the case in AAS. All three mathematical expressions explored in this study use polynomials in one form or another. Least-squares fitting is universily utilised. However, an important condition for the least-squares approach is the uniformity of the standard deviation over the range of data considered. As can be surmised from Fig. 1, this condition is not fulfilled in flame AAS. It is the *relative* standard deviation that is approximately constant over a major part of the calibration graph in AAS.[11–20]

The problem can be overcome by introducing weighting factors inversely proportional to the square of the standard deviation ($1/s_A^2$). Usually, this is cumbersome. In the present situation, where s_A is proportional to A, a similar improvement can be obtained by applying weighting factors equal to $1/A^2$ or even $1/c^2$. As the concentration of the reference solutions is precisely known beforehand, weighting factors equal to $1/c^2$ are most easily applied.

The improvement obtained by the use of weighting factors can be judged from Fig. 3. A second-degree polynomial was fitted to nine data points for Cu with and without weighting factors. The relative difference between the actual absorbance and the value read from the graph $[1-(A_i/\hat{A}_i)]$ is presented for all eight data points. Without weighting factors the fitting algorithm tends to make the absolute residuals $(A_i-\hat{A}_i)$ similar, so that invariably the lower concentrations show very large relative residuals. With appropriate weighting factors $(1/c^2)$ all relative residuals are seen to fall within 1%. Because a constant relative error is a property of the AAS technique [Fig. 1(*b*)], the advantage of weighting factors is clearly demonstrated in Fig. 3. A similar conclusion has been reported for linear calibration graphs by Bubert and Klockenkämper.[4]

Reference to equation (2) shows that the relative residuals also feature in the quality coefficient used in this study to judge how well the graph fits the data points. This criterion is preferred over lack of fit[27] or coefficient significance tests,[27, 28] because it conforms more closely to the practice of AAS analysis.

Because the quality coefficient expresses the average relative deviation of the data points from the fitted graph, we

might expect it to correspond to the noise inherent in the data. This is indeed observed, but only when weighting factors are applied. As an example, Fig. 4 presents quality coefficients for fitting various polynomials to a 20-point synthetic data set to which noise has been added. In the absence of noise the pure quadratic that underlies the synthetic data set (Syn I) is, of course, described error-free by a second-degree polynomial. With increasing noise, however, an unweighted second-degree polynomial gives rise to a more than proportionally increasing quality coefficient. This is due to the large relative deviations at low concentrations (Fig. 3). Better results are obtained when a higher degree polynomial is used, but in view of the purely quadratic basis of Syn I this is a doubtful procedure. However, when weighting factors are applied, the quality factor indeed increases proportionally with the added noise and a second-degree polynomial gives an equally good fit as higher degrees.

For the experimental data for Cu, Ni and Pb the correct degree of the polynomial is, of course, not known beforehand. Table 2 presents quality coefficients for polynomials of degree 1–4 fitted to data sets obtained from (roughly) 6, 10 and 20 reference solutions. Obviously, the linear (first-degree) calibration graph gives a very poor result in all instances. It is also clear that weighting factors significantly improve the quality coefficients. On the other hand, the quality coefficient increases (*i.e.*, becomes poorer) with an increasing number of data points. The reason lies in the location of the data points. When the data set is enlarged, more low concentrations are included (Table 1) that have a higher relative standard deviation [Fig. 1(*b*)]. Their contribution adversely affects the average relative deviation that is expressed by the quality coefficient.

The important conclusion to be drawn from Table 2 is that the quality coefficient permits a rapid decision of the lowest acceptable degree of the polynomial. Unduly high degrees may lead to oscillations and hence are to be avoided. From the results with the weighting factors applied it is clear that sufficiently low quality coefficients are obtained with a second-degree polynomial for copper and third-degree polynomials for lead and nickel. Polynomials of higher degree offer no significant improvement and only require more data points. In addition, comparison with Fig. 2 shows that polynomials of the stated degree yield quality coefficients similar to the precision reached with manually drawn calibration graphs. It therefore appears that straightforward polynomials describe the non-linear data in flame AAS very well, provided that weighting factors are applied.

When the same data are subjected to the rational function expressed by equation (3), a different picture emerges. Because the computer program used for these calculations did not accept a weighting factor of $1/c^2$, a factor of $1/c$ was used instead. Table 3 presents quality coefficients obtained by fitting the rational function to an increasing number of experimental data for Cu, Pb and Ni. In comparison with Table 2 several differences are noted.

The quality coefficient varies little with the number of data points used and the application of a weighting factor brings no improvement. This does not mean, however, that the fitted graph is unaffected by the weighting factor. Similar to Fig. 3, Fig. 5 presents relative deviations between actual lead concentrations and the fitted rational function. Without weighting factors the low concentrations again deviate appreciably, as expected. This result conforms to the findings reported by Barnett.[25] When a weighting factor is applied the situation at low concentration is improved, but simultaneously the deviations at high concentration increase appreciably. As a result, the average relative deviation (the quality coefficient) remains at practically the same high value. A similar conclusion applies to all data sets entered in Table 3. Indeed, judged from the quality coefficient values in Table 3, the fit of the rational function is significantly poorer than that of the polynomial of correct degree. It would seem, therefore, that the rational function, despite its simplicity, does not describe

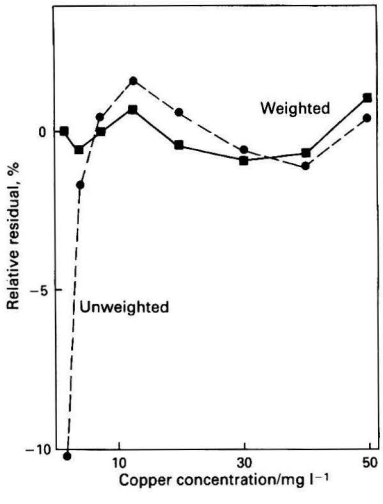


Fig. 3. Relative difference between the actual absorbance and the value read from least-squares calculated calibration graph. A second-degree polynomial was fitted to nine data points for copper with and without a weighting factor of $1/c^2$

Table 2. Quality coefficients [equation (2)] for polynomial fitting [equation (1)] of experimental flame AAS data

		Number of data points					
		Unweighted			Weighted ($1/c^2$)		
Element	Polynomial degree	6	10	20	6	10	20
Cu . .	1		43	60		15	15
	2	5	4	4	0.5	0.8	1.3
	3	4	2.5	3	0.5	0.6	1.3
	4	0.3	0.7	1.3	0.05	0.3	1.1
Pb	1		39	55		22	25
	2	14	13	25	4.9	5	4.5
	3	1	1.9	3.5	0.5	0.6	1.8
	4	1.5	1.0	11	0.5	0.4	1.7
Ni	1	48	57	60	31	35	35
	2	19	29	34	9.4	11	11.5
	3	1.5	3.3	5.7	1.1	1.3	1.9
	4	2.2	6.3	6.4	1.2	1.4	1.5

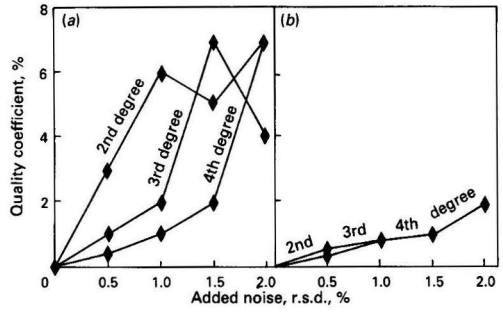


Fig. 4. Quality coefficient after equation (2) as a function of added noise. Polynomials of various degree are fitted to the 18-point synthetic data set (a pure quadratic) (*a*) without and (*b*) with a weighting factor of $1/c^2$

flame AAS data over an extended concentration range with optimum precision.

The interactive procedure used for the cubic spline did not include the calculation of quality coefficients. From visual observation it appears, however, that the experimental data approach the fitted graph to within the experimental error of about 1%. Because larger data sets are subdivided into smaller subsets of approximately constant standard deviation, weighting factors are not needed or beneficial.

The precision by which the graphs fit the data as expressed by the quality coefficient leaves the required number of data points undecided. Indeed, the data in Tables 2 and 3 only express that the relationship between any number of data points can be more or less precisely described by an appropriate mathematical expression.

The accuracy of the fitted graphs should be judged from the results obtained for the "unknowns." Because the synthetic data base was derived from a pure quadratic, it is not surprising that accurate results are found for all unknowns with either a second-degree polynomial or a cubic spline. Again, the rational function performed more poorly.

The accuracy obtained for unknowns of Cu, Pb and Ni was judged by comparing the results from computer-fitted graphs with the average values reported by the nine analysts on the basis of about 20 reference solutions (compare Fig. 2). Some representative examples are shown in Fig. 6.

The results for copper in Fig. 6(*a*) show that with straightforward polynomial fitting [equation (2)] an accuracy of about 1% could generally be achieved. In line with the preceding discussions weighting factors were used although they were actually not necessary for the moderate and high concentrations considered, but are imperative if low concentrations are to be determined with the same calibration graph.

Fig. 6(*b*) shows the results for high lead concentrations for the different mathematical expressions used. The cubic spline performed as well as the polynomial and, as remarked above, weighting factors are superfluous in this approach. In contrast, for the rational function [equation (3)] the application of a weighting factor is actually deleterious for high concentrations. However, for low concentrations weighting factors would again be mandatory.

The most striking observation from Fig. 6 and all similar data is the unnoticeable influence of the number of data points. It would appear that with appropriate precautions computer-calculated graphs yield accurate results with a surprisingly small number of reference solutions. This observation is in marked contrast to that for manually drawn graphs, where about eight reference solutions are needed and hence recommended for obtaining reliable data for sample solutions.

However, we express caution against generalisation of this

Table 3. Quality coefficients [equation (2)] for fitting a rational function [equation (3)]

Element	Data points	Unweighted	Weighted (1/*c*)	Polynomial* weighted (1/*c*²)
Cu	6	2.0	1.7	0.5
	10	2.2	1.9	0.8
	20†	4.8	7.6	1.3
Pb	6	3.8	2.9	0.5
	10	3.2	2.7	0.6
	20	3.3	2.5	1.8
Ni	6	4.2	3.4	1.1
	10	4.0	3.1	1.3
	20	3.2	2.7	1.9

* Data taken from Table 2 for second-degree (Cu) and third-degree (Pb, Ni) polynomials.

† When two extremely poorly fitted data are deleted the quality coefficient improves from 4.8 to 2.1 (for the unweighted) and 7.6 to 3.2 (for the weighted).

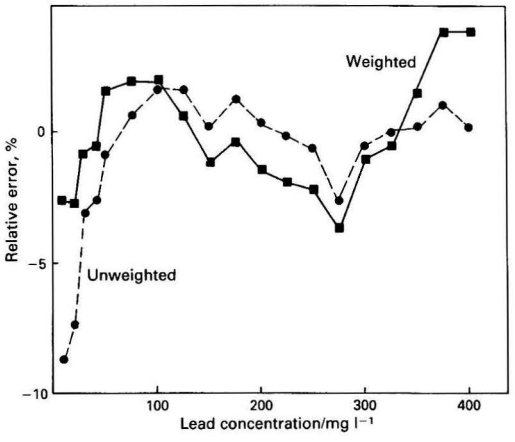


Fig. 5. Relative difference between the actual lead concentrations and the values read from the fitted rational function with and without a weighting factor 1/*c*

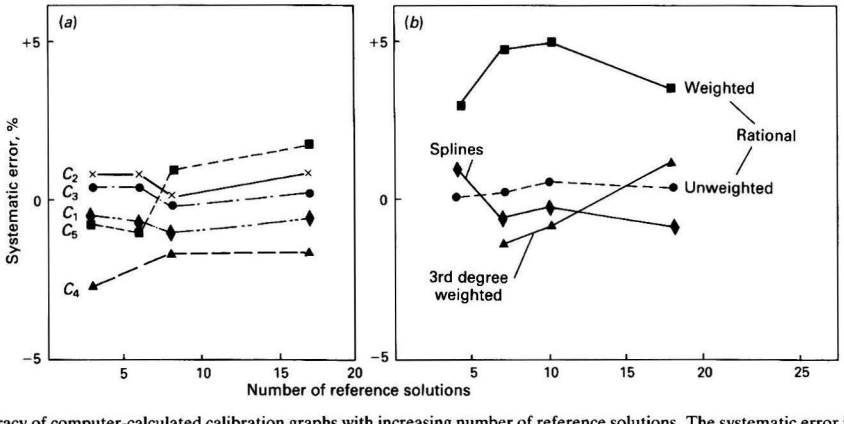


Fig. 6. Accuracy of computer-calculated calibration graphs with increasing number of reference solutions. The systematic error is expressed as the difference between the average value reported by the nine analysts (using 20 reference solutions) and the results obtained from graphs fitted to an increasing number of reference solutions. (*a*) Weighted second-degree polynomial for copper; C_1–C_5 correspond to about 6, 15, 25, 29 and 49 mg l^{-1}. (*b*) Various mathematical expressions for lead; results refer to a high lead concentration of about 385 mg l^{-1}

conclusion, which is based on only three elements and moderate to high "unknown" concentrations. If non-linear calibration graphs are to be used over their full range, a minimum number of five reference solutions is recommended for mathematical fitting procedures.

Conclusions

The main conclusion drawn from this study is that at least in flame AAS severely curved calibration graphs can be used safely provided that a few precautions are taken. Manually drawn graphs should be based on eight reference solutions well distributed over the concentration range considered. Equally good results can be obtained with least-squares fitted mathematical expressions provided appropriate weighting factors are used to accommodate the low concentration range. The number of reference solutions might then be reduced to a minimum of five.

Of the three mathematical expressions tested, the rational function that fits the concentration to the absorbance is the simplest, but yields inferior results. The cubic spline is reliable and very flexible but prone to oscillations. These are easily corrected in the presently applied interactive procedure, but require further constraints in a completely automated version.

There is, however, no reason to reject the straightforward polynomial. Indeed, polynomials of moderate degree were found to describe calibration graphs in flame AAS very well, even for nickel where the slope was allowed to decrease to 10% of its value at the origin. A suitable weighting factor ($1/A^2$ or $1/c^2$) is easily incorporated and the quality coefficient provides a simple expedient to decide the appropriate degree. This study has shown that in connection with a previous study[11] in flame AAS precise and accurate analytical results can be obtained within one calibration graph over a dynamic range of two decades.

This paper owes much to the calculations of Dr. W. B. Barnett (Perkin-Elmer, Ridgefield, CT, USA) on the rational functions and Drs. J. H. H. G. van Willigen (Technische Hogeschool Twente, The Netherlands) on the cubic splines. We stress that the conclusions drawn from their data are entirely our responsibility. We gratefully acknowledge the diligence and patience of the analysts in our laboratory in drawing numerous calibration graphs.

References

1. Agterdenbos, J., *Anal. Chim. Acta*, 1979, **108**, 315.
2. Agterdenbos, J., Maessen, F. J. M. J., and Balke, J., *Anal. Chim. Acta*, 1981, **132**, 127.
3. Mitchell, D. G., and Garden, J. S., *Talanta*, 1982, **29**, 921.
4. Bubert, H., and Klockenkämper, R., *Spectrochim. Acta, Part B*, 1983, **38**, 1087.
5. Christian, S. D., and Tucker, E. E., *Int. Lab.*, 1984, **14**, 10.
6. Thompson, M., *Analyst*, 1982, **107**, 1169.
7. de Galan, L., and Samaey, G. F., *Spectrochim. Acta, Part B*, 1969, **24**, 679.
8. van Gelder, Z., *Spectrochim. Acta, Part B*, 1970, **25**, 669.
9. Wagenaar, H. C., Novotny, I., and de Galan, L., *Spectrochim. Acta, Part B*, 1974, **29**, 301.
10. ISO 4940 and 4943; and Euronorms 136, 177 and 181.
11. Roos, J. T. H., *Spectrochim. Acta, Part B*, 1969, **24**, 255.
12. Roos, J. T. H., *Spectrochim. Acta, Part B*, 1970, **25**, 539.
13. Roos, J. T. H., *Spectrochim. Acta, Part B*, 1973, **28**, 407.
14. Ingle, J. D., Jr., *Anal. Chem.*, 1974, **46**, 2161.
15. Bower, N. W., and Ingle, J. D., Jr., *Anal. Chem.*, 1976, **48**, 686.
16. Bower, N. W., and Ingle, J. D., Jr., *Anal. Chem.*, 1977, **49**, 574.
17. Bower, N. W., and Ingle, J. D., Jr., *Anal. Chem.*, 1979, **51**. 72.
18. Steglich, F., and Stahlberg, R., *Fresenius Z. Anal. Chem.*, 1983, **314**, 402.
19. Steglich, F., and Stahlberg, R., *Fresenius Z. Anal. Chem.*, 1983, **315**, 321.
20. van Dalen, H. P. J., and de Galan, L., *Analyst*, 1981, **106**, 695.
21. Marshall, J. C., *At. Absorpt. Newsl.*, 1969, **8**, 85.
22. Goode, S. R., and Northington, J. W., *Appl. Spectrosc.*, 1979, **33**, 12.
23. Tyson, J. F., *Analyst*, 1984, **109**, 313.
24. Knegt, J., and Stork, G., *Fresenius Z. Anal. Chem.*, 1974, **270**, 97.
25. Barnett, W. B., *Spectrochim. Acta, Part B*, 1984, **39**, 829.
26. Boor, C. de, "A Practical Guide to Splines," Springer Verlag, New York, 1978.
27. Draper, N. R., and Smith, H., "Applied Regression Analysis," Second Edition, Wiley, New York, 1981.
28. Liteanu, C., and Rica, I., "Statistical Theory and Methodology of Trace Analysis," Ellis Horwood, Chichester, 1980.

Paper A4/314
Received September 6th, 1984
Accepted November 29th, 1984

Rapid Sequential Determination of Chromium(III) - Chromium(VI) by Flow Injection Analysis - Inductively Coupled Plasma Atomic-emission Spectrometry

Alan G. Cox, Ian G. Cook and Cameron W. McLeod*
Department of Chemistry, Sheffield City Polytechnic, Sheffield S1 1WB, UK

A rapid and sensitive method for the sequential determination of Cr(III) - Cr(VI) based on flow injection analysis - inductively coupled plasma atomic-emission spectrometry (FIA - ICP-AES) has been developed. A micro-column of activated alumina was used in the FIA manifold to separate and pre-concentrate Cr(VI) from Cr(III) in synthetic aqueous solutions before ICP detection at 267.72 nm. Linear calibration for Cr(III) and Cr(VI) was established over the concentration range 0–1000 μg l^{-1}. The relative standard deviations at the 10 μg l^{-1} level for a 2-ml sample injection were 2.2% for Cr(III) and 1.1% for Cr(VI) and the corresponding limits of detection were 1.4 and 0.20 μg l^{-1}, respectively. Determinations of Cr(III) and Cr(VI) at the μg l^{-1} level in reference waters of the National Bureau of Standards and the British Geological Survey were demonstrated.

Keywords: *Cr(III) - Cr(VI) speciation; flow injection analysis; inductively coupled plasma; atomic-emission spectrometry*

There is an ever increasing demand by environmental scientists for rapid analytical procedures that can determine the chemical forms of essential and toxic elements in biological and environmental samples. Few analytical techniques are capable of direct differentiation of the chemical forms of elements and, as a result, preliminary species separation is normally carried out before detection by sensitive elemental analysis techniques.[1] The combination of high-performance liquid chromatography or gas - liquid chromatography with atomic spectrometric detection is an important and powerful speciation tool[2] and numerous applications were reported in a review by Fernandez.[3] Speciation of inorganic chromium has been studied by various groups and novel combined procedures based on preliminary electrochemical separation,[4] solvent extraction,[5–7] solvent extraction and high-performance liquid chromatography,[8] ion exchange [9–13] and chelating resins [14–16] have been developed for the differential determination of Cr(III) - Cr(VI). Although many of the above schemes are highly selective and sensitive, the procedures are time consuming and prolonged sample manipulation may disturb the natural chromium speciation state.[1] More recently, the potential of flow injection analysis (FIA) for rapid inorganic speciation has been highlighted[17,18] and novel FIA procedures with spectrophotometric detection have been developed for the determination of Cr(VI).[17,19,20]

In this study, an FIA manifold incorporating a micro-column of activated alumina was combined with inductively coupled plasma atomic-emission spectrometry (ICP-AES) for the rapid sequential determination of Cr(III) and Cr(VI) in synthetic aqueous solutions and reference water samples. The FIA - ICP-AES procedure exploited the fact that activated alumina (acid form) has a high affinity for anionic Cr(VI) species, in contrast to that for Cr(III) (cationic or neutral), and this enabled time-resolved emission for the two chromium oxidation states to be monitored after injection of Cr(III) - Cr(VI) solutions. Development of this rapid speciation procedure was based on recent FIA - ICP-AES experiments, which demonstrated that activated alumina (acid form) has a high affinity for oxyanion species, including arsenate, molybdate, phosphate and vanadate. In particular, the high affinity of activated alumina for phosphate anions enabled a rapid, interference-free method to be developed for the determination of phosphorus in simple steels.[21]

* To whom correspondence should be addressed.

Experimental

Reagents and Materials

Chromium(III) and Cr(VI) stock solutions (1000 μg ml^{-1}) were prepared from chromium(III) nitrate and potassium dichromate (BDH Chemicals, AnalaR grade), respectively. Standard solutions of Cr(III) and Cr(VI) (single or mixed), prepared daily by appropriate dilution of stock solutions, were acidified using nitric acid (BDH Chemicals, Aristar grade, final acid strength *ca.* 0.01 M). Stock hydroxide solutions (2 M) were prepared from potassium hydroxide and ammonia solution (BDH Chemicals, Aristar). High-purity water was used throughout and stock and standard solutions were stored in pre-cleaned polypropylene (Nalgene) containers. Activated alumina (BDH Chemicals, Brockman Grade I, acidic form, particle size range 75–120 μm) was used for column packing.

Instrumentation and Apparatus

The ICP spectrometer (Jarrell-Ash ICAP 9000) utilised the Cr 267.72-nm line. Typical plasma operating conditions were as follows: forward power, 1.1 kW; observation height, 15 mm; coolant, argon, 20 l min^{-1}; nebuliser, argon, 1 l min^{-1}. An Apple II microcomputer was used to control data acquisition and emission - time peak profiles and signal integration were accomplished using standard software. The FIA manifold, described elsewhere,[21] consisted of a peristaltic pump, a rotary injection valve, a micro-column of activated alumina (2.5 cm, i.d. 1.5 mm) and a cross-flow nebuliser. In operation, a Cr(III) - Cr(VI) standard solution (200 μl–2 ml) was loaded into the injection valve by syringe and then injected into the carrier stream (0.01 M HNO_3, flow-rate 1 ml min^{-1}). Cr(VI) was deposited on the column whereas Cr(III) was rapidly carried to the ICP. Next, ammonia solution (200 μl, 1 M) was injected to elute Cr(VI) from the column. The recovery of Cr(VI) was checked either by calculating the ratio of peak areas of Cr(VI) to Cr(III) for 200-μl injections of Cr(III) - Cr(VI) solutions (*e.g.*, 1.0 μg ml^{-1}) or by calculating the ratio of peak areas for a 200-μl injection of Cr(VI) solution (*e.g.*, 1.0 μg ml^{-1}) with and without the alumina column in the FIA manifold.

Results and Discussion

Method Development

A typical emission - time response at 267.72 nm for a 200-μl injection of 0.1 μg ml^{-1} Cr(III) - Cr(VI) solution followed by injection of ammonia solution (200 μl, 1 M) is given in Fig. 1 to illustrate the nature of the FIA - ICP-AES experiment. Time resolution of the Cr(III) and Cr(VI) emission signals was dependent on achieving efficient deposition and elution of Cr(VI) from the activated alumina column and on ensuring that Cr(III) did not deposit. The experimental conditions employed differed from those adopted for the phosphate system.[21] An acid carrier stream (0.01 M HNO_3) was used in this work instead of distilled water to maintain the column acidic at all times. Under such conditions Cr(III) did not deposit and Cr(VI) deposition was quantitative and independent of the sample acidity. Carrier streams of high acid strength (up to 0.8 M HNO_3) were equally effective but owing to the possibility of column deterioration with prolonged operation their use was discontinued. Ammonia solution and potassium hydroxide solution of concentration >1 M were equally effective in stripping Cr(VI) from the alumina column. This finding was in contrast to that for the phosphate system where ammonia solution was an inefficient eluent.[21] Simultaneous monitoring of the Al channel (308.22 mm) during elution of Cr(VI) indicated that Al species were stripped from the column on injection of both potassium hydroxide and ammonia solution. The Al concentrations were approximately 30-fold greater with potassium hydroxide (60 *vs.* 2 μg ml^{-1}), reflecting significant chemical attack of the alumina column by this reagent. Ammonia solution (200 μl, 1 M) was used in all further work to minimise possible changes in column performance over extended periods of time. Recovery experiments (as specified under Experimental) indicated that 86% (relative standard deviation = 2% for ten injections selected at random over a 3-week period) of the Cr(VI) deposited was eluted with a single injection of ammonia solution (200 μl, 1 M). A further two injections of ammonia solution (200 μl, 1 M) ensured complete removal of Cr(VI) before a new sample was injected.

Analytical Performance

Calibration graphs for Cr(III) and Cr(VI) prepared from 200-μl injections of mixed standard solution (10, 50, 100, 500 and 1000 μg l^{-1}) gave good linearity with correlation coefficients of 0.99998 and 0.99997, respectively. At the 10 μg l^{-1} level the relative standard deviations (ten injections) were 10% for Cr(III) and 12% for Cr(VI), and this indicated that the precision was not significantly impaired by Cr(VI) deposition and elution. Limits of detection, calculated as twice the standard deviation of the background noise level, were 1.0 μg l^{-1} for Cr(III) and 1.4 μg l^{-1} for Cr(VI). Improved detection performance for Cr(VI) was obtained by increasing the sample injection volume. This is illustrated in Fig. 2 where emission - time profiles are given for 200 μl, 1-ml and 2-ml injections of 20 μg l^{-1} Cr(VI) (in each instance 200 μl of 1 M ammonia solution was used for elution). The pre-concentration capability and reliability of the FIA procedure is emphasised by the calibration graphs for Cr(VI) for the concentration range 5–50 μg l^{-1} as given in Fig. 3. For comparison purposes the corresponding data for Cr(III) (not amenable to pre-concentration) are presented. The 1- and 2-ml sample injections both gave rise to a steady-state signal that was equivalent to that for conventional nebulisation and therefore improved sensitivity at the larger injection volume was not realised. The 200-μl sample injection, in not attaining the steady-state level, gave a reduced sensitivity, in agreement with an earlier FIA - ICP-AES study.[22] The relative standard deviations (ten injections) at the 10 μg l^{-1} level for a 2-ml sample injection were 2.2% for Cr(III) and 1.1% for Cr(VI) and the corresponding limits of detection were 1.4 and 0.20 μg l^{-1}, respectively.

Determination of Cr(III) and Cr(VI) in Reference Waters

The FIA - ICP-AES procedure was applied to the determination of Cr(III) and Cr(VI) in NBS Trace Elements in Water (CRM 1643a) and a reference water (C2) of the British Geological Survey.[23] Only a total Cr concentration (17 ± 2 ng g^{-1}) is specified for the NBS sample and a certified value is not available for the BGS reference water. Analysis based on a 200-μl sample injection revealed that for both samples the predominant oxidation state was Cr(III) and that pre-concentration was required to detect Cr(VI). The emission - time responses for a 0.1 μg ml^{-1} Cr(III) - Cr(VI) standard solution and the NBS reference water are given in Fig. 4. Analytical data for both samples based on 200-μl and 2-ml sample injections are presented in Table 1.

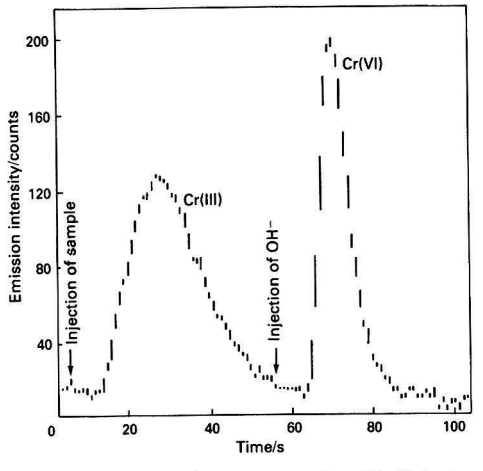


Fig. 1. Emission (267.72 nm) - time response for a 200 μl injection of Cr(III) - Cr(VI) solution (0.1 μg ml^{-1}) and a 200-μl injection of 1 M ammonia solution

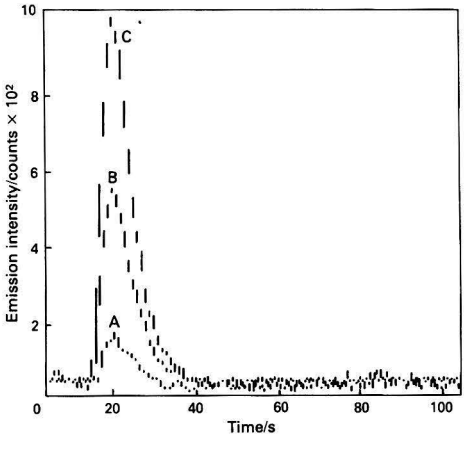


Fig. 2. Emission (267.72 nm) - time response for elution (200 μl of 1 M ammonia solution) of Cr(VI) for various injection volumes of Cr(IV) solution: A, 200 μl; B, 1 ml; and C, 2 ml

Table 1. Determination of Cr(III) and Cr(VI) in reference waters. Number of injections for each measurement, 7; uncertainty limits, ±2σ; integration time, 30 s

	Concentration/μg l⁻¹			
	NBS 1643a*		BGS C2	
Sample injection volume	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
200 μl	15.0 ± 2.1	Not detected†	13.5 ± 1.8	Not detected
2 ml	14.8 ± 1.0	1.96 ± 0.32	13.5 ± 1.4	0.51 ± 0.22

* Certified value, 17 ± 2 ng g⁻¹ total Cr.
† Limit of detection, 1.4 μg l⁻¹.

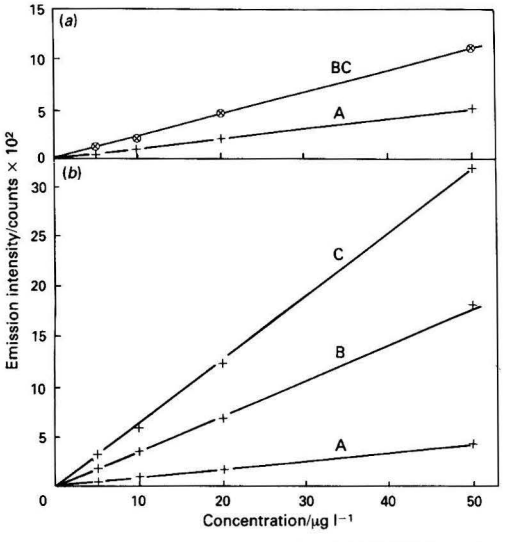


Fig. 3. Calibration graphs for (*a*) Cr(III) and (*b*) Cr(VI) for various injection volumes of Cr(III) - Cr(VI) solution: A, 200 μl; B, 1 ml; and C, 2 ml. Elution, 200 μl of 1 M ammonia solution; number of injections for each measurement, 3; integration time, 30 s

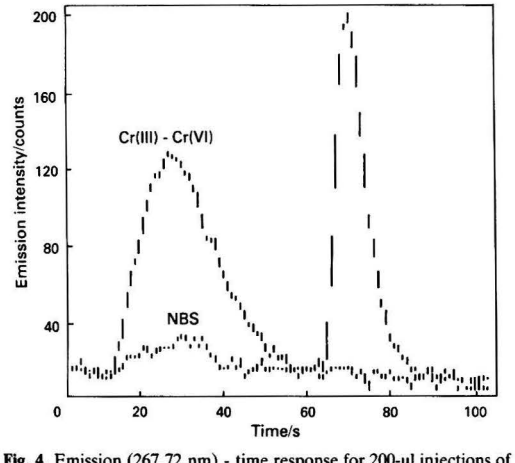


Fig. 4. Emission (267.72 nm) - time response for 200-μl injections of Cr(III) - Cr(VI) solution (0.1 μg ml⁻¹) and NBS 1643a. Elution, 200 μl of 1 M ammonia solution

Conclusion

An FIA manifold incorporating a micro-column of activated alumina has been combined with ICP-AES and used successfully for rapid sequential determinations of Cr(III) and Cr(VI) in synthetic aqueous solutions and reference waters. Attractive features of this novel method include the rapid and quantitative separation of Cr(III) - Cr(VI), a pre-concentration capability for Cr(VI) and long-term reliability of the alumina micro-column under conditions of widely different pH (*e.g.*, a single column served for the duration of this 4-month study). The method is judged to have considerable analytical potential in those fields requiring a knowledge of Cr(III) - Cr(VI) concentrations and for ultra-trace determinations of chromium. Application of the technique to the determination of Cr in natural waters and biological fluids is in progress.

References

1. Florence, T. M., and Batley, G. E., *CRC Crit. Rev. Anal. Chem.*, 1980, August, 219.
2. Van Loon, J. C., *Anal. Chem.*, 1979, **51**, 1139A.
3. Fernandez, F. J., *At. Absorpt. Newsl.*, 1977, **16**, 33.
4. Batley, G. E., and Matousek, J. P., *Anal. Chem.*, 1980, **52**, 1570.
5. de Jong, G. J., and Brinkman, U.A.Th., *Anal. Chim. Acta*, 1978, **98**, 243.
6. Rao, V. M., and Sastri, M. N., *Talanta*, 1980, **27**, 771.
7. Whiteley, R. V., and Merrill, R. M., *Fresenius Z. Anal. Chem.*, 1982, **311**, 7.
8. Schwedt, G., *Fresenius Z. Anal. Chem.*, 1979, **295**, 382.
9. Pankow, J. F., and Janauer, G. E., *Anal. Chim. Acta*, 1974, **69**, 97.
10. Pankow, J. F., Leta, D. P., Lin, J. W., Ohl, S. E., Shum, W. P., and Janauer, G. E., *Sci. Total Environ.*, 1977, **7**, 17.
11. Cresser, M. S., and Hargitt, R., *Anal. Chim. Acta*, 1976, **81**, 196.
12. Mazzucoletti, A., Minoia, C., Pozzoli, L., and Arvati, L., *At. Spectrosc.*, 1983, **4**, 182.
13. Battistoni, P., Bompadre, S., Fava, G., and Cobbi, G., *Talanta*, 1983, **30**, 15.
14. Leyden, D. E., Channell, R. E., and Blount, C. W., *Anal. Chem.*, 1972, **44**, 607.
15. Miyazaki, A., and Barnes, R. M., *Anal. Chem.*, 1981, **53**, 364.
16. Isozaki, A., Kumazai, K., and Utsumi, S., *Anal. Chim. Acta*, 1983, **153**, 5.
17. Lynch, T. P., Kernoghan, N. J., and Wilson, J. N., *Analyst*, 1984, **109**, 839.
18. Pacey, G. E., and Bubins, B. P., *Int. Lab.*, 1984, September, 26.
19. Jørgensen, S. S., and Regitano, M. A. B., *Analyst*, 1980, **105**, 292.
20. Yamane, T., and Mottola, H. A., *Anal. Chim. Acta*, 1983, **146**, 181.
21. McLeod, C. W., Cook, I. G., Worsfold, P. J., Davies, J. E., and Queay, J., *Spectrochim. Acta, Part B*, 1985, **40**, 57.
22. McLeod, C. W., Worsfold, P. J., and Cox, A. G., *Analyst*, 1984, **109**, 327.
23. Miles, D. L., and Cook, J. M., *in* Egan, H., and West, T. S., *Editors*, "IUPAC Collaborative Interlaboratory Studies in Chemical Analysis,' Pergamon Press, Oxford, 1982, pp. 143–149.

Paper A4/358
Received October 15th, 1984
December 4th, 1984

Continuous Flow Molecular Emission Cavity Analysis of Sulphite and Sulphur Dioxide

Nikos Grekas and Antony C. Calokerinos*
Laboratory of Analytical Chemistry, University of Athens, 106 80 Athens, Greece

A continuous flow method for the determination of sulphite (1–50 μg ml^{-1}) by molecular emission cavity analysis is described. The sample is mixed with an excess of orthophosphoric acid in a flow system and the sulphur dioxide evolved is continuously transferred into the cavity for generation of the S_2 molecular emission. The analysis is completely automated, requires no sample pre-treatment and samples can be analysed at a rate of 24 per hour with a relative error of 1–2%. Various anions and cations interfere and attempts have been made to eliminate their effect on the final measurement. The results obtained by the proposed procedure compare well with the expected values when the method was applied to soft drinks, wines and air samples.

Keywords: *Molecular emission cavity analysis; continuous flow system; sulphite determination*

Sulphur dioxide is a major air pollutant, which arises mainly from the combustion of fossil fuels and various other human activities, such as petroleum refining and non-ferrous smelting.[1] Sulphite is used in food products and beverages to prevent oxidation and bacterial growth. Numerous selective and sensitive methods have been developed for monitoring both forms of sulphur(IV) in various samples. The West - Gaeke method is most commonly used for sampling and measurement of sulphur dioxide in air.[2,3] The disproportionation of mercury(I) to mercury(0) and mercury(II) promoted by the complexation reaction of sulphite with mercury(II) has been proposed for the determination of low concentrations of sulphur dioxide and sulphite,[4] either by flow injection analysis with potentiometric measurement[5] or by a mercury vapour detector.[6]

Conventional molecular emission cavity analysis (MECA) has been proposed for the determination of sulphur dioxide in air.[7] Sulphur dioxide is absorbed on silica gel, which is then transferred quantitatively into a specially designed cavity. Sulphite can be determined successfully in mixtures with other sulphur ions either in aqueous solutions[8] or in solids.[9–11] Reduction of the S_2 emission due to matrix interferences within the cavity can be eliminated by using the vaporisation system for generation of sulphur dioxide after acidification of the sulphite sample and purging of the pure gas into the cavity.[12,13] Nevertheless, the procedure is manual and time consuming. In this paper, the use of the cavity as a detector for sulphur dioxide generated from a segmented continuous flow system is investigated. Results show that the continuous flow molecular emission cavity analyser (CF-MECA) developed can be successfully used for the determination of sulphite and of sulphur dioxide after fixation as disulphitomercurate(II).

Experimental

Apparatus

The MECA detector previously described[14] was used. Damping was provided by inserting an RC circuit between the photometric readout and the recorder. The continuous flow system consisted of a Technicon Proportioning Pump III and a sampler with 40 samples capacity (A 40 Sampler II, Hook and Tucker Instruments Ltd.) (Fig. 1).

* To whom correspondence should be addressed.

Reagents

All solutions were prepared from analytical-reagent grade materials using de-ionised distilled water.

Alkaline solution, 1 M *NaOH* - 0.1 M *EDTA.* Dissolve 40 g of sodium hydroxide and 37 g of EDTA (Titriplex III, Merck) in water and dilute to 1 l.

Stock sulphite solution, 1000 μg ml^{-1}. Dissolve 1.574 g of sodium sulphite, transfer into a calibrated flask together with 10.00 ml of the alkaline solution and dilute to 1 l. The solution was stable for one week. More dilute solutions were prepared daily by the least number of dilution steps possible.

Orthophosphoric acid, 3 M.

Procedure

Initiate the instrument set under the optimised conditions, shown in Fig. 1 and Table 1, but keep the sampling needle always in the "wash" position. Ignite the flame and establish the base line on the recorder. Allow 0.5–1 ml of stock sulphite solution to enter into the system and generate intense S_2 emission within the cavity. After re-establishment of the base line, activate the sampler and the analysis proceeds automatically. Construct the calibration graph, emission intensity [I (mV)] *versus* μg ml^{-1} of sulphite (C), or preferably, the log I - log C graph[14] and determine the sulphite content of the samples. Include a control standard solution for every 12 samples.

Table 1. Experimental parameters for CF-MECA analysis of sulphite. Other parameters as in Fig. 1

Parameter	Description
Cavity	Design *b*, Fig. 3
Cavity position	Flame centre, 18 mm above burner head
Cooling water flow	130 ml min^{-1}
Hydrogen flow-rate	1.0 l min^{-1}
Nitrogen flow-rate	2.1 l min^{-1}
Photomultiplier voltage	900 V
Wavelength	384 nm
Slit width	2 mm (4 nm spectral band width)
Orthophosphoric acid	3 M
Temperature of mixing coil	50 °C
Nitrogen carrier gas flow-rate	100 ml min^{-1}
Sample time	75 s
Wash time	75 s

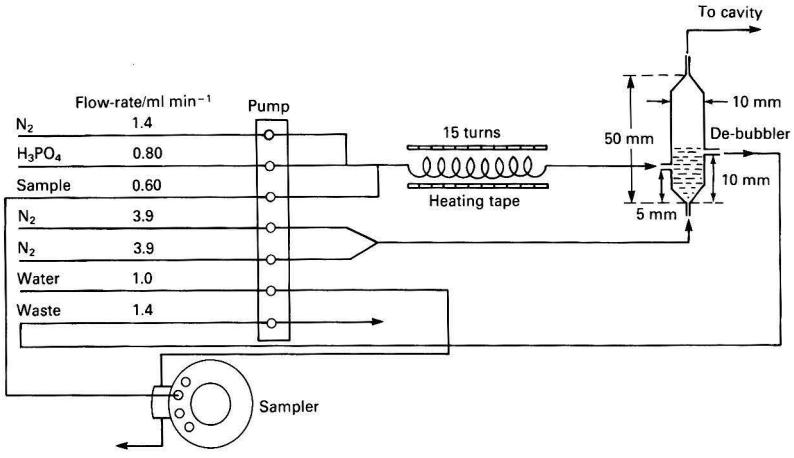


Fig. 1. Schematic diagram of the continuous flow molecular emission cavity analyser (not to scale)

Determination of sulphur dioxide in air

Transfer 40 ml of 0.04 M tetrachloromercurate(II) (TCM) solution into one of the Drechsel flasks of the air-sampling apparatus (AGL, Hitchin, Hertfordshire) and pump air for 24 h from a minor road of the city centre at 89.6 l h^{-1}. Take all special precautions required to remove trace amounts of hydrogen sulphide and solid particulate matter prior to introduction of air into the TCM solution. Restore any losses of solution due to evaporation by pure TCM solution after termination of sampling. Dilute 12.00 ml of the final solution and 2.00 ml of 0.6% *m/V* sulphamic acid with water to 25.00 ml and determine sulphite by CF-MECA.

Results and Discussion

Optimisation of Instrumental Parameters

The manifold tube sizes were selected by trial and error until optimum sensitivity was attained. The sample and wash timers of the sampler were set to 75 s to give sharp and smooth peaks, thus allowing measurement of the sulphite content of 24 solutions per hour. The wash time was not long enough to allow establishment of the base line between consecutive peaks but peak heights were measured over the initially established base line, which was found to be stable over long periods of operation.

When ordinary de-bubblers, used by other workers[15] for sweeping hydrides into an electrothermal atomiser, were tried, trace amounts of water were carried into the duct leading to the cavity and broad irregular peaks were obtained. When water was eventually pushed into the cavity, intense sodium emissions occurred. A specially designed de-bubbler (Fig. 1) was therefore used, which eliminated completely the carryover of water into the cavity. The purging rate of sulphur dioxide from the reaction mixture was increased by introducing nitrogen gas from the bottom of the de-bubbler. Nitrogen gas was also introduced into the continuous flow system, instead of air, as oxygen reduces the S_2 emission.[14] Nitrogen was supplied to the manifold through a Drechsel flask, serving as a pressure relief device. The S_2 emission intensity was independent of nitrogen supplied to the manifold at flow-rates in the range 100–300 ml min^{-1}. At flow-rates <100 ml min^{-1}, the content of the de-bubbler was sucked into the lower nitrogen inlet, while at flow-rates >300 ml min^{-1} the pressure of the system increased severely and retention times of sulphur dioxide within the cavity were reduced with subsequent reduction of the emission intensity.

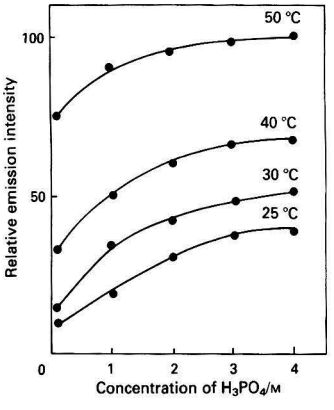


Fig. 2. Effect of the concentration of orthophosphoric acid and the temperature of the mixing coil on the emission intensity from 50 μg ml^{-1} of sulphite

The amount of sulphur dioxide evolved from a given sulphite solution depends on the molarity of orthophosphoric acid introduced into the manifold, as shown in Fig. 2. Further, by maintaining the temperature of the mixing coil above ambient with a heating tape, increased S_2 emissions and sharper peaks were obtained (Fig. 2). This was due to the reduction of the viscosity of the reaction mixture and the increase of the evolution rate of sulphur dioxide. If the temperature of the solution inside the mixing coil is above 50 °C, then water vapour forms inside the de-bubbler and after prolonged use of the system, condensation occurs within the duct to the cavity.

Optimisation of Cavity Design and Flame Composition

The size of the cavity controls the concentration of flame radicals and gases that come into contact with the analyte vapours, the internal coating may promote or reduce a specific emission, and the temperature characteristics of the metal and the over-all design severely affect the sensitivity of the measurement due to the Salet phenomenon.[16]

Two cavity designs were evaluated for the determination of sulphite by CF-MECA (Fig. 3). Cavity (*a*) consists of a stainless-steel tube with the cavity drilled at one end. The tube

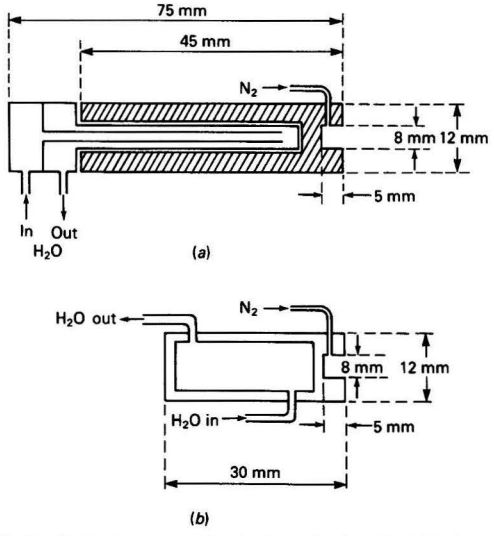


Fig. 3. Cavity designs used for the determination of sulphite (not to scale). For details of (*a*) and (*b*), see text

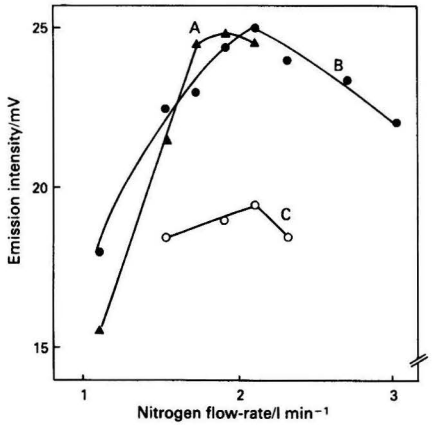


Fig. 4. Effect of nitrogen flow-rate on 50 μg ml^{-1} of sulphite for various flow-rates of hydrogen: at A, 0.70; B, 1.0; and C 1.8 l min^{-1}

slides on to a cold finger, which consists of two concentric tubes. Cooling water enters through the inner tube, hits the bottom of the outer one and flows out of the system. Cavity (*b*) consists of an aluminium alloy rod through which cooling water flows.

Cooling of the internal cavity space was more effective in cavity (*b*) than in cavity (*a*) because in (*a*) the incoming water is heated by the outgoing water. Further, aluminium removes heat from the cavity faster than stainless steel, the cavity remains cool and the S_2 emission enhancement due to the Salet phenomenon is more effective. Cavity (*b*) also gave more reproducible results than cavity (*a*). Cooling water was adjusted at a flow-rate just before condensation of water vapour from the flame occurs. It has previously been reported that treatment of the cavity with the concentrated stock solution of the analysed sulphur compound increases the S_2 emission probably owing to the formation of a solid deposit.[13,14,16] In this work, treatment of the cavity with 1000 μg ml^{-1} of sulphite increased the emission intensity from 50 μg ml^{-1} sulphite by *ca.* 40%.

The burner used was a 13-hole circular emission burner (outer diameter 15 mm, height 155 mm) with seven holes blocked to reduce flame size and improve flame stability. Hydrogen and nitrogen were only supplied to the burner. The effect of flame composition is shown in Fig. 4. All measurements were made at 384 nm with the maximum slit width available as no other potential emitters are generated from the system described.

The optimum conditions for the determination of sulphite by CF-MECA are summarised in Table 1.

Analytical Parameters

Fig. 5 shows a typical recording for a series of sulphite standards carried out by the proposed procedure. The calibration graph (*I versus C*) was sigmoidal, as expected,[13,14] and the log *I versus* log *C* calibration graph was linear in the range 1–50 μg ml^{-1} of sulphite ($\log I = -1.10 + 1.31 \log C$, $r = 0.9996$) which was used for all analytical measurements. The limit of detection (signal to noise ratio = 2) was 1 μg ml^{-1} of sulphite and the coefficients of variation for 4 and 12 μg ml^{-1} of sulphite were 1 and 0.4% ($n = 5$), respectively. When aqueous solutions of sulphite (4–18 μg ml^{-1}) were analysed by the proposed procedure, the average error was ±2% and the correlation coefficient was $r = 0.9991$.

Interferences

Interferences from anions were investigated by determining 25 μg ml^{-1} of sulphite in the presence of 10 and 50 μg ml^{-1} of

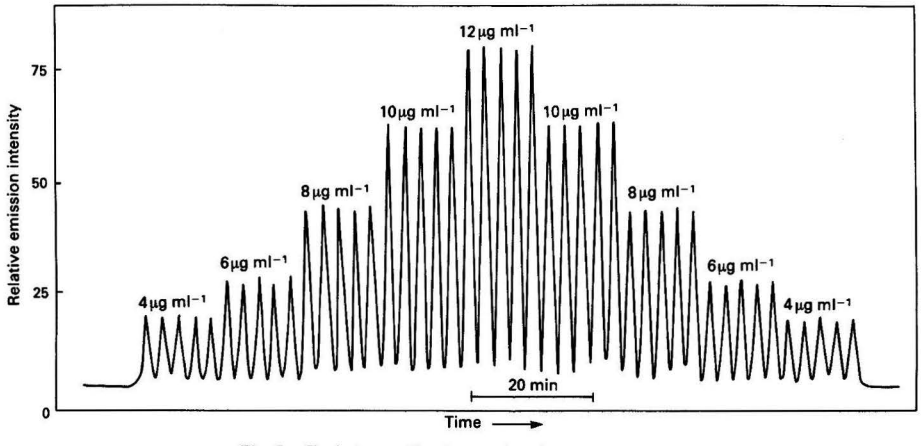


Fig. 5. Typical recording for a series of sulphite standards

Table 2. Effect of foreign ions in aqueous and EDTA solutions of sulphite (25 μg ml^{-1})

Ion	Concentration/μg ml^{-1}	Change in emission intensity, %		
		Ion + sulphite	Ion + sulphite +0.01 M EDTA	Ion + sulphite +0.1 M EDTA
Ca^{2+}	50	*	0	0
	200	−67	0	0
Ba^{2+}	10	*	0	0
	50	−66	0	0
Cr^{3+}, Zn^{2+}	50	*	0	0
Pb^{2+}	10	†	0	0
	50	†	0	0
Cd^{2+}	10	−100	−33	0
	50	−100	0	0
Fe^{3+}	10	−100	−57	−36
	50	−100	−100	−93
Co^{2+}	10	−100	−100	−50
	50	−100	−72	−33
Ni^{2+}	10	−100	−100	−56
	50	−100	−100	−33
Cu^{2+}	5	−100	−33	−56
	10	−100	−78	−67
	50	−100	−56	−50
Mn^{2+}	10	−100	−100	−100
	50	−100	−100	−100

* No significant interference.
† Precipitate formed after mixing of solutions.

anion. The responses were compared with those obtained from an uncontaminated sulphite solution. No effect was observed from thiocyanate, thiosulphate, dithionate and carbonate. A 10 μg ml^{-1} sample of sulphide generated an emission 3.5 times more intense than 25 μg ml^{-1} of sulphite and must be absent from the analysed solution. Nitrite reduced completely the emission from sulphite as a result of the oxidation to sulphate.

Most cations suppress the emission from sulphite, owing to the formation of stable complexes or insoluble compounds. An attempt was made to eliminate the effect by making the final solution 0.01 and 0.1 M in EDTA and the results are shown in Table 2. The effect of calcium(II), barium(II) and lead(II) was eliminated by 0.01 M EDTA and the effect of cadmium(II) was eliminated by 0.1 M EDTA but the interference from other cations was only partially eliminated. Manganese(II) was the most severe interferent of all the cations examined. The reduction of copper(II) to copper(I) by sulphite is probably responsible for the anomalous effect of the cation on the emission. Preliminary work using other methods indicated the formation of stable complexes between cations (*e.g.*, Cd^{2+}) and sulphite, but work is still under development.

Recovery of Sulphite from Soft Drinks

As the main ingredients of soft drinks (sugar, citric acid) were found not to affect the CF-MECA determination of sulphite, it was decided to investigate the application of the method to spiked sulphite-free solid concentrated drinks (Table 3). Aqueous solutions of samples (3) and (4) showed poor recoveries (<80%), which improved in the presence of 0.01 M EDTA. Poor recoveries from sample (4) are probably due to the presence of trace amounts of metals in the solid sample; 0.1 M EDTA did not improve the recoveries from sample (4).

Recovery of Sulphite from Wines

Organic compounds severely quench the S_2 emission owing to the formation of the thermodynamically stable gaseous carbon - sulphur species.[17] Further, ignition of a volatile organic compound, such as ethanol, within the cavity increases the temperature and alters the characteristics of the inner surface of the cavity. These problems make MECA

Table 3. Recovery of sulphite from soft drinks

Sample*	Sulphite/μg ml^{-1} Added	Found	Recovery, %
(1) Apricot	4.00	4.10	102.5
	6.00	5.95	99.2
	8.00	7.90	98.8
	10.00	9.95	99.5
	12.00	12.16	101.3
(2) Peach	4.00	3.92	98.0
	6.00	6.00	100.0
	8.00	8.30	103.8
	10.00	10.23	102.3
	12.00	11.80	98.3
(3) Apple	4.00	4.26	106.5
	6.00	5.75	95.8
	8.00	7.85	98.1
	10.00	9.50	95.0
	12.00	11.75	97.9
(4) Grapefruit	4.00	3.63	90.8
	6.00	5.75	95.8
	8.00	7.40	92.5
	10.00	9.20	92.0
	12.00	10.96	91.3

* 0.24 g of sample was diluted to 100.0 ml with 0.1 M NaOH - 0.01 M EDTA solution.

inapplicable to the determination of sulphite in wines. Nevertheless, with the CF-MECA method it was found that 1% *m/V* ethanol did not affect the emission from 10 μg ml^{-1} of sulphite; 2, 5 and 10% *m/V* ethanol reduced the emission intensity by 30, 40 and 70%, respectively and the reproducibility of the measurement deteriorated severely. Therefore, sulphite can be determined in wines by CF-MECA if the sample is diluted to a solution containing ≤1% *m/V* ethanol.

Table 4 shows the results for the determination of total sulphite in white and rosé wines. No sulphite was detected from dilute solutions of red wines, even after the addition of 15 μg ml^{-1} of sulphite. The effect was found to be due to the presence of tannic acid in these wines; 13, 25 and 38 μg ml^{-1} of tannic acid were found to reduce the S_2 emission intensity from 10 μg ml^{-1} of sulphite by 60, 95 and 100%, respectively, probably owing to the oxidation of the anion. Therefore, the method is not applicable to red wines.

Table 4. Recovery of sulphite from wines

Sample*	Sulphite/μg ml^{-1} Initially present	Added	Found	Recovery, %
1	7.20	4.00	11.22	100.5
		6.00	13.03	97.2
		8.00	15.30	101.2
2	8.60	4.00	12.50	97.5
		6.00	14.80	103.3
		8.00	16.60	100.0
3	4.00	4.00	8.20	105.0
		6.00	10.00	100.0
		8.00	11.80	97.5
4	3.20	4.00	7.10	97.5
		6.00	9.10	98.3
		8.00	11.10	98.8
5	10.35	4.00	14.45	102.5
		6.00	16.40	100.8
		8.00	18.20	98.1

* Samples (1) and (2) are white wines, sample (3) is rosé wine and samples (4) and (5) contain pine resin; analysis was performed after dilution of 10.00 ml of wine to 100.0 ml with 0.1 M NaOH - 0.01 M EDTA solution.

Table 5. Determination of sulphur dioxide in air

Sulphur dioxide/μg m^{-3} Photometric method	CF-MECA method	Difference, %
52.8	52.3	−0.9
59.5	58.9	−1.0
61.9	62.1	+0.3
63.1	63.8	+1.1
67.7	67.0	−1.0
79.6	78.9	−0.9
85.6	86.3	+0.8
	Average	0.9

Determination of Sulphur Dioxide in Air

Mercury(II) in concentrated tetrachloromercurate(II) (TCM) solutions forms insoluble salts with concentrated orthophosphoric acid, which reduce the evolution of sulphur dioxide.[13] TCM concentrations in the range 0.01–0.1 M did not affect the emission intensity from 10 and 50 μg ml^{-1} of sulphite while concentrations >0.02 M reduced the emission intensity from 5 μg ml^{-1} of sulphite by 33%. Therefore, complete recovery of sulphite occurs from TCM concentrations ≤0.02 M. In the West - Gaeke method, sulphamic acid is added to the disulphitomercurate(II) solution to reduce trace amounts of nitrite that interfere with the determination. As nitrite interferes with the CF-MECA method proposed, the effect of sulphamic acid on the sulphite determination was investigated. Sulphamic acid (0.048% *m/V*) was found to reduce the emission intensity from 2 and 4 μg ml^{-1} of sulphite by 30 and 60%, respectively, and was therefore added to all sulphite standards used for construction of the calibration graph for the determination of sulphur dioxide in air. The results were compared with the results from the pararosaniline method, which was carried out after dilution of 10.00 ml of the absorption solution to 25.00 ml[18] (Table 5).

Conclusion

The proposed continuous flow molecular emission cavity analyser for the determination of sulphite is an automated alternative to the manual determination of the anion by the same technique. It has the advantages of good sampling rate, simplicity and automation and compares well with the expected results when applied to the determination of sulphite in soft drinks, white and rosé wines and of sulphur dioxide after fixation as disulphitomercurate(II).

The authors thank M. Koupparis for stimulating discussions.

References

1. Cullis, C. F., *Chem. Br.*, 1978, **14**, 384.
2. West, P. W., and Gaeke, G. C., *Anal. Chem.*, 1956, **28**, 1816.
3. Scaringelli, F. P., Saltzman, B. E., and Frey, S. A., *Anal. Chem.*, 1967, **39**, 1709.
4. Marshall, G., and Midgley, D., *Anal. Chem.*, 1981, **53**, 1760.
5. Marshall, G. B., and Midgley, D., *Analyst*, 1983, **108**, 701.
6. Marshall, G., and Midgley, D., *Anal. Chem.*, 1982, **54**, 1490.
7. Kouimtzis, T. A., *Anal. Chim. Acta*, 1977, **88**, 303.
8. Al-Abachi, M. Q., Belcher, R., Bogdanski, S. L., and Townshend, A., *Anal. Chim. Acta*, 1976, **86**, 139.
9. Schubert, S. A., Clayton, J. W., and Fernando, Q., *Anal. Chem.*, 1979, **51**, 1297.
10. Schubert, S. A., Clayton, J. W., and Fernando, Q., *Anal. Chem.*, 1980, **52**, 963.
11. Tzeng, J.-H., and Fernando, Q., *Anal. Chem.*, 1982, **54**, 971.
12. Bogdanski, S. L., Townshend, A., and Yenigul, B., *Anal. Chim. Acta*, 1980, **115**, 361.
13. Calokerinos, A. C., and Townshend, A., *Fresenius Z. Anal. Chem.*, 1982, **311**, 214.
14. Calokerinos, A. C., and Hadjiioannou, T. P., *Anal. Chim. Acta*, 1983, **148**, 277.
15. Subramanian, K. S., and Méranger, J. C., *Analyst*, 1982, **107**, 157.
16. Bogdanski, S. L., Calokerinos, A. C., and Townshend, A., *Can. J. Spectrosc.*, 1982, **27**, 10.
17. Fredriksson, S.-A., and Cedergren, A., *Anal. Chim. Acta*, 1978, **100**, 429.
18. Katz, M., *Editor*, "Methods of Air Sampling and Analysis," Second Edition, American Public Health Association, Washington, DC, 1977.

Paper A4/345
Received October 3rd, 1984
Accepted December 3rd, 1984

Flow Injection Amperometric Determination of Ascorbic Acid and Dopamine at a Sessile Mercury Drop Electrode without Deoxygenation

Arnold G. Fogg and Abdulhadi M. Summan
Chemistry Department, Loughborough University of Technology, Loughborough, Leicestershire LE11 3TU, UK
and Miguel A. Fernández-Arciniega
Departamento de Química Analítica, Facultad de Ciencias, Universidad Autónoma de Madrid, 28034 Madrid, Spain

Ascorbic acid can be determined by flow injection amperometry at a sessile mercury drop electrode without the need to deoxygenate the eluent or sample. The determination is made at +0.19 V *vs.* S.C.E. in pH 5.5 acetate buffer. The size of the blank signal is equivalent to about 0.01 μg ml^{-1} of ascorbic acid and the signal is rectilinear up to about 60 μg ml^{-1}. Chloride gives an oxidation signal at the mercury electrode but at a more positive potential and at the 1 μg ml^{-1} level of ascorbic acid 1000 μg ml^{-1} of chloride ion did not interfere.

Hydrodynamic voltammograms of dopamine [2-(3,4-dihydroxyphenyl)ethylamine] show a plateau distinct from that of ascorbic acid and the mercury oxidation cut-off. Calibration graphs obtained at +0.26 V *vs.* S.C.E. for dopamine are rectilinear in the range 0.1–60 μg ml^{-1}. At +0.26 V chloride ion gives a signal approximately 1% of that of dopamine on a molar basis and interferes at higher ratios.

Keywords: *Ascorbic acid determination; dopamine determination; flow injection analysis; amperometry; sessile mercury drop electrode*

Ascorbic acid is oxidised at a dropping mercury electrode with a half-wave potential of +0.06 V in pH 5.5 acetate buffer. Several procedures for determining ascorbic acid polarographically have been proposed.[1–3] We used differential-pulse polarography to monitor both ascorbic acid and food colouring matters during their interaction.[4–6] In all these methods the polarographic solutions were deoxygenated as reduction of dissolved molecular oxygen occurs at or near 0 V.

Recently, we described the construction of an amperometric detector cell that could be used either with a solid electrode or with a sessile mercury drop electrode and illustrated its use by determining the food colouring matters oxidatively at a glassy carbon electrode and reductively at a sessile mercury drop electrode.[7] For the reductive determination of the food colours the eluent and sample were deoxygenated with nitrogen gas and when Britton - Robinson buffers of pH >7 were used a small amount of sodium sulphite was added to both eluent and sample to lower the oxygen concentration even further. By this means, 0.1 μg ml^{-1} of the food colours were determined and the signal was rectilinear from 0.1 to 60 μg ml^{-1}.

Further work is being carried out in this laboratory to evaluate the suitability of flow injection amperometry using a sessile mercury drop electrode for use in pharmaceutical and other quality control applications and in monitoring drug compounds in relatively simple chemical systems such as those used in dissolution tests. In studying the determination of ascorbic acid a lower detection limit was obtained than had been obtained previously when determining the food colours with added sulphite, owing to the fact that dissolved molecular oxygen did not interfere at the measuring potential used (+0.19 V) and deoxygenation of the sample and the eluent proved to be unnecessary.

In biological systems dopamine [2-(3,4-dihydroxyphenyl)ethylamine] is determined voltammetrically at carbon electrodes in the presence of ascorbic acid, which is widely distributed in nerve tissues.[8] Electrochemical pre-treatment of carbon fibre electrodes is essential in order to produce an electrode surface on which the ascorbic acid oxidation is sufficiently fast to allow separation of the ascorbic acid and dopamine oxidation processes. For this reason, the possibility of determining ascorbic acid and dopamine in admixture at the sessile mercury electrode was investigated. Chloride ion produces an oxidation wave at mercury electrodes owing to the formation of mercury(I) chloride and as chloride is generally present in biological samples its likely interference was investigated.

Experimental

Reagents

Acetate buffer solution (pH 5.5). Dilute 62.5 ml of 1 M acetic acid solution and 50 ml of 1 M sodium acetate solution to 1 l and adjust the pH to 5.5 with 1 M sodium hydroxide solution.

Standard ascorbic acid solutions. Dilute 0.100 g of ascorbic acid and 2.5 mg of EDTA (disodium salt) to 100 ml in a calibrated flask. Dilute 5 ml of this solution to 100 ml in a second calibrated flask to give a 50 μg ml^{-1} solution. Aliquots of this solution (or of more dilute solutions) are diluted with the addition of 20 ml of acetate buffer to 100 ml in a calibrated flask. The EDTA is added to stabilise the ascorbic acid solutions but the solutions are preferably freshly prepared.

Standard dopamine and chloride solutions, 100 μg ml^{-1}. These were prepared in acetate buffer using dopamine base and potassium chloride. The dopamine solutions were freshly prepared. As catecholamines are fairly readily oxidised by air it can be an advantage to prepare standard dopamine solutions in buffer previously deoxygenated with nitrogen gas.

Apparatus

The flow injection system was used as described previously[7] for the reductive determination of food colouring matters. The laboratory-built detector cell and sessile mercury drop electrode holder were also as described.[7] The cell is used partially immersed in electrolyte, pH 5.5 acetate buffer. A three-electrode system (sessile mercury drop electrode, platinum counter electrode and calomel reference electrode) was controlled by means of a PAR 174A polarographic analyser (Princeton Applied Research). Injections (120 μl)

were made by means of a low-pressure Rheodyne 5020 valve. The valve was connected to the detector cell with 1 m of 0.58 mm bore tubing. Neither eluent (pH 5.5 acetate buffer) nor sample was deoxygenated or degassed. A flow-rate of 6 ml min^{-1} was maintained using an Ismatec Mini-S pump. Current peaks were recorded on a Linseis L650 recorder.

Results

Typical hydrodynamic voltammograms, obtained by injecting dopamine solution and a mixture of ascorbic acid and dopamine solutions into pH 5.5 acetate buffer and measuring the signals obtained at various potentials, are given in Fig. 1. The complete separation of the ascorbic acid and dopamine oxidation processes from each other and from the mercury oxidation cut-off is clearly seen. Typical signals at +0.19 V for obtaining a calibration graph for the determination of ascorbic acid at low levels are given in Fig. 2. Coefficients of variation obtained on five determinations were typically less than 1%. The means of three signals obtained for each of 4, 20, 50, 100 and 200 μg ml^{-1} of ascorbic acid were 0.83, 4.17, 10.4, 20.8 and 38.9 μA, respectively. The signals at +0.26 V for dopamine were similar but the lowest amount determinable was about 0.1 μg ml^{-1}.

The means of three signals obtained at the 60 μg ml^{-1} level of chloride for producing a hydrodynamic voltammogram were 0 μA (0.10 V), 0.14 μA (0.20 V), 0.51 μA (0.25 V), 2.32 μA (0.30 V), 3.27 μA (0.32 V), 3.86 μA (0.35 V), 4.04 μA (0.37 V), 4.04 μA (0.40 V) and 3.92 μA (0.42 V). The background currents for the eluent above which the signals were measured were 0.2 μA (0.25 V), 1.25 μA (0.30 V) and 4.4 μA (0.35 V). At +0.26 V chloride ion gives a signal approximately 1% of that of dopamine on a molar basis. The interference from chloride clearly becomes increasingly significant at higher chloride to dopamine ratios.

Discussion

A method is presented by means of which ascorbic acid and dopamine can be determined by flow injection analysis (FIA) at a sessile mercury drop electrode without the need to deoxygenate the eluent or samples. Calibration graphs are generally rectilinear in the range from the detection limit to 60 μg ml^{-1} and the precision is good (the coefficients of variation were less than 1% on five injections). The attainment of the low detection limit is possible because dissolved molecular oxygen does not interfere at the electrode potentials used. Chloride at more than equimolar levels interferes in the determination of dopamine.

Alternatively, ascorbic acid and dopamine could be determined by differential-pulse polarography at the dropping mercury electrode. For determinations on small numbers of samples differential-pulse polarography, essentially a manual method, might be considered to be slightly more convenient than the flow injection method, but the FIA method is much more convenient when the number of samples is significantly larger. In the flow method measurements are made at a single potential and confidence in the results comes from making sufficient injections of sample and standards and from previous knowledge that the samples being determined behave well in the flow system. This latter can be verified by differential-pulse polarography and by obtaining hydrodynamic voltammograms.

The most widely used method for the determination of ascorbic acid in extracts of foods is the 2,6-dichlorophenolindophenol titrimetric method.[9] The titrant acts as its own indicator and indeed ascorbic acid can be determined indirectly by visible spectrophotometry by measuring the excess of 2,6-dichlorophenolindophenol.[9] These methods have two major drawbacks, namely the difficulties associated with working with coloured samples, which frequently occurs when working with certain types of foods such as fruit, and the lack of selectivity of the oxidation reaction, which precludes, for example, the determination of ascorbic acid and sulphite in admixture in pharmaceutical preparations. A standard method[10] using the titrimetric procedure on coloured solutions incorporates end-point detection by extraction of the indophenol into chloroform. On the other hand, the flow injection amperometric and the differential-pulse polaro-

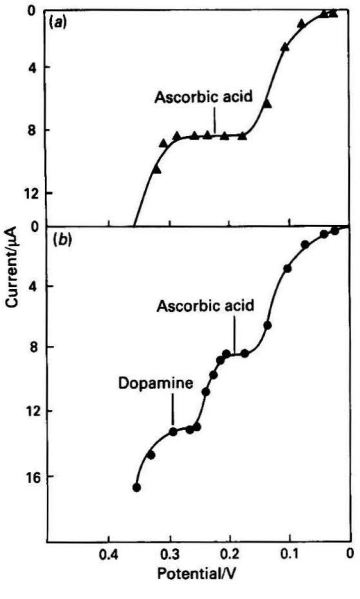


Fig. 1. Hydrodynamic voltammograms obtained for the injection of (*a*) ascorbic acid (40 μg ml^{-1}) and (*b*) a mixture of ascorbic acid (40 μg ml^{-1}) and dopamine (60 μg ml^{-1}) in pH 5.5 acetate buffer

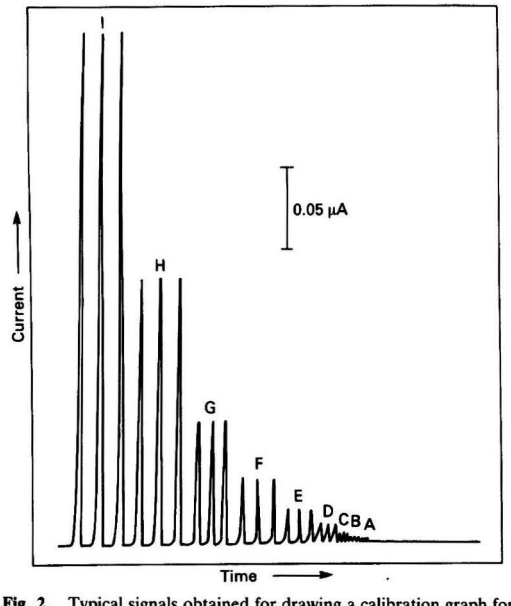


Fig. 2. Typical signals obtained for drawing a calibration graph for the determination of low levels of ascorbic acid. Ascorbic acid concentration: A, 0; B, 0.01; C, 0.025; D, 0.05; E, 0.1; F, 0.2; G, 0.4; H, 0.8; and I, 1.6 μg ml^{-1}. Potential of the working electrode, +0.19 V

graphic methods[4–6] have been used directly with coloured solutions such as those containing synthetic food colouring matters, and these methods are more selective than the titrimetric method. The shapes of polarograms and of hydrodynamic voltammograms can indicate the presence or the absence of other reductants.

A. M. S. thanks the Government of Saudi Arabia for financial support and Umm Al-Qura University for leave of absence. M. A. F.-A. thanks the British Council for a grant for this collaborative study.

References

1. Kolthoff, I. M., and Lingane, J. J., "Polarography," Volume II, Interscience, London, 1952.
2. Milner, G. W. C., "The Principles and Applications of Polarography and other Electroanalytical Processes," Longman, London, 1957.
3. Owen, R. S., and Smyth, W. F., *J. Food Technol.*, 1975, **10**, 263.
4. Fogg, A. G., and Summan, A. M., *Analyst*, 1983, **108**, 691.
5. Fogg, A. G., and Summan, A. M., *Analyst*, 1983, **108**, 1339.
6. Fogg, A. G., and Summan, A. M., *Analyst*, 1984, **109**, 743.
7. Fogg, A. G., and Summan, A. M., *Analyst*, 1984, **109**, 1029.
8. Brazell, M. P., and Marsden, C. A., *Br. J. Pharmacol.*, 1982, **75**, 539.
9. Christie, A. A., *in* Stein, M., *Editor*, "Vitamins," Churchill Livingstone, Edinburgh and London, 1971.
10. "British Pharmacopoeia 1980," HM Stationery Office, London, 1980, p. 725.

Paper A4/370
Received October 23rd, 1984
Accepted December 5th, 1984

Flow Injection Amperometric Determination of Nitroprusside at a Glassy Carbon Electrode and at a Sessile Mercury Drop Electrode

Arnold G. Fogg
Department of Chemistry, Loughborough University of Technology, Loughborough, Leicestershire LE11 3TU, UK
Miguel A. Fernández-Arciniega
Departamento de Química Analítica, Facultad de Ciencias, Universidad Autónoma de Madrid, 28034 Madrid, Spain
and Rosa M. Alonso
Departamento de Química, Universidad del País Vasco, Bilbao, Spain

Nitroprusside can be determined by flow injection analysis with amperometric detection using two different methods. In the first method nitroprusside is oxidised at a glassy carbon electrode in 0.5 M sodium hydroxide solution, and in the second method it is reduced at a sessile mercury drop electrode in pH 8 Britton - Robinson buffer. Removal of oxygen is unnecessary in the oxidative method and the rectilinear range is wider ($1 \times 10^{-6} - 5 \times 10^{-3}$ M).

Keywords: *Nitroprusside determination; flow injection analysis; glassy carbon electrode; sessile mercury drop electrode; amperometry*

Sodium nitroprusside dihydrate is a rapid-acting intravenous peripheral vasodilator that acts directly on vascular smooth muscle. It is used in cases of severe malignant hypertension and, when given intravenously, lowers blood pressure.[1]

The nitroprusside ion, $Fe(CN)_5NO^{2-}$, gives three polarographic waves[2-10] associated with the reduction of the NO^+ ligand, whereas its conjugate base, $Fe(CN)_5NO_2^{4-}$, which is formed extensively above pH 11, is not reduced at mercury.[4] At pH 9 the three nitroprusside half-wave potentials are at approximately −0.45, −0.7 and −1.0 V *vs.* S.C.E. Nitroprusside also gives an oxidation signal at mercury owing to oxidation of mercury to form insoluble mercury(II) nitroprusside.[11] An a.c. polarographic method has been reported recently for determining nitroprusside in serum.[12] The conjugate base of nitroprusside is reported to undergo oxidation by strong chemical oxidants and by dissolved molecular oxygen,[4] presumably to $Fe(CN)_5NO_2^{3-}$, but there appears to be no report of its electrochemical oxidation.

A study of the electrochemical oxidation of nitroprusside at a glassy carbon electrode using linear sweep voltammetry and cyclic voltammetry is reported here, together with the development of flow injection analysis methods for the determination of nitroprusside oxidatively at a glassy carbon electrode and reductively at a sessile mercury drop electrode.

Experimental

Electrode potentials were controlled and currents monitored by means of a PAR 174 polarographic analyser (Princeton Applied Research). A Houston Instruments Omnigraphic 2000 *X* - *Y* recorder was used to record the voltammograms and a Leeds and Northrup Speedomax *y* - *t* recorder to monitor the flow injection signals. A three-electrode operation was used with a glassy carbon or sessile mercury drop working electrode, a platinum counter electrode and a saturated calomel reference electrode. All potentials are given relative to the saturated calomel electrode.

Linear sweep and cyclic voltammograms were made at a sweep rate of 10 mV s^{-1} and a low pass filter value of 0.3 was used. In the flow injection experiments an Ismatec Mini-S pump was used to maintain the eluent flow and sample injections were made with a low-pressure Rheodyne 5020 injection valve with an injection volume of 75 µl. The injection valve was connected to the detector by means of 1 m of 0.58 mm bore tubing.

The detector cell used in the flow injection experiments consisted of a laboratory-built wall-jet detector, which can be used either with a glassy carbon electrode or with a sessile mercury drop electrode.[13] The detector is used partially immersed in electrolyte of the same composition as the eluent in order to allow electrical contact with conventional counter and reference electrodes immersed in the same electrolyte solution.[13]

Removal of oxygen was unnecessary in the flow injection experiments using the glassy carbon electrode oxidatively, but when carrying out flow injection experiments with the sessile mercury drop electrode the eluent and sample solutions were deoxygenated with nitrogen gas and by adding small amounts of sulphite. In the cyclic voltammetric experiments with glassy carbon the solutions were deoxygenated in order to observe the re-reduction peaks at negative potentials.

Reagents

The main reagents were prepared as follows.

Standard sodium nitroprusside solution, 0.01 M. Dissolve 0.745 g of sodium nitroprusside dihydrate (analytical-reagent grade) in water and dilute to 250 ml in a calibrated flask. Prepare more dilute solutions from this freshly prepared stock solution.

Standard sodium hydroxide solution, 1.0 M. Dissolve 40.0 g of sodium hydroxide (analytical-reagent grade) in water and dilute to 1 l in a calibrated flask. Prepare the eluent for the oxidative studies by diluting this solution with an equal volume of water.

Britton - Robinson buffer, pH 8.0. To a solution 0.04 M in each of orthophosphoric acid, acetic acid and boric acid add sufficient 0.2 M sodium hydroxide solution to bring the pH to 8.0.

Results

Linear Sweep and Cyclic Voltammetry

Linear sweep voltammetric studies on nitroprusside solutions of various pH values indicated that only the conjugate base of the nitroprusside ion, *viz.*, the pentacyanonitroferrate(II) ion $[Fe(CN)_5NO_2^{4-}]$, is oxidised at the glassy carbon electrode. Nitroprusside solutions prepared in 1 M sulphuric acid, acetate buffer of pH 4.7, phosphate buffer of pH 7, disodium

tetraborate buffer of pH 9.2 and Britton - Robinson buffers of pH 2, 10 and 11 exhibited no oxidation wave. In pH 12 Britton - Robinson buffer a small oxidation signal was observed. This signal was found to be larger in 0.04 M sodium hydroxide solution and to increase to a steady value on increasing the sodium hydroxide concentration to about 0.5 M (Fig. 1). In the sodium hydroxide solutions of concentrations above 0.1 M another oxidative process was evident at slightly less positive potentials. In 0.5 M sodium hydroxide solution the plateau of the oxidation process at less positive potentials was at 0 to +0.16 V and the main oxidation peak was at +0.32 V.

Cyclic voltammograms at the glassy carbon electrode with the forward scan from −0.8 V to +0.5 V obtained for the conjugate base of nitroprusside in 0.5 M sodium hydroxide solution (Fig. 2) show a re-reduction process at +0.14 V on the reverse scan corresponding to the oxidation peak at +0.32 V. A second re-reduction process at −0.54 V corresponds to the oxidation process between 0 and 0.16 V. A linear sweep voltammogram from 0 to −0.8 V showed no reduction process.

Oxidative Determination of Nitroprusside by Flow Injection Analysis with Amperometric Detection at a Glassy Carbon Electrode

Linear sweep voltammetric experiments had shown that a 0.5 M sodium hydroxide medium was suitable for determining nitroprusside. A hydrodynamic voltammogram was obtained by injecting a 10^{-3} M solution of sodium nitroprusside in 0.5 M sodium hydroxide into a 0.5 M sodium hydroxide eluent (Fig. 3). A potential of 0.57 V was used subsequently for determining nitroprusside. The effect of flow-rate on signal size was studied (Table 1): a flow-rate of 6.5 ml min^{-1} was chosen as the most suitable and was used in all subsequent experiments with this system. The signal was shown to be rectilinear with change in nitroprusside concentration from 1×10^{-6} to 5×10^{-3} M (0.3 to 7500 μg ml^{-1} of sodium nitropusside dihydrate). Typical signals obtained at the 1×10^{-6}–50×10^{-6} M levels of nitroprusside are shown in Fig. 4. Coefficients of variation on ten determinations at the 5×10^{-5} and 5×10^{-4} M levels were 1.8 and 1.5%, respectively.

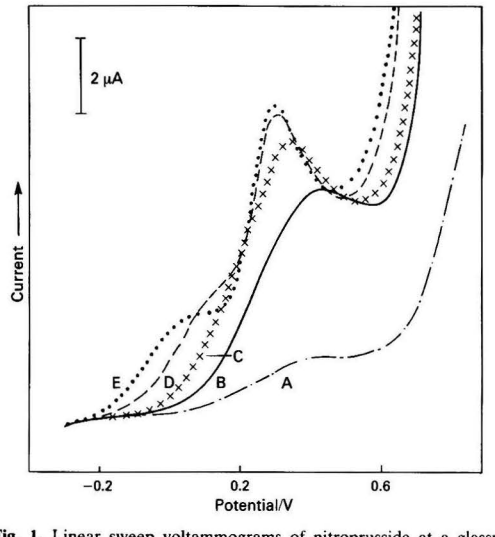


Fig. 1. Linear sweep voltammograms of nitroprusside at a glassy carbon electrode in different media. A, Britton - Robinson buffer, pH 12; and B, 0.04; C, 0.10; D, 0.25; and E, 0.5 M sodium hydroxide solution

Reductive Determination of Nitroprusside by Flow Injection Analysis With Amperometric Detection at a Sessile Mercury Drop Electrode

The nitroprusside ion is reducible at the dropping mercury electrode and the heights of the first and second polarographic waves are constant up to a pH of about 9; at higher pH they decrease in height owing to conversion of nitroprusside ion into its conjugate base.[4] Flow injection analysis was carried out here using nitroprusside solutions in pH 8.0 Britton - Robinson buffer. In solutions of pH >7 sulphite can be used to deoxygenate solutions more efficiently without interfering

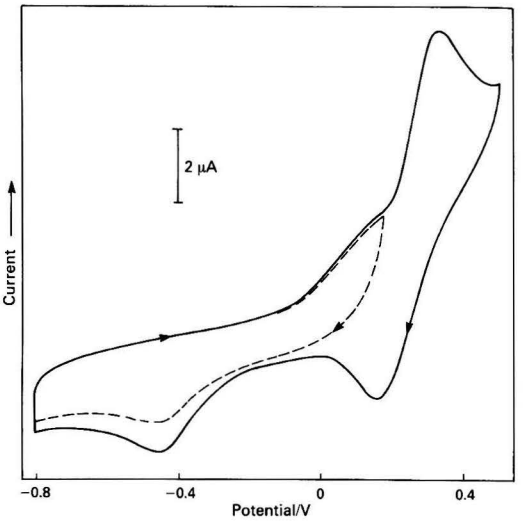


Fig. 2. Cyclic voltammograms of nitroprusside (10^{-3} M) in 0.5 M sodium hydroxide solution at a glassy carbon electrode. Scan rate, 10 mV s^{-1}

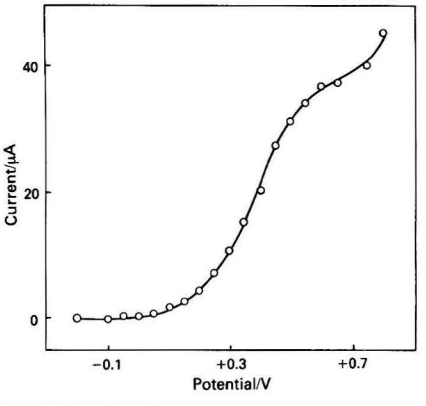


Fig. 3. Hydrodynamic voltammogram at a glassy carbon electrode obtained by injecting a 10^{-3} M nitroprusside solution (in 0.5 M sodium hydroxide) into 0.5 M sodium hydroxide eluent. Flow-rate, 4.0 ml min^{-1}

Table 1. Amperometric detection of nitroprusside at a glassy carbon electrode: effect of flow-rate

Flow-rate/ml min^{-1}	1.5	2.5	3.4	4.3	5.2	6.3	8.3
Signal/μA	3.32	3.64	4.28	4.68	5.20	5.32	5.32

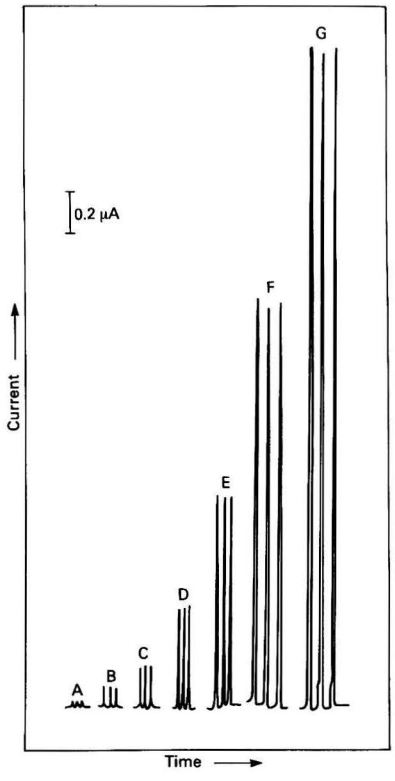


Fig. 4. Signals obtained for producing a calibration graph on the determination of nitroprusside oxidatively at a glassy carbon electrode. Nitroprusside concentration: A, 0; B, 1; C, 3; D, 7.5; E, 15; F, 30; and G, 50 × 10^{-6} M

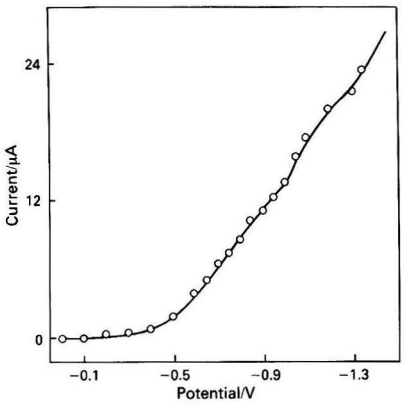


Fig. 5. Hydrodynamic voltammogram at a sessile mercury drop electrode obtained by injecting a 3 × 10^{-4} M nitroprusside solution (in pH 8 Britton - Robinson buffer) into pH 8 Britton - Robinson buffer. Flow-rate, 6.5 ml min^{-1}

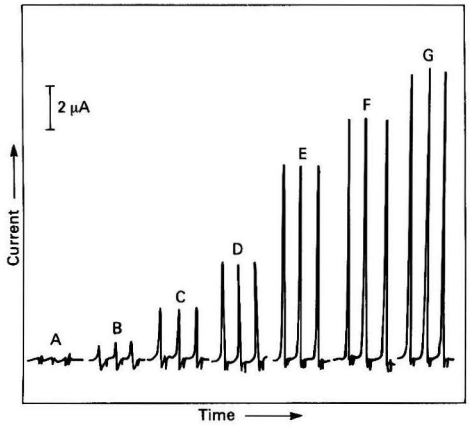


Fig. 6. Signals obtained on producing a calibration graph for the determination of nitroprusside reductively at a sessile mercury drop electrode. Nitroprusside concentration: A, 0; B, 2; C, 5; D, 10 and E, 20 × 10^{-5} M

with the polarography. The eluent was also pH 8.0 Britton - Robinson buffer. Sample and eluent were deoxygenated with nitrogen gas and then sodium sulphite heptahydrate was added to give a final concentration of 0.16%. A flow-rate of 6.5 ml min^{-1} was used as with the glassy carbon electrode, but in this instance a steady base line was obtained only after passing eluent for about 15 min. A low pass filter value of 1 was used with the mercury drop detector in order to obtain a more stable base line.

A hydrodynamic voltammogram (Fig. 5) was obtained by injecting a 3 × 10^{-4} M solution of sodium nitroprusside in pH 8.0 Britton - Robinson buffer into an eluent consisting of pH 8.0 Britton - Robinson buffer. Clearly, the three reduction processes are not as well distinguished in the flow system as they are in d.c. polarography at a dropping mercury electrode, possibly owing to lower reversibilities of the electrode reactions. A potential of −1.25 V was chosen for use in these flow injection studies. Signals were shown to be rectilinear with respect to nitroprusside concentrations from 2 × 10^{-5} to 20 × 10^{-5} M (6 to 60 μg ml^{-1} of sodium nitroprusside dihydrate). Typical signals are shown in Fig. 6. Coefficients of variation (ten determinations) were typically <2%.

Discussion

The conjugate base of the nitroprusside ion, but not nitroprusside itself, is oxidised at a glassy carbon electrode. This can be used as the basis of a flow injection analysis method of determining nitroprusside using amperometric detection. Nitroprusside ion can also be determined reductively by flow injection analysis using amperometric detection at a sessile mercury drop electrode, but this method is not as satisfactory as solutions have to be deoxygenated, the rectilinear range is narrow and detector noise is greater at the high negative potentials used.

The flow injection method using the glassy carbon electrode is simple, precise and reliable. Possible interferences can be tested for at pH 8, where the conjugate base of nitroprusside is not formed. In any column chromatographic determination of nitroprusside the addition of sodium hydroxide after the column could serve as a post-column derivatisation reaction prior to amperometric detection.

The nitroprusside ion undergoes many unusual reactions involving reaction of the coordinated NO^+ with species such as sulphide, sulphite, ketones and other compounds with acidic hydrogens.[14] Possible flow injection analysis methods involving derivatisation with nitroprusside are being investigated.

R. M. A. thanks the Patronato de la Universidad del País Vasco, Bilbao, for financial support.

References

1. Reynolds, J. E. F., "Martindale, the Extra Pharmacopoeia," Twenty-eighth Edition, Pharmaceutical Press, London, 1982, p.166.
2. Lanza, P., and Corbellini, A., *Ric. Sci.*, 1952, **22** (Suppl. A), 127.
3. Kolthoff, I. M., and Torren, P. E., *J. Am. Chem. Soc.*, 1953, **75**, 1197.
4. Zuman, P., and Kabát, M., *Collect. Czech. Chem. Commun.*, 1954, **19**, 873.
5. Kashyap, R. S., Sharma, P. N., and Kumar, A., *Indian J. Chem.*, 1980, **19A**, 535.
6. Kashyap, R. S., Sharma, P. N., and Kumar, A., *Indian J. Chem.*, 1982, **21A**, 543.
7. Mašek, J., Bapat, M. G., Ćosvić, B., and Dempír, J., *Collect. Czech. Chem. Commun.*, 1969, **34**, 485.
8. Mašek, J., and Dempír, J., *Collect Czech. Chem. Commun.*, 1969, **34**, 727.
9. Mašek, J., and Maslova, E., *Collect. Czech. Chem. Commun.*, 1974, **39**, 2141.
10. Cheney, R. P., Simic, H. G., Hoffman, M. Z., Taub, I. A., and Asmus, K. D., *Inorg. Chem.*, 1977, **16**, 2187.
11. Mori, H., Sugawara, M., and Kambara, T., *Denki Kagaku Oyobi Kogyo Butsuri Kagaku*, 1978, **46**, 442.
12. Alkayer, M., Vallan, J. J., and Pegon, Y., *Anal. Lett.*, 1981, **14**, 399.
13. Fogg, A. G., and Summan, A. M., *Analyst*, 1984, **109**, 1029.
14. Swinehart, J. H., *Coord. Chem. Rev.*, 1967, **2**, 385.

Paper A4/371
Received October 23rd, 1984
Accepted December 5th, 1984

Unusual Electrochemical Behaviour of a New Phenothiazine*

Jean-Michel Kauffmann, Gaston J. Patriarche† and Jean-Claude Viré
Free University of Brussels, Campus Plaine 205/6, Boulevard du Triomphe, 1050 Brussels, Belgium
and William R. Heineman
Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221, USA

Cyclic voltammetry of a new derivative of the phenothiazine family quisultidine, 2-(*N,N*-dimethylsulphamoyl)-1-(3-quinuclidinyl)phenothiazine, exhibits an unusually positive oxidation wave for this class of compound. The oxidation product undergoes cleavage of the side-chain to liberate the parent 2-(*N,N*-dimethylsulphamoyl)phenothiazine. The electrochemical behaviour of quisultidine is probably related to the unusual pharmacological properties of this drug.

Keywords: *Cyclic voltammetry; phenothiazine; quisultidine*

Electroanalytical techniques have proved effective in the investigation of numerous types of drugs.[1] This is especially true of phenothiazines,[1-5] for which electrochemistry is a convenient means of generating cation radicals for subsequent investigation. The importance of reversible and fairly stable cation radical formation in the phenothiazines has been pointed out by electrochemists and pharmacologists, who attribute a large part of the psychotropic activity to this oxidation product.[6-8]

We have recently had the opportunity to study a new derivative of the phenothiazine family quisultidine, 2-(*N,N*-dimethylsulphamoyl)-1-(3-quinuclidinyl)phenothiazine, from H. Koch Pharmacy International.[9] According to this report, the drug has been found to have virtually none of the antihistaminic and anticholinergic activity usually associated with the phenothiazines. However, its antisecretory action appears to be unique and is due to a mechanism that is yet to be fully understood.

We present here information about the electrochemical oxidation of quisultidine that explains some of the uniqueness of its pharmacological behaviour.

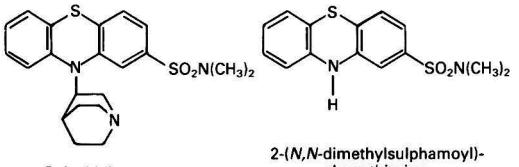
Experimental

Cyclic voltammograms were recorded with a Model 175 universal programmer/Model 174 potentiostat (Princeton Applied Research) and a PAR RE0074 recorder.

All voltammetric measurements were carried out using a conventional three-electrode design at 25 ± 0.1 °C. Working electrodes were either a platinum disc (Tacussel EDI), glassy carbon (Metrohm) or the modified carbon - polyethylene 30% *m/m* electrode.[10] Quisultidine and 2-(*N,N*-dimethylsulphamoyl)phenothiazine were a gift from Pharmuka Laboratories, Gennevilliers, France. All chemicals were of pure grade (Merck, analytical-reagent grade).

Results

The structures of the compounds investigated are shown below.



Quisultidine

2-(*N,N*-dimethylsulphamoyl)-phenothiazine

Quisultidine is surprisingly difficult to oxidise, as shown by the anodic peak potentials in Table 1 from cyclic voltammograms recorded in various concentrations of sulphuric acid (0.1–3 M). For example, oxidation of quisultidine in 0.1 M H_2SO_4 + 20% methanol occurs with a peak potential (E_{P_a}) at +1.120 V *vs.* S.C.E., as shown by the first scan in the cyclic voltammogram in Fig. 1(*a*). By comparison, the analogous compound unsubstituted at the ring nitrogen, 2-(*N,N*-dimethylsulphamoyl)phenothiazine, is oxidised with a peak potential (A) at +0.630 V *vs.* S.C.E., as shown by the first scan in Fig. 1(*b*). Of equal interest is the observation that the oxidation wave for quisultidine is irreversible over the range of scan rates investigated (10–500 mV s^{-1}), as evidenced by the absence of a corresponding cathodic peak on the reverse scan. By comparison, the unsubstituted analogue exhibits a cathodic peak A′ on the reverse scan.

Continued cycling of quisultidine reveals the appearance of new reversible redox couples A/A′, B/B′ and C/C′ in a less positive potential region [Fig. 1(*a*)]. Peaks A/A′ correspond to the redox couple of the parent 2-(*N,N*-dimethylsulphamoyl)phenothiazine, whereas couples B/B′ and C/C′ are identical with those obtained for the second scan on the parent compound as shown in Fig. 1(*b*). These results suggest that a significant amount of the unsubstituted analogue is formed during the oxidation of quisultidine.

Similar behaviour is observed in 3 M H_2SO_4. With quisultidine, one irreversible peak E_{P_a} [Fig. 1(*c*)] is observed for the first positive potential scan as in 0.1 M H_2SO_4, and new redox couples appear in a less positive potential region on subsequent scans. The peak current of peak E_{P_a} is linearly related to $V^{\frac{1}{2}}$, in the whole range of scan rates investigated (10–500 mV s^{-1}), indicating a process controlled by diffusion of the quisultidine. Peak E_{P_a} is completely irreversible in 3 M H_2SO_4 within the range 10–50 mV s^{-1}. However, at scan rates higher than 50 mV s^{-1} a corresponding cathodic peak could be detected. The oxidation of the unsubstituted phenothiazine occurs classically [Fig. 1(*d*)]; peak A splits and the reaction proceeds via the reversible cation radical (a_I/c_I).

The matching of the cyclic voltammogram of the unsubstituted phenothiazine and the less positive redox couples of quisultidine oxidation was confirmed in solutions containing a mixture of both compounds. Fig. 2(*a*) shows the cyclic voltammogram of 5×10^{-4} M quisultidine in 3 M H_2SO_4. Fig. 2(*b*) represents the mixture of 5×10^{-4} M quisultidine and 2.5×10^{-5} M parent compound; peaks a_I and a_{II} have increased. Similar behaviour is observed in 0.1 M H_2SO_4; the addition of 2.5×10^{-5} M parent compound [Fig. 2(*d*)] gives rise to an increase in peaks A/A′.

Discussion

The unusually positive oxidation wave for quisultidine is probably due to steric hindrance caused by the 3-quinuclidinyl

* Presented in part at the 165th Meeting of the Electrochemical Society, Cincinnati, OH, USA, May 1984.

† To whom correspondence should be addressed.

Table 1. Comparative peak potentials for cyclic voltammetry at various electrodes in 3 M H_2SO_4 - 20% CH_3OH. Drug concentration, 5×10^{-4} M; scan rate, 20 mV s^{-1}; potential *vs.* S.C.E.

Compound	Electrode	E_{P_a}	a_I	c_I	a_{II}
Quisultidine	Platinum	+1.010			
	Glassy carbon	+1.010			
	Carbon - polyethylene	+1.030			
2-(*N,N*-Dimethylsulphamoyl)phenothiazine . .	Platinum		+0.470	+0.410	+0.730
	Glassy carbon		+0.500	+0.420	+0.745
	Carbon - polyethylene		+0.495	+0.430	+0.740

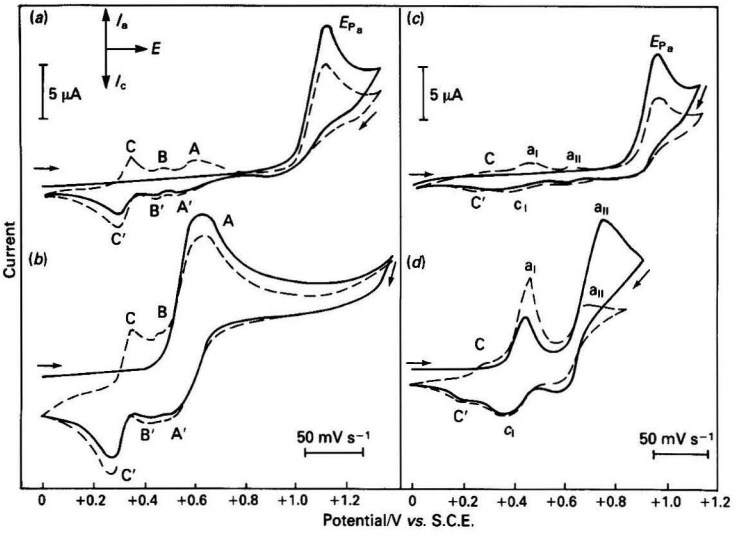


Fig. 1. Cyclic voltammograms of 5×10^{-5} M quisultidine (*a* and *c*) and 5×10^{-5} M 2-(*N,N*-dimethylsulphamoyl)phenothiazine (*b* and *d*) in 0.1 M H_2SO_4 + 20% CH_3OH (*a* and *b*) and 3 M H_2SO_4 + 20% CH_3OH (*c* and *d*). Working electrode: carbon - polyethylene. Broken lines: second scan

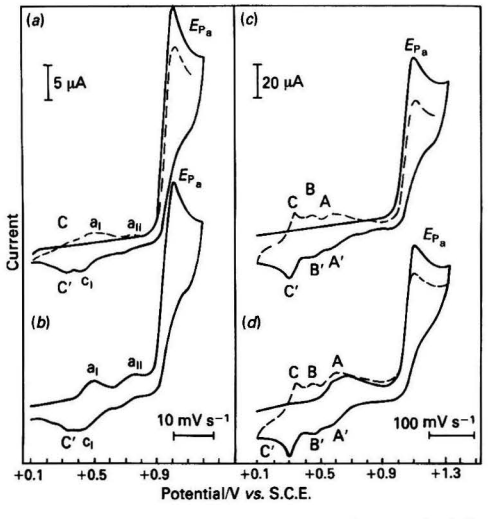


Fig. 2. Matching cyclic voltammetry at a carbon - polyethylene electrode for 5×10^{-4} M quisultidine. (*a*) Quisultidine alone in 3 M H_2SO_4 + 20% CH_3OH; (*b*) mixture of quisultidine and 2.5×10^{-5} M 2-(*N,N*-dimethylsulphamoyl)phenothiazine in 3 M H_2SO_4 + 20% CH_3OH; (*c*) quisultidine alone in 0.1 M H_2SO_4 + 20% CH_3OH; and (*d*) mixture of quisultidine and 2.5×10^{-5} M 2-(*N,N*-dimethylsulphamoyl)phenothiazine in 0.1 M H_2SO_4 + 20% CH_3OH. Broken lines: second scan

side-chain, which hinders formation of a coplanar structure for the electrogenerated radical cation. The absence of a reversible wave suggests that coplanarity is achieved by cleavage of the side-chain to liberate the 2-(*N,N*-dimethylsulphamoyl)phenothiazine. This idea is substantiated by the appearance of coupled waves that are consistent with the formation of this parent compound.

The electrochemical behaviour of quisultidine is unusual for phenothiazines and is probably related to the unusual pharmacological properties of this drug. The relationship between stable cation radical formation and psychotic activity of the phenothiazines is well documented.[6-8] The low psychotic activity of quisultidine is thus related to the difficulty in oxidation and the reactivity of the resulting radical. Administration of quisultidine probably results in the *in vivo* liberation of 2-(*N,N*-dimethylsulphamoyl)phenothiazine, which should be responsible for the negligible anticholinergic properties.

The results suggest the predictive value of cyclic voltammetry of phenothiazines for evaluating psychotic activity.

Thanks are expressed to Pharmuka Laboratories, Gennevilliers, France, for providing samples of quisultidine and to Le Fonds National Belge de La Recherche Scientifique (FNRS), Belgium, for financial support (to G. J. P.).

References

1. Patriarche, G. J., Chateau-Gosselin, M., Vandenbalck, J. L., and Zuman, P., *in* Bard, A. J., *Editor*, "Electroanalytical Chemistry," Volume 11, Marcel Dekker, New York, 1979.

2. Patriarche, G. J., and Lingane, J. J., *Anal. Chim. Acta*, 1970, **49**, 25.
3. Jarbawi, T. B., and Heineman, W. R., *J. Electroanal. Chem.*, 1982, **132**, 323.
4. Jarbawi, T. B., Heineman, W. R., and Patriarche, G. J., *Anal. Chim. Acta*, 1981, **126**, 57.
5. Bidan, G., and Genies, M., *N. J. Chim.*, 1981, **5**, 117.
6. Mahood, J. S., Packer, J. E., Searle, A. J. F., Willson, R. L., and Wolfenden, B. S., *in* Usdin, E., Eckert, H., and Forrest, I. S., *Editors*, "Phenothiazines and Structurally Related Drugs: Basis and Clinical Studies," Volume 7, Elsevier - North Holland, Amsterdam, 1980, p. 103.
7. Lovstad, R. A., *Biochem. Pharmacol.*, 1974, **23**, 1045.
8. Fenner, H., *in* Usdin, E., Forrest, I. S., and Carr, C. J., *Editors*, "The Phenothiazines and Structurally Related Drugs," Volume 5, Raven Press, New York, 1974.
9. Koch, H., *Pharm. Int.*, 1984, **1**, 6.
10. Prete, M., Kauffman, J.-M., Vire, J.-C., Patriarche, G. J., Debye, B., and Geuskens, G., *Anal. Lett.*, 1984, **17** (B12), 1391.

Paper A4/363
Received October 17th, 1984
Accepted November 7th, 1984

Studies of Calcium Ion-selective Electrodes in the Presence of Biochemical Materials

Sadjedah A. H. Khalil, Gwilym J. Moody and J. D. R. Thomas
Applied Chemistry Department, Redwood Building, UWIST, P.O. Box 13, Cardiff CF1 3XF, UK

Interferences by biochemical materials of responses towards calcium ions of calcium ion-selective electrodes have been studied for PVC matrix membrane electrodes based on calcium bis{di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate} sensor with dioctyl phenylphosphonate (I), tripentyl phosphate (II) or trioctyl phosphate (III) as solvent mediator. Experiments have also been carried out on electrodes made from commercial calcium ion-selective electrode membranes based on an ionophore sensor (electrode IV). E.m.f. changes of <0.5 mV were observed for up to 10^{-3} M starch, sucrose, uric acid, creatinine and bilirubin. Larger e.m.f. changes were observed for cholic acid, cholesterol, lecithin and vitamin D_2 (each of which were tested as solutions in ethanol or propan-1-ol added to the calcium ion containing solution in water or 0.15 M sodium chloride solution). Electrode III showed the greatest resistances to interferences by the biochemical components, followed by electrode IV. Interferences by urea and glucose were less than 1 mV for electrodes I–III and slightly more for electrode IV.

Keywords: *Calcium ions; body fluids; ion-selective electrodes; calcium determination; interferences*

Calcium levels in body fluids, such as plasma and serum, are closely controlled by several homeostatic mechanisms so that only accurate and precise determinations of calcium will reveal a loss of steady-state control and yield useful diagnostic information.[1] In this respect, the determination of the physiologically active ionised calcium can be effectively carried out only with calcium ion-selective electrodes (ISE), which have now eclipsed the methods of bioassay and spectrophotometry.[2] The several such studies with calcium electrodes up to 1976 have been reviewed[3] and subsequent studies and results may be gleaned from itemised lists.[4–8]

Many of the investigations on calcium ion measurements and their levels in biological fluids recognise the limitations and difficulties relating to the calibration of calcium electrodes. To help with electrode reproducibility and to decrease equilibration time, calcium electrodes for use in blood fluids have been standardised, for example, with buffers of ionised calcium standards in trypsin and triethanolamine buffers containing 0.150 M sodium chloride.[9–11] More recently, the semi-automated equipment now available for calcium ion measurements has been standardised and commercial assemblies compared by means of aqueous solutions prepared from analytical reagents and with pooled human serum.[2]

Many studies have been made to relate clinical observations to calcium ion-selective electrode data and to the determination of which measuring technique provides the most accurate values for clinical use.[12] Related to these are studies on the effect of protein concentration on ion-selective electrode measurements of ionised calcium.[13] Interferences of proteins in determinations of ionised calcium by the use of Orion SS-20, Nova 2 and Radiometer ICA1 analysers are considered to give differences between results for the original serum sample and an ultrafiltrate that are attributed to a predictable consequence of the Donnan equilibrium and not due to protein interferences.[14–16]

Investigations of the above kind are complemented by studies of calcium ion-selective electrodes with complexing ligands in calcium ion containing solutions.[17,18] However, on the whole there has been relatively little study of interference effects on calcium ion-selective electrodes by individual species of biochemical interest that parallel those with surfactants, which showed rather unusual interferences of calcium ion-selective electrodes by commercially available anionic surfactants.[19,20] This is relevant as components of body fluids can have surfactant properties and fears have been expressed[21] of the possibility of them interfering. Studies are described here on the effect of a selection of biochemicals on poly(vinyl chloride) (PVC) membrane calcium ion-selective electrodes containing a sensor of calcium bis{di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate} in conjunction with each of dioctyl phenylphosphonate, trioctyl phosphate and tripentyl phosphate as plasticising solvent mediators. Experiments have also been carried out on electrodes made from membranes obtained from a commercial supplier.

Experimental

Electrodes

Poly(vinyl chloride) matrix membrane electrodes with inner solutions of 10^{-1} M calcium chloride were assembled by the general procedure described previously[22,23] and the membrane compositions and electrode characteristics are summarised in Table 1. Membranes were cast in the normal way,[22,23] except that membrane IV was used as supplied.

Electrodes were conditioned overnight in 10^{-1} M calcium chloride solution and calibrated with serially diluted calcium chloride in the 10^{-1}–10^{-5} M range.

Reagents and Materials

All materials, except the following, were of the best analytical grade available.

Calcium bis{di[4-(1,1,3,3-tetramethylbutyl)phenyl]-phosphate},[24] which has the formula $Ca\{[CH_3C(CH_3)_2CH_2C(CH_3)_2C_6H_4O]_2.P(O)O\}_2$, and dioctylphenyl phosphonate,[25] which has the formula $C_6H_5P(O)[O(CH_2)_7CH_3]_2$, were prepared as described previously. Dioctyl phenylphosphonate is also listed by Lancaster Synthesis and in the Alfred Bader Rare Chemicals List of the Aldrich Chemical Company. Di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphoric acid is available commercially in admixture with the monoester (70 + 30 mixture) from the Mobil Chemical Company, Richmond, VA, USA, and procedures for separating the diester and preparing the calcium salts have been described.[24,26]

Procedure

The various electrodes (Table 1) were tested for interference by the biochemical materials in conjunction with a Corning Model 476002 reference electrode. The e.m.f. measurements were made with a Corning EEL Model 112 digital millivolt-

Table 1. Composition of master membranes and electrode characteristics. Standard deviations are given in parentheses for $n = 5$ membranes

		Electrode characteristics			
		$CaCl_2$ solutions		Solutions of $CaCl_2$ in 0.15 M NaCl	
Membrane No.	Composition*	Slope at 25 °C/mV	$E°$/ mV	Slope at 25 °C/mV	$E°$/ mV
I	0.36 g dioctyl phenylphosphonate	28.5 (±1.7)	61.5 (±0.3)	29.3 (±0.2)	58.1 (±0.5)
II	0.36 g tripentyl phosphate	27.3 (±1.1)	59.4 (±0.2)	28.8 (±0.1)	64.4 (±0.2)
III	0.36 g trioctyl phosphate	29.3 (±1.5)	57.6 (±0.5)	28.7 (±0.1)	56.1 (±0.2)
IV	Philips IS 561/SP Ca^{2+} membrane	26.3 (±1.5)	53.3 (±0.3)	26.6 (±1.1)	55.2 (±0.5)

* Membranes I–III also contained 0.04 g of calcium bis{di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate} in 0.17 g of PVC.

meter - pH meter, used in conjunction with a Servoscribe Model RE 4541 potentiometric chart recorder. The various test solutions were maintained at 25 ± 0.1 °C.

Normally, the effect of added component was examined in four background solutions, namely 10^{-2} and 10^{-3} M calcium chloride solution, and 10^{-2} and 10^{-3} M calcium chloride in a background of 0.15 M sodium chloride solution. Thus, aliquots (0.05 cm^3) of a solution of the component (5×10^{-2} M) under study were added to the appropriate background solution (25 cm^3) in which the calcium and reference electrode pair had been equilibrated to a steady response. E.m.f. readings were noted for each aliquot added and further aliquots were added until the background solution had reached at least 10^{-3} M concentration for the component. Each full run was duplicated by using a new calcium ISE. Duplicate runs agreed to better than 2%.

Results

The responses of calcium ISEs based on membranes I–IV to added surfactants have already been documented.[20] These have been repeated here for sodium dodecylsulphate (SDS) and extended to dodecylbenzene sodium sulphonate (DBSS) for comparison with the various biochemical materials studied. E.m.f. changes of smaller than 0.5 mV were observed for up to 10^{-3} M starch solution, sucrose, uric acid, creatinine and bilirubin solutions for all four electrodes. Table 2 summarises the e.m.f. changes corresponding to the presence of 10^{-3} M of the added material and Figs. 1 and 2 illustrate the change in e.m.f. pattern for selected materials on adding 0.05-cm^3 aliquots of the 5×10^{-2} M solution in ethanol (Fig. 1) or in propan-1-ol (Fig. 2) to calcium chloride solutions.

Because of limited solubility in water, some of the materials in Table 2, cholic acid, lecithin, cholesterol and vitamin D_2 (those for which data are illustrated in Figs. 1 and 2), were disssolved in ethanol and propan-1-ol. However, cholesterol is only slightly soluble in ethanol. The effect of added alcohol alone on electrode response in the various solutions is shown in Figs. 3 (ethanol) and 4 (propan-1-ol).

Discussion

The four calcium ion-selective electrodes chosen for study include three (I–III) based on calcium bis{di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate} with solvent mediators known to promote calcium ion selectivity.[27] Electrode IV is based on a neutral carrier ionophore. In all instances, interferences are expressed in terms of e.m.f. changes on bringing the various background solutions up to 10^{-3} M or slightly more in added component (Table 2 and Figs. 1 and 2). In practice, however, the levels can be different, *e.g.*, cholesterol is present in blood serum and plasma at 3.6 to 6.7 mM,[28] but some components will have concentrations of 10^{-3} M in these fluids.

Anionic surfactants were included for study in order to link their known effects on the calcium ion-selective electrodes with the new observations of this study. Thus, the general effect of added surfactant, even at about 2×10^{-5} M, is to lower the e.m.f. response of calcium ion-selective electrodes when in contact with various test solutions, regardless of whether or not calcium is present in the cell test solutions.[19,20] This mechanism is believed not to involve complexation with calcium ions,[19] and the surfactants promote the leaching of membrane components.[20]

Previous studies[19,20] have shown that the effect of surfactant is less pronounced for certain membrane compositions and, in particular, calcium ion-selective electrodes based on the calcium bis{di[4(1,1,3,3-tetramethylbutyl)phenyl]phosphate} sensor with trioctyl phosphate solvent mediator is much superior to other membrane systems in resisting interference by anionic surfactants.[20]

The utility of trioctyl phosphate solvent mediator in the composition (electrode III) is also shown in this study (Table 2 and Figs. 1 and 2). Thus, the extent of interference by the various biochemical components that are constituents of body fluids is less for electrode III than for electrodes with either dioctyl phenylphosphonate or tripentyl phosphate as solvent mediators (electrodes I and II). However, there must be regard to the effect of ethanol and propan-1-ol interference on electrode response (Figs. 3 and 4), which will be superimposed on the interferences of the biochemical solute.

Propan-1-ol seriously affects electrodes I and II, but the effect in the other circumstances (Figs. 3 and 4) is <5 mV for 0.5 cm^3 of added alcohol to 25 cm^3 of test solution (*ca.* 2%). Differing from the other electrodes, the e.m.f. of electrode III increases when alcohol is added (Figs. 3 and 4) and this observation should not be ignored in relation to the apparently small interferences by the biochemical components recorded in Table 2 and Figs. 1 and 2.

It is only for electrode II that the direction of e.m.f. change (an increase) corresponds to that observed[29,30] for solid-state cation-responsive electrodes. For the other three electrodes, the presence of alcohol gives a decrease in the e.m.f., as has been previously observed for up to 20% ethanol with a barium -

Table 2. E.m.f. changes for cells with calcium ISEs (I–IV) corresponding to various components added to calcium chloride test solutions

	ΔE caused by added components to solutions/mV															
	DOPP (I)*				TPP (II)†				TOP (III)‡				Philips membrane electrodes (IV)			
	$CaCl_2$ in water		$CaCl_2$ in 0.15 M NaCl		$CaCl_2$ in water		$CaCl_2$ in 0.15 M NaCl		$CaCl_2$ in water		$CaCl_2$ in 0.15 M NaCl		$CaCl_2$ in water		$CaCl_2$ in 0.15 M NaCl	
Component	10^{-2} M	10^{-3} M	10^{-2} M	10^{-3} M	10^{-2} M	10^{-3} M	10^{-2} M	10^{-3} M	10^{-2} M	10^{-3} M	10^{-2} M	10^{-3} M	10^{-2} M	10^{-3} M	10^{-2} M	10^{-3} M
Component added as 5×10^{-2} M solution in water or alcohol to 10^{-3} M in the calcium chloride solution—																
SDS	−55.0	−65.8	−85.0	−85.7	−63	−32.0	−40.0	−15.0	−9.0	−17.3	−2.8	−7.2	−102	−73.5	−26.5	−25.1
DBSS	−9.1	−3.3	−12.5	−9.7	−35.9	−17.0	−41.5	−24.0	−2.0	−2.0	−3.8	−1.5	−55.9	−93.3	−39.8	−59.8
Cholic acid (in propanol)	−10.4	−6.7	−24.3	−8.8	−26	−16.9	−10.0	−12.7	−4.0	−0.42	−2.2	−2.8	−2.4	−0.5	−1.2	−1.6
Cholic acid (in ethanol)	−17.1	−16.3	−13.1	−17.4	−9.6	−11.6	−6.8	−11.3	−0.8	−2.6	+0.5	+1.9	+0.3	+3.3	−1.6	+0.4
Cholesterol (in propanol)	−3.0	−11.2	−3.1	−2.3	−21	−8.8	−12.6	−10.1	−2.0	+0.4	−0.6	+1.2	−7.1	−1.7	−2.2	−0.3
Cholesterol (in ethanol)	−3.1	−5.1	+4.1	+2.5	−2.8	−3.2	−0.6	−3.5	+1.1	+1.6	+1.7	+0.2	−3.5	−0.6	0.0	−1.8
Lecithin (in propanol)	−8.6	−3.7	−6.4	−6.4	−10.0	−9.2	−7.3	−10.9	−5.0	+1.6	−0.2	+1.3	−1.0	−2.6	−2.2	+0.6
Lecithin (in ethanol)	−2.4	−13.7	−5.6	−6.5	−5.4	−5.3	−10.5	−9.8	+1.0	+1.6	+0.2	+0.2	−0.6	+0.8	+0.4	+3.3
Vitamin D_2 (in propanol)	−3.5	−8.5	−5.3	−5.3	−10.3	−6.2	−11.2	−10.9	−4.1	−5.6	−0.2	−7.6	−3.6	−4.0	−1.5	−3.1
Vitamin D_2 (in ethanol)	−2.4	−3.8	−3.0	−2.8	−5.0	−3.5	−6.5	−7.0	+2.0	−7.8	+1.0	+2.8	+1.0	+2.0	+5.7	+1.7
Urea	0.0	+0.1	−0.9	+0.2	+0.3	−0.8	+0.8	−0.1	0.0	−0.1	−0.2	+0.2	−1.7	+0.9	−0.1	−0.8
Glucose	0.0	+0.2	−0.2	−0.1	0.0	0.0	−1.1	+0.4	0.0	−0.3	−0.3	−0.1	−4.5	0.0	−0.5	−0.5
Ethanol or propan-1-ol added to give 2% solution—																
Ethanol	−2.8	−2.7	−4.8	−3.8	−2.6	−3.8	−2.5	−3.5	+3.7	+4.8	+2.1	+3.3	−0.3	+0.2	+0.5	+0.1
Propan-1-ol	−6.7	−13.0	−9.1	−8.2	−10.5	−15.6	−8.0	−10.2	+3.4	+5.1	+3.2	+2.2	−2.3	−2.0	−2.0	−3.0

* Dioctyl phenylphosphonate electrodes.
† Tripentyl phosphate electrodes.
‡ Trioctyl phosphate electrodes.

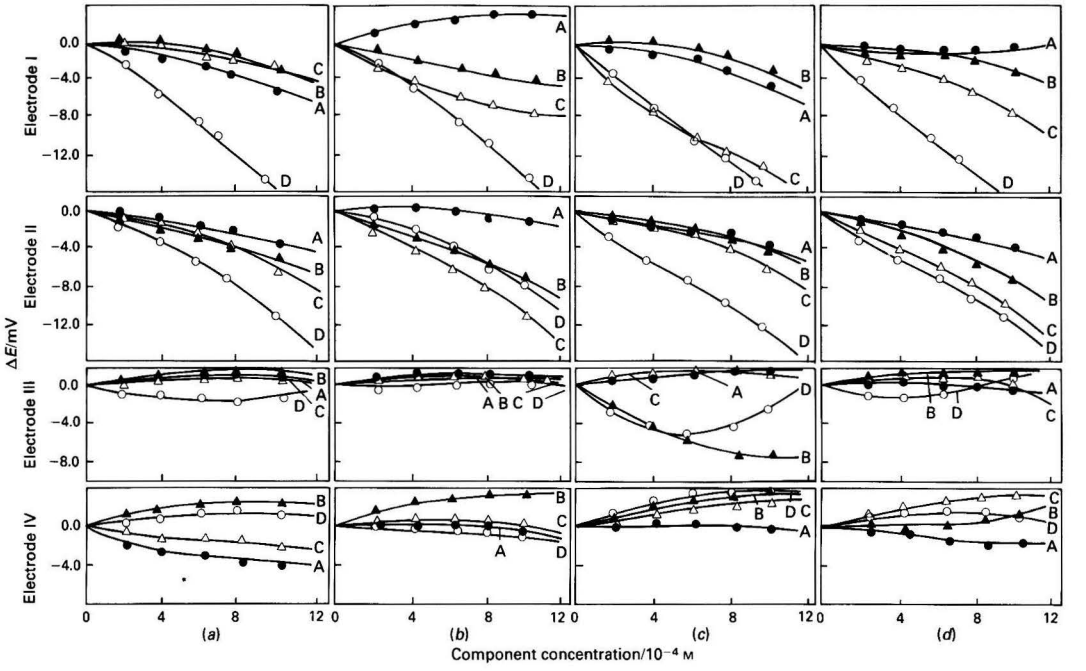


Fig. 1. Illustrative e.m.f. change patterns (electrodes I–IV) for A, cholesterol; B, vitamin D_2; C, lecithin; and D, cholic acid added in ethanol to 25 cm³ of 10^{-2} M calcium chloride in (*a*) water, (*b*) 10^{-2} M calcium chloride in 0.15 M sodium chloride, (*c*) 10^{-3} M calcium chloride in water and (*d*) 10^{-3} M calcium chloride in 0.15 M sodium chloride

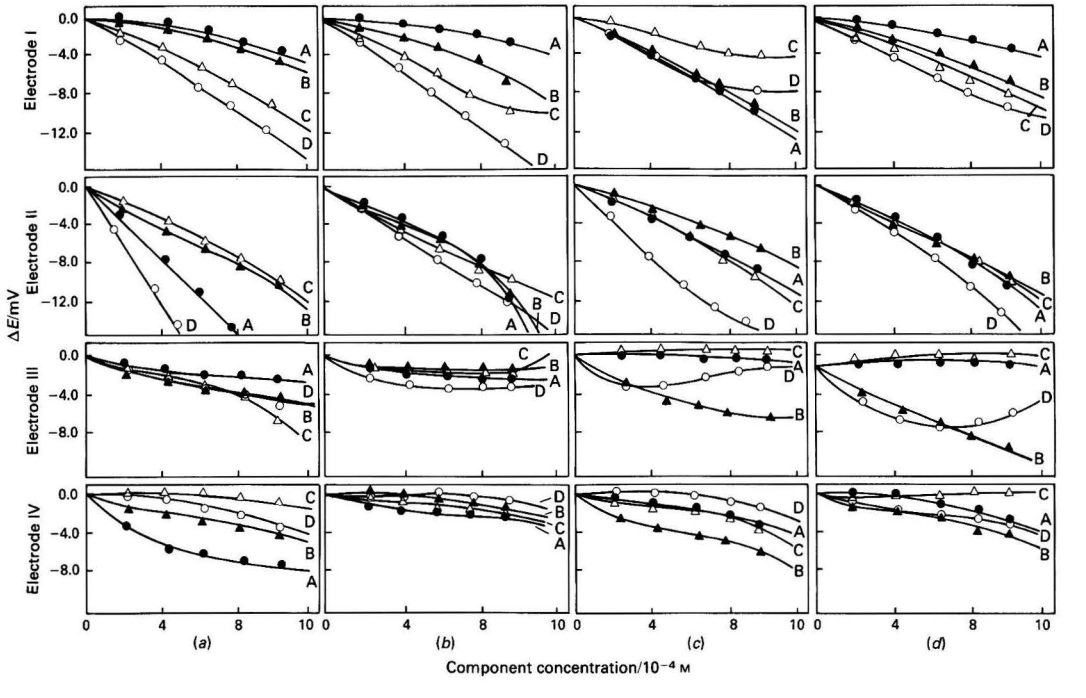


Fig. 2. Illustrative e.m.f. change patterns (electrodes I–IV) for A, cholesterol; B, vitamin D_2; C, lecithin; and D, cholic acid added in propan-1-ol to (*a*) 25 cm³ of 10^{-2} M calcium chloride in water, (*b*) 10^{-2} M calcium chloride in 0.15 M sodium chloride, (*c*) 10^{-3} M calcium chloride in water and (*d*) 10^{-3} M calcium chloride in 0.15 M sodium chloride

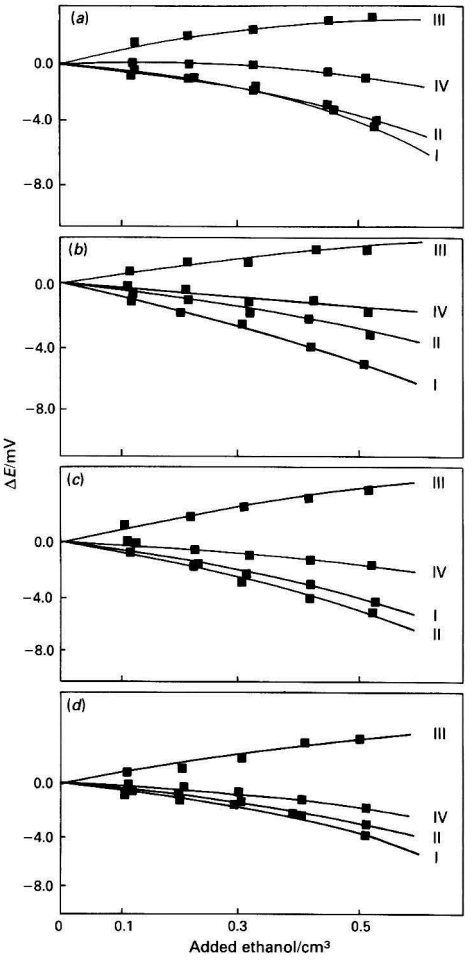


Fig. 3. E.m.f. change patterns (electrodes I–IV) for ethanol added to solutions (*a*)–(*d*) specified in Fig. 1

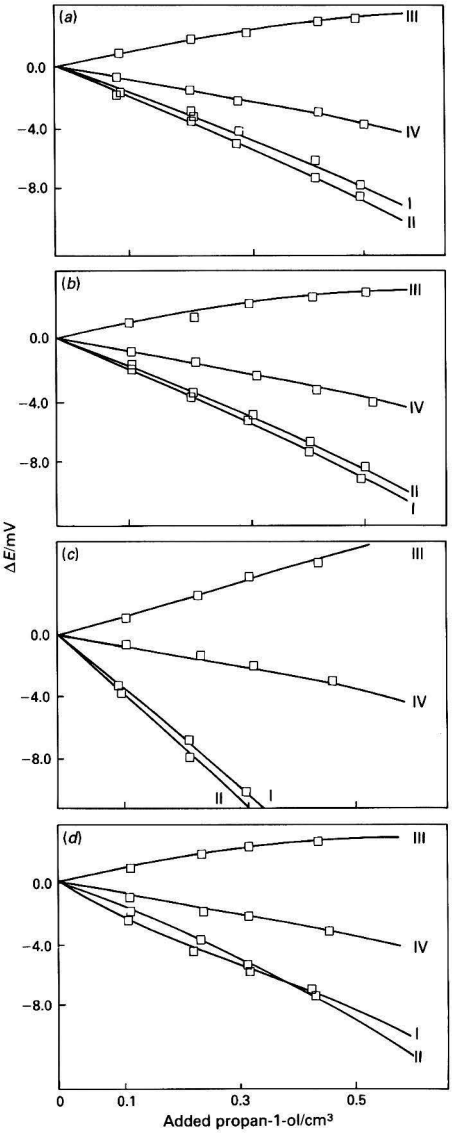


Fig. 4. E.m.f. change patterns (electrodes I–IV) for propan-1-ol added to solutions (*a*)–(*d*), specified in Fig. 2

PVC matrix membrane electrode based on the tetraphenylborate salt of the barium complex with nonylphenoxypolyethoxylate as sensors with 2-nitrophenyl phenyl ether as solvent mediator.[31] However, the energetic and solution considerations will be much more complicated for polymer - sensor plasticising solvent mediator membranes than for the more straightforward solid-state ion-selective electrode membranes.

The reason for the interferences by the biochemical components may be the presence of calcium binding sites supplemented by the surfactant properties of some of the components, *e.g.*, lecithin. In this respect the fall of e.m.f. induced by the various interferents is in the same direction as observed by Burr[32] for organic bases, namely, triethanolamine, morpholine, ethanolamine and piperidine for an Orion Model 99–20 flow-through calcium ion-selective electrode. Burr observed increases in e.m.f. with lutidine and imidazole.

Electrode IV, which is characterised by gross interferences by anionic surfactants (reference 20, Table III and Figs. 1 and 2) is similar to electrode III with regard to relative freedom from interference by the biochemical components studied here (Table 2 and Figs. 1 and 2). This suggests a different mechanism for interfering biochemical materials than for anionic surfactants. As mentioned above, electrode IV is also similar to electrode III by being relatively resistant to the effect of alcohol, but the e.m.f. changes being in the opposite direction (Figs. 3 and 4).

Conclusion

Similarly to the observations with anionic surfactants,[20] the replacement of the normally recommended dioctylphenyl phosphonate solvent mediator by trioctyl phosphate in the PVC calcium ion-selective electrode based on bis{di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate} sensor, as in electrode III, can reduce interferences from components likely to occur in body fluids. Electrodes made from commercial calcium ion-selective electrode membranes (electrode IV)

behave similarly. However, the efficacy of these systems needs to be tested on a calcium ion-free type of blood fluid.

The authors thank the Foundation of Technical Institutes, Baghdad, Iraq, for paid leave of absence and a studentship (granted to S. A. H. K.). Dr. G. de Oliveira Neto, University of Sao Paulo, Brazil, is thanked for preliminary experimental data with electrode II obtained during study leave at UWIST by the sponsorship of the British Council, London, and the Conselho Nacional de Desenvolvimento Cientifica e Technologica Brazil. Dr. J. L. F. Costa Lima, University of Oporto, Portugal, is also thanked for discussions made possible by a travel grant from the North Atlantic Treaty Organisation.

References

1. Lott, J. A., *CRC Crit. Rev. Anal. Chem.*, 1972, 41.
2. Marshall, R. W., RSC International Symposium on Electroanalysis in Clinical, Environmental and Pharmaceutical Chemistry, UWIST, Cardiff, 13–16 April, 1981, Paper 2.
3. Moody, G. J., and Thomas J. D. R., *Prog. Med. Chem.*, 1977, **14**, 51.
4. Moody, G. J., and Thomas, J. D. R., *Ion-Sel. Electrode Rev.*, 1979, **1**, 251.
5. Moody, G. J., and Thomas, J. D. R., *Ion-Sel. Electrode Rev.*, 1980, **2**, 219.
6. Moody, G. J., and Thomas, J. D. R., *Ion-Sel. Electrode Rev.*, 1981, **3**, 209.
7. Moody, G. J., and Thomas, J. D. R., *Ion-Sel. Electrode Rev.*, 1982, **4**, 233.
8. Moody, G. J., and Thomas, J. D. R., *Ion-Sel. Electrode Rev.*, 1983, **5**, 243.
9. Hattner, R. S., Johnson, J. W., Bernstein, D. S., Wachman, A., and Brackman, J., *Clin. Chim. Acta*, 1970, **28**, 67.
10. Li, T.-K., and Piechocki, J. T., *Clin. Chem.*, 1971, **17**, 411.
11. Seamonds, D., Towfighi, J., and Arvan, D. A., *Clin. Chim. Acta*, 1972, **18**, 155.
12. Ladenson, J. H., *Anal. Proc.*, 1983, **20**, 554.
13. Payne, R. B., RSC International Symposium on Electroanalysis in Biomedical, Environmental and Industrial Sciences, UWIST, Cardiff 5–8 April 1983, Paper 13.
14. Thode, J., Fogh-Andersen, N., Siggaard Andersen, M., and Siggaard Andersen, O., *Ann. Clin. Biochem.*, 1983, **20**, 271.
15. Thode, J., Fogh-Andersen, N., and Siggaard-Andersen, O., *Clin. Chem.*, 1983, **29**, 1554.
16. Maas, A. H. J., Kofstad, J., Siggaard-Andersen, O., and Kokholm, G., "Ionised Calcium, Sodium and Potassium by Ion-Selective Electrodes," Priv. Press, Copenhagen, 1984.
17. Craggs, A., Moody, G. J., and Thomas, J. D. R., *Analyst*, 1979, **104**, 412.
18. Craggs. A., Moody, G. J., and Thomas, J. D. R., *Analyst*, 1979, **104**, 961.
19. Craggs, A., Moody., G. J., Thomas, J. D. R., and Birch, B. J., *Analyst*, 1980, **105**, 426.
20. Frend, A. J., Moody, G. J., Thomas, J. D. R., and Birch, B. J., *Analyst*, 1983, **108**, 1072.
21. Birch, B. J., and Clarke, D. E., *Anal. Chim. Acta*, 1973, **67**, 387.
22. Moody, G. J., Oke, R. B., and Thomas, J. D. R., *Analyst*, 1970, **95**, 910.
23. Craggs, A., Moody, G. J., and Thomas, J. D. R., *J. Chem. Educ.*, 1974, **51**, 541.
24. Craggs, A., Delduca, P. G., Keil, L., Key, B. J., Moody, G. J., and Thomas, J. D. R., *J. Inorg. Nucl. Chem.*, 1978, **40**, 1483.
25. Craggs, A., Delduca, P. G., Keil, L., Moody, G. J., and Thomas, J. D. R., *J. Inorg. Nucl. Chem.*, 1978, **40**, 1943.
26. Cattrall, R. W., Drew, D. M., and Hamilton, I. C., *Anal. Chim. Acta*, 1975, **76**, 269.
27. Moody, G. J., Nassory, N. S., and Thomas, J. D. R., *Analyst*, 1978, **103**, 68.
28. Whitby, L. G., Percy-Robb, I. W., and Smith, A. F., "Lecture Notes on Clinical Chemistry," Second Edition, Blackwells, Oxford, 1980, p. 16.
29. Rechnitz, G. A., and Zamochnik, S. B., *Talanta*, 1964, **11**, 979.
30. Kakabadse, G. J., *Ion-Sel. Electrode Rev.*, 1981, **3**, 127.
31. Sanghera, G. S., and Thomas, J. D. R., unpublished data.
32. Burr, R. G., *Clin. Chim. Acta*, 1973, **43**, 311.

Paper A4/379
Received October 10th, 1984
Accepted December 5th, 1984

Ion-selective Determination of Total Metal Ion Concentration in an Excess of Complexing Ligand Using the Standard Additions Method

Liliana Ilcheva, Maria Polianova, Jordan Dalukov* and Brian R Chapman†
Analytical Chemistry Department, Higher Chemical-Technological Institute, 8, Kliment Ohridski St., Sofia, 1156, Bulgaria

An ion-selective potentiometric determination of total calcium and silver ion in the presence of an excess of complex-forming ligands, nitrilotriacetic acid and sodium thiosulphate, respectively, is described. A standard additions method with a Philips plastic calcium and solid-state silver ion-selective electrodes was used. This method has been applied to 1 mg l^{-1} of calcium and 10^{-3}–10^{-5} mol l^{-1} silver solutions containing an ionic strength adjustor and an excess of complex-forming ligand. Calcium measurements in solutions containing an excess of ligand and other complexing ions are reported, *e.g.*, HPO_4^{2-} and NO_3^-. This method has also been applied to the determination of total silver in black and white photographic paper containing 0.8 g m^{-2} of silver. Dissolution of the paper's silver in an excess of thiosulphate is followed by a standard additions procedure. Suitable conditions for calcium and silver determinations are reported and advantages gained in precision of measurement, electrode sensitivity and speed of analysis are discussed.

Keywords: *Ion-selective potentiometry; calcium and silver determination; standard additions; photographic paper*

The ion-selective potentiometric determination of the total metal ion in many inorganic matrices is severely handicapped by the ability of the metal ion to form strong complexes with common anions. For example, in the photographic industry, direct potentiometric determination of total silver in thiosulphate fixing solutions using a silver ion-selective electrode is not practicable because of the formation of silver thiosulphate complexes such as $Ag(S_2O_3)^-$. Free silver ion concentrations less than an electrode's practical limit of detection, 10^{-7} mol l^{-1}, and only a small fraction of the total metal in solution are commonly found. This difficulty applies equally to the calcium ion and has restricted the application of an otherwise fast, inexpensive and non-destructive potentiometric technique using calcium, silver and other metal ion-selective electrodes. Removal of complexing anions is often not practicable or involves several time-consuming sample preparation steps, *e.g.*, elution through an ion-exchange column. In several applications, complexation of the measured ion can be prevented by the addition of strong "decomplexing" agents to the sample. For example, fluoride measurement in potable water[1] commonly involves addition of cyclohexylenediaminetetraacetic acid to complex Fe^{3+}, Mg^{2+} and Al^{3+} preferentially. Unfortunately, specific "decomplexing" agents for many common inorganic anions suitable for use with the calcium and silver ion-selective electrodes have not yet been reported.

In 1971, Moody and Thomas[2] outlined the use of the standard additions procedure to determine the total metal ion, both free and complexed, in the presence of an excess of complexing ligand. A method based on the standard additions technique was originally proposed by Orion Research and describes the determination of total silver in photographic fixing solutions.[3] However, neither this method[3] nor a recent review of the application by Mascini[4] include results to demonstrate the efficacy and conditions suitable for accurate determination of total silver in thiosulphate. Similar analytical problems have been encountered with the rapid determination of total silver in coated photographic papers or emulsions. Numerous potentiometric, titrimetric, polarographic and X-ray fluorescence methods have been published[5–18] and regularly used in the photographic industry. Many of them are either time consuming or require expensive and, in some instances, toxic reagents, making them unsuitable for routine quality assurance purposes.

A recent potentiometric method[19] for photographic emulsions and fixing-baths involves dissolution of the silver in thiosulphate and titration with thioacetamide solution using a silver ion-selective electrode as an end-point indicator. The plastic calcium ion-selective electrode has also been the subject of several recent studies involving complex-forming ligands. In particular, the use of calcium ion buffer solutions based on complexing ligands nitrilotriacetic acid (NTA) and ethylenediaminetetraacetic acid (EDTA) for linear calibration of calcium ion-selective electrodes has been reported.[20,21] For example, an excess of NTA as a ion buffer allows the direct measurement of free calcium ion activity down to 10^{-7} mol l^{-1}. Experiments with carboxylates[22,23] have confirmed this. However, the determination of total calcium concentration is essential for many applications, particularly when traditional photometric methods have been previously and routinely applied. In conclusion, the standard additions procedure for the determination of total metal concentration with its advantages in accuracy, reliability and time saving has not been tested on the calcium electrode in the presence of complex-forming ligands or on the silver electrode in photographic papers.

This paper describes the conditions for the determination of the total calcium and silver concentration, both free and complexed ion, in the presence of an excess of complex-forming ligands, nitrilotriacetic acid (NTA) and thiosulphate, respectively. The standard or known additions method involves the addition of a known volume of a standard calcium or silver solution to each sample. The electrode potential change after addition is related to the sample's original calcium or silver concentration. This technique has been applied to solutions containing 1 mg l^{-1} of calcium both with and without an excess of NTA. The importance of ionic strength adjustment and pH control of the sample was investigated. Comparison of the calcium results from ion-selective measurement using an excess of NTA with those without ligand and from titrimetric analysis showed an improvement in reproducibility and measuring range for the calcium electrode. Further measurements were made on calcium solutions containing high concentrations of complex-

* Present address: Fohar Ltd., Sofia 1616, Bulgaria.

† To whom correspondence should be addressed at the following address: Applications Laboratory, Pye Unicam Ltd., York Street, Cambridge CB1 2PX, UK.

ing ions such as HPO_4^{2-} and NO_3^-. In addition, total silver concentrations between 10^{-3} and 10^{-5} mol l^{-1} were determined in solutions containing an ionic strength adjustor and thiosulphate levels in the range 70–11 400-fold higher than those of silver. This standard additions method has also been applied to the determination of total silver in commercially available black and white photographic papers containing 0.8 g m^{-2} of silver. Dissolution of silver in the emulsion coating is achieved with an excess of sodium thiosulphate and followed by measurement using a standard additions procedure. Comparison of the silver results from ion-selective measurement with those from traditional titrimetric analysis gave satisfactory results. The standard additions method in the presence of an excess of complex-forming ligand enables total calcium and silver to be determined with improved accuracy of measurement.

Experimental

Apparatus

Philips plastic calcium (IS561-Ca) and solid-state silver (IS550-Ag) ion-selective electrodes were used; they were stored in a 10^{-3} mol l^{-1} solution of either calcium or silver between measurements and overnight. A double-junction calomel reference electrode (Philips Type R44/2-SD/1) contained a salt bridge of 1 mol l^{-1} potassium nitrate. All ion-selective measurements were made with a Philips Model PW9416 microprocessor Ion-Selective Analyser, using the standard additions mode. The pH measurements were made with a Seibold Model G104 pH meter. All solutions were stirred continuously using a magnetic stirrer with small PTFE stirring bars.

Reagents

All solutions were prepared in doubly distilled water from analytical-reagent grade materials.

Calcium standard solutions, 1.000 and 10.00 mg l^{-1}. Prepared from calcium carbonate according to the method described by Midgley and Torrance.[24] These solutions were standardised titrimetrically with EDTA solution using Calcon indicator as described by Gelo.[25]

Nitrilotriacetic acid (NTA) solution, 0.1 mol l^{-1}. Prepared by dissolving 19.115 g of NTA in 1 l of 1.0 mol l^{-1} sodium hydroxide solution.

Buffer solutions

Disodium tetraborate buffer, pH 11.023. Prepared by dissolving 6.183 g of boric acid in 0.5 mol l^{-1} sodium hydroxide solution and diluting with water to 0.5 l.

Ammonia buffer, pH 10.00. Prepared by mixing 142 ml of concentrated ammonia solution (density 0.88 g cm^{-3}) with 17.5 g of ammonium chloride and diluting with water to 250 ml.

Acetate buffer, pH 5.50. Prepared by mixing 73.4 ml of 1.0 mol l^{-1} acetic acid and 50.0 ml of 1 mol l^{-1} sodium hydroxide solution and diluting with water to 1 l.

Aminoacetic acid buffer, pH 5.02. Prepared by dissolving 7.50 g of aminoacetic acid and 5.85 g of sodium chloride in water and diluting the solution to 1 l.

Complex-forming ions of HPO_4^{2-} and NO_3^- for calcium. Prepared from analytical-reagent grade H_3PO_4 and KNO_3.

Silver stock solution, 0.1 mol l^{-1}. Prepared by dissolving 17 g of silver nitrate in water and diluting the solution to 1 l. The stock solution was standardised titrimetrically with KSCN using an indicator of Fe^{3+}, as described by Vogel.[5]

Silver solutions, 10^{-3}–10^{-5} mol l^{-1}. Prepared by sequential volume dilution of the stock solution.

Sodium thiosulphate solutions, 1.5 and 2.5 mol l^{-1}. Prepared by dissolving 375 and 625 g of $Na_2S_2O_3.5H_2O$ in water and diluting the solution to 1 l. Both solutions were standardised iodimetrically.[26]

Ionic strength adjustors

Potassium chloride, sodium and ammonium perchlorate.

Photographic paper

Black and white, 10 × 10 cm, from Fohar Ltd., Sofia, Bulgaria.

Determination of the Electrode Sensitivity

A preliminary calibration of the calcium ion-selective electrode was made by measuring the electrode potential of 1.000 and 10.00 mg l^{-1} standard solutions of calcium. The pH was maintained and ionic strength adjusted by addition of the buffer and either KCl, $NaClO_4$ or NH_4ClO_4, respectively (see Table 1).

The silver ion-selective electrode was calibrated using 10^{-3} and 10^{-2} mol l^{-1} standard solutions of silver. Sufficient $NaClO_4$ or NH_4ClO_4 was added to each standard solution to maintain a constant ionic strength of 0.1 mol l^{-1}. Electrode sensitivities (millivolt per decade change in concentration) were determined automatically by the PW9416 Ion-Selective Analyser using the two-point calibration mode. This was carried out before each series of replicate analyses on both 1.000 mg l^{-1} calcium and silver solutions using the standard additions procedure.

Standard Additions Procedure

Calcium measurements

A 25.00-ml portion of each 1.000 mg l^{-1} calcium solution containing buffer, ionic strength adjustor, with and without NTA, was pipetted into a 50-ml plastic beaker. Sufficient 0.1 mol l^{-1} NTA was added to samples 3 and 7 to provide a molar ratio of NTA to calcium of 40:1; solution compositions are included in Table 1. The electrode pair was immersed in the solution and the electrode potential allowed to equilibrate for about 2–3 min and recorded. A 0.50-ml or 1.00-ml portion of a 100 mg l^{-1} calcium standard solution was immediately pipetted into the 1.000 mg l^{-1} solution using a micropipette and the electrode potential change recorded. A similar standard additions procedure was followed for 1.000 mg l^{-1} calcium solutions containing known concentrations of complexing ions HPO_4^{2-} and NO_3^- in the presence of an excess of NTA.

Silver measurements in thiosulphate solutions

Stock silver and sodium thiosulphate solutions were transferred into a 100.00-ml calibrated flask in proportions to provide molar ratios of thiosulphate to silver of 70, 100, 700, 1400, 10 000 and 11 400. Sufficient sodium or ammonium perchlorate was added to maintain a constant ionic strength of 0.1 mol l^{-1} and each solution diluted with water to 100.00 ml. A 25.00–30.00-ml portion of each thiosulphate - silver solution was pipetted into a 50-ml plastic beaker. The electrode pair was immersed in the solution and the electrode potential allowed to equilibrate for about 2–3 min and recorded. A 0.1–0.8-ml portion of a known concentrated silver standard solution was immediately pipetted into the silver and thiosulphate solution using a micropipette and the electrode potential change recorded. Standard silver concentrations approximately 100 times more concentrated than the expected silver concentration result were used, *i.e.*, 0.1–10^{-3} mol l^{-1} Ag.

Determination of silver in photographic papers

A black and white photographic paper (10 × 10 cm) was cut into 1 × 1 cm pieces and transferred into a 250.00-ml calibrated flask. A 100.00-ml volume of the 1.5 mol l^{-1} sodium thiosulphate solution was pipetted into the flask and the contents were shaken vigorously for 5 min to dissolve the silver. The solution was diluted with water to 250.00 ml and a 50.00-ml aliquot pipetted into a 100-ml plastic beaker. The electrode pair was immersed in the solution and the electrode potential allowed to equilibrate for about 2–3 min and

recorded. Either a 0.3- or 0.4-ml portion of a 9.94×10^{-2} mol l^{-1} silver standard solution was immediately pipetted into the thiosulphate solution containing dissolved silver by a micropipette and the electrode potential change recorded.

Determination of total calcium and silver by standard additions in the presence of an excess of complexing ligand

The standard additions method requires the addition of a known volume of a standard solution containing the sensed ion to a known volume of sample solution. A subsequent increase in the ion concentration of the sample solution is sensed by the ion-selective electrode and a change in the electrode potential incurred. The theoretical relationships for a standard addition in the presence of an excess of complexing ligand have previously been outlined by several workers.[2,4]

In the presence of an excess of complexing ligand the initial electrode potential (E) is proportional to the free metal ion concentration (C_{total}) and described by equation (1), which was derived from the Nicolskii expression for a cation-selective electrode:

$$E = E_0 + S\log[fC_{Total} + K_i^{Pot} f_i C_i^{Z/Z_i}] \quad .. \quad (1)$$

where S is the electrode sensitivity or slope (millivolts per decade change in concentration) obtained from electrode calibration immediately before measurement; E_o is the standard potential for the electrode pair; C_{total} is the total concentration of metal in the sample solution, both complexed and free ion; and f is the fraction of the total concentration of metal present in the form of free metal ions. The contribution to electrode potential from interfering ions is given by the product of its selectivity coefficient K_i^{Pot}, interferent concentration (C_i), charges of the measured ion (Z) and interferent ion (Z_i), where f_i is the fraction of the total interferent concentration present as the free ion. The change in electrode potential (ΔE) after standard additions is proportional to the increase in metal concentration from the standard solution, ΔC (grams of ion per litre). In the absence of any interfering ions, ΔE is described by equation (2), which was derived from equation (1) without the $K_i^{Pot} f_i C_i^{Z/Z_i}$ term:

$$\Delta E = S\log f\frac{(C_{Total} + \Delta C)}{fC_{Total}} \quad .. \quad .. \quad (2)$$

In the presence of an excess of complexing ligand, the fraction of free metal ion to total metal in solution (f) is assumed to be constant before and after standard additions. Rearrangement of equation (2) and elimination of fraction f enables the total metal concentration, C_{Total} (mol l^{-1}), of the sample to be calculated from equation (3):

$$C_{Total} = \frac{C_{St.}\,V_{St.}}{V_{Sample}}\left[10^{\Delta E/S}\left(\frac{V_{St.}}{V_{Sample}} + 1\right) - 1\right]^{-1} \quad .. \quad (3)$$

where $V_{St.}$ (l) is the volume of added standard solution containing a concentration $C_{St.}$ (mol l^{-1}) of the ion to be measured and V_{Sample} (l) the volume of sample solution. The PW9416 Ion-Selective Analyser automatically calculated and displayed the total calcium and silver concentrations, both free and complexed ion (C_{Total}), using equation (3) immediately after standard additions and a stable electrode potential change was recorded. The conversion of calcium concentration results in mol l^{-1} into mg l^{-1} was also provided by the PW9416 Ion-Selective Analyser. Silver results from coated photographic emulsions were converted from mol l^{-1} in thiosulphate solution into g m^{-2} of silver on the original paper.

Confirmatory test for the determination of total silver in photographic paper

The silver content of the photographic paper was determined using a titrimetric method.[5,27] A 10 × 10 cm photographic paper was exposed to light, developed, washed, dried and cut into pieces. The silver was dissolved in 6.0 mol l^{-1} nitric acid and titrated with 0.01 mol l^{-1} NH_4SCN using $NH_4Fe(SO_4)_2.12H_2O$ as the indicator. The measured silver content was calculated in g m^{-2} on the original photographic paper.

Results and Discussion

The standard additions method was applied to the analysis of a 1.000 mg l^{-1} calcium solution both with and without the presence of an excess of complex-forming ligand nitrilotriacetic acid. A similar procedure was adopted for the determination of total silver in solutions containing 9.94×10^{-6}–9.994×10^{-4} mol l^{-1} of Ag in the presence of an excess of thiosulphate ligand as well as black and white photographic papers containing 0.8 g m^{-2} of Ag.

An accurate determination of electrode sensitivity was made before each fresh series of replicate analyses. This procedure helped to establish the best accuracy of measurement. Only small changes in calcium and silver electrode sensitivities were noted during each replicate sequence of measurements. These were typically in the range 24.2–27.8 and 52.8–56.2 mV decade^{-1} for the calcium and 9.940×10^{-4} mol l^{-1} silver solutions, respectively. Similarly, no significant change in electrode sensitivity was noted during 12 replicate analyses on photographic papers.

None of the complexing ligands, nitrilotriacetic acid and sodium thiosulphate buffers, ionic strength adjustors and other complexing ions were shown to have any serious detrimental effects on either calcium or silver ion-selective response. In general, stable electrode potential changes after standard additions in the range 10–15 and 9.5–32.6 mV were achieved for calcium and silver solutions, respectively, after waiting times of less than 2 min. A typical change of 27 mV was recorded during the photographic paper analysis.

All electrode potential readings were taken as constant when a change of 0.1 mV or less was observed over a 30-s period.

Results for the determination of total calcium in 1.000 mg l^{-1} solutions containing different buffers and ionic strength adjustors, both with and without an excess of NTA are given in Table 1. Four buffers of acetate, aminoacetic acid, ammonia and disodium tetraborate were used. The average calcium concentration in mg l^{-1} from between 4 and 18 replicate analyses is presented for each calcium solution with its coefficient of variation.

Results for total silver in 9.994×10^{-4}, 9.940×10^{-4} and 9.940×10^{-6} mol l^{-1} silver solutions containing an ionic strength adjustor and an excess of thiosulphate 70–11 400-fold higher than the silver concentration are reported in Table 2. The average silver concentration in mol l^{-1} is presented for each solution with its coefficient of variation. The effects of complex-forming ions HPO_4^{2-} and NO_3^- on the calcium determination for a 1.000 mg l^{-1} solution with and without an excess of NTA are shown in Table 3. Results from the determination of total silver in black and white photographic paper using an excess of thiosulphate ligand are summarised in Table 4. A comparison of the ion-selective measurement results with those of a typical titrimetric method[5,27] is also made in Table 4.

The results from both calcium and silver measurements with an excess of complexing ligand confirm that total metal ion, both free and complexed, is determined by the standard additions method. This is clearly shown for 1.000 mg l^{-1} calcium in nitrilotriacetic acid, sample solutions 3 and 7 in Table 1, and for silver solutions of 9.940×10^{-4} and 9.94×10^{-6} mol l^{-1} with a 70- and 100-fold excess of sodium thiosulphate in Table 2. This agrees with the proposal given in references 3 and 4 to determine both free and complexed ion by the standard additions relationship outlined in equations(1)–(3). These results clearly demonstrate the advantages gained by the addition of an excess of complexing ligand to a solution in order to determine its total metal content. The

Table 1. Determination of total calcium in a 1.000 mg l^{-1} solution containing buffer, ionic strength adjustor with and without NTA by standard additions

Sample	Medium	Sensitivity (S)/ mV decade^{-1}	No. of measurements	Concentration of calcium in sample/mg l^{-1}	Coefficient of of variation,* %
1	KCl + NaOH; pH 10.5	24.2	4	1.57	57
2	KCl + $Na_2B_4O_7$ - HCl; pH 10.498	27.8	3	1.10	10
3	$NaClO_4$ + $Na_2B_4O_7$ - HCl; molar ratio of NTA to Ca, 40 : 1; pH 10.498	27.8	10	0.985	1.5
4	KCl + NH_2CH_2COOH; pH 5.02	26.2	3	1.09	1.44
5	KCl + CH_3COOH - CH_3COONa; pH 5.5	25.8	5	1.11	11
6	KCl + NH_3 - NH_4Cl; pH 10.027	25.4	3	1.04	4.32
7	NH_4ClO_4 + NH_3 - NH_4Cl; pH 10.027; molar ratio of NTA to Ca, 40 : 1; pH 10.027	25.4	18	0.998	0.1

* The coefficient of variation is described by Dean and Dixon[30] when the number of measurements is less than 6.

Table 2. Determination of total silver in solutions containing an excess of thiosulphate and ionic strength adjustor by standard additions

		Standard additions solution					
Concentration of silver solution/ M	Molar ratio of $Na_2S_2O_3$ to Ag	[Ag] ($C_{St.}$)/ mol l^{-1}	Volume ($V_{St.}$)/ml	Volume of sample (V_{Sample})/ml	No. of replicates	Total silver in sample/ mol l^{-1}	Coefficient of variation, %
9.940×10^{-6}	100	9.940×10^{-4}	0.4–0.8	30.0	4	1.22×10^{-5}	
9.940×10^{-4}	70	9.940×10^{-2}	0.3–0.4	30.0	6	9.23×10^{-4}	3.97
9.994×10^{-4}	700	9.994×10^{-2}	0.2–0.3	25.0	2	8.03×10^{-4}	
9.994×10^{-4}	1400	9.994×10^{-2}	0.1–0.4	25.0	7	8.441×10^{-4}	8.8
9.994×10^{-4}	10000–11400	9.994×10^{-2}	0.15–0.4	25.0	4	7.66×10^{-4}	

reported work with the plastic calcium electrode and NTA substantiates and extends the earlier work with silver and fluoride solid-state electrodes.[3,4,28] Calcium results in disodium tetraborate and ammonia buffers, samples 3 and 7 in Table 1, containing an excess of NTA show improved accuracy of measurement over their respective samples 2 and 6 without complexing ligand. The reproducibility of the standard additions method using NTA is good with coefficients of variation as low as 1.5 and 0.1%. An excess of NTA substantially improved the electrode potential stability of the calcium electrode in 1 mg l^{-1} solutions and extended its practical working range in the region of 0.04 mg l^{-1} limit of detection. Measurements using the silver electrode show that results for total silver concentration were largely unaffected by a large variation in an excess of thiosulphate concentration spanning the thiosulphate to silver molar ratio range 700–11 400 (Table 2). Lower silver concentrations than expected were noted for some solutions, which may be attributed to a change in the silver thiosulphate equilibrium after addition of a standard silver solution. The subsequent variation in the ratio of free silver ion to total silver concentration, f in equation (1), will induce an error in the calculation of the result from equation (3), which assumes that the ratio f is constant throughout standard additions. The effects of this equilibrium change were further demonstrated by repeated additions of silver providing progressively lower total silver concentrations per addition, calculated on the basis of a Gran plot.[29] These observations highlight the role and importance of establishing the correct thiosulphate concentration, thereby fixing f prior to measurement.

Common anions such as HPO_4^{2-} and NO_3^- form stable complexes with Ca^{2+} and reduce the level of ionised calcium that can be detected by an ion-selective electrode. The results in Table 3 indicate that up to a 1000-fold excess of a relatively weak complexing anion such as phosphate or nitrate does not significantly disturb the calcium - NTA equilibrium so as to interfere with the determination of total calcium, provided an excess of NTA is present and a standard additions procedure is adopted.

Table 3. Effect of HPO_4^{2-} and NO_3^- on the determination of total calcium in a 1.000 mg l^{-1} solution containing buffer, ionic strength adjustor and an excess of NTA by the standard additions method

Complex-forming anion	Molar ratio of anion to Ca	Calcium in sample/ mg l^{-1}
HPO_4^{2-}	10	1.23
	100	1.32
NO_3^-	100	0.94
	1000	1.30

Finally, the standard additions procedure reported here has, for the first time, been applied to the determination of total silver in black and white photographic paper. This important analysis in the photographic industry is traditionally carried out by titrimetry. Silver results from ion-selective potentiometry and titrimetry included in Table 4 show a satisfactory agreement between the different methods; however, titrimetric analysis provided a systematic 10% lower result, a consequence of silver lost from the associated complex preliminary treatment of the paper[27] and adsorption phenomena.[16] For example, incomplete development of the paper will lead to silver loss from subsequent washing and acid dissolution stages of pre-treatment while silver is slowly adsorbed on the walls of the container. Earlier silver ion-selective measurements in thiosulphate solutions pointed to a thiosulphate to silver molar ratio of approximately 2000 as suitable for the thiosulphate extraction step from the photographic paper. In addition, no significant change in the total silver results was noted when shaking times of 3–60 min were applied during this thiosulphate extraction step. The reproducibility of the standard additions method is twice as good as titrimetric analysis with coefficients of variation of 2.2 and 4.5%, respectively. A fast analysis time of only 10 min by standard additions compares favourably with the 60–70 min required for titrimetry. The

Table 4. Determination of the total silver in photographic paper using an excess of thiosulphate and standard additions: comparison with titrimetric method[5,27]

Method	No. of replicate analyses	Molar ratio of $Na_2S_2O_3$ to Ag	Total silver in thiosulphate solution/ mol l^{-1}	Total silver in HNO_3 solution/ mol l^{-1}	Total silver in photographic paper/g m^{-2}	Coefficient of variation, %
Ion-selective measurement using standard additions ..	12	1917	3.130×10^{-4}	—	0.843	2.2
Titrimetric	10	—	—	2.822×10^{-4}	0.761	4.5

results clearly demonstrate the advantages gained in reproducibility and speed of analysis by the addition of an excess of complexing ligand, sodium thiosulphate, to a coated photographic paper followed by a standard additions procedure for total silver. Further advantage is gained by microprocessor-based ion-selective instrumentation, *e.g.*, the Philips PW9416 Ion-Selective Analyser, which provides an immediate readout of sample concentration by standard additions.

The standard additions procedure in combination with addition of an excess of complex-forming ligand looks to extend the range of application of the calcium and silver ion-selective electrodes and no more so than within the photographic industry. Further, this incremental method may now be applied to the determination of other metal ions capable of anionic complexation.

References

1. Harwood, J. E., *Water Res.*, 1969, **3**, 273.
2. Moody, G. J., and Thomas, J. D. R., "Selective Ion-Sensitive Electrodes," Merrow, Watford, 1971, p. 51.
3. "Determination of Silver in Photographic Fixing Solutions," Application Bulletin No. 14, Orion Research, Cambridge, MA, 1970.
4. Mascini, M., *Ion-Sel. Electrode Rev.*, 1980, **2**, 30.
5. Vogel, A. I., "A Text Book of Quantitative Inorganic Analysis," Third Edition, Longman, London, 1961, p. 77.
6. Pyatnitskii, I. V., and Sukhan, V. V., "Analiticheskaya Khimiya," Akademiya Nauk SSSR, Moscow, 1975, Chapter VI.
7. "American National Standard Method for the Determination of Silver in Photographic Films, Papers, Fixing Baths, Sludges, or Residues," American National Standards Institute, New York, 1975.
8. Creyf, S., and Roosens, L., *Photogr. Korresp.*, 1970, **106**, 12.
9. Gansel, E. E., *Anal. Chem.*, 1959, **31**, 3.
10. Shulman, V. M., Saveleva, Z. A., and Igrukova, L. F., *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1966, **11**, 2.
11. Gorohovski, V. M., "Primenenie Polyarograficheskih Metodov v Fotograficheskoi Khimii," *Nauchn. Prikl. Fotogr. Kinematogr.*, 1967, Znipfik 12.
12. Kunimine, N., Ugazin, H., Yabe, K., and Asada, E., *Bunseki Kagaku*, 1964, **13**, 679.
13. "Manual for Processing Colour Films, Analytical Reagents and Procedures," Eastman Kodak Inc., Rochester, NY, 1975.
14. Bush, D. G., Zuehlke, C. W., and Ballard, A. E., *Anal. Chem.*, 1959, **31**, 1368.
15. "Testblad K. C. CH. 1307," Gavaert Photo Producten, Leverkusen, 1962.
16. Wenzel, F., *Z. Wiss. Photogr. Photophys. Photochem.*, 1950, **45**, 32.
17. Velichko, G. V., and Sheinis, E. G., *Technika Kino Televidenie*, 1980, **3**, 17.
18. Kirov, M., and Fabrikant, A., "Chimia na Otrovnite Vechtestva," Nauka i Iskustvo, Sofia, 1956, p. 377.
19. "Titration for Silver in Photographic Solutions," Technical Service Note, Orion Research, Cambridge, MA, 1983.
20. Moody, G. J., and Thomas, J. D. R., *Ion-Sel. Electrode Rev.*, 1979, **1**, 3.
21. Craggs, A., Moody, G. J., and Thomas, J. D. R., *Analyst*, 1979, **104**, 961.
22. Birch, B., Craggs, A., Moody, G. J., and Thomas, J. D. R., *in* Pungor, E., *Editor*, "Ion-Selective Electrodes," Akademiai Kiado, Budapest, 1978, p. 335.
23. Birch, B., Craggs, A., Moody, G. J., and Thomas, J. D. R., *J. Chem. Educ.*, 1978, **55**, 740.
24. Midgley, D., and Torrance, K., "Potentiometric Water Analysis," Wiley, Chichester, 1978.
25. Gelo, J., *Przegl. Papier.*, 1969, **25**, 109.
26. Williams, W. J., "Handbook of Anion Determination," Butterworth, London, 1979, p. 601.
27. "Determination of Silver," Fohar Ltd., Sofia, 1983, unpublished method.
28. Rix, C. J., Bond, A. M., and Smith, J. D., *Anal. Chem.*, 1976, **48**, 1236.
29. Gran, G., *Analyst*, 1952, **77**, 11.
30. Dean, R. B., and Dixon, W. J., *Anal. Chem.*, 1951, **23**, 636.

Paper A4/228
Received July 9th, 1984
Accepted December 5th, 1984

Amperometric Oxygen Sensors: Problems with Cathodes and Anodes of Metals Other than Silver

Imanuel Bergman
Health and Safety Executive, Broad Lane, Sheffield S3 7HQ, UK

The ideal diffusion geometry of the metallised membrane electrode is used to show that silver is the ideal material for oxygen measurement with an amperometric sensor. Gold by itself has no particular advantages over silver, while the commonly used platinum suffers both from a low overvoltage for hydrogen evolution and usually from low catalytic activity for oxygen reduction to hydroxide ion. Problems arising from the use of a two-electrode "galvanic" system are discussed.

Keywords: *Amperometric oxygen sensors; galvanic sensors; silver cathodes; gold cathodes; platinum cathodes*

Even in the nineteenth century it was realised that atmospheric oxygen could play a crucial part in the performance of some electrochemical cells.[1] However, no oxygen sensors based on this phenomenon appeared for several decades.

"Polarography" as invented by Heyrovsky was essentially triangular-wave voltammetry with a dropping-mercury electrode; the reduction of dissolved atmospheric oxygen at the dropping-mercury electrode has been much studied.[2] Indeed, for studies of other species, oxygen needs to be purged from the solution, as its reduction waves cover much of the usable voltage range. The two reduction waves correspond to an initial reduction to peroxide, followed by a reduction to hydroxide ion. In this work, electrodes of other metals intended for use in fixed-voltage oxygen monitors have been studied with polarographic techniques.

Toedt[3] described changes in corrosion currents due to changes in electrolyte oxygenation, and subsequently over several decades developed galvanic oxygen sensors based on platinum cathodes.

The ideal polarographic sensor is one in which the diffusion current plateau stretches over a wide range; the output current is independent of voltage over this range. With noble metal cathodes in most aqueous electrolytes hydrogen evolution imposes a limit at cathodic potentials. Unfortunately the overvoltage for hydrogen evolution is low on platinum.

Kolthoff and Jordan[4] showed that the separation between oxygen reduction and hydrogen evolution was greater on gold than on platinum; the former has a large overvoltage for hydrogen evolution.

The anodic end of the diffusion current plateau is limited by the low catalytic efficiency normally shown by platinum for the reduction of molecular oxygen direct to hydroxyl ion.

Hersch[5] studied a number of galvanic cells for sensing oxygen, and decided on silver as the optimum cathode. This metal was also adopted by Mackereth[6] for his widely used dissolved oxygen sensor.

A major advance in the use of amperometric sensors for the monitoring of dissolved oxygen was made by Clark,[7] who introduced a non-porous, but gas-permeable polymer membrane between the electrodes and electrolyte on the one hand, and the medium being studied on the other. Sensing electrodes derived from Clark's invention are usually made by embedding a wire or rod in glass or a polymer. Before polymers for this purpose were available, platinum was an obvious choice for the cathode; its coefficent of thermal expansion matched that of soda glass. A stable seal was relatively easy to prepare. However, nowadays most glass-blowing is carried out with borosilicate glass, whose expansion coefficient does not match that of platinum. The polymers used for fabricating most sensors are even worse in this respect. Clark, in his patent, confirmed the superiority of gold over platinum in respect of its greater overvoltage for hydrogen evolution. However, despite these factors and its great cost, platinum is still often used for amperometric oxygen sensors.

Hahn *et al.*[8] used rotating ring disc electrodes to study the reduction of oxygen at platinum or gold electrodes. They concluded that it was desirable to obtain a flat polarogram. This could be achieved with a system of high pH, and one that kept the production of peroxide at a low level.

The metallised membrane oxygen electrode[9] gave a very flat polarogram. Silver was used on the cathode, resulting in low peroxide production. Although no alkali was added to the electrolyte initially, the region near the sensing cathode rapidly became alkaline in operation. Any alkali produced by the reduction of oxygen could diffuse away from the cathode only very slowly, because the cathode was tightly clamped against a dialysis membrane, usually made of regenerated cellulose.

In a cell in which the material of interest was being oxidised, an aqueous electrolyte would normally be made acidic, to minimise the contribution to the background current of oxygen evolution. If water was involved in the oxidation, the electrolyte near the sensing anode would become even more acidic, once again tending to lower the background current even further.

As well as the sensing electrode discussed so far, an ideal amperometric system needs a reference electrode and an auxiliary electrode. The reference electrode serves as a datum with respect to which to set the energy of the electrons to be transferred to the oxygen molecules being sensed. The auxiliary electrode carries the current through the system. The voltage and current control is carried out by means of a potentiostat system, which can be made from a single operational amplifier.[10] However, most commercially available oxygen sensors use an anode that combines the functions of auxiliary and reference electrodes, usually satisfactorily.

Perhaps because the most common electrochemical cells have always been power sources, a number of amperometric sensors were developed as "galvanic" cells—two-electrode cells that needed no externally applied voltage to set the electron energy to the appropriate level. The disadvantages of galvanic and other two-electrode systems are discussed below.

Experimental

This study was based entirely on triangular-wave voltammetry. The potential was cycled through the voltage range shown in the polarogram of the appropriate figure, until two successive scans could not be distinguished from each other. The voltage cycling was then continued overnight, to see whether this procedure caused any significant alteration in the polarogram. The scan rate used was 20 mV s^{-1}. The ramp generator, potentiostat and current to voltage converter were

designed at the Health and Safety Laboratories (Sheffield). Temperature stabilisation to 25 ± 0.1 °C was achieved in an air thermostat.

Auxiliary electrodes consisted of 0.5 mm diameter wires of the same metal as the working electrode. Reference electrodes were miniature mercury - mercury(I) sulphate electrodes manufactured by EIL Ltd. (Richmond, Surrey). The reference electrode chambers were separated from the electrolyte reservoirs by 3 mm bore polyethylene tubes 100 mm long containing 0.1 M sodium sulphate, to minimise the chances of contamination of the working electrodes with mercury.

Gold and silver on gold metallised membrane electrodes were made by evaporation of layers about 50 nm thick for each metal from molybdenum boats. Platinum layers of about the same thickness were made by upward sputtering at powers of 50 W to avoid any damage to the PTFE membranes.

The cells used in this study were of the design described by Bergman and Windle[11] (Fig. 1), but the cell body was machined from poly(methyl methacrylate) (Perspex). The dialysis membrane consisted of a disc of regenerated cellulose set into a recess 6.5 mm in diameter. The background currents were, therefore, related to a projected area of 33 mm^2. The gas diffusion limiting Vyon porous polyethylene disc set into the Viton rubber gasket was 5 mm in diameter. The gas electron transfer currents were, therefore, related to a projected area of 19.5 mm^2.

Analytical-reagent grade materials were used: AnalaR-grade sodium hydroxide and sulphate (Hopkin & Williams Ltd., London). The gases were obtained from BOC Ltd. (Rotherham, South Yorkshire).

The electrolyte, 0.1 M sodium hydroxide in 0.1 M sodium sulphate, was chosen because alkali is generated at the cathode during the reduction of oxygen and sulphate is less likely than, for instance, chloride to interact specifically with the surface of one of the metals studied to modify its catalytic activity. In addition, the mercury - mercury(I) sulphate reference electrode could be used in this medium.

Results and Discussion

The gas diffusion limiting membrane used in the cell shown in Fig. 1 was made of PTFE because of its high chemical stability and high permeability for oxygen. The thinnest PTFE available at the time (3 μm) was used so that the oxygen reduction current would be limited by the diffusion of oxygen through the membrane only when the electrode metal had high catalytic activity for this reduction.

The electrochemical reduction of oxygen can proceed in two stages, first to peroxide and then to hydroxide. Such reduction, as takes place on mercury electrodes, gives two narrow diffusion current plateaux for which the current is relatively independent of voltage. Silver metal is known to be a catalyst for the decomposition of peroxide to oxygen. It is, therefore, not surprising that with a silver-containing cathode the reduction of oxygen gives a single wave (Fig. 2), suggesting reduction direct to hydroxide. There is no wave corresponding to a peroxide intermediate. The results are identical with those obtained with a cathode of silver alone.

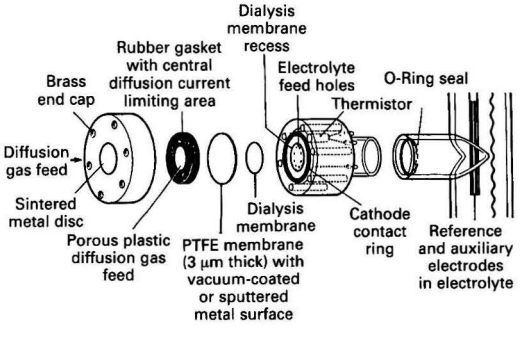


Fig. 1. Metallised membrane electrode cell Mark M4

The polarogram for a gold electrode shown in Fig. 3 has a cathodic limit as high as that for silver - gold; however, the wave shows a shorter plateau. If the cycling is continued overnight, the polarogram shown in Fig. 4 is obtained. The electrocatalytically activated gold electrode has a plateau as wide as that of the silver - gold.

Fig. 5 shows the polarogram of a platinum electrode cycled through the same potential range as the silver - gold and gold electrodes. The polarograms for both air and the inert gas used (nitrogen) are complicated by the oxidation peak of the hydrogen evolved at the extreme cathodic potentials, and then adsorbed on to the electrode. Fig. 6 shows polarograms with a voltage range restricted to minimise this complication. Not only is the cathodic limit severely restricted by hydrogen evolution, but no plateau anywhere near horizontal is present.

The low overvoltage for hydrogen evolution on platinum is reflected in a "background current" (current in the absence of oxygen) that is elevated compared with background currents on gold or silver - gold. In addition, the exposure of oxygen sensors with platinum cathodes to gases containing carbon dioxide tends to give elevated currents. The presence of the

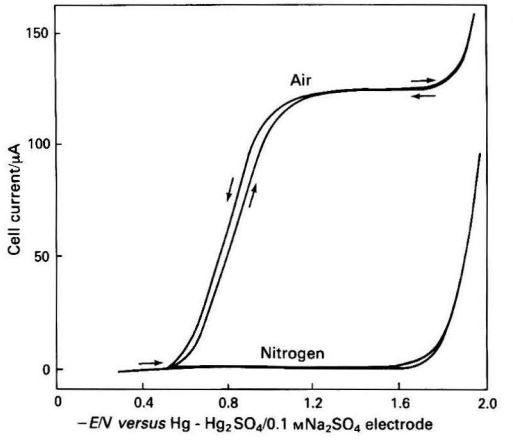


Fig. 2. Oxygen in air polarogram on an Ag - Au metallised membrane electrode in 0.1 M Na_2SO_4 - 0.1 M NaOH

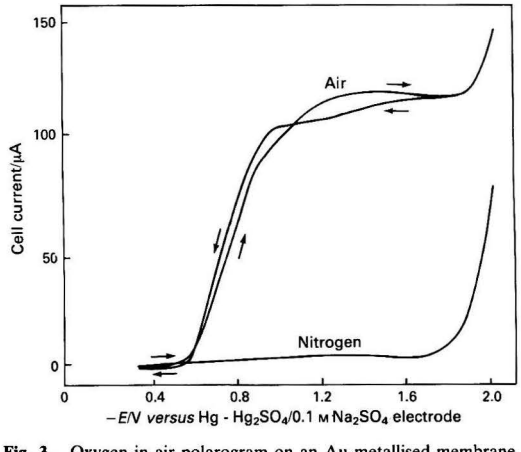


Fig. 3. Oxygen in air polarogram on an Au metallised membrane electrode in 0.1 M Na_2SO_4 - 0.1 M NaOH

carbon dioxide lowers the pH of the solution covering the cathode, and enhances the hydrogen evolution-related background current. This effect is not seen on silver - gold metallised membrane electrodes.

Platinum has a high adsorptive capacity for many gases, even those as unreactive as methane. A sudden change in the partial pressure of such a gas impinging on a platinum cathode oxygen sensor will give a current transient, because of changes in the electrode double layer.

Most platinum cathode oxygen sensors show sloping plateaux like those in Fig. 6. However, some platinum electrodes have been described as giving polarograms for oxygen reduction with flat plateaux. There are two possible explanations, one related to diffusion geometry, the other to contamination.

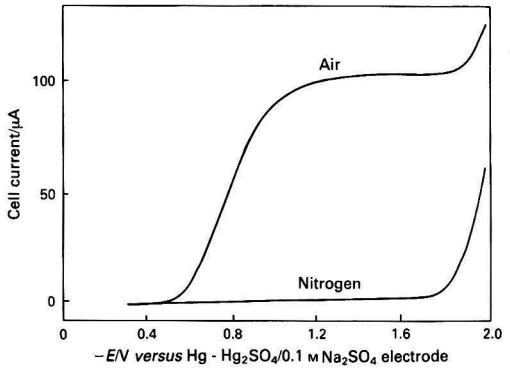


Fig. 4. Oxygen in air polarogram on a Au metallised membrane electrode in 0.1 M Na_2SO_4 - 0.1 M NaOH after overnight cycling

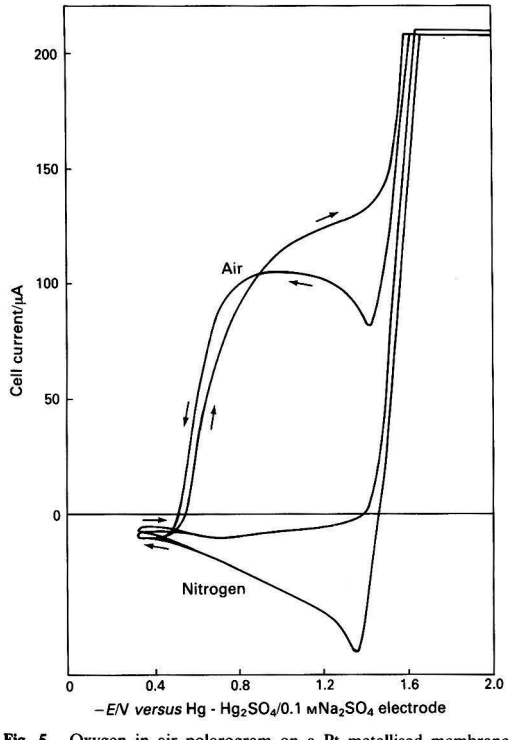


Fig. 5. Oxygen in air polarogram on a Pt metallised membrane electrode in 0.1 M Na_2SO_4 - 0.1 M NaOH

The first explanation applies to electrodes made of wires or foils as thin as a few micrometres. They are sometimes made thin so that they can be inserted into small biological structures. Another reason for making electrodes small is so that the diffusion gradient will be set up in the layer of electrolyte adhering to the electrode surface, and be relatively independent of movement of electrolyte. As an electrode becomes smaller and approximates to a point, the geometry of diffusion of oxygen to the electrode changes from linear to hemispherical diffusion. An increasing proportion of the diffusion layer will thus also be part of this viscous layer. As the diffusion becomes more limited by viscosity, the less will be the electrocatalytic effect of the electrode metal.

Often the ability of a silver - silver chloride electrode to carry current while maintaining its advantages as an effective reference electrode is used in a Clark-type electrode with this silver electrode mounted close to a cathode of platinum or gold. The latter electrodes are likely to take on at least some of the characteristics of silver electrodes, possibly within hours, by transfer of silver ions from the anode. This effect was clearly shown by Albery *et al.*[12] That dinitrogen oxide could be reduced on a silver cathode was known by about 1970. The use of metallised membrane electrode oxygen sensors made by Draeger was restricted to gases other than those containing dinitrogen oxide because of this effect.[13] Albery *et al.*[12] showed that sensors with platinum and gold cathodes, stated by them normally to be insensitive to dinitrogen oxide, could be sensitised by the transfer of silver from the anode to the cathode. However, the wide diffusion current plateau given by a metallised membrane electrode made of silver on gold enables oxygen to be monitored accurately, even at voltages at which dinitrogen oxide is reduced to a negligible extent. Such an electrode would almost certainly be preferable to one made of platinum.

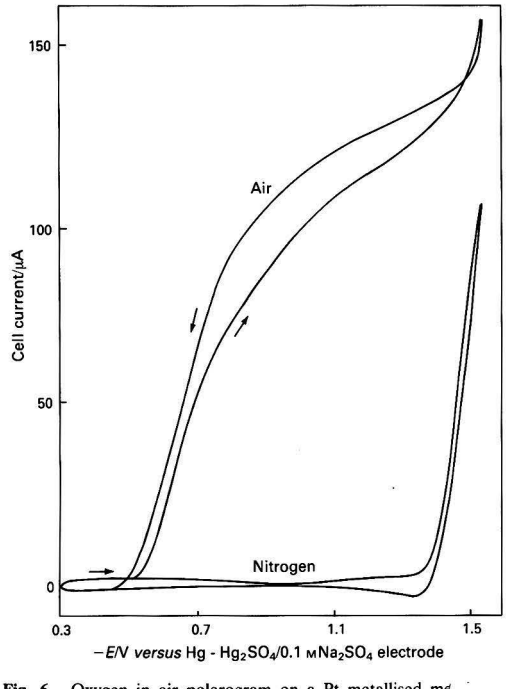


Fig. 6. Oxygen in air polarogram on a Pt metallised me electrode in 0.1 M Na_2SO_4 - 0.1 M NaOH

Anodes for Two-electrode Sensors

The silver - silver chloride electrode is the most convenient reference electrode for most sensors; it remains an effective reference electrode even when carrying the currents required by most oxygen sensors. However, in order that an electrode should serve as such a combined reference - auxiliary electrode, it also needs to remain of relatively low impedance. Otherwise the current × resistance (*IR*) voltage drop developed across the resistive layer formed on the electrode may lead to non-linearity of the signal and slow response to changes in oxygen partial pressure. High currents may take the sensing-electrode potential off the "knee" at the anodic end of the diffusion current plateau. LeFevre[14] has reported non-linearity of response in two-electrode oxygen sensors, associated with over-thick silver chloride layers on combined auxiliary - reference electrodes.

If the cell potential is set too near the cathodic end of the plateau with an over-resistive two-electrode cell, a lowering of current may take the sensing electrode potential into the region where the electrolyte decomposition gives a significant background signal. Such changes of potential may also effectively slow down the cell response, by inducing a transient component of the cell current related not only to the cell capacitance, but also to the pseudocapacitance associated, for instance, with the adsorption and desorption of impurities in the electrolyte.

The earliest versions of the metallised membrane electrode sensors developed in the author's laboratory and taken up by a manufacturer, incorporated a combined auxiliary - reference electrode consisting of a silver wire 500 mm long, of 0.5 to 1.0 mm diameter, formed into a coil of about 10 mm diameter. The amount of silver in this electrode was calculated with Faraday's laws to be equivalent to the volume of 1 M sodium chloride held in the cell reservoir. The cells were run in air at currents of between 50 and 100 μA. The nominal limit of cell life was the conversion of all the silver into silver chloride, and the concomitant conversion of the sodium chloride into sodium hydroxide. It was expected that the cell resistance would become unacceptably high long before this nominal limit had been reached. In the event, the cells were usually functional until the nominal limit had been reached, and beyond. It is possible that as the electrolyte became ever more alkaline, a proportion of the current was accounted for by oxygen evolution, rather than by conversion of silver into silver chloride.

However a problem sometimes arose if portable sensors were kept in service after the counter electrode resistance had risen to about 1 kΩ. Such a sensor might be tested and found satisfactory, then sent through the post. The resulting jolting sometimes broke the silver chloride part of the wire before the sensor arrived at its destination. Sensors taken out of service once the counter electrode resistance had reached 1 kΩ did not suffer from this problem.

Layers of silver chloride with resistances up to 30 kΩ have been found in a few commercial oxygen sensors, perhaps because of contamination from components of the polymers or adhesives used to fabricate them. Any problems caused by such resistivity can be removed by the use of a potentiostat system.

Galvanic Anodes

There is a particular class of two-electrode cells that are described as "galvanic." This is usually construed as meaning that the voltage of the working electrode with respect to the solution is set entirely by the choice of the joint auxiliary - reference electrode, without the need for any external voltage to be applied. Often, however, the potential of this galvanic electrode is not optimal,and the range of current over which the device gives an output that is linear with concentration is severely limited. There is a certain elegance in a device consisting simply of a cell and a meter, with no other power requirements; however, if a better reference electrode is available, the cost of such elegance should be carefully considered. When the device includes circuitry for amplifiers or alarms, then the best electrodes for the purpose should be chosen, irrespective of whether an external potential needs to be applied to the cell. A variety of cheap low-power devices are now available for controlling electrode potentials.

A number of materials have been used as galvanic anodes in oxygen sensors. Zinc often gives too cathodic a potential, resulting in an elevated background current, almost certainly owing to hydrogen evolution. Cadmium and lead can give potentials that are too anodic, resulting in a slow, non-linear response and a lack of stability.

One problem is common to all galvanic sensors that permit transfer of even traces of the anode metal to the sensing cathode. Small changes in potential, for instance resulting from changes in resistance, can oxidise these traces, or reduce the oxidised form. Transient currents or distortion of the cell response with time may result.

The ideal anode - cathode combination is where both are made of the same metal, as with most commercially available metallised membrane electrode oxygen sensors. However, even if the metals differ, and some anode metal is transferred to the cathode, a sufficiently large external potential applied between them will prevent small changes in electrode potential oxidising or reducing the material transferred.

Metallised Membrane Electrode

The metallised membrane electrode was developed because there was a need for a diffusion geometry for the oxygen molecules to reach the electrode - electrolyte interface through the electrode itself. It was known that, at elevated temperatures at least, oxygen would diffuse through silver metal. In the event, once a layer of silver had been evaporated on to a non-porous PTFE membrane, the oxygen output current proved to be independent of the thickness of the silver layer over the nanometres to micrometres range. It became evident that oxygen did not need to diffuse through the silver. The evaporated layers were sufficiently porous, even if thickened by electroplating, for electrolyte to seep through to the interface of the silver with the membrane. The oxygen thus needed to diffuse through only this membrane, and underwent electron transfer as soon as it reached the moist metal. The bonus from using silver was a diffusion current plateau covering a wider voltage range than almost all of the other metals normally used.

Evaporated layers of gold appeared to adhere better to the PTFE than those of silver. The eventual design of a layer of silver deposited on top of one of gold had the added advantage that, in the rare event that a silver electrode needed electrocatalytic activation, the silver could be converted into its chloride or oxide, then back to metal, with little risk of loss of electrical continuity.

Possible Disadvantages of Silver Sensing Cathodes

One way in which silver might be inferior to gold or platinum as a sensing cathode is in its lower resistance to corrosion. It will not normally be corroded during operation at a cathodic potential. Surface chlorides, oxides and sulphides are normally reduced during operation. Obviously, electrolytes in which silver can form soluble complex ions, for instance concentrations of chlorides greater than 1 M, should be avoided. However, even in such electrolytes, problems arise more frequently from the transfer of silver from a silver - silver chloride anode to the cathode, than from the loss of metal from the cathode. In a metallised membrane electrode the dialysis membrane clamped tightly against the metallised layer not only minimises the transport of interfering materials from

the electrolyte to the electrode but also minimises the loss of electrode metal or metal ions.

References

1. Walker, W., Jr., Wilkins, F. R., and Lones, J., *US Pat.*, 524229, 1894.
2. Heyrovsky, J., *Arch. Chem. Pharm. (Zagreb)*, 1931, **5**, 162.
3. Toedt, F., *Korrosion*, 1928, **3**, 37.
4. Kolthoff, I. M., and Jordan, J., *Anal. Chem.*, 1952, **24**, 1071.
5. Hersch, P., *UK Pat.*, 707323, 1954.
6. Mackereth, F. J. H., *J. Sci. Instrum.*, 1964, **41**, 38.
7. Clark, L. C., Jr., *US Pat.*, 2913386, 1959.
8. Hahn, C. E. W., Davis, A. H., and Albery, W. J., *Respir. Physiol.*, 1975, **25**, 109.
9. Bergman, I., *Nature (London)*, 1968, **218**, 266.
10. Bergman, I., *Proc. Conf. Environ. Sensors Appl.*, 1974, **29**, 67, Institution of Electronic and Radio Engineers, London.
11. Bergman, I., and Windle, D. A., *Ann. Occup. Hyg.*, 1972, **15**, 329.
12. Albery, W. J., Brooks, W. N., Gibson, S. P., and Hahn, C. E. W., *J. Appl. Physiol., Respir. Environ. Exercise Physiol.*, 1978, **45**, 637.
13. "Instruction Manual for E12 Oxygen Alarm," Draeger Manufacturing, Blyth, Northumberland, 1970.
14. LeFevre, M. E., *J. Appl. Physiol.*, 1969, **26**, 844.

Paper A4/279
Received August 10th, 1984
Accepted November 28th, 1984

Comparison of Tubular Polymeric pH and Ammonium Ion Electrodes as Detectors in the Automated Determination of Ammonia

Heung Lark Lee* and Mark E. Meyerhoff†
Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109, USA

The analytical response properties of two types of flow-through ammonia gas sensing electrode systems are examined and directly compared under the same experimental conditions. Both detection systems involve the use of continuous flow gas dialysis in conjunction with tubular polymer membrane electrode detectors. In one method, the pH of the flowing recipient stream leaving the dialyser is monitored; in the second, ammonium ions are detected. For each of five sample diluents studied, the latter method is shown to offer a greater than 100-fold improvement in detection limits over the more classical pH monitoring technique. Further studies clearly show that the ammonium electrode detection mode also offers superior selectivity over volatile amines (*e.g.*, methylamine, ethylamine, etc.). The reasons for these enhanced analytical characteristics are discussed in terms of ammonia flux through the gas-permeable membrane of the dialyser and appropriate solution equilibria.

Keywords: *Ammonia-nitrogen determination; continuous flow analysis; tubular pH and ammonium polymer membrane electrodes; gas dialysis*

The determination of total ammonia-nitrogen (*i.e.*, free ammonia gas plus ammonium ions; NH_3-N) remains an important test in many clinical, industrial and environmental laboratories. Aside from direct measurements of free NH_3-N, many indirect methods for important biochemical and other organic species involve the ultimate detection of NH_3-N following appropriate hydrolysis reactions (*e.g.*, enzymatic, base hydrolysis, etc.).

Over the past 15 years, commercial potentiometric gas sensors, based on inner glass membrane pH electrodes, have received considerable attention as simple devices for the measurement of NH_3-N.[1-3] While inexpensive and convenient to use, these probes are known to suffer significant interference from volatile amines,[4-8] a serious problem when measuring NH_3-N in waste waters and other environmental samples. In addition, incorporation of these sensors into automated flowing arrangements by means of flow-through cap assemblies results in systems with higher detection limits and poor sample throughput capabilities owing to slow recovery times. The latter limitation can be overcome by using a continuous flow gas dialysis approach in which the pH of a flowing recipient ammonium chloride solution is monitored as a function of the NH_3-N concentration in the sample.[9] This dialysis concept has also been applied to the measurement of carbon dioxide[9,10] and is not limited to the use of electrodes for the detection of the pH change. Indeed, incorporation of pH indicator dyes into the flowing stream, and subsequent passage of this stream through a spectrophotometer cell, can also be employed.[11,12] While sample throughputs can be enhanced, the ultimate detection limits of the system can become worse owing to the non-equilibrium nature of the flowing dialysis process. The effect of this non-equilibrium detection scheme on the response of such systems to volatile acids and bases, compounds that interfere with static, equilibrium-type gas sensors, has not been reported.

For automated NH_3-N determinations, we recently suggested that it would be advantageous to sense ammonium ions formed within a buffered recipient stream of a continuous flow gas dialysis arrangement[13] and further demonstrated that this approach offers high selectivity over volatile amines.[4] The purpose of this paper is to substantiate these claims further by directly comparing the performance of the two possible types of electrode-based ammonia detection systems; one using a buffered recipient stream and an ammonium ion electrode detector and a second system utilising an ammonium chloride recipient solution and a pH electrode detector (see Fig. 1). The latter arrangement is analogous to using photometric detection and pH indicator dyes. Both ammonia detectors are evaluated using exactly the same AutoAnalyzer manifold arrangement. Five sample diluents, each with a different pH, are examined. In addition, the response of each NH_3-N detection system towards a variety of volatile amines are presented. It will be shown that the new ammonium electrode based approach offers dramatic improvements in detection limits and selectivity and that these enhanced analytical response properties can be readily explained in terms of the flux of ammonia gas through the dialyser membrane and appropriate solution equilibria.

Experimental

Apparatus

A schematic diagram of the AutoAnalyzer manifold used to evaluate the two types of ammonia detectors is shown in Fig. 2. As illustrated, a constant 1 + 1.9 dilution of the sample took place within the network of the system for all the investigations reported here. A 12-in Technicon dialyser assembly (Tarrytown, NY) fitted with a 0.2 μm pore size PTFE membrane (W. L. Gore and Associates, Elkton, MD) served as the gas dialyser.

All potentiometric measurements were made with a Fisher Accumet Model 620 pH - millivolt meter and recorded on a Linear Instruments Corporation Model 555 strip chart recorder (Reno, NV). Tubular polymer membrane pH and ammonium ion-selective electrodes were prepared as described previously[9,13] using the neutral carriers tridodecylamine and nonactin, respectively. The flow-through electrode - reference electrode unit configuration was the same as that used earlier.[13]

Reagents

All chemicals used were of analytical-reagent grade. Standards and buffer solutions were prepared with distilled, de-ionised water. The five diluent solutions examined are listed in Table 1.

* On leave from the Department of Chemistry, Kyungpook National University, Daegu, 635 Korea.

† To whom correspondence should be addressed.

Table 1. Detection limits for the automated electrode based ammonia sensing systems using various diluents*

No.	Diluent	Ammonium electrode system/ 10^{-6} M	pH electrode system/ 10^{-4} M	
			0.01 M NH_4Cl	0.001 M NH_4Cl
1	0.015 M Tris - HCl, pH 8.5	4.4	6.9	9.6
2	0.015 M NaOH - boric acid, pH 9.7	3.3	2.6	1.9
3	0.015 M NaOH - Na_2HPO_4, pH 11.1	2.8	1.8	1.4
4	0.0015 M NaOH, pH 11.1	2.1	1.5	1.4
5	0.015 M NaOH, pH 12.2	1.4	1.7	1.3

* As defined in reference 14.

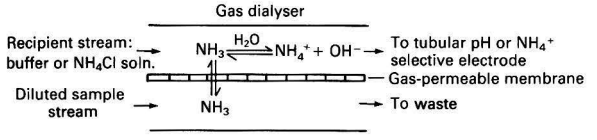


Fig. 1. Schematic representation of the two possible electrode-based flow-through ammonia detection systems using a gas-dialysis concept

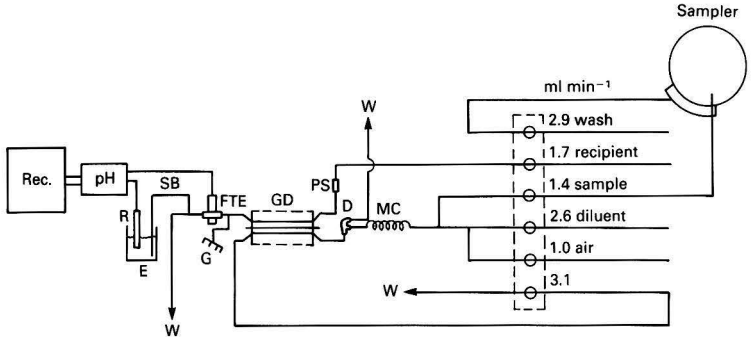


Fig. 2. Schematic diagram of the AutoAnalyzer manifold arrangement used to evaluate both types of ammonia detectors: MC, mixing coil; D, de-bubbler; PS, pulse suppressor; GD, gas-dialyser unit; G, electrical ground; FTE, flow-through tubular polymer electrode unit; SB salt bridge; E, electrolyte; R, saturated calomel reference electrode; pH, pH - mV meter; Rec., strip-chart recorder; and W, waste

Methylamine (40% in water), dimethylamine (40% in water), trimethylamine, ethylamine (70% in water), diethylamine, cyclohexylamine and morpholine were obtained from Fluka Chemical Co. (Hauppauge, NY), ethanolamine from Sigma Chemical Co. (St. Louis, MO), and triethylamine from Aldrich Chemical Co. (Milwaukee, WI). Cyclohexylamine, morpholine, ethanolamine and triethylamine were freshly distilled prior to use.

For the ammonium electrode based system, a 0.01 M (molarity refers to total ionic strength) tris(hydroxymethyl)-aminomethane - hydrochloric acid buffer (Tris - HCl) of pH 7.5, was used as the recipient stream solution. For the pH electrode based arrangement, two solutions, 0.01 and 0.001 M NH_4Cl, were studied.

Procedure

Upon reaching a stable base line potential, ammonium chloride or amine standards were introduced into the systems at a rate of 30 h^{-1} by means of an AutoAnalyzer Sampler 2. Peak heights, in millivolts (ΔE) were measured and plotted *versus* the logarithm of the NH_3-N concentration in the standards. Evaluation of the detection limits for each system with the various diluents and recipient stream compositions was carried out in accordance with the IUPAC recommended method for ion-selective electrode work.[14]

Results and Discussion

Calibration Graphs for NH_3-N

Fig. 3(*a*)–(*e*) directly compares the potentiometric response of both types of automated gas sensing electrode configurations to varying concentrations of NH_3-N using the five different diluent reagents. As expected, for both types of ammonia detectors, detections limits (see Table 1) improve as the pH of the diluent becomes more basic, indicating the increased level of free ammonia gas in the sample stream (*i.e.*, the detected form of NH_3-N). In addition, for diluents 3 and 4, it is observed that the response to NH_3-N levels off at high concentrations (10^{-2} M) no matter which of the two types of detectors are used. This is simply due to the inability of these diluents to adequately adjust this concentration of NH_4Cl to the respective pH values (*i.e.*, poor buffer capacity or base strength). Obviously, for real sample determinations, care must be taken to ensure that the diluent used has ample buffer capacity to adjust the pH of the sample to the same values as the diluted standards.

The data shown in Fig. 3(*a*)–(*e*) were obtained from potentiometric strip chart recordings of the membrane potentials of the flow-through tubular electrodes for each of the two systems. Fig. 4. shows a typical trace of such an output for some of the data plotted in Fig. 3(*e*). It can be seen that peak potentials for standards run in triplicate are reproducible, with

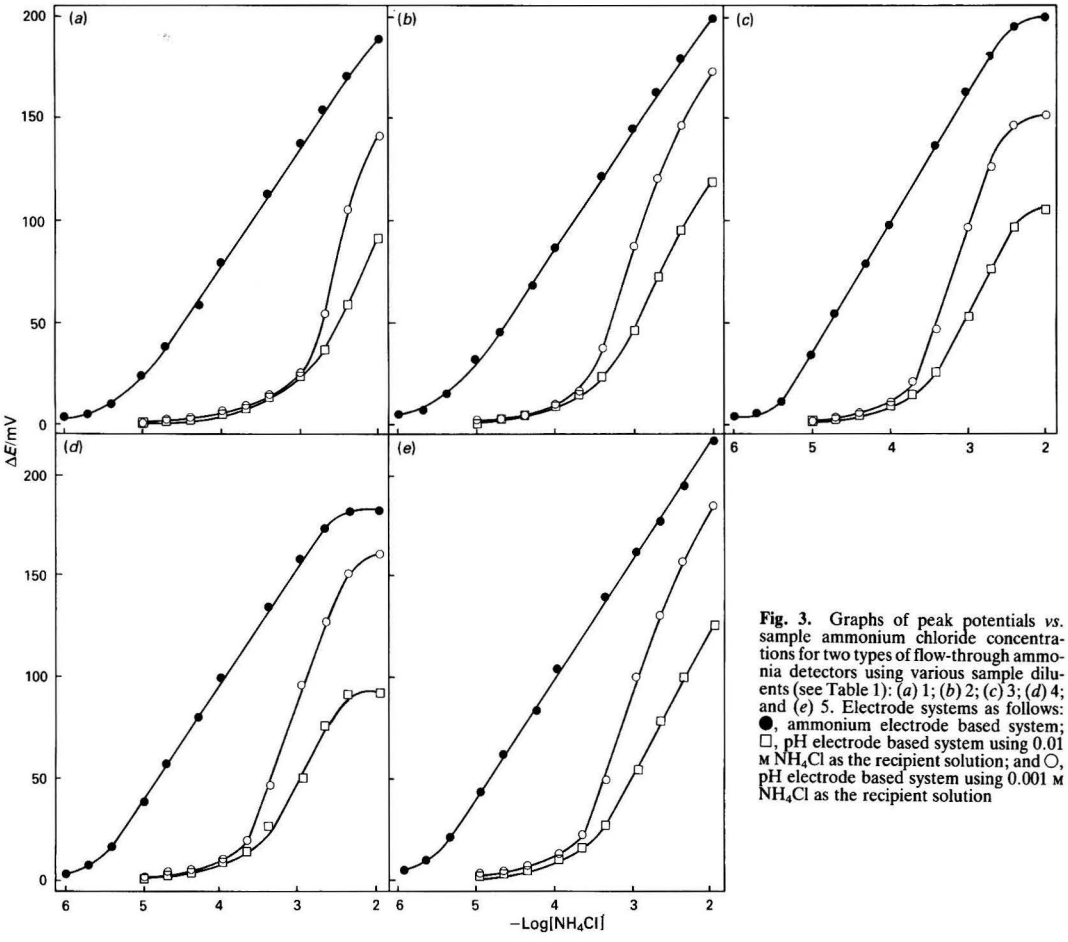


Fig. 3. Graphs of peak potentials *vs.* sample ammonium chloride concentrations for two types of flow-through ammonia detectors using various sample diluents (see Table 1): (*a*) 1; (*b*) 2; (*c*) 3; (*d*) 4; and (*e*) 5. Electrode systems as follows: ●, ammonium electrode based system; □, pH electrode based system using 0.01 M NH_4Cl as the recipient solution; and ○, pH electrode based system using 0.001 M NH_4Cl as the recipient solution

standard deviations ranging from 0.2 to 1.1 mV over the concentration range examined for both types of NH_3-N detectors. This corresponds to relative precisions of $\pm \leq 4.4\%$ for determinations of ammonia at any concentration with either detection system. In general, the ammonium electrode based detector was more reproducible than the pH sensing arrangement with relative precisions of $\leq 3.2\%$ for all standards tested. In addition, as seen in Fig. 4, return to base line between each sample (washout) is also good even at low NH_3-N concentrations and reasonably high sampling rates (30 h^{-1}). It should be noted that within the AutoAnalyzer arrangement there is a constant 1 + 1.9 dilution of the sample with diluent and, therefore, detection limits for the actual detection process are really about one-third of those values reported in Table 1.

With regard to the differences in the response of the two types of detectors, several observations can be made. Firstly, for all diluents tested, total NH_3-N can be detected at levels more than 100-fold lower by using the ammonium electrode buffered recipient stream approach. Secondly, linear response ranges with respect to peak potentials *versus* the logarithm of NH_3-N concentrations are nearly Nernstian (58–64 mV per decade) for the ammonium electrode based system but considerably above Nernstian for the pH sensing method (*i.e.*, 66–120 mV per decade). Indeed, calibration graphs for the pH electrode system are sigmoidal in shape with only limited ranges of linearity. Finally, changing the concentration of NH_4Cl in the flowing recipient stream does not significantly alter the detection limits but does change the sensitivity and response range of the pH sensing method.

These observations can be partially explained by examining the processes and equilibria reactions that give rise to the measured pH and ammonium ion concentration changes in the recipient stream (see Fig. 1). In both instances, the diffusion of ammonia gas through the membrane of the dialyser causes a change in concentration of the indicator species (pH or ammonium ions). For both systems, this diffusion is caused by the concentration gradient of dissolved ammonia gas between the sample stream and the recipient stream. In the pH sensing approach, the diffusing ammonia shifts the pH of the NH_4Cl recipient stream in accordance with the Henderson - Hasselbach equation:

$$pH_r = pK_a - \log[NH_4^+]_r + \log[NH_3]_r \quad .. \quad (1)$$

where r denotes the recipient stream values. The final concentration of ammonia in the recipient stream as it flows from the dialyser to the membrane electrode detector is dependent on the efficiency of the dialyser. That is

$$[NH_3]_r = f[NH_3]_s \quad .. \quad .. \quad .. \quad (2)$$

where s denotes the concentration of species in the sample stream as it enters the dialyser and f is the fractional efficiency of the dialysis process. Naturally, f is dependent on the flow-rates of the two streams, the gas permeability and

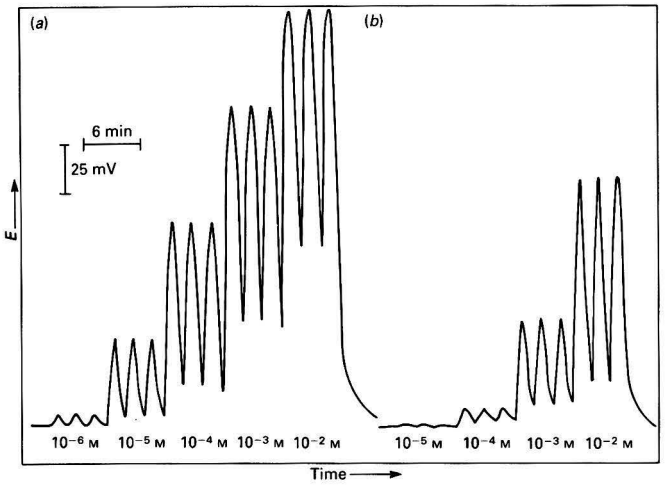


Fig. 4. Typical strip chart recordings for two types of automated ammonia detectors using the same diluent (diluent 5), identical manifold arrangement and different electrode systems. (*a*) Ammonium electrode based system; (*b*) pH electrode based system

porosity of the membrane, the diffusion coefficient of NH_3 in the membrane and solutions and the length of the dialysis chamber.

When the recipient stream is an NH_4Cl solution (pH mode), there is already a background level of free ammonia gas in the recipient stream resulting from the hydrolysis of ammonium ions. Indeed, for a 10^{-3} M solution, $[NH_3]_r = 7 \times 10^{-7}$ M, and for a 10^{-2} M solution, $[NH_3]_r = 2 \times 10^{-6}$ M. Thus at low sample NH_3-N concentrations, the concentration gradient between the sample and recipient streams is small and this results in a low flux of NH_3 gas through the membrane. Further, in this detection mode, ammonia gas diffusing across the membrane into the recipient stream hydrolyses to produce the observed pH change; however, as the equilibrium constant for the hydrolysis of NH_3 is small ($pK_b = 1.8 \times 10^{-5}$), almost all of the ammonia gas remains as dissolved ammonia. Thus, as the sample stream passes through the dialyser, ammonia gas builds up in the recipient stream thereby continuously reducing the concentration gradient across the membrane. The result of this process is that detection limits are much higher for this non-equilibrium detection scheme than for the conventional static types of ammonia sensors that utilise internal glass pH electrodes[2,15] (in the latter devices, the internal recipient solution is a static thin film of NH_4Cl and equilibrium is achieved between the sample and this film by simply allowing the probe to contact the sample for several minutes). In addition, the non-equilibrium process in the dialyser apparently causes the dialysis efficiency f [in equation (2)] to increase as a function of the $[NH_3]_s$. This is evident from the above Nernstian behaviour of the pH electrode based system [see Fig. 3(*a*)–(*e*)]. Differences in the ionic strengths of the NH_4Cl standards used for the calibration range tested could have caused this effect; however, experiments in which all standards contained a constant NaCl background (0.1 M) still produced above Nernstian behaviour. It is well known that diffusion coefficients change with concentration and this may account, in part, for the observed behaviour. Clearly, further studies need to be undertaken to fully elucidate the origin of this effect.

The dramatic improvement in detection limits and the nearly Nernstian behaviour of the ammonium ion detection mode can be attributed to the different mechanism by which the ammonia gas is sensed. In this instance, when ammonia gas diffuses through the dialyser's membrane, it is immediately converted into ammonium ions by the flowing recipient buffer stream (0.01 M Tris - HCl, pH 7.5). As no ammonia originally exists in the stream, for a given $[NH_3]_s$, a larger initial concentration gradient exists across the membrane. Further, as the diffusing ammonia is continuously converted into NH_4^+ by the buffer trap effect, this large gradient is maintained throughout the dialysis process. The net result is that more total molecules of ammonia gas transfer across the membrane for the given flow-rates used (these were optimised in accordance with continuous dialysis theory in earlier work[13]) than in the pH-sensing approach. As can be seen from Fig. 3(*a*)–(*e*) and Table 1, this continuous and higher flux of ammonia results in considerably improved detection limits and linearity of the calibration graphs. Further, in view of the Nernstian behaviour of this system, it appears that the efficiency of the dialysis process is independent of sample ammonia concentration over several orders of magnitude under these buffer trap conditions.

Selectivity

It is known that conventional ammonia gas sensors have large responses to volatile amines.[4–8] The amines pass through the gas-permeable membrane of the static sensor and change the pH of the internal NH_4Cl electrolyte layer. As, in many instances, the amines are stronger bases than ammonia, response is even greater towards these species than ammonia and severe positive interferences can result.

For the automated NH_3-N arrangement based on the same pH detection mechanism as the conventional static gas sensors, considerable response to the amines was also observed. Figs. 5(*b*) and 6(*b*) show the calibration graphs obtained for the system toward nine different amines using diluents 2 and 5. As a significant response was observed at higher amine concentrations, the total response was much less than that for ammonia [see Fig. 3(*b*) and (*e*)]. The improved selectivity over the static gas sensors can be once again explained by the non-equilibrium process that takes place in the dialyser. As the amines have smaller diffusion coefficients than ammonia, both in solution and in the air pores of the dialyser membrane,[16] the efficiency of the dialysis process under given flow conditions, etc., is much lower for the amines. Hence, only at high concentrations is the fraction transferred enough to cause large pH changes in the flowing

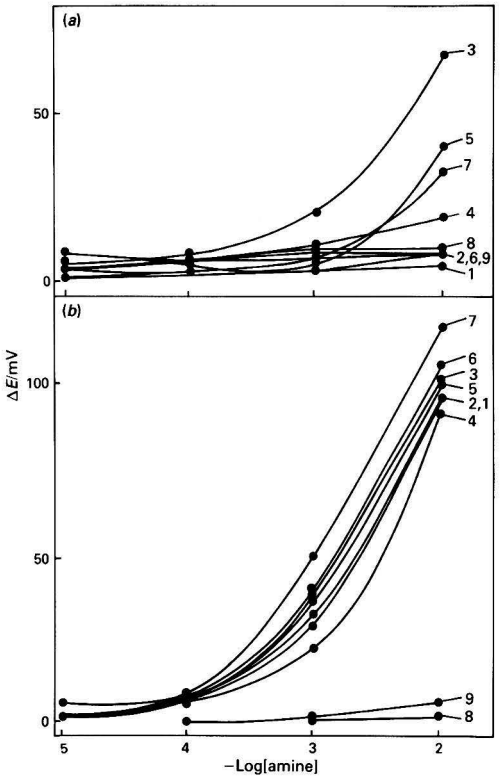


Fig. 5. Response of two types of ammonia detectors towards various amines using diluent 5. (*a*) Ammonium electrode system; (*b*) pH electrode system. Amines tested: 1, diethylamine; 2, triethylamine; 3, dimethylamine; 4, cyclohexylamine; 5, ethylamine; 6, methylamine; 7, trimethylamine; 8, ethanolamine; and 9, morpholine

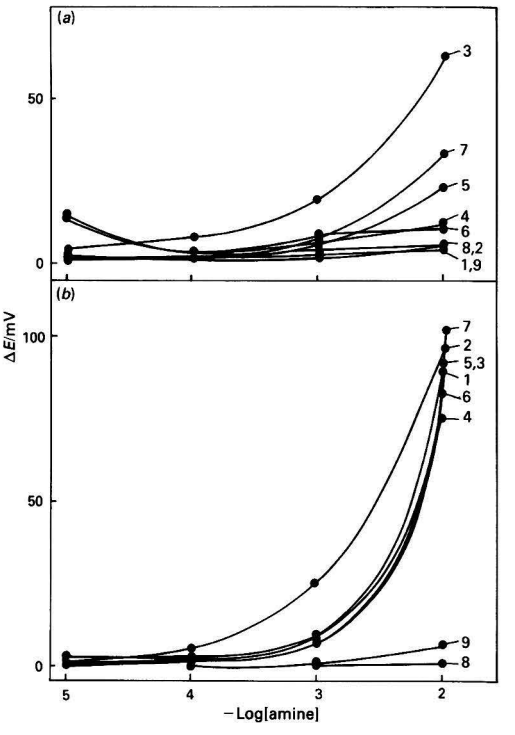


Fig. 6. Response of ammonia detectors to various amines using diluent 2. (*a*) Ammonium electrode system; (*b*) pH electrode system. Amines are as given in Fig. 5

NH_4Cl recipient stream. Consequently, only concentrations of amines above 10^{-3} M will cause substantial interference with the automated NH_3-N system based on pH detection.

For the automated system based on ammonium ion detection, even greater selectivity over volatile amines is observed. As shown in Figs. 5(*a*) and 6(*a*), concentrations of most amines up to 10^{-2} M do not yield significant responses. This is because the nonactin-based polymer membrane electrode displays high selectivity for ammonium ions over protonated forms of the amines.[4] Thus, even though the amines enter the recipient buffer stream during the dialysis process, they go undetected by the final transducer. Surprisingly, small positive signals were first observed for the lowest concentrations of methyl- and ethylamine [*i.e.*, 10^{-5} M in Figs. 5(*a*) and 6(*a*)]. These signals were found to be partially due to trace NH_3-N impurities in these particular standards. With fresh standards we observed that these signals were reduced (to $\Delta E = 7$ mV) but not completely eliminated. Difficulties in preparing standards of these two amines, which contain no ammonia impurities, makes it hard to assess the causes of these small positive signals. Nonetheless, it is evident that even these two highly volatile amines are not significant positive interferents over the concentration range evaluated.

While our data indicate that there is no positive interference due to the volatile amines, such substances can cause negative biases in the measurement of NH_3-N if their levels are substantially above those of the NH_3-N in the sample. This is because high concentrations of the amines can override the buffer capacity of the recipient stream solution (*i.e.*, increase pH) causing a shift in the equilibrium of NH_3-N favour of ammonia gas (decreased NH_4^+). Use of higher ionic strength Tris - HCl recipient buffers can overcome this problem and allow for the accurate measurement of NH_3-N in the presence of an excess of amines.[4] Alternatively, the use of a lower pH diluent can partially eliminate any effects of the amines by taking advantage of the generally higher pK_a values for these species compared with NH_3-N.

Conclusions

The data presented here clearly show that automated NH_3-N measurement systems based on the final detection of ammonium ions in a buffered recipient stream offer significantly improved detection limits, linear response ranges and selectivity over similar flow-through systems designed with pH detectors. In view of the simplicity of the required tubular ammonium selective membrane electrode, it seems likely that this new approach will replace the traditional colorimetric and potentiometric methods at present employed for automated NH_3-N determinations. Indeed, advantageous use of this concept in conjunction with automated enzymatic assays has already been proposed.[17]

The authors gratefully acknowledge the support of the National Institutes of Health, USA (GM-28882-04). In addition, Dr. H. L. Lee acknowledges support from the Korea Science and Engineering Foundation.

References

1. Bailey, P. L., "Analysis with Ion-Selective Electrodes," Heyden, London, 1980, pp. 158–181.

2. Riley, M., *in* Covington, A. K., *Editor*, "Ion-Selective Electrode Methodology," Volume 1, CRC Press, Boca Raton, FL, 1979, pp. 1–22.
3. Ma, T. S., and Hassan, S. S. M., "Organic Analysis Using Ion-Selective Electrodes," Volume 1, Academic Press, London, 1982, pp. 114–126.
4. Fraticelli, Y. M., and Meyerhoff, M. E., *Anal. Chem.*, 1981, **53**, 1857.
5. Lopez, M. E., and Rechnitz, G. A., *Anal. Chem.*, 1982, **54**, 2085.
6. Midgley, D., and Torrance, K., *Analyst*, 1972, **97**, 626.
7. Beckett, M. J., and Wilson, A. L., *Water Res.*, 1974, **8**, 333.
8. Banwart, W. L., Tabatabai, M. A., and Bremner, J. M., *Comm. Soil Sci. Plant Anal.*, 1972, **3**, 449.
9. Opdycke, W. N., Parks, S. J., and Meyerhoff, M. E., *Anal. Chim. Acta*, 1983, **155**, 11.
10. Scott, W. J., *in* Symth, W. F., *Editor*, "Electroanalysis in Hygiene, Environmental, Clinical and Pharmaceutical Chemistry," Elsevier, Amsterdam, 1980, pp. 47–60.
11. Baadenhuijsen, H., and Seuren-Jacobs, H. E. H., *Clin. Chem.*, 1979, **25**, 443.
12. Karlberg, B. I., and Anfalt, T., Paper No. 82, Presented at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March, 1980, Atlantic City, NJ.
13. Fraticelli, Y. M., and Meyerhoff, M. E., *Anal. Chem.*, 1981, **53**, 992.
14. Guilbault, G. G., Durst, R. A., Frant, M. S., Freiser, H., Hansen, E. H., Light, T. S., Pungor, E., Rechnitz, G. A., Rice, N. M., Rohm, T. J., Simon, W., and Thomas, J. D. R., *Pure Appl. Chem.*, 1976, **48**, 127.
15. Bailey, P. L., and Riley, M., *Analyst*, 1977, **102**, 213.
16. Bolz, R. E., and Tuve, G. L., *Editors*, "Handbook of Tables for Applied Engineering Science," CRC Press, Cleveland, OH, 1973, p. 546.
17. Fraticelli, Y. M., and Meyerhoff, M. E., *Anal. Chem.*, 1983, **55**, 359.

Paper A4/325
Received August 9th, 1984
Accepted November 20th, 1984

Comparison of Inhaled Furfuryl Alcohol Vapour with Urinary Furoic Acid Excretion in Exposed Foundry Workers by Chromatographic Techniques

Pirkko Pfäffli, A. Tossavainen and H. Savolainen
Department of Industrial Hygiene and Toxicology, Institute of Occupational Health, Haartmaninkatu 1, SF-00290 Helsinki, Finland

A gas-chromatographic method using a ^{63}Ni electron-capture detector was developed for furfuryl alcohol vapour. The detection limit for the vapour was 0.017 mg m^{-3} (0.004 cm^3 m^{-3}), indicating a superior sensitivity compared with older techniques. Furfuryl alcohol exposure was measured by personal sampling of six foundry workers. Their excretion of urinary furoic acid after alkaline hydrolysis of its glycine conjugates was also determined by liquid chromatography. The exposure was compared with their furoic acid excretion 2 h after tasks involving hardening of core sand with furfuryl alcohol as a binding agent. The product of the sampling time and vapour concentration predicted accurately the urinary fuoric acid excretion, so that the latter can be used as a biological indicator of exposure to the vapour.

Keywords: *Furfuryl alcohol; furoic acid; chromatography; electron-capture detector; biological exposure test*

Considerable interest has recently been shown in alkylfuran compounds because of their wide occurrence and high toxicity.[1-3] Experiments with animals indicate that the furan ring may be opened by the drug-metabolising enzyme systems of exposed subjects, which produces alkyl butenedials.[4] These dialdehydes have a high inherent reactivity towards biological macromolecules.[4]

Our work has shown that furfuryl alcohol is extensively metabolised to furoic acid, which is later conjugated with glycine and excreted in the urine.[5] This seems to be the major metabolic pathway for furfuryl alcohol, apparently aimed at its effective removal from the reaction sphere of toxicological significance. Despite its rapid metabolism, furfuryl alcohol is toxic to exposed animals,[5] which is reflected in low LC_{50} values for animals (233 cm^3 m^{-3}) and in low allowable human exposure levels (5–10 cm^3 m^{-3}).

Nevertheless, furfuryl alcohol is a versatile binding agent for core sand in foundries. It has a low boiling-point, which restricts the availability of its vapour to polymerisation of the cores. The reaction is exothermic enough to evaporate unreacted alcohol from the resin. Hence the occupational exposure periods may be brief although significant vapour concentrations can be reached. These peak vapour concentrations are easily overlooked in routine hygiene surveys with time averaging of the detected exposure. Therefore, we investigated whether personal exposure might be better detected through the urinalysis of furoic acid. In the course of these studies, we also developed a method for the analysis of furfuryl alcohol vapour.

Experimental

Analysis of Furfuryl Alcohol Vapour

Air samples (6–15 l) were drawn through personal sampling tubes fixed near the breathing zone of six foundry workers. The tubes were filled with a solid adsorbent (Porapak Q, SKC 70/35 mg; SKC Inc., Eighty Four, PA, USA) to trap the furfuryl alcohol.[6] SKC personal pumps (SKC 222-3) were operated at a suction flow-rate of 0.2 l min^{-1}. The furfuryl alcohol was desorbed from the adsorbent with 1 ml of absolute ethanol. Identical amounts of the adsorbent were added to standard liquids prepared with freshly distilled furfuryl alcohol (analytical-reagent grade; Fluka, Buchs, Switzerland) in ethanol. Overnight equilibration at room temperature was carried out before the analysis. The desorption efficiency by this method was 98.5% (n = 10, 5 μg of furfuryl alcohol per tube). The test concentration was similar to that found in tubes at the lowest actual exposure in the foundries.

A 1-μl volume of the desorption solution was injected into a Hewlett-Packard Model 5790A gas chromatograph equipped with a capillary column (25 m × 0.2 mm, i.d.; SE-54 phenyl silicone on fused silica). The column temperature was programmed from 60 to 145 °C at 20 °C min^{-1}. The temperature of the injector was 235 °C and that of the ^{63}Ni electron-capture detector was 325 °C. Ultrapure nitrogen was used as a carrier gas. The retention time of furfuryl alcohol was 2.2 min (Fig. 1). The coefficient of variation in the sampling and analysis was 0.015.

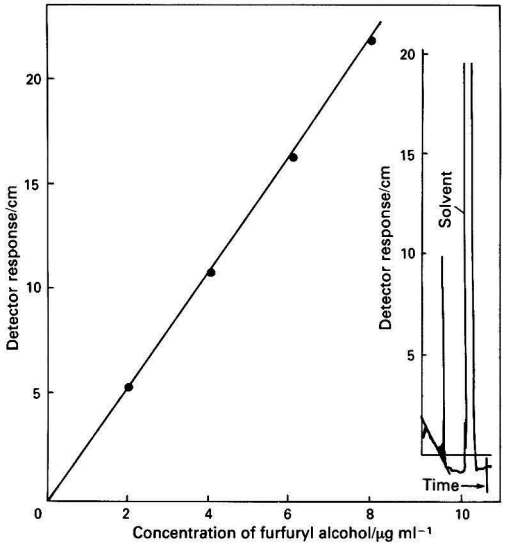


Fig. 1. Gas-chromatographic analysis of furfuryl alcohol vapour using a ^{63}Ni electron-capture detector. The detector response (y, cm) is linearly correlated with the concentration (x, μg ml^{-1}): $y = 2.75x - 0.13$. Other impurities in the foundry air sample (3.8 μg ml^{-1} of furfuryl alcohol, shaded peak) do not interfere with the analysis (inset)

Urinalysis of Furoic Acid

Urine voided 2 h after exposure to furfuryl vapour was used. Aliquots (5 ml) were boiled with 5 ml of 50% sodium hydroxide solution for 1 h to hydrolyse the furoic acid - glycine conjugate.[5] The hydrolysates were neutralised with 10 ml of 19% sulphuric acid and extracted three times with 10 ml of dichloromethane. The extracts were combined and dried with sodium sulphate before evaporation of dichloromethane under vacuum. The residue was dissolved in 2 ml of acetonitrile - acetic acid - water (14 + 1 + 85).

The furoic acid was analysed by liquid chromatography (Pye Unicam) using a Shandon Hypersil ODS column (RP_{C18} with octadecylsilyl groups, particle size 5 μm, column length 15 cm). Isocratic elution was carried out with acetonitrile - acetic acid - water (14 + 1 + 85). The sample volume was 10 μl and the flow-rate was 0.6 ml min^{-1}. A UV detector (240 nm) was employed (Fig. 2).

The recovery in the extraction and analysis of furoic acid was 90% (n = 10, 15 μg l^{-1}) with a coefficient of variation of 0.014. The test furoic acid concentration corresponded to that found in controls and in workers the next morning before the new work shift.

The furoic acid excretion was corrected in each instance for the excretion of creatinine determined by the alkaline picric acid method. The least-squares procedure was used to find the correlation between the product of furfuryl vapour concentration and sampling time and urinary furoic acid excretion.

Results

Analysis of Furfuryl Alcohol Vapour

The detector response was linearly correlated with the desorbed furfuryl alcohol (Fig. 1). The smallest amount detected was 0.1 μg ml^{-1} in the desorption solution, which corresponded to 0.017 mg m^{-3} (0.004 cm^3 m^{-3}) in the air. These figures were reached at a detector temperature of 325 °C. The detector temperature correlated with the electron-capture coefficient (K, Fig. 3) up to the maximum operating temperature (352 °C).

The furfuryl alcohol vapour exposure was best characterised by multiplication of the detected vapour concentration and the sampling time. Our pilot experiments indicated that inhalation of the vapour was almost always associated with tasks involving core sand polymerisation so that only personal sampling was applicable. This view was verified by very low furfuryl alcohol concentrations detected by static sampling for the whole shift.

Furoic Acid Excretion

Furoic acid was excreted by control subjects (15 ± 11 μmol $mmol^{-1}$ of creatinine) and by foundry workers the morning before the work shift (18 ± 16 μmol $mmol^{-1}$ of creatinine). The difference was without statistical significance. The amount of furoic acid excreted by the six workers who participated in the experiment in the morning urine after exposure on the previous day was not greater than that excreted by the control subjects. The maximum furoic acid concentration was invariably found 2 h after the end of the polymerisation task, so this was used as a standard sampling point for the urinalysis.

The product of furfuryl alcohol vapour concentration (μmol l^{-1}) and time (min) accurately predicted the furoic acid excretion (Fig. 4). No apparent correlation was found if the vapour exposure was averaged over 8 h. The same occurred if the urine was sampled at the end of shift, allowing differing time lapses after the actual exposure period, sometimes lasting only 10–20 min.

Discussion

The sensitivity of the furfuryl alcohol detection method is superior to that obtained with a flame-ionisation detector.[6] The older methods are hardly suitable for detecting exposure at the current hygiene levels, *i.e.*, 20 mg m^{-3} in most European countries or 40 mg m^{-3} in the USA.

The improved sensitivity is clearly achieved by the employment of the electron-capture detector, as the analytical

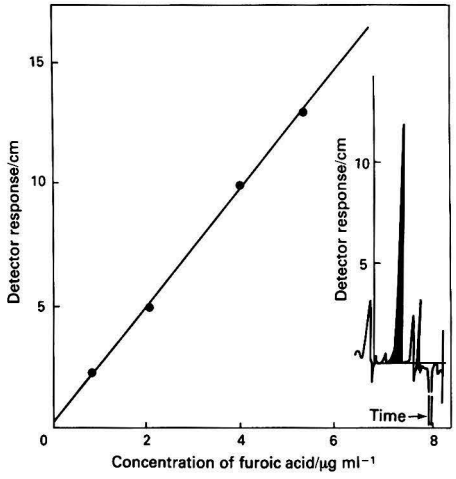


Fig. 2. Liquid-chromatographic analysis of furoic acid with a UV detector operated at 240 nm. The detector response (y, cm) is linearly correlated with the furoic acid concentration (x, μg ml^{-1}): $y = 2.42x + 0.20$. Other urinary constituents do not interfere with the determination of furoic acid (shaded peak, 5.3 μg ml^{-1}) in the urine from an exposed worker (inset)

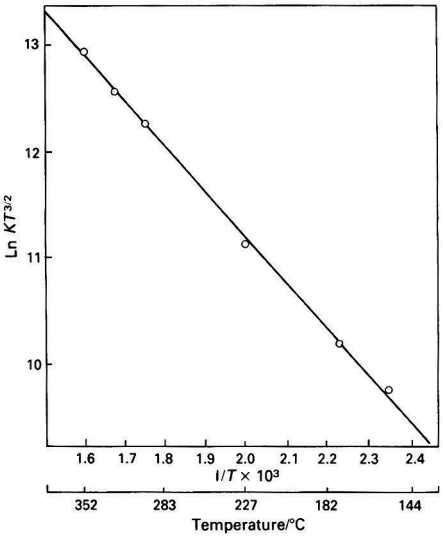


Fig. 3. Correlation of the electron-capture coefficient (K) of the ^{63}Ni detector towards the furfuryl alcohol with the detector temperature (T, K). The detector response was studied at a standard alcohol concentration (9 μg ml^{-1}). For clarity, the detector operation temperature range is given in °C

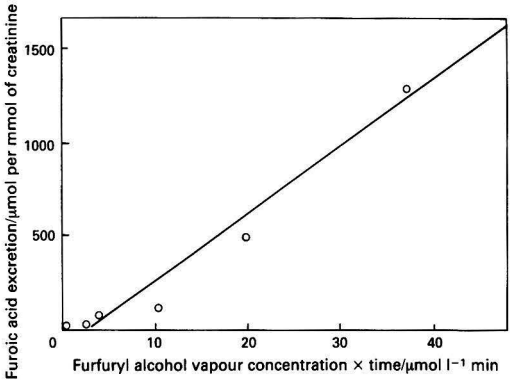


Fig. 4. Correlation of the urinary furoic acid excretion with the product of furfuryl alcohol vapour exposure (μmol l^{-1}) and time (min) 2 h after exposure to the vapour in a linear fashion given by the equation $y = 34.82x - 90.66$; $r = 0.95$

procedure is otherwise similar to older techniques.[6] Furfuryl alcohol seems to absorb thermal electrons so that the electron-capture coefficient (K, Fig. 3) could be related to the detector temperature.[7] For practical reasons, the maximum detector temperature of our apparatus was not used, as 325 °C proved highly sensitive with good reproducibility.

For the correlation of furoic acid excretion with exposure to furfuryl alcohol vapour, the vapour concentrations were expressed in molar terms. This facilitates the understanding of the uptake functions and metabolic transformation capacity. The pulmonary ventilation during moderately heavy work may extend from 25 to 35 l min^{-1}. Assuming complete uptake in the lungs, the urinary output would account for 80% of the absorbed dose in the period examined. If the uptake in the lungs is less, the urinary metabolite excretion would account for an even greater proportion of the inhaled dose. No allowance for the change in the relative molecular mass from furfuryl alcohol to furoic acid is needed if molar amounts are used. A more conclusive definition of the metabolic rate must await, for example, experiments with labelled furfuryl alcohol. However, our estimate would agree very well with the minimum amounts of furoic acid detected at the later time points.

In conclusion, the furfuryl alcohol vapour concentration can be reliably determined at very low concentrations by using gas chromatography with a ^{63}Ni electron-capture detector. An accurate idea of the exposure of workers can be achieved by urinalysis of its metabolite provided that representative samples can be obtained.

We thank Mrs. Pirjo Toropainen and Mr. Yrjö Peltonen for their excellent technical assistance.

References

1. Haschek, W. M., Morse, C. C., Boyd, M. R., Hakkinen, P. J., and Witschi, H. P., *Exp. Mol. Pathol.*, 1983, **39**, 342.
2. Gammal, L. M., Wiley, R. A., Traiger, G., Haschek, W. M., and Baraban, S., *Toxicology*, 1984, **30**, 177.
3. Wiley, R. A., Traiger, G. J., Baraban, S., and Gammal, L., *Toxicol. Appl. Pharmacol.*, 1984, **74**, 1.
4. Ravindranath, V., Burka, L. T., and Boyd, M. R., *Science*, 1984, **224**, 884.
5. Savolainen, H., and Pfäffli, P., *Environ. Res.*, 1983, **31**, 420.
6. National Institute of Occupational Safety and Health, "Manual of Analytical Methods," Volume 4, Department of Health, Education and Welfare, Cincinnati, OH, 1978, p. S365.
7. Wenworth, W. E., and Chen, E. C. M., *in* Zlatkis, A., and Poole, C. F., *Editors*, "Electron Capture in Theory and Practice in Chromatography," Elsevier, Amsterdam, 1981, p. 27.

Paper A4/380
Received November 2nd, 1984
Accepted November 21st, 1984

Gas Chromatographic - Mass Spectrometric Determination of Muramic Acid Content and Pyrolysis Profiles for a Group of Gram-positive and Gram-negative Bacteria

Larry W. Eudy,* Michael D. Walla and Stephen L. Morgan†
Department of Chemistry, University of South Carolina, Columbia, SC29208, USA

and Alvin Fox
Department of Microbiology and Immunology, School of Medicine, University of South Carolina, Columbia, SC 29208, USA

Two gas chromatographic - mass spectrometric methods, one based on chemical derivatisation and the other on analytical pyrolysis, were employed for the characterisation of a diverse group of bacteria. The muramic acid content of some Gram-positive and Gram-negative bacteria (including legionellae) was determined by aldononitrile acetate derivativisation and capillary GC - MS analysis. Muramic acid levels were generally higher in Gram-positive than in Gram-negative bacteria. When applied to the same set of bacteria, pyrolysis GC - MS and non-linear mapping were also able to distinguish Gram-negative from Gram-positive bacteria. The potential of GC - MS techniques for the direct chemical characterisation of bacteria is discussed.

Keywords: *Gas chromatography - mass spectrometry; pyrolysis; derivatisation; microorganisms*

Wide variations in structural composition occur among different groups of bacteria. Although these structural differences have been employed, together with physiological and morphological characteristics, for taxonomic purposes, the direct analysis of bacterial structure has not been routinely employed for bacterial identification primarily because of the complexity of the procedures involved.[1] The development of simple chromatographic approaches for bacterial identification has been the concern of much recent work. The first step in differentiating bacteria is usually the Gram stain. Whether instrumental methods, such as those based on gas chromatography (GC) or gas chromatography - mass spectrometry (GC - MS), can provide information similar to that provided by the Gram stain is the question addressed in this paper.

Capillary GC and GC - MS can provide relatively simple analysis of the structural composition of bacterial cells,[2] but both of these techniques require the sample to be volatile. Chemical derivatisation and thermal fragmentation (pyrolysis) are two complementary approaches for volatising bacterial components. Chemical derivatisation[3–6] usually involves several manual sample treatment steps: hydrolysis to release intact monomeric units, followed by reaction with suitable reagents to inhibit hydrogen bonding or ionic interactions. Pyrolysis [7–10] can be applied directly to the sample with minimum pre-treatment, but the biopolymer is thermally degraded into fragments that do not necessarily retain the monomeric structure of bacterial macromolecule.

The objective of the work reported here was to determine whether GC - MS techniques based on derivatisation or analytical pyrolysis have the potential to discriminate Gram-negative and Gram-positive bacteria. Derivatisation GC analysis of muramic acid content[11–13] was performed because muramic acid is a component of bacterial peptidoglycan, which is known to be present in larger amounts in Gram-positive than Gram-negative bacteria.[14] Analytical pyrolysis GC - MS was performed as a follow-up to earlier work in our laboratories[15] in which the monitoring of only two pyrolysis products from a small group of bacteria was found to provide some separation of Gram-negative from Gram-positive bacteria. This approach using GC - MS to monitor specific chemical markers for bacterial structure might be further extended to develop a chemotaxonomic scheme or decision tree as an alternative to traditional approaches for the classification of bacteria.

*Present address: Bowman-Grey Technical Center, R. J. Reynolds Tobacco Company, Winston-Salem, NC, USA.
†Author to whom correspondence should be addressed.

Experimental

Reagents and Standards

The following glass-distilled chromatographic solvents and derivatisation reagents were purchased: chloroform and methanol (Burdick and Jackson, Muskegon, MI, USA), pyridine and acetic anhydride (Applied Science Laboratories, State College, PA, USA). Analytical-reagent grade *N,N*-dioctylmethylamine was obtained from ICN (Plainfield, NY, USA) and sulphuric acid and hydroxylamine from Fisher Scientific (Fairlawn, NJ, USA). The hydroxylamine was washed with chloroform before use. Muramic acid, *N*-methylglucamine, D-ribose, D-arabinose, D-xylose and 2-ketodeoxyoctonic acid were obtained from Sigma (St. Louis, MO, USA) and L-rhamnose, L-fucose, D-mannose, D-glucose and D-galactose from Supelco (Bellefonte, PA, USA). Clin Elut and Bond Elut extraction columns were purchased from Analytichem International (Lawndale, CA, USA). All glassware was washed with acid and chloroform prior to use.

Organisms

Gram-positive and Gram-negative bacteria, including several members of the family Legionellaceae, were prepared as follows. The cells were collected by centrifugation at 10 000*g*, washed three times with distilled water, killed by heating and lyophilised. The strains and species of organisms employed in this study, and also their growth conditions, are listed below.

Staphylococcus aureus (ATCC 25923), *Staphylococcus epidermidis* (ATCC 14990), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 27736), *Proteus vulgaris* (ATCC 6380), *Enterobacter cloacae* (ATCC 13047) and *Bacillus globigii* (a gift from Dr. Gene Mayer, Department of Microbiology and Immunology, University of South Carolina, Columbia, SC, USA) were cultured overnight in tripticase soy broth (Difco,

Detroit, MI, USA). *Streptococcus pyogenes* (ATCC 10389) was grown in Todd Hewitt broth for 72 h. *Propionibacterium acnes* (strain 418) was obtained from Dr. J. L. Cantrell (National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, MT, USA) and was cultured in NIH thioglycollate broth for 72 h at 37 °C. *Clostridium perfringens* was obtained from Ms. Patsy Meade (Richland Memorial Hospital, Columbia, SC, USA) and grown overnight in NIH thioglycollate broth. *Legionella pneumophila* Philadelphia 1, *Legionella pneumophila* Knoxville, *Fluoribacter* unclassified E-327 F, *Fluoribacter dumoffii* NY-27, *Tatlockia micdadei* PPA-EK and *Tatlockia micdadei* PPA-GL were incubated for 3 d on buffered charcoal yeast extract agar in air at 37 °C and were gifts of Dr. Arnold Brown, William Jennings Bryan Dorn Veterans Administration Hospital, Columbia, SC, USA. *Micrococcus lysodeikticus* (ATCC 4698), *Bacillus subtilis* (ATCC 6633), *Azobacter vinelandii* (ATCC 12518), *Pseudomonas fluorescens* (ATCC 13430) and *Aerobacter aerogenes* (type III) were purchased from Sigma.

Derivatisation GC - MS

The aldononitrile acetate derivatisation method employed to determine the muramic acid content of bacteria was modified from that described by Fazio *et al.*[11] A 1-mg amount of each bacterial sample was hydrolysed in 0.5 ml of 2 N sulphuric acid under vacuum in a reaction tube with an adjustable PTFE plunger (Pierce, Rockford, IL, USA). The sample was heated at 100 °C in a heating module (Pierce) for 3 h. Following hydrolysis, the acid solution was cooled in an ice-bath and neutralised with 2.5 ml of 20% *N,N*-dioctylmethylamine in chloroform and 6 μg of *N*-methylglucamine in 50 μl of water were added. The aqueous phase was placed on a C-18 Bond Elut column that had been pre-wetted with 2 ml of methanol followed by 2 ml of distilled water. The sample was pulled through the column by vacuum into a reaction vial (Pierce) and evaporated to dryness under reduced pressure at 60 °C. After adding 200 μl of 15 mg ml^{-1} hydroxylammonium chloride solution in pyridine, the sample was heated at 70 °C in a heating module for 4 h. The sample was then evaporated to dryness and 30 μl of pyridine and 300 μl of acetic anhydride were added. The sample was allowed to react at 100 °C for 2 h, then evaporated to dryness. Water (1 ml) and chloroform (1 ml) were added and the mixture was placed on a Clin Elut column. The sample was eluted with chloroform, then evaporated to dryness. Approximately 25 μl of chloroform were added and the sample was analysed by GC - MS. Standards of muramic acid were included for calibration with each batch of bacterial samples. These standard amounts of muramic acid were placed in sulphuric acid solution and carried through the entire derivatisation procedure with the exception of the initial heating step.

The mass spectra of derivatised muramic acid and *N*-methylglucamine were obtained by capillary GC - MS on a Finnigan Model 4021 system (Finnigan, Palo Alto, CA, USA) on a fused-silica column coated with SE-52. The column was threaded through the GC - MS transfer line so that the column effluent passed directly into the ion source of the mass spectrometer. Routine muramic acid analyses were performed on a Model 5992A GC - MS instrument (Hewlett-Packard, Palo Alto, CA, USA) with a glass capillary column that had been coated with SP-2330 in our laboratory.[16] The oven was programmed at 15 °C min^{-1} from an initial temperature of 100 °C to a final temperature of 240 °C. Ions of *m/e* 86 and 115 were monitored to detect the *N*-methylglucamine internal standard and muramic acid, respectively; integrated areas of respective peaks at the appropriate retention times were computed for later comparisons.

Pyrolysis GC - MS

A Pyroprobe 100 ribbon pyrolyser (Chemical Data Systems, Oxford, PA, USA) was coupled to the Hewlett-Packard HP 5992A GC - MS system. Samples were prepared by suspending the bacteria in distilled water and storing them at −10 °C. After thawing, bacterial samples were sonicated and 200 μg of bacterial cells were placed on the platinum pyrolysis ribbon. The water was removed by heating the ribbon to 100 °C. The probe was inserted into the pyrolysis interface and the system was purged with helium for 1 min. The pyrolysis ribbon was ramped at 75 °C ms^{-1} to a final temperature of 800 °C and held at this temperature for 5 s. The pyrolysate was swept on to a fused-silica capillary column coated in our laboratory with Superox-4 according to the method reported by Arrendale *et al.*[17] The GC oven was held at 60 °C for 3 min, then ramped at 15 °C min^{-1} to a final temperature of 240 °C. The mass spectrometer was operated under "Autotune" conditions and integrated peak area counts were obtained by selected ion monitoring (SIM). Peak areas were stored in data files for off-line processing on an Amdahl 470/V6 time-sharing computer system using FORTRAN programs developed in our laboratory.[7] The peak-area data were normalised and autoscaled prior to further treatment. Eight pyrolysis products were selected as having apparent discriminating ability in separating the groups of microorganisms. A Euclidean distance similarity matrix describing the similarities between the pyrolysis patterns of the group of microorganisms was produced. A two-dimensional non-linear map, whose similarity matrix best matched the similarity matrix of the original eight-dimensional data, was plotted on a Hewlett-Packard Model 7225B graphics plotter.

Results and Discussion

Selection of Organisms for Study

Although GC and GC - MS have been widely applied to differentiating bacteria methods based on derivatisation GC or pyrolysis GC, it is not clear that a diverse group of organisms grown on different media and under different growth conditions can be distinguished by these analytical techniques. The effect of growth conditions on pyrograms has been the topic of continuing research interest. In some instances (*e.g.*, reference 18), characteristic profiles remain unaffected by changes in culture media, inoculation and collection times and storage time; in other instances (*e.g.*, reference 19), differences in the relative amounts of certain pyrolysis products have been noted as a result of variations in growth conditions. Many different growth media and conditions are employed in the routine microbiological laboratory. Although universal growth conditions and media would be preferred, this is not practical. Groups of organisms including strict aerobes and strict anaerobes cannot be grown under the same conditions; additionally, certain fastidious bacteria will not grow on commonly used bacteriological media. In this study, a group of organisms with wide-ranging growth requirements were purposefully selected. The group of organisms included obligate anaerobes, facultative anaerobes and obligate aerobes. Several of the organisms selected had extremely stringent growth requirements; for example, the *Legionella* organisms do not grow on conventional media.[20] Legionellae have been classified as Gram-negative organisms based on electron microscopy[21,22] and by isolation of lipopolysaccharide.[23] The organisms listed in Table 1 were analysed by derivatisation GC - MS for muramic acid content and were also subjected to pyrolysis GC - MS profiling.

Determination of Muramic Acid by GC - MS with Selective Ion Monitoring

In preliminary experiments, muramic acid and *N*-methylglucamine were derivatised and mass spectra obtained (Figs. 1 and 2). Subsequent analyses were performed on the HP-5992 GC - MS instrument operated in the SIM mode. Ions of *m/e* 115 and 86 were monitored to detect muramic acid and the internal standard, respectively, as shown in Fig. 3 for one of the organisms, *Legionella pneumophila* Philadelphia 1.

A calibration graph of the peak-area ratio for muramic acid to the internal standard, methylglucamine, against the amount of muramic acid derivatised was linear from 500 ng to 50 μg with a coefficient of determination (R^2) of 0.99. Levels of muramic acid determined for the 22 bacterial strains are reported in Table 1. Generally, the Gram-positive bacteria contained higher amounts of muramic acid than Gram-negative bacteria. Moriarty[13] has also reported that Gram-positive bacteria have higher levels of muramic acid than Gram-negative bacteria. The absolute variability in the amount of muramic acid found was greater for the Gram-positive than for the Gram-negative organisms, but the relative standard deviations for most replicate analyses were within the range 5–12%. It is interesting that there was overlap

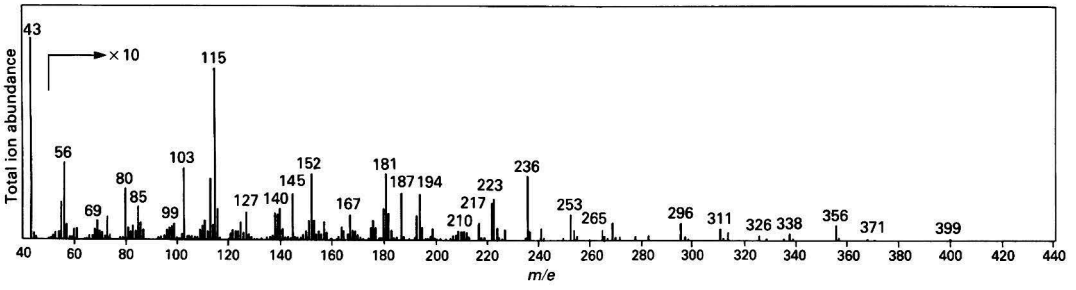


Fig. 1. Electron ionisation mass spectrum of the aldononitrile acetate of muramic acid

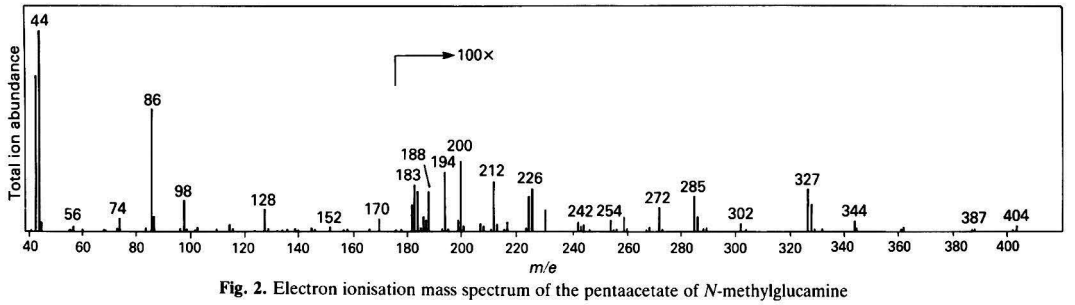


Fig. 2. Electron ionisation mass spectrum of the pentaacetate of *N*-methylglucamine

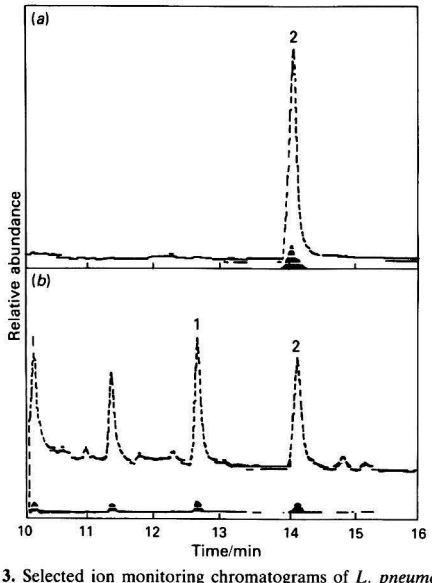


Fig. 3. Selected ion monitoring chromatograms of *L. pneumophila* Philadelphia 1: (*a*) *m/e* 86 and (*b*) *m/e* 115. Peak identification: 1, muramic acid; and 2, *N*-methylglucamine

Table 1. Results of muramic acid determinations for Gram-positive, Gram-negative and *Legionella* bacteria

Type		Bacteria	% dry mass
Gram-positive	A	*M. lysodeikticus*	3.9
	B	*S. aureus*	2.9
	C	*S. epidermidis*	1.9
	D	*B. globigii*	0.56
	E	*S. pyogenes*	0.53
	F	*B. subtilis*	0.48
	G	*C. perfringens*	0.29
	H	*P. acnes*	0.16
Gram-negative	I	*A. vinelandii*	0.38
	J	*E. cloacae*	0.29
	K	*A. aerogenes*	0.29
	L	*K. pneumoniae*	0.26
	M	*P. fluorescens*	0.18
	N	*E. coli*	0.17
	O	*P. aeruginosa*	0.14
	P	*P. vulgaris*	0.14
Legionellaceae	Q	*F.* unclassified E-327F	0.30
	R	*L. pneumophila* Knoxville	0.29
	S	*T. micdadei* PPA-EK	0.28
	T	*T. micdadei* PPA-GL	0.25
	U	*F. dumoffii* NY-23	0.20
	V	*L. pneumophila* Philadelphia 1	0.19

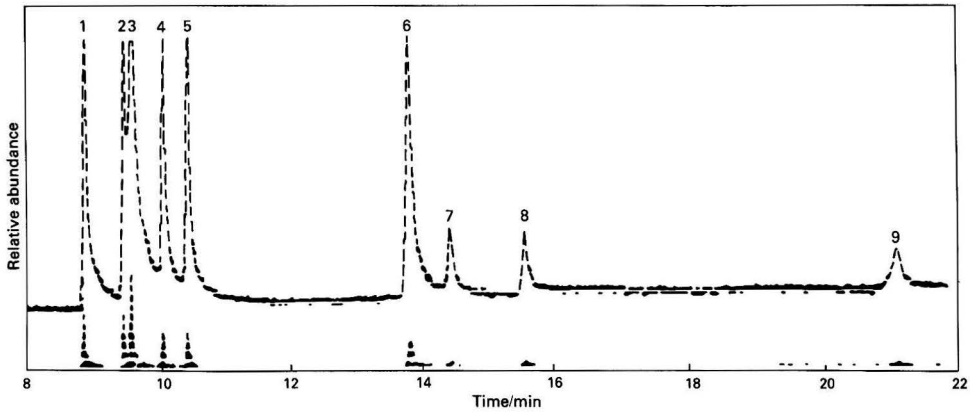


Fig. 4. Selected ion monitoring chromatogram (*m/e* 145) of several derivatised sugars. Peak identification: 1, rhamnose; 2, fucose; 3, ribose; 4, arabinose; 5, xylose; 6, mannose; 7, glucose; 8, galactose; and 9, KDO

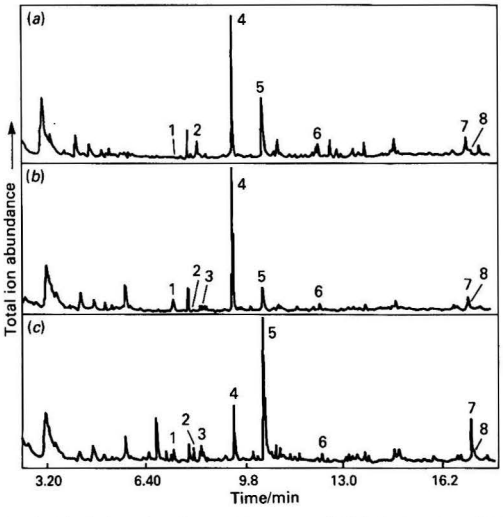


Fig. 5. Total ion abundance pyrograms of: (*a*) *L. pneumophila* Knoxville; (*b*) *P. aeruginosa*; and (*c*) *S. aureus*. Peak identification is given in Table 2

in the amount of muramic acid found in Gram-negative and Gram-positive bacteria, confirming previous suggestions that it is not merely the peptidoglycan content that determines the Gram type of an organism but that other structural characteristics are also important.[1] Finally, it should be noted that the amounts of muramic acid in legionella were within the lower range for Gram-positive organisms or within the range of Gram-negative organisms.

Although these results suggest the potential for Gram typing based on the direct analysis of bacteria, further studies analysing additional discriminating chemical "markers" on a wider variety of bacteria would be helpful. One such marker is 2-ketodeoxyoctonic acid (KDO), a carbohydrate found only in the LPS of Gram-negative bacteria. Fig. 4 illustrates the SIM GC - MS analysis of a sample containing several sugars including KDO. The aldononitrile acetate method is simple to perform, but extraneous peaks occur in the chromatogram when flame-ionisation detection or total ion abundance GC - MS is employed. The use of SIM simplifies the chromatogram by eliminating these extraneous peaks. Amino sugars other than muramic acid are difficult to analyse by the aldononitrile acetate method because of their high polarity.

Table 2. Ions monitored, retention times and tentative identification of pyrolysis products

Peak	Ion (*m/e*)*	Retention time/min	Tentative identity
1	54	7.6	2-Furancarboxaldehyde
2	67′	8.6	Unknown
3	98′	8.7	Unknown
4	98	9.7	Furfuryl alcohol
5	59	10.7	Acetamide
6	117	12.6	Benzeneacetonitrile
7	117′	17.6	1*H*-Indole
8	99	17.9	2,5-Pyrrolidinedione

*The primes refer to a second pyrolysis product detected at a later retention time using the same ion.

The alditol acetate method may therefore be preferred for the general profiling of carbohydrates in bacteria.[4–6]

Classification of Bacteria by Pyrolysis GC - MS with Selected Ion Monitoring

The potential for the pyrolysis GC - MS differentiation of bacteria and the complexity of bacterial thermal degradation patterns is illustrated in Fig. 5, which compares total ion abundance pyrograms of *L. pneumophila* Knoxville, *P. aeruginosa* and *S. aureus*. Acetamide is the most prominent pyrolysis product in the pyrogram of *S. aureus* and many other Gram-positive bacteria; furfuryl alcohol is the major product of *P. aeruginosa* and many Gram-negative bacteria. The structural origin of certain pyrolysis products, including acetamide and furfuryl alcohol, has been the subject of previous work.[15,24,25]

Each bacteria was pyrolysed in duplicate runs. By monitoring only a selected number of ions, the chromatogram was simplified, components of interest were definitively identified and the amount of data to be managed was decreased. Fifteen compounds were quantitated by monitoring ten ions at different retention times. These fifteen pyrolysis products were ranked with respect to their individual ability to discriminate the groups of microorganisms. A reduced data set was then selected, consisting of the intensities of eight pyrolysis products at *m/e* 98′, 99, 117′, 54, 59, 98, 67′ and 117 (where the primes refer to a second pyrolysis product detected at a later retention time using the same ion). The retention times and tentative identities of these pyrolysis products are summarised in Table 2.

Visual comparison of pyrograms becomes difficult as the number of peaks in each pyrogram and the total number of

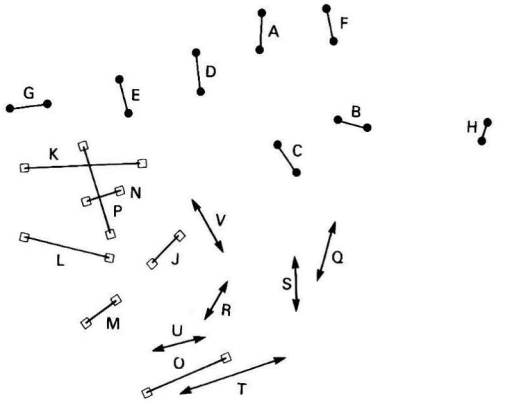


Fig. 6. Non-linear map of a group of Gram-positive and Gram-negative bacterial pyrograms. Points represent pyrograms of the bacteria listed in Table 1 (excluding *A. vinelandii*). Points representing replicate measurement are connected by lines: ●, Gram-positive; □, Gram-negative; and ▲, Legionellaceae

pyrograms to be compared increase. The current data set of 44 pyrograms with peak intensities for eight peaks can be represented as a set of 44 data points in an eight-dimensional Euclidean space. Non-linear mapping, a technique that reduces a multi-dimensional data set to a two-dimensional display approximating the structure and interpoint distances in the higher dimensional space, is a popular technique for the display of pyrolysis data.[26,27] The peak intensities for the eight pyrolysis products in the pyrograms were first normalised, then autoscaled.[7] A non-linear map that included the two data points from the pyrolysis of *A. vinelandii* showed an unacceptable lack of fit and these two points were excluded from further treatment. The pyrograms of *A. vinelandii* could be easily distinguished from the other pyrograms in the data set. The resulting non-linear map for the 21 remaining bacterial strains is shown in Fig. 6. Data points representing replicate measurements are connected by lines. The relative distance between these replicates is indicative of the reproducibility of the pyrolysis GC - MS experiment; the relative standard deviation for peak areas in replicate experiments was typically less than 10%. The non-linear map shows that differentiation between Gram-positive and Gram-negative organisms is possible. Gram-positive bacteria lie in the upper half of the map, whereas Gram-negative bacteria lie in the bottom half of the map. The legionella data cluster in a region of the non-linear map overlapping with one of the Gram-negative bacteria (*P. aeruginosa*). These results agree with morphological and biochemical studies cited above, indicating that legionellae are Gram-negative bacteria.

Conclusions

The two approaches presented here for bacterial characterisation, based on derivatisation and pyrolysis, demonstrate the potential of capillary GC - MS for the direct characterisation of microorganisms. The advantage of derivatisation is that the amount of a monomeric constituent of a microorganism is directly measured. Derivatisation suffers from the disadvantage of requiring manual sample handling. Pyrolysis GC - MS has the advantage of simplicity in sample preparation and speed of analysis, but the integrity of the monomeric structure of the bacterial sample is destroyed. The two techniques are complementary to one another and both provide information of potential value for the differentiation and identification of microorganisms. A detailed chemotaxonomic scheme could be based on the qualitative and/or quantitative analysis of bacterial constituents or pyrolysis products in addition to those studied in this work. We have considered the GC - MS differentiation of organisms by their Gram type as a potential first stage of such a chemotaxonomic scheme. Continuing work in our laboratories is concerned with the further development and application of this strategy.

This work was supported in part by National Institutes of Health (NIH) Grant No. GM 27135 (to S. L. M.), by NIH Biomedical Research Support Grant No. 2 SO7 RR07160-07 (University of South Carolina, to S. L. M. and A. F.) and by NIH Grant No. EY04715 (to A. F. and S. L. M). The assistance of Matthew Przybyciel and Joseph R. Hudson (Department of Chemistry, University of South Carolina) in the preparation of capillary columns and the assistance of Michael Lema and Dr. Arnold Brown in the preparation of bacterial samples is also gratefully acknowledged. The Finnigan GC - MS instrument was purchased with funds from the National Science Foundation through the EPSCOR programme and from the University of South Carolina.

References

1. Wiegel, J., *Int. J. Syst. Bacteriol.*, 1981, **31**, 88.
2. Drucker, D. B., *"Microbiological Applications of Gas Chromatography,"* Cambridge University Press, Cambridge, 1981.
3. Alvin, C., Larsson, L., Magnusson, M. S., Mardh, P. A., Odham, G., and Westerdahl, G., *J. Gen. Microbiol.*, 1983, **129**, 401.
4. Fox, A., Morgan, S. L., Hudson, J. R., Zhu, Z.-T., and Lau, P. Y., *J. Chromatogr.*, 1983, **256**, 429.
5. Fox, A., Lau, P., Brown, A., Morgan, S. L., Zhu, Z.-T., Lema, M., and Walla, M. D., *J. Clin. Microbiol.*, 1984, **19**, 326.
6. Walla, M. D., Lau, P. Y., Morgan, S. L., Fox, A., and Brown, A., *J. Chromatogr.*, 1984, **288**, 399.
7. Morgan, S. L., and Jacques, C. A., *Anal. Chem.*, 1982, **54**, 741.
8. Abbey, L. E., Highsmith, A. K., Moran, T. F. and Reiner, E. J., *J. Clin. Microbiol.*, 1981, **13**, 313.
9. Gutteridge, C. S., and Norris, J. R., *Appl. Bacteriol.*, 1979, **47**, 5.
10. Irwin, W. J., *"Analytical Pyrolysis: A Comprehensive Guide,"* Marcel Dekker, New York, 1982.
11. Fazio, S. D., Mayberry, W. R., and White, D. C., *Appl. Environ. Microbiol.*, 1979, **38**, 349.
12. Fox, A., Schwab, J. H., and Cochran, T., *Infect. Immun.*, 1980, **29**, 526.
13. Moriarty, D. J. W., *Oecologia (Berlin)*, 1977, **26**, 317.
14. Schleifer, K. H., and Kandler, O., *Bacteriol. Rev.*, 1972, **36**, 407.
15. Hudson, J. R., Morgan, S. L., and Fox, A., *Anal. Biochem.*, 1982, **120**, 59.
16. Hudson, J. R., Morgan, S. L., and Fox, A., *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 1982, **5**, 285.
17. Arrendale, R. F., Severson, R. F., and Chortyk, O. T., *J. Chromatogr.*, 1983, **254**, 63.
18. Reiner, E. J., and Ewing, W. H., *Nature (London)*, 1968, **217**, 191.
19. Gutteridge, C. S., and Norris, J. R., *Appl. Environ. Microbiol.*, 1980, **40**, 462.
20. Brown, A., *Lab. World*, 1981, **32**, 38.
21. Keel, J. A., Finnerty, W. R., and Feeley, J. C., *Ann. Intern. Med.*, 1979, **90**, 652.
22. Rogers, F. G., and Davey, M. R., *J. Gen. Microbiol.*, 1982, **128**, 1547.
23. Wong, K. H., Moss, C. W., Hochstein, D. H., Arko, R. J., and Schalla, W. O., *Ann. Intern. Med.*, 1979, **90**, 624.
24. Simmonds, P. G., *Appl. Microbiol.*, 1970, **20**, 567.
25. Eudy, L. W., Walla, M. D., Hudson, J. R., Morgan, S. L., and Fox, A., *J. Anal. Appl. Pyrolysis*, in the press.
26. Eshuis, W., Kistemaker, P. G., and Meuzelaar, H. L. C., *in* Jones, C. E. R., and Cramers, C. A., *Editors*, "Analytical Pyrolysis," Elsevier, Amsterdam, 1977, pp. 151–166.
27. MacFie, H. J. H., and Gutteridge, C. S., *J. Anal. Appl. Pyrolysis*, 1982, **4**, 175.

Paper A4/262
Received August 1st, 1984
Accepted December 3rd, 1984

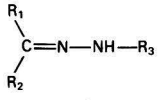
Extraction - Spectrophotometric Determination of Palladium(II) with Thiazole-2-carbaldehyde 2-Quinolylhydrazone

Toshiyuki Nakagawa, Kunio Doi and Makoto Otomo*
Department of Synthetic Chemistry, Nagoya Institute of Technology, Showa-ku, Nagoya 466, Japan

Thiazole-2-carbaldehyde 2-quinolylhydrazone (TAQH) was prepared as a reagent specifically for the spectrophotometric determination of trace amounts of palladium(II) following an extraction process. In the presence of chloride ions, palladium(II) reacts with TAQH in the wide acidity range to form a 1 : 1 metal to ligand complex extractable into benzene. The extracted species has absorption maxima at 588 and 625 nm with a molar absorptivity of 1.93×10^4 l mol^{-1} cm^{-1} at the latter wavelength and obeyed Beer's law over the range 0–4.2 μg ml^{-1} of palladium at 625 nm. The method is reasonably selective and sensitive for palladium(II) and was satisfactorily applied to the determination of palladium in some palladium catalysts.

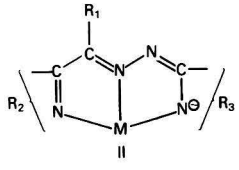
Keywords: *Palladium determination; thiazole-2-carbaldehyde 2-quinolylhydrazone; heterocyclic-substituted hydrazones; spectrophotometry; solvent extraction*

Tridentate heterocyclic substituted hydrazones characterised by the structure shown in **I** [R_1, hydrogen, alkyl or



I

aromatic groups; R_2, R_3, nitrogen heterocycles or their ring-substituted derivatives (in which the nitrogen atoms to be coordinated to a given metal ion are positioned to give two five-membered chelating rings by complexation)] have been widely used for the synthesis of organic reagents applied in photometric inorganic analysis, because they can be readily synthesised in a laboratory and give highly sensitive chromogenic reactions with some transition metal ions. A comparative study of a large number of metal complexes with these types of hydrazones suggested that an increasing double bonding characteristic of the linkage between the carbon atom attached to the ketonic carbon and the coordinating nitrogen atom in the R_2 heterocyclic ring is preferable for obtaining desirable spectral properties of the complex with respect to the position of maximum absorption and molar absorptivity. The above study also suggested that replacement of the R_2 or the R_3 heterocyclic ring by a more extended π-system improves the spectral properties of the complex to a considerable extent, the improvement being more marked when the R_2 ring is replaced. This statement is best supported by the spectral properties of the zinc(II) complexes with a series of heterocyclic-substituted hydrazones in benzene.[1] Thus the spectral properties of the complex formed can be reasonably explained by assumption of the conjugated system (**II**) as a result of the redistribution of the electron pair previously shared by the imino nitrogen.



II

* To whom correspondence should be addressed.

On the basis of these studies we synthesised a new hydrazone, thiazole-2-carbaldehyde 2-quinolylhydrazone (R_1 = hydrogen, R_2 = 2-thiazolyl and R_3 = 2-quinolyl in **I**; abbreviated to TAQH) as a potential chromogenic reagent for several transition metal ions. In this paper an extraction - spectrophotometric method for the determination of up to 4.2 μg ml^{-1} of palladium(II) with TAQH and the nature of the complex formed are described. The method has been applied satisfactorily to some real samples.

Experimental

Reagents

Unless otherwise stated all reagents were of analytical-reagent grade.

TAQH. This was synthesised by refluxing for 2 h equimolar amounts (0.01 mol) of thiazole-2-carbaldehyde (prepared by oxidation of 2-methylthiazole[2] with selenium dioxide by a modification of the method of Kaplan[3]) with 2-hydrazinoquinoline (freshly prepared from 2-chloroquinoline and hydrazine in the usual way) in ethanol (50 ml) containing a drop of glacial acetic acid. The crude compound that separated on cooling was recrystallised from ethanol to give yellow needles of melting-point 215 °C. Analytical results: found, C 61.66, H 3.78, N 22.06, S 12.41%; calculated for $C_{13}H_{10}N_4S$, C 61.40, H 3.96, N 22.03, S 12.61%. A 1×10^{-3} M TAQH solution was prepared by dissolving the required amount of the ligand in benzene. (**Caution**—Benzene is highly toxic and appropriate precautions should be taken.) This solution was stable for at least 1 week, if kept in the dark.

Palladium(II) stock solution, ca. 1×10^{-2} M. Prepared by dissolving palladium(II) chloride in 1 M hydrochloric acid. This solution was standardised gravimetrically as the dimethylglyoximate.[4]

Apparatus

A Nippon Bunko Uvidec-1 digital spectrophotometer and a Union Giken SM-401 high-sensitivity recording spectrophotometer with matched 1.00-cm quartz cells were used. A Toa Dempa HM-6A pH meter was used with a combination electrode. In determinations of the acidity constants of the ligand in aqueous 20% *V/V* dioxane solution, the pH readings were not corrected.

All measurements were made at 25 °C.

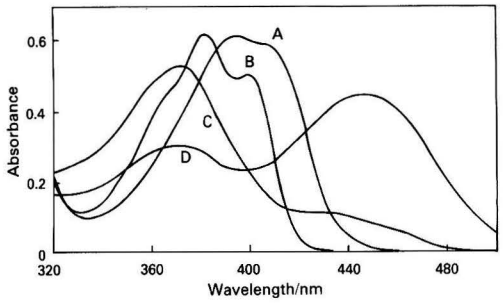


Fig. 1. Absorption spectra of TAQH in 20% *V/V* aqueous dioxane at different acidities. A, 1.4 M HCl; B, pH 2.50; C, pH 9.41; and D, 0.4 M NaOH. [TAQH], 2.5×10^{-5} M

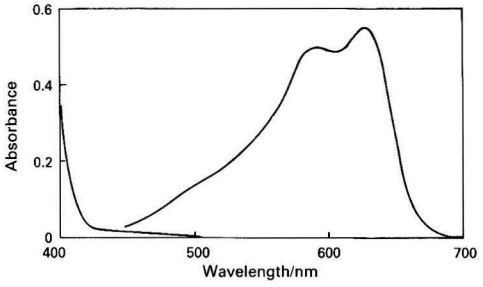


Fig. 2. Absorption spectra of TAQH and its palladium(II) complex extracted into benzene. A, Pd(II) - TAQH complex *versus* reagent blank; B, reagent blank *versus* benzene. [Pd(II)], 3.0×10^{-5} M; [TAQH], 1.0×10^{-3} M; HCl, 0.3 M

Procedure

General procedure for the determination of palladium

Into a 50-ml separating funnel, transfer a suitable aliquot of sample solution containing up to 42 μg of palladium with 3 ml of 1 M hydrochloric acid. Dilute to 10 ml with doubly distilled water and equilibrate with exactly 10 ml of 1×10^{-3} M TAQH solution in benzene by mechanical shaking for 10 min. Measure the absorbance of the benzene extract at 625 nm against the reagent blank.

Determination of palladium in real samples

Dissolve 0.1 g of the powdered sample in 10 ml of distilled water plus 10 ml of aqua regia and evaporate to dryness on a water-bath. Add 10 ml of 1 M hydrochloric acid and again evaporate to dryness. Add 20 ml of hot, 1 M hydrochloric acid, remove insoluble residue, if present, by filtration and dilute to 200 ml with distilled water. Use a suitable aliquot of this solution for the determination.

Results and Discussion

Proton Dissociation of TAQH

The ligand TAQH, which is believed to exist as the *anti*-isomer, is nearly insoluble in water but soluble in many water-immiscible organic solvents. Fig. 1 shows the absorption spectra of TAQH in 20% *V/V* aqueous dioxane at different acidities. The proton dissociation constants were estimated at $\mu = 0.2$ (NaCl) from the equation

$$\varepsilon = \frac{K\varepsilon_L + \varepsilon_{HL}[H^+]}{K + [H^+]} \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

by means of a microcomputer by applying the least-squares treatment, where ε_{HL}, ε_L and ε denote, respectively, the molar absorptivities of the conjugated acid and base and their mixture at a selected wavelength. The values obtained were $pK_3 = 0.0$ (caused by the protonation of the thiazole nitrogen), $pK_2 = 5.20$ (caused by the protonation of the pyridine nitrogen) and $pK_1 \approx 14$ (due to the proton dissociation of the imino group).

Characteristics of Palladium(II) Complex

The blue palladium(II) complex formed with the reagent is sparingly soluble in water, but readily soluble in various organic solvents such as aromatic hydrocarbons, halogenated hydrocarbons, acetate esters, ketones and aliphatic alcohols. The spectral characteristics of the complex depend on the solvent used. Benzene was chosen as the extractant because the highest absorbance of the complex in the visible region was obtained in this solvent. Under the optimum conditions of the reagent and chloride ion concentrations, acidity and shaking period, palladium(II) can be quantitatively extracted from aqueous solution with one 10-ml portion of TAQH in benzene over the concentration range studied. The absorption spectra of the reagent and the palladium(II) complex in benzene are shown in Fig. 2, the absorption maxima of the complex against the reagent blank being found at 588 and 625 nm. The absorbance measurements were made at the latter wavelength unless stated otherwise.

Effects of Experimental Conditions

The absorbance of the organic phase was measured as a function of the acidity of the aqueous phase. The data are plotted in Fig. 3, indicating that maximum and constant absorbance is obtained between pH 4.3 (in the presence of chloride ions) and 0.5 M in hydrochloric acid. In less or more acidic solutions, the absorbance decreases because of slow complex formation and of relatively rapid colour fading, respectively.

If a suitable amount of chloride is present in the aqueous phase, hydrochloric acid can be replaced by sulphuric acid of the same concentration.

A molar-ratio study demonstrated that only a three-fold molar excess of the ligand in the organic phase is necessary for maximum and reproducible absorbance. Addition of more reagent did not interfere with the formation and extraction of the complex.

Palladium(II) is quantitatively extracted into the organic phase by shaking for more than 5 min; a shaking period of 10 min was selected. The colour intensity of the complex thus obtained is constant for at least 24 h. The absorbance of the organic phase (10 ml) was also constant when the aqueous phase was varied between 5 and 40 ml.

Stoicheiometry

The method of continuous variations as modified by Vosburgh and Cooper[5] was used to evaluate the stoicheiometric ratio of metal to ligand in the complex. The results represented in Fig. 4 establish the 1 : 1 molar ratio.

As mentioned above, the presence of chloride ions was important for the formation of the extractable palladium(II) - TAQH complex. In the presence of chloride ions, the log - log plot of the distribution ratio of palladium(II) against the chloride ion concentration of the aqueous phase acidified with sulphuric acid is linear with a slope of nearly unity (Fig. 5). This means that the fourth co-ordination site of palladium(II) is occupied by a chloride ion to form a 1 : 1 : 1 mixed-ligand complex, as described earlier.[6]

It was also found that a similar mixed-ligand complex is formed in the presence of bromide or nitrate ions in place of

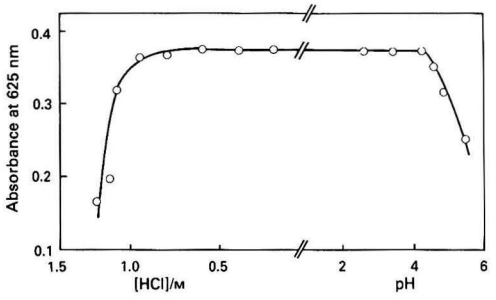


Fig. 3. Dependence of palladium(II) - TAQH complex formation on acidity. [Pd(II)], 2.0×10^{-5} M; [TAQH], 1.0×10^{-3} M

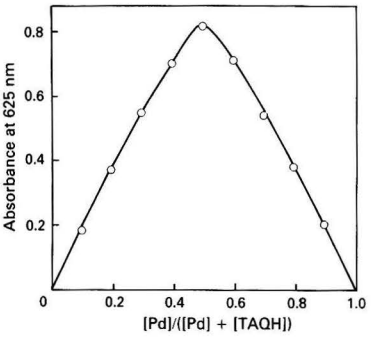


Fig. 4. Continuous variations plot for the palladium(II) - TAQH system. [Pd(II)] + [TAQH], 1.0×10^{-4} M; HCl, 0.3 M

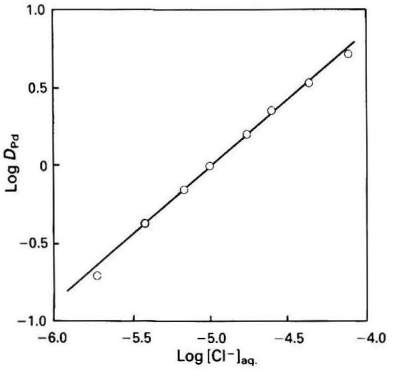


Fig. 5. Log - log plot of palladium(II) distribution *versus* chloride ion concentration. [Pd(II)], 2.0×10^{-3} M; [TAQH], 1.0×10^{-3} M; H_2SO_4, 0.15 M

chloride ions, although a minor shift in the position of the maximum absorption of the complex is observed.

Conformance to Beer's Law

A calibration graph for the determination of palladium was prepared under the optimum conditions. Good linearity was obtained between the absorbance of the organic phase and the concentration of palladium(II) up to about 4×10^{-5} M (4.2 μg ml^{-1}) at 625 nm. The molar absorptivity of the complex, calculated from the calibration graph data, is 1.93×10^4 l mol^{-1} cm^{-1}. The precision of the method was estimated for 20.8 μg of palladium, the coefficient of variation for eight determinations being 0.48%.

Comparison of Sensitivity

Table 1 presents a comparison of the spectral properties of the palladium complexes with several heterocyclic-substituted hydrazones as tridentate ligands. The position of the maximum absorption and the molar absorptivity of the TAQH complex seems to reflect the increased double-bonding characteristic of the linkage between the carbon atom at the 2-position and the co-ordinating nitrogen atom in the thiazole ring. Compared with benzothiazole-2-carbaldehyde 2-quinolylhydrazone, as may be expected, TAQH is slightly less sensitive for palladium(II); however, the method with the former ligand is influenced by chloride ions even when present at relatively low concentrations.

Effect of Diverse Ions

The possible interference of various ions was examined by introducing them into a solution containing 21.1 μg of palladium. The tolerance limit of an ion was fixed as the maximum amount causing an error not greater than 2% in the absorbance of the extract solution. The results are summarised in Table 2. Under the experimental conditions of the determination, the colour reaction of TAQH is specific for palladium(II), except for platinum(II), which slowly forms a blue complex with the ligand. However, the tolerance limits for mercury(II), gold(III), rhenium(III), irridium(III) and tungsten(VI) are low; these ions form insoluble yellow precipitates on the boundary of the two phases, giving negative errors. Iodide, thiocyanate and EDTA must be absent.

Application to Real Samples

In order to confirm the usefulness of the proposed method, it was applied to the determination of palladium in three palladium catalysts. The results are listed in Table 3 together with those obtained by a gravimetric method. Both sets of the results are in good agreement.

Table 1. Spectral properties of palladium(II) complexes with different nitrogen-containing heterocyclic hydrazones as tridentate ligands

Hydrazone $[R_1(R_2)C{=}NNHR_3]$						
R_1	R_2	R_3	Solvent	$\lambda_{max.}$/nm	$\varepsilon_{max.}$ / l mol^{-1} cm^{-1}	Reference
H	2-Pyridyl	2-Pyridyl	*o*-Dichlorobenzene	562	1.65×10^4	7
H	2-Pyridyl	2-Quinolyl	Chloroform	594	1.15×10^4	8
H	2-Pyridyl	1-Phthalazinyl	Chloroform	537	1.24×10^4	9
H	2-Quinolyl	2-Pyridyl	Benzene	615	1.6×10^4	7
H	2-Quinolyl	1-Phthalazinyl	Chloroform	580	1.37×10^4	9
H	6-Phenanthridyl	2-Pyridyl	Benzene	635	2.0×10^4	7
H	2-Thiazolyl	2-Quinolyl	Benzene	625	1.93×10^4	This work
H	2-Benzothiazolyl	1-Phthalazinyl	Chloroform	587	1.68×10^4	9
H	2-Benzothiazolyl	2-Quinolyl	Benzene	647	2.14×10^4	6

Table 2. Effect of foreign ions on the determination of 21.1 μg of palladium

Tolerance limit ([ion]/[Pd])	Ion
≥10 000	Cl^-, Br^-, ClO_4^-, NO_3^-, SO_4^{2-}, $C_2O_4^{2-}$, PO_4^{3-}, tartrate, citrate, ascorbate
≥100	Al, As(V), Ba, Be, Bi,* Ca, Cd, Co, Cr(III), Cu(II), Fe(II,III) Mg, Mn(II), Ni, Pb, Sc, Sr, Th, Ti, Tl(I), U(VI), V(V), Zn, Zr
≤50	Os(VIII), Cr(VI),† Hg(II), CN^-
≤10	Ag, Au(III),† Pt(IV), Re, Rh
≤3	Pt(II), Ru(III), W(VI),‡ SCN^-
≤1	Ir(III), I^-, NO_2^-

* Hydrochloric acid concentration of aqueous phase was 0.1 M.
† A 1-ml volume of 1% ascorbic acid was added.
‡ A 2-ml volume of 1 M tartaric acid was added.

Table 3. Determination of palladium in palladium catalysts*

Catalyst	Pd found, %	
	Proposed method	Gravimetric method[4]
Pd - $CaCO_3$	4.91, 4.93, 4.93, 4.93	4.95, 4.92, 4.95, 4.96
Pd - $CaCO_3$ - Pb	5.00, 4.97, 4.99	4.94, 4.95, 4.97
Pd - Al_2O_3	4.95, 4.90, 4.91	4.95, 4.86, 4.90

* Purchased from Aldrich Chemical Co.

Conclusions

The newly synthesised heterocyclic hydrazone, TAQH, as an *N,N,N*-tridentate ligand, is a reasonably sensitive and very selective reagent for palladium(II). On the basis of the extractability of the palladium(II) - TAQH - chloride mixed-ligand complex into benzene, a simple and reproducible method for the spectrophotometric determination of trace amounts of palladium has been devised and applied successfully to the analysis of palladium catalyst samples.

References

1. Zatka, V., Abraham, J., Holzbecher, J., and Ryan, D. E., *Anal. Chim. Acta*, 1971, **54**, 65.
2. Erlenmeyer, H., Weber, O., Schmidt, P., Küng, G., Zinsstag, Chr., and Prijs, B., *Helv. Chim. Acta*, 1948, **31**, 1142.
3. Kaplan, H., *J. Am. Chem. Soc.*, 1941, **63**, 2654.
4. Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorganic Analysis," Second Edition, Wiley, New York, 1953, p. 379.
5. Vosburgh, W. C., and Cooper, G. R., *J. Am. Chem. Soc.*, 1941, **63**, 437.
6. Otomo, M., and Kodama, K., *Bunseki Kagaku*, 1977, **26**, 455.
7. Quddus, M. A., and Bell, C. F., *Anal. Chim. Acta*, 1968, **42**, 503.
8. Heit, M. L., and Ryan, D. E., *Anal. Chim. Acta*, 1965, **32**, 448.
9. Otomo, M., and Nakayama, I., *Microchem. J.*, 1980, **25**, 75.

Paper A4/390
Received November 12th, 1984
Accepted December 3rd, 1984

Extractive Spectrophotometric Determination of Mercury(II) with 4-(2-Pyridylazo)resorcinol and a Long-chain Quaternary Ammonium Salt

Yerramilli Anjaneyulu,* P. Chandra Mouli, M. Ravi Prakasa Reddy and Kavi Purapu Chandra Sekhar
Department of Chemistry, Nagarjuna University, Nagarjuna Nagar-522 510, Andhra Pradesh, India

Mercury(II) can be extracted quantitatively as a 1 : 1 : 2 mercury - 4-(2-pyridylazo)resorcinol - cetyldimethylbenzylammonium chloride ternary complex in the pH range 7–8. This ternary system obeys Beer's law in the range 0.5–6.25 μg ml^{-1} of mercury. 1,10-Phenanthroline completely masks the extraction of Hg(II) in the presence of 4-(2-pyridylazo)resorcinol and cetyldimethylbenzylammonium chloride, whereas other divalent metal ions are quantitatively extractable, which can be exploited as an efficient technique for the separation of mercury from other divalent metal ions. Based on these results, useful procedures for the determination of mercury in medicinal samples and synthetic mixture were developed.

Keywords: *Mercury(II) determination; extractive spectrophotometry; ternary complexes; 4-(2-pyridylazo)resorcinol; cetyldimethylbenzylammonium chloride*

The complex formation and spectrophotometric determination of Hg(II) using 4-(2-pyridylazo)resorcinol (PAR) has been studied by several workers,[1-3] but the extraction of Hg(II) complexes has not been studied so far. Because of their bulky organic nature, quaternary ammonium cations act as good extracting agents for a number of anionic complexes and owing to the simplicity of extraction a number of extractive spectrophotometric studies have been made on the anionic complexes of PAR with various divalent metal ions.[4-11]

Although a number of other quaternary ammonium salts, such as tetradecylbenzylammonium chloride, tetraphenylarsonium chloride and tetraphenylphosphonium chloride, extract the mercury - PAR anionic complex very effectively, cetyldimethylbenzylammonium chloride (CDBAC) was chosen because of ease of separation of the two phases and higher colour sensitivity of the extract.

In this paper we report the results of an investigation of the extraction of the Hg(II) - PAR complex with CDBAC.

Experimental

Apparatus

Absorbance measurements were made with a Systronics Model 106 Mk(II) digital spectrophotometer. pH measurements were made on an Elico Model 335 expanded pH meter.

Reagents

Mercury(II) stock solution. A 1 × 10^{-2} M stock solution of Hg(II) is prepared by dissolving approximately 3 g of mercury(II) nitrate (BDH Chemicals) in 1 l of water to which a few drops of nitric acid are added in order to prevent hydrolysis. The mercury solution is standardised with standard EDTA solution using xylenol orange as indicator.[12] Solutions of the required strength are obtained by diluting this stock solution accurately.

4-(2-Pyridylazo)resorcinol (PAR). A fresh 1 × 10^{-2} M solution of PAR monosodium salt is prepared by dissolving the compound (Riedel-de Haën) crystallised twice from ethanol - water (1 + 1), in de-mineralised water.

Cetyldimethylbenzylammonium chloride (CDBAC). A 2 × 10^{-3} M solution of CDBAC is prepared by dissolving 0.082 g of the salt (Fluka) in 100 ml of doubly distilled water.

1,10-Phenanthroline solution. A 1 × 10^{-2} M solution of 1,10-phenanthroline is prepared by dissolving 0.9921 g of the compound (Sisco) in 500 ml of doubly distilled water containing 1.5 ml of nitric acid.

Buffer solution. A buffer solution of pH 7.2 is prepared from 0.0666 M disodium hydrogen phosphate and 0.0666 M potassium dihydrogen orthophosphate solutions.

Chloroform. Doubly distilled chloroform (BDH Chemicals) was used in all the investigations and in view of its toxic nature all extractions were carried out in a fume-cupboard.

Procedure

Pipette 5 ml of sample solution containing 0.25–2.00 μg ml^{-1} of mercury into a 125-ml separating funnel. Add 5.0 ml of phosphate buffer solution (pH 7.2), 2.0 ml of 1 × 10^{-2} M PAR solution, 5.0 ml of 2 × 10^{-3} M CDBAC solution and dilute with water to 20.0 ml. Add an exactly measured 10.0-ml portion of chloroform and shake the funnel vigorously for about 2 min. Allow the layers to separate clearly and, after 5 min, run the orange - red organic extract into a 10-mm glass cell. Measure the absorbance at 525 nm against a reagent blank as reference solution.

Results and Discussion

Optimum Solvent for Extraction

Of the various solvents studied, only chloroform, butan-1-ol and trichloroethane extract the Hg - PAR - CDBAC ternary complex effectively and, owing to the ease of separation and the high colour sensitivity, chloroform was used in all subsequent investigations.

Absorption Spectra

The absorption spectrum of the mercury(II) - PAR - CDBAC ternary complex in chloroform has an absorption maximum at 525 nm. The colour of the system is stable up to 20 min after separation of the two phases.

Optimum Conditions

A study of the effect of pH on the formation and extraction of the ternary complex showed the formation of at least two Hg(II) - PAR complexes extracted with CDBAC, one in the pH range 4.5–7.7 with $\lambda_{max.}$ at 525 nm and the other in the pH range 8–9.5 with $\lambda_{max.}$ at 530 nm. However, at pH values greater than 8 the absorbance decreased markedly owing to hydrolysis. As the colour sensitivity was greatest between pH

* To whom correspondence should be addressed.

7 and 7.5, our investigations were carried out at pH 7.2 using phosphate buffer. A 20-fold excess of both PAR and CDBAC over Hg(II) was found to be necessary in order to achieve maximum absorbance for the ternary system; at higher reagent concentrations no further change in the absorbance value was observed. However, a very large excess of CDBAC will retard the rate at which the two phases separate and remain clear.[13]

Beer's Law and Sensitivity

The system obeyed Beer's law in the range of 0.5–6.25 μg ml^{-1} of Hg(II) at 525 nm, with a molar absorptivity of 3.06×10^4 l mol^{-1} cm^{-1}.

Composition of the Hg(II) - PAR - CDBAC Complex

The composition of the extracted species was determined by Job's method of continuous variations, which revealed a 1:1:2 ratio for the Hg(II) - PAR - CDBAC ternary system.

From these results, the extraction of Hg(II) with PAR and CDBAC as a ternary complex can be explained by the following scheme:

$$[Hg(H_2O)_4]^{2+} + H_2R \rightleftharpoons [Hg(H_2O)R] + 2H^+ + 3H_2O$$

$$[Hg(H_2O)R] + 2CDBAC \underset{\text{phase}}{\overset{\text{organic}}{\rightleftharpoons}} [Hg(R)(CDBAC)]_{CDBAC\ solvent} + H_2O$$

where H_2R = PAR. In this scheme, in the extracting species one CDBAC moiety acts as a neutral coordinating molecule, expelling water from the coordinating sphere, and the other as a solvating molecule. Such a coordination by quaternary ammonium bases on valency-saturated but coordination-unsaturated chelates was reported by Nishimura *et al.*[14] Further, we observed that at higher concentrations of Hg(II) the complex separates as a solid in the aqueous phase, which also indicates that the ternary complex must exist as a neutral species in the aqueous phase also. As reported earlier for other complexes,[15] it is considered that PAR acts as a tridentate ligand, coordinating to Hg(II) through its pyridyl nitrogen, azo nitrogen and the *o*-hydroxy group.

Analytical Applications

Analysis of synthetic mixtures

It was observed that 1,10-phenanthroline, even when present in low concentrations, completely inhibits the extraction of the Hg - PAR - CDBAC system, whereas the other divalent metals that are normally associated with Hg(II) in commercial samples, such as Cd(II), Zn(II), Ni(II), Mn(II) and Pb(II), are quantitatively extractable as the corresponding metal - PAR - CDBAC ternary systems, particularly in the presence of high concentrations of 1,10-phenanthroline. This is understandable if we compare the formation constants (log β_2) of the 1:2 complexes of the above metals with 1,10-phenanthroline (Cd = 10.82; Mn = 7.61; Ni = 17.10; Zn = 12.25; Hg = 19.65) and with PAR (Cd = 21.60; Mn = 18.90, Ni = 26.00; Zn = 25.30; Hg = 11.00).[16–19] This situation was exploited for the selective separation of Hg from other divalent metals.

Procedure for the selective separation of Hg(II) from other divalent metal ions. To a synthetic mixture containing 15 μg of Hg(II) and various divalent metal ions in a 125-ml separating funnel, add 2.0 ml of 1,10-phenanthroline, 2.0 ml of 1×10^{-2} M PAR solution and 2.0 ml of 2×10^{-3} M CDBAC solution. Maintain the pH at 7.2 by adding 5.0 ml of phosphate buffer, make the volume up to 20.0 ml with distilled water, then equilibrate with 20.0 ml of chloroform for 5 min and remove the organic phase after clear separation of the two phases. Repeat this extraction process with another fresh aliquot of chloroform (20.0 ml) and collect the organic phase. In this process all divalent metal ions will pass into the organic phase as metal - PAR - CDBAC ternary systems and Hg(II) will be in the aqueous phase. Adjust the pH of the aqueous phase to 1.5 by adding dilute sulphuric acid and remove the excess of 1,10-phenanthroline by equilibrating with 10.0 ml of chloroform for 2 min. Determine the Hg(II) present in the aqueous phase by following the general extraction procedure.

Typical results are presented in Table 1.

Analysis of medicinal samples

Mercury-containing drugs should be carefully digested because prolonged digestion results in a significance loss of volatile substances. The procedure given below for the digestion of the following samples is found to give satisfactory results.

Ointments. Accurately weigh a portion of ointment containing 5 mg of mercury after thorough mixing and transfer into a 50-ml beaker. Add 5 ml of water - hydrochloric acid - nitric acid (4 + 3 + 1), cover with a watch-glass and heat on a steam-bath for 30 min. Cool to room temperature, swirl the beaker to coagulate the fat, decant the solution and rinse the residue three times with 10-ml portions of water into a 50-ml calibrated flask. Add 2 ml of 5% potassium dichromate solution and dilute to the mark with water after thorough mixing. Determine the mercury present by following the general extraction procedure.

Preservatives. Pipette duplicate aliquots containing 5–50 μg of mercury into a 25-ml calibrated flask. Place on a steam-bath and evaporate almost to dryness in a current of air. Add 5 ml of water - hydrochloric acid - nitric acid (4 + 3 + 1) and heat on a steam-bath for 30 min. Blow air into the flask for 2–3 min to expel amine oxides. After cooling to room temperature, add 30 ml of water and 2 ml of 5% potassium dichromate solution and dilute to the mark with water. Determine the mercury present by following the general extraction procedure.

The results are presented in Table 2.

Conclusion

Hg(II) can be quantitatively extracted into chloroform as the Hg - PAR - CDBAC ternary complex. The extraction of mercury is completely inhibited in the presence of an excess of 1,10-phenanthroline, whereas other divalent metal ions are extracted quantitatively into the organic phase as metal -

Table 1. Determination of Hg(II) in some synthetic mixtures containing divalent metals using PAR - CDBAC after prior separation

Mixture/μg	Hg(II) added/μg	Hg(II) found/μg	
		Hg - PAR - CDBAC method	Dithizone method
Hg(II)	15	14.8	14.60
Hg(II) + Zn(II) (100), Cd(II) (50)	15	14.6	15.14
Hg(II) + Ni(II) (100), Mn(II) (100)	15	14.9	14.80
Hg(II) + Mn(II) (100), Pb(II) (100)	15	15.2	15.40

Table 2. Determination of Hg(II) in some medicinal samples using PAR - CDBAC

Sample	Initially found,* %	Added, %	Total found,* %	Recovery, %
Yellow mercury(II) oxide, ophthalmic ointment, 1%	1.02400	2.1800	3.14120	98.04
Dexamethazone eye drops containing mercury as phenylmercury(II) nitrate as a preservative:				
Mycidex (0.001%) [Mac. Lab. (P) Ltd., Bombay]	0.00120	0.0124	0.012700	93.38
Pyrimon (0.002%) (Fairdeals Corp. Pvt. Ltd., Madras)	0.00180	0.0156	0.01790	102.87
Dexoren-S (0.002%) (Warren Pharmaceutical Pvt. Ltd., Bombay)	0.00185	0.0132	0.01590	105.65
Dexaber-C (0.002%) (Ebers Pharmaceutical Pvt. Ltd., Bombay)	0.00191	0.0109	0.01210	94.46
Soframycin (0.002%) (Roussel 1 Pharmaceutical Pvt. Ltd., Bombay)	0.00188	0.0189	0.01960	94.32
Otek (0.1%) (Fairdeals' Corp. Pvt. Ltd., Madras)	0.09600	0.1210	0.2240	103.23

* Average of three determinations.

PAR - CDBAC ternary complexes. This situation can be exploited for the selective separation of Hg(II) from other divalent metals and Hg(II) can be determined by the proposed method.

One of us (P.C.M.) thanks the University Grants Commission, New Delhi, for providing financial assistance through the Faculty Improvement Programme.

References

1. Ueda, J., *Nippon Kagaku Zasshi,* 1971, **92**, 418.
2. Funahashi, S., Tabata, M., and Tanaka, M., *Anal. Chim. Acta,* 1971, **57**, 311.
3. Eswar, M. C., and Nagarkar, S. G., *Z. Anal. Chim.,* 1972, **260**, 289.
4. Yotsuyanagi, T., Takeda, Y., Yamashite, R., and Aomura, K., *Anal. Chim. Acta,* 1973, **67**, 297.
5. Yotsuyanagi, T., Yamashite, R., and Aomura, K., *Fresenius Z. Anal. Chem.,* 1971, **253**, 301.
6. Nishimura, M., Matsunaga, K., Kudo, T., and Obara, F., *Anal. Chim. Acta,* 1973, **65**, 466.
7. Yotsuyanagi, T., Hoshino, H., and Aomura, K., *Anal. Chim. Acta,* 1974, **71**, 349.
8. Hoshino, H., Yotsuyanagi, T., and Aomura, K., *Anal. Chim. Acta,* 1976, **83**, 317.
9. Yotsuyanagi, T., Yamashite, R., Hoshino, H., and Aomura, K., *Anal. Chim. Acta,* 1976, **82**, 431.
10. Nonova, D., Menov, V., and Lihareva, N., *Talanta,* 1976, **23**, 679.
11. Papp, E., and Inczedy, J., *Talanta,* 1980, **27**, 49.
12. "Handbook of Organic Reagents in Inorganic Analysis," Wiley, New York, 1972, pp. 281–341.
13. Monova, D., and Pavlova, S., *Anal. Chim. Acta,* 1981, **123**, 289.
14. Nishimura, M., Noriki, S., and Muramoto, S., *Anal. Chim. Acta,* 1974, **70**, 121.
15. Flaschka, H. A., and Barnard, A. J., "Chelates in Analytical Chemistry," Marcel Dekker, New York, 1972, p. 128.
16. Anderegg, G., *Helv. Chim. Acta,* 1963, **46**, 2197.
17. Hnilickova, M., and Sommer, L., *Collect. Czech. Chem. Commun.,* 1961, **26**, 2189.
18. Corsini, A., Yih, I. N. L., Fernando, Q., and Freiser, H., *Anal. Chem.,* 1962, **34**, 1090.
19. Malysz, D., *Chem. Anal. (Warsaw),* 1972, **17**, 155.

Paper A4/256
Received July 30th, 1984
Accepted November 26th, 1984

Spectrophotometric Methods for the Determination of *o*-Dihydroxybenzene Derivatives

Chilukuri Suryaprakasa Sastry, V. Gurucharana Das and K. Ekambareswara Rao
Food and Drugs Laboratories, School of Chemistry, Andhra University, Waltair-530 003, India

Methods are described for the determination of *o*-dihydroxybenzene derivatives using *p*-aminoacetophenone (for catechol, guaiacol, eugenol and dopamine), thiosemicarbazide or isoniazid in the presence of sodium metaperiodate. The methods are reproducible and specific for these compounds. The method using *p*-aminoacetophenone - periodate is extended to the determination of eugenol in clove oil and dopamine in pharmaceutical preparations.

Keywords: *o-Dihydroxybenzene derivative determinations; spectrophotometry; clove oil; pharmaceutical preparations; oxidation reagents*

Several spectrophotometric methods have been reported for the determination of phenolic compounds.[1,2] The well established chromogenic reagents include *p*-aminophenol, *N,N*-dimethyl-*p*-phenylenediamine, *p*-methylaminophenol sulphate (metol) or *p*-aminophenazone in the presence of oxidant. Only a few specific methods have been reported for *o*-dihydroxybenzene (catechol) and its monomethyl ethers (guaiacol and eugenol) and catecholamine (dopamine). Typical reagents include *m*-aminophenol - sodium metaperiodate,[2] *m*-phenylenediamine - sodium metaperiodate[3] and sodium nitrite - alkali.[4]

In this paper we propose three simple, rapid, sensitive and accurate methods for the determination of *o*-dihydroxybenzene derivatives using *p*-aminoacetophenone (catechol, guaiacol, eugenol and dopamine) and thiosemicarbazide or isoniazid (catechol and guaiacol) in the presence of sodium metaperiodate. The methods are reproducible and specific for these compounds, as other phenolic compounds do not interfere. The method using *p*-aminoacetophenone - periodate is also extended to the determination of eugenol in clove oil and dopamine in pharmaceutical preparations.

Experimental

Apparatus

A Shimadzu UV 140 double-beam spectrophotometer with 1-cm glass cells was used for spectral scans and absorbance measurements. A Systronics Model 325 pH meter was used for pH adjustment.

Reagents

Sodium metaperiodate solution, 0.01 M. Prepared by dissolving 1.07 g of analytical-reagent grade sodium metaperiodate (BDH Chemicals) in 500 ml of distilled water.

o-Dihydroxybenzene derivative standard solutions. Prepared by dissolving 50 mg of each compound (catechol, guaiacol, eugenol and dopamine) separately in 100 ml of distilled water or methanol (if the compound is insoluble in water). Solutions of lower concentrations (100 μg ml^{-1}) were prepared by diluting aliquots of the stock solution.

p-Aminoacetophenone (AAP, 0.1%), isoniazid (INH, 0.2%) and thiosemicarbazide (TSC, 0.1%) solutions. Prepared by dissolving the requisite amount of each compound separately in 100 ml of distilled water or methanol (if insoluble in water).

Potassium hydrogen phthalate (pH 3.4), borate (pH 6.8) and *glycine (pH 8.5) buffer solutions.* Prepared as described.[5]

General Procedure

A 15-ml volume of the buffer solution and requisite volumes of AAP, INH or TSC and IO_4^- solutions were placed in a 25-ml calibrated flask to which 0.4–5.0 ml of the *o*-dihydroxybenzene derivative solution was added and the mixture was diluted to the mark with distilled water. The absorbance of the coloured species formed was measured during the stability period at $\lambda_{max.}$ (shown in Table 1) against a reagent blank prepared in a similar manner. The amount of compound was

Table 1. Experimental conditions

Dihydroxybenzene derivative	Reagent	pH	Volume of AAP (0.1%), INH (0.2%) or TSC (0.1%)/ml	Volume of 0.01 M $NaIO_4$ solution/ml	Stability/ min	$\lambda_{max.}$/ nm
Catechol	AAP - IO_4^-	3.4* (2.8–4.0)†	2.0* (1.5–2.3)†	3.0* (2.3–3.3)†	6–70	510
Guaiacol		3.4 (2.8–4.0)	2.0 (1.5–2.3)	3.0 (2.5–3.6)	17–45	500–510*
Eugenol		3.4 (2.8–4.0)	2.0 (1.5–2.3)	3.0 (2.5–3.6)	10–35	500
Dopamine		3.4 (2.8–4.0)	2.0 (1.5–2.3)	3.0 (2.3–3.3)	15–65	440
Catechol	INH - IO_4^-	6.8 (6.5–7.0)	1.0 (0.75–1.3)	3.0 (2.5–3.3)	10–40	460
Guaiacol		6.8 (6.5–7.0)	1.0 (0.75–1.3)	3.0 (2.5–3.6)	15–30	460
Catechol	TSC - IO_4^-	8.5 (8.0–8.8)	1.0 (0.7–1.25)	1.0 (0.5–1.2)	12–22	570
Guaiacol		8.5 (8.0–8.8)	1.0 (0.7–1.25)	1.0 (0.75–1.5)	15–40	570

* Used in the suggested procedure.
† Range for maximum absorbance and stability.

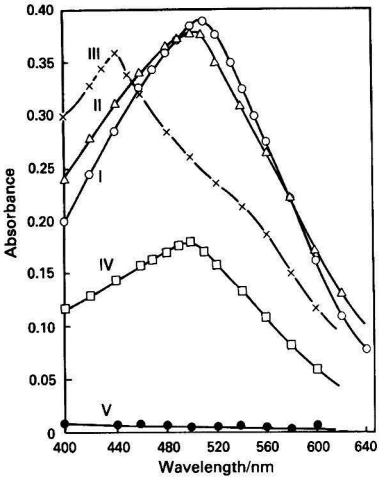


Fig. 1. Absorption spectra of I, catechol - AAP - IO_4^-; II, guaiacol - AAP - IO_4^-; III, dopamine - AAP - IO_4^-; IV, eugenol - AAP - IO_4^- (all against reagent blank); and V, AAP - IO_4^- - distilled water. [Catechol] = 7.29×10^{-5} M; [guaiacol] = 8.0×10^{-5} M; [dopamine] = 12.0×10^{-5} M; [eugenol] = 7.3×10^{-5} M; [AAP] = 5.92×10^{-4} M; and [IO_4^-] = 12.0×10^{-4} M

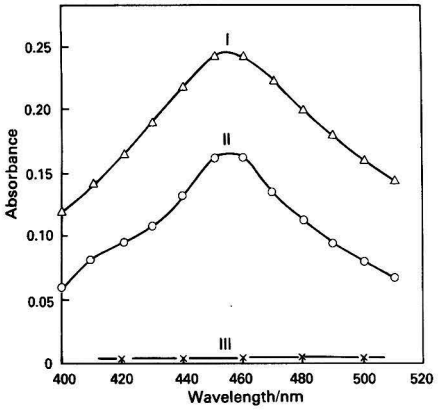


Fig. 2. Absorption spectra of I, catechol - INH - IO_4^- (against reagent blank; II, guaiacol - INH - IO_4^- (against reagent blank); and III, INH - IO_4^- (against distilled water). [Guaiacol] = 1.612×10^{-4} M; [INH] = 5.839×10^{-4} M; [IO_4^-] = 1.20×10^{-4} M; and [catechol] = 0.727×10^{-4} M

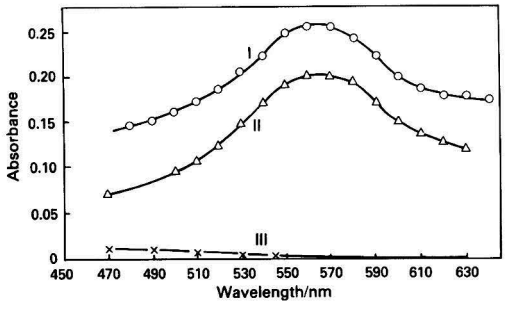


Fig. 3. Absorption spectra of I, catechol - TSC - IO_4^- (against reagent blank); II, guaiacol - TSC - IO_4^- (against reagent blank); and III, TSC - IO_4^- (against distilled water). [Guaiacol] = 1.612×10^{-4} M; [TSC] = 4.405×10^{-4} M; [catechol] = 0.727×10^{-4} M; and [IO_4^-] = 4.0×10^{-4} M

deduced from a calibration graph prepared from standard solutions under identical conditions.

The optimum and experimental conditions for each method are shown in Table 1.

Procedure for the Determination of Eugenol in Clove Oil

Clove oil was dissolved in methanol and diluted further to bring the eugenol concentration to 100 μg ml^{-1}; the general procedure was then followed. The amount of eugenol was determined from a calibration graph.

Procedure for the Determination of Dopamine in Pharmaceutical Preparations

Injection solution was appropriately diluted with distilled water to bring the dopamine concentration to 100 μg ml^{-1} and the general assay procedure was followed. The amount of dopamine was calculated from a calibration graph.

Results and Discussion

The absorption spectra of the coloured species (Figs. 1–3) show characteristic maxima (Table 1), whereas the oxidant with AAP (INH or TSC) or phenolic compounds show no absorbance in these regions. Beer's law limits, molar absorptivities and Sandell's sensitivity values are given in Table 2. The optimum conditions such as maintenance of pH and concentration of AAP, INH or TSC and IO_4^- necessary for attaining maximum absorbance and stability of the colour were established through control experiments and these conditions are incorporated in Table 1. The order of addition of reagents did not have any influence on the colour development, if mixing was carried out immediately. However, any delay in mixing *o*-dihydroxybenzene derivative to AAP, INH or TSC - IO_4^- reagent caused a considerable decrease in absorbance.

Other oxidising agents such as MnO_4^-, $Fe(CN)_6^{3-}$, Fe(III), OCl^-, CAT (chloramine T) and H_2O_2 were tried instead of IO_4^- and found to be less effective.

The precision and accuracy of the proposed and reported methods were studied by measuring the absorbance of six replicates containing 150 μg of the phenolic compound in each instance. The standard error, relative standard deviation and range of error (95% confidence limits) are given in Table 3.

The results obtained for the analysis of clove oil for eugenol and dopamine formulations are given in Table 4. Recovery experiments were carried out by adding 5 mg of accurately weighed eugenol and dopamine to previously analysed clove oil and dopamine injections, respectively, and analysed again. The recoveries obtained are given in Table 4.

Interference Studies

The interference due to other phenols in the determination of catechol (150 μg) with AAP - IO_4^- was studied; 5 mg of phenol, resorcinol, hydroquinone, 2-naphthol or phlorogluci-nol, 2 mg of 1-naphthol and 1 mg of pyrogallol did not interfere.

The excipients and other active ingredients usually present in formulations of dopamine did not interfere, whereas other catecholamines (adrenaline, noradrenaline, dopa, methyl-dopa and isoprenaline sulphate) interfered seriously.

Vicinal dihydroxybenzene derivatives (catechol and dopamine) and other monomethyl ethers (guaiacol and eugenol) were readily oxidised to *o*-benzoquinone (and methanol for monomethyl ethers) by periodate. AAP (or INH or TSC), by virtue of its strong electron-donating groups, couples with *o*-benzoquinone leading to the formation of oxidative-coupled products, *viz.*, indophenols (Fig. 4).

Table 2. Optical characteristics

Hydroxybenzene derivative	Reagent	Beer's law range/ μg per 25 ml	Molar absorptivity/ l mol^{-1} cm^{-1}	Sandell's sensitivity/ μg cm^{-2} per 0.001 absorbance unit
Catechol	AAR - IO_4^-	20–300	5.5×10^3	0.02
Guaiacol		30–250	4.65×10^3	0.026
Eugenol		50–350	2.7×10^3	0.061
Dopamine		50–600	2.85×10^3	0.066
Catechol	INH - IO_4^-	30–200	3.368×10^3	0.033
Guaiacol		100–500	1.056×10^3	0.117
Catechol	TSC - IO_4^-	30–200	3.575×10^3	0.03
Guaiacol		100–500	1.24×10^3	0.1

Table 3. Precision and accuracy of the methods*

Hydroxybenzene derivative	Reagent	Standard error,%	Relative standard deviation,%	Range of error (95% confidence limit),%
Catechol	AAP - IO_4^-	0.72	0.81	±0.85
Guaiacol		0.92	1.26	±1.32
Eugenol		1.17	1.69	±1.78
Dopamine		1.44	0.16	±1.08
Catechol	INH - IO_4^-	1.03	1.15	±1.25
Guaiacol		1.45	1.56	±1.75
Catechol	TSC - IO_4^-	1.14	0.57	±0.70
Guaiacol		1.26	1.38	±1.55
Catechol	Results of reported	0.6	0.78	±1.01
Guaiacol	methods[2]	0.9	0.10	±0.97
Eugenol		0.7	0.58	±0.61
Dopamine		1.5	0.60	±1.91

* Data obtained from six replicate samples containing 150 μg of phenolic compound in each instance.

Table 4. Results of analysis of real samples and recovery experiments

Sample	Labelled amount	Amount found* Proposed method	Amount found* Reported method[2]	Recovery, %
Clove oil I	—	28.2%	28.1%	98.8
Clove oil II	—	28.3%	28.0%	98.6
Injections:				
DH,† 200 mg per 5 ml	200 mg	197.7 mg	197.2 mg	98.0
DH, 200 mg per 5 ml	200 mg	197.4 mg	196.8 mg	98.2

* Each value is an average of three individual determinations.
† DH, Dopamine hydrochloride.

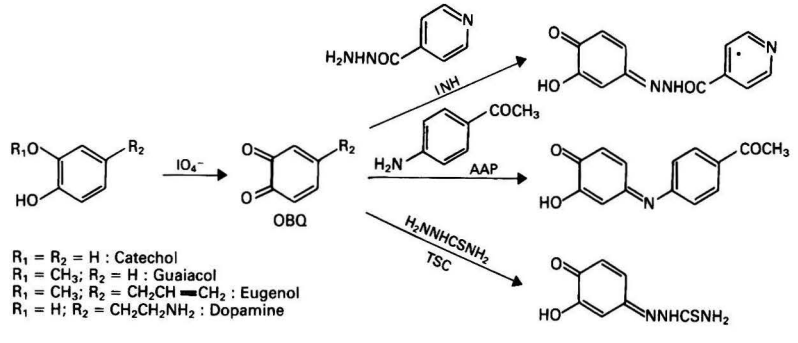


Fig. 4. Oxidation reactions of vicinal dihydroxybenzene derivatives (catechol and dopamine) and other monomethyl ethers (guaiacol and eugenol)

We are grateful to the authorities of Andhra University and to the Council of Scientific and Industrial Research, India for awarding a senior research fellowship to K.E.R.

References

1. Cheronis, N. D., and Ma, T. S., "Organic Functional Group Analysis by Micro and Semimicro Methods," Wiley, New York, 1964, p. 454.
2. Sastry, C. S. P., Rao, K. E., and Prasad, U. V., *Talanta*, 1982, **29**, 917, and references cited therein.
3. Rao, K. E., and Sastry, C. S. P., *Mikrochim. Acta*, 1984, **I**, 313.
4. Waite, J. H., and Tanzer, M. L., *Anal. Biochem.*, 1981, **111**, 137.
5. Lurie, Yu., "Handbook of Analytical Chemistry," Mir, Moscow, 1975, p. 255.

Paper A4/341
Received November 2nd, 1984
Accepted December 3rd, 1984

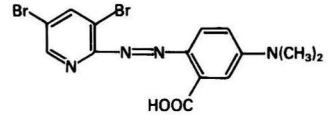
Spectrophotometric Determination of Cobalt in Pepperbush Leaves and Coal Fly Ashes Using 2-(2-Benzothiazolylazo)-5-dimethylaminobenzoic Acid

Takeo Katami and Tomokuni Hayakawa
Gifu Prefectural Research Institute for Environmental Pollution, 8 chome, Yabuta, Gifu-shi, 500 Japan
and Masamichi Furukawa and Shozo Shibata
Government Industrial Research Institute, Nagoya, Kita-ku, Nagoya-shi, 462 Japan

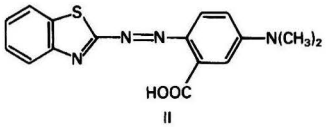
An extraction - spectrophotometric method has been developed for the determination of cobalt using 2-(2-benzothiazolylazo)-5-dimethylaminobenzoic acid (BTAMB). The method has been applied to the determination of cobalt in pepperbush leaves and coal fly ashes with good precision and accuracy.

Keywords: *Cobalt determination; 2-(2-benzothiazolylazo)-5-dimethylaminobenzoic acid; spectrophotometry; pepperbush leaves; coal fly ashes*

Several chromogenic reagents, for example nitroso-R salt and 1-(2-pyridylazo)-2-naphthol, are available for the spectrophotometric determination of cobalt, but they are not very sensitive. In a study of sensitive organic reagents that have molar absorptivities of the order of 10^5 l mol^{-1} cm^{-1} for different metals, 2-[2-(3,5-dibromopyridyl)azo]-5-dimethylaminobenzoic acid (3,5-diBr-PAMB) (**I**) was evaluated as a sensitive chromogenic reagent for cobalt.[1] However, the tolerance limit for the iron is low for coal fly ash analysis and an extraction method with cupferron was therefore necessary to remove large amounts of iron. 2-(2-Benzothiazolylazo)-5-dimethylaminobenzoic acid (BTAMB) (**II**), however, was easily synthesised and the synthesis cost of BTAMB was cheaper than that of 3,5-diBr-PAMB.[2]



I



II

In this work, an extraction - spectrophotometric method has been developed for the determination of cobalt using BTAMB. BTAMB reacts with only a few metal ions and large amounts of iron do not interfere. In this respect the selectivity of BTAMB is excellent and the analytical procedure is straightforward. The proposed method has been applied to the determination of microamounts of cobalt in pepperbush (*Clethra barbinervis Sieb*) leaves and coal fly ashes with good precision and accuracy.

Experimental

Apparatus

Spectrophotometric measurements were carried out using Hitachi Model 101 and 356 spectrophotometers with 10-mm glass cells. Extractions were carried out by shaking with an Iwaki Model KM shaker. The pH measurements were carried out with a Denki-Kagaku Model HG-2 pH meter.

Reagents

2-(2-Benzothiazolylazo)-5-dimethylaminobenzoic acid (BTAMB). The reagent was obtained by coupling diazotised 2-aminobenzothiazole with 3-dimethylaminobenzoic acid in methanolic solution at 0–5 °C as described previously.[2] This reagent can be purchased commercially from Dojindo Laboratories, Kumamoto, Japan.

BTAMB dimethylformamide solution, 0.05% *m/V*. Prepare by dissolving 0.05 g of BTAMB in 100 ml of dimethylformamide. This solution was stable for several months when stored in an amber-coloured bottle.

Cobalt standard solution. Dissolve 1.00 g of 99.99% cobalt metal in 20 ml of nitric acid (1 + 1) by heating and dilute to 1000 ml with re-distilled water to give the stock solution (1 mg ml^{-1}). Dilute 2000-fold with re-distilled water to give a working solution (0.5 μg ml^{-1}).

Buffer solution, pH 4.0. A 1 M sodium acetate - 1 M hydrochloric acid solution system was used.

Potassium periodate solution, 0.4% *m/V*.

Disodium 4,5-dihydroxybenzene-1,3-disulphonate (Tiron) solution, 1% *m/V*.

Sodium pyrophosphate solution, 5% *m/V*.

Glycine solution, 5% *m/V*.

Procedure

Transfer 20 ml of the slightly acidic sample solution containing up to 4.0 μg of cobalt into a 100-ml separating funnel. Add 5 ml of pH 4 acetate buffer solution and 5 ml of 0.4% *m/V* potassium periodate solution followed by 5 ml of re-distilled water. Add 0.6 ml of 0.05% *m/V* BTAMB solution. Mix well and allow to stand for 5 min. Add 10 ml of 1,2-dichloroethane and shake vigorously for 2 min. Transfer the organic layer into a 10-mm glass cell, after drying by passage through cotton-wool, and measure the absorbance at 705 nm against a reagent blank.

Results and Discussion

Absorption Spectra, Organic Solvent and Molar Absorptivity

Cobalt(III) and BTAMB form a blue complex that can be extracted into various organic solvents whilst cobalt(II) and BTAMB form a blue complex in aqueous methanol solution; however, the cobalt(II) complex cannot be extracted into other organic solvents. The cobalt(III) complex was extracted into 1,2-dichloroethane, chloroform and nitrobenzene. In this work, we chose 1,2-dichloroethane as the solvent because it gave good reproducibility. The cobalt(III) complex in 1,2-

dichloroethane exhibited an absorbance maximum at 705 nm, where the apparent molar absorptivity of the complex was found to be 1.18×10^5 l mol^{-1} cm^{-1}.

Effect of pH, Reagent Concentration and Shaking Time

The absorbance of the cobalt complex was found to reach a constant value over the pH range 4.0–4.5. Subsequent determinations were carried out at pH 4.0. The effect of an excess of the oxidising agent was examined. It was found that 2 ml of 0.4% *m/V* potassium periodate solution sufficed to oxidise 4.0 μg of cobalt. Next, the effect of an excess of the chromogenic reagent was examined. It was found that 0.4 ml of 0.05% *m/V* BTAMB solution sufficed to complex 4.0 μg of cobalt; with higher concentrations the absorbance was essentially constant. The minimum standing time for complete colour development of the cobalt complex was found to be 5 min at room temperature. The minimum shaking time for complete extraction of the cobalt complex was found to be 1 min at room temperature. The absorbance at 705 nm was stable for at least 7 h.

Linearity of Calibration, Precision and Nature of the Complex

The calibration graph obtained by the procedure showed good linearity over the range 0–4.0 μg of cobalt per 10 ml of 1,2-dichloroethane. Reproducibility tests for 20 results at the 3.0 μg of cobalt level showed a relative standard deviation of 0.7%. The Sandell sensitivity was 5.0×10^{-4} μg cm^{-2} of cobalt. Job's method of continuous variations showed that a 1:2 (Co to reagent) complex is formed. Similar results were obtained from a molar ratio plot.

Effect of Diverse Ions

The selectivity of BTAMB for cobalt(III) is excellent. For the determination of 3.0 μg of cobalt by this method, the limiting value of the concentration of foreign ions (including the masking agents) was indicated as that amount to cause an error of ±2% in the absorbance. The results were as follows: Cl^-, NO_3^-, ClO_4^-, SO_4^{2-}, sodium pyrophosphate, glycine and phenol, 150 mg; potassium thiocyanate, sodium sulphite, sulphosalicylic acid and sulphamic acid, 50 mg; Tiron, 10 mg; Al(III), As(III), Ba(II), Ca(II), Cd(II), Cr(VI), Hg(II), Li(I), Mg(II), Mo(VI), Pb(II), Rb(I), Sb(III), Se(IV), Si(IV), Sr(II), Tl(I), V(V), Zn(II) and W(VI), 1 mg; Ni(II), 0.5 mg; and Ag(I), 0.2 mg. Copper, nickel, palladium and vanadium formed intensely coloured complexes with BTAMB, but nickel and vanadium complexes were not extracted into 1,2-dichloroethane at pH 4, therefore, interference could be avoided by the addition of 2 ml of 0.05% *m/V* BTAMB solution. Only bismuth(III), copper(II), iron(III), manganese(II), palladium(II), tin(IV) and titanium(IV) interfered in the general procedure for the determination of cobalt, but, these interferences could be reduced by the addition of appropriate masking agents, as shown in Table 1. The analysis of a synthetic sample solution (15 ml), which contained 3.0 μg of cobalt, 10.0 μg of copper, 7 mg of iron, 50.0 μg of manganese and 0.5 mg of titanium, using various amounts of glycine, sodium pyrophosphate and Tiron was found to yield satisfactory results. From the above studies, the following procedure was formulated.

Procedure for the Analysis of Sample Solutions

Transfer 20 ml of sample into a 50-ml beaker. Add 5 ml of pH 4 acetate buffer solution, 3 ml of 5% *m/V* sodium pyrophosphate solution, 1 ml of 1% *m/V* Tiron solution, 2 ml of 5% *m/V* glycine solution and 6 ml of 0.4% *m/V* potassium periodate solution. Adjust the pH to 4.0 with 10% *m/V* sodium hydroxide solution. Then, transfer the solution into a 100-ml separating funnel. Add 0.6 ml of 0.05% *m/V* BTAMB solution and allow to stand for 5 min. Add 10 ml of 1,2-dichloroethane and shake vigorously for 2 min. Allow the phases to separate and transfer the organic layer into a 10-mm glass cell after drying by passage through cotton-wool. Measure the absorbance at 705 nm against a reagent blank.

Applications

The BTAMB method has been applied satisfactorily to the determination of cobalt in pepperbush leaves and coal fly ashes.

Determination of cobalt in pepperbush leaves

Dry a 1.0-g sample of pepperbush leaves at 85 °C for 4 h in an oven and transfer the dried sample into a PTFE beaker, add 20 ml of nitric acid (1 + 1) and allow to stand overnight. After heating on a hot-plate for 1 h, add 5 ml of nitric acid and 15 ml of hydrofluoric acid, then evaporate to 10 ml. After cooling, add 10 ml of perchloric acid and evaporate to 2 ml. Add 10 ml of re-distilled water and allow to stand for 1 h. Next, transfer the solution into a 50-ml calibrated flask. The determination was carried out according to the procedure for the analysis of sample solutions.

Pepperbush, a National Institute for Environmental Studies (NIES) standard reference material, was analysed by the proposed method and the results of six determinations were indicated: mean, 22.9 μg g^{-1}, standard deviation, 0.74 μg g^{-1}. The results obtained by the proposed method agreed with the certified value (23 ± 3 μg g^{-1}) of the NIES standard reference material.

Determination of cobalt in coal fly ashes

The coal fly ash sample preparation was carried out as described previously.[1] The determination was carried out according to the procedure for the analysis of sample solutions.

Table 1. Elimination of interferences by the addition of masking agents. The solution contained 3.0 μg of cobalt(II)

Ion	Amount tolerated/μg: Without masking agent	With masking agent	Masking agent and amount/mg
Bi(III)	50	500	Tiron (10)
Cu(II)	5	100	Glycine (100)
Fe(III)	300	10000	Sodium pyrophosphate (150) + Tiron (10)
Mn(II)	0.5	1000	Sodium pyrophosphate (150)
Pd(II)	1.0	30	Potassium thiocyanate (50)
Sn(IV)	50	200	Phenol (100)
Ti(IV)	50	1000	Tiron (10)

Table 2. Determination of cobalt in coal fly ashes

Sample	Cobalt content/μg g^{-1}: BTAMB method Average*	BTAMB method Standard deviation	Atomic-absorption spectrophotometric method Average*	Atomic-absorption spectrophotometric method Standard deviation
NBS SRM-1633a†	43.9	0.55	44.2	1.55
Coal fly ash A	20.6	0.57	20.3	1.05
Coal fly ash B	19.7	0.32	20.8	0.89

* Average of six determinations.
† Reference value, 46 μg g^{-1}.

Several standard and sample coal fly ashes were analysed by the proposed method and the results are presented in Table 2. The results obtained by the proposed method agreed with the results obtained by an atomic-absorption spectrophotometric method and also agreed with the reference value of the NBS standard reference material.

Conclusion

BTAMB reacts with cobalt(III) to form an intensely coloured complex that can be extracted into 1,2-dichloroethane. The cobalt(III) complex formed is very stable in the organic phase and the stoicheiometric ratio is 1:2 (Co to reagent). The calibration graph is linear over the range 0–4.0 μg of cobalt in 10 ml of 1,2-dichloroethane and the apparent molar absorptivity is 1.18×10^5 l mol^{-1} cm^{-1} at 705 nm. BTAMB reacts with only a few metal ions. The interferences due to copper, iron, manganese and titanium can be masked by glycine, sodium pyrophosphate and Tiron. Therefore, the method is free from the most common interferences. Satisfactory results were obtained when the method was applied to the determination of cobalt in pepperbush leaves and coal fly ashes.

References

1. Katami, T., Hayakawa, T., Furukawa, M., and Shibata, S., *Analyst*, 1983, **108**, 864.
2. Furukawa, M., *Anal. Chim. Acta*, 1982, **140**, 281.

Paper A4/188
Received May 24th, 1984
Accepted October 25th, 1984

Limit of Detection in Barium Sulphate Gravimetry for Water Analysis

Ricard Ferrús and Francesc Torrades
Escola Tècnica Superior d'Enginyers Industrials de Terrassa, Universitat Politècnica de Catalunya, Terrassa (Barcelona), Spain

Sulphate ions in the 1–5 mg l^{-1} SO_4^{2-} range can be determined gravimetrically in 200-ml samples using a large excess of barium chloride. From ten determinations at each of three concentration levels of standard sodium sulphate solution, the analytical calibration function calculated by weighted least squares is

$$x_c \text{ (mg } l^{-1} SO_4^{2-} \text{ found)} = (0.093 \pm 0.064) + (1.008 \pm 0.039)c \text{ (mg } l^{-1} SO_4^{2-} \text{ added)}$$

with 95% confidence intervals. The limit of detection is $c_L = 1$ mg l^{-1} SO_4^{2-} ($k = 3$). A systematic approach is suggested for determining the limit of detection, by considering the variance of both the blank and the calibration function.

Keywords: *Sulphate determination; limit of detection; barium sulphate gravimetry; water analysis*

Barium sulphate gravimetry is generally considered as the reference method for the determination of sulphate.[1] However, a perusal of standard procedures using this technique for sulphate in waters shows that the lower limits of the analytical ranges, for which the procedure is intended, frequently lie above the analyte concentration in real samples. This situation led to an investigation of the limit of detection[2] of the sulphate ion when using the gravimetric technique for its determination. Among the well established standard procedures, two especially attracted our attention, the ASTM D 516 Standard Test Method A[3] and that recommended by the National Water Council (NWC),[1] which cover the 20–100 and 50–250 mg l^{-1} ranges, respectively. The latter was finally chosen as a starting point for this work, in spite of its higher range. The reason for this choice was that the NWC procedure substitutes the drying of barium sulphate precipitate at 105 °C for ignition at 800 °C. Precipitates dried at 105 °C may contain several tenths of 1% of water[4] but this is a minor effect which, for our purposes, is outweighed by the simplicity of drying the precipitate instead of igniting it.

In this work results are reported in the 1–5 mg l^{-1} SO_4^{2-} range and discussed in the light of the limit of detection.

Experimental

Reagents

All chemicals were from Merck, analytical-reagent grade, except for ethylenediaminetetraacetic acid (EDTA) disodium salt dihydrate, monoethanolamine and ethanol, which were of laboratory-reagent grade. De-ionised water, distilled in an all-glass apparatus, was used throughout. The sodium sulphate concentration in the standard solution was chosen such that 50 ml gave the upper limit of the analytical range when diluted to 200 ml.

Procedure

The procedure used was essentially that recommended by the NWC.[1] To a boiling 200-ml sample of aqueous solution containing a known amount of sodium sulphate and 0.06 M in hydrochloric acid were added dropwise 10 ml of hot, just boiled, 10% *m/V* barium chloride dihydrate solution. Boiling was continued for 20 min, and the precipitate and solution thus formed were allowed to stand at room temperature for 12 h or more. The barium sulphate precipitate was gathered in a glass filter crucible (No. 4 pore size), filtered by suction, washed, dried at 105 °C and weighed.

Changes introduced and details not specified in the NWC procedure[1] were as follows. Working standard solutions were prepared according to Gottschalk[5] from just one sodium sulphate standard solution dispensed with a 50-ml burette. Hot barium chloride solution was added dropwise from a cheap burette reserved for this purpose, the total addition time being 5 min. Precipitation was carried out in 400-ml tall-form beakers, and the glass filter crucibles used were 10 ml in volume; the meticulous use of a rubber-tipped glass rod ("policeman") was of paramount importance for the quantitative transfer of the precipitate into the filter. After washing with water until free of chloride, the precipitate was washed again with three 5-ml portions of 96% *V/V* ethanol, dried for 2 h at 105 ± 2 °C, cooled for 45 min in a desiccator containing silica gel and weighed with a Mettler HK-60 balance readable to 0.01 mg. Preliminary trials showed that a 2-h period was sufficient for constant mass to be reached, a result that agrees with that of Nishimura.[6]

A series of thirty analyses were carried out in random order, ten at each of three equally spaced analyte concentration levels. After each analysis the barium sulphate was removed by soaking the glass filter crucible for 12 h or more in an EDTA - monoethanolamine solution, which was discarded after use. Next, 200 ml of 0.06 M hydrochloric acid were passed through the filter, the chloride was removed by washing with water and the crucible dried at 105 °C and kept in a desiccator over silica gel until the next determination.

Results

Calibration Range

A first estimation of the limit of detection was obtained from $n = 60$ blank measurements giving a mean ($\bar{x}_{bl}$) of −0.035 mg and a standard deviation (s_{bl}) of 0.16 mg $BaSO_4$. A chi-squared test for goodness of fit[7,8] to a Gaussian distribution with $\mu = -0.035$ mg $BaSO_4$ and $\sigma = 0.16$ mg $BaSO_4$, gave $\chi^2 = 3.07$, $\nu = 5$, so no significant lack of fit could be established at the 5% significance level. Assuming a Gaussian distribution and applying a *t*-test ($n = 60$, $\alpha = 0.05$), it was found that $\bar{x}_{bl}$ did not differ significantly from zero. This conclusion would hold even if the Gaussian distribution was not obeyed, as in that instance $k = 3$ should be substituted for $t = 2.00$ (where k is the constant introduced with Tschebyscheff's inequality[9]). However, we shall keep $\bar{x}_{bl} = -0.035$ mg in order to present a more generalised treatment. According to the IUPAC definition,[10] the limit of detection is $x_L = \bar{x}_{bl} + ks_{bl} = -0.035 + (3 \times 0.16) = 0.445$ mg $BaSO_4$ ($k = 3$), expressed in analytical signal

units, and $c_L' = 0.445 \times 0.486^{-1} = 0.92 \approx 1$ mg l^{-1} SO_4^{2-} ($k = 3$) is obtained as a provisional value by conversion of x_L into concentration units applying the stoicheiometric gravimetric factor for barium sulphate. Although the final c_L value might be different from the provisional one c_L' it was decided that the 1–5 mg l^{-1} SO_4^{2-} calibration range would be adequate to obtain c_L from x_L under the expectation that the IUPAC x_L value would fall within such a range. The results for the calibration work are given in Table 1, and are "raw" values (x_c) without blank correction.

Relative Standard Deviation

The data in Table 1 reveal that the relative standard deviation, $s_c\bar{x}_c^{-1}$, does not vary. It should be pointed out that in another series of measurements carried out in the 5–50 mg l^{-1} SO_4^{2-} range[11] the standard deviation (s_c) at the 5 mg l^{-1} level was 0.10 mg $BaSO_4$.

Bias

The results in Table 1 show positive deviations of mean values from the stoicheiometrically expected ones, those at 1 and 3 mg l^{-1} SO_4^{2-} being significant (t-test, $\alpha = 0.05$). This situation prevents us from applying barium sulphate gravimetry as an absolute method in the 1–5 mg l^{-1} SO_4^{2-} range, so a calibration function is required.

Calibration Function

The data in Table 1 are adjusted on a straight line, with the estimates for intercept (b_0) and slope (b_1) given by[12]

$$b_0 = \frac{\Sigma wx_c\Sigma wc^2 - \Sigma wc\Sigma wcx_c}{\Sigma w\Sigma wc^2 - (\Sigma wc)^2} \text{ and } b_1 = \frac{\Sigma w\Sigma wcx_c - \Sigma wc\Sigma wx_c}{\Sigma w\Sigma wc^2 - (\Sigma wc)^2}$$

where w is the statistical weight, s_c^{-2}; the estimate of σ^2 (such that the variance of x_c for c_i be $\sigma^2 w_i^{-1}$) is

$$s^2 = \frac{\Sigma wx_c^2 - b_0\Sigma wx_c - b_1\Sigma wcx_c}{n-2} = 0.996$$

where n is the number of standards analysed to obtain the calibration relationship, $x_c = f(c)$. Finally, the variances (V) for b_0 and b_1 are[13]

$$V(b_0) = \frac{\Sigma wc^2}{\Sigma w\Sigma wc^2 - (\Sigma wc)^2}s^2 \text{ and } V(b_1) = \frac{\Sigma w}{\Sigma w\Sigma wc^2 - (\Sigma wc)^2}s^2$$

Table 1. Experimental results for x_c(mg $BaSO_4$) for the determination of sulphate in the range 1–5 mg l^{-1} SO_4^{2-} using barium sulphate gravimetry

	Analyte concentration added, c*/ mg l^{-1} SO_4^{2-}		
	0.996	2.989	4.981
	0.51	1.51	2.52
	0.53	1.46	2.21
	0.50	1.43	2.38
	0.56	1.68	2.58
	0.58	1.55	2.49
	0.56	1.60	2.56
	0.54	1.61	2.54
	0.49	1.54	2.32
	0.51	1.42	2.27
	0.53	1.61	2.60
Mean, $\bar{x}_c$	0.531	1.541	2.447
Expected stoicheiometric value	0.484	1.452	2.420
Standard deviation, s_c	0.03	0.09	0.14
Statistical weight, $w = s_c^{-2}$	1111.1	123.5	51.0

* Calculated using 200 ml as the nominal initial volume.

Applying the above expressions to the data in Table 1 we arrive at equations (1) and (2):

$$x_c \text{ (mg } BaSO_4) = (0.045 \pm 0.031) + (0.490 \pm 0.019)c \quad (\text{mg } l^{-1} SO_4^{2-} \text{ added}) \quad (1)$$

and

$$x_c(\text{mg } l^{-1} SO_4^{2-} \text{ found}) = (0.093 \pm 0.064) + (1.008 \pm 0.039)c \quad (\text{mg } l^{-1} SO_4^{2-} \text{ added}) \quad (2)$$

The confidence intervals have been calculated from $V(b)^{\frac{1}{2}}t$, where $t = 2.05$ for 28 degrees of freedom and $\alpha = 0.05$. It can be seen from equation (2) that the sensitivity agrees with the expected value of 1, within the confidence interval. If we substitute $k = 3$ for $t = 2.05$, because of a possible non-Gaussian distribution, the new "confidence" intervals are $3 \times 2.05^{-1} = 1.5$ times larger.

Limit of Detection Predicted from the Regression Line

Once the analytical calibration function is known, we can predict the concentration c_L corresponding to $x_c = x_L = 0.445$ mg $BaSO_4$, as $c_L = (0.445 - 0.045) \times 0.490^{-1} = 0.82 \approx 1$ mg l^{-1} SO_4^{2-} ($k = 3$). This result for the limit of detection is the same as the provisional c_L' value, because the empirical calibration function does not differ greatly from the expected one.

Limit of Guarantee for Purity Predicted on the Lower Confidence Boundary

Related to the limit of detection is the limit of guarantee for purity, c_G,[9,14,15] or the c value corresponding to $x_c = x_L$ and predicted from the lower "confidence" boundary relationship

$$x_c = 0.045 + 0.490c - ks\left[\frac{s_c^2}{m} + \frac{1}{\Sigma w} + \frac{(c\Sigma w - \Sigma wc)^2}{(\Sigma w)^2\Sigma wc^2 - \Sigma w(\Sigma wc)^2}\right]^{\frac{1}{2}} \quad (3)$$

Equation (3) is the same as equation (7.382) in reference 16 adapted to the weighted least-squares fitting, without assuming a Gaussian distribution, and aimed at the analyst who is willing to obtain x_c as the average of m replicates on a sample. s_c^2 is an estimate of the variance of x_c. Substituting $x_L = 0.445$ mg $BaSO_4$ for x_c in equation (3) and solving c by iteration, we have $c_G = 1.24 \approx 2$ mg l^{-1} SO_4^{2-}, when $m = 1$.

The approach used here to calculate c_L and c_G from the analytical signal x_L is the same as that followed by Hubaux and Vos,[17] but there is a difference in experimental design, as they obtained x_L by extrapolating to $c = 0$ the upper confidence boundary of the analytical calibration function, while according to the IUPAC definition x_L depends on $\bar{x}_{bl}$ and s_{bl}, but not on the variance inherent in the calibration relationship. In addition, the IUPAC approach does not require the calibration function to be known down to $c = 0$, but only to $c = c_L$.

Effect of Amount of Barium Chloride

The amount of precipitating reagent, barium chloride, greatly affects the results; in this work we have maintained the 10 ml of 10% m/V barium chloride dihydrate solution, originally designed[1] for determinations in the range 50–250 mg l^{-1} of SO_4^{2-}, and applied here in the 1–5 mg l^{-1} of SO_4^{2-} range. The large excess of precipitating reagent is essential to obtain meaningful results, as shown by means of a 2×3 factorial experiment.

The results in Table 2 show only small relative errors for 10 ml of 10% solution, but when the amount of barium chloride is reduced 100-fold (either by reducing the volume of the 10% solution or by using 0.10% m/V barium chloride dihydrate solution) the results are 70–90% lower than the expected values. The effect is so evident that there is no need to implement the statistical analysis of data in Table 2. It should be pointed out, however, that an amount of precipitat-

Table 2. Effect of the amount of barium chloride. Results are in mg of $BaSO_4$

Concentration of SO_4^{2-} added*/ mg l^{-1}	Amount of barium chloride: 10 ml of 10% *m/V*†	0.10 ml of 10% *m/V*	10 ml of 0.10% *m/V*	Stoicheiometrically expected
0.995	0.45	0.10	0.03	0.48
4.978	2.38	0.81	0.57	2.42

* Calculated on 200 ml as nominal initial volume.
† As recommended in the NWC procedure.

ing reagent 100 times lower than that recommended in the NWC procedure would still be sufficient to precipitate as much as four times the sulphate ion present at the upper limit in the 1–5 mg l^{-1} SO_4^{2-} concentration range.

Discussion

The limit of detection is generally viewed as a performance characteristic of analytical instruments and methods, but it is also an effective tool in the hands of the problem-solving analyst. To illustrate this point we shall consider the results reported in this paper. When the NWC procedure is applied to a sample and the glass filter crucible increases its mass by ks_{bl} = 0.48 mg or more, we can be sure that some sulphate ion is present[19] with only a small chance of being incorrect (the first type of error). However, if the crucible mass increases by less than 0.48 mg over the blank, we can only say that either there is no sulphate ion at all or, if present, it cannot be above a given concentration c_G, called the limit of guarantee for purity. Again, a small probability of error, the second type of error,[20] should be allowed for in making this assumption. Of course, the possibility of matrix interferences is not considered here.

The limit of detection and the limit of guarantee for purity are frequently confused. The former is primarily an analytical signal,[9,17,21] expressed for convenience in concentration or amount units, while the latter is always a concentration or amount. Perhaps the misunderstanding originates in Kaiser's statement: "*Dies* (c_L) *ist also der niedrigste Konzèntrationswert, den das gegebene Analysenverfahren überhaupt liefern kann*"[14] if one fails to realise that c_L is a calculated value derived from x_L. The prediction of c_L from x_L is the final step in the analytical procedure, and analytical results (not real concentrations) below c_L are indistinguishable from zero. Even Kaiser seemed to overlook this as, further on, he wrote, "*Welchen geringsten Gehalt kann man mit einem Analysenverfahren noch 'sicher nachweisen'? Antwort*: c_L." Afterwards[9] he reasserted the correct definition: "*The limit of detection is the lowest value of concentration which the particular analytical procedure can ever yield.*" However, the limit of guarantee for purity, c_G, may be defined like Feigl's *Erfassungsgrenze*,[21] as the minimum analyte concentration or amount that ensures, with a high probability, *e.g.*, 95%, an analytical signal higher than the limit of detection, x_L.

In this situation, c_L and c_G have practically the same value, because the random errors from the blank measurement are larger than those originated in the empirical calibration function; however, with lower limits of detection such as those in instrumental trace analysis, the reverse situation might be true.[22] In that event the approach followed here would be useful.

Considering the results in Table 2, we are reminded of the possibility that the analytical calibration function $x_c = b_0 + b_1 c$ decays into $x_c = x_{bl}$ near the limit of detection, in which instance the conversion of x_L into c_G has no meaning. This is why the calibration range must be so located that it comprises the c_L and c_G values, in order to make sure that the sensitivity S is not zero. The situation where c_L and c_G become larger

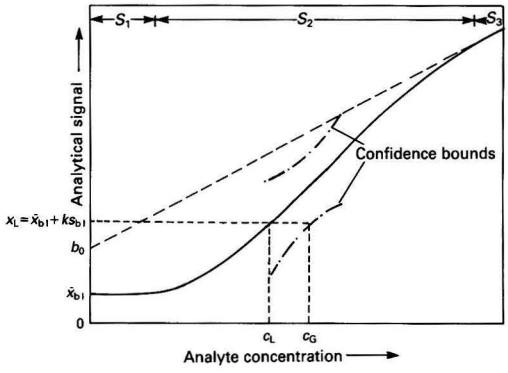


Fig. 1. Limit of detection and sensitivity. S_1, sensitivity $S = 0$; S_2, sensitivity $S > 0$, constant within small concentration intervals only; S_3, sensitivity $S > 0$, constant over a large concentration interval. Solid line, empirical calibration graph; dashed line, extrapolated calibration straight line from the S_3 region. x_L, IUPAC limit of detection (analytical signal units); c_L, limit of detection (analyte concentration units); c_G, limit of guarantee for purity

because the analytical responses are lower than expected is illustrated in Fig. 1. This is not so for the 1–5 mg l^{-1} SO_4^{2-} range considered in our work, as the experimental calibration line is close to the expected line.

The preceding discussion has been kept within the boundaries of the IUPAC definition of the limit of detection, which requires a knowledge of $\bar{x}_{bl}$ and s_{bl} as a first step. In the IUPAC definition the blank value $\bar{x}_{bl}$ is considered as a characteristic of the analytical procedure, which may be determined once and for all. This requires that the measurement of s_{bl} be made under such conditions that all causes of perturbation involved in the analytical procedure can play their full part.[9] Accordingly, we have carried out a large number (60) of blank analyses spaced over several months. The resulting standard deviation, s_{bl} = 0.16 mg $BaSO_4$, is much larger than s_c = 0.03 mg $BaSO_4$, the value obtained from determinations at 1 mg l^{-1} SO_4^{2-}; the reason might be that the s_c values in Table 1 are calculated from fewer (10) analyses performed within 2 weeks only. The aim of the experimental work that gave the results in Table 1 was to obtain a calibration line to predict c_L and c_G from x_L as more work was considered necessary to obtain the values of $\bar{x}_{bl}$ and s_{bl} needed to calculate x_L according to the IUPAC definition. Another approach is the one followed by Luckow[23] and Montag.[24] These workers do not consider the blank value as a characteristic of the analytical procedure, but the result of a control measurement to be run in parallel with the measurement on a sample containing the analyte, within a paired observation experimental design.[25] After determining the analytical calibration function $x_c - x_{bl} = Sc$, Montag defines c_L as the c value for which the lower $x_c - x_{bl}$ confidence boundary reaches zero.

Conclusion

The following procedure is suggested for determining the limit of detection: after 20 or more blank measurements, $\bar{x}_{bl}$, s_{bl} and $x_L = \bar{x}_{bl} + 3s_{bl}$ are calculated, a calibration range is chosen comprising x_L and 10 or more replicate analyses are performed at each of three equally spaced analyte standard concentrations. The calibration function is found by weighted least squares, and c_L is predicted from x_L on the fitted line, while c_G results from prediction of the lower "confidence" boundary (k = 3).

References

1. National Water Council, Department of the Environment, "Sulphate in Waters, Effluents and Solids," HM Stationery Office, London, 1979, p. 4.
2. Liteanu, C., and Rica, I., "Statistical Theory and Methodology of Trace Analysis," Ellis Horwood, Chichester, 1980, Chapter 7.
3. ASTM, "1980 Annual Book of Standards, Part 31, Water," American Society for Testing and Materials, Philadelphia, PA, 1980, p. 592.
4. Kolthoff, I. M., Sandell, E. B., Meehan, E. J., and Bruckenstein, S., "Quantitative Chemical Analysis," Fourth Edition, Macmillan, London, 1969, p. 603.
5. Gottschalk, G., *Fresenius Z. Anal. Chem.*, 1975, **276**, 81.
6. Nishimura, M., *Anal. Chim. Acta*, 1966, **34**, 246.
7. Bajpai, A. C., Calus, I. M., and Fairley, J. A., "Statistical Methods for Engineers and Scientists," Wiley, Chichester, 1979, p. 243.
8. Dixon, W. J., and Massey, F. J., Jr., "Introduction to Statistical Analysis," Fourth Edition, McGraw-Hill, New York, 1983, pp. 279–280.
9. Kaiser, H., *Anal. Chem.*, 1970, **42** (4), 26A.
10. International Union of Pure and Applied Chemistry, "Compendium of Analytical Nomenclature. Definitive Rules 1977," Pergamon Press, Oxford, 1978, p. 117.
11. Ferrus, R., and Torrades, F., unpublished work, 1983.
12. Garden, J. S., Mitchell, D. G., and Mills, W. N., *Anal. Chem.*, 1980, **52**, 2310.
13. Box, G. E. P., Hunter, W. G., and Hunter, J. S., "Statistics for Experimenters. An Introduction to Design, Data Analysis, and Model Building," Wiley, New York, 1978, p. 505.
14. Kaiser, H., *Fresenius Z. Anal. Chem.*, 1965, **209**, 1.
15. Kaiser, H., *Fresenius Z. Anal. Chem.*, 1966, **216**, 80.
16. Woodward, R. H., *in* Davies, O. L., and Goldsmith, P. L., *Editors*, "Statistical Methods in Research and Production," Fourth Edition, Longman, London, 1980, p. 196.
17. Hubaux, A., and Vos, G., *Anal. Chem.*, 1970, **42**, 849.
18. Davies, O. L., *Editor*, "The Design and Analysis of Industrial Experiments," Second Edition, Longman, London, 1979, Chapter 8.
19. Kaiser, H., and Specker, H., *Fresenius Z. Anal. Chem.*, 1956, **149**, 46.
20. Nalimov, V. V., Nedler, V. V., and Menshova, N. P., *Zavod. Lab.*, 1961, **27**, 861.
21. Svoboda, V., and Gerbatsch, R., *Fresenius Z. Anal. Chem.*, 1968, **242**, 1.
22. Long, G. L., and Winefordner, J. D., *Anal. Chem.*, 1983, **55**, 712A.
23. Luckow, V., *Fresenius Z. Anal. Chem.*, 1980, **303**, 23.
24. Montag, A., *Fresenius Z. Anal. Chem.*, 1982, **312**, 96.
25. Davies, O. L., *Editor*, "The Design and Analysis of Industrial Experiments," Second Edition, Longman, London, 1979, Section 2.24.

Paper A4/165
Received April 25th, 1984
Accepted November 12th, 1984

SHORT PAPERS

A Simple Supersonic Jet Fluorimeter with a Xenon Arc Lamp*

Sunao Yamada,† Benjamin W. Smith, Edward Voigtman and James D. Winefordner‡

Department of Chemistry, University of Florida, Gainesville, FL 32611, USA

A simple, inexpensive supersonic jet fluorescence system, involving a 4-in diffusion pump, a supersonic nozzle, a xenon arc lamp and a photomultiplier tube with a current to voltage converter, is described. The system was evaluated with anthracene. The spectral resolution was better than that obtained by low-temperature fluorescence methods where organic glasses are used. Initial results indicated a sensitivity of considerably greater than 10 μg s^{-1}.

Keywords: *Supersonic jet fluorimeter; xenon arc lamp; anthracene*

Supersonic jet expansion with laser excitation is one of the most attractive and powerful tools for obtaining highly resolved molecular spectra, as the supersonic jet produces "isolated" molecules that have been cooled to far below their (bulk phase) boiling-points but that still remain in the gas phase.[1] Several forms of spectroscopy have been carried out in the supersonic jet.[1] Most of the work to date has been with laser-induced fluorescence and/or fluorescence excitation spectroscopy.

Recently, several research groups have indicated that the supersonic jet can be used for the sensitive and selective determination of polycyclic aromatic hydrocarbons (PAHs). Small and co-workers[2,3] constructed an analytically useful supersonic jet apparatus including an Nd - YAG pumped dye laser for excitation of fluorescence, two 4-in diffusion pumps, a 50 l s^{-1} mechanical forepump and a Pyrex nozzle connected to a gas chromatograph. Amirav *et al.*[4] used a nitrogen-pumped dye laser, a 6-in diffusion pump, a 500 l min^{-1} rotary pump and a stainless-steel or tantalum nozzle for their apparatus. Imasaka *et al.*[5] reported on a system consisting of a nitrogen-pumped dye laser and an excimer pumped dye laser, a 6-in diffusion pump, a 1500 l min^{-1} booster pump, a 300 l min^{-1} rotary pump and a stainless-steel nozzle.

Basic components of any supersonic jet fluorescence apparatus are a light source, a detection system, a pumping system and a supersonic nozzle. However, the systems using dye lasers seem to us to be too complicated, too costly and too large for practical analytical use. Although dye lasers have several advantages as light sources, such as a high photon flux, narrow frequency band width, small divergence and good collimation, they make the system complicated and time consuming to use, *e.g.*, exchange of the dye solution, and expensive. Conventional (non-laser) sources, on the other hand, are simple, inexpensive and easy to use as compared with dye lasers. Previously, Leopold *et al.*[6] measured the absorption spectra of several jet-cooled molecules with the use of a deuterium lamp. More recently, a xenon arc lamp[7] and a pulsed xenon lamp[8] have been used for spectroscopic studies of some aromatic molecules seeded in supersonic jets.

An Eimac pre-collimated xenon arc lamp has found widespread use in atomic[9] and molecular[10] spectroscopy. Therefore, the application of this lamp to supersonic jet spectrometry is attractive.

As most PAHs fluoresce with reasonable quantum yields, fluorimetry is widely used for their analysis. However, they have low vapour pressures at room temperature and therefore they require substantial heating of the nozzle and the sample in order to attain continuous sample vaporisation. Although a metallic nozzle can be heated to a sufficiently high temperature, this is complicated and inconvenient for clean-up and nozzle exchange. As a result, we have constructed a simple supersonic jet apparatus, which consists of an Eimac xenon arc lamp as an excitation light source, a small pumping system, a quartz nozzle and a medium aperture monochromator system.

Experimental

The supersonic jet chamber was fabricated from a 4-in stainless-steel tube. The simple pumping system, consisting of a 4-in diffusion pump (CVC Products, Rochester, NY) backed by a 36 l s^{-1} rotary pump (Stokes), maintained an internal pressure of 10^{-2}–10^{-1} Torr during measurements.

The excitation source was a 300-W xenon arc lamp (Cermax 300 UV, ILC Technology, Sunnyvale, CA). The light source was focused to a spot of *ca.* 1.5 mm diameter by a 6-in focal length quartz lens outside the vacuum chamber and by a 1-in focal length quartz lens inside the chamber. The source beam crossed perpendicular to the supersonic beam; the beam crossing occurred 1–3 mm from the nozzle. The resulting fluorescence was collected at right-angles to both the xenon light beam and the supersonic beam by a 4-in focal length quartz lens outside the vacuum chamber and by 1-in focal length quartz lens inside the chamber, and the radiation was focused on to the entrance slit of a 1-m monochromator (Jobin-Yvon, 8 Å mm^{-1}). The output signal from a photomultiplier tube (EMI 6256B) was monitored by a home-made current-to-voltage converter and then recorded by a strip-chart recorder. No output low pass filtering was used. Fig. 1 shows a schematic diagram of the experimental system.

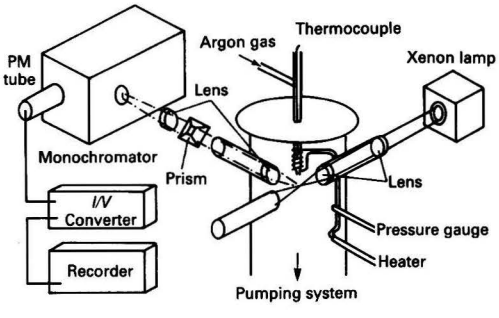


Fig. 1. Schematic diagram of the experimental system

* Research supported by NIH-GM-11373-21.

† On leave from Kyushu University, Department of Molecular Science and Technology Kasuga-Shi, Fukuoka 816, Japan.

‡ To whom correspondence should be addressed.

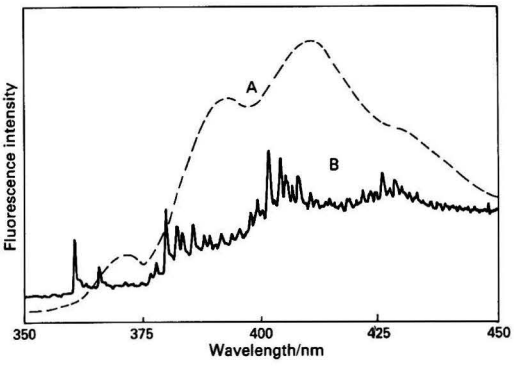


Fig. 2. Fluorescence spectra of anthracene: A, high temperature (*ca.* 420 K); B, supersonic jet (sample heated to *ca.* 520 K, seeded into argon at 600 Torr), $\Delta\lambda_{em.} = 3.2$ nm

The high-temperature supersonic nozzle was constructed by heating and drawing out the open end of a quartz tube (o.d. 8 mm; i.d. 5.5 mm). The excess of quartz was ground away, leaving an orifice with a diameter of *ca.* 80 μm. This tube was inserted 3–4 in inside the vacuum chamber and was fixed by a Cajon connector. A Cajon T connector attached to other end of this tube to both an iron - constantan thermocouple and an argon gas outlet.

The sample container was made of a quartz tube (o.d. 5 mm; i.d. 3 mm; height 15–20 mm), which was placed inside the large end of the supersonic nozzle, *ca.* 10 mm above the orifice. The tube inside the chamber was heated with a Nichrome wire that was wrapped around the tube.

Anthracene was obtained from Eastman Kodak and used without further purification. The fluorescence spectrum of anthracene at high temperature (*ca.* 420 K) was measured in a quartz cell. Several milligrams of the sample were placed in the cell and sealed after evacuation.

Results and Discussion

A typical fluorescence spectrum of anthracene obtained with the supersonic jet apparatus is shown by the solid line in Fig. 2. In Fig. 2, a number of sharp vibrational lines are shown, which were unresolved in a conventional high-temperature (*ca.* 420 K) spectrum of anthracene vapour as shown by the broken line in Fig. 2. Under the present conditions, the Mach number and the translational temperature of the measurement region in the jet are estimated to be 13–38 and 5–1 K, respectively.[11] The sharp line at 361 nm corresponds to the 0–0 transition.[4] The base line increases gradually with increasing wavelength; this is mainly a result of scattering of the excitation light.

The spectral resolution is better than those obtained for anthracene in organic glasses at low temperature.[12,13] The solid-state low-temperature spectra also show 15–20 nm red shifts because of solvent effects.[12,13] In addition, sample preparation and the cooling procedure are tedious and time consuming in solid-state low-temperature spectrometry. Supersonic jet spectrometry will overcome these problems, as this technique requires no solvent and cooling is based on a adiabatic expansion of the gas.

The supersonic jet is also attractive for combination with flowing systems, such as gas chromatography.[3] In this study, the sample (*ca.* 40 mg) lasted for *ca.* 1 h, which corresponds to an anthracene flow-rate of *ca.* 10 μg s^{-1}.

For analytical use of the supersonic jet, all previous studies have involved the time discrimination technique (pulsed beam, pulsed light source and/or pulsed detection system) in order to increase the signal to noise ratio.[2–5] However, this makes the instrument more complicated and requires longer times for measurement.

We have demonstrated in this study that the xenon arc lamp is useful as the excitation light source for supersonic jet fluorescence spectrometry. Skilful design of the nozzle and the light optics should further simplify the apparatus; such work is now in progress.

The authors thank Drs. Ishibashi and Imasaka of Kyushu University for sending their results prior to publication. One of them (E.V.) thanks Drs. Small and Hayes for the opportunity of seeing their supersonic beam apparatus and for advice on nozzle fabrication.

References

1. Levy, D. H., *Science*, 1981, **214**, 263.
2. Warren, J. A., Hayes, J. M., and Small, G. J., *Anal. Chem.*, 1982, **54**, 138.
3. Hayes, J. M., and Small, G. J., *Anal. Chem.*, 1982, **54**, 1202; 1982, **55**, 565A.
4. Amirav, A., Even, U., and Jortner, J., *Anal. Chem.*, 1982, **54**, 1666.
5. Imasaka, T., Fukuoka, M., Hayashi, T., and Ishibashi, N., *Anal. Chim. Acta*, 1982, **156**, 111.
6. Leopold, D. G., Hemley, R. J., Vaida, V., and Roebber, J. L., *J. Chem., Phys.*, 1981, **75**, 4758.
7. Zwier, T. S., Carrasquillo, M. E., and Levy, D. H., *J. Chem. Phys.*, 1983, **78**, 5493.
8. Amirav, A., and Jortner, J., *Chem. Phys. Lett.*, 1983, **94**, 545.
9. Ullman, A. H., Pollard, B. D., Boutilier, G. D., Bateh, R. P., Hanley, P., and Winefordner, J. D., *Anal. Chem.*, 1976, **51**, 3282.
10. Vo Dinh, T., Lue Yen, E., and Winefordner, J. D., *Anal. Chem.*, 1976, **48**, 1186.
11. Pauly, H., and Toennies, J. P., *in* Bederson, B., and Fite, W. L., *Editors*, "Methods in Experimental Physics," Volume 7, Part 1, Chapter 3.1, Academic Press, New York, 1968, p. 227.
12. Kirkbright, G. F., and de Lima, C. G., *Analyst*, 1974, **99**, 338.
13. Brown, J. C., Edelson, M. C., and Small, G. J., *Anal. Chem.*, 1978, **50**, 1394.

Paper A4/334
Received September 26th, 1984
Accepted November 7th, 1984

Thiophene-2-hydrazide as a Reagent for the Spectrophotometric Determination of Vanadium in Aqueous Solution

Khalid A. Abdullah, A. G. M. Al-Daher and W. A. Bashir*
Department of Chemistry, College of Education and College of Science, Mosul University, Mosul, Iraq

A new chromogen, thiophene-2-hydrazide, for the determination of trace amounts of vanadium in aqueous solution has been developed. The intense yellow, water-soluble, stable and binary complex, formed in acidic medium, is suitable for the determination of 0.5–5 p.p.m. of vanadium ion, with a molar absorptivity of 12.1×10^3 l mol^{-1} cm^{-1}, at 410 nm, relative error −0.6 to +4.0% and relative standard deviation 0.3–0.8%, depending on the concentration level of the determinant. Moreover, the colour formation is very fast. Interferences due to foreign ions have been examined.

Keywords: *Vanadium determination; aqueous solution; thiophene-2-hydrazide reagent; spectrophotometry*

Although there are a number of recent methods proposed for the spectrophotometric determination of vanadium,[1–10] none of them seems to be completely satisfactory. During our studies on the use of organic reagents in analysis, we observed that when vanadium and thiophene-2-hydrazide are mixed in the presence of dilute acidic solution, an intense yellow complex, with maximum absorption at 410 nm, was formed immediately. This observation led us to develop the method described below.

Experimental

Apparatus

Absorption measurements were performed with 1-cm matched silica cells on a Shimadzu UV-210A digital double-beam spectrophotometer.

Reagents

All chemicals used were of analytical-reagent grade.

Standard vanadium solution, 1000 μg ml^{-1}. A 0.2296-g amount of ammonium metavanadate is dissolved in distilled water and the volume is completed to 1000 ml with distilled water.

Thiophene-2-hydrazide reagent solution, 0.3% *m/V*. A 0.3-g amount of the reagent is dissolved in 40 ml of ethanol and the volume is diluted to 100 ml with distilled water.

Buffer solution. To 12.5 ml of 0.2 M potassium chloride solution is added 3.25 ml of 0.2 M hydrochloric acid and the volume is diluted to 50 ml. This gives a solution of pH 2.

Foreign ion solution, 1 mg ml^{-1}. A solution of this concentration is prepared for each foreign ion to be tested.

Procedure

Transfer increasing volumes of standard vanadium solution, covering the range from 12.5 to 125 μg, into a series of 25-ml calibrated flasks. Add 5 ml of the hydrazide reagent, 3 ml of the buffer solution and then make up the volume to the mark with distilled water. The yellow colour develops immediately and is stable for 30 min. Measure the absorbance at 410 nm against a reagent blank, prepared in the same way but containing no vanadium, using 1-cm cells. A straight line passing through the origin should be obtained. The apparent molar absorptivity (referred to vanadium) in the region of least photometric error, and at the wavelength of maximum absorption, was found to be 12.1×10^3 l mol^{-1} cm^{-1}.

Results and Discussion

For the following investigations, 50 μg of vanadium were taken and the final volumes were 25 ml.

* To whom correspondence should be addressed.

Absorption Spectra

When the dilute vanadium solution and thiophene-2-hydrazide reagent were mixed in the presence of acidic buffer solution, an intense yellow complex was formed instantly, in contrast to the colourless reagent blank solution, which gave an almost flat spectrum in the visible region. Fig. 1 shows the absorption spectra of the complex and of the reagent blank. The maximum absorption at 410 nm, characteristic of the complex, was used for all subsequent measurements.

Effect of Reagent Concentration

The concentration effect of the reagent for maximum absorbance was studied. A 4–6-ml volume of 0.3% reagent solution gave maximum colour intensity, and 5 ml was selected for the procedure.

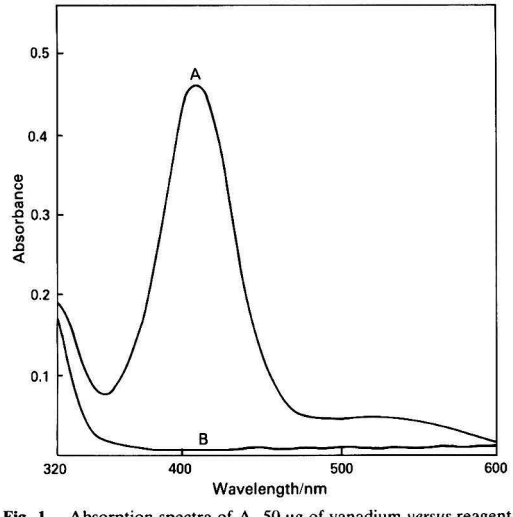


Fig. 1. Absorption spectra of A, 50 μg of vanadium *versus* reagent blank treated as given under Procedure; and B, reagent blank *versus* distilled water

Table 1. Accuracy and precision of the method

Amount of vanadium taken/μg	Relative error, %	Relative standard deviation, %
12.5	+4.0	0.8
62.5	+1.0	0.5
125.0	−0.6	0.3

Table 2. Characteristics of vanadium - hydrazide complexes

Reagent	Optimum acidity	$\lambda_{max.}$/ nm	Molar absorptivity/ l mol^{-1} cm^{-1}	M : L ratio	Reference
Anthranilic acid isopropylidene hydrazide	0.2–1.0 N H_2SO_4	525	5.1×10^3	1 : 2	8
Nicotinic acid hydrazide	pH 1.8–2.2	420	0.75×10^3	1 : 1	10
Thiophene-2-hydrazide	pH 1.8–2.2	410	12.1×10^3	1 : 1	This work

Table 3 Effect of foreign ions on vanadium determination

Foreign ion	Added as	Amount added/μg	Interference, %
Ba(II)	$BaCl_2.2H_2O$	1000	+2.0
Cd(II)	$CdCl_2.2H_2O$	1000	−0.8
Ca(II)	$Ca(CH_3COO)_2$	200	+2.0
Cr(VI)	K_2CrO_4	100	−0.2
Co(II)	$CoCl_2.6H_2O$	100	+1.7
Cu(II)	$CuSO_4$	200	−1.1
Fe(III)	$(NH_4)_2SO_4.Fe_2(SO_4)_3.24H_2O$	125	−2.0
Mg(II)	$MgSO_4.7H_2O$	1000	−1.7
Mn(II)	$MnCl_2.4H_2O$	50	+1.7
Hg(II)	$HgCl_2$	125	−0.8
Ni(II)	$NiCl_2.6H_2O$	500	+0.4
U(VI)	$UO_2(CH_3COO)_2$	25	−1.3
Zn(II)	$ZnSO_4.7H_2O$	1000	0.0
CH_3COO^-	$CH_3COONa.3H_2O$	400	−1.7
HCO_3^-	$NaHCO_3$	100	−1.5
BO_3^{3-}	H_3BO_3	800	−1.7
Br^-	KBr	1000	−1.7
CO_3^{2-}	Na_2CO_3	25	−2.0
CN^-	KCN	200	−2.0
F^-	NaF	30	−2.0
IO_3^-	KIO_3	100	+0.4
I^-	NaI	10000	−0.6
NO_3^-	$NaNO_3$	5000	+0.2
NO_2^-	$NaNO_2$	500	−2.0
$C_2O_4^{2-}$	$Na_2C_2O_4$	100	−2.0
ClO_4^-	$LiClO_4$	500	−1.7
MnO_4^-	$KMnO_4$	25	−2.0
$S_2O_8^{2-}$	$K_2S_2O_8$	25	−2.0
PO_4^{3-}	Na_3PO_4	25	−2.0
SO_4^{2-}	K_2SO_4	1000	−1.7
S^{2-}	$Na_2S.2H_2O$	1000	−2.0
SO_3^{2-}	Na_2SO_3	1000	−0.8
$S_2O_3^{2-}$	$Na_2S_2O_3.5H_2O$	1000	−1.1

Effect of Buffer Solution

To investigate the effect of pH on maximum colour intensity, a series of buffer solutions (pH 0–6) was prepared. A 3-ml volume of pH 1.8–2.2 solution gave maximum intensity. The mentioned volume of pH 2 buffer solution is recommended for the procedure.

Order of Addition of Reagent

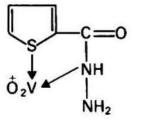
For maximum absorbance, the order of addition should be sample solution, reagent solution and then buffer solution. The absorbance is decreased to nearly half its value if the order of addition of the last two solutions is reversed.

Rate of Reaction

The reaction rate was followed experimentally by measuring the absorbance of the coloured complex, at 410 nm and against the reagent blank, *versus* time. The experimental data show that the complex was formed immediately and remained stable for 30 min, after which time gradual fading occurred. Trials (such as dilution with solvents other than water, use of surfactants, use of reducing agents and solvent extraction) attempted to increase the stability period further were unsuccessful.

Stoicheiometry of the Complex

The stoicheiometric ratio of metal to ligand in the complex, using the continuous variations method, is 1 : 1. The formula of the complex, assuming vanadium to be present in aqueous solution as VO_2^+,[11] may be postulated as:



Accuracy, Precision and Sensitivity of the Method

Under the conditions described above, the accuracy and precision (five replicates) of the method were checked. The results, shown in Table 1, indicate a reliable method. The sensitivity of the method was calculated according to the following equation:

$$S = \frac{C}{A} l \times 10^{-3}$$

where S is the sensitivity index, C is the final concentration of the determinant (p.p.m.), A is the corresponding absorbance, l is the light path length (cm) and 10^{-3} is a conversion factor. The sensitivity was found to be 0.0043 μg cm^{-2} in a 1-cm cell at 410 nm. The method is more sensitive than many existing methods, and it was compared with other methods previously reported that use related ligands (Table 2).

Interferences

To show the selectivity of the present method, the effect of foreign ions was examined by carrying out determinations of 50 μg of vanadium in the presence of each of these ions in a final volume of 25 ml. The results are summarised in Table 3.

References

1. Reddy, S. N., and Reddy, V. D., *J. Indian Inst. Sci., Sect. B*, 1980, **62**, 47.
2. Bermejo-Barrera, P., Bermejo-Barrera, A., and Bermejo-Martinez, F., *Microchem. J.*, 1980, **25**, 458.
3. Montelonga, F. G., Arias, J. J., and Jimenez, F., *Microchem. J.*, 1980, **25**, 410.
4. Tamhina, B., Vojkovic, V., and Herak, J., *Microchem. J.*, 1980, **25**, 8.
5. Kharasan, R. S., Patel, K. S., and Mishra, R. K., *Talanta*, 1979, **26**, 254.
6. Yatirajam, V., and Arya, S. P., *Talanta*, 1979, **26**, 60.
7. Kharasan, R. S., Patel, K. S., and Mishra, R. K., *Talanta*, 1979, **26**, 50.
8. Dolgorev, A. V., and Karpova, O. I., *Zavod. Lab.*, 1974, **40**, 771.
9. Pandu, V., and Anjaneyulu, Y., *Mikrochim. Acta*, 1973, 481.
10. Krych, Z., and Lipiec, T., *Chem. Anal. (Warsaw)*, 1967, **12**, 535.
11. Cotton, F. A., and Wilkonson, G., "Advanced Inorganic Chemistry," Third Edition, Wiley, New York, 1972, p. 822.

Paper A4/232
Received July 12th, 1984
Accepted September 26th, 1984

Electrochemical Behaviour and Simultaneous Determination of Copper(II) and Palladium(II) at a Dropping Mercury Electrode in the Presence of Salicylaldehyde Tris(hydroxymethyl)methylamine

R. Sreenivasulu,* V. S. Raghavendra Rao and K. Hussain Reddy
Department of Chemistry, Sri Krishnadevaraya University, Anantapur 515 003, India

The polarographic behaviour of copper(II) and palladium(II) has been studied using salicylaldehyde tris(hydroxymethyl)methylamine (ST) as a complexing agent in a 0.1 M ammonia - ammonium chloride (supporting electrolyte) medium. Well defined diffusion-controlled waves were obtained for both systems. Reversible and irreversible waves were observed for copper and palladium systems, respectively. The concentration ranges of copper(II) and palladium(II) studied were 0.15–1.6 and 0.5–1.8 mM, respectively. A method is suggested for the simultaneous determination of these metals when they are present together in pure solutions as the difference in their $E_{\frac{1}{4}}$ values is sufficient for the purpose. The method has also been applied to the simultaneous determination of these metal ions in some synthetic samples and in alloys.

Keywords: *Copper determination; palladium determination; polarography; salicylaldehyde tris(hydroxymethyl)methylamine; alloy analysis*

Schiff bases have theoretical and experimental significance in polarographic studies of metals. The importance of these compounds may be mainly due to their specific and selective reaction with metal ions. A survey of the literature revealed that salicylaldehyde tris(hydroxymethyl)methylamine (ST) has been used for the potentiometric[1] and spectrophotometric[2] determination of copper(II). The ligational behaviour of ST towards some transition metal ions has been confirmed by Rustagi and Rao,[3] but the utility of this Schiff base (ST) in the polarographic determination of copper(II) and palladium(II) has not been reported so far. In this work the polarographic characteristics of copper(II) and palladium(II) using ST as a complexing agent have been investigated and suitable conditions have been suggested for the determination of these metal ions individually and in the presence of each other. The procedure developed has been applied to the simultaneous determination of copper and palladium in alloys.

Experimental

Apparatus

A Toshniwal Model CLO 2A manual polarograph coupled with a Type MG-2 galvanometer was used to record the current *versus* voltage graphs. A saturated calomel electrode, connected to the polarographic cell through a potassium chloride - agar bridge, was used as the reference. Oxygen was expelled by passing a stream of purified nitrogen through the test solution for 10 min. Doubly distilled mercury was used. The capillary characteristics of a dropping mercury electrode at constant height (76.5 cm) of mercury head were as follows: $t = 3.8$ s; $m = 2.482$ mg s^{-1}; and $m^{2/3} t^{1/6} = 2.29$ $mg^{2/3}$ $s^{-1/2}$ in 0.1 M KNO_3 solution with an open circuit (m = mass of mercury drop per second). A Lingane Type H-cell and a Model LI-120 Elico pH meter were also used. A Toshniwal Type GL 15 thermostat was used to regulate the temperature at 26 ± 0.1 °C.

Reagents

All reagents were of AnalaR grade unless stated otherwise.

Salicylaldehyde tris(hydroxymethyl)methylamine (ST) solution, 1 M. The reagent was prepared according to the literature procedure[3] and the solution by dissolving it in dimethylformamide.

* To whom correspondence should be addressed.

Copper(II) standard solution, 1×10^{-2} M. Prepared from analytical-reagent grade copper sulphate pentahydrate and then standardised titrimetrically.[4]

Palladium(II) solution 1×10^{-2} M. Prepared from analytical-reagent grade palladium chloride and standardised gravimetrically.[4] Solutions of lower concentration were prepared by diluting this solution as required.

Results and Discussion

Polarograms were recorded at various pH values keeping other factors [such as metal ion concentration (1.0 mM) and ligand concentration (0.1 M)] constant. A well defined wave was obtained in the pH range 9.5–12.5. The diffusion current in both instances did not change in this pH range. The linear dependence of the limiting current on the square root of the height of the mercury column indicates that the rate of reduction of the metal - ST complexes is a diffusion-controlled process. A graph of $\log [(i/i_d) - i]$ *versus* $E_{d.e.}$ (potential of the dropping electrode) is a straight line in both instances. The values of the slopes and $E_{1/2}$ for various concentrations of ST are given in Tables 1 and 2 and indicate the reversible and irreversible reduction of Cu - ST and Pd - ST complexes, respectively, at the dropping mercury electrode.

Table 1. Effect of ligand concentration in the copper complex system. [Cu(II)] = 0.2 mM; pH = 10.5; and ionic strength, 0.1 M (ammonia - ammonium chloride solution)

ST concentration/M	$E_{\frac{1}{4}}$/V *vs.* S.C.E.	i_d/μA	Slope of log plot/mV
0.04	0.611	1.235	59.14
0.06	0.623	1.188	59.14
0.08	0.631	1.156	59.12
0.10	0.638	1.080	59.04
0.20	0.654	0.941	59.08
0.30	0.661	0.984	59.12

Table 2. Effect of ligand concentration in the palladium complex system. [Pd(II)] = 1.0 mM; pH = 10.5; ionic strength, 0.1 M (ammonia-ammonium chloride solution)

ST concentration/M	$-E_{\frac{1}{4}}$/V *vs.* S.C.E.	i_d/μA	Slope of log plot/mV
0.04	0.938	5.458	82.18
0.06	0.940	5.412	82.42
0.08	0.945	5.368	82.58
0.10	0.950	5.334	82.62
0.20	0.954	5.312	82.48
0.30	0.956	5.301	82.52

Effect of Metal Ion Concentration

Calibration graphs were constructed for the copper ion and palladium ion concentrations *versus* diffusion current using 0.1 M ST with 0.1 M ammonia - ammonium chloride solution and pH 10.5, for both systems. The concentration ranges of copper(II) and palladium(II) studied were 0.5–1.6 and 0.5–1.8 mM, respectively. The polarographic results can be used for their quantitative determination and also for their differentiation, as their half-wave potentials differed by more than 0.3 V.

Recommended Procedure for the Determination of Copper(II) and Palladium(II)

Polarograms for different concentrations of each metal ion were obtained under the described conditions. The diffusion currents were measured by the extrapolation method and calibration graphs were constructed in each instance. To determine the metal ion content in an unknown solution, the polarogram was obtained and the diffusion current (i_d) of this wave was referred to its calibration graph, which was linear. The results are given in Table 3.

Mixed Polarograms of Copper(II) and Palladium(II)

From the individual polarograms of copper and palladium, it was possible to differentiate the two metal ions in ST solution because their half-wave potentials differed by more than 0.3 V. Consequently, a series of polarograms were recorded with synthetically mixed solutions of cations and the i_d values referred to the respective calibration graph. The results obtained in the simultaneous determination of these metal ions are given in Table 4.

Determination of Palladium in an Oakay Alloy

A sample containing a 0.9-g amount of the alloy (Oakay alloy or Pt - Ir alloy) was dissolved in concentrated hydrochloric acid in the presence of a few drops of nitric acid. The solution was heated until the volume had been reduced to about 5 ml, then cooled; 10 ml of hydrochloric acid were added and the volume was made up to 100 ml in a calibrated flask with distilled water. An aliquot of this solution was taken and palladium was determined as described above. The results are given in Table 5.

Simultaneous Determination of Copper and Palladium in a Pt - Ir Alloy

The present method was applied to the simultaneous determination of copper and palladium in a Pt - Ir alloy. The results are presented in Table 6.

Tolerance Limits of Foreign ions

The tolerance limits of various anions and metal ions at a fixed concentration of copper and palladium (1.0 mM) were determined. In the presence of nitrate, acetate, sulphate and oxalate the $E_{1/2}$ values remained almost constant in all instances. Among the metal ions examined only Pb(II), Cd(II), Sb(III) and In(III) interfered in the determination of coppper or palladium. Tl(I), Zn(II), Mn(II), Fe(II) and V(V) did not interfere when they were present in up to a 5-fold excess. Platinum group and alkaline earth metal ions [Pt(IV), Ir(III), Rh(III), Ca(II), Ba(II), Mg(II) and Sr(II)] did not interfere in either determination for up to a 25-fold exess.

The authors thank the authorities of Sri Krishnavdevaraya University, Anantapur, for providing facilities for this work.

Table 3. Determination of metal ions with ST as a complexing agent. [ST] = 0.1 M; pH = 10.5; and sensitivity = 0.05

Metal ion	Amount of metal ion/mg Taken	Found	Error, %
Copper	0.397	0.395	−0.51
	0.794	0.799	+0.39
	1.598	1.604	+0.04
	2.223	2.210	−0.60
	2.542	2.523	−0.72
Palladium	0.665	0.670	+0.75
	1.330	1.321	−0.67
	2.660	2.645	−0.52
	3.193	3.214	+0.65
	3.724	3.750	+0.70

Table 4. Simultaneous determination of copper and palladium in a mixture. [ST] = 0.1 M; pH = 10.5; and sensitivity = 0.05

Sample No.	Amount of metal added/mg Copper	Palladium	Amount of metal found/mg Copper	Palladium	Error, % Copper	Palladium
1	1.588	1.064	1.574	1.061	−0.93	−0.28
2	1.271	1.596	1.276	1.582	+0.45	−0.85
3	0.953	1.330	0.948	1.340	−0.52	+0.75
4	0.635	2.660	0.636	2.687	+0.18	+1.03

Table 5. Determination of palladium in an Oakay alloy*

Amount of palladium/mg Taken	Found	Average found/mg	Error, %
2.667	2.665	2.666	0.037
	2.668		
	2.667		
	2.669		
	2.661		

* Certified composition: Ni, 60; Pt, 20; V, 9.5; and Pd, 10.5%.

Table 6. Simultaneous determination of copper and palladium in Pt - Ir alloy*

Amount taken/mg Copper	Palladium	Amount found/mg Copper	Palladium	Error, % Copper	Palladium
0.392	0.685	0.395	0.679	+1.02	−0.88
0.784	1.370	0.778	1.378	−0.76	+0.54
1.568	2.740	1.570	2.760	+0.03	+0.74
2.352	4.110	2.341	4.082	−0.47	−0.68

* Composition of Pt - Ir alloy: Pt, 55; Ir, 28; Rh, 7; Cu, 3; Fe, 3.5; Pd, 3.5%.

References

1. Trivedi, T., Patel, M. S., and Vyas, D. N., *Indian J. Chem.*, 1978, **16**, 449.
2. Trivedi, T., Patel, M. S., and Vyas, D. N., *Indian J. Chem.*, 1978, **16**, 26.
3. Rustagi, S. C., and Rao, G. N., *Indian J. Chem.*, 1975, **13**, 702.
4. Vogel, A. I., "A Text Book of Quantitative Inorganic Analysis," Third Edition, Longman, London, 1961, pp. 358 and 511.

Paper A4/173
Received May 8th, 1984
Accepted November 19th, 1984

BOOK REVIEWS

Principles and Practice of Analytical Chemistry. Second Edition

F. W. Fifield and D. Kealey. Pp. xii + 462. International Textbook Company. 1983. Price £13.95. ISBN 0 7002 0283 8.

The number of text-books by British authors continues to reflect the extent to which analytical chemistry is taught as a coherent subject in institutes of higher and further education in the UK, so to say that this is the best British book may be construed in a manner which is not intended. This revised and expanded version of the 1975 First Edition contains some 85 extra pages mainly concerning HPLC, ISEs, ICP-OES, X-ray methods and RIA, and naturally the impact of the microcomputer has been dealt with as well. To a large degree the coverage of the various techniques reflects the extent to which they are used in real analytical laboratories (perhaps molecular luminescence methods will appear in the Third Edition), and it is thus refreshing to see that electroanalytical methods get cut down to size (less than 10%) and spectroscopic methods (35%) and separation techniques (22%) get the appropriate coverage. The authors have adopted the fairly well tried approach to the subject matter and the book is entirely technique orientated. There is approximately one technique per chapter or section of a chapter with introductory chapters on the scope of analytical chemistry, assessment of analytical data and solution equilibria. As a variation on this theme, "Separation Techniques," which includes a substantial section on various instrumental chromatographics is placed before "Titrimetry and Gravimetry." One can see the reasoning behind the format in that it presents the material in a reasonably logical format and thus probably parallels a lecture course in the subject, and packages the topics so that students not being taught analytical chemistry as such can recognise them. The chapters are, to a large extent self-contained and each section starts with a definition of terms (not always in a language that students will readily appreciate) and a summary of the salient features of the particular technique; good for note-making and ultimately revising for exams, helpful to those students who do not start their reading by surveying the material.

There are no major sections on the application of analytical techniques, just brief indications on the way through and a short section on dealing with real samples, some 400 pages into the book.

Words like steel, water, environmental, surface and so on do not appear in the index, and of course it is tempting to argue that one cannot teach about the applications of techniques until the techniques themselves have been taught. I am not entirely convinced this is true and a bit more space devoted to explaining what analytical chemists do (and how important they are!) might be a worthwhile investment in terms of catching students' interest and firing their enthusiasm. I am not yet so cynical to think that this cannot be done with the present generation of chemistry students.

There are relatively few errors and most topics are explained clearly. The worst chapter is that on electrochemical techniques. I have always found this subject difficult and this chapter has not improved the situation, I am baffled by inclusion of the symbol for the units of volts into practically every version of the Nernst equation, as I am sure some students will be. I also found the use throughout the book of the equals sign instead of arrows in chemical equations somewhat irritating. However, these are minor criticisms and are heavily outweighed by the book's good points. I am happy to recommend the book to my students.

J. F. Tyson

Modern Methods of Particle Size Analysis

Edited by Howard G. Barth. *Chemical Analysis, Volume 73.* Pp. x + 309. Wiley–Interscience. 1984. Price £58.50. ISBN 0 471 87571 6.

The emphasis in this book is on methods of particle size analysis that are in the development stage. Most of these techniques are mainly suitable for sub-micron particles and one of the general chapters concentrates on the application of these and other methods to sub-micron sizing of emulsions and droplets.

The strength of the book lies in the chapters written by specialists, such as the excellent discussion of field flow fractionation by Caldwell.

Chapters 5 and 6 deal with instruments based on Fraunhofer diffraction, the former concentrating on the Malvern instruments and the latter on the Leeds and Northrop Microtracs. The Cilas instrument is also described, but the chapter was obviously written before the HELOS instrument became available. Leschonski claims that this is the only instrument in this range that provides a direct solution of the diffraction pattern.

Hydrodynamic chromatography is described in Chapter 9 and the limitations of the detection systems for HDC and size exclusion chromatography (SEC) are extremely well discussed in Chapter 8. Photon correlation spectroscopy is the subject of Chapter 3; this includes a full theoretical treatment together with a description of available instruments.

The weakness of the book lies in its lack of critical comment in the discussions on more established techniques. A bare description of the Coulter principle is presented in Chapter 1. Gravitational sedimentation theory is presented, followed by descriptions of photosedimentation and X-ray sedimentation techniques. A discussion of concentration effects and lower size limit would have been useful here. Both the German and British Standards recommend a maximum of 0.2–0.25% by volume for the former and 1–2 μm for the latter. Centrifugal sedimentation techniques are then described, concentrating mainly on photocentrifuges.

T. Allen

Advances in Materials Characterization

Edited by David R. Rossington, Robert A. Condrate and Robert L. Snyder. *Materials Science Research, Volume 15.* Pp. xii + 680. Plenum. 1984. Price $89.50. ISBN 0 306 41347 7.

This book is the latest edition in a series of monographs dealing with advances in glasses, ceramics and material sciences and is the proceedings of a conference on "Advances in Materials Characterisation," held at the New York State College at Alfred, Alfred, New York in 1982. The conference was the 18th in the "University Series on Ceramic Science" sponsored by both governmental and industrial research institutions. The 49 papers presented at the conference and subsequently edited by Drs. Rossington, Condrate and Snyder cover many aspects of surface and bulk characterisation of solids. It is well known, by readers of scientific journals, that since there is such a wide range of scientific disciplines used in the characterisation of granular, powdered or bulk materials it is virtually impossible for all characterisation techniques to be included in one proceedings and also at times, with such a broad remit, overlaps occur amongst the techniques described for inorganic material characterisation.

The Editors have, however, divided the scientific contents into seven general areas, which have been overviewed by experts in the various different experimental techniques of characterisation.

In the field of Surface Spectroscopy individual overviews were given by Drs. C. Pantano, I. S. T. Tsong and R. Conzernius on the quantitative analyses of glasses, ion-beam techniques as applied to ceramics and spark source and laser mass spectrometry, respectively. The techniques of vibrational spectroscopy, in terms of Fourier Transform Interferometry instrumentation (Dr. J. Ferrano) and acoustic characterisation of structural ceramics (Dr. B. Khuri-Yakub) has also been sectionalised, described and reviewed. It was disconcerting, however, to read only a brief abstract on the properties and characterisation of surface oxides, the overview paper on Electron Optical Methods by Dr. Wefer, rather than the full text, which has been published elsewhere.

In the General Crystallographic section, the importance, computer development and recent applications of X-ray powder diffraction to solid characterisation were outlined by Dr. R. Snyder. The final two sections, with no review papers, were on Surface Techniques and General Glass Characterisation.

For a book on material characterisation there was a noticeable lack of contributions on surface techniques, there being only one paper on adsorption characterisation and two papers on mercury porosimetry. The concept of applying pore potential to mercury porosimetry experimental data in an analogous manner to that of vapour adsorption (Lowell and Shield) was, however, of great interest. The over-all scope of this book is such that it should be on the shelves of any library that contributes to journals specialising in the fields of solid inorganic, powdered or bulk material characterisation. The book is well edited with clearly notated figures and diagrams. There is however a slight annoyance in the clarity of print, because in an all too common method these days, reproduction of the various authors contributions has been achieved by photolithographic techniques from camera-ready manuscripts, which vary in quality. Both the author and subject indexes, essential in this type of book, are comprehensive.

The book is highly recommended to scientists who are interested in any of the topics covered. It has a wealth of information. *N. G. Stanley-Wood*

Analysis of Volatiles. Methods and Applications. Proceedings, International Workshop, Würzburg, Federal Republic of Germany, September 28–30, 1983
Edited by Peter Schreier. Pp. xii + 469. Walter de Gruyter. 1984. Price DM190. ISBN 3 11 009805 9.

The book contains the 26 papers presented at the above Workshop, divided into three sections covering sample preparation, analytical techniques and applications.

The section on sample preparation is much shorter than the other two, containing only three papers. This is understandable, as the trend in the analysis of volatiles is to use in-line trapping techniqes, which are described in several papers in the following section. However, the paper in the first section devoted to a study of recoveries using model systems is particularly interesting.

The following two sections are, perhaps predictably, largely devoted to volatiles from foods and much use is made of capillary column gas chromatography, in some instances in multi-dimensional modes to increase the resolution. The paper by Jennings and Takeoka gives a particularly good description of the state of the art, with much attention to the practicalities involved. Throughout the whole book, the quality of information given and the attention to practical detail are excellent, but because of the format it cannot be considered as a textbook on the subject of volatiles analysis, but more as a reference work for specific applications.

These applications are again rather predictable, concentrating as they do on food-related topics, but there are two papers devoted to general odour problems and another two giving a refreshing change by dealing with the subject of separating optically active compounds. One further paper that deserves particular mention is Kubeczka's paper on ^{13}C NMR analyses, which gives a very good explanation of the rationale of the technique.

All in all, this is a very informative book that should be treated more as a reference work than a textbook and that provides a lot of detail that is directly valuable, as well as providing a stimulus to the lateral-thinking analyst faced with his own particular problem. *A. M. Humphrey*

Head-Space Analysis and Related Methods in Gas Chromatography
B. V. Ioffe and A. G. Vitenberg (translated by Ilya A. Mamantov). Pp. xviii + 276. Wiley–Interscience. 1984. Price £57. ISBN 0 471 06507 2.

This book covers the theory, instrumentation and applications of gas chromatography in the headspace mode. A basic knowledge of both conventional gas chromatography and the physical chemistry of phase equilibria has been assumed. Particular attention has been given to the determination of distribution coefficients and literature values for a number of organic solvents over the temperature range 10–30 °C are tabulated. Methods of calibration are discussed, together with techniques for increasing the sensitivity of headspace analysis for the determination of trace constituents. The chapter on instrumentation covers the design and use of equipment offered by the major manufacturers that is currently on the market. Pictures of the instruments together with schematic diagrams of the sampling systems are presented.

The part devoted to applications is divided into sections dealing with (i) water and aqueous solutions, (ii) biological systems, (iii) polymers, (iv) food products and (v) gases in solution. Most areas are well covered, but the section on food chemistry is less than 2 pages long out of 67 for the whole chapter. The importance of the subject merits a more detailed discussion, although such applications have been discussed elsewhere. A separate chapter is devoted to the determination of impurities in gases by thermodynamic equilibrium with a liquid phase. This is, in effect, headspace analysis in reverse and has some advantages over traditional methods of concentration, provided that a favourable distribution coefficient can be found. The use of both volatile and non-volatile liquids is described. A final chapter covers some other applications of headspace analysis. In particular, qualitative analysis using specific reagents for functional group modifications in the vapour phase is comprehensively reviewed. The determination of relative molecular masses, activity coefficients and ionisation constants and the calibration of gas-chromatographic instruments are also covered.

The book is well produced, well referenced and indexed and the translation appears to be excellent. The definition of detection limits as $10^{-N}\%$ is rather strange but there are few errors in text or tables. The original work was published in 1982 and covers the literature up to about 1978. Inevitably, the price is rather high and may well confine this book to the library rather than personal use. *N. T. Crosby*

Wilson and Wilson's Comprehensive Analytical Chemistry. Volume XII. Thermal Analysis. Part D. Thermophysical Properties of Solids, Their Measurements and Theoretical Thermal Analysis

Jaroslav Šesták. Pp. xx + 440. Elsevier. 1984. Price $115.50; Dfl300; $103.75; Dfl270 (Subscription). ISBN 0 444 99653 2.

The series "Comprehensive Analytical Chemistry" is too well known to need any introduction. This volume is a partly re-written, modernised and extended English version of the author's earlier work published in Czech. The original volume was generally recognised as a source book in this field.

In the first chapter the author defines his area, and defends his decision to present the work essentially as a series of reviews unified by a single author. He indicates why he has concentrated on the study of oxides and why he does not consider materials such as polymers and metals. He then proceeds to summarise the remaining chapters. These form three loosely connected parts. The first includes the description of the techniques, including the characterisation and preparation of solid samples, a very essential chapter on measurement regulation and calibration of the temperature of the system.

The second part discusses, in a series of inter-related chapters, the theoretical aspects, so much so that at least three chapters could be considered to be purely theoretical physical chemistry or physics, with no significant mention of application to real analysis. Whilst such a treatment has uses, it seems to be out of line with the general philosophy of the series, which has concentrated more on the practitioner than on the theoretician.

The third part deals with the treatment of results, and one chapter, "Mathematical Analysis of Curves and the Use of Computers," has very little direct reference to theoretical analysis except for a few illustrative examples. It is not detailed enough for use without reference to the original literature and seems to be un-connected to the rest of the book.

Overall, the book makes a good contribution to the current literature on thermal analysis. *L. S. Bark*

Environmental Chemistry. Volume 3. A Review of the Literature published up to end 1982

Senior Reporter H. J. M. Bowen. *A Specialist Periodical Report.* Pp. x + 144. Royal Society of Chemistry. 1984. Price £41; $74. ISBN 0 85186 775 8; ISSN 0305 7712.

The present publication is Volume 3 of one of the Royal Society of Chemistry's *Specialist Periodical Reports,* Volume 1 having been published in 1975 and Volume 2 in 1980. It is a fairly small, slim volume and convenient to handle and to read. Four topics are covered in four chapters, as follows: tropospheric ozone (by I. Colbeck and R. M. Harrison); environmental chemistry of organotin compounds (by S. J. Blunden, L. A. Hobbs and P. J. Smith); determination of heavy metals in sewage sludge (by S. A. Katz); and inorganic deposits in invertebrate tissues (by M. G. Taylor and K. Simkiss).

It is evident that all four chapters have been researched carefully and the result is interesting and readable reviews that are more than just lists of references. An index of authors is provided but there is no subject index (the Table of Contents, however, is helpful in this connection, as each chapter is sub-divided in some detail).

The chapter on ozone provides particularly interesting and topical reading, and the account of its formation and destruction in polluted air is more than adequate. The review of organotin compounds includes a description of toxicology and (among other items that could be helpful) a tabulation of the toxicities of a number of these compounds for fish.

Various aspects of the subjects are considered but the content relating in particular to analysis is not considerable (the references concerned represent about 15% of the total). Although every chapter includes some reference to analytical methods, it is usually subordinate to other considerations. Although the determination of heavy metals in sewage sludge is not a particularly novel subject, this chapter (as might be expected) does contain rather more analytical detail. Procedures are described for the collection, preservation and preparation of samples, together with methodology (including methods employed by UK, US and other overseas control agencies).

The critical review volume no doubt supplies a need, although it may well become increasingly difficult as time goes on to provide such a service at acceptable cost (even at the Member's price this one is an expensive item for an individual to purchase for his own use). The book can be recommended strongly for the technical library—especially for institutes and organisations with environmental interests and activities—because even though it is expensive in this format it does provide a compact and helpful synthesis from the reference data. *D. Simpson*

Chromatography of Antibiotics. Second, Completely Revised Edition

Gerald H. Wagman and Marvin J. Weinstein. *Journal of Chromatography Library, Volume* 26. Pp. xviii + 504. Elsevier. 1984. Price $113.50 (USA & Canada); Dfl295 (Rest of World). ISBN 0 444 42007 X.

Because of the medical and commercial importance of antibiotics for the treatment of infectious diseases, it is understandable that the volume chosen to inaugurate the *Journal of Chromatography Library* in 1973 was Wagman and Weinstein's "Chromatography of Antibiotics." This compilation of data from the literature fulfilled its authors' aim of providing a reference source for the specific chromatographic identification of antibiotics. The second edition has now appeared as Volume 26 of the Library in a slightly smaller format but with the listing of chromatographic data, arranged alphabetically and by code number, more than doubled in size.

The first edition contained data almost exclusively for paper and thin-layer chromatography, high-performance liquid chromatography (HPLC) then being at a relatively early stage of development. In the intervening period, HPLC has assumed a dominant role for specific identification (as here), for control of impurities and with the prospect of serving for assay in place of microbiological methods. The present volume reflects this, containing a mass of HPLC data. "Chromatography" has been interpreted liberally by the authors, as separations by electrophoresis and counter-current distribution are included. Changes between the two editions are illustrated by the general entry for tetracyclines, which formerly occupied 10 pages with information about 24 paper and 12 thin-layer chromatographic methods and 2 counter-current systems. This entry now contains, in 15 pages, details of an additional 1 paper, 5 thin-layer, 10 HPLC and 6 electrophoretic methods. Division of the liquid chromatographic data into "high performance" and "high speed" seems an unnecessary complication.

The 1984 edition reflects such enlarged entries as well as incorporation of data for many new antibiotics. The index is more than twice as long as that in the first edition. Some entries in the first edition compiled from incomplete details in the original literature have now been omitted. Although the main data have been revised and updated, the introductory chapter and that on detection are almost unchanged. This last is particularly disappointing, as a discussion of problems of detection in HPLC, for example of the macrolide group, and, given the authors' background, of the relative merits of pre- and post-column derivatisation for the aminoglycosides would have enhanced the book's value.

"Chromatography of Antibiotics" has an additional function as a literature source for new and uncommon antibiotics because chromatographic data are frequently included in reports of their first recognitions. It is essentially a laboratory aid and not armchair reading. For this reason, and because of its price, it is unlikely to be purchased by an individual but for any laboratory concerned with the isolation, identification and analysis of antibiotics it is a worthy successor to the first edition.

D. H. Calam

Organic Trace Analysis

K. Beyermann (translation editor R. A. Chalmers). *Ellis Horwood Series in Analytical Chemistry.* Pp. 365. Ellis Horwood. 1984. Price £35. ISBN 0 85312 638 0 (Ellis Horwood); 0 470 20077 4 (Halsted Press).

This is a most useful description of the whole field of organic trace analysis and is a most welcome addition to the literature, as most books that deal with trace analysis discuss mainly or solely the inorganic aspects. This book redresses the balance and in structure follows the logical steps of analysis; although procedures are not set out in detail, the comprehensive index of compounds, matrices, etc., allows their location from the literature cited. The literature coverage is both extensive and intensive; some 2991 papers are cited and the additional literature since the first German edition in 1982 represents 40% of the references in the current volume.

The "Introduction" (7 refs.) sets the scene to the present expanding interest in organic trace analysis. The second chapter, "General aspects of organic trace analysis" (203 refs.), produced with the assistance of Gorbach, deals with the difficulties, aims, methods, calibration and checking and also inter-laboratory control and collaborative studies of organic trace analysis. This chapter brings out some simple yet elegant points in data evaluation and provides an introduction to good laboratory practice, its only weakness being with regard to safety, where it omits to mention sources of UK and USA regulations and procedures, and might be taken to imply that MAC (maximum allowable workplace concentrations) had replaced TLVs. The labile nature of many sample types is given attention in "Sampling in organic trace analysis" (317 refs.), in addition to the sampling techniques themselves. Data on storage, contamination and preparation of samples for analysis are highly condensed and much is presented in tabular form in an invaluable chapter for beginners in the field, "Treatment of samples before analysis" (159 refs.). "Separation methods and concentration steps" (923 refs.) is a major chapter and deals with all the current separation procedures and prior derivatisation procedures. Having achieved separation, "Methods for the determination or detection of compounds" are then discussed (1121 refs.), the main attention being given to those proved, by a survey of publications, to be the most popular. The last chapter, "Special topics in organic trace analysis" (261 refs.), deals with a variety of items varying from determinations without separations, methods to improve detection limits and specificity, local and surface analysis to telemetry.

This is an inspiring, and in places awe-inspiring, important book, one which should be available to every teacher and student of analytical chemistry at the tertiary level, preferably as a personal copy. The author is to be congratulated on completing this major review, as is the translation editor (R. A. Chalmers), for what is a highly condensed yet readable monograph.

D. Thorburn Burns

CUT HERE

FIRST FOLD HERE

THE ANALYST READER ENQUIRY SERVICE

For further information about any of the products featured in the advertisements in this issue, write the appropriate number on the postcard, detach and post.

FOLD HERE

THE ANALYST READER ENQUIRY SERVICE

APR'85

For further information about any of the products featured in the advertisements in this issue, please write the appropriate number in one of the boxes below.
Postage paid if posted in the British Isles but overseas readers must affix a stamp.

PLEASE USE BLOCK CAPITALS LEAVING A SPACE BETWEEN WORDS *Valid 12 months*

1 NAME

2 COMPANY

PLEASE GIVE YOUR BUSINESS ADDRESS IF POSSIBLE. IF NOT, PLEASE TICK HERE ☐

3 STREET

4 TOWN

5 COUNTY, POST CODE

6 COUNTRY

7 DEPARTMENT/ DIVISION

8 YOUR JOB TITLE/ POSITION

9 TELEPHONE NO.

OFFICE USE ONLY REC'D PROC'D

FOLD HERE

Postage will be paid by Licensee

Do not affix Postage Stamps if posted in Gt. Britain, Channel Islands, N. Ireland or the Isle of Man

BUSINESS REPLY SERVICE
Licence No. WD 106

2

Reader Enquiry Service
The Analyst
The Royal Society of Chemistry
Burlington House, Piccadilly
LONDON
W1E 6WF
England

A201 for further information. See page iv.

ANALAO 110(4) 321–416 (1985) April 1985

The Analyst

The Analytical Journal of The Royal Society of Chemistry

CONTENTS

Electronically typeset and printed by Heffers Printers Ltd, Cambridge, England