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# THE ANALYST

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# Summaries of Papers in this Issue

## Polarographic Study of Certain Progestogens and Their Determination in Oral Contraceptive Tablets by Differential Pulse Polarography

A polarographic method has been developed for the assay of four progestogens as the pure drugs and their pharmaceutical dosage forms. The results obtained agreed reasonably well with the analytical data supplied by the manufacturers. The differential pulse mode was employed because it offers increased sensitivity and ease of measurement over conventional d.c. polarography.

The reduction at the dropping-mercury electrode for the four steroids was investigated and was confirmed to be a one electron per mole process for each substance. The peak potential was determined graphically and the diffusion coefficient calculated for each substance.

*Keywords*: *Progestogen determination*; *oral contraceptives*; *differential pulse polarography*

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*Analyst*, 1977, **102**, 323–327.

## Assay of Nystatin Based on the Measurement of Potassium Released from *Saccharomyces cerevisiae*

A method is described for the assay of the polyene antibiotic nystatin, based on the measurement, by means of atomic-absorption spectrophotometry, of the potassium released from *Saccharomyces cerevisiae* when treated with the antibiotic.

*Keywords*: *Nystatin assay*; *potassium*; Saccharomyces cerevisiae; *atomic-absorption spectrophotometry*

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*Analyst*, 1977, **102**, 328–332.

## Microbiological Assay of the Polyene Antifungal Antibiotics Amphotericin B, Candicidin and Nystatin

The suggested procedures of the British Pharmacopoeia for the microbiological assay of the polyene antifungal antibiotics amphotericin B, candicidin and nystatin have been compared. A simpler procedure has been examined and shown to be suitable for the microbiological assay of all three antibiotics. The increased sensitivity of the assay is due to the enhanced solubility of the antibiotics in the diluents used for preparing the assay solutions. The assay has been shown to be accurate and precise.

*Keywords*: *Amphotericin B*; *candicidin*; *nystatin*; *polyene antifungal antibiotics*; *microbiological assay*

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*Analyst*, 1977, **102**, 333–339.

## Sulphonated Alizarin Fluorine Blue (AFBS)

### Part III. Lanthanum(III) Complexes of 1-Hydroxyanthraquinone-2-sulphonate, Alizarin-5-sulphonate and AFBS, the Fluoride Complex of AFBS - Lanthanum and a Suggested Method for Fluoride Determination

An attempt is made to understand the reaction for ternary complex formation between sulphonated alizarin fluorine blue (AFBS), lanthanum and the fluoride ion through study of complex-forming reactions between lanthanum and 1-hydroxyanthraquinone-2-sulphonate (HA2S), alizarin-5-sulphonate (A5S) and sulphonated alizarin fluorine blue, successively. Comparison of experimental molar-ratio and Job plots with those derived from theory reveals that HA2S forms 1:1 and 1:2 lanthanum - HA2S complexes of $K_{11}^{abs} = 1.85 \times 10^7$ l mol$^{-1}$ and $K_{12}^{abs} = 1.29 \times 10^7$ l mol$^{-1}$; A5S forms 1:1, 1:2 and 2:2 lanthanum - A5S complexes and AFBS forms 1:1, 2:1 and 2:2 lanthanum - AFBS complexes. Fluoride ions react to form FR and $FR_2$ complexes, where R represents a particular mixture of AFBS - lanthanum complexes. Conditions under which AFBS can best be used for the determination of fluoride are explored and such an analysis is compared statistically with one using the original alizarin fluorine blue; the latter performs well. The use of other lanthanoid ions is investigated.

*Keywords*: *Sulphonated alizarin fluorine blue*; *lanthanum(III) complexes*; *fluoride determination*; *spectrophotometry*; *composition and stability of complexes*

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*Analyst*, 1977, **102**, 340–366.

## Determination of Saccharin in Soft Drinks by a Spectrophotometric Method

Saccharin reacts quantitatively with phenothiazine (thiodiphenylamine) and copper(II) acetate dissolved in 50% *V/V* ethanol at 70 °C. The reaction product can be dissolved in an organic solvent and has a characteristic colour. The colour reaction is sensitive and the absorbance, at 510 nm, of the extract in 5 ml of xylene obeys Beer's law at saccharin concentrations between 20 and 400 $\mu$g ml$^{-1}$. Cyclamates, sorbic acid, benzoic acid, 4-hydroxybenzoic acid and dehydroacetic acid do not interfere. Recoveries of saccharin from soft drinks were satisfactory. A procedure suitable for routine use is proposed.

*Keywords*: *Saccharin determination*; *soft drinks*; *spectrophotometry*

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*Analyst*, 1977, **102**, 367–370.

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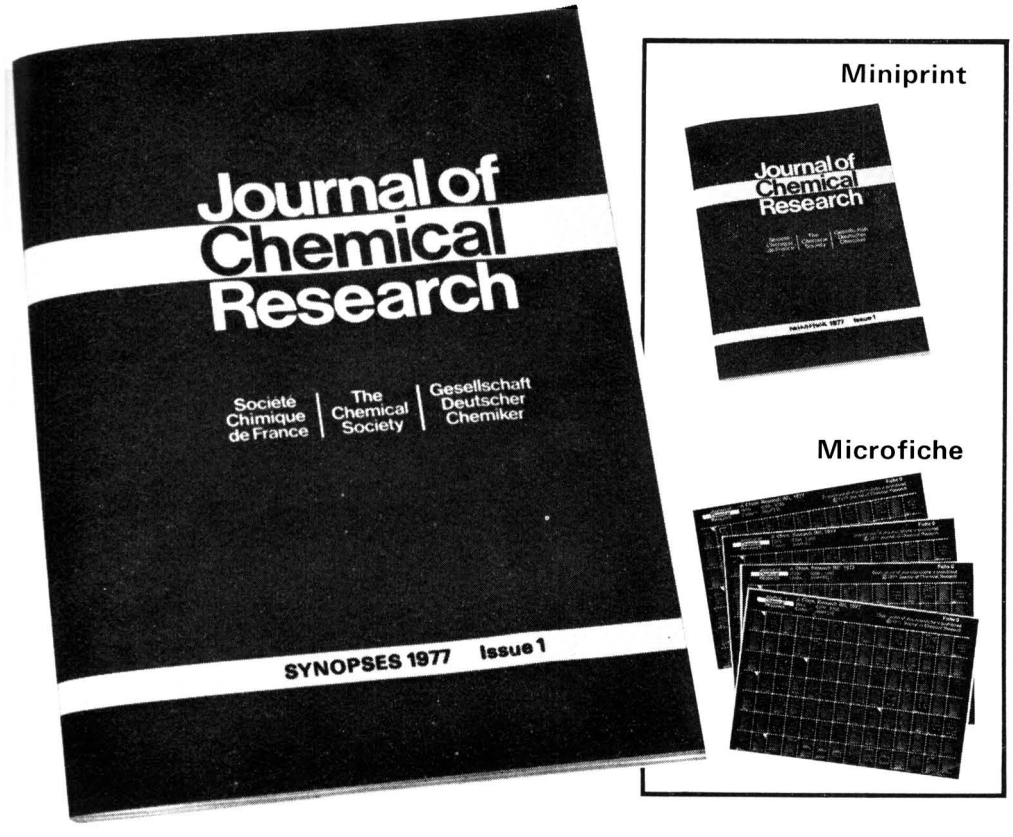


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The Analyst

## Editorial

# *The Analyst* — Progress and Development

In recent years, changes to *The Analyst* of importance to both authors and readers have taken place and it is the purpose of this editorial to review recent progress, to suggest areas for future development and to encourage more authors to submit papers to the journal.

One major change has been that since January 1975, following the amalgamation of the Society for Analytical Chemistry and the Chemical Society, *The Analyst* has been one of the primary journals published by the Chemical Society. It has thus been possible to take advantage of the benefits associated with the larger editorial unit and marketing services within a major publisher of scientific journals of the highest standard and reputation.

In both 1975 and 1976, there were increases of 5–6% in the number of manuscripts received. Nevertheless, the median time between submission of a manuscript and its publication has been progressively reduced, and is now 28½ weeks compared with 33 weeks in 1973. Naturally, many papers require little or no amendment by the authors and a minimum of editorial attention, and pass through the system more rapidly. Table I illustrates the competitive publication schedule.

Facilities for even more rapid publication exist in two special categories. Firstly, material on a topic of immediate importance to analytical chemists can appear as a *Communication*, without formal refereeing. Once accepted, express publication is guaranteed and some *Communications* have appeared 5 weeks after receipt. Secondly, a *Short Papers* category has been introduced. Although these shorter manuscripts are subjected to the normal dual refereeing system, it should be possible to complete both the refereeing and editorial stages more quickly than with full-length papers and publication will be more rapid.

*The Analyst* is an international journal with respect to both source of manuscripts and circulation. The proportion of papers received from outside the UK is 50–55%, with papers coming from over 30 different countries. A typical breakdown is shown in Table II.

The readership is world-wide (Table III), 72% of the copies going to more than 90 countries outside the UK. The circulation is over 5700, which is high for a learned journal and undoubtedly reflects both the quality and attractive subscription price of *The Analyst* in comparison with those of its commercial competitors. Papers published in *The Analyst* are therefore readily accessible to analytical chemists throughout the world, rather than having a more limited audience in one of the higher priced journals with a lower circulation.

Now that *The Analyst* is established within the publishing operations of the Chemical Society, there are opportunities for increasing the present rate of expansion in size and, with this in mind, the submission of more papers of high quality from analytical chemists in all countries is sought.

*The Analyst* covers all aspects of analytical chemistry. Traditionally there has been a high proportion of papers on applied aspects, but papers that describe fundamental or theoretical principles of analytical techniques have always been encouraged and the submission of a larger number of such papers would be particularly welcome.

Papers on new and developing areas of analytical chemistry are also desirable. Examples are the application of computer techniques and microprocessors, automated analytical methods for on-line process control, high-performance liquid chromatography, analytical isotachophoresis and isoelectric focusing, acoustical methods, sources in spectroscopy, laser techniques, environmental analysis and the use of enzymes. By publishing some of the best work on these and other topics at the forefront of developments in analytical chemistry, *The Analyst* will continue to fulfil its role of providing an unparalleled service to the analytical community and to authors, and will consolidate its position as one of the primary journals of the Chemical Society. It is our aim to continue to broaden the coverage of *The Analyst* and to improve the service provided to authors and readers, and it is hoped that more authors will now take advantage of these opportunities and contribute to the further development of the journal.

## Table I

### Speed of publication in *The Analyst* (1976)

| Time to publication/weeks | Proportion of papers, % | Time to publication/weeks | Proportion of papers, % |
|---|---|---|---|
| <10 | 6.1 | 31–35 | 20.8 |
| 11–15 | 1.3 | 36–40 | 6.7 |
| 16–20 | 3.4 | 41–45 | 4.7 |
| 21–25 | 20.8 | 46–50 | 4.0 |
| 26–30 | 30.9 | >50 | 1.3 |

Over-all median time to publication: 28½ weeks.

## Table II

### Sources of papers submitted to *The Analyst* (1976)

| Country | Proportion, % | Country | Proportion, % |
|---|---|---|---|
| UK | 46.1 | Italy | 2.6 |
| India | 8.8 | Israel | 2.6 |
| USA | 8.3 | Poland | 2.1 |
| Canada | 5.2 | The Netherlands | 1.6 |
| Australasia | 5.2 | South Africa | 1.0 |
| Japan | 3.1 | Malaysia | 1.0 |
| Scandinavia | 3.1 | Others | 9.3 |

## Table III

### Distribution of *The Analyst* (1976)

The total circulation of *The Analyst* in 1976 was 5718.

| Country | Proportion of subscribers, % | Country | Proportion of subscribers, % |
|---|---|---|---|
| UK | 28 | India | 4 |
| Western Europe | 21 | South Africa | 2 |
| North America | 20 | Latin America | 2 |
| Japan | 8 | Others | 9 |
| Australasia | 6 | | |

*Analyst, May, 1977, Vol. 102, pp. 323–327* 

# Polarographic Study of Certain Progestogens and Their Determination in Oral Contraceptive Tablets by Differential Pulse Polarography

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A polarographic method has been developed for the assay of four progestogens as the pure drugs and their pharmaceutical dosage forms. The results obtained agreed reasonably well with the analytical data supplied by the manufacturers. The differential pulse mode was employed because it offers increased sensitivity and ease of measurement over conventional d.c. polarography.

The reduction at the dropping-mercury electrode for the four steroids was investigated and was confirmed to be a one electron per mole process for each substance. The peak potential was determined graphically and the diffusion coefficient calculated for each substance.

*Keywords*: *Progestogen determination*; *oral contraceptives*; *differential pulse polarography*

Oral contraceptive preparations are now widely prescribed and as a result there has been considerable interest in their analyses. Over the years, a large number of papers have been published on the determination of these important substances. A recent report by Chatten *et al.*[1] includes a survey of the various methods that have been proposed for the assay of one or both of the ingredients of these preparations. Studies in this laboratory involving infrared spectroscopy[1] for the assay of the progestogens revealed that some excipients interfered in the analysis by causing a shift in the base-line. Consequently, for all of the progestogens, it was necessary to establish a correction for this anomaly. It was impossible to perform an analysis on some commercial products by infrared spectroscopy because certain unknown excipients interfered.

In view of these problems, it was of interest to investigate other methods of analysis for these substances. The applicability of polarographic techniques to the analysis of pharmaceutical dosage forms is well recognised.[2–5] In addition, the polarographic behaviour of the $\Delta^4$-3-ketosteroids has been well documented.[6–12] Accordingly, the purpose of this investigation was to examine the electroreduction of four progestogens, namely norethisterone (norethindrone), norethisterone acetate, dimethisterone and norgestrel, and to determine the suitability of differential pulse polarography for their determination in pharmaceutical dosage forms.

## Experimental

### Apparatus and Conditions for Polarographic Analysis

A PAR, Model 174, polarograph equipped with a drop timer was used in conjunction with a three-electrode polarographic cell consisting of a dropping-mercury electrode (D.M.E.), a saturated calomel electrode (S.C.E.) and a platinum wire as the auxiliary electrode. The entire cell was surrounded by a constant-temperature bath maintained at $25 \pm 1$ °C. The drop time was 2 s and the flow-rate was 1.34 mg $s^{-1}$. The scan rate was 2 mV $s^{-1}$, the applied potential range was $-1.0$ to $-1.95$ V and the modulation amplitude was set at 50 mV. The cell was the conventional H-type with a salt - agar bridge and an internal diameter of 2.3 cm. All of the polarograms were recorded on a Houston Omnigraphic, Model 2000, *X* - *Y* recorder. A Fisher, Model 320, pH meter fitted with a glass - calomel electrode system was employed to measure the pH of the solutions. A Buchler flash evaporator, Can. Lab. No. E5203-2, was used as an alternative means of reducing the volume of dimethylformamide solutions.

### Reagents

All reagents were of analytical-reagent or reagent grade. The following were used: disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate, dimethylformamide and 95% ethanol. Sörensen's phosphate buffer (pH 6.0) was prepared in distilled water obtained from an all-glass still.

### Reference Standards

The following pure steroids were obtained from the respective manufacturers and were used as standards without further purification: dimethisterone, norethisterone, norethisterone acetate and norgestrel. In addition, placebos were supplied by some manufacturers containing all of the ingredients found in the tablets except the active constituents. In some instances the colouring agents were also omitted.

### Preparation of Calibration Graphs

A stock solution of each steroid was prepared at the concentration of $1 \times 10^{-2}$ M. Dimethylformamide was the solvent for dimethisterone and norgestrel, while 95% ethanol was used for norethisterone acetate and norethisterone. A range of $1 \times 10^{-3}$ to $1 \times 10^{-4}$ M was employed for the preparation of the peak current *versus* concentration calibration graphs. An accurately measured volume of the stock solution of the steroid was placed in the polarographic cell, a sufficient amount of the appropriate organic solvent (95% ethanol or dimethylformamide) was added to produce a volume of 7 ml and 13 ml of Sörensen's phosphate buffer, pH 6.0, were then added.

All samples were de-oxygenated with nitrogen for 10 min prior to obtaining the polarograms and a stream of nitrogen was allowed to flow gently over the surface of the solution during the electroreduction. Differential pulse polarography, using the described parameters, was used.

### Analysis of Pharmaceutical Dosage Forms

Twenty tablets were weighed and finely powdered. A sample of the powder was taken which, according to the label claim, would give a peak current of such height that it would fall within the middle section of the calibration graph. The sample was weighed into a 150-ml beaker and was stirred magnetically for 30 min with 25 ml of the appropriate solvent. During this time the solution was warmed to a temperature below the boiling-point of the solvent. The solution was then suction-filtered through a Whatman No. 1 paper, the residue was washed with a further 10 ml of solvent and the filtrate was concentrated by heating to a volume of approximately 6.0 ml. An alternative method of removing dimethylformamide involved the use of a flash evaporator at 50 °C. This concentrate was transferred quantitatively into a 10.0-ml calibrated flask and made up to volume with the appropriate solvent. Depending upon the content of the steroid present, a suitable aliquot was taken from this sample and analysed by the same procedure as described under Preparation of Calibration Graphs, starting from "An accurately measured volume of the stock solution of the steroid. . . ." When possible, the procedure was repeated using a further aliquot of the same sample. Five samples were run on each dosage form.

## Results and Discussion

Differential pulse polarography possesses certain advantages over conventional d.c. polarography. Not only is the determination of the peak current very simple when compared with the rather elaborate measurements required for the d.c. mode, but differential pulse polarography often exhibits a sensitivity that is $10^3$–$10^4$ times greater than that of d.c. polarography.

As norethisterone acetate and norethisterone are soluble in ethanol and norgestrel and dimethisterone are readily soluble in dimethylformamide, these solvents were found to be convenient for the preparation of calibration graphs and for extraction of the steroids from their respective formulations. It was observed that a final volume of 7.0 ml of the solvent would keep the steroid in solution and this was combined with 13 ml of buffer. When combined with the appropriate organic solvent, Sörensen's phosphate buffer at pH 6.0 gave a well resolved differential pulse polarographic wave for each progestogen. Although the d.c. wave for norethisterone produced a good limiting current plateau, the pen oscillations were uneven owing to drop irregularities. These oscillations can be eliminated by the addition

of one drop of 1% gelatin but this addition results in a less well resolved limiting current plateau. The addition of a maximum suppressor is not necessary when differential pulse polarography is used.

Fig. 1 illustrates the d.c. and differential pulse waves for dimethisterone in a dimethylformamide - Sörensen phosphate buffer (pH 6.0) system. It is apparent that the d.c. wave is poorly resolved and cannot be used for quantitative purposes, whereas the differential pulse wave for this substance is smooth and well resolved. Table I shows the peak potentials, the number of electrons and diffusion coefficients for the steroids under these conditions. The number of electrons involved in the reduction process was determined by two different methods. In the first instance, logarithmic analyses were performed on the d.c. waves of the steroids. From the linear plot of log $i/(i_d-i)$ *versus* $E$, it was deduced that the reduction probably involved a one-electron, one-proton reversible process per molecule. The one-electron process was confirmed further by comparing the diffusion currents of the steroids with those of three compounds in which the number of electrons is known. The reference compounds chosen for this comparison were benzophenone, cinnamaldehyde and nitrobenzene.

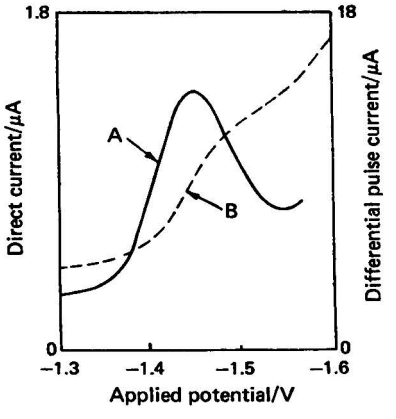


Fig. 1. Typical differential pulse and d.c. polarograms for dimethisterone. A, Differential pulse wave; B, d.c. wave with 1 drop of 1% gelatin solution added; concentration in both instances, $1 \times 10^{-4}$ M.

In all instances, identical solvent conditions and the appropriate amount of Sörensen's phosphate buffer (pH 6.0) were employed. These conclusions are in agreement with those postulated by Kabasakalian and McGlotten,[13] who provided evidence that the process is a one-electron bimolecular reduction in which the final product is a pinacol.

Calibration graphs were prepared for the four progestogens over the range $1 \times 10^{-4}$ to $1 \times 10^{-3}$ M. The dimethylformamide - Sörensen's phosphate buffer combination was employed for dimethisterone and norgestrel while ethanol - buffer was used for norethisterone acetate. These solvent - buffer combinations were equally successful for establishing the

TABLE I

PHYSICAL CONSTANTS OBTAINED FOR THE POLAROGRAPHIC REDUCTION OF PROGESTOGENS

| Steroid | Peak potential *versus* S.C.E./V | Number of electrons | Diffusion coefficient/cm$^2$ s$^{-1}$ |
|---|---|---|---|
| Dimethisterone .. | −1.46 | 1 | $4.2 \times 10^{-6}$ |
| Norethisterone acetate | −1.42 | 1 | $3.4 \times 10^{-6}$ |
| Norethisterone .. | −1.44 | 1 | $3.7 \times 10^{-6}$ |
| | | | $3.6 \times 10^{-6}$ |
| Norgestrel .. .. | −1.48 | 1 | $4.9 \times 10^{-6}$ |

norethisterone calibration graph. The correlation coefficient ($r$) for all graphs was calculated to be 0.99.

It was found that improved recoveries were obtained for the tablets when the solvent was warmed during the stirring process. An excess of solvent was employed and, consequently, after the filtration step it was necessary to concentrate the filtrate before transferring the solution into a 10-ml flask. When the solvent was ethanol, removal of the solvent was not a problem. When dimethylformamide was used, however, vigorous boiling for 25 min was required in order to reduce the volume to about 6 ml. Removal of dimethylformamide was also accomplished by employing a flash evaporator at 50 °C. Several determinations were made on tablet placebos to which known amounts of dimethisterone had been added. No difference in recovery could be detected by the two techniques. In all instances, the recoveries ranged from 100 to 101.6% with a mean recovery of 100.9 ± 0.7%.

The peak height of the polarogram was measured and compared with the values from the appropriate calibration graph. A sample of 20 tablets was used for all products except that which contained dimethisterone. Only five tablets of this preparation were available and this small sample may account for the low recovery. Unfortunately, the manufacturer's analysis was not available, although Chatten *et al.*[1] did obtain a recovery of 95% for the same product by their infrared method. In addition, the results obtained with the norethisterone products of one manufacturer were consistently low when compared with their analytical results. Presumably, the formulation prevented complete extraction of the progestogen from the powdered tablet mass. Comparative figures were supplied by the manufacturers' quality control laboratories and are included in Table II. In most instances, the recoveries obtained by the proposed method are in reasonable agreement with those supplied by the manufacturer.

TABLE II

ASSAY OF PROGESTOGENS IN ORAL CONTRACEPTIVE TABLETS

| Trade name | Nominal steroid content/mg | Steroid present | Steroid recovered, % | |
|---|---|---|---|---|
| | | | Average ± standard deviation | Manufacturers' control figures |
| Norlestin .. .. | 1.0 | Norethisterone acetate | 104.3 ± 0.4 | 102.0 |
| Norlestin .. .. | 2.5 | Norethisterone acetate | 107.2 ± 0.5 | 105.2 |
| Loestrin.. .. .. | 1.5 | Norethisterone acetate | 102.4 ± 0.8 | 100.6 |
| Logest .. .. .. | 1.5 | Norethisterone acetate | 101.7 ± 0.8 | 102.0 |
| Logest .. .. .. | 1.0 | Norethisterone acetate | 98.8 + 0.8 | 100.0 |
| Ovral .. .. .. | 0.5 | Norgestrel | 96.2 ± 1.3 | 99.6 |
| Oracon .. .. .. | 25 | Dimethisterone | 91.3 ± 0.8 | 95.0* |
| Norquen .. .. | 2 | Norethisterone | 96.2 ± 2.2 | 96.1 |
| Ortho-Novum .. .. | 2 | Norethisterone | 91.5 ± 5.2 | 100.0 |
| Ortho-Novum .. .. | 5 | Norethisterone | 91.2 ± 1.3 | 102.3 |

* Determination by Chatten *et al.*[1]

The effect of certain tablet excipients, fillers and lubricants on the polarographic determination of the steroids was studied. Certain inert tablet materials (in amounts exceeding the mass of the tablet samples employed) were subjected to the procedure used in the tablet assay. The components examined were acacia, lactose, magnesium stearate, starch, sucrose and talc. The last five did not exhibit any reduction at the D.M.E. Acacia, although not electroreducible, changed the pattern of the polarogram obtained with both solvents and the buffer alone.

Tartrazine and FD and C Blue 1 were investigated and it was observed that they do exhibit some degree of reducibility at the D.M.E. The small amount present in tablets, however, did not appear to be sufficient to cause any significant error in their analysis. This effect was demonstrated when known amounts of norethisterone acetate were added to the placebo supplied by the manufacturer in order to simulate the composition of the commerical tablets. Based on the average of three determinations, the recovery of norethisterone acetate was 101.4 ± 0.8%, which is within the limits of experimental error.

Diffusion dependency studies were carried out for each of the steroids, according to Zuman's outline.[14] Plots were made of diffusion current *versus* $\sqrt{h_{(corr.)}}$ for each steroid. Although a good linear relationship was obtained in each instance, the extrapolated line did not pass

through the origin. It is apparent, therefore, that while the reduction process is largely diffusion dependent, kinetic factors also influence the mechanism of reduction. For the four steroids studied, however, plots of peak current *versus* concentration were linear over the previously stated range.

Winkel and Proske[15] studied the behaviour of a number of ketones, diketones and aldehydes at the D.M.E. From these studies it was evident that only those compounds in which the keto group was conjugated with a double bond gave rise to polarographic waves. This conclusion was supported by a subsequent investigation of Adkins and Cox[16] on a larger number of aliphatic and aromatic ketones and their mixtures. The double bond and 3-keto group in ring A of norethynodrel are not conjugated and this study confirmed that this substance is not electroreducible. Eisenbrand and Picher[17] explained the polarographic conditions for hormones of the $\Delta^4$-3-ketosteroid group and showed that saturated 17-keto-steroids do not interfere.

The oestrogens mestranol and ethinyloestradiol do not possess an electroreducible functional group. These substances will continue to be determined by an ultraviolet procedure such as that reported by Keay.[18] Bjornson and Ottesen,[19] however, have reported the polarographic analysis of the Girard T and nitrosophenol derivatives of a number of oestrogens.

The authors gratefully acknowledge the samples of pure steroids and commercial products that were generously supplied by the following pharmaceutical manufacturers: Lederle Products Department (Cyanamid), Montreal, Quebec; Mead Johnson Canada, Belleville, Ontario; Ortho Pharmaceutical (Canada), Don Mills, Ontario; Parke Davis & Co., Brockville, Ontario; Syntex Ltd., Montreal, Quebec; and Wyeth Ltd., Windsor, Ontario. The work was supported in part by Medical Research Council Grant MA 3078.

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# Assay of Nystatin Based on the Measurement of Potassium Released from *Saccharomyces cerevisiae*

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A method is described for the assay of the polyene antibiotic nystatin, based on the measurement, by means of atomic-absorption spectrophotometry, of the potassium released from *Saccharomyces cerevisiae* when treated with the antibiotic.

*Keywords*: *Nystatin assay*; *potassium*; Saccharomyces cerevisiae; *atomic-absorption spectrophotometry*

Polyene antifungal antibiotics inhibit the growth of some fungi and yeasts. The initial reaction of the antibiotic with the sensitive cell involves binding with the sterol component of the cytoplasmic membrane. It is envisaged that aqueous pores are formed in the membrane as a result of the reaction between the antibiotic and the sterol.[1,2] The formation of these pores results in a change in the permeability of the membrane, which induces a loss of cellular components, including potassium.[3,4] The rate of release of potassium, as monitored by use of ion-selective electrodes,[5,6] by measurement of conductivity[7] and by atomic-absorption spectrophotometry,[8–10] has been shown to be dependent upon the antibiotic concentration. A method of assay for nystatin by direct measurement of potassium efflux has been developed.

## Experimental

### Materials

*Test organism*

*Saccharomyces cerevisiae* (NCYC 87) was grown on slopes of medium D (ref. 11) at 32 °C for 18 h. The yeast cells were washed off the slopes with glass-distilled water, centrifuged at 4 968 *g* for 10 min at 4 °C, re-suspended and centrifuged twice with ten volumes of distilled water and finally suspended in 0.03 M tris - hydrochloric acid buffer solution, pH 7.4. The absorbance of the suspension was adjusted to 1.0 (10-mm cell) at 650 nm, which corresponded to $3.30 \times 10^8$ viable cells $ml^{-1}$.

*Antibiotic solutions*

A freshly prepared solution of the International Standard for Nystatin (1 mg $ml^{-1}$) in dimethylformamide was diluted with distilled water and protected from light throughout the experiment. The International Standard for Nystatin (3 000 I.U. $mg^{-1}$) was used in the preliminary work and as the standard for the assay of commercial samples of nystatin.

### Apparatus

*Atomic-absorption spectrophotometer.* The instrument (Perkin-Elmer 303.0152) was operated according to the manufacturer's instructions and was calibrated against a standard solution of potassium chloride, the response being linear up to a concentration of 2 $\mu$g $ml^{-1}$ of potassium ions.

### Method

For routine use 3 volumes of the concentrated suspension of yeast cells were diluted with 7 volumes of the buffer solution, pH 7.4, and 4.5-ml volumes of the diluted suspension were placed in 10-ml test-tubes in a water-bath at 30 °C. After 30 min for temperature equilibration 0.5 ml of nystatin solution was added to each tube. The tubes were gently shaken

during a 60-min reaction period after which they were centrifuged at 3 420 *g* for 15 min and the potassium content of the supernatant was determined. Each antibiotic concentration was tested in triplicate.

Three concentrations of the standard and test solutions were tested for the assay. The highest concentration used in the reaction mixture was 3.0 I.U. $ml^{-1}$ and further dilutions were made in the ratio 1:1.25. The antibiotic solutions were added to the test-tubes in a random order and the test-tubes were randomly sited in the water-bath. Test-tubes containing no added antibiotic solution were used as controls to detect any non-antibiotic-induced leakage of potassium.

## Results and Discussion

### Cultivation of *S. cerevisiae*

Use of yeast cells in the stationary phase grown at 30–32 °C was most satisfactory as the amount of potassium ions released was then proportional to the concentration of nystatin. Cells in the exponential phase of growth incubated at 20 and 37 °C were found by other workers to give a non-linear response to nystatin when the loss of rubidium was measured.[12] Cells grown at 20 °C have been shown to be more sensitive to nystatin but when grown at 40 °C the cells were fairly insensitive to nystatin, as assessed by the nystatin-induced leakage of potassium ions.[13]

### Effect of Cell Density and Age of Stock Yeast Suspension

The amount of potassium ions released from cell suspensions of different densities is shown in Table I. A cell suspension of about $1.0 \times 10^8$ viable cells $ml^{-1}$ was chosen for experimental use, as the amount of potassium ions released would then be within the linear range of the calibration graph for potassium ions. The growth medium used to prepare the yeast was not supplemented with potassium salts, which may explain why the cells yielded about 50 times fewer potassium ions than previous workers had reported.[9,10]

TABLE I

POTASSIUM IONS RELEASED FROM SUSPENSIONS OF *S. cerevisiae* AFTER CONTACT WITH A 1.25 $\mu$g $ml^{-1}$ SOLUTION OF NYSTATIN FOR 60 min AT 30 °C

| Cell density/viable cells $ml^{-1} \times 10^7$ | 9.9 | 6.6 | 4.9 | 3.3 | 0.6 |
|---|---|---|---|---|---|
| $[K^+]$/$\mu$g $ml^{-1}$ .. .. .. | 1.80 | 1.06 | 0.86 | 0.49 | 0.09 |

A stock suspension of yeast cells (about $1.0 \times 10^8$ viable cells $ml^{-1}$) was stored at 4 °C for up to 1 week before dilution to prepare working suspensions. The amount of potassium ions released by suspensions of different ages after treatment with nystatin is shown in Table II. During storage there was a decrease in the sensitivity of yeast cells to nystatin as assessed by release of potassium ions, probably owing to the progressive leakage of potassium ions. Freshly prepared suspensions should be used for the assay.

TABLE II

POTASSIUM IONS RELEASED FROM SUSPENSIONS OF *S. cerevisiae* PREPARED FROM A STOCK SUSPENSION STORED AT 4 °C

Contact with nystatin was for 60 min at 60 °C.

| | $[K^+]$/$\mu$g $ml^{-1}$ | |
|---|---|---|
| Time stored/d | 1.25 $\mu$g $ml^{-1}$ nystatin | 0.5 $\mu$g $ml^{-1}$ nystatin |
| 0 | 1.30 | 0.65 |
| 2 | 1.07 | 0.52 |
| 4 | 1.02 | 0.45 |
| 8 | 1.01 | 0.42 |

## Reaction Time

The reaction between nystatin and the yeast cells was found to be time and temperature dependent. The amount of potassium ions released after contact with nystatin for various periods of time is given in Table III. A temperature of 30 °C was tried as the polyene antibiotic candicidin induced complete potassium-ion depletion from *S. cerevisiae* within 20 min at this temperature.[9] Our results indicated that potassium-ion leakage was complete within 40 min with nystatin and a reaction period of 60 min at 30 °C was chosen for the assay. These conditions compare with those used by other workers, *e.g.*, 60 min at 34 °C[10] and 30 min at 37 °C.[12]

TABLE III

POTASSIUM IONS RELEASED FROM A SUSPENSION OF *S. cerevisiae* AFTER CONTACT WITH A 1.0 $\mu$g ml$^{-1}$ SOLUTION OF NYSTATIN AT 30 °C

| Reaction time/min | 0 | 5 | 10 | 20 | 30 | 40 | 60 | 80 | 120 |
|---|---|---|---|---|---|---|---|---|---|
| [K$^+$]/$\mu$g ml$^{-1}$ | 0.27 | 0.62 | 0.92 | 1.46 | 1.48 | 1.55 | 1.53 | 1.58 | 1.56 |

## Potassium Content of Nystatin Samples

The presence of potassium ions in nystatin would possibly invalidate an assay based on the nystatin-induced release of potassium ions from yeast cells. Twenty-five samples of nystatin from several different sources were examined, the highest concentration of potassium ions found being 3 $\mu$g per milligram of nystatin, which represented about 0.4% of the response produced by the release of potassium ions.

## Accuracy and Reproducibility of the Assay

There was a linear relationship between the amount of potassium ions released and the concentration of the nystatin standard between 1.0 and 0.512 $\mu$g ml$^{-1}$ (Fig. 1). Although the actual amount of potassium ions released varied with each suspension of *S. cerevisiae* used, a linear response was obtained, which is demonstrated by the standard deviations of response and the coefficients of linear correlation (see Table IV). As the responses varied from day to day, a nystatin standard was included in each assay in order to ensure that the assay results were comparable.

The accuracy of the method was tested by assaying a nystatin solution that was a known dilution of the nystatin standard solution. The nystatin standard solution contained 100

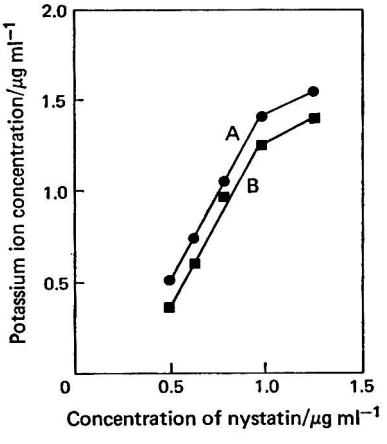


Fig. 1. Dose-response graph: nystatin concentration *versus* mean concentration of potassium ions in the supernatant. A, standard solution; B, test solution (containing 90% *V*/*V* of standard).

TABLE IV

DOSE RESPONSE OF POTASSIUM IONS ($\mu$g ml$^{-1}$) RELEASED FROM SIX FRESH SUSPENSIONS OF *S. cerevisiae* BY NYSTATIN

| Nystatin/$\mu$g ml$^{-1}$ | Suspension number | | | | | | Standard deviation/ $\mu$g ml$^{-1}$ |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| 1.000 | 1.43 | 1.33 | 1.58 | 1.67 | 1.25 | 1.36 | 0.159 |
| 0.800 | 1.04 | 0.98 | 1.25 | 1.34 | 0.93 | 1.04 | 0.161 |
| 0.640 | 0.74 | 0.67 | 0.97 | 1.06 | 0.67 | 0.79 | 0.162 |
| 0.512 | 0.51 | 0.42 | 0.75 | 0.86 | 0.46 | 0.57 | 0.173 |
| Coefficient of linear correlation | 0.9997 | 0.9745 | 0.9999 | 0.9999 | 0.9999 | 0.9998 | — |

$\mu$g ml$^{-1}$ of nystatin in 10% $V/V$ aqueous dimethylformamide. The parent test solution was a 9 + 1 dilution of the standard solution. Subsequent dilutions of standard and test solutions were made with distilled water to give solutions of concentration between 1.25 and 0.512 $\mu$g ml$^{-1}$ of nystatin. In the first two assays the measured potency ratio (0.864 6 and 0.858 7) was less than expected. The only difference between the standard and test assay solutions was the concentration of dimethylformamide that each contained; the standard solution at a nystatin concentration of 1 $\mu$g ml$^{-1}$ contained 0.1% $V/V$ of dimethylformamide and the test solution at a notional nystatin concentration of 1 $\mu$g ml$^{-1}$ contained 0.09% $V/V$ of dimethylformamide. It was not anticipated that this difference would influence the results, as at concentrations of 1% $V/V$ and below, dimethylformamide did not induce any potassium-ion leakage. When the concentration of dimethylformamide in the parent test solution was adjusted to 10% $V/V$ so that there was no difference in the concentration of dimethylformamide in the solutions for assay of standard and test, three independent assay results were obtained (Table V).

TABLE V

MEASURED POTENCY RATIOS OF SOLUTIONS OF NYSTATIN KNOWN TO CONTAIN 90% $V/V$ OF THE STANDARD NYSTATIN SOLUTION

| Potency ratio | Fiducial limits ($P$ = 0.95), % |
|---|---|
| 0.903 0 | 98.15–101.9 |
| 0.915 2 | 96.78–103.3 |
| 0.894 6 | 97.79–101.3 |

Though low concentrations of dimethylformamide do not induce a loss of potassium ions, the activity of nystatin was apparently influenced by the dimethylformamide. The above results indicate that the measurement of potassium-ion loss from cells of *S. cerevisiae* is a reliable means of comparing the potency of identical materials.

Three different samples of nystatin were assayed by measuring potassium-ion loss and by a microbiological diffusion assay using *S. cerevisiae* as the test organism.[14] The same material was used as the nystatin standard for both methods; the potency results obtained by use of the two methods were compared (Table VI). The results obtained showed good agreement for two of the samples. Nystatin has been shown to be a mixture of at least two tetraenes and the presence of a heptaene has been noted,[15] so it would not be surprising if the results of the two

TABLE VI

MEASURED POTENCY OF SAMPLES OF NYSTATIN OBTAINED BY MICROBIOLOGICAL ASSAY AND BY THE ASSAY BASED ON LOSS OF POTASSIUM IONS

| Sample | Microbiological assay | | Potassium-ion loss assay | |
|---|---|---|---|---|
| | Potency/ I.U. mg$^{-1}$ | Fiducial limits ($P$ = 0.95), % | Potency/ I.U. mg$^{-1}$ | Fiducial limits ($P$ = 0.95), % |
| A | 4861 | 97.09–102.9 | 4752 | 97.66–102.9 |
| B | 3655 | 97.93–102.1 | 4564 | 98.27–101.8 |
| C | 4541 | 97.49–102.6 | 4374 | 98.16–101.9 |

methods did not agree as the two assays measure different parameters in very different environments.

## Conclusions

The assay of nystatin based on the loss of potassium ions from yeast cells provides an additional biological system for comparing the potencies of samples of nystatin. The work involved in preparing solutions of nystatin for assay is similar for both a microbiological assay and an assay based on loss of potassium ions. The results of an assay of the latter type are ready for reading after a reaction period of 60 min whereas the microbiological diffusion assay requires an 18-h incubation period. The fiducial limits of error of the measured potency, about $\pm 2\%$, were obtained with both methods. The microbiological assay used three concentrations of standard and test, each replicated on 12 Petri dishes, and the proposed assay also used three concentrations of standard and test, but each replicated only three times.

The need to prepare a fresh culture of *S. cerevisiae* for an assay by measurement of potassium-ion loss may be a disadvantage; for a microbiological assay, a stock suspension can be used over a 4-week period if it is stored at 4 °C. The variation in the amount of potassium ions released by nystatin from different yeast suspensions can be reduced by use of a synthetic medium of known potassium content and the rigorous standardisation of the growth and preparation of the yeast suspension. The use of a nystatin standard will enable the results of assays carried out at different times to be compared.

Nystatin samples with a high potassium content would invalidate the result of an assay carried out by measurement of potassium-ion loss. The potassium content of the sample should be known as it can be taken into account, if necessary, when interpreting the assay result.

The method proposed for the assay of nystatin is simple and rapid with a precision similar to that of a microbiological assay. It would be suitable for the assay of other polyene antifungal antibiotics, such as amphotericin B and candicidin, that induce the leakage of potassium ions from yeast cells.

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# Microbiological Assay of the Polyene Antifungal Antibiotics Amphotericin B, Candicidin and Nystatin

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The suggested procedures of the British Pharmacopoeia for the microbiological assay of the polyene antifungal antibiotics amphotericin B, candicidin and nystatin have been compared. A simpler procedure has been examined and shown to be suitable for the microbiological assay of all three antibiotics. The increased sensitivity of the assay is due to the enhanced solubility of the antibiotics in the diluents used for preparing the assay solutions. The assay has been shown to be accurate and precise.

*Keywords: Amphotericin B; candicidin; nystatin; polyene antifungal antibiotics; microbiological assay*

The agar diffusion method used for the microbiological assay of the polyene antifungal antibiotics has presented difficulties owing to the nature of the antibiotics. They consist of heterogeneous mixtures of closely related compounds, which are poorly soluble in aqueous diluents, diffuse poorly in agar, are unstable in bright sunlight, and produce zones of inhibition that may be neither clear nor proportional in size to the logarithm of antibiotic concentration.

Three polyene antibiotics are included in the British Pharmacopoeia[1,2] and diffusion assays are described for each; although a common test organism is used, there are slight differences in the preparation of the assay solutions. The properties of these solutions have been investigated and a single procedure for preparing assay solutions of polyene antibiotics developed and used in the microbiological assay of amphotericin B, candicidin and nystatin. Factors affecting the assays have been investigated.

## Experimental

### Materials

*Assay organisms*

*Saccharomyces cerevisiae* (NCYC 87) was grown on slopes of the assay medium at 35 °C for 18 h. The cells were washed off with sterile distilled water and the absorbance of the suspension was adjusted to 0.7 in a 10-mm cell at 650 nm (approximately $2.3 \times 10^8$ viable cells $ml^{-1}$). The suspension can be used for 4 weeks if stored at 4 °C.

*Paecilomyces varioti* (MSSC 5605 NIAID) was supplied by Dr. S. Shadomy, Virginia Commonwealth University, USA. Slope cultures on the medium glucose 0.1, dipotassium hydrogen orthophosphate 0.1, calcium carbonate 0.2, tomato paste 2.0, peptone 0.1 and agar 1.5% *m/V* in water were incubated at 30 °C for 5–7 d until the culture consisted of mature spores, lemon yellow in colour, which are more sensitive to the polyene antibiotics than the immature hyaline spores. The spores were washed off with sterile distilled water and the absorbance of the suspension was adjusted to 0.7 in a 10-mm cell at 650 nm. The suspension can be used for 5 d if stored at 4 °C.

*Assay medium*

The following solution in water was prepared: peptone, 0.94; yeast extract, 0.47; beef extract, 0.24; sodium chloride, 3.0; glucose, 1.0; and agar, 1.5% *m/V*. The medium had a pH of 6.1.

*Antibiotics*

The potencies of the antibiotics used were amphotericin B 940 I.U. $mg^{-1}$, candicidin 2 042 U $mg^{-1}$ and nystatin 3 000 I.U. $mg^{-1}$.

*Reagents*

Ethylenediaminetetraacetic acid disodium salt (EDTA) was of AnalaR grade and dimethylformamide, dimethyl sulphoxide and 2-t-butyl-4-methoxyphenol (butylated hydroxyanisole) were of laboratory grade.

## Procedure

*Preparation of assay solutions*

Antibiotic solutions were prepared for assay (1) as described in the relevant pharmacopoeial monograph or (2) by the addition of 3 ml of dimethylformamide to 2.5 mg of antibiotic, this solution being left for 20 min in the dark at room temperature and then diluted with dimethylformamide to 50 $\mu$g ml$^{-1}$ (amphotericin B or nystatin) or 100 U ml$^{-1}$ (candicidin). If any particles were visible at this stage the dilution was placed in an ultrasonic cleaning bath [KS 201 (Kerry Ultrasonics Ltd., Hitchin)] and agitated ultrasonically for 2 min in order to disperse the particles. The procedures used for preparing the antibiotic assay solutions are summarised in Table I.

TABLE I

PROCEDURES FOR THE PREPARATION OF ANTIBIOTIC ASSAY SOLUTIONS

Abbreviations: DMF = dimethylformamide; DMSO = dimethyl sulphoxide; BHA = butylated hydroxyanisole.

| Antibiotic | Method 1 (pharmacopoeial method) | Method 2 (this work) |
|---|---|---|
| Amphotericin B | 6 mg. Add 10 ml of DMF.<br>Dilute 1 + 9 with DMF to 60 $\mu$g ml$^{-1}$.*<br>Dilute with solution A† to 0.058–0.94 I.U. ml$^{-1}$. | 2.5 mg. Add 3 ml of DMF (20 min).<br>Dilute with DMF to 50 $\mu$g ml$^{-1}$.*<br>Dilute with 80% *V*/*V* DMSO in $H_2O$ to 0.058–0.94 I.U. ml$^{-1}$. |
| Candicidin | 2.5 mg (5000 U approx.).<br>Add 3 ml of DMSO containing 0.1% *m*/*V* BHA.<br>Dilute with phosphate buffer,‡ pH 6, to 25 ml.*<br>Dilute with phosphate buffer, pH 6, to 0.0625–1.0 U ml$^{-1}$. | 2.5 mg. Add 3 ml of DMF (20 min).<br>Dilute with DMF to 100 U ml$^{-1}$.*<br>Dilute with 80% *V*/*V* DMSO in $H_2O$ to 0.0625–1.0 U ml$^{-1}$. |
| Nystatin | 7.5 mg. Add 5 ml of DMF.*<br>Dilute 1 + 19 with solution B,§ 75 $\mu$g ml$^{-1}$.<br>Dilute with solution B containing 5% *V*/*V* DMF in $H_2O$ to 4.5–75 I.U. ml$^{-1}$. | 2.5 mg. Add 3 ml of DMF (20 min).<br>Dilute with DMF to 50 $\mu$g ml$^{-1}$.*<br>Dilute with 80% *V*/*V* DMSO in $H_2O$ to 4.5–75 I.U. ml$^{-1}$. |

* Primary solution.
† Solution A: $K_2HPO_4$, 35 g; 1 M NaOH, 20 ml; DMF, 80 ml; $H_2O$ to 1 l.
‡ Phosphate buffer, pH 6: 0.2 M $KH_2PO_4$, 250 ml; 0.2 M NaOH, 28.5 ml; $H_2O$ to 1 l.
§ Solution B: $KH_2PO_4$, 95.6 g; 1 M KOH, 115 ml; $H_2O$ to 1 l.

*Assay technique*

Diffusion assays were carried out in Petri dishes with solutions applied in either fish-spine beads or holes in the agar layer. Each assay was designed to contain sufficient information to provide, from its own internal evidence, a measure of the potency of the "Test" in terms of the "Standard" and the fiducial limits to that measurement. Three dose levels of the "Standard" and three dose levels of the "Test" were tested simultaneously, *i.e.*, on each Petri dish. Six replicate dishes were used for each assay.

Aliquots of 500 ml of the assay medium were melted by steaming for 1 h, mixed, cooled to 40–45 °C and inoculated with the test organism, using either 0.4% *V*/*V* of the suspension of *S. cerevisiae* or 1.0% *V*/*V* of the suspension of *P. varioti*. Immediately after inoculation 25-ml volumes of inoculated medium were dispensed into each Petri dish (90 mm diameter, Sterilin Ltd., Teddington). The antibiotic solutions were prepared as described in Table I, any deviations from these procedures being given in the text. These solutions were protected from light. In the initial experiments the antibiotic solutions were applied to the surface of the assay medium with fish-spine beads, Number 3 (Taylor Tunnicliffe Ltd., Longton); these

beads held about 50 $\mu$l of solution. Because the zones of inhibition obtained using this technique were poorly defined, punched holes in the agar were used in later experiments, 70 $\mu$l of solution being delivered into each hole. The holes were punched in the media and solutions applied automatically.[3] In initial experiments a pre-incubation diffusion period of 2 h at room temperature was used, the plates being protected from light. When the "critical time" (the time after incubation is begun, by which the zone size and edge are fixed) was found to be 10.5 h the pre-incubation diffusion period was omitted. The Petri dishes were incubated in the dark at 32 °C for 18 h, after which the areas of the zones of inhibition were measured in arbitrary units and recorded with a Quantimet 720P Automatic Petridish Analyser, using a line graticule (Image Analysing Computers Ltd., Royston).

The conventional statistical method for parallel line assays was used for the analysis of the experimental results of assays. The area of inhibition of growth was taken as the response parameter and was analysed in relation to the logarithm of the dose. Potency ratios were measured with related confidence limits for each individual assay and the significance of departures from parallelism and linearity of dose - response regression lines were statistically tested.

## Results and Discussion

The diffusion of candicidin from fish-spine beads was less reproducible than from punched holes, resulting in non-linear dose-response lines (Fig. 1). Punched holes were therefore used to apply the antibiotic solutions to the assay dishes.

*Paecilomyces varioti* was found to be four times more sensitive than *S. cerevisiae* to candicidin (Fig. 2) but, because the preparation of suspensions was simpler and the suspensions were more stable when using *S. cerevisiae*, the latter was chosen for those assays in which sensitivity was not a limiting factor.

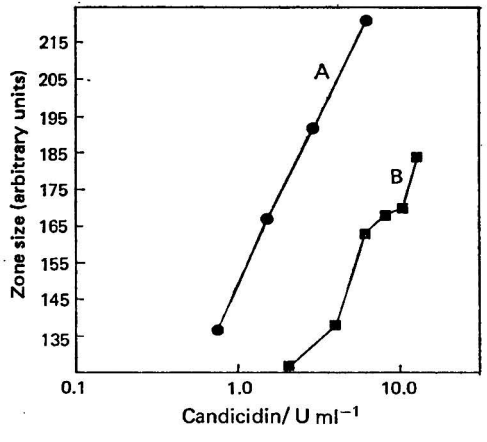


Fig. 1. Log dose-response graph for candicidin assayed against *S. cerevisiae*. A, Punched holes; and B, fish-spine beads.

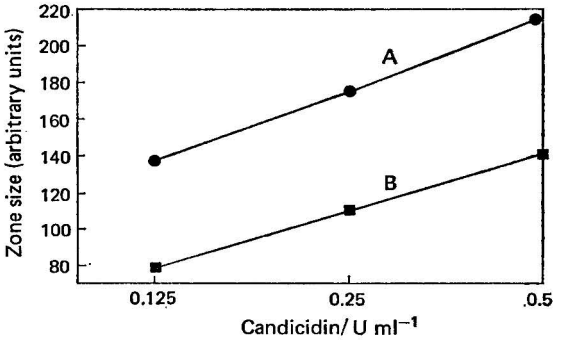


Fig. 2. Log dose-response graph for candicidin. A, Assayed against *P. varioti*; and B, against *S. cerevisiae*.

The assay medium used was similar to that recommended for the assay of amphotericin B and nystatin[1] except that the concentration of agar was reduced from 2.35 to 1.5% *m/V*. Increasing the concentration of sodium chloride from 1.0 to 3.0% *m/V* doubled the sensitivity of *S. cerevisiae* to candicidin; in addition to being larger, the zones of inhibition of growth were sharper. The addition of monovalent cations to the assay medium is thought to reduce the non-specific absorption of the polyene antibiotic and so produce larger zones of inhibition.[4,5]

In aqueous diluents, the polyene antibiotics exist as micellular suspensions that may not diffuse as readily in an agar gel as true molecular solutions. To investigate the solubility of the antibiotics in the diluents recommended for the biological assay, antibiotic "solutions" at the highest concentrations used [amphotericin B, 1 $\mu$g ml$^{-1}$ (0.94 I.U. ml$^{-1}$); candicidin, 0.489 $\mu$g ml$^{-1}$ (1.0 U ml$^{-1}$); and nystatin, 25 $\mu$g ml$^{-1}$ (75 I.U. ml$^{-1}$)] were filtered through

fluorocarbon (PTFE) membranes [Fluropore filter discs, pore size 0.2 μm, Millipore (UK) Ltd.]. The antibiotic contents of the solutions before and after filtration were determined biologically and spectrophotometrically (Table II). The decrease in antibiotic content of the pharmacopoeial assay solutions resulting from filtration indicated the micellular nature of the solutions. No loss of antibiotic was recorded when solutions of the same concentration, but dissolved in 80% *V*/*V* dimethyl sulphoxide in water, were filtered through the same membranes.

TABLE II

AMOUNT OF ANTIBIOTIC RETAINED BY PTFE MEMBRANE (0.2-μm PORE SIZE) AFTER FILTRATION OF ANTIBIOTIC ASSAY SOLUTIONS PREPARED BY METHOD 1.

| | | Antibiotic retained, % | |
|---|---|---|---|
| Antibiotic | Concentration/ μg ml$^{-1}$ | Determined biologically | Determined spectrophotometrically |
| Amphotericin B .. .. | 1.0 | 61.5 | 67.9 |
| Candicidin .. .. .. | 0.489 | 100.0 | 98.6 |
| Nystatin .. .. .. | 25.0 | 44.9 | 38.1 |

The critical time when using *S. cerevisiae* was determined. A large assay plate of seeded agar was poured, wells were punched in the agar and at varying times after the start of incubation constant volumes of antibiotic solution were placed in the wells. Solutions introduced after the critical time should not produce a zone of inhibition, but in practice small zones were produced, probably owing to the lysis of established growth. Zone diameters were projected and magnified ten-fold and were measured to the nearest millimetre. The critical time using *S. cerevisiae* was found to be 10.5 h at 32 °C.

TABLE III

DIFFUSION COEFFICIENTS, *D*, OF POLYENE ANTIBIOTICS IN ASSAY AGAR

| | *D* at 32 °C/cm$^2$ h$^{-1}$ | |
|---|---|---|
| Antibiotic | Method 1 | Method 2 |
| Amphotericin B .. .. .. | 0.011 | 0.015 |
| Candicidin .. .. .. .. | 0.009 | 0.011 |
| Nystatin .. .. .. .. | 0.014 | 0.015 |

Diffusion coefficients (*D*) for the three polyene antibiotics in assay medium using solutions prepared for assay by the pharmacopoeial method and by our method were calculated from the slope of the regression line of the logarithm of concentration against the area of the zone of inhibition of growth, which gives $DT_0$, and as the critical time, $T_0$, is known, *D* can be determined (Table III).[6,7]

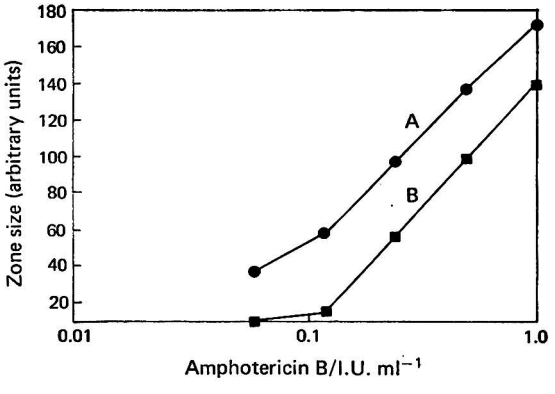


Fig. 3. Log dose-response graph for amphotericin B, assayed against *S. cerevisiae*. A, Assay by method 2; and B, by method 1.

The rate of diffusion was increased when the antibiotics were in molecular solution instead of a micellular suspension. There was then no difference between the diffusion coefficients of amphotericin B and nystatin, reflecting the similar relative molecular masses of 924 and 926, respectively.

Dose - response regressions obtained using *S. cerevisiae* and antibiotic solutions prepared by the two methods are shown in Figs. 3, 4 and 5. Antibiotic concentrations giving a linear dose response were selected for further bioassays as follows: amphotericin B 0.94, 0.47 and 0.235 I.U. $ml^{-1}$; candicidin 1.00, 0.66 and 0.44 U $ml^{-1}$; nystatin 75.00, 37.50 and 18.75 I.U. $ml^{-1}$.

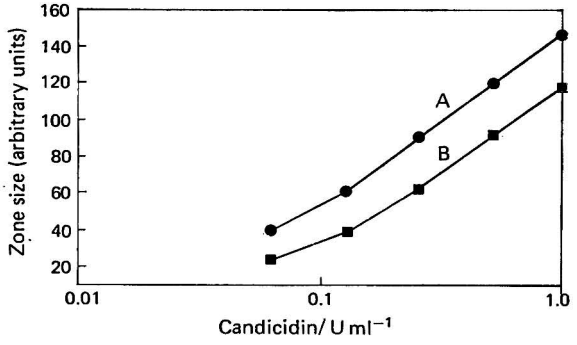


Fig. 4. Log dose - response graph for candicidin assayed agaiast *S. cerevisiae*. A, Assay by method 2; and B, by method 1.

To determine whether exposure to daylight caused any inactivation of the antibiotic, under the conditions of the assay, samples of the chosen concentrations were divided into two portions. One set was kept in the dark, the other was exposed to daylight on the laboratory bench under normal operating conditions for 3 h prior to being assayed, one against the other. Assay solutions with and without EDTA (final concentration 0.1% *m/V*) were similarly compared by assay to determine whether the inclusion of a chelating agent prevented inactivation. The results are shown in Table IV.

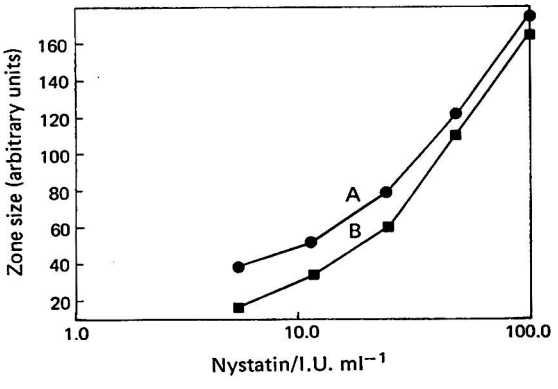


Fig. 5. Log dose - response graph for nystatin assayed against *S. cerevisiae*. A, Assay by method 2; and B, by method 1.

All three antibiotics were inactivated by exposure to daylight, amphotericin B being the most stable. Inactivation occurred regardless of the method used to prepare the solutions. Candicidin solutions prepared by the pharmacopoeial method were most unstable despite the presence of the antioxidant butylated hydroxyanisole. The addition to the assay solution of EDTA did not improve the stability of the antibiotic solutions.

TABLE IV

EFFECT OF EXPOSURE TO DAYLIGHT ON THE STABILITY OF ANTIBIOTIC SOLUTION WITH AND WITHOUT ADDITION OF EDTA

The solutions were exposed to daylight for 3 h at room temperature; 0.1% *m*/*V* EDTA added.

| Antibiotic solution | Loss of activity, %: Preparation by method 1 | Loss of activity, %: Preparation by method 2 |
|---|---|---|
| Amphotericin B | 7.40 | 8.25 |
| Amphotericin B + EDTA | 7.75 | 8.02 |
| Candicidin | 72.67 | 12.28 |
| Candicidin + EDTA | 60.00 | 9.91 |
| Nystatin | 45.65 | 43.18 |
| Nystatin + EDTA | 63.89 | 44.75 |

Primary solutions (Table I) of the antibiotics were kept for 2 weeks at 4 °C in the dark in order to determine if they could be stored without loss of activity. EDTA (0.1% *m*/*V*) was added to one set. Each week, solutions for assay were prepared from the stored primary solutions and assayed against freshly prepared solutions. The results are shown in Table V. There was some change of potency of the solutions stored at 4 °C regardless of the method used for their preparation. The marked increase in the potency of the candicidin primary solution prepared in accordance with the suggested pharmacopoeial method cannot be explained. The addition of EDTA to the primary solution of candicidin prepared by method 1 (Table I) stabilised the potency of this solution, but the storage of primary solutions cannot be recommended.

TABLE V

EFFECT OF STORAGE IN THE DARK ON THE STABILITY OF ANTIBIOTIC PRIMARY SOLUTIONS WITH AND WITHOUT ADDITION OF EDTA

The solutions were stored in the dark at 4 °C; 0.1% *m*/*V* EDTA added.

| Antibiotic solution | Change in activity, %: Preparation by method 1: 1-week storage | Change in activity, %: Preparation by method 1: 2-weeks storage | Change in activity, %: Preparation by method 2: 1-week storage | Change in activity, %: Preparation by method 2: 2-weeks storage |
|---|---|---|---|---|
| Amphotericin B | −2.91 | −18.03 | −2.91 | −5.66 |
| Amphotericin B + EDTA | −7.40 | −16.66 | −4.16 | −8.25 |
| Candicidin | +29.87 | +33.33 | −45.94 | −59.83 |
| Candicidin + EDTA | +1.01 | 0.00 | −53.05 | −65.98 |
| Nystatin | −11.50 | −15.96 | −1.96 | −9.09 |
| Nystatin + EDTA | −11.50 | −15.96 | −5.66 | −6.54 |

In order to test the reliability of the assay procedure single sets of antibiotic solutions were divided in two and used as the "Standard" and "Test," so that a potency ratio of unity would be expected, with no departure from parallelism. The three antibiotics were assayed using both methods of preparing the solutions, which were freshly prepared and protected from light; the results are shown in Table VI.

The results of assaying identical solutions of "Standard" and "Test" showed that both methods used for preparing the solutions gave reliable results as judged by the measured potency ratios. The deviations from parallelism and linearity were associated with very small variance errors, which is a common problem with very precise assays.[8,9]

The alternative method described for the preparation of assay solutions of polyene antibiotics amphotericin B, candicidin and nystatin is simpler than those methods described in the British Pharmacopoeia and the one method is suitable for all three antibiotics. The new procedure results in a slightly increased sensitivity of the assay organism *S. cerevisiae*, probably as a result of the improved solubility. The accuracy and precision of assays using the improved solutions are comparable with those obtained with the solutions described in the present British Pharmacopoeia.

TABLE VI

ASSAY OF IDENTICAL SOLUTIONS AS "STANDARD" AND "TEST"

| Antibiotic | Method of preparation of the solutions | Potency ratio | Confidence limits at 95% level | Probability (%) of— | | |
|---|---|---|---|---|---|---|
| | | | | non-parallelism | non-linearity of— | |
| | | | | | "Standard" | "Test" |
| Amphotericin B .. | 1 | 1.011 | 0.974–1.049 | NS* | NS | NS |
| | 2 | 1.004 | 0.980–1.030 | 99.9 | NS | NS |
| | 2 | 1.007 | 0.987–1.028 | 99.9 | NS | NS |
| Candicidin .. .. | 1 | 0.979 | 0.951–1.007 | 99.0 | 99.9 | 99.9 |
| | 1 | 0.992 | 0.978–1.007 | NS | 99.9 | 99.9 |
| | 2 | 0.998 | 0.967–1.033 | NS | NS | NS |
| | 2 | 0.997 | 0.983–1.011 | 95.0 | NS | NS |
| Nystatin .. .. | 1 | 0.990 | 0.965–1.017 | 99.9 | NS | 99.9 |
| | 1 | 1.000 | 0.987–1.013 | 99.0 | NS | 99.9 |
| | 2 | 0.995 | 0.978–1.013 | 99.0 | 99.0 | NS |

* NS = not significant.

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# Sulphonated Alizarin Fluorine Blue (AFBS)

## Part III.* Lanthanum(III) Complexes of 1-Hydroxyanthraquinone-2-sulphonate, Alizarin-5-sulphonate and AFBS, the Fluoride Complex of AFBS - Lanthanum and a Suggested Method for Fluoride Determination

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An attempt is made to understand the reaction for ternary complex formation between sulphonated alizarin fluorine blue (AFBS), lanthanum and the fluoride ion through study of complex-forming reactions between lanthanum and 1-hydroxyanthraquinone-2-sulphonate (HA2S), alizarin-5-sulphonate (A5S) and sulphonated alizarin fluorine blue, successively. Comparison of experimental molar-ratio and Job plots with those derived from theory reveals that HA2S forms 1:1 and 1:2 lanthanum - HA2S complexes of $K_{11}^{abs} = 1.85 \times 10^7$ l mol$^{-1}$ and $K_{12}^{abs} = 1.29 \times 10^7$ l mol$^{-1}$; A5S forms 1:1, 1:2 and 2:2 lanthanum - A5S complexes and AFBS forms 1:1, 2:1 and 2:2 lanthanum - AFBS complexes. Fluoride ions react to form FR and $FR_2$ complexes, where R represents a particular mixture of AFBS - lanthanum complexes. Conditions under which AFBS can best be used for the determination of fluoride are explored and such an analysis is compared statistically with one using the original alizarin fluorine blue; the latter performs well. The use of other lanthanoid ions is investigated.

*Keywords*: *Sulphonated alizarin fluorine blue*; *lanthanum(III) complexes*; *fluoride determination*; *spectrophotometry*; *composition and stability of complexes*

The synthesis, fundamental solution chemistry and a superficial description of its reactions with lanthanum and fluoride ions have been given for sulphonated alizarin fluorine blue {3-[*NN*-di(carboxymethyl)aminomethyl]-1,2-dihydroxyanthraquinone-5-sulphonic acid} (AFBS) in Parts I and II of this series.[1,2] The reagent behaves in a broadly similar manner to alizarin fluorine blue (AFB) but the increased solubility of AFBS and its complexes facilitates a detailed study of the formation of complexes of interest. Past literature has offered much conjecture on the nature of AFB - lanthanum and AFB - lanthanum - fluoride complex formation; in this paper we try to make more definite deductions concerning the nature of AFBS and related complexes.

We have approached the problem by studying complex formation between lanthanum ions and simple precursors of AFBS, namely 1-hydroxyanthraquinone-2-sulphonate (HA2S) and 1,2-dihydroxyanthraquinone-5-sulphonate (alizarin-5-sulphonate, A5S), before moving on to an examination of AFBS itself. For each complex formation reaction we plotted the usual pH *versus* absorption spectrum, pH *versus* absorbance (at $\lambda_{max.}$), molar-ratio and Job plot graphs, and from these proposed a tentative reaction scheme. Theoretical curves were then produced from assumed conditional stepwise formation constants $K_{mn}$ and molar absorptivities $\epsilon_{M_mR_n}$ until a good match was obtained between theory and experiment. Such an approach is time consuming if only modest computational facilities are used (in this instance a Hewlett-Packard HP35) but the results are far more useful than those so often obtained from cursory examination of Job and molar-ratio plots. Thus, the stepwise formation of complexes so often ignored in studies of this type can be readily appreciated.

### A. Lanthanum Complexes of 1-Hydroxyanthraquinone-2-Sulphonate (HA2S)

#### Theory

Consideration of the nature of the ligand and of experimental curves such as those in Figs.

* For details of Part II of this series, see reference list, p. 366.

1–4 led to the belief that complex formation occurred in two steps:

$$M + R \rightleftharpoons MR \qquad K_{11} = \frac{[MR]}{[MR]\,[R]} \quad .. \quad .. \quad .. \quad (1)$$

$$MR + R \rightleftharpoons MR_2 \qquad K_{12} = \frac{[MR_2]}{[MR]\,[R]} \quad .. \quad .. \quad .. \quad (2)$$

$$\beta_{12} = \frac{[MR_2]}{[M]\,[R]^2} = K_{11}K_{12} \quad .. \quad .. \quad (3)$$

where $M \equiv La^{3+}$ and $R \equiv$ 1-hydroxyanthraquinone-2-sulphonate, irrespective of ionised form. $K_{11}$, $K_{12}$ and $\beta_{12}$ are conditional constants varying with pH. $[R] \equiv [R]'$ (see below), but here the prime is omitted for clarity. If $C_M$ is the total lanthanum concentration, then

$$C_M = [M] + [MR] + [MR_2]$$

and, from equations (1), (2) and (3)

$$\frac{C_M}{[M]} = 1 + K_{11}[R] + \beta_{12}[R]^2 \quad .. \quad .. \quad .. \quad (4)$$

$$\frac{C_M}{[MR]} = \frac{1}{K_{11}[R]} + 1 + K_{12}[R] \quad .. \quad .. \quad .. \quad (5)$$

$$\frac{C_M}{[MR_2]} = \frac{1}{\beta_{12}[R]^2} + \frac{1}{K_{12}[R]} + 1 \quad .. \quad .. \quad .. \quad (6)$$

Similarly, if $C_R$ represents the total amount of HA2S present, then

$$C_R = [R] + [MR] + 2[MR_2]$$

$$\frac{C_R}{[R]} = 1 + K_{11}[M] + 2\beta_{12}[M]\,[R] \quad .. \quad .. \quad .. \quad (7)$$

$$\frac{C_R}{[MR]} = \frac{1}{K_{11}[M]} + 1 + 2K_{12}[R] \quad .. \quad .. \quad .. \quad (8)$$

$$\frac{C_R}{[MR_2]} = \frac{1}{\beta_{12}[M]\,[R]} + \frac{1}{K_{12}[R]} + 2 \quad .. \quad .. \quad .. \quad (9)$$

Also, from equation (7)

$$[M] = \frac{\left(\frac{C_R}{[R]} - 1\right)}{(K_{11} + 2\beta_{12}[R])} \quad .. \quad .. \quad .. \quad .. \quad (10)$$

Considering the ionisation of HA2S

$$HR^- \rightleftharpoons H^+ + R^{2-} \qquad K_a = \frac{[H^+]\,[R^{2-}]}{[HR^-]} \quad .. \quad .. \quad .. \quad (11)$$

and, if $[R]' = [HR^-] + [R^{2-}]$, then from equation (11)

$$\frac{[R]'}{[R^{2-}]} = \alpha_R = \frac{[H^+]}{K_a} + 1 \quad .. \quad .. \quad .. \quad .. \quad (12)$$

If absolute formation constants are defined by the relationships

$$M + R^{2-} \rightleftharpoons MR \qquad K_{11}^{abs} = \frac{[MR]}{[M]\,[R^{2-}]} \quad .. \quad .. \quad (13)$$

$$MR + R^{2-} \rightleftharpoons MR_2 \qquad K_{12}^{abs} = \frac{[MR_2]}{[MR]\,[R^{2-}]} \quad .. \quad .. \quad (14)$$

and

$$M + 2R^{2-} \rightleftharpoons MR_2 \qquad \beta_{12}^{abs} = \frac{[MR_2]}{[M]\,[R^{2-}]^2} \quad .. \quad .. \quad (15)$$

(we are not concerned at this stage with the charge on the complex), then the conditional constants $K_{11}$, $K_{12}$ and $\beta_{12}$ can now be related to absolute formation constants through the above side-reaction coefficients, $\alpha_R$, *i.e.*, from equations (1), (2), (3) and (12):

$$K_{11} = \frac{[MR]}{[M]\,[R]'} = \frac{[MR]}{[M]\alpha_R[R^{2-}]} = \frac{K_{11}^{abs}}{\alpha_R} \quad .. \quad .. \quad .. \quad (16)$$

$$K_{12} = \frac{[MR_2]}{[MR]\,[R]'} = \frac{[MR_2]}{[MR]\alpha_R[R^{2-}]} = \frac{K_{12}^{abs}}{\alpha_R} \quad .. \quad .. \quad (17)$$

$$\beta_{12} = \frac{[MR_2]}{[M]\,([R]')^2} = \frac{[MR_2]}{[M]\alpha_R^2[R^{2-}]^2} = \frac{\beta_{12}^{abs}}{\alpha_R^2} \quad .. \quad .. \quad .. \quad (18)$$

This treatment follows the approach of Ringbom to such problems.[3]

## Molar-ratio Graphs

For the addition of HA2S to a fixed and known concentration of lanthanum ($C_M$), equations (4), (5) and (6) were used to produce the theoretical graphs. At the chosen wavelength and at various pH values over the range of complex formation, values were chosen for $K_{11}$, $K_{12}$, $\epsilon_R$, $\epsilon_{MR}$ and $\epsilon_{MR_2}$. Appropriate values of [R] were substituted into equations (5) and (6) to yield values for [MR] and [$MR_2$]. $\Sigma R = [R] + [MR] + 2[MR_2]$ gave $C_R$, the total concentration of reagent added. The concentrations of absorbing species [R], [MR] and [$MR_2$] were then multiplied, respectively, by $\epsilon_R l$, $\epsilon_{MR} l$ and $\epsilon_{MR_2} l$ (where $l$ is the path length of the cell used) to give individual absorbances $A_R$, $A_{MR}$ and $A_{MR_2}$, and these were summed to give $\Sigma A$, which was plotted against $C_R$. Note that at any pH $\epsilon_R$ can be obtained unequivocally from the pH - absorbance graph for HA2S at the wavelength used (Fig. 2) and that $\epsilon_{MR}$ and $\epsilon_{MR_2}$, which should not change with pH, can be guessed reasonably accurately from the family of pH - absorbance graphs plotted at various metal to ligand ratios.

For molar-ratio graphs involving the addition of lanthanum to a fixed concentration of HA2S equations (7), (8) and (9) were used, but because these are functions of [M] and [R] the procedure is more complex. Suitable values of [R] were chosen and the corresponding values of [M] determined from equation (10). Then [MR] and [$MR_2$] were calculated from equations (8) and (9) or from (1), (2) and (3).

From the best $K_{11}$ and $K_{12}$ values found at various pHs, sets of $K_{11}^{abs}$ and $K_{12}^{abs}$ values were calculated from equations (16), (17) and (12). Mean values for $K_{11}^{abs}$ and $K_{12}^{abs}$ were obtained and used to re-calculate $K_{11}$ and $K_{12}$ at the pHs employed in producing the molar-ratio plots. Theoretical graphs were re-drawn and compared with the original experimental graphs in order to give a unified assessment of the whole complex-forming process. Fits using the unified $K_{11}$ and $K_{12}$ values were not as good as those using the originally chosen values.

## Job (Continuous Variation) Plots

At the pH of choice, good values were taken for $K_{11}$, $K_{12}$, $\epsilon_R$, $\epsilon_{MR}$ and $\epsilon_{MR_2}$ from experience gained with the molar-ratio graphs. The values of $C_R$ and $C_M$ were noted. A reasonable value for [R] was fed into equations (5) and (6), using the known value of $C_M$. [MR] and [$MR_2$] were calculated and $\Sigma R$ found from $\Sigma R = [R] + [MR] + 2[MR_2]$. If $\Sigma R$ was less than $C_R$, [R] was increased and the calculation repeated and *vice versa*. From a few trials

$$\begin{array}{ll} [R] & \ldots\ldots\ldots \Sigma R \\ [R]' & \ldots\ldots\ldots (\Sigma R)' \\ [R]'' & \ldots\ldots\ldots (\Sigma R)'' \end{array}$$

$\Sigma R = C_R$ up to 1% could be obtained readily by interpolation or extrapolation. When the choice of [R] gave a mutually correct set of equations, individual absorbances were obtained from $A_R = \epsilon_R l[R]$, etc., and total absorbance was plotted against $C_M$ and $C_R$ in the usual way.

Certain molar-ratio graphs gave the impression that a further stepwise addition, $MR_2 + R \rightleftharpoons MR_3$, needed to be invoked to obtain a good match. Equations to cover this case were developed in a manner identical with that just described.

### Theoretical pH - Absorbance Graphs

Here parameters $C_R$ and $C_M$ were fixed for one graph but could be varied to produce a set of graphs. By using values of $K_{11}^{abs}$ and $K_{12}^{abs}$ obtained from earlier work, $K_{11}$ and $K_{12}$ were calculated at various pHs from equations (16), (17) and (12). Then, using equations (5) and (6), an iterative process similar to that described under the Job theory was used to find a value of [R] to make $\Sigma R \approx C_R$. Then, from the now known values of [R], [MR] and [$MR_2$], the total absorbance could be calculated.

## Experimental

Sodium 1-hydroxyanthraquinone-2-sulphonate was prepared by sulphonation of 1-hydroxyanthraquinone[4] and checked by electrophoresis[1] and elemental analysis. Required for $C_{14}H_7O_6SNa$, carbon 51.54%, hydrogen 2.16%; found, carbon 51.39%, hydrogen 2.21%.

### Reagents

*Sodium 1-hydroxyanthraquinone-2-sulphonate solution*, $5.0 \times 10^{-4}$ M. Dissolve 0.163 1 g of HA2S in distilled and de-ionised water and dilute the solution to 1 l.

*Lanthanum nitrate solutions*, $2.00 \times 10^{-2}$ *and* $5.0 \times 10^{-4}$ M. Dissolve 4.330 3 g of $La(NO_3)_3.6H_2O$ in distilled, de-ionised water and dilute the solution to 1 l. Standardise it against EDTA solution (pH 5.5, xylenol orange indicator), then dilute the appropriate volume to 1 l in order to obtain the more dilute solution.

*pH control.* Solutions (0.025 M) of hexamine (prepared fresh daily), collidine and ammonia were used as appropriate to assist in adjustment of the pH.

*Sodium nitrate solution*, 1.00 M. This solution is preferred to potassium nitrate solution for fixing the ionic strength because of the insolubility of potassium anthraquinone sulphonates.

### Instrumentation

Absorption spectra were run on a Perkin-Elmer 402 or Pye Unicam SP8000 instrument; absorbance values at a fixed wavelength were measured on a Pye Unicam SP600 instrument. pH measurements were made by using an EIL 39A pH meter.

### Procedure

For all absorbance experiments the required volume of HA2S and 5.0 ml of 1.00 M sodium nitrate solution, at 20 °C, were pipetted into a 50-ml calibrated flask and diluted to approximately 50 ml minus the required volume of remaining reagents. The flask and contents were allowed to stand in a water-bath at 20 °C for 30 min, after which 1.0 ml of the appropriate pH control solution was added and the contents of the flask were transferred into a 100-ml beaker. The pH was set approximately to the desired value by use of dilute nitric acid or sodium hydroxide solution, then the required volume of lanthanum solution added and the pH adjusted accurately. The solution was again transferred into the 50-ml flask, diluted to the mark and allowed to stand for 10 min in the thermostatically controlled water-bath. The pH was again checked just before and after measurement of the absorbance.

## Results

A graph of pH *versus* absorption spectrum for HA2S alone at $C_R = 5.0 \times 10^{-5}$ M showed a normal transition from the $HR^-$ to the $R^{2-}$ form with a $\lambda_{max.}$ [$HR^-$] of 403 nm and $\lambda_{max.}$ [$R^{2-}$] of 506 nm. The usual log $(A - A_{min.})/(A_{max.} - A)$ graph taken from the pH *versus* absorbance graphs run at 403 and 506 nm gave ($\mu = 0.1$ mol $l^{-1}$) a $K_a$ value of $3.02 \times 10^{-10}$ mol $l^{-1}$, a $pK_a$ value 9.52 and a gradient of 0.99, indicating a one-proton ionisation. From this, a table of $\alpha_R$ values at various relevant pHs was drawn up by using equation (12).

Fig. 1 shows the change in the absorption spectrum on adding lanthanum to HA2S. Formation of a multi-ligand complex is suggested by the poor isosbestic point and the drift of $\lambda_{max.}$ for the complex from 495 to 486 nm.

Fig. 2 shows a family of pH *versus* absorbance graphs at 495 nm for various ratios of HA2S to

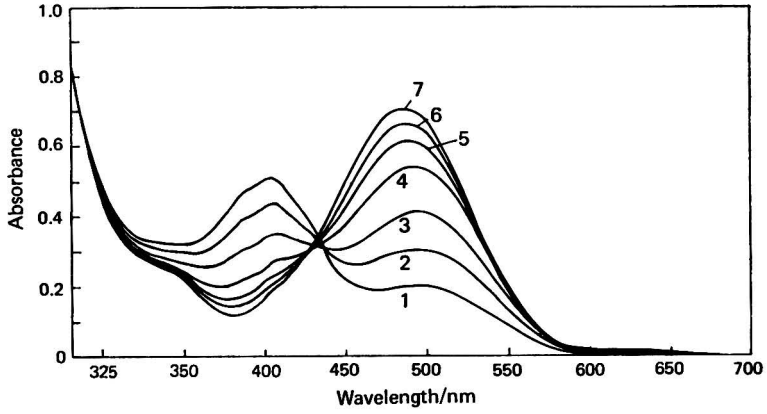



Fig. 1. The addition of lanthanum to 1-hydroxyanthraquinone-2-sulphate (HA2S). pH, 6.95; cell path, 10 mm; $C_{HA2S}$, $10^{-4}$ M; reference, water. $C_{La}$: 1, $2 \times 10^{-5}$; 2, $3.33 \times 10^{-5}$; 3, $5 \times 10^{-5}$; 4, $10^{-4}$; 5, $2 \times 10^{-4}$; 6, $3 \times 10^{-4}$; and 7, $5 \times 10^{-4}$ M.

lanthanum, $C_R$ being held constant. The shape of the graph remains almost constant with a decreasing proportion of metal until the 1:0.33 HA2S:lanthanum ratio is reached, indicating that $MR_2$ is probably the highest complex formed.

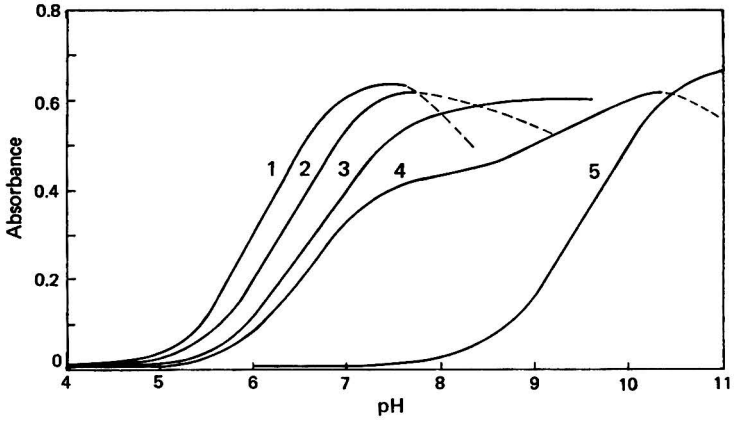



Fig. 2. pH *versus* absorbance graphs for various mixtures of lanthanum with 1-hydroxyanthraquinone-2-sulphonate (HA2S). Wavelength, 495 nm; cell path, 10 mm; reference, water. $C_{HA2S}$, $10^{-4}$ M. $C_{La}$: 1, $2 \times 10^{-4}$; 2, $10^{-4}$; 3, $5 \times 10^{-5}$; 4, $3.33 \times 10^{-5}$; and 5, 0 M. ---- indicates the onset of precipitation.

Although many molar-ratio graphs were produced, Fig. 3 shows the result at pH 7.8 when using $K_{11} = 3.5 \times 10^5$ and $K_{12} = 2.4 \times 10^5$ l mol$^{-1}$, values obtained from final values of $K_{11}^{abs}$ and $K_{12}^{abs}$.

Fig. 4 shows a Job plot at pH 7.8, obtained by using the same values, while Fig. 5 shows experimental and theoretical pH *versus* absorbance graphs for a $C_M:C_R$ ratio of 1:2. Absorbances due to individual species are shown in Figs. 3, 4 and 5.

The following fundamental values were obtained: $K_{11}^{abs} = 1.85 \times 10^7$ l mol$^{-1}$; $K_{12}^{abs} = 1.28 \times 10^7$ l mol$^{-1}$; $\epsilon_{MR} = 6.2 \times 10^3$ l mol$^{-1}$ cm$^{-1}$; and $\epsilon_{MR_2} = 1.29 \times 10^4$ l mol$^{-1}$ cm$^{-1}$. It should be pointed out that this result breaks the rule $K_{11} \not< 4K_{12}$. The formation of an $MR_3$ complex was undetectable.

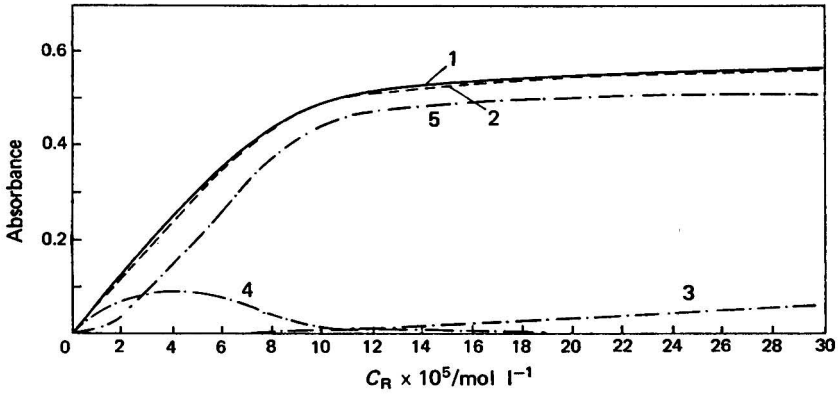



Fig. 3. Molar-ratio graph for addition of 1-hydroxyanthraquinone-2-sulphonate to lanthanum. pH, 7.8; wavelength, 495 nm; cell path, 10 mm; reference, water. $C_{La} = 4 \times 10^{-5}$ M. 1, Experimental curve; 2, theoretical curve for $K_{11} = 3.5 \times 10^5$, $K_{12} = 2.4 \times 10^5$, $\epsilon$ values as in text; 3, absorbance due to free HA2S; 4, absorbance due to La.HA2S; 5, absorbance due to $La(HA2S)_2$.

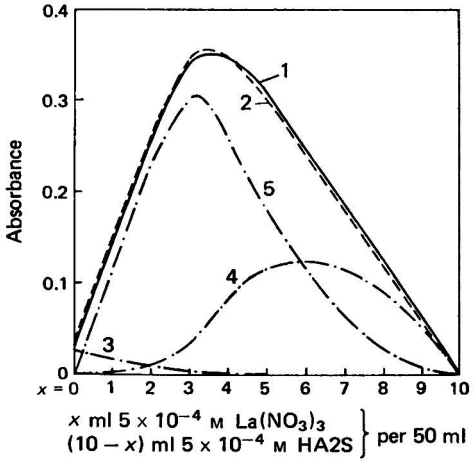



Fig. 4. Job plot for mixtures of lanthanum with 1-hydroxyanthraquinone-2-sulphonate. pH, 7.8; wavelength, 495 nm; cell path, 10 mm; reference, water. $C_{La} + C_{HA2S} = 10^{-4}$ M. 1, Experimental curve; 2, theoretical curve for $K_{11} = 3.5 \times 10^5$, $K_{12} = 2.4 \times 10^5$, $\epsilon$ values as in text; 3, absorbance due to free HA2S; 4, absorbance due to La.HA2S; 5, absorbance due to $La(HA2S)_2$.

## Discussion

Theory - experiment fits for molar-ratio graphs and Job plots were good but that for the pH *versus* absorbance plot (Fig. 5) was disappointing. We tried to correct the poor fit at pH values greater than 8.4 by introducing an $La^{3+} - OH^-$ side reaction [$\beta_1(LaOH) = 10^{3.9}$][3] but this technique was ineffective as the released reagent absorbed light to almost the same extent as the complex.

Note that in this general approach satisfactory $K_{11}$, and therefore $K_{11}^{abs}$, values can only be obtained when $K_{11}$ has a small to intermediate value. At high pH values, where $K_{11} \rightarrow K_{11}^{abs}$, the initial molar-ratio gradient reaches a steady value and becomes insensitive to $K_{11}$. $K_{12}$, however, is better determined in regions of higher stability.

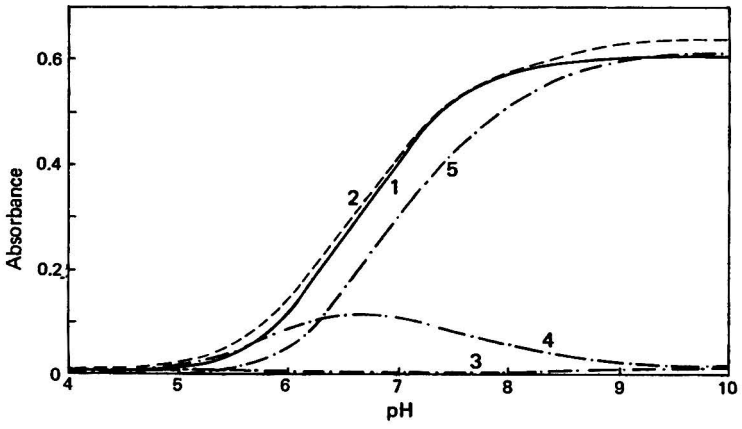
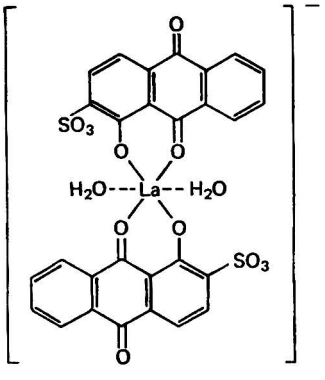



Fig. 5. pH *versus* absorbance graphs (experimental and theoretical) for a 2 : 1 mixture of 1-hydroxyanthraquinone-2-sulphonate and lanthanum. Wavelength, 495 nm; cell path, 10 mm; reference, water. $C_{HA2S}$, $10^{-4}$; $C_{La}$, $5 \times 10^{-5}$ M. 1, Experimental curve; 2, theoretical curve for $K_{11}^{abs} = 1.85 \times 10^{7}$, $K_{12}^{abs} = 1.28 \times 10^{7}$; 3, absorbance due to free HA2S; 4, absorbance due to La.HA2S; 5, absorbance due to $La(HA2S)_2$.

Prolonged investigation may have led to better theory - experiment fits and a better $K_{11}^{abs}$ : $K_{12}^{abs}$ ratio, but the object of the exercise was to determine the nature of the HA2S - lanthanum interaction and undoubtedly this results in the formation of 1:1 and 1:2 metal - ligand complexes; the final complex must then be




## B. Lanthanum Complexes of 1,2-Dihydroxyanthraquinone-5-Sulphonate (A5S)

### Theory

A study of molar-ratio graphs and Job plots for this system at low to intermediate pH values, in particular in regions of concave upward curvature at low $C_R$ and $C_M$ values (see Figs. 9 and 11), led to the belief that the system was more complicated than mere MR and $MR_2$ stepwise formation. This belief was supported by work discussed in reference 4. It was first of all imagined that multi-centre complex formation occurred thus:

$$M + R \rightleftharpoons MR \qquad K_{11} = \frac{[MR]}{[M][R]}$$

$$2MR + M \rightleftharpoons M_3R_2 \qquad K_{32} = \frac{[M_3R_2]}{[MR]^2[M]}$$

$$MR + R \rightleftharpoons MR_2 \qquad K_{12} = \frac{[MR_2]}{[MR][R]}$$

$$M_3R_2 + R \rightleftharpoons 3MR \qquad K_{11}^* = \frac{[MR]^3}{[M_3R_2][R]} = \frac{K_{11}}{K_{32}}$$

$$M_3R_2 + 4R \rightleftharpoons 3MR_2 \qquad K_{12}^* = \frac{[MR_2]^3}{[M_3R_2][R]^4} = \frac{K_{12}^3 \times K_{11}}{K_{32}}$$

Here again, $K$ values are conditional and $[R] \equiv [R]'$, the equilibrium concentration of uncomplexed reagent regardless of protonated form.

$$C_M = [M] + [MR] + 3[M_3R_2] + [MR_2]$$

$$\frac{C_M}{[M]} = 1 + K_{11}[R] + 3K_{32}K_{11}^2\,[M]^2[R]^2 + K_{12}K_{11}\,[R]^2 \quad \ldots\ldots (19)$$

$$\frac{C_M}{[MR]} = \frac{1}{K_{11}[R]} + 1 + 3K_{32}K_{11}\,[M]^2[R] + K_{12}\,[R] \quad \ldots\ldots (20)$$

$$\frac{C_M}{[M_3R_2]} = \frac{1}{K_{32}K_{11}^2\,[M]^2[R]^2} + \frac{1}{K_{32}K_{11}[M]^2[R]} + 3 + \frac{K_{12}}{K_{32}K_{11}[M]^2} \quad \ldots\ldots (21)$$

$$\frac{C_M}{[MR_2]} = \frac{1}{K_{12}K_{11}[R]^2} + \frac{1}{K_{12}[R]} + \frac{3K_{32}K_{11}[M]^2}{K_{12}} + 1 \quad \ldots\ldots (22)$$

Re-arrangement of equation (19) gives the quadratic in [R]:

$$[R]^2(3K_{32}K_{11}^2[M]^2 + K_{12}K_{11}) + [R]\,K_{11} + \left(1 - \frac{C_M}{[M]}\right) = 0 \quad \ldots\ldots (23)$$

In order to produce theoretical molar-ratio graphs for the addition of A5S to a fixed concentration of lanthanum, trial values were taken for $K_{11}$, $K_{32}$ and $K_{12}$ and reasonable values of [M] were substituted into equation (23), solution of which then gave a value for [R]. Values for $C_M$, [M] and [R] were fed into equations (20), (21) and (22) to give values for the absorbers [MR], $[M_3R_2]$ and $[MR_2]$. Trial $\epsilon$ values then gave the total absorbance ($l = 1$ cm):

$$\Sigma A = \epsilon_R\,[R] + \epsilon_{MR}\,[MR] + \epsilon_{M_3R_2}\,[M_3R_2] + \epsilon_{MR_2}\,[MR_2]$$

Finally, $\Sigma A$ was plotted against $C_R$ ($= [R] + [MR] + 2[M_3R_2] + 2[MR_2]$).

An alternative scheme which could account for the concave upward nature of molar-ratio graphs and Job plots is

$$M + R \rightleftharpoons MR \qquad K_{11} = \frac{[MR]}{[M][R]} \quad \ldots\ldots (24)$$

$$MR + MR \rightleftharpoons M_2R_2 \qquad K_{22} = \frac{[M_2R_2]}{[MR]^2} \quad \ldots\ldots (25)$$

$$MR + R \rightleftharpoons MR_2 \qquad K_{12} = \frac{[MR_2]}{[MR][R]} \quad \ldots\ldots (26)$$

$$M_2R_2 + 2R \rightleftharpoons 2MR_2 \qquad K_{12}^* = \frac{[MR_2]^2}{[M_2R_2][R]^2} = \frac{K_{12}^2}{K_{22}}$$

$$C_M = [M] + [MR] + 2[M_2R_2] + [MR_2] \quad \ldots\ldots (27)$$

$$\frac{C_M}{[M]} = 1 + K_{11}\,[R] + 2K_{22}K_{11}^2\,[M][R]^2 + K_{12}K_{11}[R]^2 \quad \ldots\ldots (28)$$

$$\frac{C_M}{[MR]} = \frac{1}{K_{11}[R]} + 1 + 2K_{22}\,K_{11}\,[M][R] + K_{12}[R] \quad \ldots\ldots (29)$$

$$\frac{C_M}{[M_2R_2]} = \frac{1}{K_{22}K_{11}^2\,[M]\,[R]^2} + \frac{1}{K_{22}K_{11}[M][R]} + 2 + \frac{K_{12}}{K_{22}K_{11}[M]} \quad \ldots\ldots (30)$$

$$\frac{C_M}{[MR_2]} = \frac{1}{K_{12}K_{11}[R]^2} + \frac{1}{K_{12}[R]} + \frac{2K_{22}K_{11}[M]}{K_{12}} + 1 \quad .. \quad .. \quad .. (31)$$

From equations (24), (25) and (26)

$$\frac{[M_2R_2]}{[MR]} = K_{22}[MR] = K_{22}K_{11}[M][R]$$

and

$$\frac{[MR_2]}{[M_2R_2]} = \frac{K_{12}}{K_{22}K_{11}[M]} = \frac{K_{12}}{K_{22}} \times \frac{[R]}{[MR]}$$

Also, from equation (28)

$$[R]^2(2K_{22}K_{11}^2[M] + K_{12}K_{11}) + [R]K_{11} + \left(1 - \frac{C_M}{[M]}\right) = 0 \quad .. (32)$$

Note that

$$2M + 2R \rightleftharpoons M_2R_2 \qquad \beta_{22} = \frac{[M_2R_2]}{[M]^2[R]^2} = K_{22}K_{11}^2$$

This relationship is the main controller of the shape (sharpness of break) of the graph.

To produce a theoretical molar-ratio graph, assume $K$ and $\epsilon$ values and then substitute reasonable values of [M] into equation (32) using the particular experimental value of $C_M$. Calculate [R] and hence obtain [MR], $[M_2R_2]$ and $[MR_2]$ from equations (29), (30) and (31) or (24), (25) and (26). Calculate $\Sigma A$ and $C_R$ (equation 33) as in earlier instances.
For the addition of lanthanum to A5S

$$C_R = [R] + [MR] + 2[M_2R_2] + 2[MR_2] \quad .. \quad .. \quad .. (33)$$

From equations (24), (25), (26) and (33)

$$\frac{C_R}{[R]} = 1 + K_{11}[M] + 2K_{22}K_{11}^2[M]^2[R] + 2K_{12}K_{11}[M][R] \quad .. \quad .. (34)$$

$$\frac{C_R}{[MR]} = \frac{1}{K_{11}[M]} + 1 + 2K_{22}K_{11}[M][R] + 2K_{12}[R] \quad .. \quad .. \quad .. (35)$$

$$\frac{C_R}{[M_2R_2]} = \frac{1}{K_{22}K_{11}^2[M]^2[R]} + \frac{1}{K_{22}K_{11}[M][R]} + 2 + \frac{2K_{12}}{K_{22}K_{11}[M]} \quad .. \quad .. (36)$$

$$\frac{C_R}{[MR_2]} = \frac{1}{K_{12}K_{11}[M][R]} + \frac{1}{K_{12}[R]} + \frac{2K_{22}K_{11}[M]}{K_{12}} + 2 \quad .. \quad .. \quad .. (37)$$

and, from equation (34)

$$[M]^2(2K_{22}K_{11}^2[R]) + [M](2K_{12}K_{11}[R] + K_{11}) + \left(1 - \frac{C_R}{[R]}\right) = 0 \quad .. (38)$$

In order to produce a molar-ratio graph, substitute reasonable values of [R] into equation (38) and using the experimentally known $C_R$ determine [M]. Hence, determine [MR], $[M_2R_2]$ and $[MR_2]$ from equations (24), (25) and (26) or (35), (36) and (37).

To produce a theoretical Job plot, note the particular experimental values for $C_R$ and $C_M$, *e.g.*, $C_R = 9.0 \times 10^{-5}$ M and $C_M = 1.00 \times 10^{-5}$ M. Choose a likely value for [R] and substitute this value into equation (38) to give [M]. Then determine [MR], $[M_2R_2]$ and $[MR_2]$ as before. Check $\Sigma R = C_R$ [equation (33)]. Next determine $\Sigma M$ [equation (27)] and compare this value with the experimental value for $C_M$. If these values differ by more than 1% change the chosen value of [R] and repeat the operation. A few trials and judicious additive interpolation will yield a good match between $\Sigma M$ and $C_M$. Now, by using the consistent values of [M] and [R], determine [MR], $[M_2R_2]$ and $[MR_2]$ and hence the absorbance.

## Experimental

Sodium alizarin-5-sulphonate (Bayer, Leverkusen, W. Germany) was recrystallised from distilled water, employing a careful intermediate filtration. Recovered solid was dried at 70 °C under vacuum in the presence of phosphorus(V) oxide and subjected to elemental analysis in triplicate. Required for $C_{14}H_7O_7SNa.H_2O$, carbon 46.48%, hydrogen 2.52%; found, carbon 46.71, 46.71 and 46.13%, hydrogen 2.79, 2.77 and 2.69%. The presence of one molecule of water of crystallisation was confirmed by thermogravimetric analysis.

### Reagents

*Sodium alizarin-5-sulphonate (A5S) solution*, $5.0 \times 10^{-4}$ M. An amount of 0.180 1 g of this material was weighed out and dissolved by repeated additions of water at 70 °C. The combined decanted solutions were cooled and diluted to 1 l.

Lanthanum nitrate, sodium nitrate and pH-control solutions were as previously described.

Solutions for spectrophotometry were prepared as in the 1-hydroxyanthraquinone-2-sulphonate experiments. All of the metal complex studies were impaired by the low solubility of one of the complex species.

### Procedure

Absorbance readings were taken as soon as was consistent with steadiness of value (usually a 10-min wait) but, even so, solutions were often slightly turbid at measurement. Consequently, absorbance readings under certain conditions were not reproducible and, in some instances, the expenditure of excessive effort in matching theoretical and experimental curves was not deemed worthwhile.

## Results

The change in absorption spectrum with pH of A5S is shown in Fig. 6.

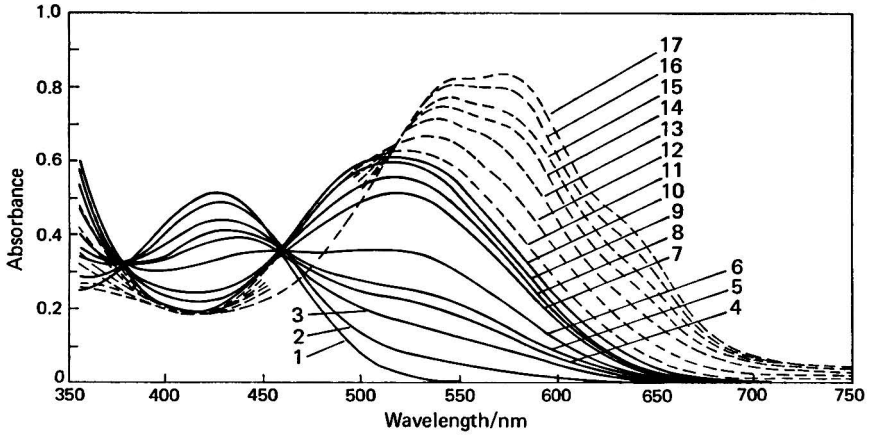



Fig. 6. Absorption spectra of alizarin-5-sulphonate (A5S) at various pH values. Cell path, 20 mm; reference, water. $C_{A5S} = 5 \times 10^{-5}$ M, $\mu = 0.1$. pH values: 1, 3.39 and 4.16; 2, 6.00; 3, 6.46; 4, 6.82; 5, 7.05; 6, 7.30; 7, 7.94; 8, 8.27; 9, 9.40; 10, 10.15; 11, 10.42; 12, 10.85; 13, 11.33; 14, 11.83; 15, 12.21; 16, 12.86; and 17, 13.40.

Two clear isosbestic points show the non-overlapping stepwise ionisations $H_2R^- \rightleftharpoons H^+ + HR^{2-}$ and $HR^{2-} \rightleftharpoons H^+ + R^{3-}$; $\lambda_{max.}(H_2R^-) = 430$ nm, $\lambda_{max.}(HR^{2-}) = 520$ nm, $\lambda_{max.}(R^{3-}) = 575$ nm. Log $(A - A_{min.})/(A_{max.} - A)$ graphs at the above wavelengths gave the following dissociation constants ($\mu = 0.1$ mol l$^{-1}$):

$$K_1 = 1.23 \times 10^{-7} \text{ mol l}^{-1} \quad pK_1 = 6.91$$

and

$$K_2 = 9.55 \times 10^{-12}\ \text{mol l}^{-1} \quad pK_2 = 11.02$$

$$\alpha_{R^{3-}} = \frac{C_R}{[R^{3-}]} = \frac{[H^+]^2}{K_1K_2} + \frac{[H^+]}{K_2} + 1$$

and

$$\alpha_{HR^{2-}} = \frac{C_R}{[HR^{2-}]} = \frac{[H^+]}{K_1} + 1 + \frac{K_2}{[H^+]}$$

(no metal present) were tabulated for pH values subsequently to be used experimentally.

The change in the absorption spectrum of A5S following the addition of lanthanum at pH 6.6 is shown in Fig. 7, which clearly indicates the presence of a variety of complexes.

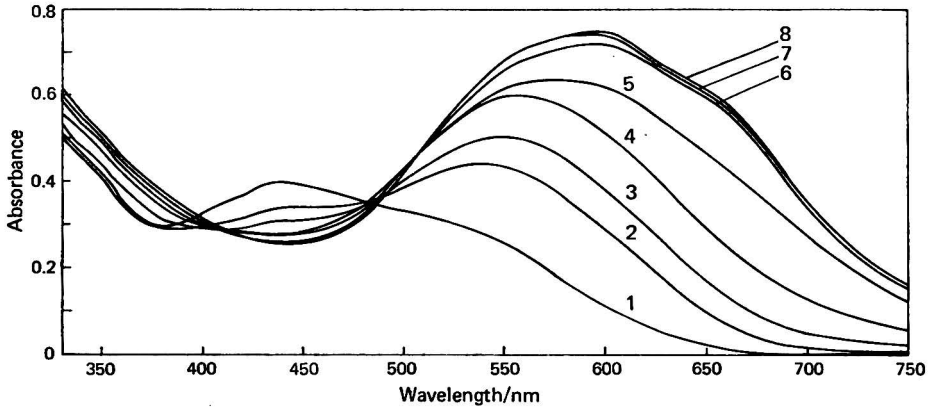



Fig. 7. Absorption spectra of mixtures of alizarin-5-sulphonate with lanthanum. pH, 6.60; cell path, 20 mm; reference, water. $C_{A5S} = 5 \times 10^{-5}$ M. $C_{La}$: 1, 0; 2, $10^{-5}$; 3, $1.67 \times 10^{-5}$; 4, $2.5 \times 10^{-5}$; 5, $3.33 \times 10^{-5}$; 6, $5 \times 10^{-5}$; 7, $10^{-4}$; and 8, $2.5 \times 10^{-4}$ M.

Fig. 8 shows pH - absorbance graphs at 580 nm for various ligand to metal ratios at constant $C_R$. These graphs are of conventional shape for A5S to lanthanum ratios of 1:5, 1:2 and 1:1, but distortion and a lowering of $A_{max.}$ set in at the 1:0.5 ratio, indicating that $M_mR_n$ ($m = n$) is probably the predominating form.

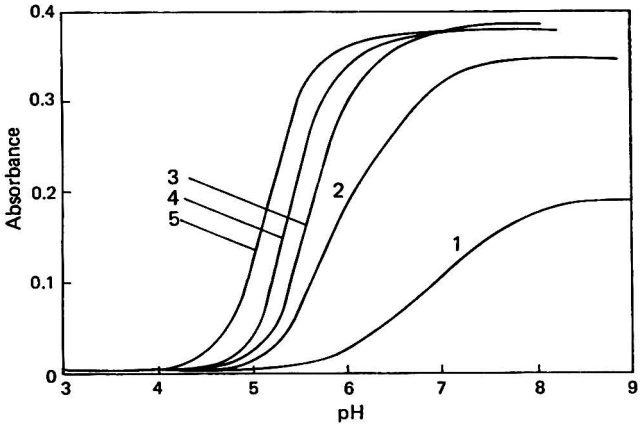



Fig. 8. pH *versus* absorbance graphs for various mixtures of alizarin-5-sulphonate with lanthanum. Wavelength, 580 nm; cell path, 10 mm; reference, water. $C_{A5S} = 5 \times 10^{-5}$ M. $C_{La}$: 1, 0; 2, $2.5 \times 10^{-5}$; 3, $5 \times 10^{-5}$; 4, $10^{-4}$; and 5, $2.5 \times 10^{-4}$ M.

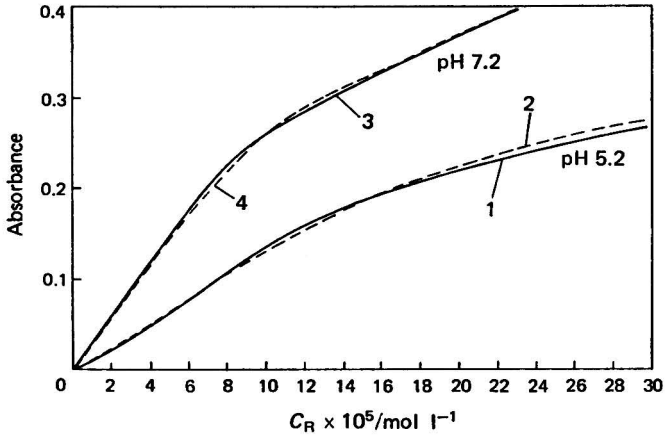



Fig. 9. Molar-ratio graphs at pH 5.2 and pH 7.2 for the addition of alizarin-5-sulphonate to lanthanum. Wavelength, 580 nm; cell path, 10 mm; reference, water. $C_{La} = 5 \times 10^{-5}$ M. 1, Experimental curve, pH = 5.2; 2, theoretical curve, pH 5.2 for $K_{11} = 2.0 \times 10^3$, $K_{22} = 2.4 \times 10^5$, $K_{12} = 2.0 \times 10^3$, $\epsilon$ values as in text; 3, experimental curve, pH = 7.2; 4, theoretical curve, pH 7.2 for $K_{11} = 2.8 \times 10^6$, $K_{22} = 2 \times 10^6$, $K_{12} = 6 \times 10^6$.

Molar-ratio plots for the addition of A5S to lanthanum were run at pH values of 5.2, 5.5, 5.7, 6.0, 6.5 and 7.2. The results for pH values 5.2 and 7.2 are illustrated in Fig. 9. Molar-ratio graphs for the addition of lanthanum to A5S were produced at pH 6.2 and 7.2; the former is shown in Fig. 10 but the latter match was as good.

A Job plot was carried out at pH 5.8 (Fig. 11).

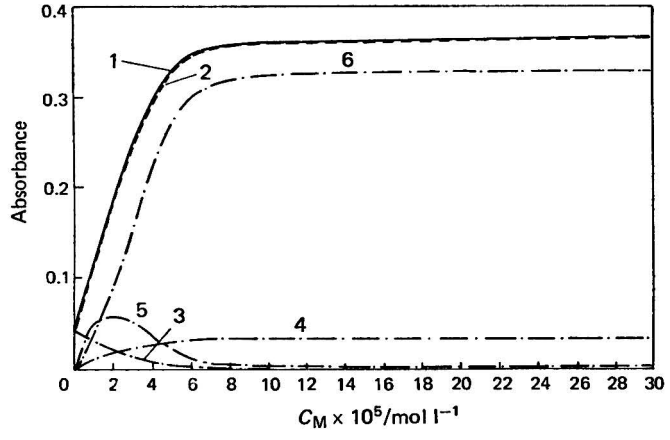



Fig. 10. Molar-ratio graph at pH 6.2 for the addition of lanthanum to alizarin-5-sulphonate. Wavelength, 580 nm; cell path, 10 mm; reference, water. $C_{A5S} = 5 \times 10^{-5}$ M. 1 (——), Experimental curve; 2 (– – – –), theoretical curve for $K_{11} = 1.5 \times 10^5$, $K_{22} = 5 \times 10^5$, $K_{12} = 5 \times 10^4$, $\epsilon$ values as in text; 3, absorbance of free A5S; 4, absorbance of La.A5S; 5, absorbance of $La(A5S)_2$; 6, absorbance of $La_2(A5S)_2$.

## Molar Absorptivity Values at 580 nm (l $mol^{-1}$ $cm^{-1}$)

$\epsilon_R$ varies with pH and can readily be obtained from the pH - absorbance graph (Fig. 8).

$\epsilon_{MR} = 5.0 \times 10^3$
$\epsilon_{M_2R_2} = 1.50 \times 10^4$ at pH less than 6.5 and $1.54 \times 10^4$ at pH greater than 6.5
$\epsilon_{MR_2} = 1.31 \times 10^4$ at pH less than 7.2 and $1.34 \times 10^4$ at pH greater than 7.2

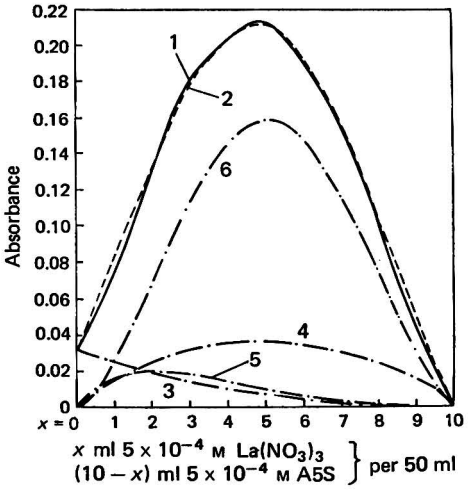
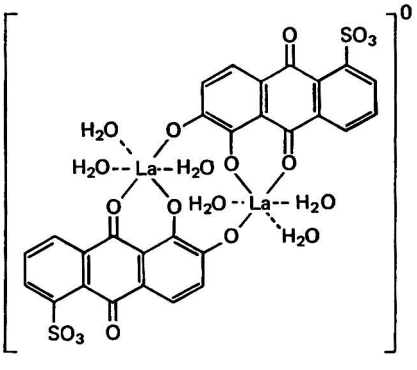



Fig. 11. Job plot for mixtures of lanthanum with alizarin-5-sulphonate. pH, 5.8; wavelength, 580 nm; cell path, 10 mm; reference, water. $C_{La} + C_{A5S} = 10^{-4}$ M. 1, Experimental curve; 2, theoretical curve for $K_{11} = 1.7 \times 10^4$, $K_{22} = 2 \times 10^5$, $K_{12} = 5 \times 10^3$, $\epsilon$ values as in text; 3, absorbance of free A5S; 4, absorbance of La.A5S; 5, absorbance of $La(A5S)_2$; 6, absorbance of $La_2(A5S)_2$.

## Discussion

Attempts to obtain good theoretical - experimental curve fits for $M + R \rightleftharpoons MR$, $MR + R \rightleftharpoons MR_2$ and $M + R \rightleftharpoons MR$, $2MR + M \rightleftharpoons M_3R_2$ systems, both of which are feasible, failed. Curve fits for the $M + R \rightleftharpoons MR$, $2MR \rightleftharpoons M_2R_2$, $MR + R \rightleftharpoons MR_2$ systems were generally good, bearing in mind the turbidity problem.

A structure for the $M_2R_2$ complex is shown below.




This suggestion is supported by the proposed structure of the 1-hydroxyanthraquinone complex and the analysis figures given below. The zero charge would suggest the source of the turbidity problems.

The insolubility was turned to good account in that four samples of the complex were readily prepared as shown in Table I.

TABLE I

PREPARATION OF COMPLEX SAMPLES

| Sample number | $5 \times 10^{-4}$ M A5S/ml | $10^{-2}$ M $La(NO_3)_3$/ml | pH | A5S:La ratio |
|---|---|---|---|---|
| 1 | 500 | 20 | 6.0 | 1:0.8 |
| 2 | 2 000 | 100 | 5.3 | 1:1 |
| 3 | 500 | 50 | 6.0 | 1:2 |
| 4 | 500 | 100 | 6.0 | 1:4 |

A 10.0-ml volume of 0.025 M acetic acid was introduced into each solution in order to provide easy adjustment of the pH and all of the mixtures were allowed to stand overnight. Solutions 1, 3 and 4 were diluted to about 1 500 ml before the addition of lanthanum. Precipitates were centrifuged off, washed with a small volume of water, and then dried at 65 °C under vacuum over phosphorus(V) oxide. The elemental analysis is recorded in Table II.

TABLE II

ELEMENTAL ANALYSIS

| Formula | Sample number | Carbon, % | Hydrogen, % | Lanthanum, % | Sulphur, % |
|---|---|---|---|---|---|
| | | *Found*— | | | |
| — | 1 | 33.2 | 1.95 | 28.8 | 6.4 |
| — | 2 | 33.7 | 1.97 | 29.1 | 6.3 |
| — | 3 | 35.3 | 1.49 | 28.4 | 6.3 |
| — | 4 | 34.4 | 1.64 | 29.0 | 6.4 |
| | | *Required*— | | | |
| $(A5S)_2La_2.6H_2O$ | — | 33.0 | 2.17 | 27.2 | 6.3 |
| $(A5S)_2La_2.5H_2O$ | — | 33.6 | 2.01 | 27.7 | 6.4 |
| $(A5S)_2La_2.4H_2O$ | — | 34.2 | 1.84 | 28.2 | 6.5 |
| $(A5S)_2La_2.3H_2O$ | — | 34.8 | 1.67 | 28.8 | 6.6 |
| $(A5S)_2La_2.2H_2O$ | — | 35.5 | 1.49 | 29.3 | 6.8 |

The amount of lanthanum was determined by nitric acid followed by wet oxidation with perchloric acid then photometric titration with EDTA, using xylenol orange as indicator. Sulphuric acid must be absent.

Carbon analyses were difficult to carry out, possibly owing to the formation of a refractory carbide with lanthanum; prolonged burning in a Perkin-Elmer 240 was necessary. We hope to make this, thankfully rare, phenomenon the subject of a brief later study. There is no logical drift in the analysis figures; indeed, they are satisfactorily consistent considering the variation in sample production conditions. Allowing for some uncertainty in the proportion of water of hydration, agreement between theory and experiment is gratifying.

The calculation of $K^{abs}$ values is difficult because of the complicated nature of the loss of hydrogen ions in the formation of the various complexes, *i.e.*, the relationship between the conditional $K$ values and $\alpha_{R^{3-}}$ and $\alpha_{HR^{2-}}$ is obscure. However, Table III shows the expected,

TABLE III

SUMMARY OF CONDITIONAL FORMATION CONSTANTS (l $mol^{-1}$) FOR LANTHANUM - A5S COMPLEXES AS OBTAINED FROM LIGAND-ADDITION MOLAR-RATIO GRAPHS (UNLESS OTHERWISE STATED)

| pH | $K_{11}$ | log $K_{11}$ | $K_{22}$ | log $K_{22}$ | $K_{12}$ | log $K_{12}$ |
|---|---|---|---|---|---|---|
| 5.2 | $2.0 \times 10^3$ | 3.3 | $2.4 \times 10^5$ | 5.4 | $2.0 \times 10^2$ | 2.3 |
| 5.5 | $4.0 \times 10^3$ | 3.6 | $5.0 \times 10^5$ | 5.7 | $2.5 \times 10^3$ | 3.4 |
| 5.7 | $1.0 \times 10^4$ | 4.0 | $6.4 \times 10^5$ | 5.8 | $2.0 \times 10^3$ | 3.3 |
| 5.8* | $1.7 \times 10^4$ | 4.2 | $2.0 \times 10^5$ | 5.3 | $5.0 \times 10^3$ | 3.7 |
| 6.0 | $5.0 \times 10^4$ | 4.7 | $2.5 \times 10^5$ | 5.4 | $5.0 \times 10^3$ | 3.7 |
| 6.2† | $1.5 \times 10^5$ | 5.2 | $5.0 \times 10^5$ | 5.7 | $5.0 \times 10^4$ | 4.7 |
| 6.5 | $3.1 \times 10^5$ | 5.5 | $7.1 \times 10^5$ | 5.9 | $8.0 \times 10^4$ | 4.9 |
| 7.2† | $3.0 \times 10^6$ | 6.5 | $2.0 \times 10^6$ | 6.3 | $1.6 \times 10^6$ | 6.2 |
| 7.2 | $2.8 \times 10^6$ | 6.5 | $2.0 \times 10^6$ | 6.3 | $6.0 \times 10^6$ | 6.8 |

* Job plot.
† Molar-ratio graph, addition of lanthanum.

but in some instances rather erratic, increase of $K$ values with pH while Fig. 12 shows graphs of log $K$ values plotted against pH and compares these with log $\alpha$ graphs drawn to the same scale.

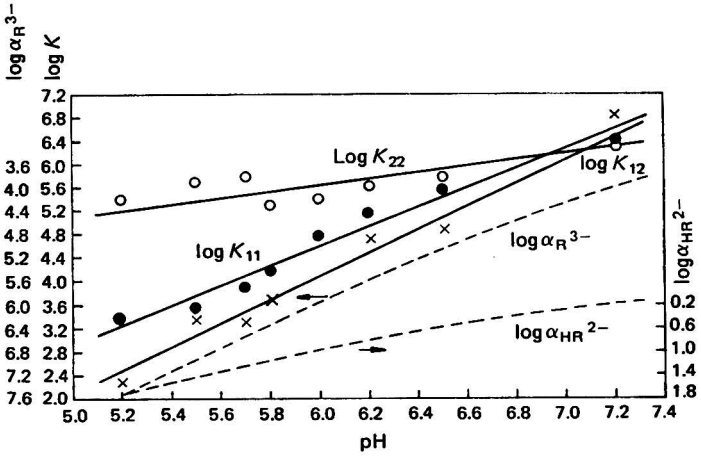



Fig. 12. Alizarin-5-sulphonate - lanthanum system. Variation of log $K$ and log $\alpha$ values with pH. ●, Log $K_{11}$; ×, log $K_{12}$; ○, log $K_{22}$.

From Fig. 12, it would appear that 1 : 1 and 1 : 2 metal to ligand complex formation is coupled with the loss of two protons, while dimer formation is associated with the loss of one proton per molecule. This assumption is reasonable as the complex monomer cannot have more than one proton to lose, whereas the free ligand molecule can lose two. Taking log $K$ values at pH 7 from Fig. 12 and using equation (16), the $K^{abs}$ values shown in Table IV are suggested.

TABLE IV

LOG $K^{abs}$ VALUES (l mol$^{-1}$) FOR A5S - LANTHANUM COMPLEX FORMATION AS CALCULATED FROM LOG $K^{abs}$ = LOG $K$ + LOG $\alpha$

$K$ values are those at pH 7.0.

| | Log $K_{11}$ | Log $K_{11}^{abs}$ | Log $K_{22}$ | Log $K_{22}^{abs}$ | Log $K_{12}$ | Log $K_{12}^{abs}$ |
|---|---|---|---|---|---|---|
| Log $\alpha_{R^{3-}}$ = 4.28 .. | 6.3 | 10.6 | — | — | 6.1 | 10.4 |
| Log $\alpha_{HR^{2-}}$ = 0.26 .. | — | — | 6.2 | 6.4 | — | — |

## C. Lanthanum Complexes of Sulphonated Alizarin Fluorine Blue (AFBS)

The acid - base ionisation properties of AFBS have been discussed in Part II[2] and some features of lanthanum - AFBS complex formation mentioned in Part I[1]; in particular, $\lambda_{max.}$ for the principal complex is at 545 nm.

Many spectrophotometric experiments of the usual type involving attempts at curve matching on the basis of simple M + R ⇌ MR or M + R ⇌ MR, MR + M ⇌ $M_2R$ or M + R ⇌ MR, 2MR ⇌ $M_2R_2$ equilibria led eventually to the conclusion that all of these reactions were involved.

### Theory

The final proposed scheme is

$$M + R \rightleftharpoons MR \qquad K_{11} = \frac{[MR]}{[M][R]} \quad .. \quad .. \quad .. \quad (39)$$

$$MR + MR \rightleftharpoons M_2R_2 \qquad K_{22} = \frac{[M_2R_2]}{[MR]^2} \quad .. \quad .. \quad .. \quad (40)$$

$$MR + M \rightleftharpoons M_2R \qquad K_{21} = \frac{[M_2R]}{[MR][M]} \quad .. \quad .. \quad .. (41)$$

Also,

$$M_2R + R \rightleftharpoons M_2R_2 \qquad K_{22}{}^* = \frac{[M_2R_2]}{[M_2R][R]} = \frac{K_{22}K_{11}}{K_{21}}$$

$$M_2R_2 + 2M \rightleftharpoons 2M_2R \qquad K_{21}{}^* = \frac{[M_2R]^2}{[M_2R_2][M]^2} = \frac{K_{21}^2}{K_{22}}$$

$$2M + 2R \rightleftharpoons M_2R_2 \qquad \beta_{22} = \frac{[M_2R_2]}{[M]^2[R]^2} = K_{22}K_{11}^2 \quad .. \quad .. (42)$$

$K$ terms are again conditional constants, valid only at a fixed pH value.

$$C_R = [R] + [MR] + [M_2R] + 2[M_2R_2]$$

and, from equations (39), (40) and (41)

$$\frac{C_R}{[R]} = 1 + K_{11}[M] + K_{11}K_{21}[M]^2 + 2K_{11}^2K_{22}[M]^2[R] \quad .. \quad .. (43)$$

$$\frac{C_R}{[MR]} = \frac{1}{K_{11}[M]} + 1 + K_{21}[M] + 2K_{11}K_{22}[M][R] \quad .. \quad .. \quad .. (44)$$

$$\frac{C_R}{[M_2R_2]} = \frac{1}{K_{11}^2K_{22}[M]^2[R]} + \frac{1}{K_{11}K_{22}[M][R]} + \frac{K_{21}}{K_{22}K_{11}[R]} + 2 \quad .. \quad .. (45)$$

$$\frac{C_R}{[M_2R]} = \frac{1}{K_{11}K_{21}[M]^2} + \frac{1}{K_{21}[M]} + 1 + \frac{2K_{22}K_{11}[R]}{K_{21}} \quad .. \quad .. \quad .. (46)$$

Re-arranging equation (43):

$$[M]^2(2K_{11}^2K_{22}[R] + K_{11}^2K_{21}) + [M]K_{11} + \left(1 - \frac{C_R}{[R]}\right) = 0 \quad .. \quad .. (47)$$

Molar-ratio graphs were produced by substituting values of [R] into equation (47), thus obtaining [M] by quadratic solution. [MR], $[M_2R_2]$ and $[M_2R]$ were found by using equations (39), (40) and (41). The Job plot was produced in a similar manner, the value of [R] required to give the correct $C_M$ figure being found by iteration.

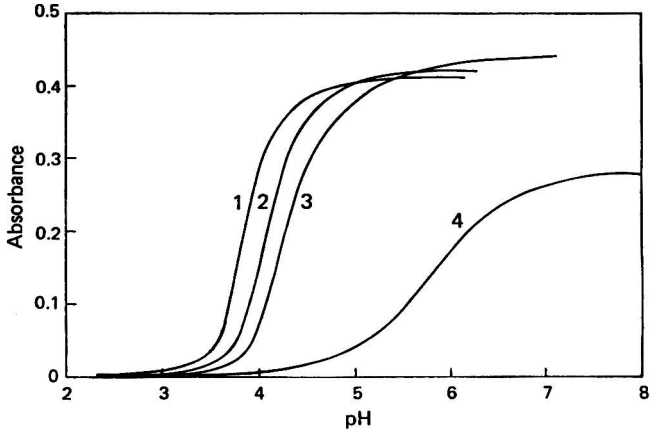



Fig. 13. pH *versus* absorbance graphs for various mixtures of lanthanum with sulphonated alizarin fluorine blue (AFBS). Wavelength, 545 nm; cell path, 10 mm; reference, water. $C_{AFBS} = 5 \times 10^{-5}$ M. $C_{La}$: 1, $2.5 \times 10^{-4}$; 2, $10^{-4}$; 3, $5 \times 10^{-5}$; and 4, 0 M.

## Experimental

*Sulphonated alizarin fluorine blue solution*, $5.0 \times 10^{-4}$ M. This solution was prepared by treating the zwitterion form with two equivalents of sodium hydroxide solution, as is described in Part II.[2]

Lanthanum nitrate and pH-control solutions were prepared as described previously; potassium nitrate can be substituted for the sodium nitrate.

Solutions were mixed as appropriate and diluted to 50 ml after adjustment of the pH. They were left to stand at 20 °C for 10 min and the pH values were checked immediately on completion of the absorbance readings. Complex formation was virtually instantaneous.

## Results

The change of absorption spectrum with pH of a 6:5 $C_M : C_R$ mixture is given in Fig. 3 in Part I.[1]

Fig. 13 here shows pH-absorbance graphs at 545 nm for various lanthanum to AFBS ratios. The variation in final plateau absorbance of the three ratios used is reproducible and significant. It implies an MR + M $\rightleftharpoons$ $M_2R$ type of equilibrium where $\epsilon_{M_2R}$ is less than $\epsilon_{MR}$; indeed from the figure good values of $\epsilon_{M_2R}$ and $\epsilon_{MR}$ (really 0.5 $\epsilon_{M_2R_2}$) can be obtained. The shapes of these curves cannot be reproduced on the basis of a simple M + R $\rightleftharpoons$ MR equilibrium.

The molar-ratio graph at pH 4.4 (Fig. 14) and Job plot at pH 4.6 (Fig. 15) show concave upward curvature in their regions of low metal concentration. This curvature is indicative of dimer formation. From this evidence the scheme developed under Theory is proposed.

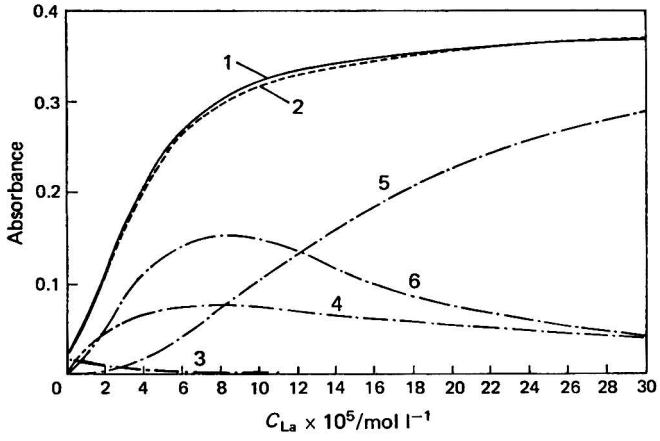



Fig. 14. Molar-ratio graph for the addition of lanthanum to sulphonated alizarin fluorine blue at pH 4.4. Wavelength, 545 nm; cell path, 10 mm; reference, water. $C_{AFBS} = 5 \times 10^{-5}$ M. 1, Experimental curve; 2, theoretical curve for $K_{11} = 8.5 \times 10^4$, $K_{22} = 2.8 \times 10^4$, $K_{21} = 1.8 \times 10^4$, $\epsilon$ values as in text; 3, absorbance of free AFBS; 4, absorbance of La.AFBS; 5, absorbance of $La_2$AFBS; 6, absorbance of $La_2(AFBS)_2$.

Fig. 16 shows a molar-ratio study carried out at pH 4.8, which is that recommended for fluoride determination.

Conditional constants and $\epsilon$ values are summarised in Table V. A steady increase in $K$

TABLE V

CONDITIONAL FORMATION CONSTANTS (l mol$^{-1}$) AND MOLAR ABSORPTIVITIES, $\epsilon$ (l mol$^{-1}$ cm$^{-1}$), FOR THE LANTHANUM - AFBS REACTION

$\epsilon_{M_2R} = 8\,120$ and $\epsilon_{M_2R_2} = 17\,600$ at all pH values.

| pH | $K_{11}$ | $K_{22}$ | $K_{21}$ | $\epsilon_R$ | $\epsilon_{MR}$ |
|---|---|---|---|---|---|
| 4.4 | $8.5 \times 10^4$ | $2.8 \times 10^4$ | $1.8 \times 10^4$ | 260 | 4 300 |
| 4.6 | $2.9 \times 10^5$ | $5.3 \times 10^4$ | $4.2 \times 10^4$ | 320 | 4 300 |
| 4.8 | $1.0 \times 10^6$ | $1.0 \times 10^5$ | $1.0 \times 10^5$ | 600 | 4 300 |
| 6.4 | $1.0 \times 10^{10}$ | $5.0 \times 10^6$ | $3.2 \times 10^6$ | 4 520 | 4 500 |

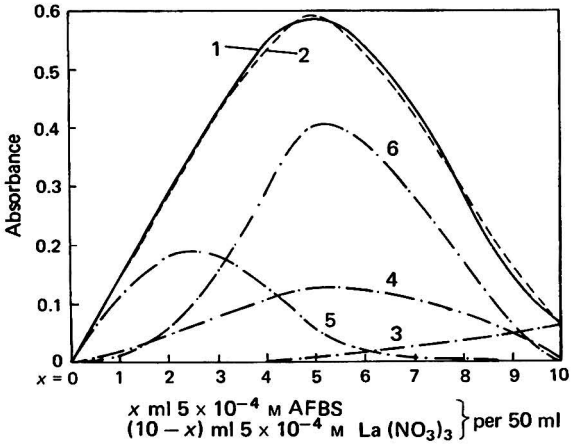



Fig. 15. Job plot for mixtures of lanthanum with sulphonated alizarin fluorine blue at pH 4.6. Wavelength, 545 nm; cell path, 20 mm; reference, water. $C_{La} + C_{AFBS} = 10^{-4}$ M. 1, Experimental curve; 2, theoretical curve for $K_{11} = 2.9 \times 10^5$, $K_{22} = 5.3 \times 10^4$, $K_{21} = 4.2 \times 10^4$, $\epsilon$ values as in text; 3, absorbance of free AFBS; 4, absorbance of La.AFBS; 5, absorbance of $La_2$(AFBS); 6, absorbance of $La_2(AFBS)_2$.

values with pH is evident but we have not attempted to calculate $K^{abs}$ values from $\alpha$ coefficients as the way in which hydrogen ions are involved in complex formation is difficult to assess. pH - absorbance graphs can be approximately reproduced by using an M + R $\rightleftharpoons$ MR log $K^{abs}$ value of 19.7.

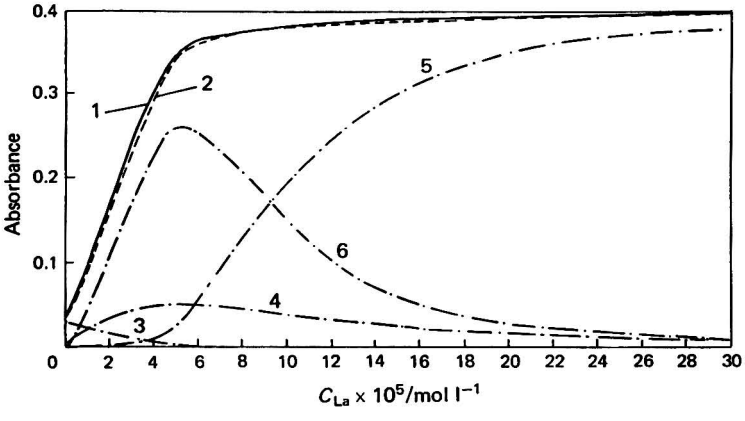



Fig. 16. Molar-ratio graph for the addition of lanthanum to sulphonated alizarin fluorine blue at pH 4.8. Wavelength, 545 nm; cell path, 10 mm; reference, water. $C_{AFBS} = 5 \times 10^{-5}$ M. 1, Experimental curve; 2, theoretical curve for $K_{11} = 10^6$, $K_{22} = 10^5$, $K_{21} = 10^5$; $\epsilon$ values as in text; 3, absorbance of free AFBS; 4, absorbance of La.AFBS; 5, absorbance of $La_2$AFBS; 6, absorbance of $La_2(AFBS)_2$.

## Discussion

Curve fits between theory and experiment for Figs. 14–17 are good, although in Figs. 15 and 16 the full sigmoidal nature of the experimental curve in the low-metal regions could not be

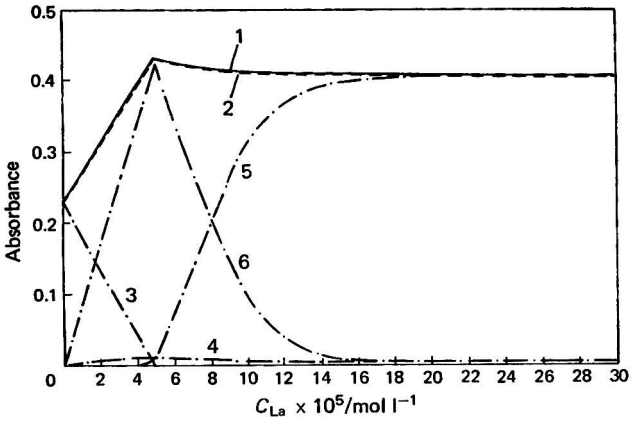
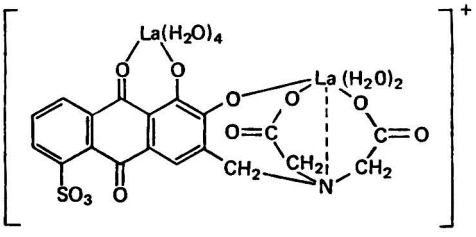



Fig. 17. Molar-ratio graph for the addition of lanthanum to sulphonated alizarin fluorine blue at pH 6.4. Wavelength, 545 nm; cell path, 10 mm; reference, water. 1 (——), Experimental curve; 2 (– – – –), theoretical curve for $K_{11} = 10^{10}$, $K_{21} = 3.2 \times 10^6$, $K_{22} = 5 \times 10^6$; 3, absorbance of free AFBS; 4, absorbance of La.AFBS; 5, absorbance of $La_2$AFBS; 6, absorbance of $La_2(AFBS)_2$.

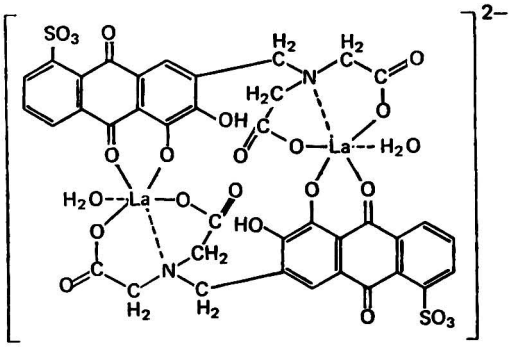
exactly reproduced by theory. This is thought to be a result of some tendency towards $MR + R \rightleftharpoons MR_2$ formation in the presence of a large excess of reagent. The studies on sulphonated 1-hydroxyanthraquinone and alizarin support the production of MR, $M_2R_2$ and possibly $MR_2$ species by AFBS.

The molar-ratio graph at pH 6.4 (the plateau region of the pH - absorbance curves) is remarkable (Fig. 17); we have come across nothing like it before. It gives strong support to the $M_2R_2 + 2M \rightleftharpoons 2M_2R$ suggestion (see Theory).

Taking the complete study into consideration, the structure of the $La_2R$ complex with AFBS is probably




while that of the $La_2R_2$ complex is the analogue of the structure proposed by Langmyhr *et al.*[5] for La - AFB, *i.e.*,



Participation of the 2-hydroxy ligand oxygen atoms is uncertain.

Fig. 18 shows spectra of a 1:1 mixture of lanthanum and AFBS at pH values greater than 6.4 where the complex is fully formed (see Fig. 13). A small shift in $\lambda_{max.}$ from 545 to 556 nm and a small increase in absorbance is evident, which may or may not be due to proton ionisation although it is interesting to note that the spectrum at pH 13 is nothing like that of fully ionised AFBS.

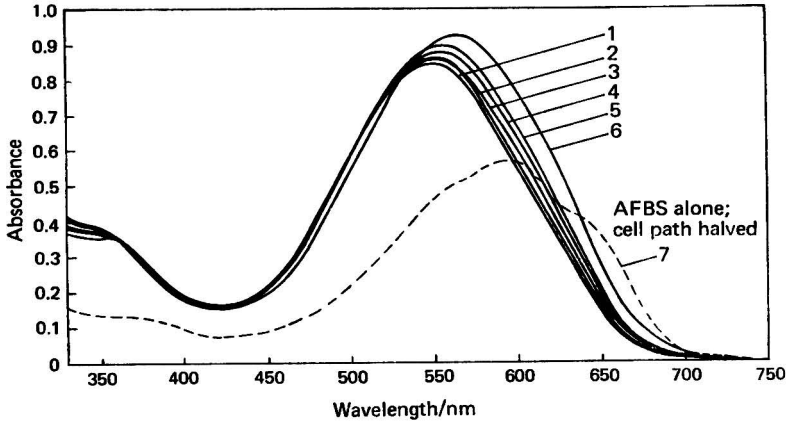



Fig. 18. Absorption spectra of a 1:1 mixture of lanthanum with AFBS at high pH values. Cell path, 20 mm; reference, water. $C_{La} = C_{AFBS} = 5 \times 10^{-5}$ M. pH values: 1, 6.37; 2, 8.29; 3, 9.36; 4, 10.78; 5, 11.80; and 6, 12.98. 7, AFBS alone, pH 12.95, 10-mm cell.

Solid samples of a lanthanum - AFBS "complex" were prepared by precipitation from equimolar or excess-metal mixtures at pH values between 3.7 and 6.4 (Table VI). Postulation of the ion pair $(La_2R_2)^{2-}.(La_2R)^{4+}_2$ gave the best explanation for microanalysis results.

TABLE VI

COMPOSITION OF VARIOUS LANTHANUM - AFBS COMPLEXES

| Sample number | Volume of $4 \times 10^{-3}$ M AFBS solution/ml | Volume of $10^{-2}$ M $La(NO_3)_3$ solution/ml | pH | AFBS to La ratio |
|---|---|---|---|---|
| 1 | 500* | 200 | 5.8 | 1:8 |
| 2 | 60 | 24 | 5.5† | 1:1 |
| 3 | 100 | 64 | 6.4 | 5:8 |
| 4 | 100 | 74.4 | 4.8 | 5:9.3 |

* $5 \times 10^{-4}$ M AFBS.
† pH reduced to 3.7 when precipitate established.

The ratios used in preparations 3 and 4 (Table VI) were thought, from the theoretical studies, to give the best opportunity for production of the ion pair. The precipitates were left overnight, centrifuged off and then dried in an oven at 70 °C. They were further dried at 70 °C under vacuum in the presence of phosphorus(V) oxide and then analysed (see Table VII). A thermogravimetric analysis of a bulked sample indicated the presence of nine molecules of water. Essentially the same material would appear to be produced over a wide range of conditions and the analytical results support the proposed reaction scheme reasonably well. The results for the percentage of nitrogen, 2.72 and 3.01, would seem to be "wild"; it is difficult to conceive of any complex with such high values.

### D. Fluoride Complexes of Lanthanum - AFBS

Absorption spectra for AFBS:lanthanum fluoride 1:1:2 and 1:2:2 mixtures have been

TABLE VII

RESULTS OF ELEMENTAL ANALYSES OF LANTHANUM - AFBS COMPLEXES

Required for $[La_2(AFBS)_2]^{2-}.[La_2(AFBS)]^{+}_{2}.10H_2O$: carbon 31.9, hydrogen 2.19, nitrogen 1.96, lanthanum 29.2 and sulphur 4.5%.

| Sample number | Carbon, % | Hydrogen, % | Nitrogen, % | Lanthanum, % | Sulphur, % |
|---|---|---|---|---|---|
| 1 | 30.1 | 2.12 | 2.72 | 30.4 | 4.5 |
| 2 | 30.0 | 2.14 | 2.24 | 28.5 | 6.1 |
| 3 | 30.4 | 2.26 | 3.01 | 29.4 | 4.9 |
| 4 | 30.4 | 2.27 | 2.43 | 30.0 | 4.2 |

published in Part I.[1] Also shown there is a pH - absorbance graph for the latter mixture. The wavelength of maximum absorption for the predominant AFBS - lanthanum fluoride complex is 585 nm; the wavelength of the highest absorbance difference between AFBS - lanthanum - fluoride and AFBS - lanthanum complexes is 635 nm. The formation of AFBS - lanthanum - fluoride is virtually complete at about pH 4.8.

## Experimental

The previously described solutions of AFBS, lanthanum nitrate, etc., were used together with a $5.0 \times 10^{-4}$ M solution of sodium fluoride (Koch-Light, electronic grade). Appropriate volumes of the solutions were mixed, the lanthanum always being added last; this follows the practice with AFB although it is less important in this instance. pH values were fixed before final dilution to 50 ml and checked again following absorbance measurement. The standing time was 20 min at 20 °C unless otherwise stated.

## Results

Figs. 19 and 20 illustrate the variation in rate of fluoride complex formation with respect to pH and AFBS to lanthanum ratio. The choices of a pH of 4.8 and ligand to metal ratio of 1:4 were heavily influenced by these studies.

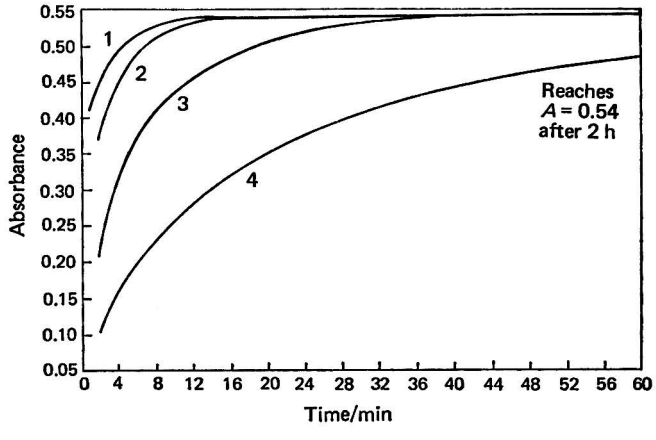



Fig. 19. Influence of pH on the rate of reaction of fluoride with a 1:4 AFBS - lanthanum mixture. Wavelength, 635 nm; temperature, 20.0 °C; cell path, 40 mm. $C_{AFBS} = 5 \times 10^{-5}$, $C_{La} = 2 \times 10^{-4}$, $C_F = 1.6 \times 10^{-5}$ M. Reference solutions as above with $C_F = 0$. pH values: 1, 4.2; 2, 4.8; 3, 5.2; and 4, 5.6.

The formation of the fluoride complex from an established AFBS - lanthanum complex at pH 4.8 is shown in Fig. 21. The clean isosbestic point and the termination of increase in absorbance at only a 3:5 ratio of fluoride to AFBS should be noted.

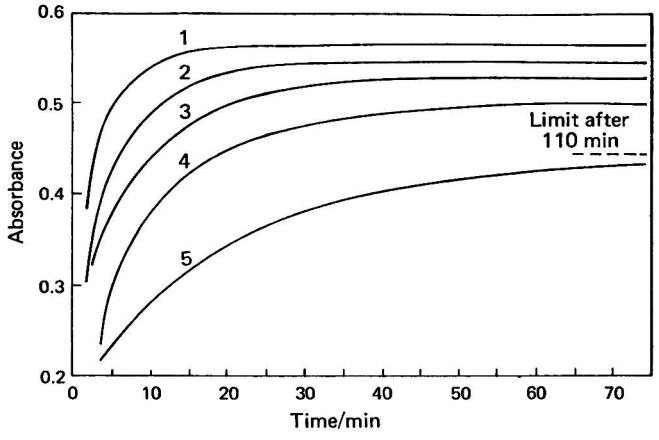



Fig. 20. Influence of AFBS to lanthanum ratio on the rate of formation of the fluoride complex. pH, 4.8; wavelength, 635 nm; cell path, 40 mm; temperature, 20.0 °C. $C_{AFBS}$ = $5 \times 10^{-5}$ M. $C_{La}$: 1, $2 \times 10^{-4}$; 2, $10^{-4}$; 3, $7.5 \times 10^{-5}$; 4, $6 \times 10^{-5}$; and 5, $5 \times 10^{-5}$ M. $C_F$ = $1.6 \times 10^{-5}$ M. Reference solutions as for particular test; $C_F$ = 0.

Fig. 22 shows AFBS - lanthanum - fluoride spectra for various AFBS to lanthanum ratios at pH 4.8 and an AFBS to fluoride ratio of 1:1. Little would appear to be gained from extending this former ratio beyond 1:2 and similar spectra at AFBS to fluoride ratios of 1:0.5 and 1:2 offer the same conclusion. AFBS - lanthanum - fluoride against AFBS - lanthanum difference spectra are shown in Fig. 23; here an AFBS to lanthanum ratio of 1:4 appears to be marginally the best.

Again, Fig. 24 shows a graph of the absorbance of AFBS - lanthanum - fluoride minus the absorbance of AFBS - lanthanum at 635 nm and pH 4.8 and 4.2 against the AFBS to lanthanum ratio. The figure shows very well the robust nature of a fluoride determination based on this chemistry in that fluoride sensitivity is virtually constant between AFBS to lanthanum ratios of 1:3 and 1:8. Also the large pH change has little effect.

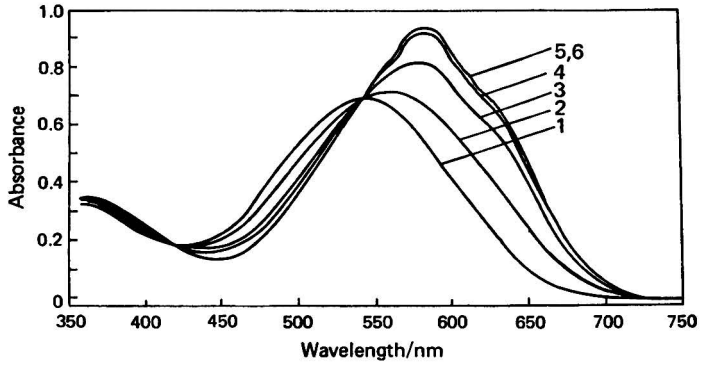



Fig. 21. Absorption spectra showing formation of the ternary AFBS - lanthanum fluoride complex at pH 4.8 from established AFBS - lanthanum. Cell path, 20 mm; reference, water. $C_{AFBS}$ = $5 \times 10^{-5}$ M; $C_{La}$ = $10^{-4}$ M. $C_F$: 1, 0; 2, $10^{-5}$; 3, $2 \times 10^{-5}$; 4, $3 \times 10^{-5}$; 5, $4 \times 10^{-5}$; and 6, $10^{-4}$ M.

## Optimum pH

The absorbances at 635 nm of AFBS to lanthanum to fluoride 1:4:0.32 against water, AFBS to lanthanum 1:4 against water and AFBS to lanthanum to fluoride 1:4:0.32 against

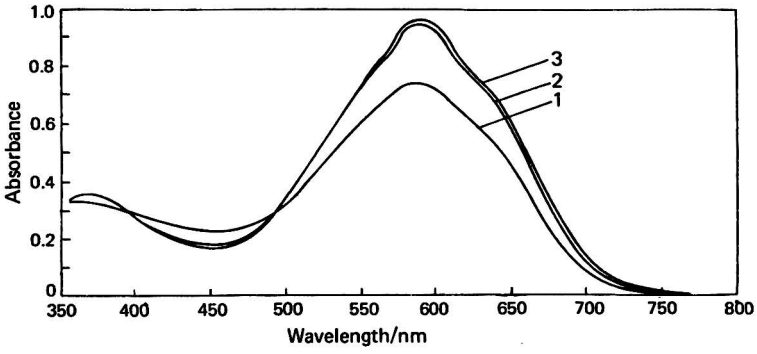



Fig. 22. Absorption spectra of 1:1 mixtures of AFBS and fluoride with various proportions of lanthanum. pH, 4.81; cell path, 20 mm; reference, water. $C_{AFBS} = 5 \times 10^{-5}$ M; $C_F = 5 \times 10^{-5}$ M. $C_{La}$: 1, $5 \times 10^{-5}$; 2, $10^{-4}$; and 3, $2.5 \times 10^{-4}$ M.

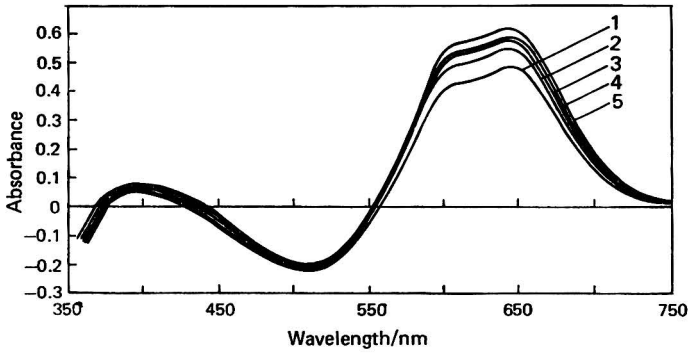



Fig. 23. Ternary complex difference spectra at pH 4.8, showing the effect of variation of ligand:metal ratio. Cell path, 40 mm. $C_{AFBS} = 5 \times 10^{-5}$ M; $C_F = 1.6 \times 10^{-5}$ M. $C_{La}$: 1, $5 \times 10^{-6}$; 2, $6 \times 10^{-5}$; 3, $7.5 \times 10^{-5}$; 4, $10^{-4}$; and 5, $2 \times 10^{-4}$ M. Reference solutions as for particular test,[5] $C_F = 0$.

AFBS to lanthanum 1:4 as a function of pH are shown in Fig. 25. Fluoride sensitivity is constant over the pH range 4.25–6.0 but it may be advantageous to operate inside the plateau regions of the individual complexes, *i.e.*, the pH range 5.0–6.0. It should be borne in mind, though, that the reaction rate slows markedly with increase in pH (Fig. 19).

Fluoride-determination calibration graphs for the best AFBS and AFB conditions are shown in Fig. 26. Linear regression analysis on these two graphs and one obtained a year previously with AFBS to lanthanum equal to 1:4, and a pH of 4.2 gives the results shown in Table VIII.

## TABLE VIII

### LINEAR REGRESSION ANALYSIS ON FLUORIDE DETERMINATION CALIBRATION GRAPHS

| | Gradient/ absorbance unit $\mu g^{-1}$ | Absorbance axis intercept | Coefficient of correlation | Standard deviation of absorbance readings from line | Standard deviation of gradient |
|---|---|---|---|---|---|
| AFBS, pH 4.8 .. .. | 0.0375 | 0.0018 | 0.9998 | 0.0040 | 0.00033 |
| AFBS, pH 4.2 .. .. | 0.0358 | 0.00014 | 0.9999 | 0.0027 | 0.00021 |
| AFB, pH 4.8 .. .. | 0.0323 | −0.0163 | 0.9999 | 0.0012 | 0.00013 |

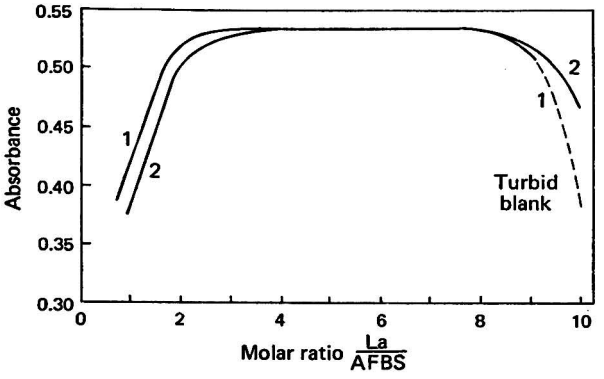



Fig. 24. Variation of sensitivity towards fluoride with change in metal to reagent ratio and pH. Wavelength, 635 nm; cell path, 40 mm. $C_{AFBS} = 5 \times 10^{-5}$ M; $C_F = 1.6 \times 10^{-5}$ M. 1, pH 4.8; 2, pH 4.2. Reference solution as for particular test; $C_F = 0$.

For this study AFBS points derived from 1.9 to 15.2 $\mu$g of fluoride and AFB points from 1.9 to 13.3 $\mu$g of fluoride were taken. For AFBS at pH 4.2 twelve replicate determinations of 13.3 $\mu$g of fluoride gave $A = 0.477$ with a standard deviation of 0.0015 and a coefficient of variation of 0.32%.

A study concerning the variation of fluoride sensitivity with atomic number of the lanthanide ion used is illustrated in Fig. 27. The result is almost identical with that produced by Greenhalgh and Riley for AFB.[6]

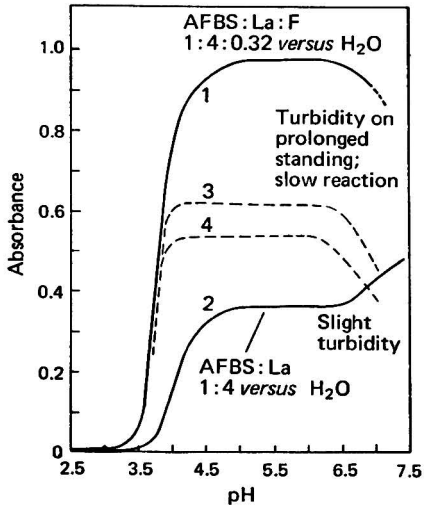



Fig. 25. Variation of sensitivity towards fluoride with change of pH at a 1:4 AFBS to lanthanum ratio. Wavelength, 635 nm; cell path, 40 mm. Curve 1, $C_{AFBS} = 5 \times 10^{-5}$ M, $C_{La} = 2 \times 10^{-4}$ M, $C_F = 1.6 \times 10^{-5}$ M, water reference. Curve 2, as above, $C_F = 0$, water reference. Curve 3 is curve 1 minus curve 2. Curve 4 is the experimental result of using solutions that gave curve 1 as tests with solutions that gave curve 2 as reference.

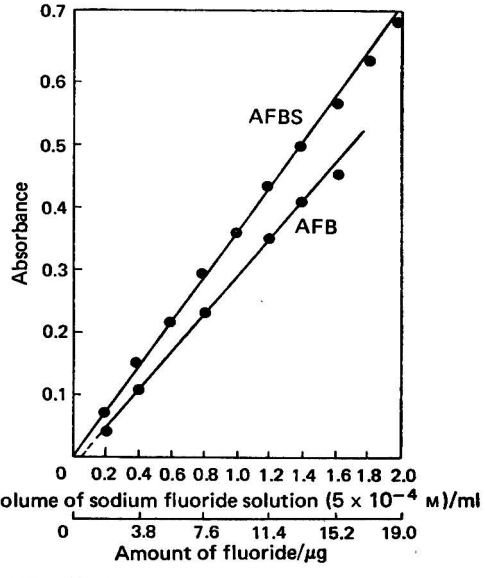



Fig. 26. Beer - Lambert or calibration graphs for the addition of fluoride to 1:4 AFBS - lanthanum and 1:1 AFB - lanthanum at pH 4.8. Cell path, 40 mm; wavelength, 635 nm (AFBS), 610 nm (AFB). $C_{AFBS} = 5 \times 10^{-5}$ M; $C_{La} = 2 \times 10^{-4}$ M (for AFBS), $5 \times 10^{-5}$ M (for AFB). Reference solutions were the relevant binary complexes under the same conditions. Final volume, 50 ml.

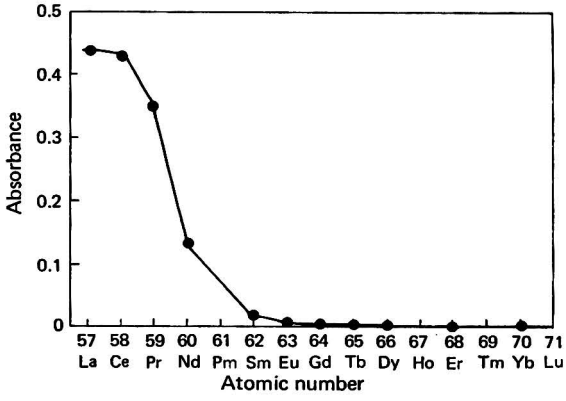



Fig. 27. Variation of complex sensitivity towards fluoride with atomic number of lanthanide ion used. pH, 4.2; cell path, 40 mm; wavelength, 635 nm. $C_{AFBS} = 5 \times 10^{-5}$ M; $C_{lanthanide} = 10^{-4}$ M; $C_F = 1.4 \times 10^{-5}$ M. Reference solution as above but $C_F = 0$.

## Nature of the Ternary Fluoride Complex

A molar-ratio graph for the addition of fluoride to 1:2 AFBS - lanthanum at pH 4.8 is shown in Fig. 28. The predominant complex would appear to be AFBS to lanthanum to fluoride 1:2:0.5. This agrees with the spectra in Fig. 21. A theoretical molar-ratio graph was constructed on the basis of

$$F + R \rightleftharpoons FR \qquad K_{11} = \frac{[FR]}{[F][R]}$$

$$FR + R \rightleftharpoons FR_2 \qquad K_{12} = \frac{[FR_2]}{[FR][R]}$$

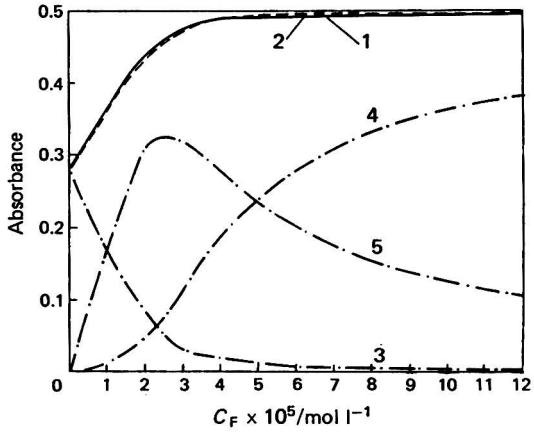



Fig. 28. Molar-ratio graph for the addition of fluoride to established AFBS - lanthanum 1:2. pH, 4.8; wavelength, 585 nm; cell path, 10 mm. $C_{AFBS} = 5 \times 10^{-5}$ M; $C_{La} = 10^{-4}$ M; reference, water. Curve 1 (——), experimental; curve 2 (– – – –), theoretical for $K_{11} = 8.0 \times 10^5$, $K_{12} = 2.2 \times 10^5$, $\epsilon$ values as in text; 3, absorbance due to AFBS - lanthanum species alone; 4, absorbance due to "FR"; 5, absorbance due to "$FR_2$".

Here R represents the mixture of metallised reagent species with which fluoride reacts. Fig. 16 reveals that at pH 4.8 and $C_{La} = 10^{-4}$ M, $C_{AFBS} = 5 \times 10^{-5}$ M; [MR] $= 8.8 \times 10^{-6}$ M, $[M_2R_2] = 8.8 \times 10^{-6}$ M and $[M_2R] = 2.35 \times 10^{-5}$ M. From theory such as that used in the 1-hydroxyanthraquinone study the equation

$$[F] = \frac{\left(\frac{C_R}{[R]} - 1\right)}{(K_{11} + 2K_{11}K_{12}[R])}$$

can be derived and used to produce the molar-ratio graph. Fig. 28 demonstrates an excellent match for

$$K_{11} = 8.0 \times 10^5, \quad K_{12} = 2.2 \times 10^5 \text{ l mol}^{-1}$$
$$\epsilon_R = 5\,640, \epsilon_{FR} = 1.0 \times 10^4, \epsilon_{FR_2} = 2.0 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$$

The experimental graph at the 1:1 AFBS to lanthanum ratio is also typical of FR, $FR_2$ formation. A Job plot at pH 4.8 (Fig. 29) further demonstrates production of a 1:2 F-R complex. This is in marked contrast to results obtained for AFB (see references in Part I[1]).

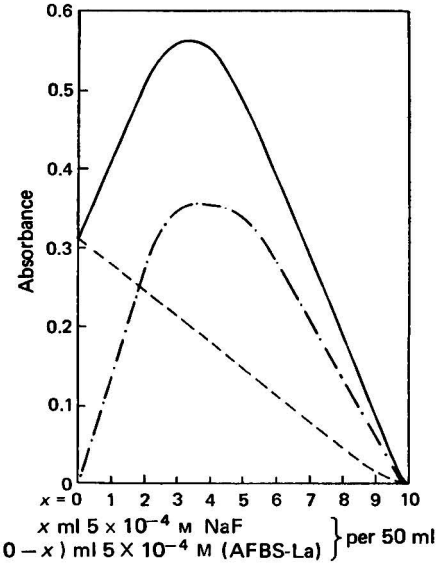



Fig. 29. Job plot for mixtures of fluoride with 1:1 AFBS - lanthanum. pH, 4.8; wavelength, 635 nm; cell path, 20 mm; reference, water. $C_{AFBS\text{-}La} + C_F = 10^{-4}$ M. ——, Total absorbance; – – – –, absorbance due to 1:1 AFBS - lanthanum; – . – . – . –, difference plot.

## Discussion

When fluoride ions enter an AFBS - lanthanum system they are faced with a choice of at least three complex species with which to react, although the increase in sensitivity that results when lanthanum is in molar excess leads to the belief that fluoride favours the $M_2R$ species. The principal fluoride absorber could be $[F(La_2R)_2]^+$ but to draw a structure for this would, on the evidence available, be guesswork.

### Fluoride Determination

Based on the results of this section of the paper a fluoride determination is as follows. To up to 25 ml of the test solution, containing 1–20 $\mu$g of fluoride, add 5.0 ml of 1 M potas-

sium nitrate solution, 2.0 ml of 0.025 M hexamine, 5.0 ml of $5.0 \times 10^{-4}$ M AFBS and adjust the pH to 4.8. Add 10.0 ml of $1.0 \times 10^{-3}$ M lanthanum nitrate solution, re-check the pH, then dilute the solution to 50 ml with water. After 20 min measure the absorbance of the solution against the appropriate blank in 4.0-cm cells at a wavelength of 635 nm.

If a slight loss in sensitivity is no problem, acetate buffer of pH 4.8 can be introduced in order to simplify control of pH. The ratio of lanthanum to reagent, and the pH, are not critical provided that they are exactly the same for test and blank. Detailed interference studies will form the basis of a separate paper.

Although the sensitivity towards fluoride of AFB is almost as good as that of AFBS, the latter is preferable for its greater range of accordance with the Beer - Lambert law and linearity, speed and ease of use. It is, however, a pity that the difference in $\lambda_{max.}$ between AFBS - lanthanum - fluoride and AFBS - lanthanum is a good deal less than that between AFB - lanthanum - fluoride and AFB - lanthanum, as is demonstrated in Fig. 1 in Part I.[1]

We thank Mr. W. J. Swindall for the elemental microanalysis results.

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NOTE—References 1 and 2 are to Parts I and II of this series, respectively.

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# Determination of Saccharin in Soft Drinks by a Spectrophotometric Method

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Saccharin reacts quantitatively with phenothiazine (thiodiphenylamine) and copper(II) acetate dissolved in 50% *V/V* ethanol at 70 °C. The reaction product can be dissolved in an organic solvent and has a characteristic colour. The colour reaction is sensitive and the absorbance, at 510 nm, of the extract in 5 ml of xylene obeys Beer's law at saccharin concentrations between 20 and 400 $\mu g\ ml^{-1}$. Cyclamates, sorbic acid, benzoic acid, 4-hydroxybenzoic acid and dehydroacetic acid do not interfere. Recoveries of saccharin from soft drinks were satisfactory. A procedure suitable for routine use is proposed.

*Keywords*: *Saccharin determination*; *soft drinks*; *spectrophotometry*

Saccharin has been widely used as an artificial sweetening agent in a variety of food products. However, in order to avoid excessive use, several countries have set tolerance limits. The literature contains numerous reports on the determination of saccharin in food and beverages. Various techniques have been used: thin-layer chromatography,[1] gravimetry,[2] polarography,[3] the use of an ion-selective electrode,[4] colorimetry,[5] infrared spectrometry,[6] gas - liquid chromatography,[7] ultraviolet spectrometry[8] and recently molecular emission cavity analysis.[9] Recently, we found that saccharin reacted with a solution of phenothiazine and copper(II) acetate dissolved in an organic solvent and produced a red colour. A colorimetric method for saccharin was reported recently by Fernandez-Flores *et al.*,[10] but this method was not suitable for routine use because of complex formation and the vigorous reaction conditions required. In this paper, we describe the development of a simple, accurate and specific reaction for saccharin, which can be used for its routine determination in soft drinks.

## Experimental

### Apparatus

*Hitachi, Model EPS-032, spectrophotometer.*

### Reagents

Analytical-reagent grade reagents are to be used throughout the determination.

*Phenothiazine solution.* Dissolve 1 g of phenothiazine (thiodiphenylamine) in 100 ml of 99.5% ethanol. Prepare the solution fresh daily.

*Acetic acid solution*, 0.5%.

*Copper(II) acetate solution*, 0.5%. Dissolve 0.5 g of copper(II) acetate in 100 ml of 0.5% acetic acid.

*Ethanol*, 50% *V/V*.

*Saccharin standard stock solution*, 1 000 p.p.m. Weigh accurately 100 mg of saccharin and dissolve it in 100 ml of 50% ethanol.

*Sodium hydrogen carbonate solution*, 1%.

*Sulphuric acid*, 10%.

*Hydrochloric acid*, 10%.

*Diethyl ether and xylene.* Very high purity grades.

### Procedure

The beverage was decarbonated by repeated shaking and pouring from one beaker to another. A 50-g amount of sample was transferred into a 300-ml separating funnel fitted with a PTFE stopcock, 30 ml of water and 5 ml of 10% sulphuric acid were added and the mixture was extracted twice with 100 ml of diethyl ether, shaking for 3 min, and then the aqueous layer

was discarded. The upper layer was extracted twice with 25 ml of 1% sodium hydrogen carbonate solution and the diethyl ether layer discarded. The aqueous layer was acidified with 10 ml of 10% hydrochloric acid, and then extracted twice with 30 ml of diethyl ether. The ether extract was washed with 10 ml of distilled water, transferred into a 100-ml flask and evaporated to a small volume at 40 °C.

The saccharin residue in the 100-ml flask was transferred into a test-tube (about 17.5 × 1.7 cm i.d.) by means of 5 ml of 50% ethanol, and 1 ml of copper(II) acetate solution and 1 ml of phenothiazine solution were added, followed by 3 ml of ethanol. The test-tube was placed in a water-bath at 65–70 °C and was held at that temperature with occasional shaking for 50 min. After being cooled to room temperature, the sample solution was transferred into a 50-ml separating funnel with 2 ml of 99.5% ethanol, and 5 ml of xylene and 15 ml of water were then added. The mixture was shaken vigorously for 5–10 min and the xylene layer separated and dried with 1 g of anhydrous sodium sulphate.

## Preparation of Calibration Graph

The maximum absorption of the colour was found to occur at 510 nm. Aliquots of the standard saccharin solution containing 0.1 and 2 mg of saccharin were placed in test-tubes and taken through the procedure described above. The absorbances of the solutions were measured at 510 nm in 10.0-mm cells. A graph of the absorbance against concentration of saccharin was a straight line passing through the origin. Beer's law was obeyed at concentrations of saccharin in the xylene solution between 20 and 400 $\mu$g ml$^{-1}$.

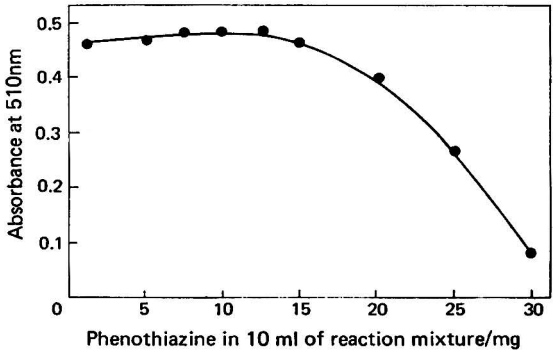


Fig. 1. Effect of amount of phenothiazine on intensity of colour. Amount of saccharin added: 1.00 mg.

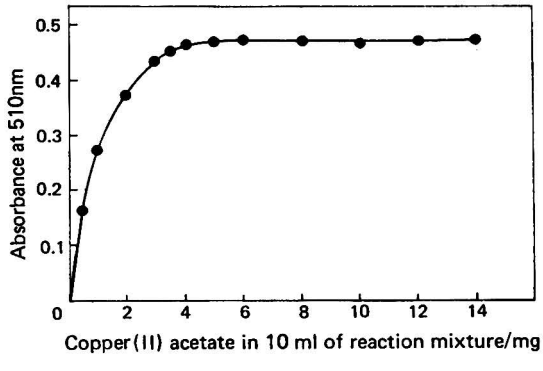


Fig. 2. Effect of amount of copper(II) acetate on intensity of colour. Amount of saccharin added: 1.00 mg.

### Determination of Saccharin in Samples

The absorbances of both standard and sample solutions were measured in 10.0-mm cells at a wavelength of 510 nm against a blank carried through the procedure simultaneously. The amount of saccharin was calculated as follows:

$$\text{Saccharin in sample (p.p.m.)} = \frac{B}{A} \times \frac{m_1}{m_2} \times 5\,000$$

where $A$ and $B$ are the absorbance values of standard and sample, respectively, $m_1$ mg is the amount of saccharin per millilitre of xylene extracted from the standard solution with absorbance $A$ corresponding to the corrected absorbance, and $m_2$ g is the amount of sample present.

## Results and Discussion

The colour reaction of saccharin with phenothiazine was studied. The maximum intensity of the colour was obtained by using 10 mg of phenothiazine, 5 mg of copper(II) acetate and 1 ml of 0.5% acetic acid, as shown in Figs. 1 and 2 and Table I. A precipitate was obtained in a neutral or alkaline solution or if an excess of copper(II) acetate was present. A study of the effect of reaction time showed that the optimum time for maximum sensitivity was 45–50 min at 70 °C, as shown in Table II. Xylene, hexane, ethyl acetate, cyclohexane, 4-methylpentan-2-one and chloroform were tried as extraction solvents. The most suitable of these solvents was xylene and the least suitable was chloroform; the absorbance of the xylene extract remained stable for at least 1 d.

TABLE I

EFFECT OF ACETIC ACID CONCENTRATION ON INTENSITY OF COLOUR

| Acetic acid concentration, % | 0.1 | 0.5 | 1.0 | 2.0 | 5.0 | 7.0 | 10.0 | 15.0 |
|---|---|---|---|---|---|---|---|---|
| Absorbance at 510 nm in 10.0-mm cell | 0.46 | 0.47 | 0.40 | 0.38 | 0.34 | 0.31 | 0.21 | 0.18 |

TABLE II

EFFECT OF TIME AT 70 °C ON INTENSITY OF COLOUR

| Time/min | 10 | 20 | 30 | 40 | 50 | 60 |
|---|---|---|---|---|---|---|
| Absorbance at 510 nm in 10.0-mm cell | 0.12 | 0.20 | 0.30 | 0.45 | 0.47 | 0.46 |

The following food additives did not interfere, as can be seen from Table III: cyclamates, sorbic acid, benzoic acid, 4-hydroxybenzoic acid and dehydroacetic acid.

TABLE III

INFLUENCE OF FOOD ADDITIVES ON RECOVERY OF SACCHARIN FROM SOLUTIONS CONTAINING 1 mg OF SACCHARIN

| Additive | Amount added/mg | Saccharin found/mg | Recovery, % |
|---|---|---|---|
| Cyclamate | 1 | 0.99 | 99 |
| | 5 | 0.98 | 98 |
| Sorbic acid | 1 | 1.00 | 100 |
| | 5 | 1.00 | 100 |
| Benzoic acid | 1 | 1.00 | 100 |
| | 5 | 1.00 | 100 |
| 4-Hydroxybenzoic acid | 1 | 1.00 | 100 |
| | 5 | 1.00 | 100 |
| Dehydroacetic acid | 1 | 1.00 | 100 |
| | 5 | 1.00 | 100 |

To check the validity of the proposed procedure for determining saccharin in soft drinks, recoveries of saccharin added to various saccharin-free soft drinks were measured (Table IV).

The recoveries ranged from 95.7 to 103.6% with an average of 99.5%. The standard deviation for the whole procedure was 0.03% for the 18 determinations given in Table IV.

TABLE IV

RECOVERY OF SACCHARIN ADDED TO SACCHARIN-FREE SOFT DRINKS AND BEVERAGES

Amount of saccharin added, 1.00 mg.

| Sample | Saccharin found/mg | | | Mean recovery, % |
|---|---|---|---|---|
| Water .. .. .. | 1.00 | 1.00 | 1.00 | 100.0 |
| Cola .. .. .. | 0.94 | 0.95 | 0.98 | 95.7 |
| Apple juice .. .. | 0.97 | 0.97 | 0.98 | 97.3 |
| Lemon drink .. .. | 0.99 | 1.03 | 1.04 | 102.0 |
| Orange drink .. .. | 1.02 | 1.01 | 1.05 | 103.6 |
| Carbonate drink .. | 0.98 | 0.99 | 1.00 | 99.0 |
| Mean .. .. .. | | 0.99 | | 99.5 |
| Standard deviation .. | | ±0.03 | | ±2.5 |

It was concluded that the reaction of saccharin with phenothiazine is quantitative and is as sensitive as previously published methods.[5,6,8,9] It gives a rapid and very simple method for the determination of saccharin. The reaction is specific for saccharin and the colour is very stable.

The authors thank Mrs. Akiko Hirose for her co-operation in carrying out this work.

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# Rapid Spectrophotometric Micro-determination of Nitrites in Water

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A method involving the use of a reagent suitable for the rapid spectrophotometric determination of nitrites in water is described. Nitrites react with resorcinol in an acidic medium and the nitroso product forms a pale yellow chelate with the zirconyl ion. The absorbance of the chelate is measured at 347 nm and the calculated molar absorptivity is $2.67 \times 10^4$ l mol$^{-1}$ cm$^{-1}$. The method is suitable for the determination of the nitrite ion in a 1.0-cm cell in the range from a few parts per hundred million to about 1 p.p.m. The relative standard deviation of the method is 1.5% at a concentration of 1 p.p.m. Some of the problematic interferents found in most of the analytical procedures for the determination of nitrites are tolerated in the present method. Features of the method include good selectivity and reproducibility, high stability of the coloured reaction product and simplicity.

*Keywords*: *Spectrophotometry*; *nitrite micro-determination*; *water*; *chelation*; *zirconyl ion*

Nitrite, when correlated with other nitrogen forms in water, can provide an index of organic pollution and oxides of nitrogen in the air are well known health hazards. General reviews dealing with the determination of nitrite can be found in the literature.[1–3] The most widely used and applied methods are those based on spectrophotometry, and they can be classified into four main groups: group 1, diazotisation of an aromatic amine and subsequent coupling to form an azo dye; group 2, oxidation of an organic molecule by nitrite; group 3, formation of free radical chromogens; and group 4, formation of nitroso compounds.

In the group 1 methods, a naphthalene derivative is usually used as the coupling agent. The original method of Griess[4] was modified several times; one of these modifications by Rider and Mellon[5] was adopted by the ASTM Committee and has became a standard procedure.[6] The procedures are generally sensitive, but are also subject to interference from various ions and require close control of the temperature. Most of the techniques have a relatively short period of colour stability. In addition, the reagent employed in the standard procedure [*N*-(1-naphthyl)ethylenediamine dihydrochloride] has been reported to be carcinogenic.[7] Methods in group 1 that involve other types of diazotising or coupling agents[8–10] also suffer the drawbacks of relatively rapid fading of colour and serious interferences, some known,[8] and some expected but not investigated.[9,10]

Methods in group 2 are non-selective and highly vulnerable to interference by oxidising and reducing agents.[11–13] While most of the methods in group 3 are very sensitive,[14] the reagents require tedious synthesis and a relatively long reaction time. Further, many inorganic oxidising agents produce the same type of spectra as nitrite. Methods in group 4 make use of, among other techniques, the nitrosation of *NN*-dimethylaniline[15] (a highly poisonous reagent) and a relatively non-specific nitrosation of 2,6-xylenol.[16]

The present paper describes a simple and rapid micro-scale method for the determination of nitrites. It is based on the formation of a chelate following a reaction with resorcinol and zirconyl ions in an acidic medium. The reagent was chosen following a preliminary investigation of phenols, naphthols and transition metals. The main advantages of this procedure are high selectivity, relatively low toxicity of the reagent, high stability of the coloured product, good sensitivity and reproducibility and ease of performance.

This method represents a novel way of determining nitrites. In principle, the reagent can be improved by use of other chelating agents.

## Experimental

### Apparatus

Spectral measurements were made with a Beckman, Model B, spectrophotometer equipped

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with 10- and 1-mm glass cells. The pH values were measured with a Radiometer Copenhagen PHM64 pH meter, equipped with a combined glass electrode.

## Reagents

All chemicals used were of analytical-reagent grade. The water was doubly distilled. Resorcinol and zirconyl chloride octahydrate were used with no additional purification.

*Reagent solutions.* Solution A: 1 g of resorcinol and 1.1 g of zirconyl chloride octahydrate are dissolved in doubly distilled water containing 7.5 ml of concentrated hydrochloric acid and the solution is diluted to 500 ml. Solution B: 1 g of sodium sulphate and 1.5 g of sodium acetate trihydrate are dissolved in 500 ml of doubly distilled water. On the day of sampling equal volumes of the two solutions are mixed. These solutions will remain stable for several weeks if stored in a refrigerator in a tightly stoppered amber-glass bottle.

*Nitrite standard solution.* A 0.5-g amount of sodium nitrite is placed in a beaker and dried to constant mass (for about 24 h) in a desiccator containing concentrated sulphuric acid. A stock solution is prepared by dissolving 0.3749 g of the dried sodium nitrite in 250 ml of doubly distilled water. This solution contains 1000 p.p.m. of nitrite ion. More dilute solutions are prepared daily by adding doubly distilled water to the stock solution. The stock solution is replaced every 2 weeks.

## Procedure

Pipette 2 ml of the sample containing nitrite in the range 0.3–5 p.p.m. into a 10-ml calibrated flask. Fill the flask to the mark with reagent solution and shake it thoroughly. After allowing 3 min for the reaction to take place, read the absorbance in a 1-cm cell at 347 nm against a similarly prepared blank. The concentration of nitrite can then be determined from a standard calibration graph, which is constructed by plotting known concentrations of nitrite against absorbance.

# Results and Discussion

## Spectral Studies

Fig. 1 shows the absorption spectrum of the coloured product at pH 1.5, the maximum absorption occurring at 347 nm. Slight absorption of the reagent blank at 347 nm indicated the need for all measurements to be made *versus* the blank. The magnitude of the blank absorbance was found to increase slightly with ageing of the reagent. The absorption of the reagent blank at 347 nm in a 1-cm cell, measured against water, was in the range 0.020–0.050, depending on the age of solution A.

Beer's law holds up to a concentration of 7 p.p.m. of nitrite, as was demonstrated by the calibration graph, which was a straight-line graph passing through the origin over this range. The coloured product developed in solutions containing up to 10 p.p.m. of nitrite could be

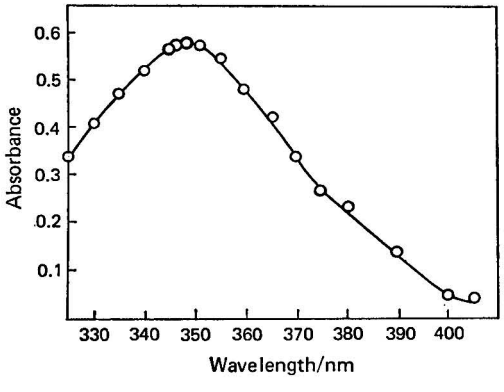



Fig. 1. Absorption spectrum of 1 p.p.m. of nitrite ion at pH 1.5 measured against reagent blank.

diluted with the reagent so as to fall within the linear range; in this event the absorbance of the samples still strictly followed the calibration graph.

The calculated molar absorptivity at 347 nm (pH 1.5) was $2.67 \times 10^4$ l mol$^{-1}$ cm$^{-1}$, and the sensitivity of the method (expressed as the amount of nitrite corresponding to an absorbance of 0.020) in a 1.0-cm cell at 347 nm was 0.035 p.p.m.

## Effect of pH

Aqueous solutions of zirconyl chloride behave like solutions of low aggregates of hydrated $ZrOOH^+$ and chloride ions. The pH is nearly the same as that of hydrochloric acid at the same molarity. The main process in the reaction sequence involving this method is the nitrosation of resorcinol, which must be carried out in an acidic medium. In order to establish the most desirable acidity, samples were studied spectrophotometrically at various pH values (Fig. 2). It was found that both the absorbance and the time required for colour development changed with the acidity. The pH range 1.3–2.4 is the optimum range from the point of view of intensity and reaction time; 2 min are required for development of full intensity. Below pH 1.3 the colour is developed almost instantly but the absorption intensity is slightly lower in comparison with the optimum range. Above pH 2.4 the rate of colour development slows, and up to pH 4 the maximum intensity is obtained within 20 min. Above this pH value, and up to 5.2, the intensity of the developed colour decreases gradually and about 24 h are needed for complete colour development. Beyond pH 5.5 formation of insoluble hydrated zirconium(IV) oxide interferes with the reaction. Changing the pH of the reagent solution with alkalis causes the formation of hydrated zirconium(IV) oxide from about pH 2.5. However, in our experiments the acidity was reduced without the formation of interference, up to a pH of 5.5, by the addition of sodium acetate. Variation of the pH did not influence the position of the absorption peak.

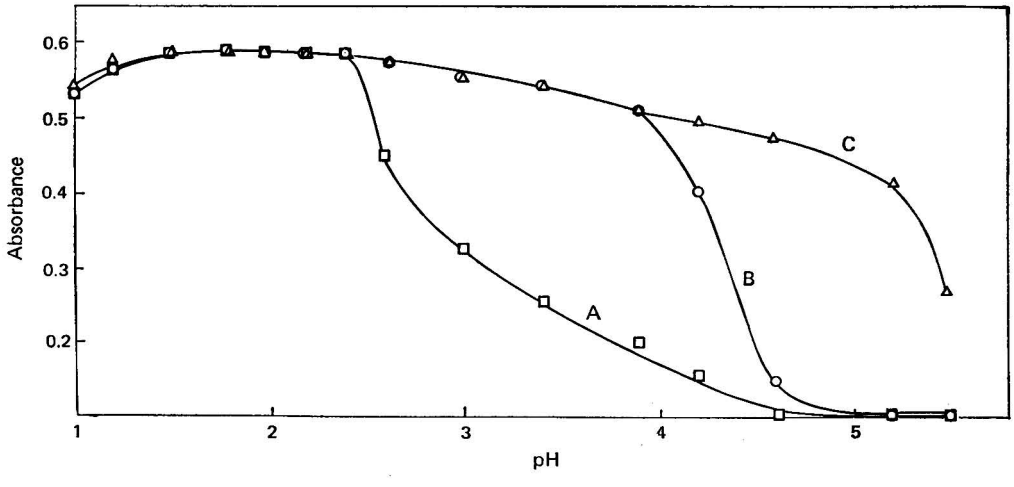



Fig. 2. Effect of pH on colour development, and the stability of the coloured product, measured at 347 nm. A, colour intensity after 3 min; B, colour intensity after 20 min; and C, colour intensity after 24 h.

## Optimum Range of Nitrite for Determination

The upper limit of nitrite concentration that can be determined by this method is fixed by the solubility of the coloured product. Using the analytical procedure recommended here, concentrations up to 15 p.p.m. of nitrite do not precipitate within 24 h. The most dependable determinations were carried out in the concentration range from a few parts per hundred million to 7 p.p.m. of nitrite ion in 1–10-mm cells.

### Effect of Reagent Concentration

A 2-ml volume of a solution containing 5 p.p.m. of nitrite ion was added to 8 ml of the recommended reagent in order to obtain the optimum results. A reagent with a five-fold excess of zirconyl chloride or resorcinol, or both, had no effect on the colour development. The role of sulphate ion is to increase the sensitivity of the reaction. A minimum amount (60 p.p.m.) of sulphate ion in the reagent solution causes an increase of 71% in the absorbance. A five-fold excess of sulphate concentration in the reagent solution does not increase the sensitivity. The presence of acetate prevents precipitation (probably of zirconatosulphates) in the reagent solution after it has stood for several hours at room temperature.

### Effect of Temperature

The effect of the temperature at which the determination was carried out was investigated. The colour development is virtually independent of temperature up to 50 °C (a high temperature for sub-tropical conditions) but the reagent turns turbid during heating. Up to 33 °C no interference occurs within 24 h. At 40 °C the reagent turns turbid within 2.5 h, while at 50 °C the turbidity occurs within 12 min. This interference is probably also caused by the formation of zirconatosulphates and the turbidity is prevented by the elimination of sulphate from the reagent solution.

### Reproducibility

A statistical study of ten independent determinations of solutions containing 1 p.p.m. of nitrite was carried out over a period of three consecutive days. The results are given in Table I.

TABLE I

REPRODUCIBILITY OF THE METHOD

Nitrate concentration, 1 p.p.m.

| Day | Absorbance value | Nitrite found, p.p.m. |
|---|---|---|
| 1 | 0.585 | 1.013 |
| | 0.570 | 0.987 |
| | 0.575 | 0.996 |
| 2 | 0.572 | 0.991 |
| | 0.580 | 1.005 |
| | 0.573 | 0.993 |
| 3 | 0.560 | 0.970 |
| | 0.590 | 1.022 |
| | 0.585 | 1.013 |
| | 0.580 | 1.005 |

| | |
|---|---|
| Mean absorbance .. .. | 0.577 |
| Standard deviation .. .. | $\pm 8.8 \times 10^{-3}$ |
| Relative standard deviation .. | $\pm 1.5\%$ |

### Colour Stability

The colour was found to be stable. Samples analysed after standing for 24 h at room temperature showed no loss of absorbance (Fig. 2).

### Interference Study

The effects of various potential interferences were investigated. The results of these experiments are shown in Table II.

Some of the problematic interferents expected in the azo dye methods, such as sulphite, copper(II), iron(II) or iodide, are tolerated in the proposed procedure.

TABLE II

EFFECT OF DIVERSE IONS

Nitrite concentration, 1 p.p.m.

| Ion investigated | Ratio of ion to nitrite (*m/m*) | Error, %* | Ion investigated | Ratio of ion to nitrite (*m/m*) | Error, %* |
|---|---|---|---|---|---|
| Acetate | 200 | n | Ammonium | 200 | n |
| Bromide | 200 | n | Cadmium | 200 | n |
| Carbonate | 200 | n | Calcium | 200 | n |
| Chlorate | 200 | −1.7 | Cerium(III) | 200 | n |
| Dichromate | 4 | +5.1 | Cobalt(II) | 200 | n |
| Fluoride | 50 | n | Copper(II) | 200 | −1.6 |
| Iodate | 200 | n† | Iron(II) | 200 | −2·3 |
| Iodide | 150 | −3.8 | Iron(III) | 8 | +5.1 |
| Manganate | 12 | +3.2 | Lead | 200 | n |
| Oxalate | 200 | n | Lithium | 200 | n |
| Perchlorate | 200 | +1.7 | Magnesium | 200 | n |
| Persulphate | 200 | −1.8 | Manganese(II) | 200 | n |
| Phosphate | 8 | +5.1 | Potassium | 200 | n |
| Sulphate | 200 | n | Sodium | 200 | n |
| Sulphite | 200 | −1.7 | Strontium | 200 | n |
| Aluminium | 200 | n | | | |

* n = negligible (less than the relative standard deviation).
† Colour turns brown - black after standing for 8 min at room temperature.

The interfering ions at pH 1.5 fall into two categories. The first group are ions that form, with the reagent, turbid products such as fluoride or phosphate. This interference is probably caused by the formation of a fluoride zirconate and diphosphatozirconic acid. The second group are ions that absorb light in the wavelength region of the reaction product, such as iron(III), dichromate and permanganate. These ions cause positive interference.

Among the ions that commonly occur in water samples, phosphate and iron(III) are the only ions that produce serious interference. However, as interference from iron(III) constitutes an inherent disadvantage its concentration must be reduced before analysis to within the tolerated concentration given in Table II. This treatment can be achieved by means of a cation-exchange resin (Amberlite IR-120), as described by Hutchinson and Boltz.[12]

The interference by phosphate ion can be prevented by the addition of suitable amounts of sulphuric acid to the mixed solutions A and B. This procedure is accompanied by a gradual decrease in absorbance as the amount of acid added is increased, as is shown in Table III.

TABLE III

ABSORBANCE OF 1 p.p.m. NITRITE SOLUTION CONTAINING PHOSPHATE AS INTERFERENT

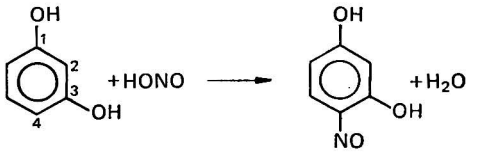
| Concentration of phosphate, p.p.m. | Volume of concentrated sulphuric acid/ml $l^{-1}$ | | |
|---|---|---|---|
| | 5 | 10 | 20 |
| 100 | Turbid | Turbid | 0.110 |
| 44 | Turbid | 0.26 | — |
| 21 | 0.480 | — | — |

Metals that are precipitated as chlorides, such as silver, are also tolerated in this method. In this instance, zirconyl nitrate must be used instead of zirconyl chloride.

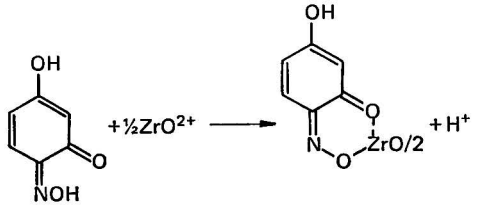
## Proposed Reaction Mechanism

The reaction sequence described in the procedure involves two steps. The first step consists in an electrophilic attack by nitrous acid on an activated aromatic ring. In this instance, position 4 in the resorcinol ring is the one most activated,[17] as a result of the presence

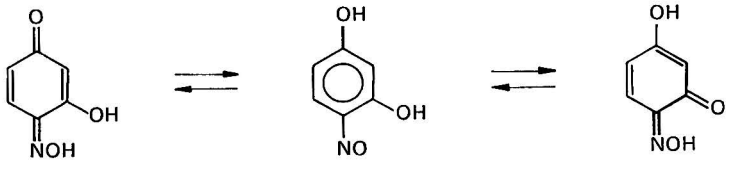
of hydroxyl groups in the 1- and 3-positions. In the presence of micro-concentrations of nitrites, resorcinol is presumed to react with nitrous acid as follows:




Nitrosation of position 2 and dinitrosation of the ring occur at higher concentrations of nitrite. The nitroso product exists in equilibrium with its tautomeric quinoidal oximes, which include a double-bond conjugated system, *i.e.*,




The presence of zirconyl ions in the second step causes complete displacement of the equilibrium towards the 1-hydroxy quinoidal oxime, owing to the rapid formation of the coloured chelate,[18] as follows:




This method, with some modifications, may also be suitable for the determination of nitrogen dioxide in air. An appropriate procedure for such a determination is currently in preparation.

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# Determination of Residues of Substituted Phenylurea Herbicides in Grain, Soil and River Water by Use of Liquid Chromatography

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A rapid and sensitive procedure is described for the determination of trace amounts of eight substituted phenylurea herbicides. High-performance liquid chromatography is used, with an ultraviolet spectrophotometric detector. The herbicides are extracted from grain and soil samples with methanol and from water samples with dichloromethane. They are chromatographed on microparticulate silica bonded with octadecyltrichlorosilane using a mixture of methanol, water and ammonia as mobile phase.

*Keywords*: *Herbicide residues determination*; *phenylurea herbicides*; *high-performance liquid chromatography*

Substituted phenylurea compounds (urons) are widely used as selective herbicides in agriculture[1] and consequently can give rise to residues in crops. In addition, some herbicide can be absorbed by the soil and can be leached out into river water. Methods for the determination of residues of phenylurea herbicides have been based on gas - liquid chromatographic or colorimetric procedures.[2] Gas - liquid chromatography of phenylurea herbicides is difficult because of their ease of thermal decomposition. Procedures have been reported in which careful control of conditions allows these compounds to be chromatographed intact.[3,4] Alternatively, the urons can be hydrolysed to the corresponding substituted anilines; these compounds are then determined by either gas - liquid chromatography, directly[5] or as derivatives,[6] or colorimetrically[7] after coupling with a suitable chromophore. Methods based on hydrolysis lack specificity and involve lengthy procedures.

These disadvantages can be overcome by using liquid chromatography. Following the earlier work of Kirkland[8] on phenylurea herbicides, Sidwell and Ruzicka[9] applied liquid chromatography to the identification and determination of active ingredient contents of phenylurea herbicide formulations. Smith and Lord[10] have used liquid chromatography for the determination of chlortoluron residues in soil, but diuron and monuron interfered in their chromatographic system. It was thought that the technique of Sidwell and Ruzicka could form the basis of a method for routine monitoring. By modification of the mobile phase a technique has been devised for the direct determination of residues of chlorbromuron, chlortoluron, chloroxuron, diuron, linuron, metobromuron, monolinuron and monuron in grain, soil and river water.

## Experimental

### Apparatus

*Liquid chromatograph.* A Waters Associates constant-volume solvent-delivery system, Model 6000, was used. A variable-wavelength ultraviolet monitor (Cecil Instruments, Model CE 212), fitted with a 10-$\mu$l flow cell and set at 240 nm was used as a detector.

*Preparation of column.* A stainless-steel column tube, 300 × 4.6 mm i.d., was washed with chloroform and methanol and polished on the inner surface. One end was fitted with a $\frac{1}{4} \times \frac{1}{16}$ in Crawford Patent column end fitting and the other was coupled to a 400-mm pre-column reservoir through a $\frac{1}{4} \times \frac{1}{4}$ in Swagelock union. A slurry of 5-$\mu$m Spherisorb ODS (Phase Separations Ltd.) was packed into the column by releasing 5 000 lb in$^{-2}$ of solvent (acetone) pressure. The column was prepared for stop-flow injection by removing the top few milli-

metres of packing and inserting a disc of stainless-steel fine-mesh gauze, of 8-μm nominal porosity, a plug of silanised glass-wool and a top plug of porous PTFE.

*Sample injection.* A Varian Associates stop-flow injector was used and samples were injected on to the stainless-steel fine-mesh gauze fitted on top of the packing. A needle guide was incorporated in the injector to ensure that samples were introduced on to the centre of the top of the column.

*Rotary evaporator.* This is used with a water-bath that can be maintained at 55 °C.

## Reagents

All reagents should be of analytical-reagent grade unless otherwise specified.

*Dichloromethane.* Laboratory-reagent grade.

*Methanol.*

*Methanol.* Spectrograde (Fisons).

*Mobile phase.* Prepare a solution of 60% methanol in water and add 0.6% *V*/*V* of ammonia solution (sp. gr. 0.88).

*Sodium sulphate, anhydrous, granular.*

*Uron standard solutions.* Prepare a standard solution in Spectrograde methanol containing 0.25 mg $l^{-1}$ of phenylurea herbicide; dilute as necessary.

## Procedure

*Grain*

Grind a 50-g sample and transfer it into a 500-ml flat-bottomed flask. Add 100 ml of methanol and shake on a wrist-action shaker for 1 h. Filter the resulting slurry through a Whatman No. 1 filter-paper using reduced pressure. Wash the flat-bottomed flask with 50 ml of methanol and add the washings to the filter-funnel, leave for 3–5 min, then apply the reduced pressure. Repeat the washing procedure with a further 50 ml of methanol. Combine the extract and washings and then remove the methanol using a rotary evaporator with a water-bath at 55 °C. Dissolve the residue in dichloromethane, using a total volume of 50 ml, and pass the dichloromethane extracts through a column of anhydrous sodium sulphate (50 g). Wash the sodium sulphate with 50 ml of dichloromethane, combine the extract and washings and evaporate to dryness at 55 °C in a rotary evaporator. Cool the flask and add 5.0 ml of methanol; swirl the flask to dissolve the residue and filter the solution through a Whatman No. 42 filter-paper. Using a flow-rate of the mobile phase of 0.6 ml $min^{-1}$, inject 5 μl of the sample solution into the liquid chromatograph. Calculate the uron content of the sample by comparing the peak height obtained with those obtained from 5-μl injections of standard solutions.

*Soil*

Air-dry a sample of soil and transfer 50 g into a 500-ml flat-bottomed flask. Extract and filter the sample using the method described under *Grain*. Remove the methanol by using a rotary evaporator with a water-bath at 55 °C. Cool the flask and add 5.0 ml of methanol, swirl to dissolve the residue and filter the solution through a Whatman No. 42 filter-paper. Using a flow-rate of 0.6 ml $min^{-1}$, inject 5 μl of extract into the liquid chromatograph and determine the uron content using the procedure described under *Grain*.

*Water*

Add 100 ml of dichloromethane to 1 l of river water in a 2-l separating funnel and shake for 30 s. Run off the dichloromethane and repeat the extraction twice, using 50-ml portions of dichloromethane. Dry the extracts by passing them through a column of anhydrous sodium sulphate (50 g) and wash the column with 50 ml of dichloromethane. Combine the extracts and washings and remove the dichloromethane in a rotary evaporator with a water-bath at 55 °C. Cool the flask and add 5.0 ml of methanol, swirl the flask to dissolve the residue and inject 5 μl of the solution into the chromatograph. Determine the uron content of the sample using the procedure described under *Grain*.

## Results and Discussion

Methanol was chosen as the extraction solvent for wheat and soil because Khan *et al.*[4] found it to be the best solvent for extraction of urons from soil. Variable recoveries were obtained

from wheat samples when a Soxhlet extraction apparatus was used and this effect was attributed to breakdown of some of the uron herbicides by prolonged heating with methanol.[11] However, satisfactory recoveries were achieved consistently by using a wrist-action shaker. An extraction time of 1 h was chosen as this time has been reported as the optimum for soil.[4] Care was taken during the evaporation of solutions of urons in methanol to ensure that the temperature did not rise above 55 °C as it has been reported that at higher temperatures degradation occurs.[10]

## TABLE I

### RECOVERY OF URONS FROM FORTIFIED SAMPLES

Five determinations were carried out on each sample. Results given are percentage recoveries.

| | Wheat | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Fortified at 5 mg kg$^{-1}$ | | Fortified at 2 mg kg$^{-1}$ | | Fortified at 0.5 mg kg$^{-1}$ | | Soil, fortified at 2 mg kg$^{-1}$ | | Water, fortified at 0.1 mg kg$^{-1}$ | |
| Uron | Range | Mean | Range | Mean | Range | Mean | Range | Mean | Range | Mean |
| Chlorbromuron .. .. | 91.0–95.5 | 92.5 | 87.5–93.0 | 91.0 | 84.5–92.5 | 88.0 | 98.0–101.0 | 99.5 | 98.0–100.0 | 99.0 |
| Chlortoluron .. .. | 89.5–94.0 | 92.0 | 87.0–92.5 | 89.5 | 85.5–94.0 | 89.0 | 100.0–103.0 | 101.5 | 97.0–100.5 | 99.0 |
| Chloroxuron .. .. | 87.5–91.5 | 89.5 | 87.0–91.0 | 88.5 | 86.5–94.0 | 89.5 | 97.0–103.0 | 99.0 | 94.5–100.5 | 97.0 |
| Diuron .. .. .. | 89.0–94.5 | 91.5 | 88.0–90.5 | 89.0 | 87.0–92.5 | 89.0 | 98.0–100.5 | 99.5 | 97.5–102.0 | 100.0 |
| Linuron .. .. | 90.5–94.5 | 92.5 | 86.5–93.5 | 89.0 | 85.5–96.0 | 91.5 | 94.0–100.5 | 97.5 | 96.0–100.5 | 98.5 |
| Metobromuron .. .. | 90.5–95.5 | 92.0 | 89.5–93.5 | 91.5 | 84.0–93.0 | 86.5 | 96.5–101.0 | 99.5 | 96.5–100.5 | 99.0 |
| Monolinuron .. .. | 90.0–95.5 | 92.5 | 89.0–94.0 | 92.5 | 87.0–95.5 | 90.0 | 100.5–103.0 | 102.0 | 97.5–100.0 | 98.5 |
| Monuron .. .. | 87.0–94.5 | 90.0 | 91.0–97.0 | 94.5 | 89.0–95.5 | 92.5 | 99.0–102.0 | 100.5 | 98.0–100.5 | 99.5 |

The eluting agent originally used for liquid chromatography was 60% methanol in water, as recommended by Sidwell and Ruzicka.[9] This solvent gave adequate resolution of all eight urons but a clean-up procedure, based on that of Onley and Yip,[12] was required in order to remove interfering co-extractives. Even with the clean-up, sample extracts of wheat injected on to the chromatograph showed an interfering co-extractive peak that had the same retention

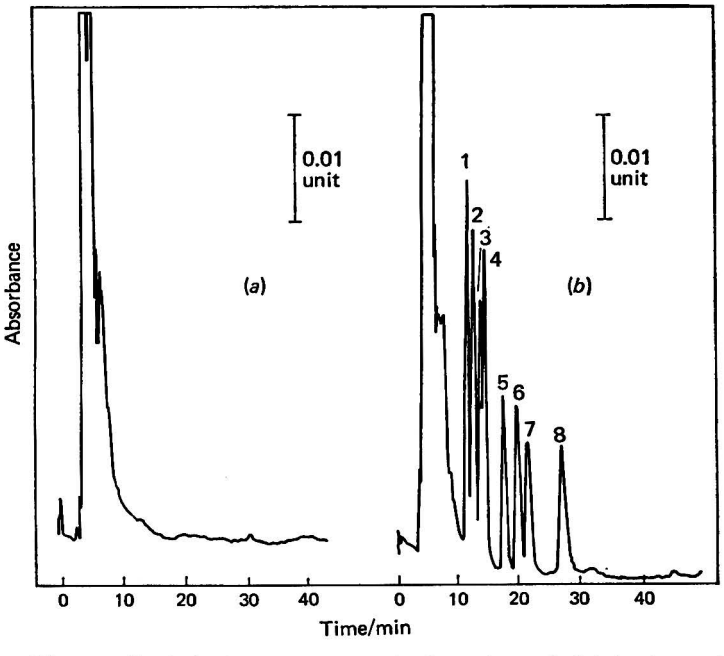


Fig. 1. Typical chromatograms obtained from 5-μl injections of wheat extracts: (*a*) unfortified; and (*b*) fortified with uron herbicides at 2 mg kg$^{-1}$. 1, Monuron; 2, monolinuron; 3, metobromuron; 4, chlortoluron; 5, diuron; 6, linuron; 7, chlorbromuron; and 8, chloroxuron.

time as chloroxuron. It was then found that the incorporation of a small amount of ammonia in the eluting agent solved the problem of co-extractive interference by moving the interfering peaks to a smaller retention volume while leaving the resolution of the urons unaltered. This

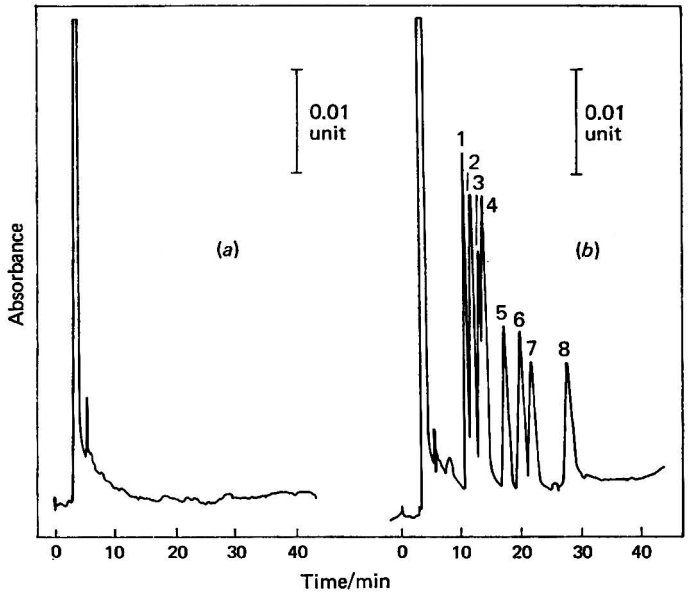


Fig. 2. Typical chromatograms obtained from 5-μl injections of soil extracts: (*a*) unfortified; and (*b*) fortified with uron herbicides at 2 mg kg$^{-1}$. 1, Monuron; 2, monolinuron; 3, metobromuron; 4, chlortoluron; 5, diuron; 6, linuron; 7, chlorbromuron; and 8, chloroxuron.

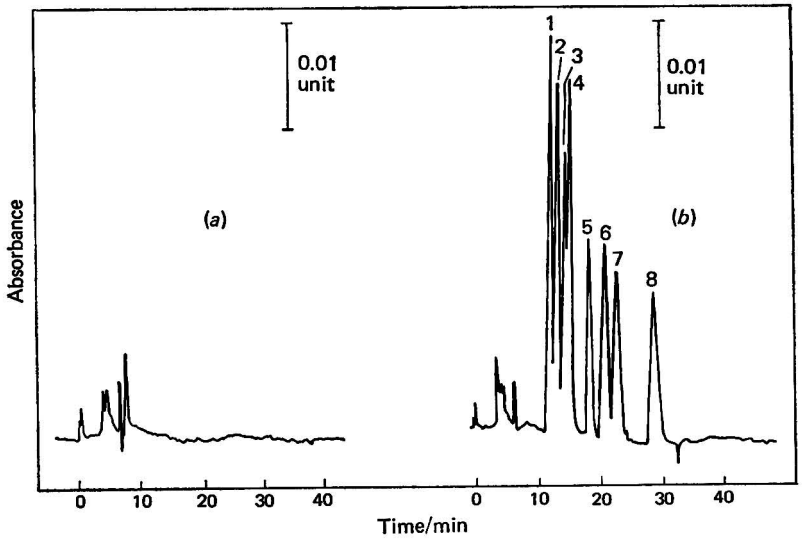


Fig. 3. Typical chromatograms obtained from 5-μl injections of river water extracts: (*a*) unfortified; and (*b*) fortified with uron herbicides at 0.1 mg kg$^{-1}$. 1, Monuron; 2, monolinuron; 3, metobromuron; 4, chlortoluron; 5, diuron; 6, linuron; 7, chlorbromuron; and 8, chloroxuron.

modification was subsequently applied to samples that had not been subjected to a clean-up procedure and it was found that satisfactory chromatographic traces could be obtained for all of the substrates, and thus the method could be simplified by incorporation of ammonia into the eluting agent. However, as the wheat extracts are oily, it is advisable to flush the column periodically with Spectrograde methanol to wash off any oils not eluted by the mobile phase.

The recoveries obtained for urons from samples of wheat, soil and water are shown in Table I. Samples of wheat and soil were fortified by adding known volumes of solutions containing (*a*) monuron, metobromuron, diuron and chlorbromuron or (*b*) monolinuron, chlortoluron, linuron and chloroxuron. The lower recoveries from wheat may be attributable to residual oil, which remains after the evaporation of the dichloromethane, increasing the volume of the solvent as this oil is miscible with methanol.

Typical chromatograms, obtained from extracts of wheat, soil and river water, are shown in Figs. 1–3. The lower limits of detection were estimated to be 0.2 p.p.m. for wheat, 0.2 p.p.m. for soil and 0.01 p.p.m. for river water; below these levels both co-extractives and signal noise interfere.

The authors thank the Government Chemist for permission to publish this paper.

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# Determination of Nanogram and Sub-nanogram Amounts of Europium and Cerium by Means of Candoluminescence Emission in the Presence of a Co-activator*

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The candoluminescence emissions of cerium and europium from a calcium oxide - calcium sulphate matrix are greatly enhanced if mineral acids are added with the activator solution to the matrix, before it is placed in a nitrogen - hydrogen - air flame. If sulphuric acid is used, 0.01–15 ng of cerium and europium in 1 μl of solution can be determined. Some metal ions interfere and an attempt is made to explain the interference mechanisms.

*Keywords*: *Europium determination*; *cerium determination*; *candoluminescence emission*

A study of the candoluminescence of trace amounts of lanthanoid elements in a calcium oxide-based matrix was reported in an earlier paper.[1] Terbium, praseodymium and europium were found to give intense emissions and cerium a much weaker emission. Other lanthanoids gave no luminescence when present in very small amounts. It was also briefly mentioned that sulphuric acid enhanced the candoluminescence intensity of europium and cerium(III). This paper describes a detailed investigation of these two activators in the absence and presence of co-activators, especially sulphuric acid, resulting in the development of more sensitive procedures for the determination of europium and cerium than were reported previously.[1]

## Experimental and Results

The Unicam SP900 flame spectrophotometer, burner, recorder and matrix holder were as described previously.[2] The band passes were 13.8 nm at 600 nm and 11.5 nm at 560 nm for europium and cerium, respectively. The matrix was also prepared and inlaid into the matrix holders as described previously.[2] All reagents used were of analytical-reagent grade, except for europium nitrate and cerium(III) nitrate, which were 99.9% (Koch-Light Laboratories Ltd.).

### Preparation of Stock Solutions

Europium and cerium solutions of concentration 1 000 μg ml$^{-1}$ were prepared by dissolving 0.2810 and 0.3095 g of $Ce(NO_3)_3.6H_2O$ and $Eu(NO_3)_3.5H_2O$, respectively, in doubly glass-distilled water and diluting to 100 ml with water. Calibration solutions were then prepared by serial dilution of the stock solution with water.

### Measurement of Candoluminescence Emission

A 1-μl volume of a test, blank or calibration solution was injected on to the centre of the matrix surface and the matrix holder was inserted into the sample holder and introduced into the flame. The change in emission intensity with time at 560 nm (cerium) or 600 nm (europium) was recorded, and the maximum candoluminescence intensity measured. The emission intensity was measured either after the initial introduction of the matrix into the flame or after thermal pre-treatment of the matrix in the flame (see below). The latter involved optimally heating the matrix in the flame for 20 s (cerium) or 40 s (europium), removing it from the flame to cool for 60 s (cerium) or 25 s (europium), then re-inserting it into the flame for candoluminescence measurement.

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## Calibration and Determination of Europium or Cerium (0.01–15 ng)

For calibration purposes, a series of standard solutions containing 0–0.2, 0–2 or 0–15 $\mu$g ml$^{-1}$ of europium or cerium were prepared by suitable dilution of the stock solution to volume in 10-ml flasks. During the dilution, 1.5 ml of concentrated sulphuric acid was added to each europium standard and 1.0 ml to each cerium standard. The candoluminescence of each solution was measured as described above, and the blank response from a solution containing no added activator was subtracted from each measurement. A graph of peak intensity *versus* amount of europium or cerium was plotted. Europium or cerium was determined by treating the unknown solutions in the same way.

## Optimisation of Flame Composition and Matrix Position in the Flame

In order to obtain the greatest emission intensity, the variation in luminescence with flame composition and matrix position was measured for cerium and europium, in the presence of sulphuric acid as co-activator. A comparison was also made with the situation when no co-activator was added. The flow-rates of hydrogen and air (Fig. 1) were fairly critical for both elements in the presence of sulphuric acid and for non-co-activated europium. Increasing the nitrogen flow-rate increased the intensity for both co-activated elements and also for europium in the absence of co-activator (Fig. 1). On the basis of these results, the optimum flow-rates were considered to be as follows: for cerium, nitrogen = 8.0, hydrogen = 2.0 and air = 2.0 l min$^{-1}$; and for europium, nitrogen = 8.0, hydrogen = 1.75 and air = 1.75 l min$^{-1}$ (with no co-activator, 2.0 l min$^{-1}$).

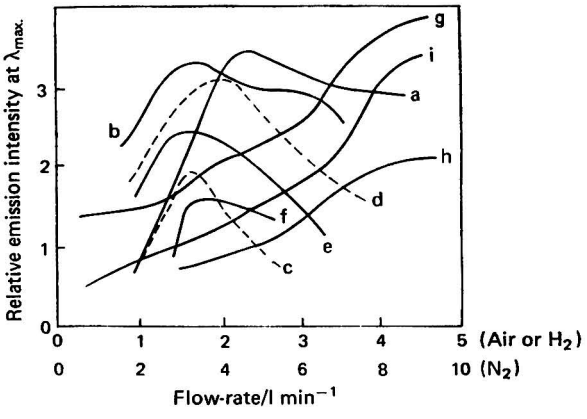


Fig. 1. Effect of flame gas flow-rates on candoluminescence intensity of 2 ng of cerium (560 nm, with sulphuric acid) and 5 ng of europium (600 nm with sulphuric acid, 585 nm without). Effects of hydrogen flow-rate on (a) Ce; (b) Eu + $H_2SO_4$ (both at $N_2$ = 7.0 and air = 2.0 l min$^{-1}$); and (c) Eu alone ($N_2$ = 8.0, air = 2.0 l min$^{-1}$). Effects of air flow-rate on (d) Ce ($N_2$ = 7.0, $H_2$ = 2.0 l min$^{-1}$); (e) Eu + $H_2SO_4$ ($N_2$ = 8.0, $H_2$ = 1.75 l min$^{-1}$); and (f) Eu alone ($N_2$ = 8.0, $H_2$ = 2.0 l min$^{-1}$). Effects of nitrogen flow-rate on (g) Ce ($H_2$ = 2.0, air = 1.75 l min$^{-1}$); (h) Eu + $H_2SO_4$ ($H_2$ and air = 2.0 l min$^{-1}$); and (i) Eu alone ($H_2$ and air = 1.75 l min$^{-1}$).

The effect of distance between matrix and burner top is shown in Fig. 2. It shows that co-activated europium reached maximum intensity higher in the flame than cerium although the cerium candoluminescence was measured in a slightly greater total gas flow. For non-co-activated europium, the distance was less than that found when the co-activator was present. The effect of horizontal distance into the flame was also measured. The optimum distances into the flame from the edge were 5 mm for europium and 8 mm for cerium. When no co-activator was present, the optimum position for europium was at the edge of the flame.

## Spectral Measurements

The spectra of sulphuric acid-co-activated cerium and europium candoluminescence were measured, as was that for europium when no co-activator was added. The spectra are shown in Fig. 3 and show that, in the presence of sulphuric acid, the peak of maximum candoluminescence intensity for europium shifts from 585 to 600 nm. It was also found that the cerium peak shifts from 570 to 560 nm. The measurement of emission intensity was made after thermal pre-treatment (see below) for both cerium and europium. The thermal pre-treatment was found not to change the wavelength of maximum intensity.

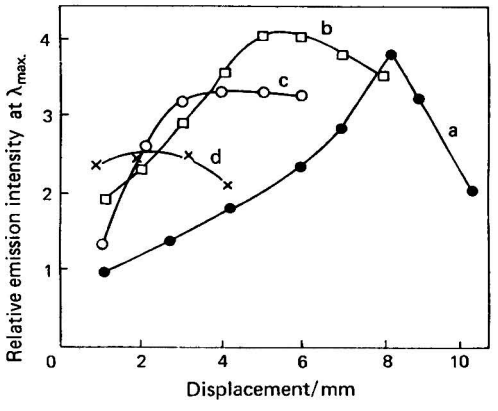


Fig. 2. Effect of matrix position on peak candoluminescence intensity of sulphuric acid-co-activated cerium (2 ng) and europium (5 ng) under recommended flame conditions. Effect of horizontal distance (mm) into flame from edge for (a) Ce (height in flame = 8 mm) and (b) Eu (height = 5 mm); effect of height in flame above burner top for (c) Ce and (d) Eu (both 5 mm into flame).

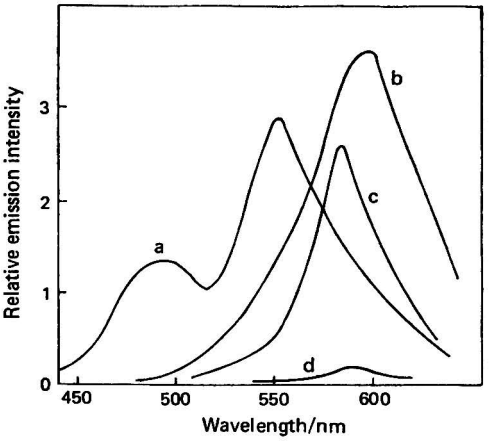


Fig. 3. Candoluminescence spectra of (a) Ce + $H_2SO_4$; (b) Eu + $H_2SO_4$; (c) Eu alone; and (d) matrix alone.

## Repeated Emissions

Two types of candoluminescence activators have been observed. The first is typified by lead in a calcium oxide-based matrix[2] and indium in a magnesium oxide-based matrix.[3] The emissions cannot be repeated when the matrix is removed from and later returned to the flame. The second type, which is more common, includes bismuth,[2] manganese,[4] europium, praseodymium and terbium[1] in a calcium oxide-based matrix. Their emission can be repeatedly stimulated, sometimes with increasing intensity, on repeated introduction of the matrix into the flame.

Sulphuric acid-co-activated cerium and europium candoluminescences are both of the second type. They display enhanced emission if the matrix is first heated in the flame and then cooled, before measuring the candoluminescence on subsequent introduction into the flame. This effect was examined by pre-heating the matrix at a constant position in the flame for different times. The results (Fig. 4) indicated that heating for 40 and 20 s gave the most enhanced intensities for europium and cerium, respectively; brief pre-heating gave much decreased intensities. It was also found that the cooling time before re-inserting the matrix into the flame influenced the candoluminescence intensity. Fig. 5 shows that cooling for 25–50 s gave the most increased intensity for europium while 40 s gave the most increased intensity for cerium, in the presence of sulphuric acid. Longer cooling of cerium-containing matrices had little additional effect on the intensity, but longer cooling of europium caused a decrease in the intensity. Fig. 5 also shows the effect of cooling the non-co-activated europium matrix; there is much less enhancement on cooling in this instance. Repeated introductions of the same matrix into the flame, after the optimum pre-heating period, gave the same emis-

sion intensity in each instance. Such re-introductions were used for measuring the spectra and the effect of flame conditions described above.

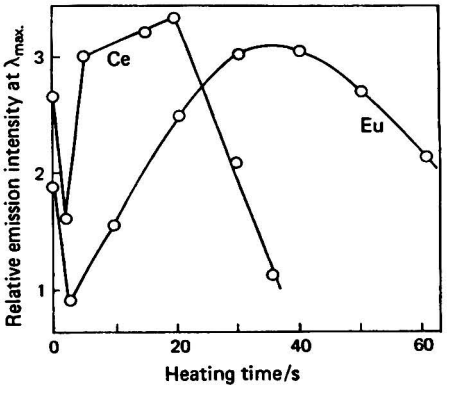


Fig. 4. Effect of pre-heating time on emissions from sulphuric acid co-activated europium (5 ng) and cerium (2 ng) (cooling times 25 and 60 s, respectively).

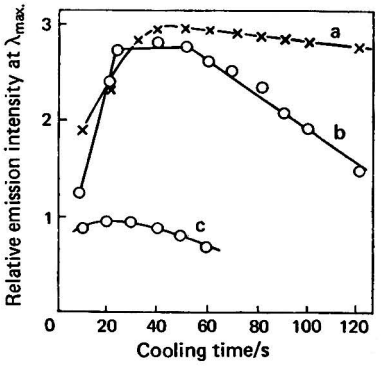


Fig. 5. Effect of cooling time after pre-heating on emissions from (a) Ce (2 ng; 20-s pre-heating) + $H_2SO_4$; (b) Eu (5 ng; 40-s pre-heating) + $H_2SO_4$; and (c) Eu (5 ng) alone.

The enhanced emissions obtained after thermal pre-treatment could have arisen either from an improved mutual interaction between activator and matrix, or from a change in physical properties of the matrix itself, thus enabling it to interact more strongly with an activator when it was added. To test this latter possibility, the matrix was pre-heated before adding cerium as activator. The results (Table I) show that an appreciable enhancement is again obtained, indicating that the enhancement arises from changes in the properties of the matrix itself on thermal pre-treatment.

TABLE I

EFFECT OF HEATING MATRIX IN FLAME FOR 20 S BEFORE ADDITION OF CERIUM - SULPHURIC ACID SOLUTION (1 $\mu$l)

| | Emission intensity (arbitrary units) | |
|---|---|---|
| Amount of cerium/ng | No pre-heating | Pre-heating |
| 0.1 | 6 | 20 |
| 0.2 | 17 | 41 |
| 0.3 | 22 | 54 |
| 0.4 | 39 | 68 |

## Effect of Co-activator

A co-activator is a species which, if also present in the matrix, enhances the emission of the activator. The co-activator effect of sulphuric acid had originally been attributed to the sulphate ion.[5] However, sodium sulphate gave no enhancement, so it was postulated that the hydrogen ion was the co-activator. This postulation was confirmed by adding other acids (perchloric, nitric, hydrochloric, hydrobromic and hydriodic acid) to the activator solution, all of which enhanced the emission intensities for both cerium and europium. The effect of various concentrations of acids on the candoluminescence intensity of europium and cerium was measured using the optimum conditions established for sulphuric acid as co-activator but without thermal pre-treatment and the results are shown in Figs. 6 and 7.

The acids also stimulated a weak yellow emission when no activator was added to the matrix. This was considered to be a blank emission and was subtracted from measurements made in the presence of an activator. Table II shows that perchloric, hydriodic, hydrochloric and hydrobromic acid gave very weak yellow emissions; that from sulphuric acid was negligible, as were those from orthophosphoric, formic and acetic acid. The spectrum of the yellow emission was similar to that of manganese candoluminescence ($\lambda_{max.} = 580$ nm).[4] It is suggested that it arises from the co-activation of manganese present as an impurity in the

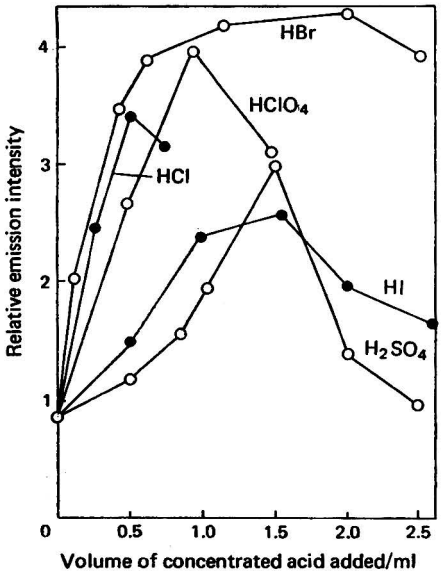


Fig. 6. Effect of acids on the peak candoluminescence intensity of europium (10 ng); no pre-heating.

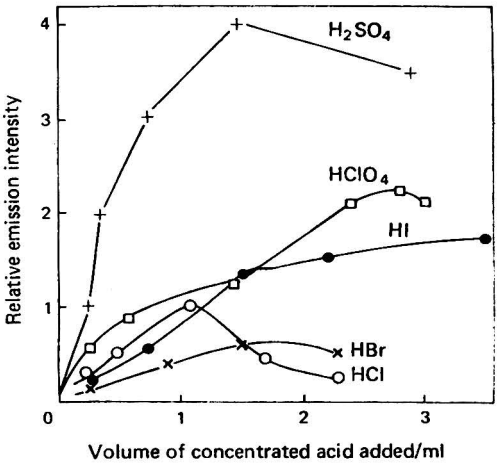


Fig. 7. Effect of acids on the peak candoluminescence intensity of cerium (10 ng); no pre-heating.

matrix. This effect has been shown to be a limiting factor in the determination of sub-nanogram amounts of manganese by candoluminescence. This suggestion is also supported by the fact that 2 N hydrochloric acid was found to be a more effective co-activator for manganese candoluminescence than 2 N sulphuric acid,[4] just as it is more effective in producing the blank emission.

TABLE II

BLANK EMISSION INTENSITY (CO-ACTIVATOR ONLY ADDED) COMPARED WITH THAT GIVEN BY ADDED CERIUM OR EUROPIUM (BOTH 10 ng), WITHOUT THERMAL PRE-TREATMENT

| Co-activator (2 N) | Emission intensity (arbitrary units) | | | |
|---|---|---|---|---|
| | At 560 nm | | At 600 nm | |
| | Blank | Ce | Blank | Eu |
| HI .. .. .. | 3 | 61 | 2 | 61 |
| HBr .. .. | — | — | 3 | 90 |
| $HClO_4$ .. .. | 4 | 66 | 6 | 81 |
| HCl .. .. | 2 | 84 | 5 | 36 |
| $H_2SO_4$ .. .. | 0 | 85 | 0 | 58 |
| $H_3PO_4$ .. .. | 0 | 4 | 0 | 7 |
| HCOOH .. .. | 0 | 1 | 0 | 6 |
| $CH_3COOH$.. .. | 0 | 1 | 0 | 6 |

After subtraction of the blank, the orders of enhancing powers were found to be $H_2SO_4 = HCl > HClO_4 \geqslant HI \gg H_3PO_4$, HCOOH, $CH_3COOH$ for cerium and $HBr > HClO_4 > HI \gg H_2SO_4 > HCl \gg H_3PO_4$, HCOOH, $CH_3COOH$ for europium. Sulphuric acid was thus selected as the most generally useful co-activator. Concentrations of 2.7 and 1.8 M sulphuric acid for europium and cerium, respectively, were used in all further studies. It was also noted that the emission from matrices co-activated with the organic acids decreased on repeated introductions into the flame, presumably as the matrix reverted to the oxide.

## Determination of Cerium and Europium

Using the optimum conditions, sulphuric acid-co-activated europium and cerium gave linear calibration graphs over the range 0.01–15 ng. A given instrumental sensitivity setting could

accommodate readily the ranges 1–10 ng (Fig. 8), 10–100 pg (Fig. 9) or 0.1–1.0 ng. Fig. 8 also emphasises the enhancement achieved by co-activation and by the pre-heating and cooling process.

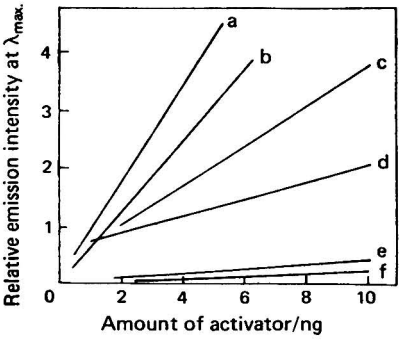


Fig. 8. Calibration graphs for Ce + $H_2SO_4$ (a) with pre-heating and (b) without pre-heating; for Eu + $H_2SO_4$ (c) with pre-heating and (d) without pre-heating; and for Eu alone (e) with pre-heating and (f) without pre-heating.

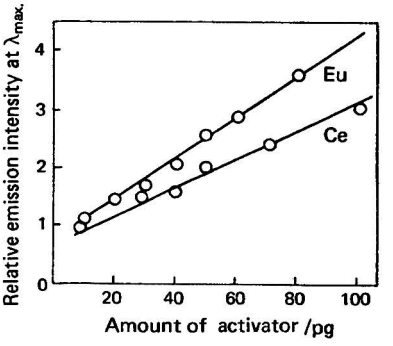


Fig. 9. Calibration graphs for sulphuric acid-co-activated cerium and europium in the 10–100-pg range.

The coefficient of variation for the determination of 5 ng of europium in a given solution was 5% (10 determinations), while for 3 ng of cerium it was 4% (10 determinations).

*Detectability*

The smallest amounts of cerium and europium that could visually be detected were found to be $10^{-12}$ and $5 \times 10^{-12}$ g, respectively, when sulphuric acid was also present in the matrix.

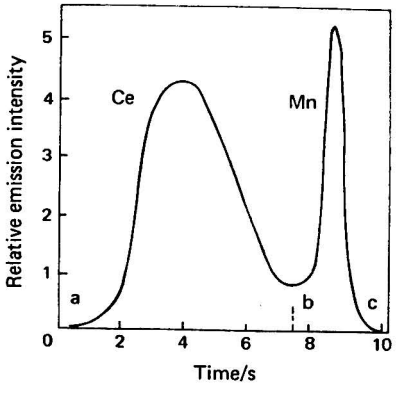


Fig. 10. Candoluminescence signals from a mixture of cerium (1 ng) and manganese (4 ng) in the presence of sulphuric acid. a–b, 550 nm; b–c, 568 nm.

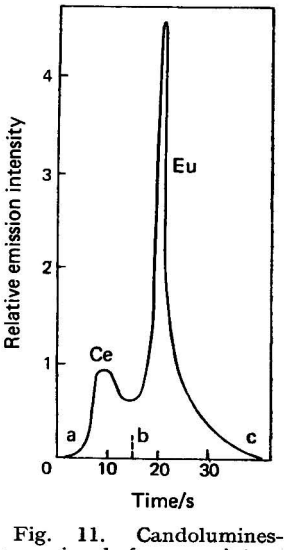


Fig. 11. Candoluminescence signals from a mixture of cerium (0.1 ng) and europium (0.1 ng) in the presence of sulphuric acid. a–b, 550 nm; b–c, 550–600 nm; scanned at the rate of 25 nm in 7 s.

When no sulphuric acid was present, the limit of visual detection for europium was $5 \times 10^{-8}$ g while that for cerium was $10^{-6}$ g.

*Interference*

Cerium,[1] europium,[1] terbium[1] and manganese[4,6] are the only metal ions that give candoluminescence emission at 600 nm in a calcium oxide matrix. By positioning the matrix in the middle of the flame, europium and cerium emit strongly, but manganese and terbium give only weak emission as their strongest emission is given at the edge of the flame.

The candoluminescence of terbium or cerium appears almost immediately the matrix is introduced into the flame (low-temperature activator), whereas that of europium or manganese appears only after about 20 or 10 s, respectively (high-temperature activator), at which time the emission of cerium or terbium has substantially diminished. The successive emissions of cerium and manganese and of cerium and europium are illustrated in Figs. 10 and 11. There is no spectral interference on cerium emission, but the manganese and cerium peaks overlap with that of europium.

As has been found for other candoluminescence activators, numerous ions, whether or not they give candoluminescence, depress or enhance the candoluminescence intensity of europium and cerium. These effects are summarised in Table III, together with the maximum amounts of these ions that can be tolerated. The results show that the determination of europium is unaffected by a large excess of many common ions, and of some lanthanoid ions, such as lanthanum, terbium, erbium, dysprosium and yttrium. Smaller amounts of gadolinium can also be tolerated. The determination of cerium is more susceptible to interference although at least a 50-fold excess of lanthanum, terbium, erbium and dysprosium can be tolerated; bismuth, cobalt, iron(III), chromium(III), praseodymium, arsenite and selenate ions cannot be tolerated at more than a 5-fold excess.

TABLE III

INTERFERENCE EFFECTS AND MAXIMUM TOLERABLE MASS PROPORTIONS OF VARIOUS IONS IN THE DETERMINATION OF CERIUM (2 ng) AND EUROPIUM (5 ng)

| Ion | Maximum tolerable amount* Eu | Ce | Ion | Maximum tolerable amount* Eu | Ce |
|---|---|---|---|---|---|
| $K^+$ | 100× (+) | 100× (+) | $Pd^{2+}$ | 10× (+) | 40× (+) |
| $NH_4^+$ | 100× (+) | 100× (+) | $As^{3+}$ | 10× (+) | 5× (+) |
| $Al^{3+}$ | 50× (+) | 10× (−) | $Bi^{3+}$ | 10× (−) | 5× (−) |
| $Be^{2+}$ | 10× (+) | 100× (+) | $Hg^{2+}$ | 100× (+) | 80× (+) |
| $Sb^{3+}$ | 80× (+) | 100× (+) | $Sr^{2+}$ | 50× (−) | 25× (−) |
| $Co^{3+}$ | 50× (−) | 5× (−) | $Cr^{3+}$ | 5× (−) | 5× (−) |
| $Cu^{2+}$ | 50× (−) | 10× (−) | $La^{3+}$ | 100× (−) | 50× (−) |
| $Fe^{3+}$ | 30× (−) | 5× (−) | $Sm^{3+}$ | 10× (−) | 40× (−) |
| $V^{5+}$ | 10× (−) | 30× (−) | $Gd^{3+}$ | 30× (−) | 10× (−) |
| $Ni^{2+}$ | 100× (−) | 10× (−) | $Tb^{3+}$ | 100× (−) | 100× (+) |
| $Zn^{2+}$ | 100× (−) | 50× (−) | $Er^{3+}$ | 100× (−) | 50× (−) |
| $Mg^{2+}$ | 100× (−) | 200× (−) | $Dy^{3+}$ | 100× (−) | 50× (−) |
| $Ag^+$ | 50× (−) | 25× (−) | $Pr^{3+}$ | 10× (−) | 5× (−) |
| $Se^{VI}$ | 10× (−) | 5× (−) | $Y^{3+}$ | 100× (−) | 10× (−) |

* (+) = enhancement; (−) = depression.

## Simultaneous Determinations

The temperature-based resolution of candoluminescence responses has been used to resolve certain mixtures, such as lead and bismuth,[2] lead and manganese[4] and praseodymium and terbium.[1] Similar resolutions can be achieved with sulphuric acid-co-activated cerium and europium.

Figs. 10, 11 and 12 show responses from mixtures of cerium and manganese, cerium and europium and terbium and europium, respectively. For example, cerium is a very fast activator compared with europium, so it is possible to measure first the green cerium emission at 560 nm and then to change the wavelength to 600 nm to measure the orange europium emission that is stimulated after 15 s (Fig. 11). Care must be taken to avoid measurement of sodium-atom emission at 589 nm during the wavelength change. To check for mutual interferences, the effects of cerium on 5 ng of europium and of europium on 2 ng of cerium were

investigated; the results are shown in Fig. 13. Up to 10 ng of europium will not interfere with cerium, but greater amounts depress the emission. Cerium was found to have a very small depressive effect on the emission of europium when present in at least a two-fold mass excess; greater amounts caused a great depression in intensity.

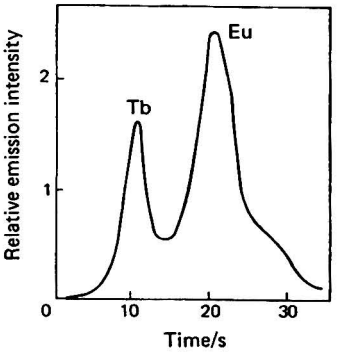


Fig. 12. Candoluminescence signals from a mixture of terbium (5 ng) and europium (10 ng) at 575 nm.

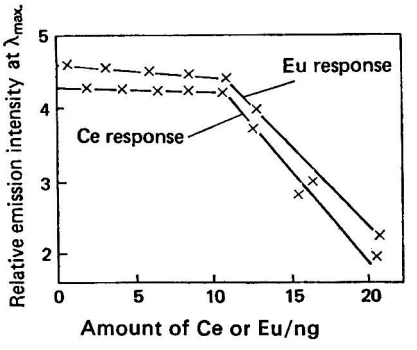


Fig. 13. Effect of cerium on europium candoluminescence intensity and *vice versa* (2 ng of Ce, 5 ng of Eu).

## Other Activator - Co-activator Systems

Since this work was begun, several other activator - co-activator systems have been discovered, including the co-activation of manganese by chloride[4] and of antimony by fluoride in a calcium oxide - calcium sulphate matrix, and of indium by halides in a magnesium hydroxide matrix. Such effects, therefore, are not uncommon, and will be described in detail in later papers.

## Discussion

Addition of strong acids to the matrix enhances the candoluminescence intensity of europium and cerium. The maximum enhancement for 10 ng of europium is about seven-fold; that for cerium is much greater, transforming an almost immeasurable signal into a signal more sensitive than that of europium. The nature of the enhancement process is not yet known, but the reaction of the matrix with the acid to give the calcium salt of the acid must be involved. Further, there is some variation in effectiveness between the various acids, indicating that the different salts formed have different efficiencies in promoting candoluminescence. It is also apparent that the order of effectiveness differs for the two activators, sulphuric and hydrochloric acids being most effective for cerium and hydrobromic acid most effective for europium. On the other hand, it should be noted that 12% of the matrix is already composed of calcium sulphate, so that the bulk presence of calcium sulphate is insufficient to stimulate candoluminescence from cerium and europium, as was proved with studies using calcium sulphate alone as a matrix. It would appear, therefore, that the *in situ* formation of the calcium salt is important for co-activation to occur. The slight change in peak wavelength could be a result of the change in the crystal environment of the activator.

As in the determination of praseodymium and terbium, there are a number of interferences that do not arise from spectral effects. Such interference effects can result in a decrease or, less commonly, an increase in the candoluminescence intensity. These effects, caused by relatively small amounts of other ions, are considered to arise from energy transfer between the luminescing ion and the interferent in the matrix. Such depressions thus arise from energy transfer from the excited, potentially luminescing ion to the interfering ion. The receptor ion may then lose this energy by a non-radiative process, or possibly by luminescence itself. Thus, for example, common transition-metal ions such as copper, iron and nickel depress the luminescence of cerium and europium, but no luminescence is observed from the interfering

ions. On the other hand, praseodymium depresses the candoluminescence of europium, while at the same time the luminescence of praseodymium is enhanced.[1] Enhanced emissions, therefore, also seem to arise by energy transfer from excited ions. Such ions may themselves be capable of losing their excitation energy radiatively, as in the praseodymium - europium example above, or in the similar enhancement of cerium luminescence by terbium (terbium emission is depressed[1]). Alternatively, they may not themselves be capable of luminescence in the particular matrix, and function only as energy transfer agents. Typical examples are the enhancing effects of lanthanum on praseodymium and terbium[1] and of beryllium on europium and cerium.

The great sensitivity of the technique for monitoring trace amounts of lanthanoid elements should be very useful for the determination of such elements in a wide range of samples. As an example, the determination of parts per million amounts of cerium in rocks has been studied and is described in a related paper in this issue.[7]

T. A. K. Nasser thanks the Iraqi Government (Ministry of Higher Education and Scientific Research) for the award of a scholarship.

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# SHORT PAPERS

## Determination of Trace Amounts of Cerium in Silicate Rocks Based on its Candoluminescence in a Calcium Oxide Based Matrix

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*Keywords: Cerium determination; silicate rocks; candoluminescence emission*

Cerium in rocks is mainly determined spectrophotometrically,[1] using reagents such as bromopyrogallol red,[2] salicylfluorone[3] and arsenazo I.[4] These reagents are not very selective and require a pre-separation of cerium from most of the common elements and other lanthanoids, thus making the methods very tedious. The direct determination of cerium by atomic-absorption spectrophotometry is very insensitive[5]; however, the sensitivity can be improved considerably by formation and extraction of ceromolybdophosphoric acid, and determination of the molybdenum by atomic-absorption spectrophotometry.[6] An ultraviolet spectrophotometric finish can also be used.

Recently, a very sensitive method for the determination of cerium (above 10 ng $ml^{-1}$) has been developed,[7] based on the measurement of the green candoluminescence produced by cerium in a calcium oxide - calcium sulphate matrix, with sulphuric acid as a co-activator, when the matrix is inserted into a hydrogen - nitrogen - air flame. This paper describes the application of this method to the determination of trace amounts of cerium in rocks. It involves the fusion of the sample with lithium metaborate,[8] and does not require the isolation of cerium from other components of the rock, before measuring the candoluminescence intensity of the cerium.

### Experimental

The spectroscopic equipment and matrix preparation were as described previously.[9]

#### Reagents

*Cerium nitrate, 99.9% pure.* Koch-Light Laboratories Ltd., Colnbrook, Bucks.

*EDTA solution.* The solution contained 2 g of the free acid and 10 ml of concentrated ammonia solution per litre.

All chemicals except cerium nitrate were of analytical-reagent grade. All solutions were prepared with distilled de-ionised water.

#### Rock Dissolution[8]

Weigh accurately 0.2 g of finely powdered rock, mix it well with 1 g of lithium metaborate and fuse the mixture in a gold or platinum crucible at 900–950 °C for 30 min. Remove the crucible from the furnace, rotate the melt over the inner wall of the crucible and cool the crucible quickly with water. Wash the outer surface of the crucible and place it on its side in a 100-ml beaker. Add 5 ml of concentrated nitric acid and 25 ml of water and heat the beaker gently until the material in the crucible has been dislodged. Add 20 ml of the EDTA solution (which helps to stabilise the trace metals in solution) and transfer the mixture into a 200-ml calibrated flask. Make up to volume with water and determine the cerium content of 1 $\mu$l of the solution as described below.

For the blank, take 1 g of lithium metaborate alone through this procedure.

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### Calibration Solutions (10–100 pg of Cerium)

To a series of six 10-ml calibrated flasks add 0.0, 2.0, 4.0, 6.0 and 8.0 ml of a 0.1 $\mu$g ml$^{-1}$ cerium solution and 1.0 ml of a 1.0 $\mu$g ml$^{-1}$ cerium solution (prepared from cerium nitrate). Add slowly to each flask 1.5 ml of concentrated sulphuric acid and make up to volume with water.

### Determination of Cerium

Inject 1 $\mu$l of one of the standard solutions on to the centre of the matrix surface. Place the surface in the flame (hydrogen flow-rate 2.0 l min$^{-1}$, air 2.0 l min$^{-1}$ and nitrogen 8.0 l min$^{-1}$) 30 mm above the burner top and 5 mm into the flame from the edge.[7] Record the emission intensity - time response at 560 nm, and measure the maximum candoluminescence intensity. Repeat the experiment at least twice and obtain the mean intensity. Carry out measurements on the other standard solutions in a similar manner and plot the average intensity (after deduction of the intensity of the solution to which no cerium had been added, which should be extremely small) against amount of cerium, for calibration purposes. Repeat the measurements for the rock and blank solutions, and obtain the cerium content of the rock solution from the calibration graph, after deducting the blank intensity from that given by the rock solution.

## Results

The procedure gives a linear calibration graph over the range 10–100 pg of cerium (equivalent to about 0.01–0.1 p.p.m. in the solution and about 10–100 p.p.m. in the rock) with a reproducibility of about $\pm 5\%$. Standard rocks G-A, G-H and W-I were subjected to the above procedure. The results obtained are given in Table I, where they are compared with the results of other workers. Very similar results were obtained by candoluminescence after subjecting the rocks to a sodium carbonate fusion. There is some uncertainty about the correct cerium contents of these rocks, but the results found by candoluminescence fall well within the range of the other workers' results.

TABLE I

DETERMINATION OF CERIUM IN STANDARD SILICATE ROCKS

| | | Cerium concentration, p.p.m. | |
|---|---|---|---|
| Sample | Main constituents | Certified results* | Candoluminescence results |
| G-A[10] | 70% $SiO_2$; 14.5% $Al_2O_3$; K, Na, Ca, Mg, Fe | 55, 58, 63, 90 | 75 $\pm$ 5%† |
| W-I[11] | 53% $SiO_2$; 15% $Al_2O_3$; 9% FeO; Na, Mg | 23 | 18 $\pm$ 5% |
| G-H[10] | 76% $SiO_2$; 12.5% $Al_2O_3$; K, Na | 30, 37, 52, 104 | 41 $\pm$ 5% |

* From various laboratories.
† Relative standard deviation (coefficient of variation).

Other methods for determining cerium are subject to severe interferences. It was expected that the amounts of iron and aluminium present would greatly suppress the candoluminescence of cerium,[7] but the results obtained indicate that their effect, if any, is very limited. This result may be a consequence of the EDTA or borate present in the solution, which inhibits the precipitation of iron and aluminium on the matrix surface. The nature of such interferences, and their elimination, in candoluminescence is only beginning to be understood,[7] but an extensive investigation into this problem is under way and the results, including those for cerium, will be published later.

T. A. K. Nasser thanks the Iraqi Government (Ministry of Higher Education and Scientific Research) for the award of a scholarship.

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# Determination of the Rate of Hydrolysis of Ytterbium Sulphide Films by Carbon Furnace Atomic-emission Spectrometry

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*Keywords: Ytterbium sulphide films; glass surfaces; rate of hydrolysis; carbon furnace atomic-emission spectrometry*

The application of carbon furnace atomisers as sources for atomic-emission measurements has been described in several recent publications.[1–6] The technique gives very low detection limits for a wide range of elements and has already been successfully applied to the determination of copper in spinach and pine needles,[4] lithium in high-purity copper[7] and minor elements in steels.[8] Ytterbium sulphide is used as a protective film in the manufacture of high-quality optical components. Although the coating formed is very hard, erosion of the surface film occurs when it comes into contact with water. As part of a project for the development of protective films, a measurement of the rate of hydrolysis of ytterbium sulphide was required. Ytterbium is a sensitive element in flame-emission spectroscopy,[9] and it also gives a very low detection limit in carbon furnace atomic-emission spectrometry.[5] The advantage of the small sample size required by the latter technique has allowed the development of a simple procedure for the determination of ytterbium in solution, following hydrolysis, from small samples of coated glass. Repetitive analysis of the solution at different times has allowed the rate of hydrolysis to be calculated.

## Experimental

### Apparatus

The instrument used was a Perkin-Elmer 306 atomic-absorption/emission spectrometer equipped with an HGA-72 heated graphite atomiser and coupled to a Servoscribe strip-chart recorder. The instrument was operated in the emission mode and was set up in the manner described previously.[1,5] Standard Perkin-Elmer HGA-72 graphite tubes were used for atomisation. The operating conditions for the determination of ytterbium were those listed in Table I.

TABLE I

OPERATING CONDITIONS

| | | | |
|---|---|---|---|
| Wavelength/nm | 398.798 | Volume of sample solution/μl | 50 |
| Slit width (setting 3)/mm | 0.3 | Scale expansion | ×1 |
| Spectral band width/nm | 0.2 | Argon flow-rate/l min$^{-1}$ | 1.5 |
| Drying conditions | 40 s at 100 °C | Argon feed pressure/p.s.i.g. | 40 |
| Atomisation conditions (setting 999 units) | 10 s at 2 400 °C under gas stop operation | Recorder chart speed/cm s$^{-1}$ | 2 |

## Reagents

High-purity distilled water was used for the preparation of all solutions.

A stock solution of ytterbium containing 1 000 $\mu$g ml$^{-1}$ was prepared by dissolving the appropriate amount of ytterbium sulphate in 0.01 M sulphuric acid. This solution was diluted to provide 10 and 1.0 $\mu$g ml$^{-1}$ stock working solutions as required. Calibration solutions in the concentration range 0.005–0.5 $\mu$g ml$^{-1}$ were prepared, immediately before use, by dilution of these stock working solutions.

## Procedure

A piece of ytterbium sulphide coated glass with a surface area of approximately 1–2 cm$^2$ was immersed in 10 ml of water. At various time intervals following immersion, 50-$\mu$l aliquots of the solution were withdrawn by use of a high-precision Oxford micropipette and their ytterbium contents determined by injection into the carbon furnace atomiser, which was operated under the conditions described above. The emission signal obtained during the atomisation stage was recorded on the recorder chart. For each aliquot the maximum peak height was measured and the emission signal obtained at the same time during the atomisation of a 50-$\mu$l water blank was subtracted from the peak value. The ytterbium content of each aliquot was obtained by comparison with a calibration graph that was constructed by use of standard ytterbium solutions representing the appropriate range of concentrations; the standard solutions were determined immediately after the sample, using the same procedure.

## Results and Discussion

The analysis of rare earth elements by atomic-emission spectrometry is well documented. Detection limits for ytterbium obtained by use of flame emission using a dinitrogen oxide - acetylene flame, given by Kniseley *et al.*,[9] were 0.002 $\mu$g ml$^{-1}$ in aqueous solution and 0.0003 $\mu$g ml$^{-1}$ in an alcoholic solution; these values compared favourably with a value of 0.04 $\mu$g ml$^{-1}$ obtained by use of a flame atomic-absorption method. Although the reported[5] detection limits for ytterbium when using carbon furnace atomic emission of 0.000 58 $\mu$g ml$^{-1}$ in standard Perkin-Elmer tubes and 0.000 36 $\mu$g ml$^{-1}$ in specially modified tubes were similar to those observed when using flame-emission spectrometry, carbon furnace atomisation was preferred for the present application as only small sample volumes (50 $\mu$l) were required. This small volume requirement allowed several tests to be made at intervals on the same experimental solution without a significant alteration to the over-all volume of the solution. The rate of hydrolysis could thus be calculated from this kinetic experiment. Under the conditions used chemical interferences were also minimised.[2] Inter-element spectral interferences were found to be negligible and the background emission signal from the carbon tube was reproducible, stable and easily subtracted.

The accuracy of the technique was checked by adding 0.25, 0.50, 0.75 and 1.00 $\mu$g of ytterbium to test solutions containing 0.18 $\mu$g of ytterbium. Recoveries of between 94 and 104% were obtained, which are consistent with the known reproducibility of carbon furnace atomisers using manual sample injection, and which suggest that the accuracy of the method is suitable for the application described.

The ytterbium content of the 10-ml sample solutions was measured at intervals for time periods up to 75 min. Results from a typical experiment are shown in Fig. 1. The slope of the graph of the increase of ytterbium concentration with time is linear, indicating a zero-order reaction. This to is be expected as the rate is dependent solely on the area of solid ytterbium sulphide film exposed to the solution, which is constant within each experiment. The zero-order rate constant, $k_0$, calculated from the slope of the graph in Fig. 1 and measurement of the surface area of the film, was found to be 2.28 $\pm$ 0.21 $\mu$g cm$^{-2}$ h$^{-1}$ for distilled water and 2.77 $\pm$ 0.23 $\mu$g cm$^{-2}$ h$^{-1}$ for Glasgow tap water. In both instances the rates of reaction were constant over a period of at least 15 h. The analytical procedure indicates the rate at which ytterbium passes into solution. If it is assumed that the film is composed only of ytterbium sulphide, then the rate of hydrolysis of the sulphide film can be simply calculated to be 2.91 $\mu$g cm$^{-2}$ h$^{-1}$ in distilled water and 3.54 $\mu$g cm$^{-2}$ h$^{-1}$ in tap water. Optical components protected by such a film could be expected to lose sulphide in an analogous manner whether exposed to rain or river water or other sources of water.

The procedure developed here demonstrates the advantages of carbon furnace atomisation in the study of the dissolution of surface films. The sensitivity for many elements in both

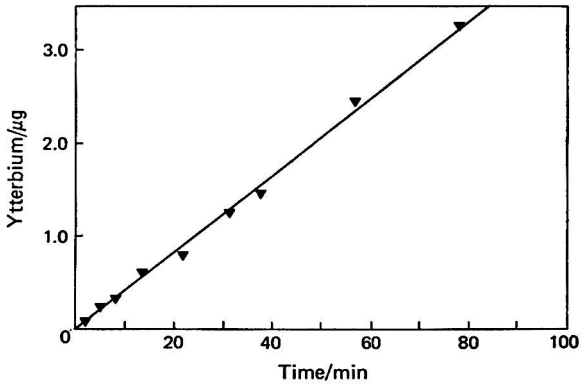


Fig. 1. Amount of ytterbium in 10 ml of distilled water *versus* time. Surface area of sample, 1.13 $cm^2$; temperature of water, 20 °C.

absorption and emission spectrometry would allow similar simple, rapid methods to be adopted that also require small test portions. The sensitivity of the emission mode for ytterbium obviates the need for an expensive hollow-cathode lamp and simplifies the procedure even further.

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# Determination of Several Elements in Lateritic Ores by Instrumental Neutron-activation Analysis

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*Keywords*: *Lateritic ores*; *nickel ores*; *metals*; *neutron-activation analysis*

Although most of the world's nickel is derived from deposits of sulphide minerals, a significant amount is extracted from lateritic ores formed in tropical regions as a result of the weathering of basic rocks.[1] Such sedentary occurrences may be of economic worth also as sources of other elements. In a geochemical study of a lateritic profile it is desirable to perform an appreciable

number of elemental analyses as a function of depth, and it was considered that instrumental neutron-activation analysis (INAA) could be of value in this context.[2,3] Chromium, cobalt, copper, iron, manganese, nickel, scandium, sodium and zinc have been determined as a matter of routine by this method. Relevant nuclear data for these elements are presented in Table I. Other gamma-rays are associated with several of the radionuclides listed but only those which can be regarded as being largely free from interference have been included, although in some instances the additional rays have provided useful confirmation of identification.[4] Because counting was performed at a considerable distance from the nuclear reactor used for the neutron activation, the use of radionuclides with half-lives of less than about 15 min was generally precluded.

TABLE I

NUCLEAR DATA FOR ELEMENTS DETERMINED

| Element | Radionuclide produced by (n,$\gamma$) reaction | Abundance of target isotope, % | Isotopic activation cross-section ($\sigma$)/barn | Half-life of product | Principal $\gamma$-ray used/MeV |
|---|---|---|---|---|---|
| Chromium .. .. | $^{51}$Cr | 4.31 | 16 | 27.8 d | 0.320 |
| Cobalt .. .. .. | $^{60}$Co | 100 | 37 | 5.26 yr | 1.173, 1.333 |
| Copper .. .. .. | $^{64}$Cu | 69.09 | 4.5 | 12.8 h | 0.511 ($\gamma\pm$), 1.345 |
| Iron .. .. .. | $^{59}$Fe | 0.33 | 1.2 | 45 d | 1.099, 1.292 |
| Manganese .. .. | $^{56}$Mn | 100 | 13.3 | 2.58 h | 0.847 |
| Nickel .. .. .. | $^{65}$Ni | 1.16 | 1.5 | 2.56 h | 1.482 |
| Scandium .. .. | $^{46}$Sc | 100 | 22 | 84 d | 0.889 |
| Sodium .. .. .. | $^{24}$Na | 100 | 0.53 | 15.0 h | 1.369, 2.754 |
| Zinc .. .. .. | $^{69}$Zn$^{m}$ | 18.56 | 0.075 | 13.8 h | 0.439 |

An important feature in instrumental activation analysis is the choice of the most appropriate irradiation and cooling time and this is made so as to maximise, as far as possible, the activities of the radionuclides of interest in comparison with those of others. The work reported here was based on one set of conditions and proved satisfactory for the determination of the elements of concern.

Activation analysis is usually a comparative method, involving simultaneous irradiation of the samples and standards that contain known amounts of the elements to be determined. In the selection of such standards for comparison it is of vital importance that neutron self-shielding should correspond to that in samples. In this work the specimens for analysis were in powdered form and consideration was given initially to using triturated US Geological Survey Standard Rock (PCC-1) as the standard. However, results quoted in the literature for the concentrations of some of the elements in this rock may vary from the average value by factors of 0.5–1.5; also, the chromium, iron and nickel concentrations are lower than in the laterites, while the concentrations of magnesium, silicon and sodium are higher. Hence, it was decided to prepare an artificial standard by mixing known amounts of elements as their oxide or chloride in such proportions as correspond to a typical lateritic matrix.

## Experimental

### Standard Mixture

A comparison standard was made by mixing together accurately weighed samples of ignited Specpure or AnalaR grade silica (25%), iron(III) oxide (48%), chromium(III) oxide (2.7%), nickel(II) oxide (2.1%), manganese oxide (3.0%), sodium chloride (0.16%), magnesium oxide (17.7%), zinc oxide (0.25%), aluminium oxide (0.66%), copper(II) oxide (0.065%), scandium-(III) oxide (0.030%) and tricobalt tetraoxide (0.20%). Admixture was achieved by mechanical shaking for 2 h, followed by grinding with a pestle and mortar. Homogeneity was confirmed by neutron irradiation of duplicate portions and comparison of the derived specific activities of the constituents; the reproducibility corresponded to expectation from calculations based on counting statistics. The mixture was used also as a standard for activation analysis of PCC-1 and yielded the results chromium 0.298%, cobalt 0.013%, copper 0.001 2%, iron 6.13%, manganese 0.098%, nickel 0.256%, scandium 0.000 87%, sodium 0.067% and zinc 0.005 3%, which are within the ranges quoted in the literature.[5]

## Irradiation

Samples of about 0.4 g of lateritic ore, and similar amounts of standard mixture, were accurately weighed into polythene tubes of internal diameter 4 mm. The sealed vials were packed together with silica-wool in a standard polythene irradiation tube and sent for irradiation in the nuclear reactor HERALD at the Atomic Weapons Research Establishment, Aldermaston. An irradiation usually involved six samples of ore, together with two portions of standard, and was of 2 h duration in a thermal flux of $2.5 \times 10^{12}$ neutrons $cm^{-2}$ $s^{-1}$.

Following delivery to our laboratory the irradiation tubes were opened and samples were transferred to clean polythene counting vials.

## Counting

Samples and standards were counted with a co-axial lithium-drifted germanium detector (Canberra) connected to a 4 096-channel pulse-height analyser (Intertechnique). The germanium(lithium) semiconductor detector has the following characteristics: diameter, 42.5 mm; length, 41.5 mm; resolution, 2.50, 2.10 and 1.42 keV (FWHM) for cobalt-60, caesium-137 and cobalt-57, respectively; and peak to Compton ratio, 22:1 at the 1.333-MeV photo-peak of cobalt-60.

The samples were placed in a conventional castle at a distance of 3.5 cm from the "head" of the counter. Calculations of peak areas were based on the methods of Covell[6] or Sterlinski[7] and, where necessary, corrections were made for decay.

Counting was generally commenced 11–14 h after the end of irradiation. The counts were initially 200, 300 or 500 s in duration (live time) and repeat measurements were made over a period of 4 d in order to establish half-lives and confirm identification.

## Results and Discussion

A typical gamma-ray spectrum is shown in Fig. 1 and results of instrumental activation analyses are presented in Table II. In several instances conventional chemical methods for the determination of metals (chromium,[8] cobalt,[9] copper,[9] iron,[8] manganese,[8] nickel[10]) in ores were also applied and the results are listed in the table. The determinations quoted are

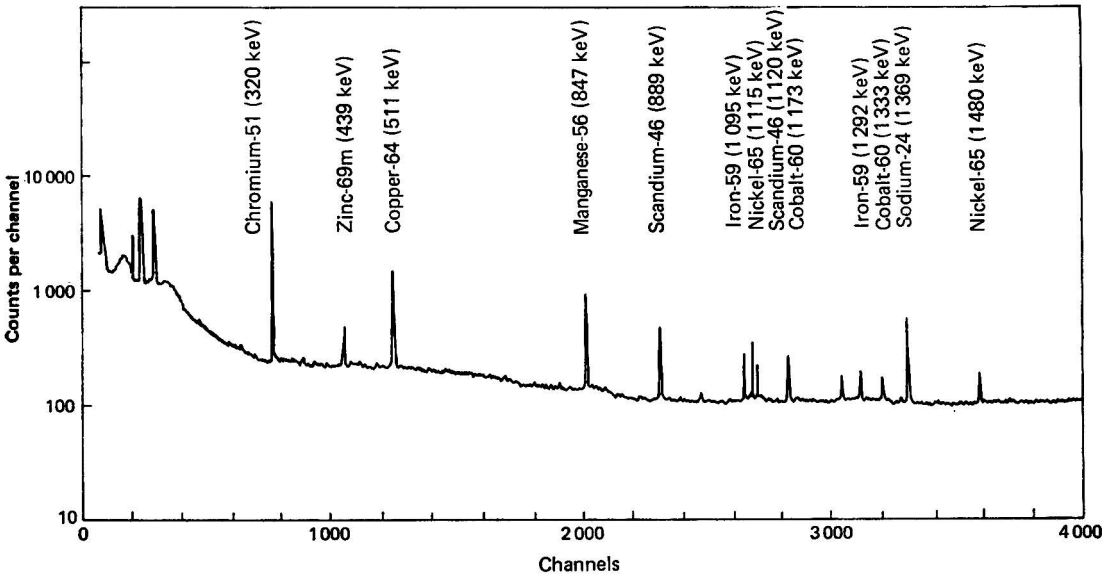


Fig. 1. Gamma-ray spectrum of sample L2 measured 15 h after the end of irradiation. Counting time 300 s.

## TABLE II

### ANALYSIS OF LATERITIC NICKEL-ORE PROFILES AS A FUNCTION OF DEPTH

Results in italic type from INAA; others from conventional chemical methods.

| Deposit | Sample number | Depth/m | Description | Metal, % Chromium | Cobalt | Copper | Iron | Manganese | Nickel | Scandium | Sodium | Zinc |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| La Gloria, Lake Izabal, Guatemala | L1 | 1.5–2 | Surficial laterite | *0.08* | *0.11* | *0.13* | *42.4* | *0.26* | *1.69* | *0.012 8* | *0.007 5* | *0.014* |
| | | | | 0.08 | 0.12 | 0.13 | 43.8 | 0.28 | 1.73 | | | |
| | L2 | 2.5–3 | Plastic laterite | *0.08* | *0.13* | *0.13* | *31.5* | *0.31* | *1.91* | *0.008 0* | *0.023 1* | *0.012* |
| | | | | 0.09 | 0.15 | 0.14 | 32.2 | 0.31 | 1.92 | | | |
| | L3 | 3.5–4 | Saprolite | *1.25* | *0.26* | *0.12* | *24.9* | *0.25* | *1.98* | *0.006 0* | *0.024 5* | *0.006 5* |
| | | | | 1.34 | 0.29 | 0.12 | 25.2 | 0.26 | 2.13 | | | |
| | L4 | 5.25–5.75 | Boulders in saprolite zone | *1.21* | *0.14* | *0.12* | *9.09* | *0.22* | *1.85* | *0.002 2* | *0.023 1* | *0.002 6* |
| | | | | 1.28 | 0.15 | 0.12 | 9.15 | 0.22 | 1.91 | | | |
| | L5 | 8–8.5 | Weathered rock | *0.68* | *0.11* | *0.11* | *10.10* | *0.12* | *1.21* | *0.002 3* | *0.028 5* | *0.003 7* |
| | | | | 0.78 | 0.12 | 0.11 | 10.53 | 0.12 | 1.18 | | | |
| | L6 | 8–8.5 | Fines of weathered rock | *1.10* | *0.19* | *0.11* | *22.2* | *0.20* | *1.45* | *0.005 5* | *0.017 5* | *0.005 6* |
| | | | | 1.30 | 0.21 | 0.11 | 22.6 | 0.20 | 1.60 | | | |
| | L7 | 10.25–10.75 | Rock | *0.65* | *0.07* | *0.01* | *7.88* | *0.09* | *0.62* | *0.002 1* | *0.023 3* | *0.002 4* |
| | | | | 0.65 | 0.08 | 0.01 | 7.95 | 0.09 | 0.65 | | | |
| New Caledonia: Lat. 22° 17′ S Long. 166° 58′ E | BNC1 | 0–2 | Ferricrete | *0.35* | *0.051* | *0.12* | *46.7* | *0.14* | *0.71* | *0.009 8* | — | *0.68* |
| | | | | 0.32 | 0.062 | 0.14 | 47.0 | 0.13 | 0.73 | | | |
| | BNC2 | 2–7 | Limonite | *0.34* | *0.077* | *0.13* | *48.5* | *0.21* | *1.47* | *0.010 8* | — | *0.63* |
| | | | | 0.34 | 0.081 | 0.15 | 49.5 | 0.21 | 1.52 | | | |
| | BNC3 | 7–11 | Limonite | *0.69* | *0.093* | *0.16* | *49.1* | *0.20* | *1.59* | *0.011 0* | *0.026* | *0.65* |
| | | | | 0.83 | 0.106 | 0.17 | 51.0 | 0.20 | 1.65 | | | |
| | BNC4 | 11–13 | Limonite | *1.47* | *0.15* | *0.24* | *49.3* | *0.25* | *1.42* | *0.008 4* | *0.012* | *0.64* |
| | | | | 1.50 | 0.15 | 0.25 | 49.5 | 0.24 | 1.41 | | | |
| | BNC5 | 13–14 | Limonite and asbolite | *1.65* | *0.27* | *0.23* | *52.0* | *1.80* | *1.71* | *0.012 2* | *0.043* | *0.94* |
| | | | | 1.75 | 0.26 | 0.27 | 52.3 | 1.80 | 1.75 | | | |
| | BNC6 | 14–17 | Limonite and asbolite | *2.04* | *0.16* | *0.21* | *38.8* | *1.69* | *3.20* | *0.009 6* | *0.066* | *0.91* |
| | | | | 2.15 | 0.16 | 0.22 | 38.1 | 1.72 | 3.21 | | | |
| | BNC7 | 17–20 | Saprolite | *1.09* | *0.08* | *0.13* | *11.3* | *0.72* | *1.56* | *0.005 5* | *0.127* | *0.07* |
| | | | | 1.05 | 0.08 | 0.16 | 12.5 | 0.67 | 1.61 | | | |
| Pamalea, Indonesia: Lat. 4° 10′ S Long. 121° 37′ E | BIP1 | 11–13 | Limonite | *1.21* | *0.12* | *0.070* | *49.1* | *0.81* | *1.40* | *0.009 0* | *0.002 1* | *0.011* |
| | | | | 1.35 | 0.12 | 0.075 | 49.1 | 0.82 | 1.46 | | | |
| | BIP2 | 13–17 | Asbolite | *0.98* | *0.059* | *0.113* | *20.8* | *0.69* | *2.71* | *0.005 6* | *0.002 7* | *0.006 6* |
| | | | | 1.05 | 0.064 | 0.115 | 19.1 | 0.64 | 2.83 | | | |
| | BIP3 | 18–19 | Saprolite and quartz boxwork | *0.35* | *0.022* | *0.048* | *9.13* | *0.27* | *2.35* | *0.001 8* | *0.001 1* | *0.001 9* |
| | | | | 0.37 | 0.031 | 0.051 | 10.15 | 0.27 | 2.43 | | | |
| Typical coefficient of variation of INAA (approx.) | | | | *±6%* | *±2%* | *±0.5%* | *±3%* | *±1%* | *±2%* | *±2%* | *±0.5%* | *±3%* |

the average of at least two analyses. Agreement between the different procedures is satisfactory, which substantiates the fact that INAA can yield sufficiently accurate results with a minimum of sample handling and only a few man-hours of work.

In addition, we have used INAA for studying the distribution of chlorine in lateritic profiles and its leaching from such ores.[11] Radiochemical neutron-activation analysis has been used for the determination of precious metals in such deposits.[12]

The authors are grateful to INCO Limited for supplying them with samples of lateritic ores. They also thank the Science Research Council for sponsoring the irradiations in the nuclear reactor at Aldermaston. An award to one of us (S.A.) from the British Council is gratefully acknowledged.

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# Rapid Thermal-decomposition Technique for the Atomic-absorption Determination of Mercury in Rocks, Soils and Sediments

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*Keywords: Mercury determination; minerals; atomic-absorption spectrophotometry; thermal decomposition*

Mercury has long had an association with geochemistry for mineral exploration purposes. As early as 1946 Saukov[1] showed that its mobility and the consequent large dispersion aureoles that it produced made it well suited for use as a geochemical indicator element. Little was contributed to this subject outside the Soviet Union until the early 1960s, when Hawkes and Webb,[2] James[3] and Hawkes and Williston[4] published reports of research carried out in the UK and USA. Determination of the small amounts of mercury present in geological samples presented few problems after the advent of cold-vapour atomic-absorption techniques, but methods of sample pre-treatment, prior to the actual measurement, varied widely.[5–8]

In order to carry out a determination, all of the mercury compounds present must be converted into metallic mercury and this conversion can be achieved by wet or dry oxidation. Existing methods for wet oxidation tend to be time consuming, resulting in too few determinations per day,[9] and dry-oxidation procedures, although generally faster, yield volatile sulphur dioxide and organic products, which absorb light at 253.7 nm. These interferents can

be removed from the gas stream by absorbents[10,11] or selectively eliminated by instrument design features.[12–16]

The method described in this paper retains the collection and release system already in use in this laboratory[9] but incorporates a thermal decomposition stage for breakdown of the sample, thus avoiding the need for the preparation of the mercury-free reaction vessels that are frequently used in wet-oxidation techniques. The ability to undertake analyses of soils that are rich in organic matter without the need to replenish the absorbents frequently is of prime importance. While numerous techniques for the thermal decomposition of samples, followed by amalgamation and subsequent release of mercury by heating, have been described and reviewed,[17] this paper describes a rapid and convenient apparatus for such determinations of mercury in geological and soil samples.

## Experimental

### Apparatus

All atomic-absorption measurements were made by using a single-beam instrument (Varian Techtron AA1200) with a peak read-out facility; a mercury hollow-cathode lamp (Q in Fig. 1) was used as the light source. The apparatus for sample decomposition, mercury collection and measurement is shown in Fig. 1. A stream of air, from which the water vapour and mercury have been removed by trap A, is passed over the sample in boat B, through the absorbents E, F and G and on to the gold collector, I. The taps C and J are used to divert the air flow into the part of the system in which it is required. Heating of the sample and heating and cooling of the gold are accomplished by sliding the 400 mm long silica tubes $T_1$ and $T_2$ into or out of the furnaces. The temperature of each furnace is independently controlled by a variable-voltage transformer. The copper cooling coils cool the gold during the sample heating stage, thereby aiding mercury collection, and the silica gel during the gold heating stage, thus minimising volatilisation of the organic combustion products along the absorption tube. After heating the gold, mercury vapour is carried through silicone-rubber tubing to the gas cell, L, and is thence exhausted via a suction pump and filter. A read-out of the absorbance value is supplied by the digital display, M. Construction of a similar furnace has been described by Nicholson and Smith in a previous paper.[18] All stoppers that come into contact with hot gases are made from silicone rubber.

### Reagents

*Silica gel.* Purified laboratory reagent, about 6–22 mesh, non-indicating silica gel was used. It was heated briefly in a silica crucible to 400 °C before use in order to expel water.

*Alumina.* Chromatographic grade, 100–200 mesh, activated, heated as above.

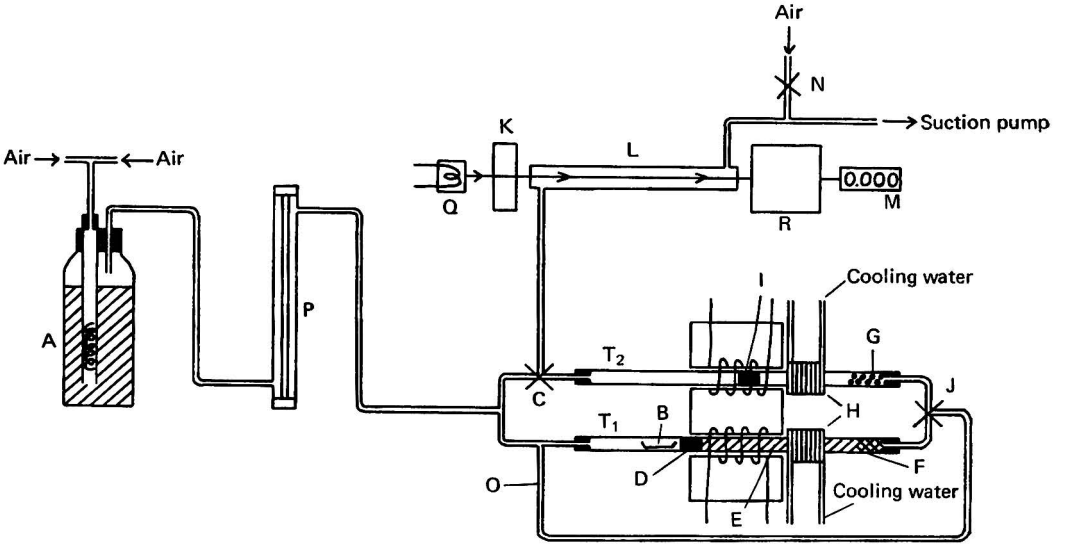
*Magnesium perchlorate.* Microanalytical reagent, 14–22 mesh.

*Mercury standards.* Standards of 0.05 and 0.5 p.p.m. were prepared by adding known amounts of standard mercury solution [prepared from mercury(II) oxide and 1 N sulphuric acid] to 10 g of ignited rock base and grinding with a mechanical agate pestle and mortar until dry.

### Procedure

Set up the apparatus as shown in Fig. 1 and switch on all supplies. Set the temperature of $T_1$ to 650 °C and of $T_2$ to 750 °C by adjustment of the transformers. Calibration of the settings is achieved by using a resistance thermometer. Turn the taps C and J so as to send the air flow through both furnace tubes, the gold being heated at the same time. Leave the apparatus for several minutes in order to remove all mercury from the system. (*N.B.* If the absorption tube has been packed with fresh reagents, several injections of mercury vapour - air are necessary to condition the system before use.) Turn tap J to divert air through the by-pass and set the flow-rate to 2.5 l $min^{-1}$ by adjusting tap N. As soon as the digital display gives zero readings, indicating that the system is free from mercury, slide the tube containing the gold out of the furnace into the cooling coil and allow it to cool for 1 min.

Weigh a convenient amount of freeze-dried sample, usually about 100 mg but dependent on its content of mercury, into a 40 × 10 mm nickel boat. Mix samples known to contain sulphide minerals with calcium oxide. Next turn tap J to send air through $T_1$, introduce




A = Gold coil and silica gel mercury and water vapour trap
B = Combustion boat
C = Two-way tap
D = Silver coil
E = Silica gel
F = Alumina
G = Magnesium perchlorate
H = Copper cooling coils
I = Gold wire collector
J = Two-way tap
K = Monochromator
L = Gas cell
M = Readout
N = Tap
O = Bypass
P = Flow meter
Q = Hollow-cathode lamp
R = Detector
$T_1$ = Furnace tube
$T_2$ = Furnace tube

Fig. 1. Diagram of the apparatus.

the boat into the end of tube $T_1$, as shown, and re-connect the system. Diversion of the air over the sample will result in a drop in flow-rate to 0.5 l $min^{-1}$. Allow the air to pass for 2 min, then close off $T_2$ by turning taps C and J and slide the tube containing the gold into the furnace; remove the boat. Air will now pass directly to the gas cell, L. After 1 min open taps C and J, via the by-pass, in order to sweep the mercury vapour into the gas cell. When the peak has been recorded, the gold is cooled for 1 min and the cycle can begin again. By using this procedure, one sample can be analysed every 4 min, which gives an acceptable 70–80 determinations per day, allowing time for cleaning of the furnace tube and calibration.

## Calibration

Calibration was carried out by injecting known concentrations of mercury vapour and air into the system, measuring the peaks and preparing a calibration graph. The prepared rock standards were checked against this graph. The calibration is linear up to 0.15 $\mu$g of mercury, which gives an absorbance peak of about 1.1 with a 175-mm cell.

## Results and Discussion

Previous work carried out in this laboratory has shown that attacking various samples with acidic permanganate solution adequately dissolves elemental mercury, its salts and organo-mercurials. However, experience gained over the past few years indicates that cinnabar is partially resistant to this procedure and Jonasson *et al.*[19] also found this to be true. Sample decomposition by heating, while possibly introducing other errors,[20] obviates any tendency towards low results caused by failure of chemical attack to dissolve completely any mercury compounds. Watling *et al.*[21] gave thermal release characteristics for a range of inorganic mercury compounds from various matrices.

The silver coil, D, is in such a position in $T_1$ as to be on the outlet side of the furnace during the combustion process and, being at a temperature of approximately 400 °C, it serves as an aid to oxidation for the volatile products.[10] The silica gel traps most of the organic material and some water vapour, while the alumina serves to cut the air flow-rate and acts as a micro-filter for the more volatile organic constituents. The magnesium perchlorate is a safeguard against the possible carryover of water vapour. Sulphur dioxide appears not to interfere provided that the gold is kept at 750 °C. Below this temperature a decrease in efficiency is noticeable if samples containing a significant amount of sulphide are analysed repetitively. Addition of calcium oxide to the sample in the boat will reduce this tendency.[22] Air suction rather than air pressure is used to avoid losses of volatile mercury compounds when the sample is introduced. Other characteristics of the system have already been discussed by Head and Nicholson.[9]

By using the recommended conditions samples containing up to 99.5% of organic matter have been satisfactorily analysed by means of this procedure, and 40 determinations per absorption tube can be carried out, without impairment of efficiency, at this level if 25-mg amounts of sample are used. Multiple replicate determinations at this low mass indicate that no significant sampling problems occur when working with the various types of material. Three samples of soil, representing a range of organic matter contents, were chosen for statistical evaluation of the proposed method. Table I gives results for these three samples for comparison with results obtained by means of wet oxidation. Thirty replicate determinations were made on each sample.

TABLE I

MERCURY CONTENT OF THREE SOIL SAMPLES

| Soil | Organic matter content, % | Mercury content by established method,[9] p.p.m. | Mercury content by proposed method, p.p.m. | Coefficient of variation, % |
|---|---|---|---|---|
| Sample 1 .. .. | 6.8 | 0.33 | 0.37 | 3.8 |
| Sample 2 .. .. | 25.9 | 0.05 | 0.06 | 9.1 |
| Sample 3 .. .. | 52.0 | 0.06 | 0.08 | 6.7 |

Provided that the air-cleaning trap, A, is replenished at regular intervals no measurable blank value is recorded.

## Conclusions

The use of thermal decomposition for the analysis of geological materials has the advantage of being relatively rapid without losing any of the accuracy of the somewhat longer wet-oxidation technique. Samples containing considerable amounts of organic material can be analysed quickly without recourse to expensive absorbents or time-consuming separations.

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# Communication

*Material for publication as a Communication 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.*

*Manuscripts are not subjected to the usual examination by referees and inclusion of a Communication is at the Editor's discretion.*

## A Simple Matrix Modification Procedure to Allow the Direct Determination of Cadmium in Blood by Flame Micro-sampling Atomic-absorption Spectrophotometry

*Keywords*: *Cadmium determination*; *blood*; *matrix modification*; *flame micro-sampling atomic-absorption spectrophotometry*

The determination of cadmium in whole blood is of importance in surveys of possible environmental pollution and in industry for monitoring personnel exposed to this metal or its compounds. Many of the analytical methods in current use are based on electrothermal atomisation and atomic-absorption spectrophotometry. Although these methods have excellent sensitivities, the rates of analysis are not usually sufficiently fast for handling large numbers of samples on a routine basis. Flame micro-sampling methods permit higher rates of analysis but cannot be used directly for determinations of cadmium in blood because of the concurrence of the cadmium atomic signal with a large molecular absorption/scatter signal produced during combustion of the sample. This communication describes the use of a simple matrix modification procedure in which the rate of formation of cadmium atoms is retarded sufficiently to resolve the cadmium atomic signal from the molecular absorption signals.

In earlier studies on flame micro-sampling techniques for the analysis of blood for lead,[1] it was noted that phosphate ions seriously retarded the rate of formation of lead atoms. The addition of phosphate, however, has proved useful for the determination of cadmium using electrothermal atomisation[2] by allowing ashing temperatures up to 900 °C to be used without loss of this metal. It was therefore decided to study the effect of phosphate on the analysis of blood for cadmium by flame micro-sampling atomic-absorption spectrophotometry. Diammonium hydrogen orthophosphate was chosen for this study as the excess of $NH_4^+$ ions might minimise the molecular absorption interference from sodium chloride.[2] The equipment used was a Perkin-Elmer 103 single-beam atomic-absorption instrument fitted with a Delves cup micro-sampling attachment with nickel cups and an aluminium oxide absorption tube. Lead and hydrogen hollow-cathode lamps were used as radiation sources and measurements were made at 228.8 nm with a 0.7-nm band pass. The absorption signals were recorded on a Perkin-Elmer, Model 56, chart recorder, input 5 mV, *i.e.*, ×2 scale expansion or 0.5 A full scale.

Standard cadmium solutions containing 0, 5, 10, 15, 20, 30 and 40 $\mu g\ l^{-1}$ in 0.1% *V/V* nitric acid were prepared by dilution of a stock 1 mg $ml^{-1}$ solution (BDH Chemicals Ltd.). Solutions of diammonium hydrogen orthophosphate in water (2% and 3% *m/V*) were prepared from analytical-grade solid reagent. Microlitre volumes of aqueous solutions and blood samples were measured with Eppendorf pipettes.

The effect of phosphate on the atomic-absorption signals from an aqueous cadmium solution (Table I) was to retard both the appearance time and the time for maximum absorbance of the

TABLE I

EFFECT OF PHOSPHATE ON CADMIUM ATOMIC-ABSORPTION PROFILE

| Volume of 2% $(NH_4)_2HPO_4$ added/$\mu l$ | $t_{app}$*/s | $t_{max}$†/s | Relative sensitivity (peak) |
|---|---|---|---|
| 0 | 0.5 | 1.75 | 100 |
| 10 | 1.5 | 3.0 | 75 |
| 20 | 2.0 | 4.0 | 65 |

* $t_{app}$, appearance time of Cd atomic signal.
† $t_{max}$, time of maximum Cd atomic signal.

cadmium atomic signal. This effect was accompanied by a reduction in peak sensitivity. As the molecular absorption/scatter signal from the combustion of 10-$\mu l$ samples of whole blood occurred almost immediately the crucible was inserted into the flame and was completed 2.0 s after insertion, the results in Table I suggested that the analysis of blood for cadmium might be feasible in the presence of added phosphate. It was found that the optimum amount of phosphate needed

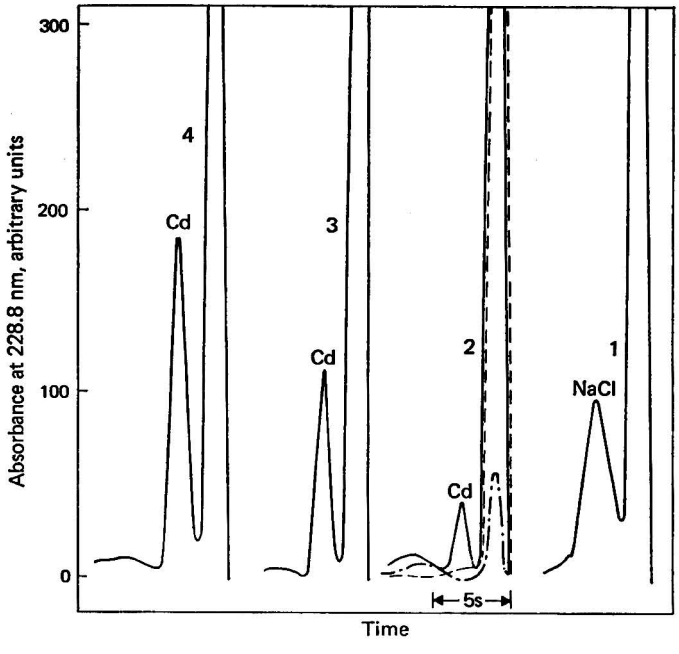


Fig. 1. Absorption signals obtained from: 1, 10 $\mu l$ of blood with no addition of phosphate; 2, 10 $\mu l$ of blood plus 20 $\mu l$ of 3% $(NH_4)_2HPO_4$ [——, Cd lamp; — — —, $H_2$ lamp; – · – · – · –, blank from 20 $\mu l$ of 3% $(NH_4)_2HPO_4$ with Cd lamp]; 3 and 4, 10 $\mu l$ of blood plus 20 $\mu l$ of 3% $(NH_4)_2HPO_4$ plus 10 $\mu l$ of 5 and 10 $\mu g\ l^{-1}$ Cd solutions, respectively. In each instance the large signal to the right of the Cd peak is due to the combustion products of the blood matrix. All signals were recorded using a Cd hollow-cathode lamp unless otherwise stated.

for complete resolution of the cadmium signal and the molecular signals from 10-$\mu$l samples of blood was provided by 20 $\mu$l of 3% *m/V* diammonium hydrogen orthophosphate solution.

A very simple procedure to determine cadmium levels in blood was to add to a nickel cup 20 $\mu$l of 3% *m/V* diammonium hydrogen orthophosphate solution and 10 $\mu$l of blood sample. Calibration was achieved by the addition of 10-$\mu$l volumes of a series of standard cadmium solutions of concentrations 0, 5, 10, 15, 20 and 30 $\mu g\ l^{-1}$ to a series of crucibles containing 20-$\mu$l volumes of the phosphate solution and 10-$\mu$l volumes of blood. The solutions were dried on a hot-plate at 150 °C (3 min) and then the cups were inserted into the air - acetylene flame immediately below the entrance hole of the absorption tube as described previously for lead.[1]

The effect of phosphate on the absorption signals obtained from 10-$\mu$l samples of blood is shown in Fig. 1. There was no discernible cadmium signal from the blood sample without added phosphate. The first peak was due to absorption/scatter of radiation by the combustion products of the sample whereas the second coincided with a yellow "sodium" emission and was presumably due to sodium chloride. There was, however, complete resolution of the cadmium signal and the molecular absorption/scatter signal in the presence of an excess of phosphate and the sodium chloride signal was eliminated. Further, there was no significant molecular absorption signal concurrent with the cadmium signal. The linearity of signal response with added cadmium is also shown. The calibration graph was linear up to 30 $\mu g\ l^{-1}$ of cadmium.

The precision of replicate analyses (Table II) was good above the 10 $\mu g\ l^{-1}$ level, but obviously requires improvement at lower levels. The sensitivity of $16 \times 10^{-12}$ g for 1% absorption was

TABLE II

PRECISION OF ANALYSES OF BLOOD FOR CADMIUM

| Sample No.* | Cd found/$\mu g\ l^{-1}$ Mean $\pm$ s.d. | No. of tests | Relative standard deviation |
|---|---|---|---|
| 1 | 6.54 $\pm$ 0.96 | 7 | 0.146 |
| 2 | 11.49 $\pm$ 0.64 | 7 | 0.056 |
| 3 | 17.09 $\pm$ 0.97 | 7 | 0.057 |
| 4 | 23.20 $\pm$ 1.45 | 8 | 0.062 |
| 5 | 26.10 $\pm$ 1.79 | 8 | 0.068 |
| 6 | 36.40 $\pm$ 2.52 | 8 | 0.069 |

* Samples 2, 3, 4, 5 and 6 were prepared by adding cadmium at concentrations of 5, 10, 15, 20 and 30 $\mu mol\ l^{-1}$, respectively, to sample 1.

reduced because of the effect of phosphate. This would, however, still allow routine analysis with 10-$\mu$l volumes of blood, which are easily obtainable even from children, and also provide good discrimination of results below 15 $\mu g\ l^{-1}$, which is the upper limit reported for persons not exposed to cadmium.[3,4]

Recovery tests *per se* are not sufficient to establish the accuracy of the method as calibration is effected by the addition of cadmium to blood. Thus the recovery data in Table II do not provide unequivocal proof of accuracy, which must be established by comparison with other analytical procedures.

The limited data presented here do indicate, however, that the procedure described forms the basis of a simple, rapid, micro-scale method for the analysis of blood for cadmium and it is hoped to report a more detailed evaluation of this method later.

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# Book Reviews

ANNUAL REPORTS ON ANALYTICAL ATOMIC SPECTROSCOPY, REVIEWING 1975. Volume 5. Edited by C. W. FULLER. Pp. viii + 267. London: Chemical Society. 1976. Price £15.

The fifth annual volume of this publication continues the collation and presentation of current information on analytical atomic spectroscopy in much the same manner as previous volumes, and once again the team behind this presentation must be congratulated on their combined effort. As Gordon Kirkbright has pointed out in the Foreword, there now seems little doubt that this publication is providing a service that is widely appreciated by practising analysts and to those who do not already have access to the series I strongly recommend that they add it to their book list.

The presentation maintains the standards of previous years and it is pleasing to note that in Part I, which is concerned with Fundamentals and Instrumentation, there appears to be a greater attempt to distinguish the more exciting and relevant developments that have occurred. As I indicated in reviewing Volume 4, this can be of considerable assistance to those who may not be in a position to make their own judgement on these matters and, additionally, it does of course provide more interesting reading than flat scientific prose. JOHN AGGETT

RADIOCHEMICAL METHODS IN ANALYSIS. Edited by D. I. COOMBER. Pp. x + 498. New York and London: Plenum Press. 1975. Price £17.33; $27.50.

Although there are now numerous published texts concerning the uses of radiotracers and tracer methodology, this book fulfils an important need for a text specifically related to radioanalytical methods.

The eleven chapters have been well written by twelve different experts and contain a total of 1 691 references. The book is very readable and the differing literary styles of the contributors have been well blended by the Editor.

The aim of the book, to give an account of the principal radiochemical methods used in chemical analysis, is basically achieved. However, a number of important topics such as radiochemical methods in enzyme and enzymatic analysis are missing in detail and the book is biased on the inorganic chemical side. I was rather disappointed to find that less than 10% of the references (about 160) were to publications beyond 1970, which suggests a much slower growth rate in the practical applications of tracer techniques in analysis than is actually occurring.

The first three chapters give a general account of methods of detection and measurement of radionuclides, including absolute counting, $\gamma$-ray spectrometry and liquid scintillation measurement. The appendix to Chapter 1, giving a summary of detectors that can be used for different types of radiation, is especially useful for quick reference. Chapter 3 includes an excellent account of Cerenkov counting, with 346 references.

The use of computers for processing data from studies with radioactivity is now often an essential requirement and Chapter 4 on this subject is a welcome inclusion. Chapter 5 gives a detailed account of separation methods for inorganic radionuclides, which is followed by an excellent chapter on radiochromatography and radioelectrophoresis. Chapter 6 includes also details of numerous problems and pitfalls in radiochromatographic analysis. Chapter 7 on activation analysis could have fitted into the book better as an earlier chapter. Chapter 8 discusses radiotracers in inorganic analysis and some of the basic theory on dilution analysis overlaps with that discussed in Chapter 9, which is an extremely good chapter dealing mainly with saturation analysis. In this chapter radioimmunoassay techniques in particular are discussed in detail and almost half of the 179 references are to publications beyond 1970, reflecting the very rapid growth in this type of analysis. Chapter 10 deals with a topic of growing importance, involving determination of radioactivity in the environment. It includes also an interesting section on isotopes in the cosmosciences but the section on isotopes in health analysis is rather poor and emphasises only the negative aspects of radioactivity and little about the benefits. Such a topic requires a full discussion to be meaningful and a comparison with other features of our environment. It is refreshing to see a chapter included on the applications of radiation sources in analytical methods, which often tend to be forgotten; this usefully concludes the descriptive part of the book. The subject index is just adequate.

I could find very few errors in this book and recommend it for most technical and specialist libraries. Teachers and other specialists will find it a useful reference book and at £17.33 it is not expensive for its contents. E. ANTHONY EVANS

ANALYTICAL CHEMISTRY OF THE CONDENSED PHOSPHATES. By S. GREENFIELD and M. CLIFT. Pp. xii + 202. Oxford, New York, Toronto, Sydney, Paris and Braunschweig: Pergamon Press. 1975. Price £6; $14.40.

The changing attitude to research in industry and the deteriorating economic situation has sadly resulted in a sharp reduction in the volume of original work published by analytical chemists in industry. It is therefore a great pleasure to review an authoritative text by two well known industrial analysts, whose experience of the analytical chemistry of the condensed phosphates must be second to none.

The book describes a wide variety of methods of determining the condensed phosphates (gravimetric, titrimetric, electrometric, chromatographic, nuclear magnetic resonance, X-ray diffraction, infrared and many more). It is not, however, a catalogue of all the methods that have been proposed, but rather a critical selection of procedures that have been proved in day-to-day use in the authors' laboratory over many years. Some of the material has not previously been published, and in such instances (for example, the automated ion-exchange procedure for mixtures of condensed phosphates) the detail is invaluable. In addition to these aspects, there are chapters on the determination of condensed phosphate esters (pesticides), on the treatment of samples, on qualitative tests for individual polyphosphates and on the preparation of pure samples of some polyphosphate salts and acids.

The book is well prepared, with a wealth of experimental detail. It is also liberally illustrated: for example, 46 powder X-ray diffraction patterns are reproduced from the authors' collection, and there are also numerous infrared and nuclear magnetic resonance spectra. It provides a good example of the contribution of modern analytical chemistry to the understanding of a very complex field of chemistry, and in this respect should be most valuable to all analytical chemists. It will be indispensable, of course, to all analysts involved in the determination of condensed phosphates.

A. TOWNSHEND

THE IDENTIFICATION OF MOLECULAR SPECTRA. Fourth Edition. By R. W. B. PEARSE and A. G. GAYDON. Pp. viii + 407. London: Chapman and Hall. New York: John Wiley. 1976. Price £20.

The First Edition of "Pearse and Gaydon" was published in 1941; since then, the work of these authors has been regarded as the standard reference book whenever the identification of diatomic and polyatomic molecules from their spectra has been concerned. The Second and Third Editions followed in 1950 and 1963, respectively; this Fourth Edition contains a great deal of new material, derived largely from the contributions made by studies of upper atmosphere chemistry and by the technique of flash photolysis. The literature references presented have been updated to about the end of 1974. The spectral data for some 490 diatomic and 127 polyatomic molecules are now listed.

This is a prestige publication. The cost is very reasonable, by current standards; every aspect of the quality of production of this book is first class and its appearance enhances the reputations of both the publisher and the authors.

D. M. W. ANDERSON

ADVANCES IN INFRARED AND RAMAN SPECTROSCOPY. Volume 2. Edited by R. J. H. CLARK and R. E. HESTER. Pp. xvi + 343. London and Rheine: Heyden. 1976. Price $35; £17.50; DM112.

This is the second volume in a new series intended to be of general interest to students, teachers, scientists and technologists within and beyond the fields of biology, chemistry and physics. This volume reinforces the excellent impression made recently by the first volume. Further, it is a pleasure to congratulate all those concerned on producing books that are really up-to-date at the time of publication—every chapter in this volume quotes from material published in 1975; two of the chapters succeed in including data published in 1976.

The chapters of this volume deal with the following: Vibrational Energy Levels in Matrix-isolated Species (J. K. Burnett, M. Poliakoff, J. J. Turner and H. Dubost); Vibrational Information from the Electronic Spectra of Transition Metal Co-ordination Compounds (C. D. Flint); Industrial Plant Applications of Infrared and Raman Spectroscopy (H. A. Willis); Time-resolved and Space-resolved Raman Spectroscopy (M. Bridoux and M. Delhaye); Vibrational Spectra of Ionic Vapours, Liquids and Glasses (J. P. Devlin); and Raman and Infrared Spectral Studies of Electrolytes (D. E. Irish and H. M. Brooker). These chapters vary in length from 13 pages (18 references) to 100 pages

(682 references); the latter (by Irish and Brooker) reviews comprehensively the progress made in the field of electrolytes since 1971 and could almost have been published as a separate monograph.

As in Volume 1, the authors and topics have been well chosen by the Editors; the standard of writing is uniformly good, and the book is well produced and free from trivial errors. Everyone concerned with this series is doing a good job: this series will quickly become one of the established features of the infrared and Raman literature. D. M. W. ANDERSON

NMR. BASIC PRINCIPLES AND PROGRESS. Volume 11. HIGH RESOLUTION NMR SPECTROSCOPY IN SOLIDS. By M. MEHRING. Pp. xii + 246. Berlin, Heidelberg and New York: Springer-Verlag. 1976. Price DM68; $27.90.

Readers of *The Analyst* are more often concerned with the NMR spectra of liquids or solutions than with those of solids. The NMR spectrum of a liquid or solution usually consists of a series of sharp lines, which can be readily assigned to precise nuclei or groups of nuclei, whereas solids give rise to wide-line spectra with broad, structureless peaks that are less easy to interpret. Wide-line spectra result from anisotropic interactions such as direct dipole - dipole interaction between magnetic nuclei in neighbouring molecules. This interaction, which is appreciable in solids where the relative orientations between molecules are fixed, is negligible in liquids and solutions where molecules are subjected to constant thermal agitation and dipole - dipole interaction is averaged to zero.

This monograph, by a Professor of Physics at Dortmund University, surveys the basic nuclear spin interactions that occur in solids and describes methods for suppressing dipole - dipole interactions and for obtaining useful information from high-resolution NMR spectra of solids. The author discusses multiple pulse methods, double resonance methods, magnetic shielding tensors and spin-lattice relaxation in line-narrowing experiments. The bibliography of literature references is remarkably up-to-date (1976).

The volume, which has been printed by photographic reproduction of typescript, has a number of proof-reading errors and, in places, the English is difficult to follow. However, the detailed mathematical treatment makes the work more suitable for physicists than for practising analytical chemists. J. E. PAGE

NMR. BASIC PRINCIPLES AND PROGRESS. Volume 12. CHLORINE, BROMINE AND IODINE NMR. PHYSICO-CHEMICAL AND BIOLOGICAL APPLICATIONS. By BJÖRN LINDMAN and STURE FORSÉN. Pp. xiv + 368. Berlin, Heidelberg and New York: Springer-Verlag. 1976. Price DM96; $39.40.

The stable isotopes of chlorine, bromine and iodine have large nuclear quadrupole moments and, in this respect, differ from the stable isotope of fluorine (fluorine-19), which has a spherically symmetric distribution of nuclear charge. Nuclei with electric quadrupole moments usually give rise to broad NMR signals, which are often too weak to be detected by conventional high-resolution NMR spectrometers, and they present special experimental problems. Thus, although chlorine-35 and chlorine-37 were first investigated in the early days of NMR spectroscopy, measurements with the quadrupolar halogen isotopes continue to be far less frequent than are those with fluorine-19.

An NMR study of quadrupolar nuclei can, however, provide useful information on physico-chemical and biological systems. The relaxation of quadrupolar nuclei, when caused by intramolecular interactions modulated by molecular motion, is easier to interpret than is that of non-quadrupolar nuclei. Investigations with chlorine-35 have given information on molecular reorientation and association in liquids, and they are of value in testing theoretical models for molecular mobility in liquids. Biochemical applications include studies of the relaxation of aqueous halide ions taking part in chemical exchange with proteins and with other macromolecules.

This monograph by two well known NMR spectroscopists from Lund University, Sweden, provides a comprehensive account of applications of chlorine-35, chlorine-37, bromine-79, bromine-81 and iodine-127 NMR in liquids and solutions and in liquid crystals. An introductory chapter on the NMR properties of quadrupolar nuclei is followed by chapters on relaxation and shielding effects in covalent halogen compounds, scalar spin couplings, relaxation and shielding of halide ions, quadrupolar splittings in liquid crystals, halide ions in biological systems and the perchlorate ion.

The volume is clearly written, appears to be free from errors and covers the published literature up to January, 1976. It provides the best account of this specialised subject that has so far appeared. J. E. PAGE

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## Rapid Spectrophotometric Micro-determination of Nitrites in Water

A method involving the use of a reagent suitable for the rapid spectrophotometric determination of nitrites in water is described. Nitrites react with resorcinol in an acidic medium and the nitroso product forms a pale yellow chelate with the zirconyl ion. The absorbance of the chelate is measured at 347 nm and the calculated molar absorptivity is $2.67 \times 10^4$ l mol$^{-1}$ cm$^{-1}$. The method is suitable for the determination of the nitrite ion in a 1.0-cm cell in the range from a few parts per hundred million to about 1 p.p.m. The relative standard deviation of the method is 1.5% at a concentration of 1 p.p.m. Some of the problematic interferents found in most of the analytical procedures for the determination of nitrites are tolerated in the present method. Features of the method include good selectivity and reproducibility, high stability of the coloured reaction product and simplicity.

*Keywords*: *Spectrophotometry*; *nitrite micro-determination*; *water*; *chelation*; *zirconyl ion*

**J. GABBAY, Y. ALMOG, (the late) M. DAVIDSON and A. E. DONAGI**

Research Institute for Environmental Health, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel.

*Analyst*, 1977, **102**, 371–376.

## Determination of Residues of Substituted Phenylurea Herbicides in Grain, Soil and River Water by Use of Liquid Chromatography

A rapid and sensitive procedure is described for the determination of trace amounts of eight substituted phenylurea herbicides. High-performance liquid chromatography is used, with an ultraviolet spectrophotometric detector. The herbicides are extracted from grain and soil samples with methanol and from water samples with dichloromethane. They are chromatographed on microparticulate silica bonded with octadecyltrichlorosilane using a mixture of methanol, water and ammonia as mobile phase.

*Keywords*: *Herbicide residues determination*; *phenylurea herbicides*; *high-performance liquid chromatography*

**D. S. FARRINGTON, R. G. HOPKINS and J. H. A. RUZICKA**

Department of Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, SE1 9NQ.

*Analyst*, 1977, **102**, 377–381.

## Determination of Nanogram and Sub-nanogram Amounts of Europium and Cerium by Means of Candoluminescence Emission in the Presence of a Co-activator

The candoluminescence emissions of cerium and europium from a calcium oxide - calcium sulphate matrix are greatly enhanced if mineral acids are added with the activator solution to the matrix, before it is placed in a nitrogen - hydrogen - air flame. If sulphuric acid is used, 0.01–15 ng of cerium and europium in 1 $\mu$l of solution can be determined. Some metal ions interfere and an attempt is made to explain the interference mechanisms.

*Keywords*: *Europium determination*; *cerium determination*; *candoluminescence emission*

**R. BELCHER, TARIK A. K. NASSER and ALAN TOWNSHEND**

Department of Chemistry, University of Birmingham, P.O. Box 363, Birmingham, B15 2TT.

*Analyst*, 1977, **102**, 382–390.

### Determination of Trace Amounts of Cerium in Silicate Rocks Based on its Candoluminescence in a Calcium Oxide Based Matrix

*Short Paper*

*Keywords*: *Cerium determination*; *silicate rocks*; *candoluminescence emission*

**R. BELCHER, TARIK A. K. NASSER, L. POLO-DIEZ and ALAN TOWNSHEND**

Department of Chemistry, University of Birmingham, P.O. Box 363, Birmingham, B15 2TT.

*Analyst*, 1977, **102**, 391–392.

### Determination of the Rate of Hydrolysis of Ytterbium Sulphide Films by Carbon Furnace Atomic-emission Spectrometry

*Short Paper*

*Keywords*: *Ytterbium sulphide films*; *glass surfaces*; *rate of hydrolysis*; *carbon furnace atomic-emission spectrometry*

**D. LITTLEJOHN and J. M. OTTAWAY**

Department of Pure and Applied Chemistry, University of Strathclyde, Cathedral Street, Glasgow, G1 1XL.

*Analyst*, 1977, **102**, 393–395.

### Determination of Several Elements in Lateritic Ores by Instrumental Neutron-activation Analysis

*Short Paper*

*Keywords*: *Lateritic ores*; *nickel ores*; *metals*; *neutron-activation analysis*

**S. AHMAD and D. F. C. MORRIS**

Nuclear Science Centre, School of Chemistry, Brunel University, Uxbridge, Middlesex, UB8 3PH.

*Analyst*, 1977, **102**, 395–399.

### Rapid Thermal-decomposition Technique for the Atomic-absorption Determination of Mercury in Rocks, Soils and Sediments

*Short Paper*

*Keywords*: *Mercury determination*; *minerals*; *atomic-absorption spectrophotometry*; *thermal decomposition*

**R. A. NICHOLSON**

Institute of Geological Sciences, Geochemical Division, 64–78 Gray's Inn Road, London, WC1X 8NG.

*Analyst*, 1977, **102**, 399–403.

### A Simple Matrix Modification Procedure to Allow the Direct Determination of Cadmium in Blood by Flame Micro-sampling Atomic-absorption Spectrophotometry

*Communication*

*Keywords*: *Cadmium determination*; *blood*; *matrix modification*; *flame micro-sampling atomic-absorption spectrophotometry*

**H. T. DELVES**

Trace Metals Laboratory, Department of Chemical Pathology, The Hospital for Sick Children and Institute of Child Health, 30 Guilford Street, London, WC1N 1EH.

*Analyst*, 1977, **102**, 403–405.

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