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THE ANALYST

EDITORIAL

Further Development of *The Analyst*

SIX months ago the Society introduced a revised "Notice to Authors" and, simultaneously, a modified system for subjecting papers to the scrutiny of referees. At the same time we mentioned in an Editorial some alterations in the appearance of *The Analyst* that had taken place at the beginning of the year. Then, after outlining the changed responsibilities of the Editorial (formerly Publication) Committee, and the formation of the Publications Policy Committee, we said that many proposals of the *Analyst* Development Committee remained to be implemented. These changes (like the change in the paper stock on which the journal is printed) could not be introduced except at the beginning of a volume, but will take place in January, 1964.

The most important of these proposals was undoubtedly one that the Proceedings of the Society, *i.e.*, the reports of the activities of the Society, its Sections and its Groups, other "domestic" news pertaining to the Society, obituaries, and certain other items, should be published separately from the scientific matter at present making up most of *The Analyst*. The Publications Policy Committee has given further consideration to this proposal and has studied possible alternatives and matters of detail. Its report has been accepted by Council, which has decided that from January *The Analyst* shall be devoted entirely to matters appropriate to the journal of a learned Society. It will contain Special Lectures, Review Papers, Original Papers (including Short Papers), Book Reviews, Communications and, when required, Editorials.

Communications are a new feature being introduced in response to requests for a medium for the prompt publication of urgent material. It is not intended for simple claims to priority, but rather for the brief description of work that has made some progress and then has stopped for lack of time, or of resources, or even of inspiration. Publication has a twofold purpose: the ideas, although not fully worked out, may yet be valuable to workers on other problems, or other workers may be able to provide the necessary inspiration or resources to permit the completion of the original piece of research.

Certain conditions must be fulfilled. Manuscripts must obviously be on urgent matters of some scientific importance (for time does not permit examination by referees), and must not exceed 300 words. They cannot include diagrams, although there will be no objection to formulae or tabular matter. Timing also precludes any question of appeal against the Editor's refusal to include a particular Communication, although a manuscript so refused can always be submitted to the Editorial Committee for its decision after the usual examination by referees. In return *The Analyst* offers publication in a minimum time from receipt of 4 to 5 weeks, which is the length of the final stage of the production schedule. To this it may be necessary to add 30 days should a script arrive 1 day too late for a particular issue, making the maximum time 9 weeks. Such rapid publication can only be achieved for a small proportion of the material in each issue.

The *Proceedings of the Society for Analytical Chemistry* will bring the rest of the traditional contents of *The Analyst* to members and non-members alike, and will also incorporate notices

of forthcoming meetings and other material that has hitherto appeared in the *Bulletin*. The Publications Policy Committee foresees the possibility of the *Proceedings* developing as a vehicle for other material worthy of the widest possible dissemination. *Proceedings* will therefore be treated from its inception as a fully-fledged journal and will be supplied in due time with a title page and index to each volume to facilitate the binding of this record of the Society's activities.

Two other changes remain to be mentioned. The cover of *The Analyst* has been re-designed slightly to lay greater emphasis on the fact that it is the journal of our Society. Also, in response to several requests, space will be found among the advertisements for duplicate copies of summaries of papers in a form in which they can readily be cut out for pasting on index cards, a service already available in other major analytical journals.

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETINGS

AN Ordinary Meeting of the Society was held at 6.30 p.m. on Wednesday, October 9th, 1963, in the Main Chemistry Lecture Theatre, Imperial College of Science and Technology, Imperial Institute Road, London, S.W.7. The Chair was taken by the President, Dr. D. C. Garratt, Hon.M.P.S., F.R.I.C.

The subject of the meeting was "Thin-layer Chromatography" and the following papers were presented and discussed: "General Aspects," by L. J. Morris, B.Sc., Ph.D.; "Specific Separations on Impregnated Thin Layers," by L. J. Morris, B.Sc., Ph.D.; "Application of Thin-layer Chromatography to Inorganic Systems," by K. Burton, B.Sc., D. Lyons, B.Sc., G. Nickless, B.Sc., Ph.D., and F. H. Pollard, D.Sc., Ph.D.; "Effects of Alkyl and Alkenyl Substitution on the Chromatography of Quinols and Related Compounds," by D. McHale, B.Sc., Ph.D., A.R.I.C.; "Separation of Steroids on Microslides," by P. Oxley, M.A., B.Sc., A.R.I.C.; "Thin-layer Chromatography of Lipids," by B. W. Nichols, M.Sc., Ph.D.

DEATHS

WE record with regret the deaths of

Leonard Balmforth
Daryl Robert O'Dea
William Stross.

MIDLANDS SECTION

AN Ordinary Meeting of the Section was held at 7 p.m. on Tuesday, October 15th, 1963, at the Nottingham and District Technical College, Burton Street, Nottingham. The Chair was taken by the Chairman of the Section, Mr. W. H. Stephenson, F.P.S., D.B.A., F.R.I.C.

The following paper was presented and discussed: "Sugar in Foodstuffs—Some Newer Methods," by J. L. Buchan, M.Sc., A.R.I.C.

MICROCHEMISTRY GROUP

THE forty-first London Discussion Meeting of the Group was held at 6.30 p.m. on Wednesday, October 16th, 1963, at "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by Mr. T. R. F. W. Fennell, B.A.

A discussion on "The Determination of Boron" was opened by H. J. Cluley, M.Sc., Ph.D., F.R.I.C., and M. R. Hayes, A.R.I.C.

Circular Dichroism

A Review*

By R. D. GILLARD

(*Department of Chemistry, Imperial College of Science and Technology, London, S.W.7*)

Plane-polarised light may be resolved into *laevo*- and *dextro*-circularly polarised light. When it is passed through an optically active medium, the amplitudes of these components are initially equal, but if the refractive indices of the medium are different for each component, there is rotation of the emergent light, which is now elliptically polarised. The phenomenon of circular dichroism (the differential absorption of *laevo*- and *dextro*-circularly polarised light) is a function of wavelength and the shape of the molecules in the medium.

This short review draws attention to a technique that may well prove invaluable in analysis.

INTEREST in optical activity has recently revived.[1] The most commonly used property of optically active molecules in research applications has been the optical rotatory dispersion curve (the variation of specific rotation of plane polarised light with the wavelength of the light). Several valuable reviews of this technique are available, for example, those of Djerassi[2] and Klyne,[3] but so far it has not attracted attention as an analytical method, probably because the rotatory dispersion curves of single substances are not easy to interpret, and it is difficult, although not impossible, to draw quantitative conclusions from the rotatory dispersion curves of mixtures.

Plane polarised light may be resolved into two circularly polarised components with equal amplitudes. Optical rotatory power is attributed to a difference in refractive indices of the optically active material for *laevo* (left) and *dextro* (right) circularly polarised light. If η_l (the refractive index for left circularly polarised light) is larger than η_d (that for right circularly polarised light), the left circularly polarised light is delayed in traversing the medium, giving rise to a rotation of the plane-polarised resultant of the left and right circular components. In passing through absorption frequencies of an optically active molecule, the absorption of the left-handed component differs from that of the right-handed component. With the usual notation of ϵ for molar extinction coefficient and with appropriate suffixes, circular dichroism at any wavelength is given by $\epsilon_l - \epsilon_d$, which we call $\Delta\epsilon$. Cotton[4] observed this in solutions of the tartrates of transition metals, and the phenomenon became known as the Cotton effect.[5] Since circular dichroism arises through electronic transitions, the spectra produced are similar to ultraviolet and visible spectra, but, for each circular dichroism band, $\Delta\epsilon$, $(\epsilon_l - \epsilon_d)$, may be either positive or negative, depending on the handedness of the molecule concerned. The requirements for the observation of a circular dichroism band are seen to be an optically active (asymmetric) molecule and an electronic absorption band. Any chromophore will give rise to circular dichroism bands; an example is the carbonyl ($>C=O$) absorption ($n \rightarrow \pi^*$) at 3000 Å. The circular dichroism of this band for a 16-ketosteroid is shown in Fig. 1.

Measurement

The most obvious method of obtaining circular dichroism, $\Delta\epsilon$, is to measure the difference in optical density for *laevo*- and *dextro*-circularly polarised light; apparatus has been described to permit this to be done.[6] However, the method generally used involves the fact that, after plane polarised light has passed through an optically active medium *in a region of electronic absorption*, the circular components have been unequally absorbed, so that on recombination of the unequal components, elliptically polarised light is obtained instead of plane, polarised light. As the ellipticity is a function of the difference in absorption of *laevo*- and *dextro*-circularly polarised light, it is directly related to circular dichroism. The molecular ellipticity is given by—

$$[\theta] = 3300\ \Delta\epsilon.$$

Most measurements of circular dichroism have been based on ellipticity determinations, as noted by Mitchell.[7] A recent commercial instrument, in which an oscillating crystal technique[8] is used, is also based on this well tried principle.

* Reprints of this paper will be available shortly. For details, please see p. 902.

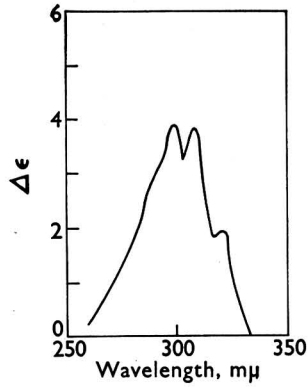

Fig. 1. Circular dichroism for ($n \rightarrow \pi^*$) band of carbonyl group in a 16-ketosteroid

CHEMICAL APPLICATIONS

The principles implicit in the previous discussion may be summarised as:

(*a*) Circular dichroism is a function of wavelength, λ, with positions of maxima coincident with visible or ultraviolet absorption bands. However, two bands that are not resolved in the ordinary electronic absorption spectrum are often separate in circular dichroism. The selection rules for circular dichroism and electronic absorption are often different, which leads to useful differences in band shapes.

(*b*) The sign of a circular dichroism peak depends on the actual shape ("handedness" or "absolute configuration") of the molecule causing it.

So far, circular dichroism has been studied in very few systems. Its uses seem likely to be in the fields detailed below:

(*i*) Analytical studies of mixtures, either from reactions or from natural sources. This will be discussed later.

(*ii*) Relative and absolute configurations of natural products such as steroids, terpenes, proteins and sugars. For example, the absolute configuration of the diterpenoid, cafestol, has been shown[9] to be the opposite to that previously deduced from optical rotatory dispersion. Several other erroneous configurations based on optical rotatory dispersion have also been corrected[9] by studies of circular dichroism.

(*iii*) Determination of structural features in complex organic molecules. It has been found[10] that the circular dichroism of the 20-cyano-derivatives of 11-ketosteroids permitted the isomers to be distinguished and formulated, whereas neither the nuclear magnetic resonance nor infrared spectra were sufficiently distinctive to be useful. Similar applications are feasible in inorganic complex compounds. Since more circular dichroism bands appeared for the (−)trisoxalatocobaltate^{III} anion, $(-)[Co(C_2O_4)_3]^{3-}$, than are expected for an ion with D_3 symmetry, the presence of some aquated ions of lower (C_2) symmetry was postulated,[11] with the formula $[Co(C_2O_4)_2(C_2O_4H)\ (OH_2)]^{2-}$.

(*iv*) Relative and absolute configurations of octahedral co-ordination compounds. It has been deduced from the equivalence of the circular dichroism curves[12] for the $(-)_{546\cdot1}[Co(EDTA)]^-$ and the $(-_{546\cdot1}[Co(+PDTA)]^-$ ions (where EDTA is ethylenediaminetetra-acetate and +PDTA is dextrorotatory propylenediaminetetra-acetate), that their relative configurations are the same. The absolute configuration of $(-)_{546\cdot1}[Co(+PDTA)]^-$ is known (Fig. 2), so that the absolute configuration of $(-)_{546\cdot1}[Co(EDTA)]^-$ is seen to be the same.

(*v*) Because of the differences in selection rules for circular dichroism compared to electronic spectra (magnetic-dipole-allowed transitions appear strongly in circular dichroism), distinctions may be made between spectroscopic bands. Confirmation was obtained in this

way of the assignment[13] of the lowest energy transition ($^1T_1 \rightarrow {}^1A_1$) in octahedral cobalt^{III} complexes. It was noticed in studies of optical rotatory dispersion that 2-α-iodocholestan-3-one gave rise to a strong Cotton effect centred at about 300 mμ, whereas no ultraviolet absorption band was observed in this region. Re-examination of the compound by Gillard (unpublished work) and Bose and co-workers[14] showed that the ultraviolet band at 298 mμ had an ϵ_{max} value of less than 4, whereas the circular dichroism band had a value for $(\epsilon_l - \epsilon_d)$ of about 1, so that the magnetic dipole character of the transition responsible for the circular dichroism band is beyond doubt.

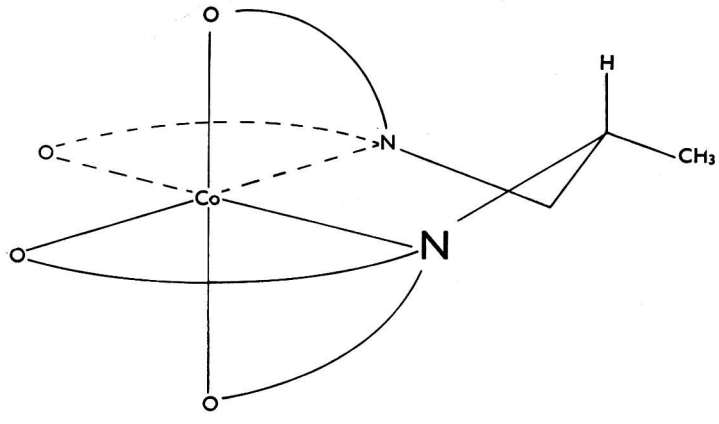

Fig. 2. The absolute configuration of $(-)_{546\cdot1}[Co(+PDTA)]^-$ (each curved chelate ring represents $-CH_2COO$)

Analytical uses

Wherever optical activity occurs, as in nearly all natural products, circular dichroism is an obvious means of measuring both chemical and optical purity, since circular dichroism is a function of the concentration of the optically active species giving rise to it. For example, in the carbohydrate field, pure methyl-2-oxo-3,4-isopropylidene-β-L-arabinoside shows a circular dichroism maximum of $\Delta\epsilon = -1{\cdot}8$ at 312 mμ. However, as in any spectrophotometric method, circular dichroism is useful only for chromophores absorbing between about 2000 and 7000 Å. Concentrations required are about the same as those used for other spectrophotometric work, and the results are accurate to approximately 1 per cent. The amount of an optically active substance in an otherwise inactive mixture may be determined; applications to such materials as pine oil distillates and pyrethrum products are obvious.

The occurence of the circular dichroism of a particular chromophore, such as carbonyl or nitro groups, within a narrow range of wavelengths offers possibilities for a "fingerprint" method of qualitative analysis for functional groups, particularly in synthetic products. So far, the characteristic circular dichroism of only a few functional groups is known, but more examples are being added rapidly. One might expect at least the chromophores listed below to find application: for α-amino-acids, the *N*-phthaloyl[15] and thiourea[16] derivatives; for cyclo-alkenes, the osmate esters[17]; for α-substituted carboxylic acids, the acylthiourea derivatives[18]; and for α-hydroxyacids the dithiocarbonate derivatives.[16] Other chromophores that have been used in optical rotatory dispersion include thiones,[19] disulphides,[20] *N*-nitroso-amides[21] and nitrites.[22] (References are to optical rotatory dispersion work.)

The cobalamin system presents many analytical problems; an interesting recent study[23] presents the circular dichroism curves for several cobalamins. These curves show much more marked differences than the absorption spectra; for example, for cyanocobalamin and aquocobalamin, which have similar absorption spectra, the circular dichroism bands at 500 mμ are, respectively, negative and positive, and the signs of several other bands are also different for the two compounds.

Conclusion

Little analytical work involving the use of circular dichroism has appeared to date, though mixtures of 11-ketosteroids and 20-ketosteroids have been analysed by circular dichroism.[24] However, circular dichroism has many potential applications, particularly in the field of natural products, for which the technique may prove useful for handling mixtures now amenable only to complicated treatments.

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Information Retrieval in the Analytical Laboratory

A Review*

By D. R. CURRY

(*Overseas Geological Surveys (Department of Technical Co-operation), 64–78, Gray's Inn Road, London, W.C.1*)

The potential applications of modern information-retrieval techniques in the analytical laboratory are outlined. A summary is given of the simple manual punched-card systems available. Attention is drawn to published material on the various aspects of the subject.

EVERY analytical laboratory deals with information, both as a receiver and as a creator. The process of analysis gives a continuing stream of new information about samples being examined. It is desirable that this information should be stored accessibly so that the effort originally expended in getting the analytical result is not wasted. At the same time the analytical chemist has, as the starting material for many of his investigations, the ever increasing scientific literature. In both these aspects the techniques of information retrieval may be useful. Information retrieval in this context is taken to mean manual systems as opposed to automatic data-processing techniques requiring the use of electronic computers. Save in exceptional circumstances the use of electromechanical card-sorters is seldom justified. They achieve little that cannot be done manually and may frequently take longer. In this paper the requirements of information retrieval are discussed under three headings—

(*a*) Laboratory records of work done;

(*b*) Reference data for instrumental methods;

(*c*) Literature.

As the analytical chemist is personally concerned in these problems, it is most strongly urged that study of the techniques available will prove worthwhile. Purely office routines are properly the concern of an O. and M. Officer, but manipulation of scientific facts should not be delegated to unqualified personnel.

THE PROBLEM OF LABORATORY RECORDS—

Most analytical laboratories, whether routine or research, have some method of recording samples, in which a costing procedure may also be incorporated. The system used should be able to provide the head of the laboratory with up-to-date information on the work in hand. At the same time records of past work done should not be left to memory, since the staff may change or retire. It should give all relevant details on previous examinations of particular types of sample; and also, for administrative purposes, show the numbers of these types of sample being analysed (*i.e.* how the flow of work is varying).

INSTRUMENTAL METHODS AND LITERATURE—

In many instrumental methods the recorded characteristics of a sample are assessed by comparison with similar records of known materials. At the same time the collection of standard records should be accessible via the various materials or classes of materials. In this way one should be able to find the characteristics that a series of organic homologues may have in common.

Many analytical laboratories are only small parts of larger organisations and their libraries may not, either through limitations of staff or knowledge of subject, be able to provide the analytical chemist with an adequate bibliographic service. Analytical information may not be found exclusively in the primary analytical literature or in abstracts, as some relevant material is bound to appear in the general literature of the field in which the analyst is working, *e.g.* petroleum, metallurgy or semi-conductors. The complexities of cross referencing often dissuade the analytical chemist from maintaining his own conventional card index. At the

* Reprints of this paper will be available shortly. For details, please see p. 902

same time the analytical chemist may partly remember the reference of a particular item of interest (*e.g.*, author or journal) and an index should also answer problems from this approach.

In all the instances mentioned above, records and information accessible in the laboratory and under the control of chemical staff are needed. Before considering detailed methods for tackling these problems, a brief outline is given of the various types of manual punched cards.

MANUAL PUNCHED CARDS

EDGE-PUNCHED CARDS—

Edge-punched cards are the simplest of manual punched cards, and their form is familiar to most people.[1 to 8] The cards have a series of holes along the edges, so that a slot may be formed by punching away the small amount of card between the hole and the edge. When a pack of cards is aligned and a sorting needle inserted through one of the holes, those cards that have been slotted in that particular position will fall away. The holes may be assigned various meanings, and a given set or "field" of holes grouped together to represent letters or figures. It is also possible to have more than one row of holes along part of the edge, to increase the number of selections available. The cards may be sorted by hand as indicated or in some systems by simple frames holding a series of needles, so that all the slots in one edge of a series of cards can be sorted simultaneously. Systems have also been devised for punching plain cards and sorting them on an array of bars.[9]

BODY-PUNCHED CARDS—

The theory of body-punched cards is the same as that of edge-punch cards. Instead of slots at the edge, however, slots are punched to join pairs of holes in the body of the card. Sorting has to be carried out in a special cradle that permits all the slots to be examined simultaneously. There is an unpunched area on the card for written information.[10]

FEATURE CARDS—

Feature cards are known by various names—coincidence cards, Batten cards or more popularly "Peek-a-boo" cards. The principle on which these operate is the reverse of edge punched item cards and is illustrated in Fig. 1. A feature card is prepared for each feature or concept considered relevant to part of a collection of items (such as abstracts). Each item is assigned a serial number, and every feature card has a number of squares, each corresponding to a particular numbered item. When a feature corresponds to a particular item a hole is punched in that item's square in the relevant feature card. When a series of feature cards is superimposed, any co-incident holes represent items possessing all the features selected. Feature cards are punched for items, whereas edge-punched item cards are slotted for features.

COMPARISON OF ABOVE SYSTEMS—

For a small collection of items not requiring complex indexing, the edge-punched cards offer economy and convenience. They have an important use, since they also reveal any concurrence of features. For example, if a series of cards is selected from a collection by sorting for holes A, C and D, and all the selected cards are also slotted in position B, this is immediately obvious as another common feature.

Feature cards are most valuable when a higher degree of cross referencing of a large number of features is essential. Speed of sorting is greater than with either of the other two systems, provided that the number of items sought is small, as the items themselves have to be in a subsidiary numerical file. The feature-card system is most adaptable to changes of emphasis as new subjects of interest develop. It is usual with edge- or body-punched cards to use custom-printed cards, so that the user does not have to refer to a book for the meanings of the various holes. Although unused holes should be available in the original design of the card their use will be limited, whereas it is a simple matter to introduce new feature cards at any time.

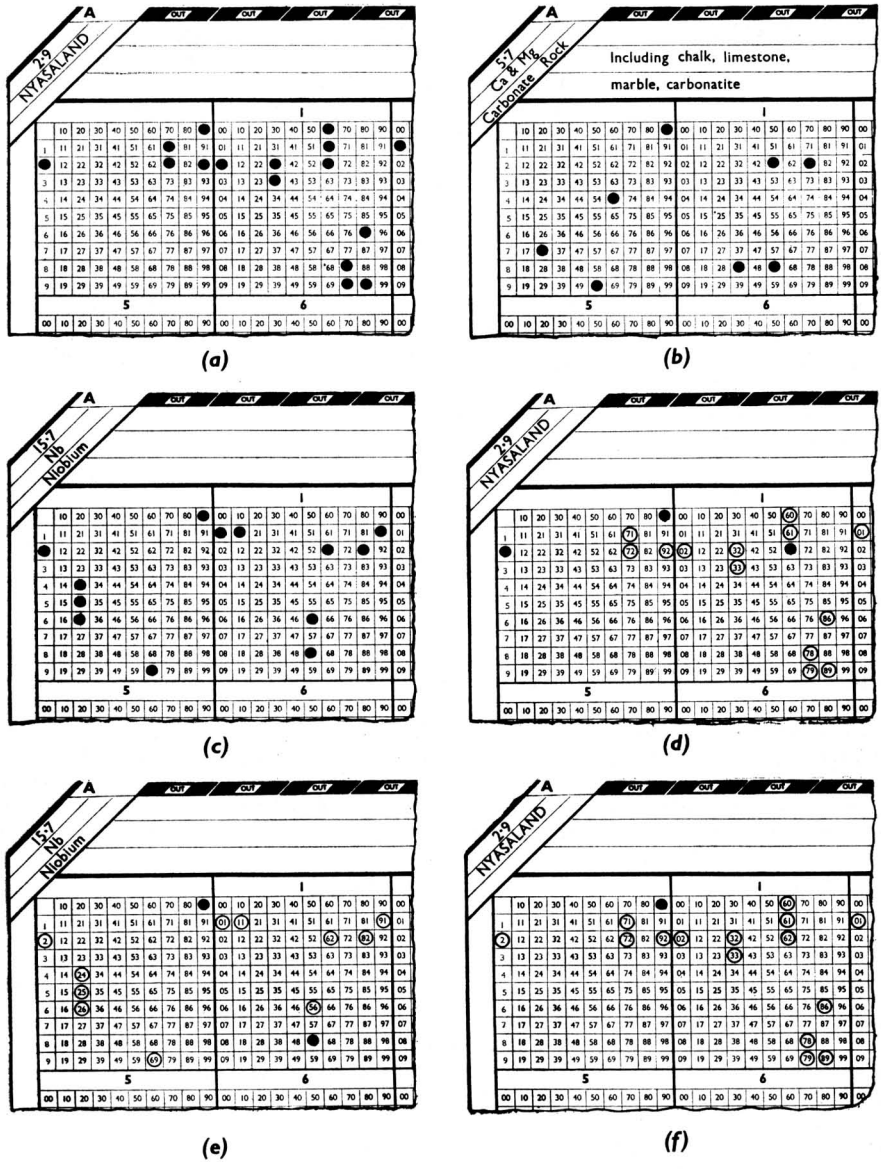

Fig. 1. Illustration of feature-card system[12]: (*a*), (*b*) and (*c*) are parts of feature cards from a sample index representing source, type of material and elements determined, respectively. Each hole in a card represents the numbers of samples having that particular feature. (*d*) shows the position when card (*a*) is superimposed on card (*c*) (*i.e.*, samples 2, 90 and 162 originated from Nyasaland and were analysed for niobium). Similarly (*e*) illustrates card (*c*) when placed on top of card (*b*), showing 90 and 158 as common holes. If all the cards are put together the result is shown by (*f*), only sample 90 being a carbonate rock from Nyasaland analysed for niobium. (Published by permission of the Controller, H.M. Stationery Office)

Laboratory records

Although there are various references to efficient laboratory organisation, for example see Kent-Jones,[11] little literature giving practical details of achieving such control has been published. One the earliest comprehensive systems used an edge-punched master card for each sample analysed[1] and for a routine laboratory, a simple system has been advocated.[2] In the United Kingdom one of the edge-punched cards in use for some years at the Macaulay Institute for Soil Research, Aberdeen, has been discussed.[3] There is an example of the use of body-punched cards for record keeping (although not specifically chemical),[10] and recently a machine has been designed[9] at the Royal Armament Research and Development Establishment, Fort Halstead, Sevenoaks, Kent for converting plain record cards for edge-notched sorting. The application of feature cards to laboratory records has also been reported.[12]

Reference data for instrumental methods

Before considering reference data in detail, it should be remembered that the quality of data retrieved can only be as good as the input. All authors, referees and editors of primary literature presenting data should give attention to this matter, which has been discussed by the Director of the Office of Critical Tables.[13]

X-ray diffraction—

Chronologically, the first reference data system available on a commercial basis was the "X-Ray Diffraction Data Index" published in 1942 by the American Society for Testing Materials. In this system the three strongest lines in terms of inter-planar spacing of the powder diffraction pattern are used to select a card giving the full details of the pattern and the reference to the source of information. When the series was initiated, plain cards were used and filed numerically, but with the use of edge-punched cards, advocated by Matthews,[4] the three strongest lines and the chemical composition could be indexed. Practical use, however, established that the cumulative index book was most efficient, except for special searches meriting the use of I.B.M. cards. More recently, a proposal by Dr. Matthews to use a feature card index has been adopted.[14] Whereas the edge-punched cards were supplied neither punched nor marked for punching, the set of 150 feature cards covering 5,698 substances are supplied punched, and it is stated that "as new sections are added to the data file the feature cards can be returned for the additional data to be entered." The 10,000 position feature cards should therefore facilitate searching the index for identification purposes.

Infrared spectra—

In the United Kingdom there are two common systems for indexing infrared spectra. The Sadtler[15] system is in book form and has various indexes covering formula, name of compound and also the "Spec-finder" for identification of unknown spectra. In this procedure the strongest bands in twelve sections of an unknown spectrum are listed and compared with a numerical list automatically prepared by computer. A separate section of the Sadtler index deals with commercial products.

The Butterworth-D.M.S.[16] system was described by Thompson[5] and involves the use of edge-punched cards, which are available punched or marked ready for punching. The strongest bands in the spectrum are indexed and also various structural features of the compound. The body of the card also gives bibliographic details and physical properties in addition to the full spectrum. In 1960 a second series of cards was started, in which the same indexing procedures for inorganic spectra was used. Concurrently with the spectral cards, a series of literature abstract cards is supplied; these are similar edge-punched cards indexing author or authors, year of publication and the topics covered (theories, apparatus, etc.). Recently, a feature-card index to the spectral series has been issued (DMS-I-Cards); 211 of these deal with an extended range of the holes in the original series, and the capacity of each card is 5000 items.

A valuable collective index[17] of available spectra has recently been compiled and will be kept up to date by annual supplements.

DIFFERENTIAL THERMAL ANALYSIS—

The latest recruit to punched card data indexes is the Scifax DTA data index issued in 1962.[18] This is based on a simple edge-punched card, the body of which contains details of the differential thermal analysis record and bibliographic references. One edge is supplied punched with principal peaks, the remaining edges being left free for the user to punch as required, although for minerals it is suggested that the Hey classification be used.

LITERATURE SYSTEMS

The universal decimal classification for analytical chemistry,[19] class 543, may serve its purpose for arranging books, but a single glance should reveal its inadequacy for indexing complex papers in the literature. The problems associated with comprehensive indexing of analytical literature have received more attention than other aspects of information retrieval previously considered. Three papers on edge punched cards[6,7,8] give various examples of the different ways information can be coded by using numbers and letters. It is possible to index authors' names, substances, subjects and dates, thus permitting a reasonable amount of cross referencing.

Recently in American literature a co-ordinate indexing system has been introduced.[20] This is based on the feature-card principle, although for economy the method suggested is the 'Uniterm' card system. In my opinion this is a false economy, since the manual effort of entering numbers on cards and recognising correlations far outweighs the additional cost of feature-card equipment if the capacity of the system is likely to exceed 500 items. A feature-card system specifically applied to inorganic analytical literature and having single sided *Analytical Abstracts* as its foundation has been described. This largely obviates the need for extensive coding of subjects, and makes the index more useful to the analytical chemist in the laboratory.[12]

CONCLUSION

Information retrieval techniques have many applications in the analytical laboratory. It is vital to determine the probable scope required before deciding on the optimum system. Factors to be considered include the type of information available, the information to be retrieved, reasons for requiring the information and the method of seeking it. If a collection of spectra is not to be used to determine the spectrum of compound X, it is unnecessary to include a formula index. When the probable size and nature of material to be included in an index have been determined, the various mechanical means of achieving an efficient form can be considered.

Small systems of transient interest and those to which reference will be made infrequently and from a particular aspect are probably most easily derived by using edge-punched cards. Feature cards are adaptable and most efficient when the population of the system is measured in thousands. The upper capacity limit of a manual system depends again on use.

Anyone considering setting up a system of any size for information retrieval will find study of two books helpful.[21,22] For those wishing to keep abreast of this rapidly growing subject there is the *Journal of Chemical Documentation* issued by the Division of Chemical Literature of the American Chemical Society. A comprehensive review of the state of the subject in the United States has been published,[23] and, in the general field of information retrieval, *ASLIB Proceedings* and the series issued by the National Science Foundation "Current Research and Development in Scientific Documentation" are of interest.

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Analytical Methods Committee

REPORT PREPARED BY THE ADDITIVES IN ANIMAL FEEDING STUFFS SUB-COMMITTEE

THE Analytical Methods Committee has received the following report from its Additives in Animal Feeding Stuffs Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

In 1958 The Society for Analytical Chemistry received a request from the Scientific Sub-Committee of the Standing Advisory Committee, Fertiliser and Feeding Stuffs Act, 1926, to the Ministry of Agriculture, Fisheries and Food for advice on the availability of suitable methods of analysis for the various types of additives commonly included in animal and poultry feeding stuffs.

The matter was referred to the Analytical Methods Committee of the Society, which appointed the Additives in Animal Feeding Stuffs Sub-Committee to study this problem. The additives involved fall into six principal groups—antibiotics, synthetic hormones, minerals, prophylactics, and water-soluble and oil-soluble vitamins. On the basis of this classification, six panels were set up by the Sub-Committee to investigate suitable methods of analysis for the selected groups of additives.

The growing practice of adding to animal rations not only vitamins and nutritional supplements, but also drugs and medicaments for the stimulation of growth or control of disease has set new problems in analysis and manufacturing control. A particular feature of this development has been the need to bring together two hitherto unrelated fields of analytical activity—that of the pharmaceutical industry on the one hand and of the animal feeding stuffs industry on the other.

Not only has the supplemented feed (as fed to the animal) been considered, but also the higher-potency preparations supplied as diet supplements (pre-mixes), either for intimate admixture by the feeding-stuffs manufacturers or for individual administration by the user. Although analytical methods exist (published or unpublished) for most, if not all, of the substances *per se* used as additives, in few instances has it been established that such methods could be applied to the determination of these additives when present in feeds. To a lesser extent, this also applies to the higher-potency supplements. Broadly speaking, there are three factors to be taken into account when applying an analytical method to feeding stuffs; these are—

(*i*) the sensitivity of the method (because the additive in question may be present at low concentration);

(*ii*) the interference from the ingredients of the basic feed;

(*iii*) the interference from other additives (in some instances, the identity of these may not be known to the analyst).

The representative sampling of feeding stuffs is of considerable importance in the analysis of the products because of the low concentrations of additives usually encountered. This aspect, however, does not strictly come within the purview of the Sub-Committee's work, and so, except in Part 5 of this Report, which deals with the determination of oil-soluble vitamins, no recommendations have been made. In order to assess the precision of any proposed method, each Panel prepared special samples, in addition to using marketed compound feeding stuffs, for collaborative investigations, because experience has shown that extreme care is required in the admixture of additives, and that the degree of fineness of milling can be critical in ensuring the production of a uniform mixed sample for analytical purposes.

This report is divided into six parts, each part dealing with the work of one of the Panels, together with their recommended methods for determining the additives allocated to the particular panel.

PART 1. REPORT OF THE ANTIBIOTICS PANEL

The Determination of Penicillin, Chlortetracycline and Oxytetracycline in Diet Supplements and Compound Feeding Stuffs

Introduction

THE Antibiotics Panel was set up under the chairmanship of Mr. S. A. Price, and its membership was: Mr. A. J. Cavell, Mrs. J. Gammon, Mr. O. Hughes, Mr. W. P. Jones, Mr. G. Sykes (deputy Miss F. N. Mulholland) and Mr. S. Varsanyi, with Miss A. M. Parry as Secretary; Mr. A. H. Sexton, Mr. J. S. Simpson and Mr. J. H. Taylor also served on the Panel. The Panel was appointed to consider methods for determining antibiotics.

Only three antibiotics—penicillin, chlortetracycline and oxytetracycline—are permitted by law to be added to feeding stuffs in Great Britain. For this purpose they are available as diet supplements, with potencies ranging from about 1 to about 250 g of antibiotic per lb, for incorporation in the feeding stuff at the rate of a few pounds per ton, and as other much less concentrated preparations for incoporation at the rate of several hundredweights per ton. Procedures for determining the three antibiotics in both high-potency and low-potency diet supplements, as well as in compound feeding stuffs, have been devised.

Experimental and results

High-potency diet supplements—

In investigations of the extraction and assay of supplements the Panel was guided by information from the manufacturers of the products. Early collaborative tests confirmed that chemical as well as microbiological methods were applicable to these relatively high-potency products. The chemical methods recommended by the Panel for each of the three anitbiotics in their respective supplements are described in Appendixes I, II and III, and the results obtained by the collaborating laboratories in a final collaborative test with these methods are summarised in Tables I, II and III. Only one figure for each laboratory is included for a given antibiotic; in some instances this was the result of one determination and in others two or more.

Table I

Results of final collaborative test of the determination of penicillin in a diet supplement by the recommended chemical method

Sample contained approximately 1 g of procaine penicillin per lb.

Laboratory	Procaine penicillin, g per lb
A	1·15
B	1·03
C	1·02
D	—
E	1·04
F	1·14
G	0·92
Mean	1·05
Standard deviation	0·085
Coefficient of variation	8·1%

From the inter-laboratory variances of these results it was considered that the methods for the assay of high-potency diet supplements are satisfactory, and that, if several laboratories examine samples of this type containing penicillin or chlortetracycline in concentrations of about 1 or 3·6 g per lb., 19 out of 20 of the results might be expected to fall within ±20 per cent. of the means; with supplements containing about 5 g of oxytetracycline per lb, 19 out of 20 of the results might be expected to fall within ±10 per cent. of the mean.

LOW-POTENCY DIET SUPPLEMENTS—

The methods recommended below for compound feeding stuffs are generally suitable for these supplements.

COMPOUND FEEDING STUFFS—

The problems associated with the assays of compound feeding stuffs are much more complex. First, the concentrations of the antibiotics (1 to 20 g per ton) are so low that chemical methods are inapplicable and it is necessary to use microbiological procedures. Second, the composition of feeding stuffs is so variable and the range of additives used is so

TABLE II

RESULTS OF FINAL COLLABORATIVE TEST OF THE DETERMINATION OF CHLORTETRACYCLINE IN A DIET SUPPLEMENT BY THE RECOMMENDED CHEMICAL METHOD

Sample contained approximately 3·5 g of chlortetracycline hydrochloride per lb.

Laboratory	Chlortetracycline hydrochloride, g per lb
A	3·37
B	3·30
C	3·10
D	—
E	3·53
F	3·52
G	2·75
Mean	3·26
Standard deviation	0·296
Coefficient of variation	9·1%

TABLE III

RESULTS OF FINAL COLLABORATIVE TEST OF THE DETERMINATION OF OXYTETRACYCLINE IN A DIET SUPPLEMENT BY THE RECOMMENDED CHEMICAL METHOD

Sample contained approximately 5 g of oxytetracycline per lb.

Laboratory	Oxytetracycline (free base), g per lb
A	4·76
B	4·81
C	4·73
D	—
E	4·83
F	4·83
G	4·73
Mean	4·78
Standard deviation	0·176
Coefficient of variation	3·7%

extensive that a method found satisfactory for one feeding stuff will not necessarily be satisfactory for another.

At the beginning of the work the Panel agreed on the use of plate, rather than tube, methods for the microbiological assays, and the use of large plates rather than Petri dishes was preferred. It was also decided that the extraction methods to be used should be those that had been found satisfactory for the respective supplements by the antibiotic manufacturers themselves. For the early investigations, these extraction methods and the organisms to be used were defined, but the details of the microbiological techniques were left to the choice of the individual analysts.

The results of these trials were encouraging with penicillin and oxytetracycline, but with chlortetracycline it was apparent that the procedure had to be investigated more fully if accurate and precise results were to be obtained. Experiments were therefore undertaken to find the optimum conditions for plate assays, particularly as applied to chlortetracycline.

Among the factors examined were the constituents of the assay medium and its depth, the cavity size, the strain of test organism and whether or not the inoculum should be vegetative or a spore suspension, and the incubation temperature. Inocula and media were exchanged, and a detailed examination was made of the size and clarity of the inhibition zones in relation to the results obtained. It was evident from this work that the methods for all three antibiotics should be specified in considerable detail.

With some samples it is desirable that an unsupplemented sample of the feeding stuff under test should be available as a blank sample; it can then be extracted by exactly the same procedure as is applied to the test sample, and the extract used as a diluent for the standard, *i.e.*, to give a "modified standard." As an unsupplemented sample is rarely available in practice, the Panel also investigated methods for destroying or removing the antibiotic from the sample or the sample extract; the procedures found satisfactory for this purpose are included in the methods recommended for the assay of compound feeding stuffs described in Appendixes IV, V and VI. The methods drafted by the Panel specify in unambiguous detail the procedure to be followed in the extraction stage, but at the same time are sufficiently flexible to be carried out in any laboratory equipped for, and experienced in, this type of work. Throughout the collaborative studies, the Panel has been impressed by the importance of experience, not only in microbiological assays in general, but in the assay of each particular antibiotic. It is assumed also that anyone undertaking such assays is familiar with the statistical design and calculations involved. The results obtained by the collaborating laboratories in a final collaborative test with these methods are summarised in Tables IV, V and VI.

TABLE IV

RESULTS OF ASSAYS OF PENICILLIN IN COMPOUND FEEDING STUFFS MEASURED AGAINST VARIOUS STANDARDS

Key to Standards—Standard 1—Unmodified.
Standard 2—Modified with unsupplemented poultry meal extract.
Standard 3—Modified with supplemented poultry meal extract treated to remove the antibiotic.
Standard 4—Modified with unsupplemented pig meal extract.
Standard 5—Modified with supplemented pig meal extract treated to remove the antibiotic.

Laboratory	Penicillin in poultry meal			Penicillin in pig meal		
	Standard 1, g per ton	Standard 2, g per ton	Standard 3, g per ton	Standard 1, g per ton	Standard 4, g per ton	Standard 5, g per ton
A	5·55	5·50	5·21	5·05	5·28	4·90
B	6·10	6·10	5·50	5·57	5·90	5·60
C	5·40	5·29	5·70	5·10	5·30	5·50
D	5·12	5·34	—	5·05	5·58	—
E	5·19	5·59	5·15	5·07	5·12	5·17
F	5·63	5·51	5·49	5·36	5·52	5·23
Mean	5·50	5·55	5·41	5·20	5·45	5·28
Standard deviation	0·35	0·29	0·23	0·22	0·28	0·28
Coefficient of variation	6·46%	5·23%	4·25%	4·17%	5·14%	5·31%

At first sight it would appear from the Tables that the results outlined with unmodified standards do not differ materially from those obtained with modified standards. In some laboratories however, the simpler procedure, in which unmodified standards were used, occasionally resulted in invalid assays, because the dose - response curves were non-parallel. Provided that there is no such deviation from parallelism between the slopes, the simpler procedure, in which the standard is diluted with buffer solution rather than with unsupplemented or treated feed extract, may be used.

Some collaborating laboratories found evidence of non-parallelism of the response curves when unmodified standards were used; the means and standard deviations of results so obtained have not, therefore, been included in the Tables.

It must be emphasised that in using these methods it is assumed that the identity of the antibiotic is known and that only one is present. The determination of two or more

antibiotics present together in a feeding stuff is likely to present special problems to the analyst, and the presence of certain prophylactics and other drugs may complicate otherwise satisfactory microbiological methods. These are problems that could well occupy attention in the future, but their complete solution will probably depend on more detailed fundamental work as well as on collaborative investigation.

From the inter-laboratory variances shown in Tables IV, V and VI it may be stated that, if several laboratories experienced in microbiological assays of this type all assay the same samples of poultry or pig meals containing 5 g of penicillin or 10 g of chlortetracycline or oxytetracycline per ton, 19 out of 20 results may be expected to be within about 10 per cent. of the mean for penicillin, 20 per cent. of the mean for chlortetracycline and 25 per cent. of the mean for oxytetracycline.

TABLE V

RESULTS OF ASSAYS OF CHLORTETRACYCLINE IN COMPOUND FEEDING STUFFS MEASURED AGAINST VARIOUS STANDARDS

For Key to Standards—See Table IV

Laboratory	Chlortetracycline in poultry meal			Chlortetracycline in pig meal		
	Standard 1, g per ton	Standard 2, g per ton	Standard 3, g per ton	Standard 1, g per ton	Standard 4, g per ton	Standard 5, g per ton
A	8·51	9·95	9·90	7·41	9·77	8·90
B	10·00	—	9·20	9·30	—	8·60
C	8·34	8·99	8·91	9·27	9·35	9·35
D	9·84	10·01	9·39	9·27	9·64	8·74
E	7·97	9·05	8·97	10·02	9·75	10·03
F	7·67	8·91	7·14	8·47	8·90	8·33
Mean	8·72	9·38	8·92	8·96	9·48	8·99
Standard deviation	0·97	0·55	0·86	0·90	0·37	0·61
Coefficient of variation	11·2%	5·87%	9·64%	10·06%	3·91%	6·79%

TABLE VI

RESULTS OF ASSAYS OF OXYTETRACYCLINE IN COMPOUND FEEDING STUFFS MEASURED AGAINST VARIOUS STANDARDS

For Key to Standards—See Table IV

Laboratory	Oxytetracycline in poultry meal			Oxytetracycline in pig meal		
	Standard 1, g per ton	Standard 2, g per ton	Standard 3, g per ton	Standard 1, g per ton	Standard 4, g per ton	Standard 5, g per ton
A	7·89	8·98	8·71	7·85	8·76	8·78
B	9·15	11·75	11·45	9·10	10·85	11·10
C	9·53	9·37	9·42	9·32	9·31	9·10
D	7·66	9·29	8·69	8·39	10·0	9·17
E	9·21	9·66	9·80	8·80	9·18	9·72
F	11·05	11·40	10·70	9·73	9·87	9·80
Mean	9·08	10·07	9·79	8·86	9·66	9·61
Standard deviation	1·23	1·18	1·10	0·67	0·74	0·83
Coefficient of variation	13·5%	11·76%	11·28%	7·61%	7·66%	8·6%

ACKNOWLEDGMENTS

The Panel is grateful to the organisations in whose laboratories the work was carried out, and in particular to Messrs. Boots Pure Drug Co. Ltd., Cyanamid of Great Britain Ltd., Distillers Company Ltd., Glaxo Laboratories Ltd., Pfizer Ltd. and Vitamins Ltd. for generously supplying materials for the collaborative tests.

Appendix I

CHEMICAL DETERMINATION OF PENICILLIN IN DIET SUPPLEMENTS

PRINCIPLE OF METHOD—

The supplement is extracted with water, and the procaine penicillin in the extract is termined by the iodimetric method of the "British Pharmacopoeia 1958"[1] for total penicillins in benzylpenicillin (see Note 1).

APPLICABILITY AND RANGE—

The method is applicable to diet supplements containing not less than 1 g of penicillin per lb.

REAGENTS—

Water—Use distilled or de-ionised water.

The other reagents required are described in the "British Pharmacopoeia 1958."[1]

PROCEDURE

EXTRACTION OF SAMPLE—

(*a*) *For supplements containing* 1 *to* 5 *g of penicillin per lb*—Weigh accurately about 25 g of sample, and transfer quantitatively to a small glass mortar. Mix with a few millilitres of water, and grind to form a smooth paste. Transfer the mixture quantitatively to a tared 250-ml conical flask, rinsing in with water, and add sufficient water to the mixture to give a total weight of 175 g. Mix thoroughly, and set aside for 5 minutes, with occasional shaking. Filter the supernatant liquid through a fluted 24-cm Whatman No. 42 filter-paper, discard the first 20 ml of filtrate, and then collect 60 ml. Determine the procaine penicillin in the filtrate by the method described below (see Note 2).

(*b*) *For supplements containing* 16 *g of penicillin per lb*—Extract the sample as described above under (*a*), but use about 12 g of sample and make the weight of the mixture up to 162 g with water.

(*c*) *For supplements containing* 224 *g of penicillin per lb*—Weigh accurately about 2·5 g of sample, and transfer quantitatively to a glass mortar. Mix with a few millilitres of water, and grind to form a smooth paste. Transfer the mixture quantitatively to a 500-ml calibrated flask, dilute to about 400 ml with water, and set aside for 5 minutes, with occasional shaking. Dilute the mixture to the mark, mix well, immediately filter through a fluted 24-cm Whatman No. 42 filter-paper, discard the first 20 ml of filtrate, and then collect 60 ml. Determine the procaine penicillin in the filtrate by the method described below (see Note 2).

DETERMINATION OF PROCAINE PENICILLIN—

Determine the procaine penicillin in a 10-ml portion of the filtrate from the extraction of the sample by the iodimetric method for total penicillins exactly as described in the "British Pharmacopoeia 1958" under "Benzylpenicillin," p. 89 (see Note 3).

RESULTS—

Express the results as grams of procaine penicillin per lb of supplement.

NOTES

1. Since this report was prepared the "British Pharmacopoeia 1963"[2] has been published, and in this new pharmacopoeia a method is included for the determination of total penicillins in procaine penicillin. The B.P., 1963, method is similar to that recommended by the Panel, except that the procaine is first removed from solution by precipitation with sodium silicotungstate; as the procaine has been removed, it is unnecessary to apply the correction factor of 1·04 (see Note 3).

2. The analysis should be completed with the minimum of delay, because the aqueous penicillin solution slowly decomposes.

3. It is necessary, in order to determine the factor applicable in each laboratory, to standardise the volumetric reagents by using a standard preparation of benzylpenicillin sodium and to convert the factor obtained to a figure applicable to procaine penicillin by multiplying by the appropriate molecular-weight ratio; the molecular weight of benzylpenicillin sodium is 356·4 and that for procaine penicillin (monohydrate) is 588·7, so that the ratio of molecular weights is 1 to 1·652. It is generally recognised that when the iodimetric method is applied to procaine penicillin it gives results that are 4 per cent. low; it is recommended, therefore, that the factor be multiplied by 1·04 to correct for this.

Appendix II

CHEMICAL DETERMINATION OF CHLORTETRACYCLINE IN DIET SUPPLEMENTS

PRINCIPLE OF METHOD—

The method is based on that of Chiccarelli, Woolford and Trombitas.[3]

REAGENTS—

Water—Use distilled or de-ionised water.

Hydrochloric acid, 5 N.

Dilute sodium hydroxide solution—Dilute 8 ml of 5 N sodium hydroxide to 100 ml with water.

Sodium metabisulphite solution—A 10 per cent. w/v solution in water. The solution must be freshly prepared.

Phosphate buffer solution (pH 7·5)—Dissolve 178 g of dipotassium hydrogen orthophosphate, K_2HPO_4, and 22 g of potassium dihydrogen orthophosphate, KH_2PO_4, in 1 litre of water. Filter the solution before use.

Stock standard chlortetracycline solution—Transfer exactly 100 mg of chlortetracycline hydrochloride B.P. to a 100-ml calibrated flask, dilute to the mark at 20° C with water, and mix well. Store the solution in an amber-glass bottle at 5° to 8° C; under these conditions the solution is stable for one week.

Working standard chlortetracycline solution—Dilute 5·0 ml of stock standard chlortetracycline solution to 100 ml at 20° C with water, and mix well;

1 ml ≡ 0·05 mg of chlortetracycline hydrochloride.

Prepare the solution immediately before use.

PROCEDURE

EXTRACTION OF SAMPLE—

Weigh accurately a portion of the sample expected to contain approximately 5 mg of chlortetracycline hydrochloride (see Note 1) into a 100-ml calibrated flask. Add about 70 ml of water and 4 ml of 5 N hydrochloric acid, shake the mixture for 10 minutes, dilute to the mark with water, and mix thoroughly (see Note 2).

Filter a portion of the mixture through Celite filter aid, discarding the first 20 to 30 ml of filtrate, collect the clear filtrate, and determine the chlortetracycline in the filtrate by the method described below.

DETERMINATION OF CHLORTETRACYCLINE—

Transfer by pipette two 10-ml portions of the filtrate from the extraction of the sample into separate 50-ml calibrated flasks; these form the sample and sample blank solutions.

Transfer by pipette two 10-ml portions of the working standard chlortetracycline solution into further separate 50-ml calibrated flasks; these form the standard and standard blank solutions.

To the solutions representing the sample and the standard add, in order, 12 ml of 5 N hydrochloric acid, 15 ml of phosphate buffer solution (pH 7·5), 2 ml of sodium metabisulphite solution and 3 ml of dilute sodium hydroxide solution, and suspend the flasks in a bath of boiling water for exactly 7 minutes, swirling the contents occasionally (see Note 3).

To the solutions representing the sample blank and the standard blank add 15 ml of phosphate buffer solution (pH 7·5), 2 ml of sodium metabisulphite solution and 3 ml of dilute sodium hydroxide solution, and suspend the flask in a bath of boiling water, swirling the contents occasionally (see Note 3). After exactly 5 minutes, add 12 ml of 5 N hydrochloric acid, and heat for an additional 2 minutes.

Immediately after the completion of the heat treatment, cool the four flasks in running water, dilute the contents of each flask to the mark at 20° C with water, and mix well.

If a cloudiness develops in the solutions at this point, spin the sample and sample blank solutions in a centrifuge until clear.

Measure the optical densities, at 445 mμ, of the solutions representing the standard and the sample against their respective blank solutions with a suitable spectrophotometer.

CALCULATION—

The amount of chlortetracycline hydrochloride in the sample is given by the expression—

Chlortetracycline hydrochloride content =

$$\frac{(A_{445}\ \text{sample}) \times 0{\cdot}01 \times (\text{dilution of sample}) \times 453{\cdot}6}{(A_{445}\ \text{standard}) \times (\text{weight of sample}) \times 1000}\ \text{g per lb,}$$

where A_{445} = optical density at 445 mμ and

0·01 = final concentration of the standard in mg per ml.

RESULTS—

Express the results as grams of chlortetracycline hydrochloride per lb of supplement.

NOTES

1. The sample taken must not weigh less than 100 mg. If high-potency material is to be assayed and a weight of 100 mg contains more than 5 mg of chlortetracycline hydrochloride, take a sample weighing 100 mg or more, and make appropriate dilutions to give a concentration of 0·05 mg per ml. Increase the amount of 5 N hydrochloric acid used in direct proportion to the dilution.

2. The concentration of this solution is approximately 0·05 mg of chlortetracycline hydrochloride per ml.

3. It is essential that the water is boiling throughout the entire heating period.

Appendix III

CHEMICAL DETERMINATION OF OXYTETRACYCLINE IN DIET SUPPLEMENTS

PRINCIPLE OF METHOD—

The method is based on the measurement of the colour produced by reaction of oxytetracycline with ferric chloride in the presence of 0·01 N hydrochloric acid.

APPLICABILITY—

Ingredients such as phosphates, fluoride, thiocyanates, etc., that combine with ferric iron, interfere with the method. These interfering substances must therefore be absent or be removed before the analysis is undertaken.

RANGE—

For supplements containing not less than 5 g of oxytetracycline per lb.

REAGENTS—

Water—Use distilled or de-ionised water.

Dilute acetic acid—A 10 per cent. v/v solution of glacial acetic acid in water.

Concentrated hydrochloric acid, sp.gr. 1·16 to 1·18.

Hydrochloric acid, 0·01 N—Adjust to pH 2·0 if necessary.

Ferric chloride solution—A 0·05 per cent. w/v solution of ferric chloride, $FeCl_3.6H_2O$, in 0·01 N hydrochloric acid.

Standard oxytetracycline solution—Dissolve sufficient oxytetracycline hydrochloride in 0·01 N hydrochloric acid, and dilute with the same acid to give a solution containing 400 μg of oxytetracycline per ml.

PROCEDURE

EXTRACTION OF SAMPLE—

(*a*) *For supplements containing 5 g of oxytetracycline per lb*—Weigh accurately about 3 g of the sample into a 250-ml beaker, add 50 ml of acetic acid, and set the mixture aside for 20 minutes, stirring gently (see Note 1). Filter the solution quantitatively through a Buchner funnel, more than once if necessary, until the filtrate is absolutely clear. Wash the filter-paper with 10 ml of water, combine the filtrate and washings, and adjust the clear mixture to pH 2·0 ± 0·05 (measured with a pH meter) with a few drops of concentrated hydrochloric acid. Transfer the solution quantitatively to a 100-ml calibrated flask, rinsing in with 0·01 N hydrochloric acid, and dilute to the mark at 20° C with the same acid (see Note 2). Determine the oxytetracycline hydrochloride in the solution by the method described below.

(*b*) *For supplements containing 10 g of oxytetracycline per lb*—Extract the sample as described under (*a*), but use about 2·5 g of sample.

(*c*) *For supplements containing 25 g of oxytetracycline per lb*—Extract the sample as described under (*a*), but use about 1 g of sample.

DETERMINATION OF OXYTETRACYCLINE—

Transfer a 5-ml portion of the solution from the extraction of sample to each of two clean test-tubes. Add to one tube 15 ml of 0·01 N hydrochloric acid and to the other tube 5 ml of 0·01 N hydrochloric acid and 10 ml of ferric chloride solution, mix, and set aside at 20° to 25° C for 20 minutes. Measure the optical density of each solution in turn at 490 mμ., in a 1-cm cell with a suitable spectrophotometer.

Prepare, in 0·01 N hydrochloric acid, dilutions of standard oxytetracycline solution covering the range 0 to 400 μg of oxytetracycline per ml, and treat 5-ml portions of each dilution as described above for the solution from the extraction of the sample. Construct a graph relating the optical densities to the number of micrograms of oxytetracycline in each dilution.

By reference to this calibration graph, determine the amount of oxytetracycline in the solution from the extraction of the sample, and hence calculate the amount of oxytetracycline in the sample.

RESULTS—

Express the result as grams of oxytetracycline (free base) per lb of supplement.

NOTES

1. A magnetic stirrer is suitable.

2. If the material being assayed contains 5 g of oxytetracycline per lb, the concentration in this solution is approximately 300 μg per ml.

Appendix IV

MICROBIOLOGICAL ASSAY OF PENICILLIN IN DIET SUPPLEMENTS AND COMPOUND FEEDING STUFFS

Normal bacteriological procedures and precautions must be adopted in preparation of cultures, sterilisation of media and glassware, etc.

REAGENTS—

Assay medium—

Peptone	5·0 g
Lab. Lemco	3·0 g
Agar	15·0 g
Glass-distilled water	to 1 litre

The recommended procedure for preparing the medium is to dissolve the peptone and the Lab. Lemco in the water, adjust the solution to pH 7·0, stir in the agar, and transfer the mixture to an autoclave. Steam for 30 minutes, and then heat at 115° C for 20 minutes. Transfer 200- to 250-ml amounts (see Note 1) to 12-oz screw-capped bottles, and sterilise at 115° C for 20 minutes.

Acetone - phosphate buffer solution (*pH* 7·5)—Dissolve 9·78 g of disodium hydrogen orthophosphate, Na_2HPO_4, and 1·85 g of potassium dihydrogen orthophosphate, KH_2PO_4, in 200 ml of hot distilled water, cool, add 250 ml of acetone, and dilute to 1 litre with distilled water.

Phosphate buffer solution (*pH* 7·0)—Dissolve 5·0 g of dipotassium hydrogen orthophosphate, K_2HPO_4, and 3·9 g of potassium dihydrogen orthophosphate, KH_2PO_4, in distilled water, and dilute to 5 litres with distilled water. The solution may be stored; if so, it should be sterilised.

ORGANISM—

The organism used in the assay is *Bacillus subtilis* (NCIB 8236), or any other suitable strain, *e.g.*, ATCC 6633.

Maintenace of culture—Maintain cultures of the organism on slopes of either of the two sterile media listed below—

Peptone	6·0 g
Casein hydrolysate (enzymic)	4·0 g
Marmite	3·0 g
Lab. Lemco	1·5 g
Dextrose	1·0 g
Agar	15·0 g
Glass-distilled water	to 1 litre

Adjust to pH 6·5 to 6·6, and sterilise by heating at 115° C for 10 minutes in 10-ml bottles.

or

Difco Penassay Seed Agar

Sterilise by heating at 115° C for 10 minutes in 10-ml bottles.

Preparation of spore suspension—Transfer 200 ml of one of the media to a Roux bottle, and sterilise by heating at 115° C for 10 minutes. Cool, inoculate with the culture, and incubate at approximately 30° C for 7 to 8 days. Wash the growth from the surface of the medium with 150 ml of sterile glass-distilled water, spin the suspension in a centrifuge, re-suspend the deposit in sterile glass-distilled water, and dilute to match approximately opacity 4 on Brown's opacity tubes. Fill 1-oz screw-capped bottles with the suspension, pasteurise at 70° to 75° C for 30 minutes, and store in a refrigerator.

PROCEDURE

PREPARATION OF PLATES—

Melt the contents of a bottle of assay medium by steaming for approximately 1 hour, and cool to about 50° C in a water bath at this temperature.

Inoculate the melted medium with the spore suspension of *B. subtilis*—dilute the suspension, if necessary, and use an inoculum density found by previous experiment to give zone diameters of suitable size (see Note 2)—mix well, and pour on to a 12-inch × 12-inch sterilised plate (see Note 3). The plate must be supported on a level surface while the medium is being poured. Leave the plate at room temperature with the lid raised until the medium has set, and store in a refrigerator for at least 1 hour.

Cut 64 cups in the solidified medium with the aid of a template; use a No. 5 cork-borer (8 mm in diameter) or nearest available size.

PREPARATION OF STANDARD SOLUTIONS—

Dissolve 50 mg of benzylpenicillin sodium of known potency in phosphate buffer solution (pH 7·0), and dilute to 500 ml with the buffer solution (see Note 4).

Take a portion of the solution containing as much antibiotic activity as is estimated to be present in the test sample (*see Note* 5), *dilute to* 100 *ml with acetone - phosphate buffer solution* (*pH* 7·5), *and add* 20 *g of the blank* (*unsupplemented*) *feed* (*see Note* 6). *Shake for* 1 *hour, and then spin in a centrifuge, or filter.* Take a portion of the solution, dilute, if necessary, with acetone - phosphate buffer solution (pH 7·5) to give a solution containing 1·0 i.u. of penicillin per ml, and from this make a further dilution in acetone - phosphate buffer solution (pH 7·5) to give a solution containing 0·2 i.u. of penicillin per ml.

PREPARATION OF TEST SOLUTIONS—

Supplements—Weigh a 1-g sample, add 250 ml of acetone - phosphate buffer solution (pH 7·5), and shake vigorously. Spin in a centrifuge, or filter, and dilute the solution with acetone - phosphate buffer solution (pH 7·5) in accordance with the expected potency to the levels required in the assay, namely, approximately 1·0 and 0·2 i.u. of penicillin per ml.

Feeding stuffs—Weigh a 20-g sample, add 100 ml of acetone - phosphate buffer solution (pH 7·5), and shake for 1 hour. Spin in a centrifuge, or filter, and dilute the solution with acetone - phosphate buffer solution (pH 7·5) according to the expected potency to the levels required in the assay, namely, approximately 1·0 and 0·2 i.u. of penicillin per ml.

ASSAY—

By means of a statistically satisfactory assay design, such as suggested by Brownlee *et al.*,[4] Lees and Tootill,[5] Price and Boucher[6] or Simpson and Lees,[7] compare the test and standard solutions in a cup-plate assay by using the previously prepared plates.

Plate out the solutions according to the chosen assay design; deliver a uniform volume of 0·05 to 0·1 ml into each cup of the prepared plates. Set aside at room temperature for 1 hour to allow pre-diffusion, and then place in an incubator at approximately 30° C.

After incubation for 18 to 20 hours, measure the diameters of the inhibition zones with finely pointed vernier callipers or a projection device.

Check the parallelism of the test and standard responses, and, if satisfactory, calculate the potency, P, of the test solution from the equation—

$$P = \text{antilog}\left\{ \frac{(T_2 + T_1) - (S_2 + S_1)}{(S_2 - S_1) + (T_2 - T_1)} \times \log 5 \right\},$$

where T_2 = the total of the responses to the high dose of test solution,

T_1 = the total of the responses to the low dose of test solution,

S_2 = the total of the responses to the high dose (1·0 i.u. per ml) of standard solution, and

S_1 = the total of the responses to the low dose (0·2 i.u. per ml) of standard solution

Calculate the potency of the sample from the dilution employed.

RESULTS—

Express the results as grams of procaine penicillin per pound of diet supplement or per ton of compound feeding stuff.

NOTES

1. The volume of the medium should be such that when poured into a 12-inch × 12-inch plate the depth of medium is 0·1 inch.

2. Zone diameters reported by the Panel members ranged from 15 to 28 mm for a dose level of 1·0 i.u. per ml and from 10 to 20 mm for a dose level of 0·2 i.u. per ml. The increase in response for this five-fold increase in dose ranged from 5 to 10 mm.

3. Sterilisation may be achieved by heating in an autoclave or by swabbing with acid alcohol.

4. For the assay of diet supplements, the instructions in *italic type* can be omitted. They may also be omitted in the assay of compound feeding stuffs if, by previous experiment, it has been found that the test response is parallel to that of an "unmodified standard."

5. Take a 0·6-ml portion for a feeding stuff estimated to contain 5 g of procaine penicillin per ton.

6. If unsupplemented feed is not available, a suitable preparation can be made by steaming a portion of the test sample for 1 hour or heating in an autoclave at 120° C for 15 minutes.

Appendix V

MICROBIOLOGICAL ASSAY OF CHLORTETRACYCLINE IN DIET SUPPLEMENTS AND COMPOUND FEEDING STUFFS

Normal bacteriological procedures and precautions must be adopted in preparation of cultures, sterilisation of media and glassware, etc.

REAGENTS—

Assay medium—

Yeast extract	1·0 g
Ammonium nitrate	5·0 g
Sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$), B.P. grade	5·0 g
Dextrose	5·0 g
Agar	15·0 g
Glass-distilled water	to 1 litre

The recommended procedure for preparing the medium is to dissolve the yeast extract, ammonium nitrate and sodium dihydrogen orthophosphate in the water, adjust the solution to pH 7·5, stir in the agar, and transfer the mixture to an autoclave. Steam for 30 minutes, and then heat at 115° C for 20 minutes. Remove from the autoclave, add the dextrose and filter clear through a prepared paper-pulp pad about 0·5 inch thick. Cool, and adjust the solution to pH 7·0. Transfer 200- to 250-ml amounts (see Note 1) to 12-oz screw-capped bottles, and sterilise at 115° C for 20 minutes. The final pH of the medium is 6·7.

Aqueous acid - acetone mixture—Mix 40 ml of hydrochloric acid, sp.gr. 1·16 to 1·18, with 1300 ml of acetone, and dilute to 2 litres with distilled water.

Sodium hydroxide, N.

Phosphate buffer solution (*pH* 4·5)—Dissolve 13·6 g of potassium dihydrogen orthophosphate, KH_2PO_4, in distilled water, and dilute to 1 litre with distilled water.

Hydrochloric acid, N.

Sodium chloride solution—A 5·85 per cent. w/v solution in distilled water.

ORGANISM—

The organism used in the assay is *Bacillus cereus* NCIB 8849 or 9231 (ATCC 11778).

Maintenance of culture—Maintain cultures of the organism on slopes of "Oxoid" Blood Agar Base ("Oxoid" Nutrient Broth + 1·5 per cent. of agar) that has been sterilised at 115° C for 10 minutes in 10-ml screw-capped bottles.

Preparation of spore suspension—Prepare the following medium—

Protone (Difco)	5·0 g
Manganese sulphate ($MnSO_4.4H_2O$)	0·001 g
Agar	20·0 g
Glass-distilled water	to 1 litre

Transfer 200-ml amounts of the medium to Roux bottles, and sterilise by heating at 120° C for 15 minutes. Cool, inoculate with the culture, and incubate at 30° C for 5 to 7 days. Wash the growth from the surface of the medium with 150 ml of sterile glass-distilled water, and spin the suspension in a centrifuge; wash the deposit with three successive 150-ml portions of sterile glass-distilled water, spinning the suspension in a centrifuge each time. Re-suspend the growth in sterile glass-distilled water, and dilute to match opacity 1, or less, on Brown's opacity tubes. Store the suspension in a refrigerator.

PROCEDURE

PREPARATION OF PLATES—

Melt the contents of a bottle of assay medium by steaming for approximately 1 hour, and cool to about 50° C in a water bath at this temperature.

Inoculate the melted medium with the spore suspension of *B. cereus*—dilute the suspension, if necessary, and use an inoculum density found by previous experiment to give zone diameters of suitable size (see Note 2), mix well, and pour on to a 12-inch × 12-inch sterilised plate (see Note 3). The plate must be supported on a level surface while the medium is being poured. Leave the plate at room temperature with the lid raised until the medium has set, and store in a refrigerator for at least 1 hour.

Cut 64 cups in the solidified medium with the aid of a template; use a No. 5 cork-borer (8 mm in diameter) or nearest available size.

PREPARATION OF STANDARD SOLUTIONS—

Dissolve 50 mg of chlortetracycline hydrochloride of known potency in phosphate buffer solution (pH 4·5), and dilute to 500 ml with the buffer solution (see Note 4).

Measure 25 ml of solution A (see below under "Preparation of Test Solutions") into a 50-ml beaker, add 2·5 ml of N sodium hydroxide, boil gently for 15 minutes, and cool. Add 2·5 ml of N hydrochloric acid, transfer the solution quantitatively to a 50-ml calibrated flask, add a portion of the standard chlortetracycline solution containing as much antibiotic activity as is estimated to be present in the test sample, and dilute to the mark with phosphate buffer solution (pH 4·5). Dilute a portion with phosphate buffer solution (pH 4·5) to give a solution containing 0·25 μg of chlortetracycline per ml, and from this make a further dilution in phosphate buffer solution (pH 4·5) to give a solution containing 0·05 μg of chlortetracycline per ml.

PREPARATION OF TEST SOLUTIONS—

Supplements—Weigh a 1-g sample, add sufficient aqueous acid - acetone mixture to give a volume of 250 ml, shake for 1 hour, and filter. Dilute the filtrate with phosphate buffer solution (pH 4·5) according to the expected potency to the levels required in the assay, namely, approximately 0·25 and 0·05 μg of chlortetracycline per ml.

Feeding stuffs—Weigh a 20-g sample, add 100 ml of aqueous acid - acetone solution, shake for 1 hour, and spin in a centrifuge. Titrate a 5-ml portion of the supernatant liquid with N sodium hydroxide, with methyl orange solution as indicator. Transfer a 25-ml portion of the supernatant liquid to a 100-ml calibrated flask, add a volume of N sodium hydroxide equivalent to five times the volume required in the titration, and dilute to the mark with phosphate buffer solution (pH 4·5). (This is Solution A.)

To 10 ml of Solution A, add 1 ml of sodium chloride solution, and dilute with phosphate buffer solution (pH 4·5) according to the expected potency to the levels required in the assay, namely, approximately 0·25 and 0·05 μg of chlortetracycline per ml, (see Note 5).

ASSAY—

By means of a statistically satisfactory assay design, such as suggested by Brownlee *et al.*,[4] Lees and Tootill,[5] Price and Boucher[6] or Simpson and Lees,[7] compare the test and standard solutions in a cup-plate assay by using the previously prepared plates.

Plate out the solutions according to the chosen assay design; deliver a uniform volume of 0·05 to 0·1 ml into each cup of the prepared plates. Set aside at room temperature for 1 hour to allow pre-diffusion, and then place in an incubator at approximately 30° C.

After incubation for 18 to 20 hours, measure the diameters of the inhibition zones with finely pointed vernier callipers or a projection device.

Check the parallelism of the test and standard responses, and, if satisfactory, calculate the potency, P, of the test solution from the equation—

$$P = 0{\cdot}25 \text{ antilog} \left\{ \frac{(T_2 + T_1) - (S_2 + S_1)}{(S_2 - S_1) + (T_2 - T_1)} \times \log 5 \right\},$$

where T_2 = the total of the responses to the high dose of test solution,

T_1 = the total of the responses to the low dose of test solution,

S_2 = the total of the responses to the high dose (0·25 μg per ml) of standard solution and

S_1 = the total of the responses to the low dose (0·05 μg per ml) of standard solution.

Calculate the potency of the sample from the dilution employed.

RESULTS—

Express the results as grams of chlortetracycline hydrochloride per pound of diet supplement or per ton of compound feeding stuff.

NOTES

1. The volume of medium should be such that when poured into a 12-inch × 12-inch plate the depth of medium is 0·1 inch.

2. Zone diameters reported by the Panel members ranged from 14 to 30 mm for dose levels of 0·25 μg per ml and from 12 to 25 mm for dose levels of 0·05 μg per ml. The increase in response for this five-fold increase in dose ranged from 2·2 to 9 mm.

3. Sterilisation may be achieved by heating in an autoclave or by swabbing with acid alcohol.

4. For the assay of diet supplements the instructions in *italic type* can be omitted. They may also be omitted in the assay of compound feeding stuffs if, by previous experiment, it has been found that the test response is parallel to that of an unmodified standard.

5. If, for example, the sample contains 10 g per ton, or 9·84 μg per g, dilute to 20 ml.

Appendix VI

MICROBIOLOGICAL ASSAY OF OXYTETRACYCLINE IN DIET SUPPLEMENTS AND COMPOUND FEEDING STUFFS

Normal bacteriological procedures and precautions must be adopted in preparation of cultures, sterilisation of media and glassware, etc.

REAGENTS—

Assay medium—

Yeast extract	1·0 g
Ammonium nitrate	5·0 g
Sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$), B.P. grade	5·0 g
Dextrose	5·0 g
Agar	15·0 g
Glass-distilled water	to 1 litre

The recommended procedure for preparing the medium is to dissolve the yeast extract, ammonium nitrate and sodium dihydrogen orthophosphate in the water, adjust the solution to pH 7·5, stir in the agar, and transfer the mixture to an autoclave. Steam for 30 minutes, and then heat at 115° C for 20 minutes. Remove from the autoclave, add the dextrose, and filter clear through a prepared paper-pulp pad about 0·5 inch thick. Cool, and adjust the solution to pH 7·0. Transfer 200- to 250-ml amounts (see Note 1) to 12-oz screw-capped bottles, and sterilise at 115° C for 20 minutes. The final pH of the medium is 6·7.

Acid methanol solution, 2 per cent. v/v—Dilute 20 ml of hydrochloric acid, sp.gr. 1·16 to 1·18, to 1 litre with methanol.

Sodium hydroxide, N.

Hydrochloric acid, N.

Phosphate buffer solution (pH 4·5)—Dissolve 13·6 g of potassium dihydrogen orthophosphate, KH_2PO_4, in distilled water and dilute to 1 litre with distilled water.

Sil Flo—Obtainable from Haller and Phillips Ltd., 68–70 Goswell Road, London, E.C.1.

Sodium carbonate—$Na_2CO_3.10H_2O$. B.P. quality.

ORGANISM—

The organism used in the assay is *Bacillus cereus* NCIB 8849 or 9231 (ATCC 11778).

Maintenance of culture—Maintain cultures of the organism on slopes of "Oxoid" Blood Agar Base ("Oxoid" Nutrient Broth + 1·5 per cent. of agar) that has been sterilised at 115° C for 10 minutes in 10-ml screw-capped bottles.

Preparation of spore suspension—Prepare the following medium—

Protone (Difco)	5·0 g
Manganese sulphate ($MnSO_4.4H_2O$)	0·001 g
Agar	20·0 g
Glass-distilled water	to 1 litre

Transfer 200-ml amounts of the medium to Roux bottles, and sterilise by heating at 120° C for 15 minutes. Cool, inoculate with the culture, and incubate at 30° C for 5 to 7 days. Wash the growth from the surface of the medium with 150 ml of sterile glass-distilled water, and spin the suspension in a centrifuge; wash the deposit with three successive 150-ml portions of sterile glass-distilled water, spinning the suspension in a centrifuge each time. Re-suspend the deposit in sterile glass-distilled water, and dilute to match opacity 1, or less, on Brown's opacity tubes. Store the suspension in a refrigerator.

PROCEDURE

PREPARATION OF THE PLATES—

Melt the contents of a bottle of assay medium by steaming for about 1 hour, and cool to about 50° C in a water bath at this temperature.

Inoculate the melted medium with the spore suspension of *B. cereus*—dilute the suspension, if necessary, and use an inoculum density found by previous experiment to give zone diameters of suitable size (see Note 2)—mix well, and pour on to a 12-inch × 12-inch sterilised plate (see Note 3). The plate must be supported on a level surface while the medium is being poured. Leave the plate at room temperature with the lid raised until the medium has set, and store in a refrigerator for at least 1 hour.

Cut 64 cups in the solidified medium with the aid of a template; use a No. 5 cork-borer (8 mm in diameter) or nearest available size.

PREPARATION OF STANDARD SOLUTIONS—

Dissolve 50 mg of oxytetracycline hydrochloride of known potency in phosphate buffer solution (pH 4·5), dilute to 500 ml with the buffer solution and prepare modified standards by either method (*i*) or (*ii*) described below (see Note 4).

(*i*) *Standard solution modified with inactivated supplemented feed*—Prepare an adsorption column, 9 to 10 cm in length, composed of a mixture of equal parts of sodium carbonate and Sil Flo, in a chromatography tube, 3 cm in diameter. Pack the column dry, and tamp down before use.

Transfer approximately 50 ml of Solution A (see below under "Preparation of Test Solutions") to the top of the dry adsorption column, allow the solution to pass through the column, and finally apply gentle suction. Pass the solution through the column once only. Adjust the pH of the eluate to pH 4·5 with N hydrochloric acid, and transfer 20 ml of the solution quantitatively to a 50-ml calibrated flask. Add a portion of the standard oxytetracycline solution, prepared as described above, containing as much antibiotic activity as is estimated to be present in 4·0 g of the test sample, and dilute to the mark with phosphate buffer solution (pH 4·5). Dilute a portion with phosphate buffer solution (pH 4·5) to give a solution containing 0·8 μg of oxytetracycline per ml, and from this make a further dilution in phosphate buffer solution (pH 4·5) to give a solution containing 0·2 μg of oxytetracycline per ml.

(*ii*) *Standard solution modified with unsupplemented feed*—Weigh a 20-g sample of the unsupplemented feeding stuff, add 100 ml of acid methanol, shake for 1 hour, and spin in a centrifuge. Adjust the supernatant liquid to pH 4·5 with N sodium hydroxide, and transfer 20 ml of the solution quantitatively to a 50-ml calibrated flask. Add a suitable portion of the standard oxytetracycline solution, and dilute as described above under (*i*).

PREPARATION OF TEST SOLUTIONS—

Supplements—Weigh a 1-g sample, add sufficient acid methanol to give a volume of 250 ml, shake for 1 hour, and filter. Dilute the filtrate with phosphate buffer solution (pH 4·5) according to the expected potency to the levels required in the assay, namely, approximately 0·8 and 0·2 μg of oxytetracycline per ml.

Feeding stuffs—Weigh a 20-g sample, add 100 ml of acid methanol, shake for 1 hour, and spin in a centrifuge. (This is Solution A.)

Transfer 10 ml of Solution A to a 25-ml calibrated flask, adjust the pH to 4·5 with N sodium hydroxide, and dilute to the mark with phosphate buffer solution (pH 4·5). If necessary, dilute portions with phosphate buffer solution (pH 4·5) according to the expected potency to the levels required in the assay, namely, approximately 0·8 and 0·2 μg of oxytetracycline per ml.

ASSAY—

Compare the test and standard solutions in a cup-plate assay; use the previously prepared plates and a statistically satisfactory assay design.

Plate out the solutions according to the chosen assay design; deliver a uniform volume of 0·05 to 0·1 ml into each cup of the prepared plates. Set aside at room temperature for 1 hour to allow pre-diffusion, and then place in an incubator at approximately 30° C.

After incubation for 18 to 20 hours, measure the diameters of the inhibition zones with finely pointed vernier callipers or a projection device.

Check the parallelism of the test and standard responses and, if satisfactory, calculate the potency, P, of the test solution from the equation—

$$P = 0{\cdot}8 \text{ antilog}\left\{\frac{(T_2 + T_1) - (S_2 + S_1)}{(S_2 - S_1) + (T_2 - T_1)} \times \log 4\right\},$$

where T_2 = the total of the responses to the high dose of test solution,

T_1 = the total of the responses to the low dose of test solution,

S_2 = the total of the responses to the high dose (0·8 μg per ml) of standard solution and

S_1 = the total of the responses to the low dose (0·2 μg per ml) of standard solution.

Calculate the potency of the sample from the dilution employed.

RESULTS—

Express the results as grams of oxytetracycline (base) per pound of diet supplement or per ton of compound feeding stuff.

NOTES

1. The volume of medium should be such that when poured into a 12-inch × 12-inch plate the depth of medium is 0·1 inch.

2. Zone diameters reported by Panel members ranged from 16 to 26 mm for dose levels of 0·8 μg per ml and from 11 to 18 mm for dose levels of 0·2 μg per ml. The increase in response for this four-fold increase in dose ranged from 3·2 to 10 mm.

3. Sterilisation may be achieved by heating in an autoclave or by swabbing with acid alcohol.

4. For the assay of supplements a standard diluted further with the buffer solution to give solutions containing 0·8 and 0·2 μg of oxytetracycline per ml may be used instead of one modified with a feeding stuff as described below. Likewise, with feeding stuffs if, by previous experiment, it has been found that the test response is parallel to that of an unmodified standard, a modified standard need not be used.

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The Determination of Small Amounts of Iron and Chromium in Sapphire and Ruby for Maser Applications

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It has been required to know the amount and distribution of paramagnetic ions in synthetic sapphire and ruby crystals used for maser devices. The extremely hard and chemically inert nature of the sample materials creates special analytical problems.

Methods, both chemical and spectrographic, have been developed for determining 0·002 to 0·2 per cent. of iron and of 0·01 to 0·5 per cent. of chromium. The methods are applicable to small samples ($\geq$ 20 mg) so that material from different regions of a crystal can be analysed to assess homogeneity.

In the spectrographic method, the crushed sample is mixed with graphite powder containing cobalt oxide as internal standard, and a d.c. arc total-combustion technique is used to determine chromium and iron. The chemical methods involve spectrophotometric determination of chromium with diphenylcarbazide and of iron with bathophenanthroline, both applied after decomposition of the crushed sample with a sodium carbonate - sodium tetra-borate flux; for determining iron at low levels rigorous purification of reagents is essential to achieve suitably low blank values.

The chemical methods have higher precision, but the spectrographic method is more rapid and is preferred for routine work. Results by the various methods are presented.

Sapphires and rubies have long been prized as gemstones. Chemically and crystallographically they are essentially identical, consisting of single-crystal α-alumina or corundum. Both owe their colour to the presence of other metallic elements as impurities; in ruby, for example, the characteristic red colour is imparted by chromium which, in the form of oxide, is incorporated in the aluminium oxide structure. In the absence of impurity elements sapphire is a colourless or "white" crystal.

Blue, red (ruby) and "white" sapphire have all been made for many years in the laboratory. Originally the intention was presumably to make available gemstones at a price much lower than the natural variety, but at a later date sapphire and ruby were made to supply industrial needs. Among the most important of these has been material for jewel bearings, manufactured for instruments, watches, etc. This use arises from the fact that corundum is extremely hard and second only to diamond on the conventional Moh's scale of hardness. Recently many new industrial applications have arisen for most of which the colourless or "white" sapphire is the material required.

The most successful and most common method for the production of these synthetic crystals is the flame-fusion process developed by Verneuil about 1904.[1,2] A large single crystal or boule, probably weighing 50 to 70 g, is the first product of the Verneuil process, and this boule can then be cut and worked to give material of the dimensions and shape required for the intended industrial use. For production of the coloured crystals the appropriate impurity is added to the aluminium oxide powder that serves as the "raw material" of the flame-fusion process; however, much of this additive, together with other accidental impurities, is driven off at the high temperature attained in the process (about 2000° C). Some degree of inhomogeneity of impurity distribution may in fact occur in the boule single crystal.

A recent and most important application of synthetic ruby is in the preparation of maser crystals. The maser is a device for microwave amplification by stimulated emission of radiation, and it has been found that the chemical and crystallographic nature of ruby

is particularly suited to a special version of this device known as the laser, in which the microwaves are replaced by optical radiation in the visible or near-visible range. One of the problems in maser studies on ruby, and in related work on "white" sapphire, is to investigate the relationship between the microwave spectral data and the nature, amount and distribution of impurities in the crystals. It is desirable for this purpose to have detailed analytical information concerning the concentration and distribution of added and accidental impurities down to the lowest detectable level.

Bearing in mind the refractory nature of sapphire and ruby, particularly in the form of a massive piece of single crystal, the analytical problem is considerable. The materials are difficult to decompose except by certain fusion methods, for which the sample must be in a finely crushed form. The extreme hardness of the materials creates special problems in the preparation of an uncontaminated finely crushed sample, particularly for the determination down to low levels of an impurity such as iron.

In the sections below are described the analytical investigations by means of which methods have been established for determining chromium, an added impurity, and iron, generally a fortuitous impurity, in single-crystal material. These methods, both chemical and spectrographic, relate to the determination of the average or total amount of chromium or iron in the whole of the sample selected for examination. In addition, an account is given of appropriate methods of sampling used to make a preliminary assessment of the distribution of chromium in ruby boule.

The analytical problem

Chromium in ruby—

Ruby crystals of different chromium contents, representing the range of colours normally used for gems or jewel bearings, are available, and it has been possible to explore the range of chromium contents in which maser action is most prominent. This has shown the need, in work on maser crystals, for analytical methods to cover the range 0·01 to 0·5 per cent. of chromium. To permit analysis of small samples, *e.g.*, in distribution studies, it was required that the method should be applicable to sample weights of about 10 mg, *i.e.*, corresponding to the determination of 1 to 50 μg of chromium. Suitable methods of measurement, both chemical and spectrographic, are, of course, available for these amounts of chromium. For the chemical determination the method adopted was decomposition of the crushed sample by fusion and subsequent development of the chromium - diphenylcarbazide colour, which lends itself to precise spectrophotometric measurement. While corrections for the blank value are essential in this method, the amounts of chromium likely to be introduced from reagents, etc., are not sufficiently large to endanger the accuracy of the determination. The real difficulty is associated with the sampling and preparation of material of such considerable hardness and of the geometrical shape of the Verneuil boule; particular care is needed to avoid the introduction of extraneous chromium during the crushing process.

Iron in sapphire and ruby—

Most of the analytical interest in iron has been concerned with the determination of the amounts present as an adventitious impurity in "white" sapphire and in ruby; these amounts, which are small compared with the chromium content of ruby, fall in the range 0·002 to 0·01 per cent. Consideration was again given to methods applicable to small weights of sample, of about 20 mg, corresponding to the determination of 0·4 to 2 μg of iron. Some determinations have been required on experimental materials having deliberate additions of iron, but even here the levels (up to 0·2 per cent.) have been lower than those mentioned in connection with chromium. Both chemical and spectrographic methods have again been used.

In the determination of iron, the problems of sample preparation are more severe than for the chromium determination. The risk of contamination with the element to be determined is much greater, and the levels to be determined are lower. For chemical determination of iron, reagents of sufficient sensitivity are available, but further problems can arise. The necessary fusion stage provides an opportunity for an exchange of iron between melt and crucible material; in addition, reagents of normal analytical purity may bring into the determination amounts of iron which in total may be as much or more than the amount of iron it is required to measure. On the other hand, a direct spectrographic technique enables the analyst to avoid these chemical difficulties, though not those of the sampling process.

THE DETERMINATION OF CHROMIUM IN RUBY

SAMPLING—

One satisfactory method involves the initial reduction of the ruby sample to a coarse powder by thermal shock. Pieces of boule, held in platinum-tipped tongs, are heated to redness in a flame and dropped into water. The resulting coarse powder is then further crushed in a mortar made from either steel or tungsten of high purity; the crushed material is sifted through a 60-mesh silk or nylon screen for the spectrographic method or through a 120-mesh screen for the chemical method; oversize material is again crushed and sifted. The powdered sample is treated with hydrofluoric and nitric acids to remove any tungsten derived from the mortar, or is boiled for a few minutes with aqua regia if a steel mortar has been used. Finally the acid-washed powder is washed three times by decantation with de-ionised water and dried at 110° C for 30 minutes.

When the determination of both iron and chromium is required on the same prepared sample, use of the tungsten mortar is essential (see section on "Determination of Iron"—"Sampling").

When knowledge about the local concentration of chromium is required a different method of sampling is employed. The boule is trepanned at each sampling point, to a depth of 3 to 4 mm, with a tool made from 16 s.w.g. steel tube. This tool is attached to a Mullard ultrasonic 60-watt drill unit; boron carbide (300 mesh) is used as the abrasive. Each cylindrical piece cut out by this technique weighs 7 to 10 mg. Each piece is crushed in a steel percussion mortar, and the resulting powder is sifted, acid-cleaned, washed and dried as described above.

CHEMICAL METHOD

The method adopted involves fusion of the prepared ruby sample in platinum with a sodium carbonate - sodium tetraborate mixture; this is in our experience the most suitable method of attack on sapphire and ruby materials. The determination is completed spectrophotometrically by a conventional diphenylcarbazide method.

REAGENTS—

Standard potassium dichromate stock solution—Dissolve 0·707 g of analytical-reagent grade potassium dichromate in water, and dilute to 1 litre.

1 ml ≡ 250 μg of chromium.

Standard potassium dichromate working solution—Prepare when required for use by diluting 1 ml of the stock solution to 100 ml with water.

1 ml ≡ 2·5 μg of chromium.

Sodium carbonate - sodium tetraborate flux—Mix intimately 10 g of analytical-reagent grade anhydrous sodium carbonate with 5 g of anhydrous sodium tetraborate (prepared by ignition of the hydrated analytical-reagent grade salt).

Aluminium potassium sulphate solution—Dissolve 1 g of analytical-reagent grade hydrated aluminium potassium sulphate in 100 ml of water.

1 ml ≡ approximately 1 mg of alumina.

Sulphuric acid, 0·4 N—Dilute 400 ml of N sulphuric acid with water to 1 litre.

Sulphur dioxide solution—Prepare a saturated solution of the gas in water.

Potassium permanganate, approximately 0·1 N—Dissolve 0·32 g of analytical-reagent grade potassium permanganate in 100 ml of water.

Sodium azide solution—Dissolve 1 g of sodium azide in 100 ml of water.

Diphenylcarbazide solution—Dissolve 0·25 g of analytical-reagent grade diphenylcarbazide in 100 ml of analytical-reagent grade acetone. Prepare fresh each day.

CALIBRATION—

In each of six 100-ml beakers place 0·1 g of flux, 10 ml of aluminium potassium sulphate solution and 25 ml of 0·4 N sulphuric acid. To the contents of the separate beakers add from a burette amounts of 0, 1, 2, 3, 4 and 5 ml of the working standard dichromate solution (1 ml ≡ 2·5 μg of chromium). Treat each solution as described below.

Add 2 drops of sulphur dioxide solution to reduce the dichromate, and boil the solution vigorously for 1 to 2 minutes to remove excess of sulphur dioxide; then add 3 drops of 0·1 N potassium permanganate, and continue boiling for 5 minutes to re-oxidise the chromium to the sexavalent state.

Cool somewhat, then add sodium azide solution dropwise to de-colorise the solution. Avoid using a large excess; 5 to 10 drops is usually adequate. Boil the solution for 1 to 2 minutes to remove hydrazoic acid, cool thoroughly, then transfer to a 50-ml calibrated flask. Add 5 ml of diphenylcarbazide solution, dilute to the mark with water, and mix the solution.

Allow 5 to 10 minutes to elapse for full colour development, and then measure the optical density of each solution at 540 mμ in a 4-cm cell, with water in the reference cell. Deduct the reading for the solution containing no added chromium from the readings for the other solutions. From these results prepare a calibration graph or calculate the mean factor relating corrected optical density to micrograms of chromium.

PROCEDURE—

On a semi-micro balance weigh out 2 to 10 mg of the prepared sample, depending on the expected chromium content. (For chromium contents up to 0·1 per cent. use approximately 10 mg of sample, for contents of 0·1 to 0·2 per cent. use approximately 5 mg and for contents of 0·2 to 0·5 per cent. use approximately 2 mg of sample.) Transfer the weighed sample portion to a small platinum crucible of about 5 ml capacity, add 0·1 g of flux, and mix throughly with a glass rod.

Heat the covered crucible over a Meker burner, gently at first, then strongly for 10 minutes. Swirl the crucible occasionally to aid attack of the sample.

Cool, then transfer the crucible and lid to a 100-ml beaker, add 25 ml of 0·4 N sulphuric acid, and heat on a hot-plate until the melt has dissolved; complete dissolution should be obtained.

Remove the crucible and lid, rinse them, add the rinsings to the solution, and heat the solution to boiling. Add 3 drops of 0·1 N potassium permanganate, and continue boiling for 5 minutes. Complete the procedure for development and measurement of the chromium colour as described under "Calibration."

Run a blank determination in parallel with the sample determination, and correct for any chromium found. Finally, calculate the chromium content of the sample.

SPECTROGRAPHIC METHOD

For the spectrographic determination of chromium, the prepared sample is mixed with a carbon-powder spectrographic buffer containing cobalt as the internal standard. The mixture is placed in a cupped graphite electrode, and the chromium is determined by a d.c. arc total-combustion technique. Standardisation is effected by comparison with mixtures prepared from ruby in which the chromium has been determined chemically.

The same spectrographic buffer and arc conditions are also used in the determination of iron; if required, chromium and iron can be determined simultaneously, provided that the method of sample preparation has been appropriate for determining iron. For convenience, details of the spectrographic procedure relating to the determination of iron are given separately under "Determination of Iron."

In experiments on the spectrographic method, particular attention was paid to assessing the effect of particle size of the sample and to observing the variation with time of the evaporation of chromium and cobalt (and also iron). No difference in evaporation behaviour was observed between samples and standards. The use of a sample of different particle size showed that material of less than 60 mesh was satisfactory, no difference being observable with more finely crushed material.

APPARATUS—

Spectrograph—Hilger large-quartz spectrograph with a mask containing a 5-mm horizontal slit adjacent to the plate.

Excitation source—A d.c. arc source capable of providing a current of 15 amps; open circuit voltage at least 120 volts.

Slit height—2 mm.

Slit width—0·015 mm.

External optics—A 30-cm quartz lens, placed adjacent to the slit so as to form an image of the arc on the collimating lens of the spectrograph.

Wavelength range—3400 to 2450 Å.

Plate calibrator—A rotating sector disc with a step-to-step ratio of 2 to 1.

Densitometer—Hilger non-recording densitometer.

Electrodes—Upper electrode (negative): a 1 inch length of 6·35-mm diameter Ringsdorff RW-O graphite rod. Lower electrode (positive): Ringsdorff RW0078 electrode.

Photographic plates—Kodak B.10.

REAGENTS—

Crushed ruby boule—Ruby boule crushed as described under "Sampling" and of known chromium content as determined by the chemical method.

Crushed sapphire boule—"White" (*i.e.*, chromium-free) sapphire boule crushed as described under "Sampling."

Spectrographic buffer—Mix 9·72 g of carbon powder, prepared by turning JMIB carbon rods, with 0·28 g of cobalt oxide in an agate mortar.

Developer—Dilute 1 part of Ilford 1D2 stock solution with 2 parts of water.

PREPARATION OF CHROMIUM STANDARDS—

Prepare in duplicate a series of standards, covering the range 0·005 to 0·25 per cent. of chromium, as described below.

Weigh out 5·0 mg of crushed, chemically analysed ruby boule, or appropriate portions of chemically analysed ruby and "white" sapphire to give a total of 5·0 mg. Mix with 5·0 mg of spectrographic buffer in an agate mortar, and transfer to an electrode.

Record the spectra of the standards as described below.

PROCEDURE—

Weigh out 5·0 mg of the prepared sample, mix in an agate mortar with 5·0 mg of spectrographic buffer, and transfer to an electrode.

Place a pair of electrodes in the arc stand with the filled one in the lower (positive) arm. Touch the electrodes together, switch on the current, separate the electrodes to give a 4-mm gap, and adjust the control rheostat to give a current of 10 amps. Run the arc for 2 minutes, maintaining an electrode separation of 4 mm.

To record a calibrating spectrum on the same plate, remove the mask, increase the slit height to 12 mm, place the sector disc in position, and photograph a 5-amp iron arc for 60 seconds.

Develop the plate for 3 minutes at 20° C, fix, wash and dry.

Measure the transmission of the chromium line at 2672·83 Å and the cobalt line at 2650·27 Å, and of the spectral background adjacent to each line.

CALCULATION OF RESULTS—

Construct a plate calibration curve from the transmission readings from the calibrating spectrum. Convert the transmission readings for the chromium and cobalt lines to relative intensity, and correct the line intensities for background intensities. Calculate the ratio of the chromium and cobalt line intensities, and convert this to percentage chromium by reference to a working curve.

The working curve is prepared on log - log paper from the spectra of the standards.

RESULTS

When ruby samples were analysed for chromium both chemically and spectrographically, satisfactory agreement between the methods was obtained. This is illustrated in Table I, which shows values obtained by both methods on samples covering a range of chromium contents.

A typical experiment to assess the distribution of chromium in a "flat" ruby boule is illustrated in Fig. 1. The samples for analysis were removed from the flat face of the boule, at the points shown, by the trepanning technique previously described under "Sampling";

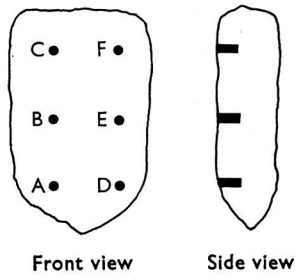

Fig. 1. Distribution of chromium in a flat ruby boule. Percentage of chromium found at sampling points: A, 0·021; B, 0·021; C, 0·021; D, 0·017; E, 0·016; F, 0·016

the chromium contents were determined chemically. As shown in Fig. 1, the results obtained covered the range 0·016 to 0·021 per cent. of chromium, although the alumina powder from which the boule was grown contained an addition corresponding to 0·1 per cent. of chromium. This illustrates the large loss of additive that can occur in the flame-fusion process.

TABLE I

COMPARISON OF CHEMICAL AND SPECTROGRAPHIC RESULTS FOR CHROMIUM IN RUBY

Sample number	Chromium content found by—	
	Chemical method, %	Spectrographic method, %
26777	0·015	0·016
26707	0·058	0·053
27153	0·10	0·11
27154	0·20	0·19

THE DETERMINATION OF IRON IN SAPPHIRE AND RUBY

SAMPLING—

Extreme care is required to avoid the introduction of extraneous iron during the preparation of the sample. The method described below was found to be satisfactory.

Heat the sample piece, held in platinum-tipped tongs, to red heat in a flame, and then drop into "pure" water to shatter the sample by thermal shock. Pour off the water, dry the sample at 110° C, and then transfer to a mortar made from tungsten of high purity and provided with a well-fitting tungsten rod as a pestle. Fit a polythene cap to the upper end of the pestle, and tap this gently with a hammer to crush the sample. Sift the fine material through a 60-mesh silk or nylon screen for the spectrographic method or through a 120-mesh screen for the chemical method. Return the oversize material to the mortar, and again crush so that all the material passes through the screen.

Transfer the sifted powder to a clean platinum crucible, add 2 ml of hydrofluoric acid and 2 ml of nitric acid, and warm the mixture for about 5 minutes to dissolve any tungsten acquired in the crushing. Dilute with water, allow the powder to settle, and carefully decant off the liquid. Wash the powder with water by decantation several times, dry at 110° C, and store the dried powder in a clean capped specimen-tube.

SPECTROGRAPHIC METHOD

In this particular context, a spectrographic determination offers great advantages over a chemical method. The relatively high blank values for iron resulting from reagents of normal purity (and the consequent need in a chemical method to purify the reagents) are

eliminated by the use of a direct spectrographic technique. It was established by experiment that the presence of chromium had no effect on the iron spectrum, so that the same technique was applicable to the determination of iron in colourless sapphire and in ruby.

Although it has been shown that the iron content of ruby and sapphire is generally in the region of 0·005 per cent., the method described below has been devised for iron contents up to 0·2 per cent.; this has permitted the analysis of experimental material containing deliberately added iron. As stated earlier, the spectrographic buffer and arc conditions used are the same as for the chromium determination, so that iron and chromium can be determined simultaneously if required.

APPARATUS AND REAGENTS—

The apparatus, spectrographic buffer, electrodes, photographic plates and developer are as described for the determination of chromium. Other reagents required specifically for the determination of iron are listed below.

Crushed boule—Ruby or sapphire boule of low iron content, crushed as described under "Sampling."

Aluminium ammonium sulphate, hydrated—Analytical-reagent grade.

Ammonium ferric sulphate, hydrated—Analytical-reagent grade.

Ammonia solution, sp.gr. 0·88—Analytical-reagent grade.

Wash solution—A 2 per cent. w/v solution of ammonium nitrate made just alkaline to methyl red by adding ammonia solution.

PREPARATION OF STOCK ALUMINA - IRON MIXTURES FOR IRON STANDARDS—

Prepare two stock mixtures of alumina, containing 0·1 and 1 per cent. of iron, as described below.

For the 0·1 per cent. mixture, weigh out and transfer to a 250-ml beaker 8·88 g of aluminium ammonium sulphate and 0·0086 g of ammonium ferric sulphate. For the 1 per cent. mixture weigh out and transfer to a second beaker 8·80 g of aluminium ammonium sulphate and 0·0861 g of ammonium ferric sulphate.

Dissolve the salts in water, and dilute to about 150 ml. Add 2 or 3 drops of methyl red indicator, heat the solution to boiling, and add ammonia solution until the indicator just turns yellow. Set the solution aside on a water-bath for 10 minutes.

Filter off the precipitate on a Whatman No. 41 or equivalent filter-paper, and wash the precipitate with hot wash solution. Transfer the paper and precipitate to a silica crucible, dry, and then ignite at a low heat until all carbonaceous matter has been removed. Finally, heat the crucible and contents in a muffle furnace at 1000° C for 1 hour. Cool, transfer the mixture to an agate mortar, and grind to a fine powder.

PREPARATION OF IRON STANDARDS—

Prepare in duplicate a series of standards, covering the range up to 0·2 per cent. of iron, as described below.

Weigh out 5·0 mg of spectrographic buffer, add the weight of alumina - iron mixture specified in Table II, and mix in an agate mortar. Add 5·0 mg of crushed boule, mix in the mortar, and transfer the mixture to an electrode.

Record the spectra of the standards as described below.

TABLE II

WEIGHTS OF ALUMINA - IRON MIXTURES FOR SPECTROGRAPHIC IRON STANDARDS

Iron content of standard, %	Weight of alumina - iron mixture added
Blank	—
0·006	0·30 mg of 0·1% mixture
0·02	1·00 mg of 0·1% mixture
0·06	0·30 mg of 1% mixture
0·20	1·00 mg of 1% mixture

PROCEDURE—

Weigh out 5·0 mg of the prepared sample, mix in an agate mortar with 5·0 mg of spectrographic buffer, and transfer to an electrode.

Excite the sample, and record the spectrum: also record a calibrating iron spectrum on the same plate, exactly as described for chromium. Develop the plate for 3 minutes at 20° C, fix, wash, and dry.

Measure the transmission of the iron lines at 2611·87 and 2621·68 Å and of the cobalt line at 2590·59 Å, and of the spectral background adjacent to each line.

CALCULATION OF RESULTS—

Construct a plate calibration curve from the transmission readings for the calibrating spectrum. Convert the transmission readings for the iron and cobalt lines to relative intensity and correct the line intensities for background intensities. Calculate the ratio of iron and cobalt line intensities and convert this to percentage iron by reference to a working curve.

The working curve is prepared on log - log paper from the spectra of the standards. For this purpose deduce from the spectra, by the method of standard additions,[3] the iron content of the crushed boule used in the standards, and correct the nominal iron content of each standard accordingly.

CHEMICAL METHOD

As described above, the preparation of standards in the spectrographic technique involves the mixing of solid materials, a crushed sapphire or ruby being "doped" with alumina - iron mixtures of known iron content. Excellent results over a range of iron contents, *e.g.*, with materials containing deliberate additions of iron, were obtained by the use of these synthetic standards. However, a possible doubt attached to the spectrographic determination of iron at the lowest levels, *e.g.*, in the purest undoped materials, as this necessarily involved an extrapolation of the working curve.

For this and other reasons, a considerable effort was put into the development of a chemical method suitable for determining iron at the lowest levels. Spectrophotometric determination with bathophenanthroline[4] was used; this is the most sensitive and selective reagent available for iron, and there was no difficulty in achieving the required sensitivity. In the development of the method, the two aspects requiring most consideration (apart from the sampling problem already discussed) were the method of decomposition and the need to attain adequately low blank values. These two points are discussed in more detail below.

DECOMPOSITION—

As in the determination of chromium, the most suitable method of decomposition involves a fusion process for attack of the sapphire or ruby. Although there are no special problems with chromium, the choice of flux and of crucible material are all-important in the determination of iron. In particular, there is a need to minimise the possibility of exchange of iron between crucible and melt (and of attack on the crucible), and it is desirable to achieve complete decomposition in the shortest possible time. After an investigation of various fluxes and crucible materials, the process finally adopted involved "sintering" in platinum at 1050° C with a small portion of flux consisting of anhydrous sodium carbonate and anhydrous sodium tetraborate in the proportion 2 to 1 by weight. Only 50 mg of flux and 20 mg of sample were used, and the mix was heated in a platinum crucible in an electric muffle furnace.

The "sinter" process was introduced by Finn and Klekotka of the National Bureau of Standards in 1930,[5] and this technique has been in use in these laboratories for many years for decomposing glass and refractory materials. In the present context, the sinter technique has several advantages—

(*i*) the process is quick; complete attack of the sample is obtained within 10 minutes' heating;

(*ii*) the small amount of flux reduces the blank value arising from iron introduced from this source;

(*iii*) both constituents of the flux are readily re-crystallised and therefore potentially amenable to purification;

(*iv*) while it is known that fusions in platinum can give rise to exchange of iron between melt and crucible, the use of a sinter rather than a fluid melt minimises the extent of this exchange process.

It has nevertheless been thought an essential precaution to do one or more blank fusions in the crucible immediately before and after each determination of iron. If results at the customary blank level are obtained, this provides an assurance that exchange of iron with the crucible has not occurred in the determination. As is our normal practice when determining small amounts of iron, a platinum crucible was reserved solely for the work on sapphire and ruby.

BLANK VALUES—

In trace analysis, the contribution from reagents and apparatus of the element to be determined should ideally be appreciably less than the amount in the sample. It may be desirable, as in our work, to adopt an analytical process permitting the use of reagents that either are available in a high state of purity or readily lend themselves to purification.

In the method devised for determining iron, the reagents used are: sodium carbonate - sodium tetraborate flux, hydrochloric acid, a combined reducing and buffer solution containing hydroxylammonium chloride and sodium acetate, bathophenanthroline solution and chloroform. In initial work on this method, only the reducing - buffer solution was purified (by development and extraction of the iron - bathophenanthroline colour). The blank values were at the level of 0·7 to 0·8 μg of iron, *i.e.*, similar to and sometimes greater than the amount of iron in a sample. Subsequently, the blank value was substantially reduced by decreasing the amount of bathophenanthroline used, by employing distilled hydrochloric acid and by purifying the constituents of the flux. Consistent blank values at the level of 0·2 μg of iron were then obtained, an amount equivalent to only a half of the smallest amount of iron determined in a sample.

For purification of the constituents of the flux, use was made of the reagent phenyl-2-pyridyl ketoxime, which has the unusual property of forming an extractable complex with ferrous iron in strongly alkaline solutions.[6,7] Separate strong aqueous solutions of sodium carbonate and of sodium tetraborate were prepared, iron was extracted as the phenyl-2-pyridyl ketoxime complex, and the sodium carbonate and tetraborate were re-crystallised from solution. Purification in this manner was more successful with sodium tetraborate than with sodium carbonate, a possible explanation being the presence of iron in an insoluble form in the sodium carbonate. Better results with the sodium carbonate were achieved by introducing a filtration step, with titanium to serve as a "collector," before the extraction.

Experimental details of the chemical method for determining iron in sapphire and ruby are given below.

PREPARATION AND PURIFICATION OF REAGENTS—

Water—Use distilled water further purified by passage through mixed-bed ion-exchange resin. Store in a polythene container.

Hydrochloric acid—Purify by isopiestic distillation[8] as described below. Clean a 10-inch vacuum desiccator with hydrochloric acid, dry the desiccator, and remove any lubricant. Into the base of the desiccator pour 500 ml of analytical-reagent grade hydrochloric acid, sp.gr. 1·18. To a polythene or polystyrene container add 100 ml of water, and support the container on glass rods above the level of the acid; close the desiccator with the lid. At daily intervals withdraw a small aliquot from the solution in the plastic container, making use of the hole in the desiccator lid, and by titration determine the strength of acid. Continue the distillation until acid of 8 N concentration is obtained (2 to 3 days). Store the distilled acid in a glass-stoppered bottle.

Bathophenanthroline solution—Dissolve 0·0668 g of bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) in 100 ml of analytical-reagent grade ethanol, and dilute to 200 ml with water. Store in a glass-stoppered bottle.

Chloroform—Analytical-reagent grade.

Reducing — buffer solution—Dissolve 20 g of analytical-reagent grade sodium acetate trihydrate and 2·0 g of analytical-reagent grade hydroxylammonium chloride in water, transfer to a separating funnel, and dilute to 200 ml. Add 2 ml of bathophenanthroline solution, mix, and set aside for 1 minute. Add 10 ml of chloroform, shake vigorously for 1 minute, allow the layers to settle, and run off the chloroform layer. Repeat the extraction, adding further bathophenanthroline solution, if necessary, until a colourless extract is obtained. Store the purified solution in a glass-stoppered bottle.

Sodium carbonate—Dissolve 10 g of analytical-reagent grade anhydrous sodium carbonate in 50 ml of hot water, and add a solution containing the equivalent of about 5 mg of titanium dioxide (prepared by dissolving 25 mg of potassium titanyl oxalate in a few drops of sulphuric acid (1 + 1), heating to fumes, then cooling and diluting with about 5 ml of water). Digest the solution at about 70° C for 30 minutes, and then filter through a coarse paper, without washing the precipitate. Re-heat the filtrate to about 70° C, add 0·2 g of sodium dithionite and 5 ml of phenyl-2-pyridyl ketoxime solution (0·2 g dissolved in 100 ml of 0·1 N hydrochloric acid), and keep the solution hot for about 5 minutes. Cool the solution, transfer to a separating funnel, and extract with successive 10-ml portions of chloroform until a colourless extract is obtained (2 or 3 extractions should suffice). Run the aqueous solution into a beaker, heat to 50° C, add 50 ml of ethanol, and cool. Pour off the alcohol layer, again heat to 50° C, add 25 ml of ethanol, cool, and discard the alcohol layer; if necessary, repeat the heating, the addition of 25 ml of ethanol and the cooling to obtain crystallisation of sodium carbonate from the aqueous solution. Filter off the crystals on a No. 4 grade sintered-glass crucible, and wash once with ethanol. Transfer the crystals to a clean platinum crucible, dry at 110° C then heat at 250° C to convert the precipitated monohydrate into anhydrous sodium carbonate. Store in a screw-capped bottle.

Sodium tetraborate—Dissolve 20 g of analytical-reagent grade sodium tetraborate in 100 ml of hot water. Add 0·2 g of sodium dithionite and 5 ml of phenyl-2-pyridyl ketoxime solution, and maintain the solution at about 70° C for 5 minutes. Cool the solution to about 40° C, transfer to a separating funnel, and extract the solution with two successive 10-ml portions of chloroform, rejecting the extracts; complete the extractions quickly so as to minimise risk of crystallisation of the sodium tetraborate. Pour the aqueous solution into a beaker, and cool the solution, stirring during the cooling to prevent the crystals adhering to the walls of the beaker. Filter off the crystals on a No. 4 sintered-glass crucible, and wash once with ethanol. Transfer the crystals to a clean platinum crucible, dry at 110° C, and then heat at 300° C to dehydrate the sodium tetraborate. Store in a screw-capped bottle.

Flux—Mix purified sodium carbonate and purified sodium tetraborate in the proportions 2 to 1 by weight. Store in a screw-capped bottle.

Standard iron stock solution—Dissolve 0·100 g of pure iron in a few millilitres of dilute sulphuric acid (1 + 6), and dilute with water to 1 litre.

$$1 \text{ ml} \equiv 100 \ \mu\text{g of iron.}$$

Standard iron working solution—Prepare when required for use by diluting 1 ml of the stock solution to 100 ml with water.

$$1 \text{ ml} \equiv 1 \ \mu\text{g of iron.}$$

CALIBRATION—

To each of four dry 50-ml separating funnels add 2 ml of water, 0·2 ml of hydrochloric acid and 50 mg of flux. To the solutions in three of the funnels add 0·5-, 1·0- and 2·0-ml portions of the working standard iron solution, corresponding to 0·5, 1·0 and 2·0 μg of iron. Dilute all four solutions to 5 ml with water, and treat each as described below.

Add 5 ml of reducing - buffer solution (the amounts of reagents used give a pH of 4·7 to 4·9 at this stage). Add 2 ml of bathophenanthroline solution, mix and set aside for 1 minute. Add from a pipette 10 ml of chloroform, shake vigorously for 1 minute, and allow the layers to settle. Run off the chloroform layer through a small roll of filter-paper in the stem of the funnel (to absorb any entrained water) into a 4-cm cell. With chloroform in the reference cell, measure the optical density of the extract at 535 mμ; use a suitable mask if the volume of solution is insufficient to fill the light path of the spectrophotometer.

From the optical densities of the solutions with added iron subtract the optical density of the solution with no added iron. From these results prepare a calibration graph or calculate the mean factor relating corrected optical density to micrograms of iron.

PROCEDURE—

To a semi-micro platinum crucible, specially reserved for the determination, add 50 mg of flux. Cover the crucible and with platinum-tipped tongs transfer it to a muffle furnace at 1050° C, taking care to handle only the outside of the crucible. Heat for 10 minutes, remove the crucible, and allow it to cool.

Add 2 ml of water and 0·2 ml of hydrochloric acid, and gently heat the covered crucible on a hot-plate for a few minutes to dissolve the fused mass. Allow the crucible to cool, transfer the solution to a dry 50-ml separating funnel, and dilute with water to 5 ml. Add 5 ml of the reducing - buffer solution and 2 ml of bathophenanthroline solution, set aside for 1 minute, and then proceed with the extraction and spectrophotometric measurement of the extract as described under "Calibration." Calculate the amount of iron found.

If the amount of iron found is at the normal level of the blank value, proceed with a determination. If an excessive amount of iron is obtained, or if a crucible is being used for the first time, repeat the blank fusion as above until the amount of iron found is at a suitably low level. (With reagents purified as described, the amount of iron customarily found in blank fusions was $0{\cdot}2 \pm 0{\cdot}05$ μg of iron.)

For the determination, add 50 mg of flux and 20 mg of the prepared sample to the crucible, and mix with a clean glass rod. Cover the crucible, and heat for 10 minutes at 1050° C in a muffle furnace. Allow the crucible to cool, add 2 ml of water and 0·2 ml of hydrochloric acid, and gently heat the covered crucible on a hot-plate until dissolution of the sintered mass is complete; heating for 15 to 20 minutes may be required. Cool the crucible, transfer the solution to a separating funnel, and proceed with the development, extraction and measurement of the iron colour as previously described. Calculate the total amount of iron found.

Without delay carry out a blank fusion in the crucible and determine the amount of iron obtained. If this amount is at the customary blank level, calculate the mean blank value from the amounts of iron found for the blank fusions made immediately before and after the determination on the sample. Subtract this mean blank value from the total amount of iron found in the determination on the sample, and hence calculate the percentage of iron in the sample.

Because of the small amounts of iron to be determined, constant precautions are needed to avoid contamination of solutions and apparatus from airborne dust, etc.

Results

To illustrate levels of the blank value and the actual amounts of iron commonly determined, a chemical determination is shown in detail in Table III. As will be seen from Table III, the blank fusions before and after the determination on the sample gave amounts of iron at the customary blank level of 0·2 μg of iron, thus providing an assurance that no significant exchange of iron between crucible and melt had occurred in the intervening determination. The material analysed in this particular determination was sapphire grown in these laboratories.

Table III

Details of a determination of iron in "white" sapphire

Operation	Optical density of extract	Iron found, μg
Blank fusion before determination	0·033	0·23
Determination on 20-mg sample	0·080	0·55
Blank fusion after determination	0·028	0·19

Mean blank value = 0·21 μg of iron
Iron in sample = 0·55 − 0·21 = 0·34 μg ≡ 0·002%

In Table IV are shown results obtained for iron by both chemical and spectrographic methods on a range of samples. The main interest in determining iron has been concerned with sapphire and ruby in which iron is present only as an adventitious impurity, and most of the results given in Table IV relate to this type of material (iron content generally 0·002 to 0·005 per cent.). Satisfactory agreement between the two methods was obtained at these levels and the chemical method, although relatively lengthy, has thus provided independent evidence that the spectrographic technique is applicable at these low levels of iron content.

An independent check by neutron-activation methods on two of the samples listed in Table IV, was arranged through Dr. C. A. Parker of the Admiralty Materials Laboratory. Results for iron were obtained by direct gamma-ray spectrometry on the irradiated sample and also by a more rigorous method in which the iron was separated chemically before being counted. The respective results by these two techniques were 0·004 and 0·001 per cent.

of iron on sapphire No. 28171 (for comparison with the figures given in Table IV, *i.e.*, mean values of 0·002 per cent. chemically and 0·002 per cent. spectrographically) and 0·002 and 0·001 per cent. on ruby No. 27154 (for comparison with figures of 0·004 per cent. chemically and 0·0045 per cent. spectrographically in Table IV). These results by activation methods, though not in precise agreement with our own values, do help to establish the general level of iron content in the sapphire and ruby materials. It should be added that in the determination of chromium (a much less exacting problem), excellent agreement was obtained between results at the Admiralty Materials Laboratory and those reported in Table I. On ruby No. 27154 results of 0·20 and 0·20 per cent. of chromium were obtained by gamma-ray spectrometry on the irradiated sample, compared with values of 0·20 and 0·19 per cent. of chromium by chemical and spectrographic methods, respectively.

To illustrate determination at higher levels, Table IV includes some results on "doped" materials, *i.e.*, with iron deliberately added during their preparation. Again, satisfactory

TABLE IV

COMPARISON OF RESULTS BY CHEMICAL AND SPECTROGRAPHIC METHODS FOR IRON IN SAPPHIRE AND RUBY

Sample	Sample number	Iron content found by— spectrographic method, %	Iron content found by— chemical method, %
"Pure" materials—			
Sapphire	28171	0·0025, 0·002, 0·002, 0·0015	0·002, 0·002
Sapphire	27309	0·004, 0·004	0·003, 0·003, 0·002
Sapphire	27308	0·003, 0·003, 0·005	0·004, 0·003, 0·003
Ruby	26707	0·004, 0·004, 0·004, 0·006	0·004
Ruby	27154	0·0045 (mean value)	0·004
Materials "doped" with iron—			
Ruby	26777	0·10	0·09, 0·09
Corundum	25163	0·19, 0·22	0·18

agreement between chemical and spectrographic methods was obtained. At these higher levels, the problems of the chemical determination of iron are greatly simplified; purification of reagents becomes unnecessary and a less sensitive reagent, *e.g.*, thioglycollic acid, can be used if required.

DISCUSSION

In the analysis of sapphire and ruby by chemical or spectrographic methods, the process of sample preparation is a critical part of the procedure, particularly in the determination of iron. In the investigation of various sampling techniques, spectrographic examination has been of value in indicating the nature and degree of any contamination that might arise at the various stages in the sampling process. We are satisfied that the techniques adopted will yield prepared sample material essentially uncontaminated with the element to be determined.

In the preceding descriptions of the analytical methods, the chemical method has been given first in the section on chromium, and the spectrographic method first in the section on iron; the order chosen is both logical and chronological. For chromium in ruby, the chemical method was developed first, and chemically analysed ruby was subsequently used as a means of calibrating the spectrographic method for chromium. With the determination of iron, the obvious difficulties of a chemical method encouraged the initial development of a spectrographic method; this in turn led to the development of a chemical method as a means of assessing the validity of the spectrographic method at low levels of iron content.

The work has shown the spectrographic technique to be applicable over an appreciable range of chromium and iron contents, and this technique has been the preferred method for routine work. Once the calibrations have been completed the procedure is rapid; iron and chromium can be determined simultaneously, and the sample preparation is a little less exacting in that material of less than 60 mesh is required instead of less than 120 mesh for the chemical methods.

The chemical method for iron has served its immediate purpose of verifying the validity of the spectrographic technique at low levels of iron content and of helping to establish the levels of adventitious iron present in sapphire and ruby. It requires considerable care and is relatively time-consuming, and is now used only for the occasional confirmatory test.

On the other hand, the chemical method for chromium has been of continuing value, because it has a higher precision than the spectrographic method. It has been used, for example, in establishing the existence of small differences in chromium contents in distribution studies of the type illustrated in Fig. 1.

The analytical techniques described in this paper have fulfilled exploratory requirements to establish the levels of chromium in ruby and of iron in sapphire and ruby, and to provide some information on their distribution. Ultimately, the ideal analytical technique would be a non-destructive test capable of giving point-to-point information on the distribution of "impurities" in the actual crystal to be used in a maser device. Preliminary work on the determination of chromium suggests that both X-ray fluorescence and optical absorption spectrophotometry may be of some value in this connection; with X-ray fluorescence, however, limitations may arise on sensitivity and from the fact that only the composition of material near the surface is assessed.

When the work described in this paper was essentially complete, a method for analysing sapphire and ruby maser crystals, involving a rather different approach from our own, was reported by Dodson.[9] In this method up to 20 mg of the crushed sample is decomposed by fusion for 2 to 2½ hours with about 1 g of potassium hydroxide in a zirconium crucible. Chromium is determined spectrophotometrically with diphenylcarbazide on an aliquot of the solution of the melt. On a separate aliquot iron is determined with 2,2′-dipyridyl; because of the pale colours obtained even with concentrated solutions, the iron colour is developed either in a small volume of solution in the depression of a white tile or on a small disc of filter-paper impregnated with the reagent, and the iron is assessed by visual comparison with standards similarly prepared.

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The Extraction and Identification of Permitted Food Colouring Matters with Special Reference to the Changes Undergone During Processing and Subsequent Storage

FIRST REPORT OF THE TRACE MATERIALS (COLOURS) COMMITTEE SET UP BY THE BRITISH FOOD MANUFACTURING INDUSTRIES RESEARCH ASSOCIATION

THE Trace Materials (Colours) Committee of the British Food Manufacturing Industries Research Association was set up in April 1959 under the Chairmanship of Dr. A. A. Houghton with the following terms of reference—

> "The Committee exists to examine any problems which may arise in the use of coal-tar colouring matters in foodstuffs as a result of the Colouring Matters in Foods Regulations.
>
> The principal interest is in the effects of processing the colours in foodstuffs and of storage of the finished products. Particularly in the formation of new coloured products and the changing of the proportion of subsidiary dyes.
>
> Inevitably this involves the consideration of the best processes for isolating colours from foods, identifying them and estimating their concentration.
>
> Changes in the percentage of subsidiary dyes are important in view of the standards now being laid down by the British Standards Institution."

The present working membership of the Committee is—Mr. A. G. Sansome (Edward Sharp and Sons Ltd.), *Chairman*; Mr. N. R. Jones (B.F.M.I.R.A.), *Secretary*; Dr. D. Dickinson, assisted by Mr. T. W. Raven (Fruit and Vegetable Canning and Quick Freezing Research Association), Messrs. K. J. Gardner (Mars Ltd.), R. A. Knight (British Baking Industries Research Association), F. J. Lipscomb (John Mackintosh and Sons Ltd.), R. C. Spalding (Kent County Council Public Analysts Department) and E. F. Williams, assisted by H. C. Hornsey (J. Sainsbury Ltd.). Dr. A. A. Houghton (Mars Ltd.) and Dr. A. G. Lipscomb (John Mackintosh and Sons Ltd.) previously served on the Committee as Chairmen.

Changes undergone by food colours during processing and subsequent storage are important from two major aspects: (1) Fading or undesirable changes in shade may be such as to affect the acceptability to the consumer if the desired appearance cannot be achieved or is lost during storage. (2) The Colouring Matters in Foods Regulations permit only certain colours to be used. If the colours undergo changes resulting in the production of new coloured substances, it might be concluded that non-permitted colours had been added to the food in the first place.

Also, should the situation eventually arise wherein food colours are required to conform to the appropriate British Standards (as is envisaged), it might happen that foodstuffs would be examined to ascertain whether or not the colouring matters in them were acceptable. In particular, the proportion of subsidiary dyes might be determined, and, therefore, changes in the proportions of the main dyes and any subsidiaries are of interest, as well as the appearance of dyes not present in the original colouring matter.

To obtain this information, it was considered vital that the methods used should remove all the colour from the foodstuff, and permit it to be recovered for subsequent examination. It was considered to be particularly important that the procedures involved should avoid any severe heat treatment or the use of strong acids or alkalis, as any changes in the dyes during extraction would invalidate the conclusions drawn. The traditional wool-dyeing technique was thought to be undesirable, as repeated boiling is required to obtain complete recovery of the dyes.

METHODS

Only the methods that have been employed in the work of the Committee will be described here, and only water-soluble dyes have been investigated.

All methods may be divided into three separate parts—

(*a*) transfer of the dyes from the foodstuff into solution,
(*b*) separation of the dye from extraneous substances and
(*c*) separation of individual dyes and identification.

These parts can be regarded as unit operations that can be combined together to suit particular products and particular colouring matters.

The Committee has examined the following products as representative of different types: boiled sweets, toffees, fondant, madeira cake, fresh sausages and fish paste.

So far, these products have been coloured with the food colours listed below, but for a few of these products the whole range of colours was not used—

Tartrazine	Red 10B	Orange G
Sunset Yellow	Red 2G	Yellow 2G
Amaranth	Ponceau MX	Blue VRS
Ponceau 4R	Ponceau SX	Indigo Carmine
Carmoisine	Red 6B	Erythrosine
Green S	Black PN	

In addition, Tartrazine was processed in canned peas.

The choice of dyestuffs was conditioned by the setting up of specifications by the British Standards Institution (B.S.I.), which gave standards of purity for the dyestuffs and suggested suitable solvent systems for separating subsidiary dyes from the principal colouring matters by paper chromatography. The dyes used were all of good commercial foodstuffs quality, and, where B.S.I. specifications had been published, they conformed to these specifications.

TRANSFER OF DYES FROM THE FOODSTUFFS INTO SOLUTION

Methods for extracting dyes from foods are described in the Association of Public Analysts (A.P.A.) Handbook[1] and in other places. These methods have proved quite satisfactory in practice, and it is necessary to give only a brief indication of the method used for most products.

Boiled Sweets and fondants—Direct dissolution in water was sufficient.

Toffees— A solution in water was made just alkaline with ammonia solution and filtered by suction, a large excess of filter-aid (*e.g.*, Hyflo Supercel) being used.

Madeira cake—Air-dried, and de-fatted with light petroleum before extracting the colour by soaking for several hours or overnight with cold 50 per cent. aqueous alcohol containing 1 per cent of ammonia solution, sp.gr. 0·88. Solutions were clarified by centrifugation, and the extractions were repeated when necessary. The final extracts were combined and concentrated.

Sausages—Extracted in ammoniacal 50 per cent. aqueous alcohol (20 g of sausages in 14 ml of water, 25 ml of ethanol and 1 ml of ammonia solution, sp. gr. 0·88) by setting the mixture aside in the cold for half an hour. The solution was then filtered and concentrated.

Fish paste—Extracted in ammoniacal 50 per cent. acetone (20 g of fish paste in 6 ml of water, 20 ml of acetone and 1 drop of ammonia solution, sp.gr. 0·88). After having been stirred for 10 minutes the solution was spun in a centrifuge and the acetone boiled off. Further extractions of the residues were often required to remove the whole of the colour. Fat was then removed by shaking with light petroleum.

Canned Peas—The samples were macerated in their own liquor. Colour was extracted directly from this macerate by a solvent.

In all these methods heating was kept to a minimum to avoid changes in the dyestuffs. In particular, the solutions should be concentrated under reduced pressure. Some of the methods used eliminated the need for concentration by evaporation.

SEPARATION OF DYES FROM EXTRANEOUS SUBSTANCES

The principles involved were those of solvent extraction, ion exchange and column chromatography. None of these methods is specific for synthetic dyes, nor is the wool-dyeing technique. It is always desirable to purify the first extract, either by repeating the process used or by using a different principle. The latter would seem most desirable as the impurities carried over in, for example, solvent extraction may not be carried over in a different process, such as ion exchange.

SOLVENT EXTRACTION—

The higher alcohols, particularly isobutanol and n-butanol, were the most useful of the solvents investigated. Dyes were normally extracted from aqueous solution under acid conditions and removed from the solvent by weak ammonia solution. Typical working conditions were those for canned peas—

> Macerate the peas with their own liquor. To 25 ml in a 50-ml stoppered cylinder add 2 ml of 25 per cent. w/v sulphuric acid. Warm to 50 to 60° C in a water-bath, add 15 ml of isobutanol, and shake vigorously. Decant off the isobutanol extract. Repeat until extracts contain no further colours. Combine the extracts.

The colour can be removed from this extract by direct chromatography on an alumina column or can be extracted into dilute ammonia solution. In the latter instance, ammonium sulphate will be present so that further purification by another method is desirable.

Similar methods were used successfully on sausages and madeira cake (prepared as described above) and, by using n-butanol, on boiled sweets and fish paste.

A method involving solvent extraction after formation of a complex with a quaternary ammonium compound was described by Drevon and Laur.[2] This method had the disadvantage that the complex needed to be broken down by the use of strong acids and high temperatures.

A modified method, however, has been found successful, although it has not yet been tried with all the colours examined. In this modified method a cetylpyridinium salt is used to form the complex, which is then broken down in the cold by careful neutralisation with a dilute solution of Teepol, *e.g.*, the procedure for toffees—

> Make the coloured syrup alkaline with ammonia solution, add Supercel, and filter; 25 ml of syrup should contain approximately 0·2 mg of colour. Adjust the pH to 9, add 10 ml of 0·1 per cent. cetylpyridinium chloride solution, and extract with 10 ml of chloroform. Repeat with a second 10-ml portion of chloroform, which should leave the aqueous phase colourless. Shake the chloroform extracts with successive 5-ml portions of 0·1 per cent. Teepol solution, rejecting the aqueous fractions until some colour is extracted. Retain this fraction in contact with the chloroform, and add 1 per cent. Teepol solution dropwise, shaking between additions, until no colour remains in the chloroform layer. Reject the chloroform. The aqueous layer contains a precipitate. Remove this precipitate by extracting twice with 10 ml of light petroleum (boiling-range, 40° to 60° C). Concentrate the clear aqueous dye solution under reduced pressure.

With some colours, *e.g.*, Yellow 2G, the colour complex formed a solid mass at the water-chloroform interface. This could be overcome by gentle extraction with n-butanol instead of chloroform.

ION EXCHANGE—

In the ion-exchange methods employed, aminoethylcellulose in the form of Whatman's AE.50 floc or powder or AE.30 paper was used. This is a weakly basic anion-exchange material that absorbs acid dyestuffs from acid solutions (at a pH below 4 to 5). After the aminoethylcellulose had been thoroughly washed, the colour was stripped with dilute ammonia solution. Hence this material acted in a manner similar to that of wool, but required no heating. It was very useful for absorbing the colour from large volumes of solution, thus achieving concentration without the use of heat. The floc and powder were used in the form of short columns (say 5 cm long, 1 to 1·5 cm in diameter), the powder being mixed with about 3 parts of Kieselguhr to improve its porosity. Typical working instructions were those for boiled sweets—

> Dissolve about 20 g of sweets in cold water, and, if not already acid, acidify with acetic acid; pass the solution through a 1-inch column of Whatman's AE.50 floc. Wash the floc well with extremely dilute acetic acid, and then with water. Strip the colour from the floc with a little dilute ammonia solution (the strength required varies with the dye from about 1 to 10 per cent.*), collecting the coloured portions only. A few millilitres only should be required, but the ammonia can be removed by aspiration or during concentration under reduced pressure.

* All concentrations of ammonia solution given in percentages refer to volumes of ammonia solution, sp.gr. 0·88, in 100 volumes of water.

Similar methods were also used for fondant, toffees, fish paste, and madeira cake. Whatman's AE.30 paper was used to purify the colour extract from toffees in the following manner after concentration under reduced pressure as described above—

> Take up the residue in a few millilitres of water, and make just acid to litmus with 1 per cent. hydrochloric acid. Pass the solution through a double disc of Whatman's AE.30 paper in a Gooch crucible under suction. Pass the filtrate repeatedly until all the dye is absorbed. Wash the filtrate with 20 ml of cold water, and strip it by passing a minimum volume of 10 per cent. ammonia solution through it.

CHROMATOGRAPHY—

Solutions of extracted dyes in isobutanol or n-butanol were sometimes purified by chromatography on an alumina column, *e.g.*, the solvent method described for canned peas can be continued—

> Pass the combined extracts through a $\frac{3}{4}$-inch bore polythene tube packed with dry alumina. Cut the column to isolate the coloured bands, and stir each fraction in a small beaker with 5 to 10 ml of 30 per cent. v/v ethanol. After the alumina has been allowed to settle, the supernatant liquors can be applied directly to chromatography paper.

APPLICABILITY OF METHODS—

All the methods were not carried out on all products containing the whole range of dyes. Alternative methods were employed only when the first method tried did not give an apparently complete recovery of the colour. For some dyes difficulty was experienced in obtaining a complete extraction into solvent, a complete take up on ion-exchange material or a complete removal from that material, but it should be emphasised that for a simple identification, not requiring complete recovery, any of these methods should be satisfactory.

With fresh sausages, methods involving the use of columns were unsatisfactory because the columns became blocked by colloidal proteins; this difficulty was not experienced with fish paste, presumably because much of the protein had been denatured by heat. Solvent methods were normally satisfactory for fresh sausages.

Ion-exchange methods were found satisfactory and very convenient with simple systems such as plain boiled sweets and fondants. With the exception of certain colours they were also found satisfactory with products, such as toffees and fish paste, containing cooked protein.

The colours that caused difficulties were Yellow 2G, Blue VRS, Black PN and Erythrosine. Erythrosine was extracted satisfactorily from an acid solution by ether. Blue VRS was found to be much more readily extracted into n-butanol than into isobutanol, and its partition was found to be strongly affected by pH, too acid a solution giving a poor extraction. Table I shows the results of solvent and cetylpyridinium methods as applied to these difficult dyes in toffees and fish paste.

TABLE I

SOLVENT AND CETYLPYRIDINIUM COMPLEX METHODS APPLIED TO TOFFEES AND FISH PASTE

	Toffees		Fish paste	
Colour	Solvents	Cetylpyridinium complex	Solvents	Cetylpyridinium complex
Blue VRS	Unsatisfactory	Satisfactory	Satisfactory with n-butanol	Satisfactory
			Unsatisfactory with isobutanol	Satisfactory
Erythrosine ..	Not tried	Satisfactory	Unsatisfactory	Not tried
Black PN	Satisfactory	Satisfactory	Unsatisfactory	Unsatisfactory
Yellow 2G ..	Not tried	Satisfactory	Satisfactory	Not tried

Once an initial separation of the colour had been made, other methods, unsatisfactory for the initial separation, were usually found quite satisfactory for further purification, suggesting that the problem was probably one of interfering impurities such as soluble proteins, amino acids, etc.

TABLE II

CHANGES OCCURRING DURING PROCESSING AND STORAGE

n.c.p.—no change on processing n.c.s.—no further change on storage C.I. numbers refer to the 1956 colour index

Colour	Original dye	Boiled sweets	Fondants	Toffees	Fish paste	Madeira cake	Fresh sausages
Tartrazine CI 19140	No subsidiaries	n.c.p.	—	n.c.p.	n.c.p. Traces of mauve and brown after 15 months' storage	n.c.p.	n.c.p.
Sunset Yellow F.C.F. CI 15985	3 subsidiaries 0·42%, 0·32%, 0·42%	n.c.p.	—	n.c.p.	n.c.p.	n.c.p.	n.c.p.
Amaranth CI 16185	2·2% of Fast Red E present	n.c.p.	—	New red produced. Fast Red E partly faded	Yellow- brown colour produced	n.c.p.	n.c.p.
Ponceau 4R CI 16255	Orange-red and yellow subsidiaries, total 0·9%	n.c.p.	—	Subsidiaries increased	Yellow subsidiary greatly increased. Pink produced. Yellow increased after 14 months' storage as intense as main colour	n.c.p.	n.c.p.
Carmoisine CI 14720	0·4% red subsidiary	Red and purple on processing	—	n.c.p.	Yellow and orange on processing. Additional yellow, orange, pink and brown after 14 months' storage	n.c.p.	n.c.p.
Red 10B CI 17200	Mauve, blue and pink subsidiaries, total 0·9%	Additional blue on processing	—	Mauve subsidiary intensified. Orange-brown formed. Some subsidiaries lost, orange produced after 9 months' storage	Mauve subsidiary intensified. Blue subsidiary lost. Orange-brown formed. n.c.s. 13 months	n.c.p.	Red formed. n.c.s. 13 months
Red 2G CI 18050	0·2% pink subsidiary	n.c.p. n.c.s. 12 months	—	n.c.p. Trace of pink after 9 months' storage	Original colour almost gone. Several yellows formed. Mauve and traces of pink after 13 months' storage	n.c.p.	n.c.p.

TABLE II (*contd.*)

Colour	Original dye	Boiled sweets	Fondants	Toffee	Fish paste	Madeira cake	Fresh sausages
Ponceau MX CI 16150	Red subsidiary	n.c.p. n.c.s. 9 months	n.c.p. n.c.s. 9 months	n.c.p. n.c.s. 9 months	Yellow formed. Yellow lost after 9 months' storage	n.c.p.	Subsidiary lost
PonceauSX CI 1470	No subsidiaries	Trace of pink n.c.s. 8 months	Trace of 3 pinks n.c.s. 8 months	n.c.p. n.c.s. 8 months	n.c.p. n.c.s. 8 months	n.c.p.	n.c.p.
Red 6B CI 18055	No subsidiaries	n.c.p. n.c.s. 9 months	n.c.p. n.c.s. 9 months	Trace of red. n.c.s. 9 months	Brownish yellow formed. Main colour lost after 6 months' storage. Yellow, orange, and more brownish-yellow formed	Trace of red	All colour lost on processing
Orange G CI 16230	Yellow and orange subsidiaries	n.c.p. Subsidiaries lost after 9 months	Trace of new colour found. Subsidiaries lost after 9 months	Red and new orange found. Subsidiaries lost after 9 months	n.c.p. Subsidiaries lost and new faint orange formed after 9 months	n.c.p.	n.c.p.
Yellow 2G CI 18965	2 yellow subsidiaries	n.c.p. n.c.s. 6 months	Traces of new colour formed	—	Subsidiaries lost n.c.s. 8 months	Trace of red	n.c.p.
Erythrosine CI 45430	At least 6 subsidiaries	n.c.p. Some subsidiaries lost after 7 months	n.c.p. Some subsidiaries lost after 7 months	n.c.p.	n.c.p. Faint brown after 7 months	Red and orange. Possibly original subsidiaries intensified	Yellowish
Blue VRS CI 42045	4 subsidiaries Mauve, blue, blue-green	n.c.p. n.c.s. 6 months	Trace of mauve	—	Trace of yellow n.c.s. 7 months	Trace of red	n.c.p.
Indigo-Carmine CI 13015	4 blue subsidiaries	—	Faint yellow. Yellow lost after 7 months	n.c.p.	n.c.p. except almost complete fading	—	n.c.p. except almost complete fading
Green S CI 44090	2 green subsidiaries	n.c.p.	n.c.p.	n.c.p.	n.c.p.	Yellow	n.c.p.
Black PN CI 28440	2 red, 1 orange 1 grey-blue subsidiaries	Yellow	Orange and yellow formed. 1 red subsidiary lost	n.c.p.	Orange	—	n.c.p.

Separation and identification of individual dyes

The final process in every investigation made use of paper chromatography. These methods are adequately described in the A.P.A. Handbook, but the following additional notes should prove helpful.

Solvents for paper chromatography—

The following solvents were used most frequently—

n-Butanol - water - glacial acetic acid—(20 + 12 + 5) by volume. (Solvent No. 5 of the A.P.A. Handbook.)

Ethyl acetate - pyridine - water—(11 + 5 + 4) by volume.

Ethyl methyl ketone - acetone - water—(7 + 3 + 3) by volume. (B.S.I. method for determining subsidiaries in Amaranth,[3] Ponceau 4R[4] and Red 10B.[5])

Ethyl methyl ketone - acetone - water - ammonia solution, sp.gr. 0·88—(700 + 300 + 300 + 2) by volume. (B.S.I. method for determining subsidiaries in Tartrazine,[6] Sunset Yellow,[7] Carmoisine,[8] Red 2G[9] and Yellow 2G.[10])

Trisodium citrate dihydrate - water - ammonia solution, sp.gr. 0·88—Trisodium citrate dihydrate (2 g) in 95 ml of water and 5 ml of ammonia solution. (B.S.I. method for determining subsidiaries in Orange G[11] and Ponceau SX.[12])

The B.S.I. solvents were used when appropriate, as the work described here was designed partly to look for changes in proportions of subsidiaries. However, it was often found desirable to extend the time for development when a more complete picture of the subsidiaries present was required; by using several different solvent mixtures, subsidiaries were often separated that would not have been found in a single solvent.

General comments on paper chromatography—

The following general observations, some of which have already been made in the A.P.A. Handbook, are emphasised.

(*i*) R_F value alone is not a safe criterion for identification, even when several different solvents are used. R_F values are affected by such factors as temperature, length of run, paper variations (thickness, texture, machine direction, batch-to-batch variations), presence of impurities and other less easily defined conditions.

(*ii*) The use of standard dyes as markers is essential, but even with this precaution, impurities extracted with the colours, etc., may cause differences between the R_F values of the markers and extracted dyes.

(*iii*) The only safe method is to superimpose standard dye and extracted dye. If no separation occurs in any solvent system the colours may be assumed to be identical. At the same time spots of standard dye and extracted dye should be run separately at similar concentrations. This is necessary because the standard dye itself may split into more than one component and give misleading results.

(*iv*) Certain colours will produce two or more spots of identical composition when run in some solvents if the concentration on the paper is too high (*e.g.*, Tartrazine in A.P.A. Solvent 5).

Results

The effects of processing the various colours so far examined are shown in Table II. No storage tests were carried out on fresh sausages or madeira cake, because both these products have only a short shelf-life.

Uncoloured material was also processed to ensure that yellow and brown spots on the chromatograms did not originate entirely from ingredients other than the dyestuffs.

Fading of colours on processing or storage is not reported here as it is almost universal, and no attempt has been made to investigate colourless substances produced by breakdown of the dyes.

Certain other changes have been reported in the literature.

Dickinson and Raven[13] reported that Ponceau 4R was reduced to a yellow colour by hydrogen sulphide or sulphur compounds liberated during the processing and storage of certain foodstuffs, and that Erythrosine lost iodine to produce fluorescein when canned cherries coloured with Erythrosine were stored in unlacquered cans.[13,14]

Ruiz and Laroche[15] found that Black PN was reduced in some sugar confectionery to give an orange colour that was not a known food dye. They also found that during extraction (on wool) this dye was partly converted to another orange colour that was not a known food dye.

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The Isolation and Separation of Dyes from Foodstuffs by Column Chromatography

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A method is described for isolating from foodstuffs, and then separating by column chromatography, twelve synthetic dyes used in Israel. Two adsorbents were used: alumina, at different pH values, and silica gel. The eluents used were water, n-butanol and methanol.

In a previous publication[1] a paper chromatographic method was described for separating and identifying a group of twelve synthetic water-soluble food dyes in use in Israel. The procedure described can be applied directly to commercial dyes. Dyes to be determined in food must be extracted and then purified before they can be identified, since the impurities associated with food often cause considerable difficulty in identifying the dyes, by either paper chromatography, spectrophotometry or any other means of identification.

Wool has been used for extracting coal-tar dyes from food-stuffs,[2,3,4] and by suitable modification of this method, basic dyes could be separated from acidic ones.[5,6] Helberg[3] suggested the use of solutions of different pH values for eluting the adsorbed dyes from the wool. This procedure involved the use of strong acids and heat, conditions liable to alter the chemical structure of the dyes. Other procedures for isolating and separating dyes include solvent extraction at different pH values[7] and column chromatography.[8,9] Kaolin, alumina and silica gel have been used as adsorbents in chromatography.[10,11,12,13,14] The ninth edition of the "Official Methods of Analysis" of The Association of Official Agricultural Chemists, describes a method for separating dyes by using a powdered-cellulose column and a series of sodium chloride solutions as eluents.[15] This technique, however, was not always found to give the desired separation.[16]

A procedure for extracting dyes from foods and separating them by column chromatography is described in this paper. By using silica gel and alumina adjusted to different pH values, and with water, butanol and methanol as solvents, the dyes tested could be separated. The adsorbents acted as partition, adsorption or ion-exchange columns, depending on the conditions used. The dyes were obtained chemically pure, and could subsequently be identified with ease.

Method

Reagents—

All reagents used were of analytical-reagent grade.

Hydrochloric acid, 2 N and N.

Ammonia solution, sp.gr. 0·88, and N.

Ammonia water—Dilute 1 ml of ammonia solution, sp.gr. 0·88, with 99 ml of water.

Methanol, redistilled.

Methanol, 90 per cent. v/v.

Ammoniacal methanol—Dilute 1 ml of ammonia solution, sp.gr. 0·88, with 99 ml of methanol.

n-Butanol, dry.

n-Butanol—Saturate with 2 N hydrochloric acid by shaking the two reagents together for a few minutes.

Chloroform, redistilled.

Acetone, redistilled.

Sodium hydroxide solution, 5 N.

Sodium acetate solutions, M, 0·5 M and 0·1 M aqueous.

DYES—

Ponceau 4R	Carmoisine	Amaranth
Red 6B	Red FB	Ponceau SX
Tartrazine	Naphthol Yellow S	Sunset Yellow FCF
Green S	Blue VRS	Indigotine

ADSORBENTS—

Silica gel—M.F.C. Materials for Chromatography, Hopkin & Williams Ltd.

Basic alumina—B.D.H. Chromatography grade.

Acid alumina—Place about 500 g of the basic alumina in a 5-litre Erlenmeyer flask, and cover with N hydrochloric acid to about 10 cm above the surface of the adsorbent. Shake the Erlenmeyer flask occasionally for about 1 hour, and set aside overnight. Decant the acid. Cover the alumina with a further portion of N hydrochloric acid. Shake again occasionally for an hour, and decant the acid. Wash the alumina thoroughly several times with distilled water until the pH of the washings reaches 3 to 3·5. Filter off the alumina on a Buchner funnel, transfer to a flat tray, and set aside to dry at room temperature.

Neutral alumina—Place about 100 g of the acid alumina in a 1-litre Erlenmeyer flask, and cover with N ammonia solution to about 3 cm above the surface of the adsorbent. Shake the Erlenmeyer flask for about an hour, set aside overnight, and decant. Cover the alumina with a further portion of N ammonia solution, and shake again for an hour. Decant and wash the alumina with warm distilled water until free of chloride, and then filter it off on a Buchner funnel. Dry the alumina first at 110° C, and then activate it at 300° C for 3 hours.

PREPARATION OF COLUMNS—

Columns of 20-cm length and 2-cm diameter were used. Place a piece of cotton- or glass-wool at the bottom of the column. Fill loosely with the appropriate adsorbent to a height of about 5 cm, and cover with another piece of wood. Wet the adsorbent with the required solvent by applying slight pressure.

PROCEDURE—

Extraction of the dyes from foodstuffs—The procedure for extracting dyes added to food stuffs differs according to the type of food, and for this purpose the foodstuffs may be divided into two main categories.

I. Foodstuffs soluble in, or miscible with water:
   (*a*) Soft and alcoholic drinks, sweets, jellies, etc.
   (*b*) Milk.

II. Foodstuffs sparingly soluble, or insoluble, in water:
   (*a*) Non-fatty foodstuffs, like cereals or jams.
   (*b*) Foodstuffs containing fat, *e.g.*, butter, sausage or cheese, and non-fatty food containing natural pigments, *e.g.*, paprika.

I (*a*) Dissolve the foodstuff in a suitable amount of water at room temperature, or on a heated water-bath. Adsorb the solution on an acid-alumina column, and wash the column with 100 to 200 ml of warm water (between 50° and 70° C); liquid preparations may be adsorbed directly on the acid-alumina column. Elute the adsorbed dyes with ammonia water, and evaporate the eluate to dryness on a water-bath.

I (*b*) Add three volumes of acetone to one volume of milk, shake and allow the precipitate to settle. Collect the precipitate on filter-paper, and wash with small amounts of acetone until the dye is completely extracted. Apply the acetone solution onto an acid-alumina column and elute with warm water and then ammonia water as described under I (*a*).

II (*a*) Crush or cut the foodstuffs into small pieces and grind them in a mortar with 2N hydrochloric acid to a homogeneous paste. Extract the paste with 10-ml portions of butanol saturated with 2 N hydrochloric acid, and decant the liquid into a separating funnel; three or four portions of acid - butanol mixture will extract most of the dyes. Only Green S and Blue VRS will remain mainly in the paste and pass only partly into the butanol. Extract these two dyes from butanol with ten to twelve 5-ml portions of 2 N hydrochloric acid.

Adsorb the butanol solution containing all the dyes (except Green S and Blue VRS) on an acid-alumina column; wash and elute as described under I (*a*).

Add the acid extract containing Green S and Blue VRS to the original paste. Triturate the paste well, and filter. Wash the precipitate with 2 N hydrochloric acid until all traces of the dyes are removed from the paste. Adjust the pH of the acidic solution to approximately 1 with 5 N sodium hydroxide, and adsorb on to a silica-gel column. Elute the dyes from the silica-gel column with ammoniacal methanol, and *immediately* evaporate to dryness on a water-bath.

II (*b*) Crush or cut the foodstuffs into small pieces, and transfer them to an Erlenmeyer flask. Extract the mass in the Erlenmeyer flask with 20-ml portions of chloroform until the latter is colourless, while the flask is being shaken mechanically. Add 20 ml of N hydrochloric acid to the food mass, and extract again in the Erlenmeyer flask with 20-ml portions of chloroform. Remove the chloroform each time by decanting into a separating funnel. Return the hydrochloric acid solution each time to the food mass in the Erlenmeyer flask, and continue the extraction until the chloroform layer is colourless. Discard the interfering natural pigments and any unlisted dyes present that are soluble in chloroform. Extract the acid solution and the food mass with 10-ml portions of butanol until all the dyes have been extracted from the food. If Green S and Blue VRS dyes are suspected to be present in the sample tested, extract the combined butanol solution with ten to twelve 5-ml portions of 2 N hydrochloric acid, and add the acid extracts to the original acid solution. Adjust the pH of the acidic solution to approximately 1 with 5 N sodium hydroxide, and proceed as outlined under II (*a*). If the dyes Green S and Blue VRS are not expected to be present, combine the butanol extracts and adsorb on an acid-alumina coloumn, wash, and elute as described under I (*a*).

Chromatographic separation of the dyes

Separation of the dyes Green S and Blue VRS—

Dissolve the dyes obtained by one of the procedures outlined above (not more than 1 mg of each dye should be present in the sample) in 10 to 20 ml of distilled water, and pour the solution onto a silica-gel column. All the dyes except Green S and Blue VRS move quickly through the column. Wash the column with 5-ml portions of distilled water. Blue VRS remains strongly adsorbed at the top of the column, while Green S moves slowly during the washing: continue until this dye reaches to about 0·5 cm from the bottom of the column. To separate all the dyes from Green S and Blue VRS, 20 to 30 ml of water are usually sufficient. However, should separation not be complete, a second silica-gel column may be inserted below the first. (This step may be omitted when Green S and Blue VRS have been extracted and separated from the food by procedure II (*a*) or II (*b*).)

The dyes Green S and Blue VRS are eluted from the silica gel with ammoniacal methanol, and the solution is *immediately* evaporated to dryness on a water-bath. To separate dyes Green S and Blue VRS from each other, dissolve the residue in aqueous butanol (1 part water *plus* 10 parts butanol), and adsorb the solution on a basic-alumina column. Wash the column with 10 ml of aqueous butanol. Blue VRS is eluted first, followed by Green S. Adsorb each dye solution separately on an acid-alumina column, and elute each with ammonia water.

The dyes eluted earlier from the silica-gel column with water (all 12 dyes except Green S and Blue VRS) are adsorbed on an acid-alumina column and eluted with ammonia water. Evaporate the solution to dryness on a water-bath, and dissolve the residue in 1·5 ml of water and then add 10 ml of butanol. Adsorb the solution on a basic-alumina column, and wash the column with butanol saturated with water. The dyes on the column separate into two distinct bands, an upper one, Fraction A (containing Ponceau 4R, Amaranth, Red 6B, Tartrazine, and Indigotine), and a lower one, Fraction B (containing Carmoisine, Red FB, Ponceau SX, Naphthol Yellow S, and Sunset Yellow FCF). Continue eluting the column with aqueous butanol until the lower Fraction B is completely eluted.

Separation of the dyes in fraction A—

Wash the column containing the remaining Fraction A with three 5-ml portions of methanol to remove the butanol, and then continue washing with 90 per cent. methanol. Indigotide, and then Red 6B are now eluted; each dye is adsorbed on a separate acid-alumina

column. Remove the remaining dyes, Ponceau 4R, Amaranth and Tartrazine, from the first column, by eluting with 10 to 15 ml of ammonia water. Evaporate to dryness. Dissolve the residue in water, and adsorb on an acid-alumina column. Elute with 0·5 M sodium acetate solution. Three distinct bands appear on the column. The first to be eluted is Tartrazine, and then Ponceau 4R; Amaranth remains adsorbed on the column. Dilute the acetate solutions containing Tartrazine and Ponceau 4R with water, (one part of acetate solution *plus* 10 parts of water), and adsorb again on acid alumina. Wash the last three acid-alumina columns with distilled water to remove the excess of sodium acetate before elution of the dyes. Finally, elute all the five dyes, now separated on individual columns, with ammonia water.

SEPARATION OF THE DYES IN FRACTION B—

Adsorb the aqueous butanol solution containing the dyes of Fraction B on an acid-alumina column, and wash with water to remove the solvent.

Elute the dyes with ammonia water, and evaporate the solution to dryness on a water-bath. Dissolve the residue in 10 ml of methanol, and adsorb the solution on a basic-alumina column. Elute Sunset Yellow FCF by washing with 90 per cent. methanol, and elute the remaining dyes from the column with 0·1 M sodium acetate. Adsorb the acetate solution on neutral alumina.

Wash the column with about 40 ml of 0·1 M sodium acetate, which will elute Naphthol Yellow S. Continue the washing with four or five 10-ml portions of M sodium acetate, which elutes Carmoisine and Ponceau SX together leaving Red FB at the top of the column.

To separate Carmoisine from Ponceau SX, dilute the 1 M acetate solution with about 200 ml of distilled water, and adsorb again on acid alumina. Wash the column with water, and extract the dyes with ammonia water. Evaporate the solution to dryness on a water-bath. Dissolve the residue first in 1·5 ml of water, and then add 10 ml of butanol. Chromatographically separate the solution on a basic-alumina column, by using aqueous butanol (1·5 parts water *plus* 10 parts butanol) as solvent. Carmoisine separates from the column.

Adsorb the eluted dyes Carmoisine, Naphthol Yellow S and Sunset Yellow FCF on acid-alumina columns, wash with water, and elute with ammonia water. Wash the column containing Red FB with water, and then elute the dye with ammonia water. Remove Ponceau SX directly from its column with ammonia water without previous washing with water.

The isolated food colours can now be identified by paper chromatography.[1]

DISCUSSION OF METHOD

Most of the synthetic dyes used for colouring foods are azo-compounds, containing various acidic and basic groups of different strength. These functional groups, their type, position and number in the dye-molecule, permit separation of the dyes by column chromatography. The two adsorbents used (silica gel and alumina, in their various forms described above), together with the three eluents (water, butanol and methanol), provide a wide range of adsorption, partition and ion-exchange conditions.

Silica gel adsorbs only dyes with basic groups from aqueous solution.

Acid alumina strongly adsorbs dyes with acid groups, and permits removal of extraneous material.

It is possible to separate the dyes into groups or individual colours by using alumina of different pH values.

By the method described, about 100 μg of a dye may be recovered from up to 500 g of food. In starchy gelatinous food a minimum of about 500 μg of dye must be present in 100 g of a sample.

The dyes are separated and isolated without application of either heat or strong reagents. This will be advantageous when working with compounds liable to decompose.

The extraction of small concentrations of dyes from food required the use of large amounts of solvents. By passing diluted solutions of dyes through appropriate columns, the dyes were adsorbed and the solvents recovered. Thus a dye solution could be concentrated without prolonged heating.

The use of small columns allowed the separated dyes to be obtained on single columns. In this way it was possible to proceed with the isolation and identification of some dyes (including those which remain strongly adsorbed on the column) before the complete separation of all the dyes had been accomplished.

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The Separation and Determination of Chromium Sesquioxide in Chrome Ores and Chrome-bearing Refractories

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Dichromate in solutions obtained from chrome ores and refractories by fusion can be almost completely removed by using liquid ion-exchange "resins" under slightly acid sulphate conditions. The chromium can then be quantitatively recovered from the organic phase by stripping with potassium hydroxide solution. After reduction, the chromium sesquioxide can then be determined spectrophotometrically as the complex with ethylenediamine-tetra-acetic acid to an accuracy within about 0·2 per cent.; this is more than adequate for routine control analysis. The aqueous phase is available for the determination of other constituents.

The analysis of chrome ores and chrome-bearing refractories has been the subject of considerable study in the past few years. The current methods for analysing these materials are tedious, and the results often show considerable discrepancies between laboratories. Consequently, a more rapid and accurate method is needed and also one more suitable for routine control testing, when some accuracy may be sacrificed for the sake of increased speed.

Bryant and Hardwick's method[1] has been tested by the Chemical Analysis Committee of the British Ceramic Research Association and has proved to be a satisfactory procedure for the direct determination of chromium sesquioxide in these materials. Also, the same Committee has evaluated and published, in a private communication to the industry, a method for the accurate and direct determination of total iron. There is still a need for a method whereby the same solution of the sample can be used for determining both chromium and the other constituents. This is important, as the decomposition of the material is difficult and time consuming, and, therefore, it is undesirable to develop a series of methods for various constituents each involving a separate decomposition.

One of the main difficulties presented by this type of material is that the large amount of chromium present complicates the determination of many of the other constituents, and it is therefore desirable to separate this element before proceeding with the rest of the analysis. This paper represents a first step in a research programme, in that it describes a method for the prior separation and determination of the chromium to an accuracy adequate for routine control analyses.

In the past, chromium has been separated in this class of materials by one of three methods: volatilisation as chromyl chloride; electrolysis with the use of a mercury cathode; or by solvent extraction with isobutyl methyl ketone. Each procedure has its disadvantages. Volatilisation as chromyl chloride involves the decomposition of the sample with perchloric acid, which is not invariably successful, and, in addition, complete removal of the chromium is rarely achieved. Separation via a mercury cathode simultaneously removes the iron and manganese, and also there is a danger that some of the silica may be precipitated. Further, the subsequent cleansing of the mercury may give rise to difficulties in some laboratories. Extraction with isobutyl methyl ketone is the most useful of these techniques, but is invariably incomplete owing to the reduction of some of the chromate by the organic phase in the presence of the necessary hydrochloric acid. Often the amount of the residual chromium is tolerable, but occasionally the amount can be high enough to introduce significant errors.

Recently, liquid ion-exchange "resins" have become available, and these appeared to offer the possibility of removing chromate from slightly acid solutions. Green's work[2] on the removal of iron, titanium and zirconium suggested that the removal of chromium would be almost specific. Stripping the chromate back from the "resin" could then be accomplished by using alkali.

Liquid ion-exchange "resins"—

The "resins" used in this work were manufactured by the Rohm and Haas Company of America and were obtained from their agents in this country, Charles Lennig and Co. Ltd.,

26–28 Bedford Row, London, W.C.1. They are Amberlite LA–1 and LA–2, liquid secondary amines insoluble in water and having a high molecular weight. The "resins" are soluble in a large range of organic solvents, and that in which they are generally supplied is stated to be toluene.

Experimental

Preliminary trials showed that when a slightly acid solution of potassium dichromate in sulphuric acid was shaken with an approximately equal volume of "resin" solution (4 per cent. in chloroform) almost all the dichromate ion was transferred to the organic phase. When this organic phase was shaken with N potassium hydroxide the chromate ion was stripped from the "resin" and returned to the aqueous phase.

The solutions used in subsequent experiments were made to simulate those that would be obtained after fusion of a 0·5-g sample in sodium carbonate and boric acid and dissolution of the melt in sulphuric acid. A blank solution slightly acidified with sulphuric acid and containing 7 g of sodium carbonate and 2·5 g of boric acid per aliquot was used.

The effects of acidity, type of "resin" and solvent were investigated. The strength of the "resin" solution was increased from that used by Green to 10 per cent. by volume in the chosen solvent.

Effect of acidity—

The pH of the blank solution used for these experiments was found to be 2·4. Separations with "resin" LA–1 were carried out by using in the first extraction 25 ml of the "resin" solution and with 0-, 10-, 20- and 30-ml additions of dilute sulphuric acid (1 + 9).

The competition of the sulphate ion reduced the efficiency of the extraction, some dichromate being left in the aqueous phase. Two further extractions, each with 10 ml of "resin" solution were therefore carried out resulting in a colourless aqueous phase. This phase was then washed twice with 25 ml of chloroform to remove traces of "resin." The aqueous phase was then tested for residual chromate and also for total chromium (see Table I).

Table I

Effect of acid concentration on the extraction of chromate

Potassium dichromate equivalent to 125 mg of chromium sesquioxide was taken for each determination

Sulphuric acid (1 + 9) added, ml	Chromate found in aqueous phase, mg of Cr_2O_3	Total chromium found in aqueous phase, mg of Cr_2O_3
0	0·42	0·45
10	0·00	1·19
20	0·00	1·49
30	0·00	1·77

The results in Table I show that more complete removal of the chromate is achieved at higher concentrations of acid, but is offset by increasing reduction of the sexavalent chromium. This reduction may be due to impurities in the "resin," *e.g.*, ferrous iron, organic reducing agent, or both. Stripping the "resin" before use to remove the ferrous iron, if present, was ineffective, and attempts to remove the organic reducing agents by treating the "resin" with permanganate revealed that the permanganate was reduced almost indefinitely; hence it was concluded that the "resin" itself was being oxidised.

To minimise this reduction and still obtain a complete removal of chromate, no acid was added until after the first two extractions, then 10 ml of sulphuric acid (1 + 9) were added before the third extraction. This technique proved effective.

The pH of the solution had an initial value of 2·4; after the first extraction this value increased to pH 4·2 and after the second extraction to pH 4·6. After addition of the sulphuric acid the pH dropped to about 1·3 and thereafter increased only slightly. This would seem to indicate a definite loss of sulphuric acid into the "resin."

Choice of "resin"—

Amberlite LA-1 and LA-2 "resins" are similar in type, but, of the two, LA-2 is the more basic, so that, provided the sulphate was not preferentially extracted, it could be expected to be the more effective.

Separations with each "resin" were carried out as indicated above. Again, total chromium and residual chromate were determined in the aqueous phases. In both instances the chromate content was negligible and the total chromium sesquioxide was 0·060 mg with LA-1 and 0·045 mg with LA-2. The latter figure is equivalent to 0·009 per cent. of chromium sesquioxide if a 0·5-g sample of material had been taken. LA-2 was chosen for the method, as it also showed less tendency to emulsify.

CHOICE OF SOLVENT—

Chloroform had been used exclusively up to this stage of the work, and as a slight reduction still occurred it was thought possible that a change of solvent might be beneficial. "Resin" having a concentration of 10 per cent. by volume was used throughout.

As the "resin" is already dissolved in toluene this was the obvious first choice. Total residual chromium sesquioxide with LA-2 was 0·015 mg. This was a considerable improvement over the results obtained with chloroform, but the use of toluene, which is lighter than the aqueous phase, introduces manipulative complications. If it had proved possible to determine the chromium sufficiently accurately for a referee method, toluene would have been the solvent chosen, but, as the final method for chromium is of routine accuracy, chloroform appears to be at least adequate.

Carbon tetrachloride was next tried, but was even less effective than chloroform, the residual chromium sesquioxide being 0·11 mg.

Mixtures of carbon tetrachloride or chloroform with toluene (so as to obtain a heavier organic phase) were not so effective, probably because separation of the phases was much slower.

POSSIBLE EXTRACTION OF OTHER CONSTITUENTS—

Silica—It was thought that silica might be partially extracted under these conditions. With the equivalent of 4·95 per cent. of silica present before the extraction, recoveries were 4·99 per cent. with Amberlite LA-1 being used and 4·90 per cent. with LA-2. These errors are probably not greater than the experimental error of the spectrophotometric method of determination.

Ferric oxide—Under the conditions of acidity used here, Green found that extraction of iron took place, but the results of experiments showed that when a blank fusion solution (see "Experimental" p. 878) was present no loss of iron occurred. Thus it may be that it is the concentration of the sulphate or competing ion that is the important factor.

Titania—No extraction of titania was observed. However, Green found that it was extracted in the presence of hydrogen peroxide, and, as this could provide the basis for a subsequent separation and determination, attempts were made to extract titanium from the solutions by this procedure. In all instances recoveries were found to be only about 50 per cent. of the amount present, so that again competition from the sulphate appears to restrict extraction.

Manganese oxide—No extraction of manganese oxide appears to occur, whether this is added in the manganous state or as permanganate.

STRIPPING THE "RESIN" PHASE—

A single extraction with N potassium hydroxide appeared to strip the "resin" completely, but a second extraction was made with the same reagent to ensure that no significant amount of chromium was left in the slightly cloudy emulsion in the organic phase.

DETERMINATION OF THE CHROMIUM SESQUIOXIDE—

Several methods for the determination of chromium were examined. Most of the accurate methods are based on its determination as chromate, but even by using such a technique as Bryant and Hardwick's, which involves an auxiliary oxidation, some chromate always appears to be reduced by the slight traces of "resin," and results were always low. Other methods for determining the chromic ion were attempted, including gravimetric techniques with 8-hydroxyquinoline and benzoic acid, but low results were recorded.

A volumetric method based on the formation of a complex with ethylenediaminetetra-acetic acid (EDTA) and back titration with zinc and with dithizone as indicator gave surprisingly sharp end-points in the strong purple solution, particularly if this is screened with Naphthol Green B to a dark blue with a slight tinge of green. But it became clear from the consistently low results that the amount of EDTA required to ensure complete reaction would mean excessively large back-titrations.

The presence of large amounts of sulphate ion rules out precipitation as barium or lead chromate.

Přibil and Klubalova's spectrophotometric method, as described by Welcher,[3] proved to be successful. The chromate is reduced to the chromic ion with sodium sulphite and the chromium complexed with a large excess of EDTA at a pH between 2 and 4. The violet colour of the complex is measured at 550 mμ.

Solutions containing various amounts of chromate, and a portion of the blank fusion solution were extracted into the "resin" and the combined organic phase stripped with potassium hydroxide. The chromium^{III} recovered from the acidified aqueous solutions was then determined spectrophotometrically. The results, expressed as percentage of chromium sesquioxide equivalent to the chromium^{III}, were—

Amount added ..	10	10	10	25	25	40	40	50	50	50
Amount recovered ..	10·0	10·0	9·8	24·9	24·9	40·1	39·9	50·0	50·0	49·8

It will be seen that the results have a slight negative bias, but the technique of calibrating the instrument on solutions of potassium dichromate would appear to produce answers accurate enough for routine control. However, Přibil states that the sulphate ion causes the results to be slightly low (an error of up to 1 per cent.), and as the solutions contain sulphate extracted into the "resin" it seems legitimate to calibrate the instrument on solutions that have been extracted into the "resin" in the presence of a blank solution. Table II shows the results on a range of samples, the results having been calibrated against pure solutions and solutions passed through the "resin."

TABLE II

DETERMINATION OF CHROMIUM SESQUIOXIDE IN DIFFERENT MATERIALS CONTAINING CHROMIUM

The results are the mean of duplicate determinations

Material	Accepted value for Cr_2O_3 content, %	Cr_2O_3 content found after calibration against— pure solutions, %	Cr_2O_3 content found after calibration against— solutions extracted into "resin," %
Mag-chrome brick, A	11·90	11·8	11·9
	11·26	11·1	11·3
Chrome-mag brick, B	23·79	23·6	23·8
	29·54	29·2	29·5
Turkish chrome-ore	39·44	39·0	39·2
Philippine chrome-ore	34·04	33·9	34·2
Grecian chrome-ore (B.C.S. 308) ..	41·50	41·1	41·3

An accuracy to within 0·5 per cent. of chromium sesquioxide is required in routine control work. The results obtained by the proposed procedure, after calibration against blank fusion solutions extracted into the "resin," had a maximum error of ±0·2 per cent.; this is not significantly different from the error in the results obtained by Bryant and Hardwick's procedure. The method takes a little longer to complete, but has the considerable advantage that other determinations can be made on the aqueous phase without recourse to a further fusion. Work is continuing on this possibility, with particular reference to the determination of lime, which has been a stumbling block in the past.

METHOD

"RESIN" SOLUTION—

Prepare a (1 + 9) solution of "resin" by adding 50 ml of Amberlite LA-2 to 450 ml of chloroform and mixing.

DECOMPOSITION OF THE SAMPLE—

Accurately weigh 0·500 g of the finely ground sample, dried at 110° C, into a platinum crucible. Add 7 g of anhydrous sodium carbonate and 2·5 g of boric acid, and mix thoroughly.

Heat over a bunsen or Meker flame, slowly raising the temperature until the mixture begins to melt; keep at this temperature until melting is complete. Then raise the temperature slowly and steadily to the full heat of the flame (about 950° C). After about 5 minutes at this temperature, swirl the contents of the crucible every 2 minutes, making sure that the particles of the sample on the side of the crucible come into contact with hot molten flux.

If swirling is begun too early, it is difficult to detach unfused particles from the side of the crucible. Continue to heat and swirl at this temperature for 25 to 45 minutes depending on the nature of the sample.

Completeness of decomposition can be checked visually by the absence of unfused particles at the bottom of the melt. The type and position of the burner should be chosen so as to maintain oxidising conditions throughout.

Cool, place the crucible and lid in a 250-ml beaker containing 85 ml of water and 15 ml of diluted sulphuric acid (1 + 1), and warm until extraction is complete. Remove the crucible and lid from the beaker, washing them with the minimum volume of water. Cool the solution.

Add diluted ammonia solution (1 + 1) until the first formation of a permanent precipitate, and then re-dissolve in 1 or 2 drops of diluted sulphuric acid (1 + 1).

EXTRACTION OF CHROMATE—

Transfer the solution to a 500-ml separating funnel, A; the volume at this stage should not exceed 150 ml. Add 25 ml of dilute resin solution (1 + 9), shake for 1 minute, and allow to separate. Transfer the organic phase to a second separating funnel, B, washing the stem of funnel A with chloroform. Repeat the extraction with 10 ml of dilute resin solution, and again transfer the organic phase to funnel B.

Add 10 ml of dilute sulphuric acid (1 + 9) to the aqueous phase, and repeat the extraction with a further 10 ml of dilute "resin" solution, again transferring the organic phase to funnel B.

Remove the traces of "resin" from the aqueous phase by two extractions with 20-ml portions of chloroform, the chloroform layers being added to the combined "resin" solution in funnel B. Reserve the aqueous phase and washings from funnel A for any other determinations, if required.

To funnel B add 50 ml of potassium hydroxide solution (approximately N), and shake for 1 minute. Allow the layers to separate, and transfer the organic layer to the now empty funnel A. Add to this funnel a further 50 ml of the potassium hydroxide solution, and again extract. Discard the organic layer, and combine the two aqueous phases. Remove traces of "resin" by shaking twice with 10-ml portions of chloroform, discarding the chloroform layers.

Transfer the alkaline aqueous phase to a 450-ml beaker, add hydrochloric acid, sp.gr. 1·18, until the solution becomes acid, and then add 6 drops in excess.

Boil the solution to remove the chloroform and to reduce the volume to about 200 ml. Transfer the solution to a 250-ml calibrated flask, and dilute to 250 ml.

DETERMINATION OF CHROMIUM SESQUIOXIDE—

If the solution appears cloudy, filter 40 to 50 ml through a dry Whatman No. 42 filter-paper, discarding the first few millilitres.

Transfer a 20-ml portion of this solution to a 250-ml beaker, add 20 drops of hydrochloric acid, sp.gr. 1·18, and then 10 ml of a 5 per cent. solution of sodium sulphite, *while stirring*. Dilute to about 50 ml with water, and boil for about 5 minutes. Cool to room temperature, add 10 ml of 5 per cent. EDTA solution, and then add ammonia solution, sp.gr. 0·88, until the first appearance of a permanent precipitate. Dissolve this precipitate by adding 20 drops of diluted acetic acid (1 + 1), and dilute to 200 ml. Boil for 10 to 15 minutes, cool, and dilute to 250 ml in a calibrated flask.

Measure the optical density of the solution against water at 550 mμ in 4-cm cells. Determine the chromium sesquioxide content of the solution by reference to a calibration graph prepared either from pure solutions of potassium dichromate or by passing dichromate solutions mixed with a blank fusion solution through the separation process.

We thank the Director of Research of the British Ceramic Research Association, for permission to publish this paper.

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The Determination of Residues of "Ruelene" in Milk

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A method is described for determining Ruelene insecticide (*O*-4-t-butyl-2-chlorophenyl methyl methylphosphoramidate) residues in milk. After initial extraction from the milk with solvent, the insecticide is separated from interfering materials by paper chromatography. The Ruelene is then determined from its phosphorus content. The method described has a sensitivity of 0·05 p.p.m., and recoveries of Ruelene added to milk are 74 per cent. at a level of 0·5 p.p.m. and 80 per cent. at a level of 1 p.p.m.

RUELENE is the trade name of the Dow Chemical Company for *O*-4-t-butyl-2-chlorophenyl methyl methylphosphoramidate. This is a systemic insecticide that gives good control of warble fly in cattle. Before its use in treating dairy cattle could be recommended, studies on the rate of appearance and concentration of residues of Ruelene in milk had to be carried out, and to this end a chemical method for determining the pesticide at residual levels in milk had to be devised. The *in vitro* stability and recovery of Ruelene from milk had been studied by Timmermann *et al.*,[1] who used radioactive Ruelene. These studies showed that Ruelene was stable in milk for at least 14 days at 12° C and that recoveries of 90 per cent. could be achieved by using methyl cyanide and chloroform to extract the residues from milk.

The method described here was therefore based on this preliminary extraction. Ruelene insecticide is fat-soluble, and it was hoped to employ the clean-up procedure, described by Laws and Webley[2] in their general method, before determining the pesticide from its phosphorus content. However, it was found that Ruelene is strongly adsorbed on to alumina and could not be eluted by light petroleum alone. The use of mixed eluting solvents tends to remove traces of other phosphorus-containing compounds from the column thus increasing the blank value. It was decided, therefore, to investigate the use of quantitative paper chromatography. This was found to be satisfactory, and it was possible to obtain good recovery of the insecticide from the paper. The Ruelene was determined as phosphorus after wet ashing the insecticide with a mixture of nitric and perchloric acids.[2,3]

METHOD

APPARATUS—

Spectrophotometer—Unicam SP500, or any similar suitable instrument.
All-glass chromatography tanks—For descending-solvent chromatography.

REAGENTS—

All reagents should be of analytical-reagent grade and low in phosphate.
Chloroform.
Methyl cyanide—Purify by percolating through a column of neutral alumina.
n-Hexane.
Solvent system for chromatography—In a separating funnel shake trimethylpentane, methanol and water in the ratio 5:4:1. Allow the phases to separate. The upper organic layer forms the mobile phase, the aqueous layer the stationary phase.
Perchloric acid, N.
Sulphuric acid, N.
Nitric acid, sp.gr. 1·42.
Hydrochloric acid, sp.gr. 1·18.
Ammonia solution, sp.gr. 0·88.
Ammonium molybdate solution—Dissolve 50 g of ammonium molybdate in 400 ml of 10 N sulphuric acid, and dilute to 1 litre with distilled water.

Stannous chloride solution, 40 *per cent. w/v*—Dissolve stannous chloride dihydrate in hydrochloric acid, sp.gr. 1·18.

Stannous chloride solution, dilute—Dilute 0·5 ml of the 40 per cent. w/v solution to 100 ml with absolute ethanol. Prepare immediately before use.

Ethanolic sulphuric acid—Mix 5 ml of concentrated sulphuric acid with 245 ml of absolute ethanol.

Isobutanol - benzene mixture, (1 + 1) *v/v*.

Ruelene, 95 *per cent. pure*—Standard solutions in acetone.

Potassium dihydrogen orthophosphate, dry—For primary standards.

PRELIMINARY EXTRACTION—

Measure out 100 ml of milk into a separating funnel. If recovery experiments are to be carried out, add the Ruelene as a solution in acetone at this stage. Then add 150 ml of methyl cyanide, and shake the mixture thoroughly; this precipitates the milk solids. (Precipitation and coagulation can be aided by holding the separating funnel under a hot tap for about a minute.) Filter the supernatant aqueous methyl cyanide through a wad of glass-wool into a second separating funnel, wash the precipitated milk solids with 150 ml of chloroform, and add the washings to the methyl cyanide extract. Shake the combined extracts, and allow the phases to separate. Transfer the lower chloroform - methyl cyanide layer to a dry 500-ml Erlenmeyer flask, and re-extract the aqueous layer with a mixture of 35 ml of methyl cyanide and 35 ml of chloroform. Combine the organic phases, and dry for 1 hour over anhydrous sodium sulphate. Filter the dried methyl cyanide - chloroform extract into a dry distillation flask, and remove the solvent in a current of air. Transfer the oily residue quantitatively to a 100-ml separating funnel with 25 ml of methyl cyanide. Extract the methyl cyanide solution three times with an equal volume of hexane to remove fats and phospholipids.

Transfer the methyl cyanide extract to a 100-ml round-bottomed flask that has a small well blown in the bottom. Remove the solvent on a steam-bath, evaporation being aided by a gentle current of air. Wash down the sides of the flask with a small amount of methyl cyanide, and evaporate the solvent. Dissolve the residue in the well of the flask in a small amount of acetone (0·1 to 0·2 ml). The extract is then ready for chromatography.

CHROMATOGRAPHY—

The solvent system used is trimethylpentane - methanol - water in the ratio 5:4:1. Use the aqueous layer as the stationary phase and the organic layer as the mobile phase. The separation is carried out on Whatman No. 1 filter-paper by descending-solvent chromatography. To ensure saturation of the atmosphere with the stationary phase, line the walls of the chromatography tank with sheets of filter-paper soaked in the stationary phase.

The method of application of the extract to the paper chromatogram can be described as a process of "wicking." Wash a strip of Whatman No. 17 filter-paper, 5 cm long and 1 cm wide, with acetone. Cut one end of this strip to a point, and by using a Pasteur pipette apply the milk extract, together with the acetone washings of the well of the flask, as a band about 2 cm behind the point. Then stand the strip, point uppermost, in a small tube and place the point of the strip in contact with the paper chromatogram, which must be supported in a horizontal plane. Mark the paper chromatogram into lanes with light pencil marks, and locate the tip of the strip on the starting line. Add a small amount of acetone to the tube, thus applying the Ruelene to the chromatogram by means of ascending chromatography or "wicking." Keep the area of the spot discrete by evaporation with a gentle current of air applied to the upper surface of the paper. Allow this process to continue for about 45 minutes, by which time any Ruelene present will have been transferred to the chromatogram.

Then transfer the chromatogram to the glass tank, and set aside overnight to equilibrate. Add the mobile phase, and allow the chromatogram to develop for 3 hours. Then remove the chromatogram from the tank, and set aside at room temperature to dry. Inspect the chromatogram under ultraviolet light; the position of Ruelene can be identified as a dark spot. This spot has an R_F value of approximately 0·6 under the conditions described in this paper. Run standard solutions of Ruelene as markers to aid location. Mark the position of the spot.

By using a template, cut an area 2 inches long by 1 inch wide from the chromatogram at the position of the spot for Ruelene. Cut a similar area from the marker spot to act as a standard; cut a similar area from in front of the marker spot to provide a paper blank.

Transfer the pieces of paper to separate 100-ml digestion flasks, and to the contents of each flask add 10 ml of glass-distilled water and then 5 ml of concentrated nitric acid. Then add 4 ml of N perchloric acid and 1 ml of concentrated hydrochloric acid, together with a glass bead. Digest the paper under reflux for 40 minutes, then drain the water from the condensers, and heat the contents of the flasks to brown fumes. Remove the condensers, and heat the contents of the flasks until white fumes of perchloric acid appear. Care must be taken at this stage when paper is being digested as the contents of the flasks may ignite; this can be avoided by adding a further 1 ml of N perchloric acid, together with 2 to 3 ml of water. Wash the walls of each condenser with about 5 ml of glass-distilled water, and add the washings to the flasks. Heat the solutions until white fumes appear.

Set the flasks aside to cool, and add 10 ml of water. Gently boil the contents of each flask under reflux for about 30 minutes; this ensures the hydrolysis of any pyrophosphate formed in the latter stages of oxidation. Set the flasks aside to cool, add 4 ml of ammonia solution, sp.gr. 0·88, and boil off the excess of ammonia.

Transfer the contents of each flask quantitatively to separate 100-ml separating funnels calibrated at 10 ml, and dilute to the mark with distilled water. Add 3 ml of ammonium molybdate solution to the contents of each separating funnel, and mix the solutions. Add 9 ml of the isobutanol - benzene mixture, and vigorously shake the separating funnels for exactly 30 seconds. Allow the phases to separate, and discard the aqueous layer. Wash the organic phase once with 5 ml of N sulphuric acid, and again discard the aqueous layer; remove as much of the aqueous phase as possible at this stage, and dry the stem of each separating funnel with a strip of filter-paper. Then transfer the organic layer to a stoppered 10-ml graduated cylinder, rinse the separating funnel with a small amount of ethanolic sulphuric acid, and add the rinsings to the cylinder. Make the contents of each cylinder up to 9·6 ml, and add 0·4 ml of ethanolic stannous chloride solution. Mix each solution thoroughly, and then measure its optical density at 730 mμ in 1-cm cells against a blank solution prepared by using glass-distilled water in place of the test solution. Prepare a standard curve from known amounts of standard orthophosphate solution.

Results

The recovery, by the proposed procedure, of Ruelene standards run as marker spots on the paper chromatograms is shown in Table I. These results have all been corrected for the paper blank value.

Table I

Recovery of ruelene standards

Ruelene present, μg	Ruelene found, μg	Recovery, %
50	45·0	90
111	121	109
50	46·5	93
53·5	48·0	90
50	51·0	102
55·5	54·5	98

The mean paper blank value (16 determinations) was found to be equivalent to 5·0 μg of Ruelene, with a range of 3·5 to 9·2 μg.

Samples (100 ml) of milk from untreated cows were analysed by the method described, and the results for the milk blank value corrected for paper blank value, were—

Ruelene equivalent, μg	4·0	8·0	4·2	2·7	1·7	5·7
Ruelene equivalent, p.p.m.	0·040	0·080	0·040	0·027	0·017	0·057

Recovery experiments were carried out on milk to which Ruelene had been added. The results of these experiments are shown in Table II, and have been corrected for both paper and milk blank values.

TABLE II

RECOVERY OF ADDED RUELENE

Ruelene added, μg	Ruelene found, μg	Recovery, %
100	80·2	80
111	88·0	79
55·5	41·5	75
55·5	40·5	73

These results show that residues of Ruelene in milk can be determined satisfactorily by this method. The mean milk blank value is equivalent to 0·044 p.p.m., which means that it should be possible satisfactorily to determine low levels of residues of Ruelene in milk. Preliminary experiments have shown that the appearance of residues of Ruelene in milk, after treatment of the cows, is transient and only low concentrations appear.

We thank Mr. F. G. Brown of Dow Agrochemicals Limited for supplying a pure sample of Ruelene and also Professor A. N. Worden and Mr. C. E. Waterhouse of the Huntingdon Research Centre for their interest and helpful discussions during the progress of this work.

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A Conductimetric Micro Method for the Simultaneous Determination of Carbon and Hydrogen in Organic Compounds

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A method is described in which the carbon dioxide and water vapour issuing from a modified Věceřa combustion train are absorbed in conductimetric cells, one containing potassium hydroxide and the other sulphuric acid; this permits carbon and hydrogen in organic compounds to be determined in 15 to 20 minutes. A similar method can be applied with a Belcher and Ingram train if nitrogen is absent.

Suggestions are made for a possible conductimetric determination of carbon, hydrogen and nitrogen.

IN two previous papers[1,2] conductimetric micro methods for determining separately carbon and hydrogen in organic compounds have been described. In the second[2] of these papers it was briefly stated that the two conductimetric cells could be placed in series and the two determinations carried out simultaneously. It was further stated that future work would be aimed at substantially reducing the time required for such analysis. This paper describes the manner in which this aim has been fulfilled.

It was decided to work with sample weights of 1 and 5 mg, the latter weight being considered preferable for routine work, and to concentrate initially on applying the conductimetric finish to the rapid empty-tube combustion method described by Belcher and Ingram.[3]

DESCRIPTION OF APPARATUS

For work on 1-mg samples the two conductimetric cells were unchanged from the previous work, with the exception of the use of a skirted joint (see Fig. 1) in the hydrogen cell to reduce dead space. The two cells were joined by a small tube containing M.A.R. anhydrone (14 to 22 mesh), the connections being made with Neoprene tubing.

For work on 5-mg samples larger conductimetric cells were constructed, of similar design, but holding approximately five times the volume of electrolyte.

HYDROGEN CELL—

The electrodes consist of bright platinum wire (0·015 inch in diameter) of which about $\frac{1}{8}$ inch protrudes into the electrolyte. The plug carrying the thermistor is made from polytetrafluoroethylene. The over-all height of the cell is 40 cm; it has a capacity of 25 ml, and the length of the bubble path is 50 cm. At a flow rate of 25 ml per minute the contact time between the liquid and the bubbles of gas is about 10 seconds, and complete circulation of the electrolyte occurs every 90 seconds. (With a flow rate of 50 ml per minute the contact time is 5 seconds and the circulation time 60 seconds.) It is essential that the bore of the delivery tube is 1·5 mm to prevent undue condensation of water. The outlet from the cell is made through a skirted joint to keep dead space over the electrolyte to a minimum.

CARBON CELL—

Each electrode consists of 10 turns of bright platinum wire (0·015 inch in diameter) wound round a mandrel of $\frac{1}{16}$-inch diameter. The plug carrying the thermistor is made from either polythene or polytetrafluoroethylene. The over-all height of the cell is 55 cm; it has a capacity of 100 ml, and the length of the bubble path is 75 cm. At a flow rate of 25 ml per minute the contact time between the liquid and the bubbles of gas is about 15 seconds, and complete circulation of the electrolyte occurs every 60 seconds. (With a flow rate of 50 ml per minute the contact time is 10 seconds, and the circulation time is 45 seconds.)

The bore of the delivery tube is 2·0 to 2·5 mm with an outside diameter of 4 mm; this tube is sealed into a second tube that then fits into the larger sized glass coil (see Fig. 2), which is ground to fit the cell.

The two cells are mounted in a Perspex box and are joined by a small tube containing M.A.R. anhydrone (14 to 22 mesh), the connections being made with ball joints that are kept lightly greased with Apiezon grease.

The rest of the apparatus is similar to that used in the previous work, conductance being measured with a Guest, Keen & Nettleford conductance meter.

ELECTROLYTE—

The electrolytes for the cells were the same as those used in the previous work, namely, 99·83 per cent. sulphuric acid for the hydrogen cell[2] and 0·05 N potassium hydroxide for the carbon cell,[1] each cell being calibrated in the same manner as before.

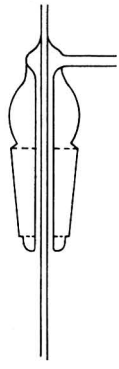

Fig. 1. Skirted joint used in hydrogen cell

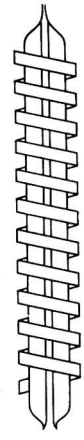

Fig. 2. Delivery tube of carbon cell

The mean factor from ten additions of dilute sulphuric acid to the hydrogen cell was 0·00480, expressed as mg of hydrogen per indicated ohm. The factor is operative up to the addition of 4·11 mg of hydrogen (36·7 mg of water), which permits the analysis of at least twelve 5-mg samples containing 5·0 per cent. of hydrogen.

The mean factor from ten additions of 0·05 N potassium carbonate to the carbon cell was 0·01696, expressed as mg of carbon per indicated ohm. The factor is operative up to the addition of 12·5 mg of carbon, which permits the analysis of three 5-mg samples containing 70 per cent. of carbon.

EXPERIMENTAL

During the work involving the use of the larger conductimetric cells it became apparent that, in this application, the Belcher and Ingram empty-tube combustion method was not suitable for the analysis of nitrogen-containing compounds; the nitrogen oxides on exit from the hot combustion chamber were not quantitatively retained by lead dioxide heated to 190° C, owing probably to the dissociation of nitrogen dioxide into nitric oxide and oxygen at temperatures in excess of 620° C, as suggested by Belcher and Ingram.[4]

$$2NO_2 \rightleftharpoons 2NO + O_2$$

These oxides of nitrogen were retained by the sulphuric acid in the hydrogen cell, resulting in high values for hydrogen. The method was satisfactory for all other types of compounds tested.

In view of this, Věceřa's combustion procedure,[5] in which cobalto-cobaltic oxide, Co_3O_4, at 620° C is used as catalyst, and an oxygen flow-rate of 20 to 25 ml per minute is maintained, was next examined as a more rapid method of pyrolysis for both 1- and 5-mg samples. This method proved to be completely satisfactory, the nitrogen oxides being quantitatively retained by lead dioxide heated to 190° C. The use of lead dioxide prolonged the recovery time of both water and carbon dioxide; without the reagent, carbon and hydrogen could be determined in

10 minutes by both the Věceřa and Belcher and Ingram methods on 1- or 5-mg samples. With the reagent in the Věceřa train, carbon and hydrogen could be determined in 15 minutes on a 1-mg sample and 20 minutes on a 5-mg sample, the limiting factor being the time taken for the combustion of the compound.

The filling used in the combustion tube for analysing 5-mg samples is shown in Fig. 3; when 1-mg samples are used, the length of the lead dioxide filling is reduced from 6 to 3 cm, the remaining space being filled with quartz chips.

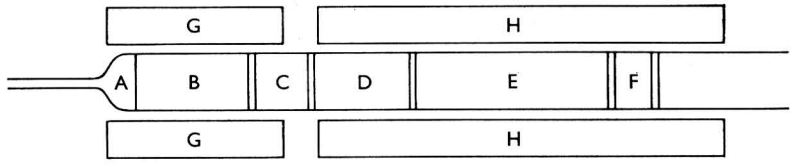

Fig. 3. Combustion tube fillings: A, quartz-wool plug; B, lead dioxide, 22 to 52 mesh (6 cm); C, quartz chips (2·5 cm); silver gauze (5·0 cm); E, cobalt oxide, 14 to 22 mesh (10·0 cm); F, silver gauze (1·0 cm). G, heating mortar; H, main furnace

Method

Assemble the apparatus as shown diagrammatically in Fig. 4. Wash and dry the cells in the manner described previously.[1,2] With oxygen flowing through the combustion tube at 25 ml per minute fill, by means of pipettes, first the hydrogen cell and then the carbon cell with the appropriate volumes of electrolyte. A piston-operated pipette fitted with a tap offers the most convenient way of dispensing the sulphuric acid. Ensure that no electrolyte enters the delivery tubes and that no air bubbles are trapped in the electrodes. Turn the main tap, F, to the by-pass position, and allow the cells to stabilise. This stabilisation period, which is only necessary when first filling the cells, takes approximately 10 minutes and is indicated by a constant reading (or drift) on the conductance meter. With oxygen flowing through the by-pass admit the sample into the combustion tube, and replace the bung. Close the main tap F in the oxygen line (see Fig. 4), and open pressurising tap, E; then open tap F to the combustion tube, and close the pressurising tap E. This procedure is essential to prevent electrolyte entering the delivery tubes of the cells. Should electrolyte

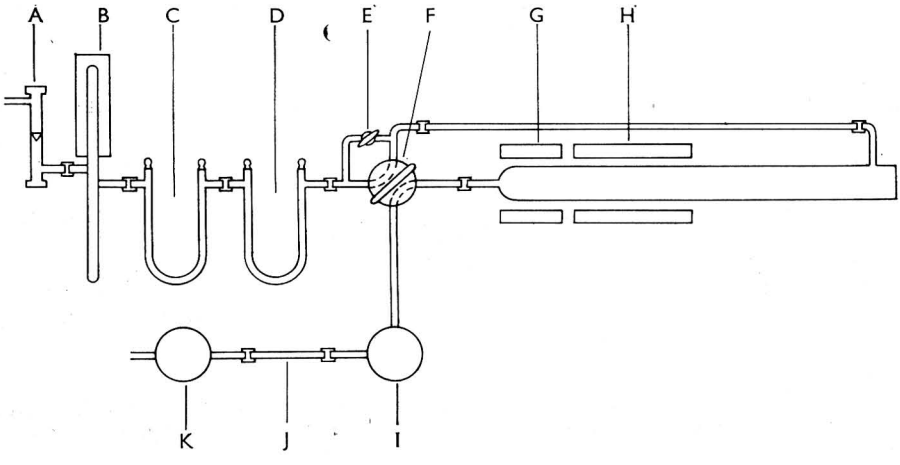

Fig. 4. Assembled apparatus

enter the delivery tubes, the cells must be emptied, dried, and re-filled before the start of any analysis. Pyrolyse the sample in the usual manner, allowing from 5 to 7 minutes to complete the oxidation; avoid violent combustion. Continue the passage of oxygen through the combustion tube until the conductance meter reading for both cells is constant or indicates the drift value. Turn tap F to the by-pass position, and remove the sample container from the combustion tube. (The passage of oxygen for several hours through the combustion tube should be avoided, as this leads to over-drying of the lead dioxide, which results in the first hydrogen value being low.)

Results

The proposed method has been applied to various organic compounds. The results for benzoic acid, indicating the standard deviation, the bias and the standard deviation of bias, are shown in Table I. The figures obtained by the gravimetric Pregl procedure are also shown for purposes of comparison. When the results of Table I are being interpreted it should be remembered that the sensitivity, and hence the accuracy and reproducibility, of the conductimetric finish can be varied by altering the strength or the volume of the electrolyte or both. The proposed method represents a compromise between the need for extreme sensitivity and the desire to do several determinations without refilling the cells.

Results for the carbon and hydrogen content of some other compounds are shown in Tables II and III.

Table I

Results for hydrogen and carbon obtained on benzoic acid by different methods

Ten determinations were made by each procedure

Weight of sample taken, mg	Method of combustion	Finish	Results for hydrogen: Standard deviation	Bias	Standard deviation of bias	Results for carbon: Standard deviation	Bias	Standard deviation of bias
1	Pregl*	G	0·176	0·08	0·056	0·980	0·67	0·312
1	Pregl†	C	0·072	0·00	0·023	0·310	0·02	0·106
1	Věceřa†	C	0·036	0·04	0·011	0·115	0·02	0·035
5	Pregl*	G	0·022	0·03	0·007	0·250	0·01	0·080
5	Pregl†	C	0·056	0·02	0·017	0·157	0·07	0·044
5	Věceřa†	C	0·033	0·02	0·010	0·061	0·02	0·019
5	Belcher and Ingram‡	C	0·036	0·03	0·012	0·149	0·02	0·049

G. Gravimetric. C. Conductimetric.
* Manganese dioxide used as absorber for nitrogen oxides.
† Lead dioxide heating-mortar used.
‡ No nitrogen absorber used.

Table II

Carbon and hydrogen found in 1-mg samples of various compounds

A Věceřa train with a lead dioxide heating mortar was used. The weights of sample taken were in the range 0·900 to 1·150 mg. The drift on the hydrogen cell was 8 ohms per hour increase, and the drift on the carbon cell was 3 ohms per hour increase. Three determinations were made on each sample

Compound	Hydrogen content: Mean found, %	Hydrogen content: Theoretical, %	Carbon content: Mean found, %	Carbon content: Theoretical, %
m-Dinitrobenzene	2·44	2·38	42·72	42·86
Triphenylphosphine	5·83	5·72	82·16	82·44
Sulphonal	7·08	7·02	36·77	36·82
Cyclohexanone-2,4-dinitrophenylhydrazone	5·02	5·07	51·75	51·86

Conclusions

The determination of carbon and hydrogen by a simultaneous conductimetric finish has been successfully accomplished by suitable adaptation of the Věceřa train and the Pregl heating mortar. The procedure is now in routine use in these laboratories and is easily carried out by relatively inexperienced staff. The number of determinations of carbon and hydrogen that can be performed has been increased to more than twice that determined by the gravimetric Belcher and Ingram procedure previously used.

Experience in the removal of nitrogen oxides has suggested a possible means of determining nitrogen conductimetrically, when complete conversion of nitrogen to its oxides can be accomplished, since these oxides are absorbed in 99·83 per cent. sulphuric acid; experiments are now being made to verify this. If this determination can be made conductimetrically we will then attempt to determine carbon, hydrogen and nitrogen simultaneously. One way of doing this is to absorb the carbon dioxide in potassium hydroxide and the nitrogen oxides

Table III

Carbon and hydrogen found in 5-mg samples of various compounds

A Věceřa train with a lead dioxide heating-mortar was used. The weights of sample taken were in the range 3·75 to 5·25 mg. The drift on the hydrogen cell was 2·0 ohms per hour increase, and the drift on the carbon cell was <1 ohm per hour (negligible over the test period)

Compound	Number of determinations	Hydrogen content		Carbon content	
		Mean found, %	Theoretical, %	Mean found, %	Theoretical, %
m-Dinitrobenzene	4	2·35	2·38	43·10	42·86
Phenylthiourea	3	5·17	5·25	55·08	55·20
Bromobenzoic acid	3	2·46	2·49	41·97	41·80
Sulphonal	3	7·04	7·02	36·88	36·82
Triphenylphosphine	3	5·72	5·72	82·25	82·44
n-Butanol	3	13·56	13·60	65·06	64·80
Cyclohexanone-2,4-dinitrophenylhydrazone	3	5·04	5·07	52·10	51·86

in sulphuric acid, after first absorbing the water vapour in a desiccant; subsequent heating of the desiccant would release the water vapour, which could then be absorbed in 99·83 per cent. sulphuric acid. Alternatively, it may be possible to split the gas stream and absorb each constituent separately.

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SHORT PAPERS

The Determination of Silica in Rocks and Minerals by Isotope Dilution with Silicon-31

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Precise and accurate methods are necessary for determining silica in rocks and minerals, particularly for purposes of rock classification. The classical gravimetric method for determining silica is time-consuming and has been shown to be subject to several sources of error.[1] Rapid spectrophotometric methods based on measurement of the blue colour of the reduced molybdosilicate complex[2] are also subject to interferences from certain ions in solution, particularly arsenate and phosphate.[3]

In the past few years, radiometric methods of chemical analysis have become common, and both neutron-activation and isotope-dilution methods have been applied to rock analysis. We have developed an extremely rapid and simple method for determining silica, which is accurate, reasonably precise and is not subject to most of the interferences affecting the gravimetric method. This method has been used for the routine determination of silica in silicates at the Mineralogical - Geological Museum, and is suitable for use wherever reactor facilities are available.

Experimental

The principle of the method is described below. A small amount of 2·6-hour silicon-31 (as active silica) is added to the powdered rock sample (ratio of active silica to silica in the sample, approximately 1 to 30); the mixture is fused with sodium hydroxide at 800° C and the cooled melt dissolved in water. The solution is acidified with hydrochloric acid and evaporated until most of the silica has precipitated. Evaporation to dryness must be carefully avoided as this increases the impurity content of the final silica. The silicic acid is removed by spinning in a centrifuge, washed thoroughly and ignited at 1000° C for 2 hours. A weighed portion of the silica is mounted on a planchet and counted with an end-window Geiger - Müller counter connected to a scaler. Standards of the active silica are also counted. The silica content of the rock is calculated from the expression—

$$\text{Percentage of silica in sample} = \frac{\left[\frac{m \times r_s \times 20}{r} - a\right] 100}{w}$$

Where, w = weight of sample taken, m = weight of silica in sample, r = count rate of silica in sample at time t, r_s = count rate of standard at time t and a = amount of active silica added.

Difficulty was found in obtaining sufficiently pure silica for irradiation. Irradiated Johnson Matthey 'Specpure' silica exhibited gamma activity, due to sodium-24, in addition to some longer lived beta activity. After irradiation the silica is fused with sodium hydroxide, the melt dissolved in water and the solution scavenged for impurities by adding ferric chloride; the resulting ferric hydroxide is removed by centrifugation. It was found that most of the foreign beta activity could be removed in this manner. The interference due to sodium-24 was eliminated by plotting the decay curves for the standards and correcting for the residual sodium-24 activity. The scavenged solution was used to prepare standards and to "spike" the samples.

In preparing the silica for counting it was found that not more than 30 mg of silica could be used, otherwise self-absorption of the beta radiation occurs. It was established experimentally that self-absorption is negligible if not more than 20 mg of silica are used.

Nuclear and irradiation data

Thermal neutron flux—approximately 10^{12} neutrons per sq. cm per second.

Reaction— $^{30}\mathrm{Si}(n,\gamma)^{31}\mathrm{Si} \xrightarrow[T_{\frac{1}{2}} = 2{\cdot}62\ \mathrm{h}]{\beta^-\ (1{\cdot}48\ \mathrm{MeV})} {}^{31}\mathrm{P}$.

Cross section of reaction—0·110 barns.

Method

Apparatus—

Thin end-window Geiger - Müller tube and scaler—Obtainable from the Nuclear Chicago Corporation.

Circular stainless-steel planchets—31 mm in diameter and having a 25-mm diameter inscribed circle.

Reagents—

Silica—"Specpure," obtainable from Johnson Matthey & Co. Ltd.
Sodium hydroxide pellets—AnalaR grade.
Ferric chloride solution, 10 per cent. w/v, aqueous.
Silver nitrate solution, 1 per cent. w/v, aqueous.
Hydrochloric acid, sp.gr. 1·18—AnalaR grade.
Dilute hydrochloric acid (1 + 9).

Procedure—

Weigh out 0·5 g of "Specpure" silica (dried at 110° C) into a small polythene tube, and heat-seal the ends. Irradiate the capsule in a nuclear reactor with a thermal neutron flux of 10^{12} neutrons per sq. cm per second for a period of 2 days. Set the material aside for several hours after irradiation to allow any short-lived activity to decay. Open the capsule, and transfer the contents quantitatively to a nickel crucible. Add 5 g of sodium hydroxide pellets, and heat the covered crucible at 800° C for 8 minutes. Allow the crucible to cool, then place it in a 200-ml beaker, add 100 ml of water, and heat on a steam-bath until the contents of the crucible have dissolved. Remove the crucible from the beaker, wash it, add 10 drops of ferric chloride solution, and heat on a steam-bath to complete precipitation of ferric hydroxide. Allow the solution to cool, filter, wash the filter-paper, and make the solution up to 250 ml in a calibrated flask.

Prepare five standards for counting by the procedure described below. Transfer 0·250 ml of scavenged silica solution by pipette on to a 25-mm diameter circle of tissue paper placed on a 31-mm diameter stainless-steel planchet. Evaporate the solution to dryness under an infrared lamp.

Weigh out 0·5 g of the sample into a nickel crucible, and by pipette add 5 ml of scavenged silica solution. Place under an infrared lamp, and evaporate slowly to dryness. Add 5 g of sodium hydroxide pellets, and fuse at 800° C for 10 minutes. Cool, add 15 ml of water, and heat the crucible gently to dissolve the cake. Transfer the contents to a 500-ml nickel evaporating dish, and wash the crucible thoroughly with dilute hydrochloric acid. Add 20 ml of concentrated hydrochloric acid to the contents of the evaporating dish, and place the dish on a steam-bath until the solution has evaporated to approximately 10 ml. (Most of the silica will have now precipitated.) Transfer the precipitate to a 15-ml centrifuge tube, and spin it. Wash the precipitate with 10 per cent. hydrochloric acid and then with water until the filtrate gives no positive test for chloride with a 1 per cent. solution of silver nitrate. Transfer the precipitate to a 15-ml quartz crucible, and ignite for 2 hours at 1000° C. Cool, and grind the silica to a fine powder in an agate mortar. Weigh out accurately 15 to 20 mg of the silica on to a steel planchet, and with water make an even deposit in the inscribed area. Dry under an infrared lamp. Prepare 10 such samples for counting.

Counting—

Immediately after their preparation, count the standards (at least 10,000 counts), and recount at frequent intervals over a period of 12 hours. Plot a curve of $\log_{10}$ activity *versus* time. Apply a correction for the longer-lived activity (^{24}Na) in the original silica solution. This correction can be calculated from the deviation of the curve from linearity. The samples should be counted as soon as possible after preparation, and not less than 4000 counts should be recorded. Apply a correction for decay of silicon-31 during counting and for coincidence, if significant. Read the count rate of the standard, at the time of counting the sample, from the decay curve of the standard. Calculate the silica content of the sample from the expression given on p. 891.

Results

Listed in Table I are results for silica in several standard reference samples together with accepted values. Relative deviations are also listed to indicate precision. It can be seen that good agreement is obtained for all samples except N.B.S. 70. No reason for this lack of agreement was found.

Precision is within 2 per cent., and this will be satisfactory for many geological purposes in view of the simplicity and rapidity of the method. Statistical evaluation of the results for G-1

TABLE I

RESULTS FOR THE DETERMINATION OF SILICA IN STANDARD SAMPLES

Standard sample	Number of determinations	Mean silica content, %	Relative deviation	Accepted value for silica content, %
G-1	39	72·56	1·70	72·65*
W-1	22	52·77	1·50	52·64*
N.B.S. 70 ..	18	67·49	1·90	66·63†
N.B.S. 99 ..	11	68·53	—	68·66†
Sulphide ore ..	2	34·28	—	34·6‡

* Values given in the "Second Report on a Co-operative Investigation of the Composition of Two Silicate Rocks."[1]

† National Bureau of Standards Certificate value.

‡ Value given in the "Report of the Nonmetallic Standards Committee," Canadian Association of Applied Spectroscopy.

show that no bias is found in the distribution of the values, as occurs in the results obtained by the normal gravimetric method.[1]

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Modification of the Scott Method for the Determination of Indole

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SCOTT[1] has published a method whereby indole is determined colorimetrically with *p*-dimethylaminocinnamic aldehyde after extraction from aqueous solutions with light petroleum. This method was shown by Scott to be 2·5 times as sensitive as that of the reaction with *p*-dimethylaminobenzaldehyde, and the green colour formed was claimed to be stable for several hours. Under the conditions in Singapore (temperature 30° C; relative humidity 80 per cent.) it was found that, although this method resulted in a far more sensitive colour development than that obtained with the *p*-dimethylaminobenzaldehyde reagent, the colour was unstable and faded at the rate 0·7 per cent. per minute after the first 15 minutes of colour development. To overcome this difficulty the procedure described below was developed; it has the advantages that the colour is stable for at least 2 hours under our conditions, and is about 4 times more sensitive than Scott's method, giving a linear graph within the range 0·5 to 5 μg or 0·05 to 0·5 μg of indole per ml in the original aqueous solution.

REAGENTS—

p-*Dimethylaminocinnamic aldehyde reagent*—A 0·5 per cent. w/v solution in n-propanol.

Sulphuric acid reagent—8 per cent. v/v of analytical-reagent grade concentrated sulphuric acid in 95 per cent. ethanol.

Acetic acid—50 per cent. v/v of analytical-reagent grade glacial acetic acid in water.

PROCEDURE—

Extract the indole from 10 ml of aqueous solution with 5 ml of toluene by shaking for 1 minute. Remove 4 ml of the toluene extract, and add to it 1 ml of the *p*-dimethylaminocinnamic aldehyde reagent. Place the mixture in a water-bath for 10 minutes at 37° C, and then add 0·5 ml of the sulphuric acid reagent, and allow the reaction to proceed for 10 minutes. Add 4 ml of the acetic acid, immediately remove the tube from the water-bath, and cool it to room temperature in tap water. Extract the colour into the acetic acid at the same time by gentle shaking for 1 minute. Allow the acid and organic layers to separate. Withdraw the lower acetic acid layer when it has

cleared, and measure the colour in 1-cm cells with a suitable spectrophotometer at 625 mμ against a reagent blank solution. If difficulty is experienced in obtaining a clear acetic acid layer, either spin the extract in a centrifuge or take an accurately measured volume of the extract and add 0·2 ml of ethanol.

Discussion of the method

The extraction of indole with toluene results in the removal of 98 per cent. of the indole originally present in the aqueous phase. This was determined by measuring the absorption of the aqueous phase at 275 mμ before and after extraction.[1] n-Propanol is used as solvent for the *p*-dimethylaminocinnamic aldehyde reagent to allow complete miscibility of the sulphuric acid reagent with toluene. Fig. 1 shows the rate of colour development at 37° C.

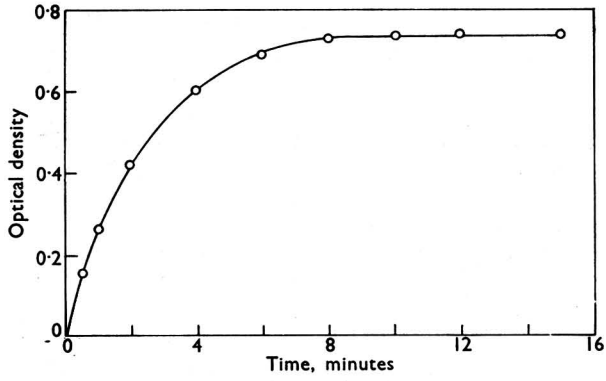

Fig. 1. Rate of development of the indole - *p*-dimethylaminocinnamic aldehyde colour at 37° C. In each test the colour development was stopped by extraction with 50 per cent. acetic acid; the optical density was then measured at 625 mμ

The use of acetic acid for the final extraction of the coloured complex has the advantage that the colour is stable in this solvent. Table I shows that the colour in toluene decreases slightly during 2 hours, whereas the colour in the acetic acid remains unchanged. Measurement of the coloured complex in toluene, before and after extraction with acetic acid, showed that 97 per cent. of the colour was extracted.

The standard colour development curve obtained after extraction of aqueous solutions containing known concentrations of indole was linear, passed through the origin and had a gradient of 0·134.

No colour was formed with any of the compounds named below: 3-methylindole, indole-3-ylpropionic acid, indol-3-ylaldehyde or indol-3-ylacetic acid. It appears, therefore, that non-substitution of the 3-position on indole is a specific requirement for the reaction, and that the method can be safely used for accurately determining indole in the presence of 3-methylindole.

Table I

Stability of indole colour complex with *p*-dimethylaminocinnamic aldehyde

The reaction was carried out with 5 μg of indole in 4 ml of toluene, and the optical densities were measured at 625 mμ with a Unicam SP1400

	Optical density of complex in toluene	Optical density of complex extracted into acetic acid
10 minutes after removal from water-bath at 37° C	0·73	0·88
2 hours later	0·68	0·88

I thank Mr. Francis Tan for his invaluable technical assistance.

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Modification to the Two-column Chromatographic Apparatus used in the Determination of Vitamin A in Edible Fats

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THE method proposed by Boldingh and Drost[1] for determining vitamin A in margarine has been adopted with slight modifications, as the official method[2] in the United Kingdom. Scott and Taylor[3] also used the modified method for determining vitamin A and carotene in butter.

A difficulty encountered in the two-column chromatographic procedure is in adjusting the rate of flow of the eluting liquid through the lower column. The apparatus is fitted with taps to control gas under pressure, which is applied to the columns to force the liquid through them. However, it is not easy to adjust the taps so as to maintain the necessary pressure differential. This becomes particularly inconvenient at the stage when the analyst is fully occupied collecting the eluate in tubes and testing their contents for the presence of vitamin A. By using a simple device, I have achieved good control over the rate of flow of the eluting liquid through the lower column.

Instead of connecting the upper and lower columns with a rubber tube, the opening for the tube in the upper column is plugged, and a 10-ml syringe (a hypodermic syringe is satisfactory) is connected to the lower column, as shown in Fig. 1.

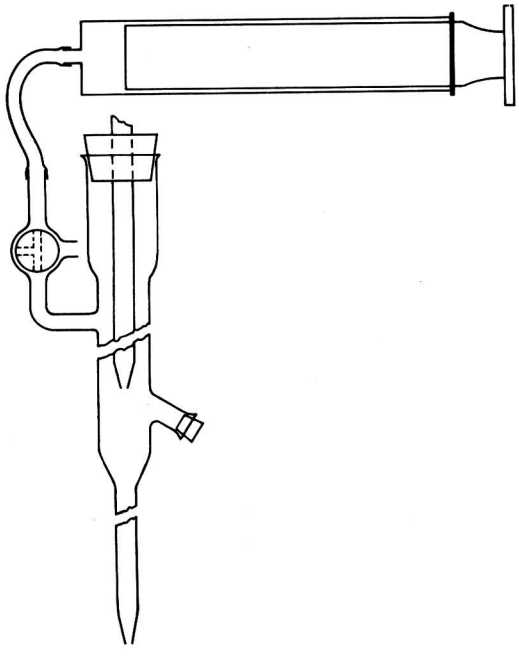

Fig. 1. Diagram showing how the syringe is connected to the lower column

Initially, the plunger of the syringe is set half way in the barrel. The level of the liquid in the lower column is raised or depressed by slight movement of the plunger. As a few seconds elapse before the new equilibrium is established, care should be taken to avoid over-adjustment.

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The Determination of *N*-Substituted Aromatic Thioureas

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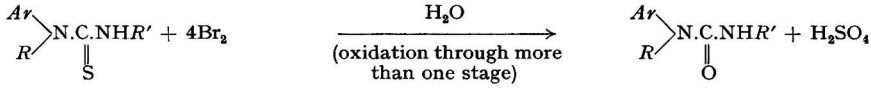
A search of the literature on the aromatic substituted thioureas revealed a lack of suitable methods for their determination. Callan and co-workers[1,2] reported that *sym*-diphenylthiourea could be titrated easily with bromine in glacial acetic acid at 25° to 30° C, 4 atoms of bromine being required per molecule of *sym*-diphenylthiourea. According to these workers substitution of bromine in the aromatic nuclei takes place. Wojahn reported that 1-naphthylthiourea is best determined argentimetrically[3] and that phenylthiourea could be determined with sodium hypoiodite.[4] Apart from these few attempts, no systematic work seems to have been carried out on the determination of aromatic thioureas (but compare other work[5,6,7,8]).

It has now been found that some aryl substituted thioureas can be determined titrimetrically with bromide - bromate mixture in an acid medium, and with iodine in a sodium hydrogen carbonate medium. The course of reaction with bromine can be represented by the general scheme shown below; the primary reaction is always removal of sulphur from the thiourea to give the corresponding urea.



where Ar = phenyl and

(*i*) R and R' = H,
(*ii*) R = H and R' = phenyl,
(*iii*) R = H and R' = methyl,
(*iv*) R = ethyl and R' = H.

The urea so produced undergoes bromination in the aromatic nucleus (or nuclei) to give the corresponding nuclear brominated products, with the exception of *N*,*N*-ethylphenylthiourea, which appears to behave like di-substituted anilines or those anilides substituted on the nitrogen attached to the aromatic nucleus.

Thus—

(*i*) $C_6H_5.NH.CS.NH_2 + 7Br_2 \xrightarrow{H_2O} C_6H_2.Br_3.NH.CO.NH_2 + H_2SO_4 + 11HBr.$

N-phenylthiourea — 2,4,6-tribromophenylurea

(*ii*) $C_6H_5.NH.CS.NH.C_6H_5 + 6Br_2 \xrightarrow{H_2O} Br.C_6H_4.NH.CO.NH.C_6H_4.Br + H_2SO_4 + 10HBr.$

N,*N*′-diphenylthiourea — 4,4′-dibromodiphenylurea

(*iii*) $C_6H_5.NH.CS.NH.CH_3 + 7Br_2 \xrightarrow{H_2O} C_6H_2Br_3.NH.CO.NH.CH_3 + H_2SO_4 + 11HBr.$

N-methyl-*N*′-phenylthiourea — *N*-methyl-*N*′-(2,4,6-tribromo)phenylurea.

(*iv*) $(C_6H_5)(C_2H_5)N.CS.NH_2 + 4Br_2 \xrightarrow{H_2O} (C_6H_5)(C_2H_5)N.CO.NH_2 + H_2SO_4 + 8HBr.$

N,*N*-ethylphenylthiourea — *N*,*N*-ethylphenylurea

According to the mechanisms outlined above, thioureas (*i*) to (*iv*) require 14, 12, 14 and 8 atoms of bromine, respectively, for complete reaction; this has been verified experimentally. The brominated products in equations (*i*), (*ii*) and (*iii*) have been isolated and identified.

Experimental

Bromimetric determination of aromatic thioureas—

In the direct titration of phenylthiourea solutions with bromine or bromide - bromate mixture in an acidic medium a sharp and reproducible end-point could not be obtained. Methyl red and phenol red, which were used as internal indicators, were readily decolorised before the true end-point was reached. Potentiometric titrations, which had been successful with thiourea and its

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alkyl homologues,[5,8] showed no improvement on the methods described above, as the changes in potential were rather slow and therefore untrustworthy. The procedure described below was found to give reproducible results.

To a known volume (about 20 ml) of a standard solution of the thiourea in water or dilute acetic acid, acidified with 5 N hydrochloric acid, was added a known excess of 0·1 N bromide - bromate solution (prepared from analytical-reagent grade materials). The solution was set aside for 20 to 30 minutes, and potassium iodide was added. The iodine liberated (by the excess of bromine) was then titrated against standard sodium thiosulphate solution, in the usual manner. The number of atoms of bromine consumed per molecule of thiourea is shown in Table I.

The influence of a large excess of bromine on the determination was tested with a solution containing 0·3324 g of *N,N'*-methylphenylthiourea per litre. Portions of this solution from 2·0 to 30·0 ml were taken, and 10 ml of 5 N hydrochloric acid and then 10 ml of 0·1 N bromide - bromate were added to each; the excess of bromine was then titrated with 0·0944 N sodium thiosulphate. The results (atoms of bromine consumed per molecule of the thiourea) ranged from 17·7 for the 2-ml portion of original solution to the theoretical 14 for the 30-ml portion, indicating that results are high when too large an excess of bromine is present.

Experiments with larger amounts of the thiourea solutions and bromide - bromate mixture allowed the brominated products to be separated. Phenylthiourea gave the expected 2,4,6-tribromophenylurea (m.p. found, >270° C[10]; bromine found, 64·89 per cent.; calculated 64·3 per cent.). Extremely small amounts of dibromophenylurea were also formed (m.p. 201° C[10]) and were separated by fractional crystallisation. *sym*-Di-phenylthiourea gave the expected 4,4'-dibromodiphenylurea (m.p. found, >300° C[11,12]; bromine found, 45·0 per cent.; calculated, 43·19 per cent.). *N*-Methyl-*N'*-phenylthiourea gave the expected *N*-methyl-*N'*-tribomophenylurea (m.p. found, 222° C; bromine found, 59·7 per cent.; calculated, 61·97 per cent.—the slightly lower value found might be due to the presence of a lower brominated product). *N,N*-Ethylphenylthiourea gave a product melting at 74° C, free from bromine and containing 17 per cent. of nitrogen. The product could not be identified fully (m.p. of ethylphenylurea, 62° to 62·5° C[13,14]).

TABLE I

BROMIMETRIC DETERMINATION OF AROMATIC THIOUREAS

Thiourea	Solvent	Range of determination, g per litre	Atoms of bromine consumed per molecule of thiourea	
			Expected	Found
N-Phenyl-	Water	0·1522 to 1·5222	14	13·5
N,N'-Diphenyl-	Acetic acid	0·9132 to 9·1324	12	11·8
N,N'-Methylphenyl- ..	Water	0·3324	14	14·0*
	Acetic acid	3·3248 to 6·6496	12	12·0
N,N-Ethylphenyl-	Water	0·1803 to 0·3604	8	8·5

* Over-bromination causing high results can occur if the excess of bromine used is too large.

IODIMETRIC DETERMINATION OF AROMATIC THIOUREAS—

Direct titrations of phenylthiourea solutions with iodine were also unsuccessful, as the consumption of iodine per molecule of phenylthiourea was found in acidic medium to be between 1 and 2 atoms and in alkaline medium between 4 and 6 atoms. Phenylthiourea, *N,N'*-methylphenylthiourea and *N,N*-ethylphenylthiourea were successfully determined as described below.

Ten millilitres of the thiourea solution of known concentration were mixed with about 20 ml of M sodium hydrogen carbonate and a known excess of standard iodine solution. After about 20 minutes the excess of iodine was titrated with 0·1 N arsenous oxide. As expected, 1 mole of the thiourea was equivalent to 8 atoms of iodine; up to a concentration of about 1 g of the thiourea per litre, quantitative results were obtained.

DISCUSSION OF THE METHOD

Aromatic thioureas are not particularly soluble in water, which imposes a limit on the concentration range in which they can be determined in aqueous solution; within this range, however, they can be accurately determined with bromide - bromate solution in acid medium or with iodine in presence of sodium hydrogen carbonate.[5] The bromine consumption in the bromimetric determination was found to be slightly less than expected (except for *N,N*-ethylphenylthiourea), but in all determinations the results were reproducible within the limits of experimental error.

Only *N,N'*-methylphenylthiourea consumed more bromine than expected, the amount depending on the excess of bromine used.

The results of determinations with excess of iodine in presence of sodium hydrogen carbonate showed excellent agreement with the amount of sample taken, especially when dilute solutions were used. *sym*-Diphenylthiourea, which is almost insoluble in water, could not be determined iodimetrically. A search for non-aqueous media for the determination of aromatic thioureas is being undertaken.

I thank Professor S. S. Joshi and Dr. R. H. Sahasrabudhey for their keen interest and valuable suggestions and Dr. G. B. Singh, Head of the Department of Chemistry, for the facilities provided during this investigation.

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Application of a Modified Stannous Chloride Reagent for determining Orthophosphate

BY A. HENRIKSEN

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Two years ago Sletten and Bach[1] suggested that the stannous chloride used as reducing agent in the molybdenum blue method for colorimetric determination of orthophosphate should be dissolved in glycerol instead of in strong mineral acid. They claimed excellent stability for this reagent. Apparently, this modification has received little attention. We have used the modified reagent in our laboratory for more than a year with good results, and we believe it provides an important improvement in the molybdenum blue method.

In our routine determinations of phosphate in large amounts of seawater and, occasionally, in sewage effluents, we differentiate between orthophosphate and acid hydrolysable phosphates. To obtain this differentiation we chose the extraction method originally published by Martin and Doty[2] and later modification by the Association of American Soap and Glycerin Producers (AASGP).[3] A further advantage of the extraction step is elimination of interferences from most materials found in water.

We have modified two of the reagents used in the method described by the AASGP Committee. First, the stannous chloride reagent was modified by dissolving 0·125 g of $SnCl_2.2H_2O$ in 100 ml of analytical-reagent grade glycerol (phosphate-free). (One millilitre of this solution was used directly for reducing the yellow molybdophosphoric acid in the organic layer.) Second, the benzene - isobutanol mixture used for extraction was replaced by pure isobutanol, to avoid the use of benzene (a health hazard). The colour was measured in an E.E.L. photometer (Evans Electroselenium Ltd.) with a deep-red filter (609) and 4-cm cells being used. The visible spectrum of the colour formed shows two peaks, at 625 and 730 mμ.

Polarographic analysis of samples of the modified reagent stored for about 5 months at room temperature showed that only 16 per cent. of the stannous ions were oxidised to stannic ions, even though no precautions were taken to protect the solution from light and air.

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Received *April 18th*, 1963

Diffusion Bottles for the Determination of Fluorine

By R. J. HALL*

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THE original bottle and cap described by Hall[1,2] for determining fluorine by diffusion as hydrofluoric acid is no longer available, but those at present supplied by Arnold Horwell Ltd., Cricklewood, London, also have a capacity of 20 ml, and can be used without modification. The sample and reagents are placed in the bottle, and the filter-paper (3 cm × 1·5 cm) is inserted into the neck so that 2 to 3 mm can be folded back over the rim. One drop of 0·1 N magnesium succinate is placed with a dropping pipette, having a fairly long capillary (7 or 8 cm), on that part of the paper that is free inside the bottle. The cap is quickly but carefully screwed on, and this alone holds the filter-paper in position. The cap is now sealed with hot wax as previously described. Recoveries from these bottles have been as quantitative and reproducible as with those formerly used.

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Received *July 23rd*, 1963

* Present address: National Agricultural Advisory Service, Kenton, Newcastle-on-Tyne.

Book Reviews

KIRK-OTHMER ENCYCLOPEDIA OF CHEMICAL TECHNOLOGY. Volume I. ABHERENTS TO ALUMINUM. Edited by HERMAN F. MARK, JOHN J. MCKETTA, JUN., DONALD F. OTHMER and ANTHONY STANDEN. Second Edition. Pp. xx + 990. New York and London: Interscience Publishers, a division of John Wiley & Sons. 1963. Price £16 18s.; price per volume for subscribers to the complete set of 18 volumes £13.

I think it was Oliver Wendell Holmes who, pondering on the remarkably well-informed conversation of a new acquaintance, suddenly realised that all the subjects discussed had started with the letter A; and that the first volume of a new encyclopedia had recently been published. He lost no time in contriving another meeting and leading the conversation to a subject lower down in the alphabetical scale—with the expected result! A conscientious reviewer of the first volume of the second edition of Kirk and Othmer might well find himself in the same position as Holmes' acquaintance, and be able to talk with full authority on subjects as widely separated but as alphabetically close as ablation, alcoholic beverages, aerosols, algal cultures and allyl compounds.

The first edition appeared in 15 volumes published between 1947 and 1956, with supplements in 1957 and 1960. The interval that has elapsed since the first edition of this encyclopedia has obviously necessitated much revision, expansion and addition, and it is claimed that the 1000 specialist authors participating in the present work have ensured the inclusion of the most recent advances in all fields of chemical technology. Emphasis is placed about equally on the scientific presentation of the physical and chemical properties of a substance and on its manufacture and uses. Fundamental concepts and unit operations of chemical engineering are dealt with in individual articles. It is intended to cover analytical methods in general articles, and there are to be numerous articles on general scientific subjects. There is an adequate bibliography for each major article.

It is difficult to assess the extent and efficiency with which all these claims have been implemented from the examination of a volume that considers only words up to AL. However from such tests as it has been possible to apply to the 35 major articles contained in the 990 pages of this volume, it would seem that the claims made as to scope, accuracy and documentation are fully justified. Moreover, if one considers the number of authors involved, the presentation is remarkably balanced and consistent, and in addition to this the layout is pleasing and easy to the eye. Indeed, for a scientific work the general standard of production may be described as almost lavish.

The analyst may be less satisfied. This volume contains no articles on analytical methods, and such references to chemical analysis as exist are relatively brief and superficial and occur in only a few of the articles. It is hoped that the subsequent volumes will restore the balance—the word "analysis" has still to come. At this stage however, I cannot offer any guide as to what help or benefit will accrue to my analyst colleagues.

Nor can I wholly subscribe to the international character claimed for this new edition. Compared with the first edition, which was intended to concentrate on United States technology, it certainly has broadened its frontiers. However, it is still predominantly American in approach and treatment, and a glance at the list of contributors and the bibliographies amply confirms this view.

One is tempted to compare this work with the nearest of its kind published in the United Kingdom, Thorpe's "Dictionary of Applied Chemistry," the last edition of which (11 volumes, each of about 600 pages) appeared spread over the years 1937 to 1954. Thorpe, when it was published, had an unassailable reputation for comprehensive treatment in a concise form combined with accuracy, and it has to be stated that in these respects it is equalled by Kirk and Othmer. However, it is important to appreciate the difference between a dictionary and encyclopedia. Thorpe combined much of the character of both; many words were defined, and selected words and subjects were discussed in articles of appropriate length. Kirk and Othmer is a true encyclopedia in that it has no specific definitions, and the articles are longer and mostly of a generalised nature, covering, as a rule, groups of substances rather than individual compounds. Thus one of the longest articles (89 pages) is headed "Alkali and Chlorine Industries," and it covers chlorine, sodium carbonate and sodium hydroxide. Under the heading "Acids," one finds only "acids carboxylic" and "acids dicarboxylic," with no reference to other acids, such as acids, aliphatic or acids, mineral. These no doubt, are discussed elsewhere. Industrial alcohol is now considered under "Ethanol" instead of under "alcohol" as in the first Edition; and in general a compound is discussed under its own name, under a group of substances or as a derivative under a parent compound. Even with the cross-references (which do not tell us where to find "alcohol"), this system may appear to make reference rather difficult when one has to handle all eighteen volumes of this size. No doubt a collective index will be available ultimately, as with the first Edition, but this of course must await the publication of the complete text.

This is obviously a very important work, and any opinion that has to be based only on the first of the 18 volumes must necessarily be guarded. This fraction however, represents a promising start and if, as appears likely, the analyst will not want to buy the series for its coverage of chemical analysis, he will certainly want to do so for the information it can give him on chemical technology as a whole. The word "want" is used advisedly because of the price consideration. Attention should however, be drawn to the substantial reduction of 23 per cent. offered by the set subscription price.

JULIUS GRANT

JOURNAL OF THE ASSOCIATION OF PUBLIC ANALYSTS. Volume 1, No. 1. First Quarter, 1963. Hon. Editor, DR. E. C. WOOD. Pp. 24. London: The Association of Public Analysts. Annual Subscription for 4 quarterly parts 30s.; single copies 7s. 6d.

A decade has passed since it was mutually agreed to separate certain functions of the Society of Public Analysts and Other Analytical Chemists by altering the name to the Society for Analytical Chemistry and by the formation of the Association of Public Analysts. Advantage has been gained by the changed emphasis of the Society and its Journal, and the Association has been able to concentrate on, and to expand, its own particular interests.

It appears, however, that for some time public analysts have felt the need for more practical interchange of ideas and for more information of what other public analysts are thinking and how they propose to tackle the various problems that concern their work. The circulation of confidential bulletins and of non-confidential monthly reports has not wholly met their needs. It was therefore decided to publish a quarterly journal, and the first number has now appeared. This contains a Foreword by the President of the Association, Special Announcements, Analytical and Technical Contributions and Items of Interest. The Special Announcements include the official view of the Association on Food Standards for Sausages and Sausage Meat, Luncheon Meat, Flavoured Milk, Marzipan, or Almond Paste, and Candied Peel or Cut Peel. Some of these standards have been arrived at by agreement with the Food Manufacturers Federation and will be of interest to all food chemists. The contributions are from public analysts only, but it is hoped that subscribers to the Journal and others interested will submit matters of mutual interest.

Public analysts were the founders of our Society and have always maintained close relations with chemists in all branches of the food industry. It is important that such liaison should be maintained, and the opportunity for food chemists to subscribe and contribute to this new journal should not be missed.

In his Foreword to this first number the President says "The Association has done much since its inception in 1953 to foster a spirit of co-operation, negotiation and mutual understanding between those responsible for making the laws relating to food and drugs, those responsible for their enforcement, and those who have to tread warily to avoid committing an offence." If, as it deserves, this Journal secures support from the wide field of food chemists, it will fill a very useful and important rôle in the public interest. J. R. NICHOLLS

PRACTICAL METHODS FOR THE MICROBIOLOGICAL ASSAY OF THE VITAMIN B-COMPLEX AND AMINO ACIDS. By E. C. BARTON-WRIGHT, D.Sc., F.R.I.C., M.I.Biol. Pp. 52. London: United Trade Press Ltd. 1963. Price 10s. 6d.

This inexpensive paper-covered book consists of a series of articles written for *Laboratory Practice* and is, in effect, a revised edition in cheaper format of the author's "Microbiological Assay of the Vitamin B-Complex and Amino Acids." The list of B-Vitamins appearing in the earlier book has been extended to include folinic acid and vitamin B_{12}, and other additions are detailed instructions for performing disc-inoculum and cup-plate microbiological assays.

The author has introduced minor modifications into some of the basal media and, in a number of assays, has changed the method of preparing the inoculum; instead of transferring the micro-organism to saline solution he dilutes the sub-culture with Ringer's solution. He recommends a change of micro-organism for four of the vitamin and four of the amino acid assays.

Chemists not widely experienced in microbiological assays can obtain through this book comprehensive instructions for the assay of B-vitamins and amino acids at very low cost, and expert microbiologists owning the earlier edition will probably be willing to spend the same small sum to learn of the author's later experiences and views, although these may not be fully in accord with their own. A. J. AMOS

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PHYSICAL CHEMISTRY. By WALTER J. MOORE. Fourth Edition. Pp. xiv + 879. London: Longmans, Green and Co. Ltd. 1963. Price 50s.

Correction

THE "Report of the Proceedings of the Thirteenth Session of the International Commission for Uniform Methods of Sugar Analysis," listed under PUBLICATIONS RECEIVED last month (page 822) is only obtainable from Monsieur R. Saunier, Secretaire, I.C.U.M.S.A., Syndicat National des Fabricants de Sucre de France, 23, Avenue d'Iena, Paris XVI^{e}, France, and **not** from I.C.U.M.S.A., Keston, Kent, as indicated in the original entry.

Report of the Analytical Methods Committee: Reprints

Nitrogen Factors for Chicken

THE Report prepared by the Meat Products Sub-Committee, "Nitrogen Factors for Chicken," reprinted from *The Analyst*, August, 1963, **88**, 583–584, is now available from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1. Price to members, 1s. 6d. each; to non-members, 2s. 6d. each.

Reports of the Analytical Methods Committee are only available from the Secretary (not through Trade Agents) and remittances, made out to the Society for Analytical Chemistry, MUST accompany orders.

Reprints of Review Papers

"Circular Dichroism"

REPRINTS of the Review Paper, "Circular Dichroism," by R. D. Gillard, published in this issue of *The Analyst*, will soon be available from the Secretary, Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1, at 2s. 6d. per copy, post free.

"Information Retrieval in the Analytical Laboratory"

REPRINTS of the Review Paper, "Information Retrieval in the Analytical Laboratory," by D. R. Curry, published in this issue of *The Analyst*, will soon be available from the Secretary, Society for Analytical Chemistry, at the above address, price 2s. 6d. per copy, post free.

A remittance for the correct amount, payable to The Society for Analytical Chemistry, MUST accompany the order; these reprints are not available through Trade Agents.

Erratum

SEPTEMBER (1963) ISSUE, foot of p. 738, present address of T. Doran. *For* "Sunbury-on-Thames, Surrey." *read* "Sunbury-on-Thames, Middlesex."

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