THE ANALYST

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

THE AMERICAN CHEMICAL SOCIETY

On Wednesday, September 5th, in New York, the President, Dr. J. R. Nicholls, C.B.E., presented the following address to the American Chemical Society on the occasion of the celebration of the 75th Anniversary of its foundation—

FROM
THE SOCIETY OF PUBLIC ANALYSTS
AND
OTHER ANALYTICAL CHEMISTS
TO
THE AMERICAN CHEMICAL SOCIETY

On the occasion of the Celebration in New York, in September, 1951, of the Seventy-Fifth Anniversary of the founding of the American Chemical Society, the President, Council and Members of the Society of Public Analysts and Other Analytical Chemists send Greetings to the Officers and Members of the American Chemical Society.

The Society sincerely welcomes the opportunity thus given to it in its own seventy-eighth year to express the friendship and goodwill of all its members to their American confreres celebrating the seventy-fifth anniversary of their Society, and offers its best wishes for the continued prosperity of the American Chemical Society.

(*Signed*) J. R. NICHOLLS (*President*).
J. H. HAMENCE (*Honorary Treasurer*).
K. A. WILLIAMS (*Honorary Secretary*).

Dated this Third day of September
Nineteen Hundred and Fifty One.

Seal of the Society of Public Analysts and Other Analytical Chemists

XIIth INTERNATIONAL CONGRESS OF PURE AND APPLIED CHEMISTRY, SEPTEMBER 10th TO 13th, 1951

THE Council of the Society of Public Analysts and Other Analytical Chemists has appointed Dr. J. R. Nicholls, C.B.E., Mr. R. C. Chirnside and Dr. K. A. Williams as delegates to the XIIth International Congress of Pure and Applied Chemistry.

BIOLOGICAL METHODS GROUP

THE Summer Meeting of the Group was held at 2 p.m. on Friday, June 1st, 1951, at the National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7.

The following papers were presented and discussed: "Errors of the Mouse Insulin Assay," by P. A. Young and G. A. Stewart; "Simplification in Statistical Computation," by D. C. Gilles; "Cup-plate Assays of Biotin and Nicotinic Acid," by S. Morris and A. Jones.

From 4 p.m. until 6 p.m. the following demonstrations were presented: "Demonstrations of Some of the Less Well-known Methods of Biological Assay in use at the National Institute for Medical Research," by W. L. M. Perry; "Assay of Digitalis," by G. F. Somers; "Assay of Vitamin B_{12} with *Euglena gracilis*," by P. Waterhouse; "Microbiological Assay of Crude Extracts Containing Several Growth Factors," by F. A. Robinson and B. W. Williams; "Apparatus for Automatic Recording of Blood-clotting Times," by S. S. Randall.

The Determination of the Acidity of Milk

By E. I. JOHNSON and J. KING

(*Presented at the meeting of the Society on Wednesday, April 4th*, 1951)

A review is given of the various methods that have been used in determining the acidity of milk. Measurements of the increase of colour with increase of pH during the addition of alkali when phenolphthalein is used as indicator have shown that the comparative method, using a fixed concentration of rosaniline acetate in the milk as a colour standard, is sound in principle and gives concordant results.

The acidity of milk is of fundamental importance to the dairy industry and many attempts have been made to standardise a method for its determination so that observers in different laboratories shall obtain concordant results. The difficulty in so doing arises mainly because milk is an excellent buffer from its normal pH to values well on the alkaline side, so that the usual sudden change in colour observable when a strong alkali is added to a strong acid in the presence of a suitable indicator does not occur. The gradual increase in colour with addition of alkali raises doubts in the mind of the operator as to whether there is any true end-point. The observed end-point usually depends not only on the acidity of the sample but also on the operator's acuity of colour perception. This depends on whether the operator has normal or abnormal colour vision, on his state of fatigue and on the method of lighting. To minimise these effects it is customary in many laboratories to match the onset of pink colour to that of a standard amount of rosaniline acetate solution added to a given amount of the same milk.[1,2] Alternatively, the amount of 0·1 *N* sodium hydroxide required to alter the pH of, say, 10 ml of the milk to a standard pH, usually 8·3, is recorded as the "acidity," the measurement of pH being performed electrometrically and not colorimetrically. The effect of adding increasing amounts of phenolphthalein has also been studied, as it is now well known that, with decreasing amounts of the indicator, the pH to which it is necessary to titrate the milk before a pink colour is given rises from about 8·3 with an indicator concentration of 0·05 g per 100 ml of milk to 8·9 when the concentration is only 0·005 g per 100 ml.[2,3] The addition of the higher amount of indicator in alcoholic solution to milk at its normal pH leads to precipitation of some of the phenolphthalein in an extremely finely divided form, and this acts as a "reservoir" that increases the amount of the soluble (coloured) form of indicator in the solution as the pH rises. The dilution of the milk by different normalities of alkali used in the titration displaces the end-point. Sommer and Minos,[4] in a review of the literature on the subject and from their own experimental work, conclude that tri-calcium phosphate is precipitated on the addition of alkali, dilution decreasing the amount affected and hence the titratable acidity. Dilution also decreases the "salt effect" and "protein effect" on the colour of the indicator. Kruisheer[5] has described six methods, which make use of various volumes of milk, normalities of alkali and concentrations of phenolphthalein, and has compared the results given by some of them in the determination of the acidity of samples of the same milk.

The measurement of colour has been intensively studied in recent years. In 1931 the Commission Internationale de l'Eclairage (C.I.E.) adopted certain resolutions for the measurement and specification of colour.[6] The Lovibond - Schofield colorimeter is a convenient

instrument for measuring colour in terms of units that are readily convertible to the C.I.E. system.[7] The conversion graph with this instrument is based on Judd's Uniform Chromaticity System,[8] which enables colour differences to be expressed in terms of numbers of "least perceptible differences." This system has been adopted in the following work, which was carried out to ascertain the theoretical basis for the "comparative" method of determining the acidity of milk.

Experimental

Apparatus—

The Lovibond - Schofield colorimeter was used throughout, measurements being transferred to the graph of equal chromaticities described by Schofield.[7] One of the blocks of magnesium carbonate was removed and a glass cell, which completely filled the aperture, was fixed in its place. The milk to which the indicator or rosaniline acetate had been added was poured into this cell and its colour measured in the normal manner.

Direct comparisons without the aid of the colorimeter were also made between a milk to which the standard rosaniline acetate had been added and the milk at various stages of titration in a divided Petri dish, constructed as follows. Molten paraffin wax was poured into a Petri dish of 6·8 cm internal diameter and 1·2 cm depth until the remaining volume was about 30 ml. Before the wax had set, a thin slip of glass measuring about 6·8 cm by 1·1 cm was held centrally in the dish to divide it into two equal semi-circular cells. The wax was then allowed to solidify and the cells were made water-tight by sealing at the ends of the partition with paraffin wax. Molten paraffin wax was then poured into each compartment until each volume was about 12 ml. For the electrometric determination of pH the meter used was of normal construction with a glass electrode; it could be read to 0·02 pH. The readings of the meter were checked at a range of pH 8·0 to 9·0 with standard buffer solutions.

Methods—

For direct comparisons, 10-ml portions of milk were delivered into both halves of the divided Petri dish. To the contents of one cell was added 1 ml of a 0·5 per cent. solution of phenolphthalein in 50 per cent. v/v alcohol and to the other 1 ml of a freshly-prepared 0·00024 per cent. solution of rosaniline acetate in 50 per cent. v/v alcohol, slightly acidified with acetic acid. A 0·1 *N* solution of sodium hydroxide was then added to the cell containing phenolphthalein, while stirring until the colour matched that of the contents of the rosaniline-acetate cell. With a little manipulation it was possible to bring the surfaces of the liquids to the top of the glass dividing partition by dropping in glass beads or by further addition of paraffin wax so that the two fields were practically touching each other with a sharp dividing line. It is possible to judge a colour match more accurately in this way than by comparing the contents of separate dishes and also more accurately than in the small fields of the colorimeter. It is therefore possible to discern the first slight change of colour at a point somewhat before that necessary to record "one least perceptible difference" by measurement in the colorimeter.

For measurement of the colours by the Lovibond - Schofield colorimeter, 50 ml of milk were used, the amount of the phenolphthalein and rosaniline acetate solutions being increased correspondingly. This bulk was necessary to provide sufficient material for a viewing cell of sufficient size and for the simultaneous measurement of pH as the titration proceeded. A 0·1 *N* solution of sodium hydroxide was added in increments of about 0·4 ml at a time and the colour and the pH were measured as rapidly as possible, because the colour fades on standing, especially as the pH approaches 9. It was found to be very difficult to measure the pink colour at a pH below 8·2 with accuracy by means of the instrument, but differences in the Petri cells were observable down to a pH of about 8·0.

The milk containing the rosaniline acetate was then poured into the cell and its colour measured in the same way as described for the milk plus phenolphthalein.

The readings from the Lovibond - Schofield colorimeter were transferred to a scale of equal chromaticity and are shown in Fig. 1. The corresponding pH of the milk for each colour reading is also shown. The lines joining the central point to "Blue," "Yellow" and "Red" represent the chromaticities of the blue, yellow and red Lovibond glasses on the equal chromaticity scale. The line on the right represents the locus of saturated colours,

i.e., of monochromatic wavelengths from about 565 mμ to 585 mμ. The colour of the milk before adding indicator (or rosaniline) is represented by a point on the line joining the central point to yellow, showing that this milk is on the yellow side of white by about two "least perceptible differences." As the alkali is added to the milk containing phenolphthalein, the milk becomes at first slightly pink and then a deeper red with increasing amounts of blue, the final colour being a red - magenta. Starting from the yellow line it will be seen that an increase of pH at first brings about very little change in chromaticity, so that there is little perceptible colour change. From about a pH of 8·4, however, there is a rapidly increasing colour change with increasing pH. This rate of colour change has been plotted in Fig. 2 to a scale of least perceptible differences, with the corresponding pH and additions of 0·1 *N* sodium hydroxide. The colour of the milk containing rosaniline is also shown on the same figure, and it is obvious from an inspection of the curves that it occurs at a point where the rate of change of colour with increasing pH is rapidly increasing. Below this pH,

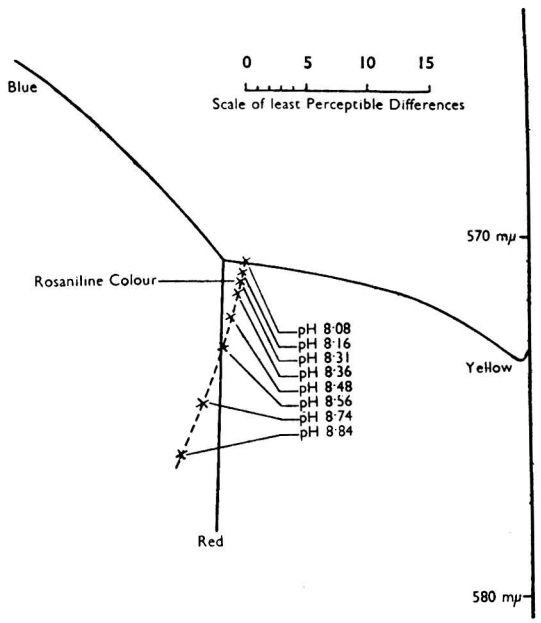

Fig. 1. Titration of 50 ml of milk containing 5 ml of 0·5 per cent w/v phenolphthalein. Locations of colours measured on Schofield's Uniform chromacity chart.

The dotted line shows alteration of colour with changing pH. The colour changes slowly at first with changing pH, from a pale yellow to pale pink, the red colour rapidly increasing with increasing pH.

the colour change is so gradual that the operator may be in doubt when to cease adding alkali, but at higher pH values the rate of change of colour is so great that a very small addition makes an appreciable colour change. It is therefore easy to obtain a colour match at the "rosaniline point."

Discussion of results

It has been a matter of dispute among laboratories as to whether more consistent results are obtained by comparison with the rosaniline acetate tinted milk or by judging the first onset of pink colour. A number of laboratories recently collaborated in a comparative study of the two methods in connection with the formulation of a standard method to be issued with a British Standard, and Mr. H. B. Hawley undertook a statistical analysis of the results. On the whole there was a decided preference for the comparative method. Barkworth and Evans[9] also reached this conclusion, but their statistical analysis referred only to results obtained by one operator working always in the same laboratory.

A study of the diagrams shows why this should be so. In order that an observer shall be in no doubt as to the "end-point" of a titration, it is essential that the rate of change of

colour shall be reasonably great compared with the rate of change of pH. In titrating strong acids with strong bases the rate of change is very great, but when the rate of change of pH is small and uniform with addition of alkali, as occurs with milk (Fig. 2), the circumstances are quite different. Here the rate of change of colour follows an asymptotic curve and does not reach a point where a change is readily observable until a pH of about 8·3 is reached. This point is approximately that of the rosaniline tinted milk on the colour curve, and this fact explains why replicates between observers and laboratories are more easily obtained by this method. It is the nearest point to the first perceptible colour change at which a rapid change of colour is observed on the addition of alkali and is probably the best point that could have been chosen for the average observer.

The milk recorded in Figs. 1 and 2 is that of a bulk-delivery milk issued in the London area by large dairy companies. Milks from Jersey cows are distinctly yellow and the curve

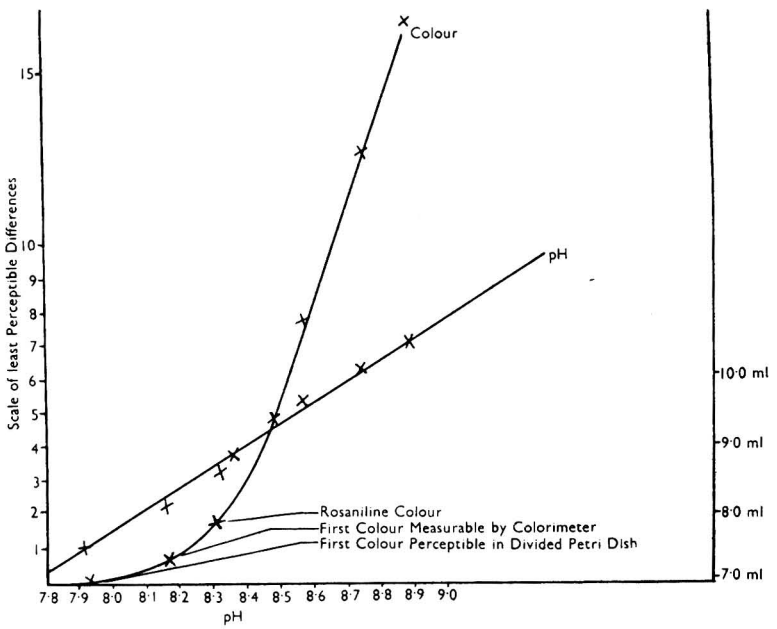

Fig. 2. Rate of colour change

(Fig. 1) indicating colour change with change of pH for these milks will be approximately parallel to that shown, but appreciably to the right.

It is unfortunate that acidity as given by the rosaniline standard is slightly higher than that judged from a direct titration, but it seems certain that greater uniformity among laboratories would be achieved by the rosaniline comparative method. In view of the fact that the "titratable acidity" of milk is an empirical value, having no exact equivalent in terms of a given acid (although usually reported as lactic acid), the use of the comparative rosaniline method is justified.

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Discussion

The President remarked that this was an important paper. The acidity of milk had often been regarded as an easy determination to make and it was usually carried out by the most junior staff of a food laboratory. But anyone who had had experience of the determination knew how relatively uncertain was the end-point and how it was dependent on the amount of indicator used. While comparative results could be obtained by the prescribed technique of any one laboratory, the results from different laboratories might differ considerably; so much so that one wondered whether there was such a thing as "acidity of milk" that could be measured by titration involving the use of indicators. The different proportions of buffering salts in different samples of milk might mean that titratable acidity had little significance. It was a good thing that the process had been submitted to critical review.

Mr. King in reply stated that any method relying on a change of pH was purely empirical and was by no means a measure of lactic acid. If, however, universally uniform results were to be attained, a carefully standardised method was essential. He welcomed any further suggestions on this point.

Dr. J. G. Davis complimented the authors on the way in which they had scientifically investigated the interesting and important phenomenon of the colour change of phenolphthalein in milk. His interest in the problem of the determination of the acidity of milk arose on visiting a creamery many years ago when he had been told that brine-cooling of the milk resulted in a fall in acidity. The basis of this statement was that the acidities measured by the creamery receiving the milk as compared with those measured before despatch were consistently lower to the extent of about 0·02 per cent. of "lactic acid." Investigation of the technique used in the two laboratories showed that the first laboratory used only three drops of phenolphthalein, which may be described as the classical method in the dairy industry, while the receiving creamery used 1 ml. This had prompted him to study the effect of concentration of indicator and it had been easy to demonstrate that the pH of the combined milk and indicator, at the point where the first faint pink colour was perceptible, varied appreciably according to the concentration of the indicator used.[1] They had therefore recommended the standard use of 1 ml of a 0·5 per cent. solution of phenolphthalein for every 10 ml of milk as a reasonable compromise. It was true that this did not settle the vexed question of the colour end-point. A variety of methods had been tried, including the use of pink stirring rods, pink tiles and a special Tintometer glass, and the best of these appeared to be the use of the Tintometer glass, perhaps because the glass could be adjusted to match the end-point colour most accurately. Unfortunately this method was very expensive and for this reason alone it had not been recommended.

In practice, experienced dairy laboratory analysts worked to a very early end-point. Thus, in titrating milk of average quality, a distinct greyness or characteristic change in colour was perceptible when the titration was at about 0·12 per cent. of "lactic acid" and the first perceptible pink colour appeared at about 0·14 per cent. The inexperienced analyst nearly always worked to a later end-point (more intense pink) and so returned a somewhat higher figure for acidity. Obviously from the scientific point of view a colour standard was required, and the rosaniline acetate method, while giving a reasonably good match, required a special solution to be available, which was a further complication in the test; for this reason it had not been generally adopted.

Dr. Davis emphasised that the test really was a complete anomaly in that the value usually stated for fresh milk (0·14 per cent.) was not really lactic acid at all, although this expression was always used. The test really measured the buffer value of the milk from its initial pH (about 6·6) to the pH of the visually detected end-point with phenolphthalein (about 8·4). If the test *really* measured lactic acid it might be worthwhile to bring out a more elaborate and accurate form of the test, but for farmers' milks he suggested that the test should be abolished.

Mastitis, which affected about one-third of the cows in this country, resulted in the lowering of the acidity of milk to a variable extent, while a high proportion of solids or feeding on certain types of land could result in a high acidity. As a measure of souring or hygienic quality for herd milks the test was, therefore, not reliable. What was wanted was a specific test for the amount of lactic acid formed in the milk, and work was proceeding on these lines. From the point of view of dairy control it would have to be a very quick test. The present official rejection test, the rapid resazurin test, took 10 minutes. The industry urgently wanted a still quicker test that would give in half or a quarter of a minute a reasonably accurate measure of the degree of souring.

These objections did not, of course, apply to bulk milks, *e.g.*, as from a 3000-gallon tanker, for the bulking levelled out the variations of acidity in individual herd milks. The titratable acidity test, therefore, was a reliable measure of souring for bulk milks provided that a standardised technique could be developed. Whatever test was recommended would have to be simple and of a type that could be used in the ordinary dairy control laboratory.

Mr. King, in his reply, agreed with Dr. Davis on the completely empirical nature of the acidity determination by any method in which phenolphthalein was used as an indicator and further agreed that a rapid and specific determination of lactic acid would afford far more information as to the condition of milk. Until phenolphthalein should cease to be used in determining milk acidity it was most desirable to standardise the procedure on a sound basis.

Dr. Egan said that it might be that some mixed indicators increased the number of least perceptible differences that accompanied the indicator colour change. Kolthoff and Stenger[2] have described the use

of phenolphthalein in conjunction with a dye, methyl green, the colour of which was complementary to magenta, for the titration of milk; unfortunately the pH at which the best colour change occurs is 9·0. Dr. Davis had described an effect that may be observed in favourable conditions without the addition of a dye or a second indicator; namely, that the yellow colour of the milk being titrated gave way to a chalky neutral tint immediately before the phenolphthalein colour appeared. Had Mr. King any experience of the colour changes or any comments to make on them?

MR. KING replied that he thought there was a sound basis for Dr. Davis's observations. Assuming that the average milk had a spectral reflection curve that was preferential in the yellow part of the spectrum, the superimposition of a slight magenta colour—which was complementary to yellow and given by the first change of phenolphthalein—would result in a chalky-white neutral colour, immediately before the pink - magenta given by a further addition of alkali to the milk. He agreed that the range of pH covered by Kolthoff's suggested mixed indicator was far too great to give agreement with the usually accepted figures for the acidity of milk.

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An Improved Volumetric Method for the Determination of Hydrogen Sulphide and Soluble Sulphides

BY J. A. KITCHENER, A. LIBERMAN AND D. A. SPRATT

(*Presented at the meeting of the Society on Wednesday, April 4th*, 1951)

The usual iodimetric method of determining hydrogen sulphide or sulphur in iron and steel is not satisfactory when accurate results are required. The use of alkaline sodium hypochlorite as combined absorbing and oxidising reagent has been investigated and found to offer many advantages. The reagent is stable to boiling and gives quantitative oxidation of sulphide to sulphate. The recommended procedure has been shown to give results correct to within 0·3 per cent. of the sulphur content.

IN the course of researches into the thermodynamical activity of sulphur in liquid iron,[1] it was necessary to make a large number of determinations of (*a*) hydrogen sulphide in mixtures of hydrogen sulphide and hydrogen and (*b*) sulphur in iron. The quantity of sulphur in the samples was generally between 1 and 10 mg, and it was required to determine it to within ± 1 per cent. A method of analysis was therefore needed that would be (*i*) reliable, both in reproducibility and absolute accuracy, (*ii*) sensitive to 0·01 mg and (*iii*) reasonably rapid.

A review of the literature suggested that, of the many and various methods that have been described for the determination of hydrogen sulphide and sulphur in iron, the commonly used volumetric method[2,3] would be the most suitable for the purpose. In this method, the hydrogen sulphide in a gas mixture, or resulting from the dissolution of a sample of iron in acid, is absorbed and then titrated by iodimetry. There are many modifications for the absorbing medium and method of titration, several of which are in common use in steelworks. Absorbing solutions include ammoniacal or buffered cadmium chloride or acetate or zinc sulphate, while in the determination of hydrogen sulphide in gases, sodium hydroxide is often used. The resulting sulphide suspension or solution may be acidified and titrated immediately with iodine or iodide - iodate mixture, but the most common procedure is to add the sulphide to an excess of acidified iodine and then titrate the residual iodine. The basic reaction is—

$$S'' + I_2 \rightarrow S \downarrow + 2I'.$$

The usual evolution method[3] is generally considered satisfactory for routine analysis of plain carbon steels, but when it was applied to a number of the hydrogen sulphide - hydrogen and pure iron - sulphur samples arising from the present work the results were not

sufficiently concordant, although several of the usual absorbents were tried. Closer examination of the ordinary volumetric method shows that it is really semi-empirical rather than accurately stoicheiometric. It is reliable only for a carefully standardised set of conditions and any variation of the conditions may lead to unexpected errors from a variety of causes. Examples of errors up to 17 per cent. arising merely from variation in the size of the sample used in the analysis have been quoted by Lundell, Hoffman and Bright.[2] In the present work it was often necessary to use very small specimens of iron because of the abnormally large proportion of sulphur in many of them (*e.g.*, about 1 per cent. of sulphur instead of the usual range of 0·01 to 0·1 per cent.). In these circumstances it would clearly be unsatisfactory to take the ordinary volumetric method on trust when the research required an *absolute* accuracy of 1 per cent., as the results might easily be subject to large unknown errors.

Sources of errors in the ordinary volumetric method

Consideration was given to the possibility of developing a more reliable volumetric method, and the potential sources of error in the existing methods were first examined. The following errors have been recognised—

Incomplete evolution of the sulphur as hydrogen sulphide during dissolution of the iron—This is a well known difficulty with certain cast irons and alloy steels, but since it does not apply to pure iron - sulphur samples or hydrogen sulphide - hydrogen mixtures it need not be discussed further here.

Incomplete absorption of the hydrogen sulphide—Rapid dissolution of the sample is usually recommended as this is found empirically to give better results.[2] Theoretically it should make no difference; that it is found to do so is another indication of the existence of weakness in the method. If the gas contains much hydrogen sulphide some of it may escape absorption and two absorption bulbs in series must then be used.[4] Loss of hydrogen sulphide was noticed with some of the high-sulphur samples when tested in conjunction with the usual absorbents. Alkali was found to be the most effective absorbent.

Errors in the titration procedures—(*a*) If cadmium solutions are used, the cadmium sulphide must be protected from sunlight since it is photo-sensitive.[5]

(*b*) With the cadmium or zinc back-titration method, the end-point is not altogether satisfactory, the colour changing from a reddish-brown to a deep blue colour. Attempts to increase the sensitivity of the titration by working with more dilute solutions gave discouraging results.

(*c*) If the sulphide solution is first acidified and then titrated directly with iodine, some hydrogen sulphide may be lost before the titration is completed.

(*d*) If the back-titration procedure is used there is a possibility of occlusion of iodine by the colloidal sulphur that is formed.[6]

(*e*) If sodium hydroxide is used as the absorbing solution, atmospheric oxygen is apt to cause rapid oxidation of the alkali sulphide in solution.

(*f*) If alkali absorbent is mixed with excess of acidified iodine there is a possibility of volatilisation of iodine owing to the rise of temperature.[7] To avoid errors (*d*) and (*f*) the solution is usually diluted considerably,[8] but, with alkali sulphides, this emphasises the danger of (*e*).

The above list gives the chief *potential* sources of error in the volumetric methods; certain others have been noted by Etheridge.[9] Although they do not necessarily vitiate the determinations, they indicate the importance of very careful control of conditions. Shaw[10] and Treadwell and Hall[11] have pointed out that differences of technique, dilution of the solution, rate of addition, and amount of potassium iodide present may produce large variations in the results.

There is clearly a need for a better method, giving greater reliability and sensitivity. In developing such a method the following points deserve attention—

(*i*) The reactions employed in the titration should be accurately stoicheiometric (no side-reactions).

(*ii*) Absorption should preferably be by alkali hydroxide, as this minimises the danger of loss of hydrogen sulphide.

(*iii*) If alkali is to be used, there must be complete exclusion of air from the apparatus and the solutions. Further, transference or dilution of the sodium hydroxide - sodium

sulphide solution or addition of large volumes of reagent solutions containing dissolved air should be avoided.

(*iv*) The neutralisation of large amounts of alkali should be avoided to minimise loss of iodine.

(*v*) If an oxidising agent could be found that would oxidise sulphide to sulphate instead of to colloidal sulphur, it would improve the sensitivity fourfold, avoid occlusion of iodine, and improve the end-point.

(*vi*) For accurate work an all-glass apparatus should be used, so avoiding rubber, which absorbs hydrogen sulphide.

Use of alkaline hypochlorite for absorption and oxidation

All the above desiderata were satisfied by the introduction of an alkaline solution of sodium hypochlorite as a combined absorbing and oxidising reagent.

Kolthoff and Sandell[12] state that sulphides are quantitatively oxidised to sulphates by calcium hypochlorite in alkaline media according to the reaction—

$$S'' + 4\,OCl' \rightarrow SO_4'' + 4\,Cl',$$

but they do not specify the conditions necessary to secure complete oxidation. The use of hypobromites and hypochlorites for the determination of sulphides was proposed by Willard and Cake.[13] Hypochlorites are the more stable, but it was found that at least 4 *N* sodium hydroxide was needed to effect oxidation at room temperature, whereas 2·5 *N* sodium hydroxide was sufficient with sodium hypobromite. Willard and Cake therefore proposed absorbing hydrogen sulphide in 2·5 *N* sodium hydroxide and then washing this into 0·3 *N* sodium hypobromite. The method to be described below avoids transferring alkali sulphide solutions (*cf.* point (*iii*) above) by using a combined absorbing and oxidising reagent. The more stable hypochlorite is used, at a concentration of 0·1 *N* instead of 0·3 *N* to give greater sensitivity. The alkali is 0·4 *N* instead of 2 *N* to reduce the heat of neutralisation (*cf.* point (*iv*) above). The use of dilute calcium hypochlorite solutions for the approximate determination of microgram quantities of sulphides has been described recently by Pepkowitz.[14]

Solutions of pure hypochlorites are remarkably stable if kept free from organic matter (dust). They can be boiled without decomposition.[15] They are more stable still in the presence of free alkali,[16,17] and such solutions can be kept for many months without appreciable change of titre, provided they are stored in dark bottles to avoid photochemical decomposition by sunlight.

When attempts were made to absorb hydrogen sulphide directly into alkaline hypochlorite it was found that some colloidal sulphur was always formed, even with fairly concentrated sodium hydroxide and a considerable excess of sodium hypochlorite. Experiments were therefore made to find a means of securing quantitative oxidation to sulphate. It was not considered desirable to use very concentrated alkali for reasons already given. The use of higher temperatures was therefore considered. Willard and Cake reported more nearly complete oxidation with hypochlorite at 45° C. Pepkowitz recommended 80° to 90° C. In the present work it was found that any colloidal sulphur formed during absorption at room temperature could be removed by subsequently heating the solution to about 70° C. However, warming just sufficiently to clear the solution of sulphur did not lead to concordant results. There must certainly be intermediates between sulphide and sulphate, and apparently some stages of the reactions are sluggish.

Finally, it was found satisfactory to *boil* the solution. This procedure led to concordant results and stoicheiometric relations corresponding to formation of sulphate. As, so far as is known, the boiling of alkaline hypochlorite solutions is a novel step in volumetric analysis the following evidence is presented to demonstrate its reliability. The validity of the procedure is also supported by the accuracy of the results of the new method as recorded below.

Solutions approximately 0·4 *N* with respect to sodium hydroxide and 0·1 *N* with respect to sodium hypochlorite were made by passing chlorine from a cylinder into the alkali until the required titre was reached. Provided there is a considerable excess of alkali and the solutions are cool and dilute, the product obtained is almost entirely hypochlorite, no appreciable amount of chlorate being formed.[18,19] It is then satisfactory to standardise the solution by the iodimetric method (see Kolthoff and Sandell,[12] pp. 587 and 640). The

solution is just acidified with hydrochloric acid, excess of potassium iodide is added, and the iodine liberated is titrated with sodium thiosulphate, using starch at the end-point. Aliquot 25-ml portions of such solutions were transferred by pipette into conical flasks and boiled briskly for different times. The flasks were then cooled under the tap, and the contents titrated as described above. Table I shows the results (in millilitres of 0·1 *N* sodium thiosulphate) of independent tests by two of the authors on three solutions prepared at different times.

TABLE I

EFFECT OF BOILING ON SOLUTIONS OF SODIUM HYPOCHLORITE

Duration of boiling	Titre of solution A (A.L.), ml	Titre of solution B (A.L.), ml	Titre of solution C (D.A.S.), ml
0 (cold)	25·45	30·00	47·05
1 min.	25·25	29·85	46·84
2 min.	25·20	29·80	46·85
5 min.	25·19	29·80	46·85
10 min.	25·20	29·85	46·81

It is seen that during the first 2 minutes' boiling there was a small loss of titre amounting to about 0·7 per cent. in each experiment. *Thereafter the titre remained constant* within the accuracy of titration (about ±0·1 per cent.) during a further 8 minutes' boiling. The initial loss is believed to be due to reaction with traces of dust. It is quite clear that alkaline hypochlorite alone is perfectly stable to boiling, and that the titre reached after, say, 5 minutes' boiling is a highly reliable figure.* It is essential, of course, to protect the solutions from bright sunlight during treatment.

Experiments in which duplicate samples of sodium sulphide solutions were added to the alkaline hypochlorite and boiled for 5 minutes gave similarly reproducible results. The rate of addition of the sodium sulphide was found to be immaterial, whereas the amount of sulphur initially precipitated and the titre in the cold were very dependent on the mixing conditions. Since sulphate could be detected after the reaction, and since all possible oxidation reactions had evidently finished after 5 minutes' boiling, it was concluded that the treatment effected complete oxidation of sulphide to sulphate irrespective of the intermediates formed. Since also the starting and final hypochlorite solutions were stable it was evident that the process could form the basis of a reliable and stoicheiometric method of determining hydrogen sulphide or any sulphides from which hydrogen sulphide could be completely evolved.

Proof of the *absolute* accuracy of the hypochlorite titration is not easy, as there is no convenient substance that can be taken as a primary standard for hydrogen sulphide. Even sulphides such as those of zinc and cadmium, which are sometimes used in the gravimetric determinations of those metals, cannot be prepared completely pure and of stoicheiometric composition.[22] It has therefore been necessary to resort to indirect checks, which are described later in this paper.

METHOD

APPARATUS—

The complete apparatus for the determination of soluble sulphides or sulphur in iron and steel is shown in Fig. 1. The chief parts, preferably made of Pyrex glass, are the evolution flask, A, which has a wide ground-glass joint carrying a small dropping funnel, B, and a spray trap and condenser (conveniently combined, C), and the absorption flask, E. Connection is made by the tube, D, which has ground cones at each end. If desired, the ground joint to flask E may be replaced by a rubber stopper. If the evolution apparatus is likely to be used in bright daylight, the absorption flask must be screened by a box of wood or cardboard. The dimensions of the apparatus are not critical.

REAGENTS—

Sodium hypochlorite, 0·1 N, in sodium hydroxide, 0·4 N—Prepare by passing chlorine from a cylinder into a 0·5 *N* solution of sodium hydroxide until the required concentration

* It is worth recording that "Chloramine T," which is a very convenient substitute for hypochlorite for titrations in the cold,[20,21] does not behave in the same way to boiling. Instead, there is a progressive loss of titre, no doubt due to reaction with the organic part of the compound.

is reached (see above). For 2 litres of solution, this takes between 5 and 7 minutes at a moderate rate of bubbling.

Sodium thiosulphate, 0·05 *N*—Prepare by dissolving 25 g of sodium thiosulphate and 7·6 g of borax (as preservative) in 2 litres of water. It is most conveniently standardised against a solution of potassium iodate (1·7 g of potassium iodate dried at 180° C for 2 hours).

Hydrochloric acid, diluted (1 + 1)—Prepare an air-free solution by diluting concentrated hydrochloric acid with its own volume of boiled, cooled water. Store in a Winchester bottle fitted with a siphon and protect the contents from oxidation by means of a bubbler containing alkaline pyrogallol.

Potassium iodide—A freshly made approximately 10 per cent. solution.

Hydrochloric acid, approximately 2 *N*.

Starch solution—A freshly made approximately 0·5 per cent. solution.

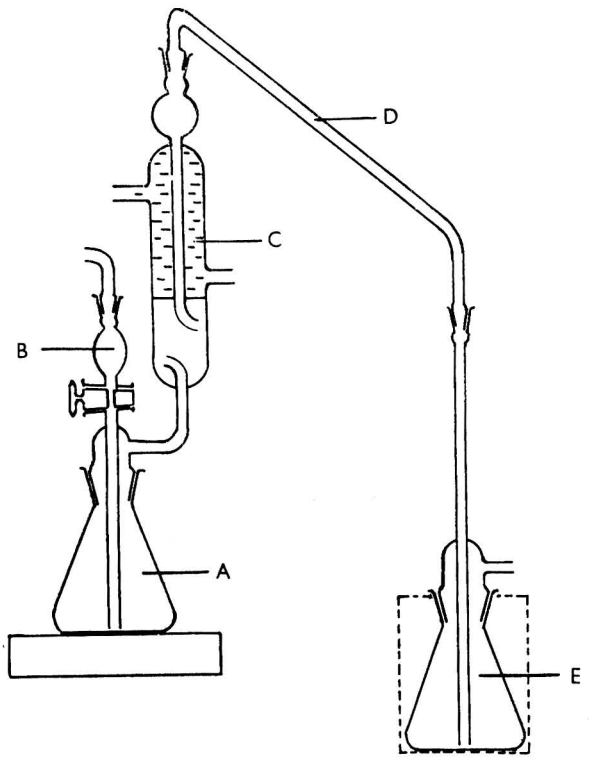

Fig. 1. Details of apparatus

PROCEDURE—

To determine, for example, sulphur in iron, first wash the whole apparatus with water, rinse it with alcohol and dry with warm air. Weigh the sample to ±0·5 mg and place it in the evolution flask. Assemble the apparatus as shown in Fig. 1, the ground joints being very lightly greased. Transfer 25 ml of standard alkaline hypochlorite solution by means of a pipette into the absorption flask and add 25 ml of water. The absorption flask must be screened from bright light. Pass a steady stream of hydrogen from a cylinder through the apparatus for 10 minutes to sweep out all air. Place a small piece of lead-acetate paper and a small piece of starch - iodide paper in the exit tube of the absorption flask to test the completeness of absorption. Admit about 20 ml of diluted hydrochloric acid (1 + 1) into the evolution flask through the tap-funnel, keeping the amount of air introduced with it to a minimum. Place a small flame or, better, an electric hot-plate under the evolution flask and maintain a continuous, slow stream of hydrogen through the apparatus. With high-sulphur irons the rate of dissolution must not be too great.

When the sample has finally dissolved (as shown for iron by means of a magnet placed near any residual particles of carbon, etc.), boil the solution in the evolution flask, A, for 5 minutes to ensure that the hydrogen sulphide has been expelled. The trap and condenser prevent hydrochloric acid from passing over into the absorption flask, E. Then disconnect the absorption flask and boil its contents steadily for 5 minutes ± 1 minute. The white colloidal sulphur that appears during the absorption is completely oxidised to sulphate and the solution becomes quite clear.

Remove the tubes from the absorption flask, E, and rinse them, collecting the rinsings in the flask. Immediately cool the flask thoroughly, *e.g.*, by covering the neck with a small inverted beaker and standing the flask under a running tap. The solutions are then ready for titration. Add 10 ml of 10 per cent. potassium iodide solution and then 20 ml of 2 *N* hydrochloric acid. Titrate the liberated iodine against 0·05 *N* sodium thiosulphate, and add 5 ml of starch solution as indicator when the end-point is nearly reached.

Determine the thiosulphate equivalent of the hypochlorite reagent in duplicate by boiling 25 ml and titrating in the same way as in a sulphur determination. The difference between this figure and that given by a sulphur determination is the volume of thiosulphate solution that is equivalent to the sulphur in the sample—

1 ml of 0·05 *N* sodium thiosulphate $\equiv$ 0·200 mg of sulphur.

Confirmation of the Absolute Accuracy of the Method

In the absence of any satisfactory primary standard for hydrogen sulphide or soluble sulphides, it was necessary to resort to indirect tests. The sulphur contents of a number of materials were therefore determined by the hypochlorite method described above and by a standard method, and the results compared.

Comparison with direct weighing method for hydrogen sulphide—

Sherman, Elvander and Chipman[23] have recently shown that hydrogen sulphide in the gases evolved from the dissolution of iron in acids can be accurately determined by first washing the gases to remove acid vapours, drying with "Anhydrone" and then absorbing and weighing the hydrogen sulphide directly in a bulb of "Ascarite." This method was used to determine the sulphur content of a sample of manganese sulphide prepared according to the method described by Biltz and Wiechmann.*[24] The samples used for the direct weighing method weighed 0·25 g, and were dissolved in the evolution flask in the usual way. Samples of the same specimen of manganese sulphide weighing 0·05 g were similarly dissolved and the hydrogen sulphide determined by the volumetric method as described in this paper. The results, expressed as percentage of sulphur in the specimen of manganese sulphide, were as follows—

	Weight of sample	Sulphur, %	Mean
By direct weighing of hydrogen sulphide ..	0·25 g	34·4, 34·4	34·4
By hypochlorite titration	0·05 g	34·1, 34·7, 34·2	34·3

From these figures it is concluded that the hypochlorite titration method is an accurate means of determining hydrogen sulphide. It gives results that are probably correct to within 0·3 per cent. of the true value.

Comparisons with the standard gravimetric (barium sulphate) method—

Borings from a special iron - sulphur ingot, prepared in the Chemistry Laboratory of the British Iron and Steel Research Association, were sampled by mixing and quartering. Twelve determinations by the new volumetric method gave a mean of 0·384(5) per cent. of sulphur. Independent umpire analyses on 5-g samples carried out by Miss M. Dalziel in

* It is noteworthy that Biltz and Wiechmann claimed that manganese sulphide so prepared is stoicheiometric. The evidence, however, was only that the manganese content was correct for manganese sulphide; the sulphide content of their sample may well have been low, as was that of the manganese sulphide used for the present test. Possibly some S (= 32) is replaced by $(OH)_2$ (= 34). The above test was not, of course, dependent upon having a stoicheiometric sulphur content.

the Advanced Analytical Laboratories of this Department, using the standard A.S.T.M. gravimetric method, gave 0·387 per cent.

Similar comparisons have also been made with mixtures of zinc sulphide and zinc (volumetric 1·14 per cent.; gravimetric 1·15 per cent.) and of ferrous sulphide and iron (volumetric 1·01 per cent.; gravimetric 1·01(5) per cent.).

These tests confirm that the evolution - volumetric method described here gives results for sulphur in iron and other metals that are in good agreement with the gravimetric method. There can be no doubt that it is reliable within the required limits of ± 1 per cent.; it is probably better than this. The data appear to suggest that the volumetric method may be giving results about 0·6 per cent. lower than the gravimetric method, but even the standard barium sulphate method is probably not of the highest absolute accuracy. The present authors do not know of any unambiguous tests of the ultimate reliability of the barium sulphate determination, whereas its liability to errors is well known.[2,25]

Time required for a determination and limit of sensitivity

A single determination of sulphur in iron can be completed in about $1\frac{1}{2}$ hours, allowing 1 hour for the dissolution of the sample. For the analysis of mixtures of hydrogen sulphide and hydrogen the time required is only about half an hour. As two determinations can easily be carried out simultaneously, an experienced worker with several sets of apparatus could perform 8 to 12 analyses in a working day.

Since the hypochlorite method is more than four times as sensitive as the ordinary method by virtue of its stoicheiometry and its better end-point, it is worth considering how small a quantity of sulphur as hydrogen sulphide or soluble sulphide can be detected. Some tests made with a small absorption flask and an ordinary 5-ml micro-burette showed that the end-point is sensitive to $\pm 0{\cdot}005$ ml of 0·062 *N* sodium thiosulphate. Hence, allowing this uncertainty on both the unknown and the blank, the uncertainty of the difference would be $\pm 0{\cdot}01$ ml. This is a *limiting sensitivity* of 0·0024 mg of sulphur, which would be 0·00024 per cent. by weight in a 1-g sample of iron, or 0·00017 per cent. by volume as hydrogen sulphide in 1 litre of a gas mixture at N.T.P.

The above estimate gives the sensitivity; the smallest amount of sulphur that could be determined with a given accuracy is, of course, correspondingly greater. For example, 0·01 per cent. of sulphur in a 1-g sample of iron could be readily determined correct to the nearest 0·001 per cent. With larger amounts of sample or higher sulphur contents the full accuracy of the method (*i.e.*, within $\pm 0{\cdot}3$ to 0·6 per cent. of the true sulphur content) can be achieved.

Two of us (A. L. and D. A. S.) thank the British Iron and Steel Research Association for B.I.S.R.A. Bursaries, during the tenure of which the above work was done.

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Discussion

Mr. F. L. Okell said that the authors were to be congratulated upon having removed one of the three long-standing difficulties of the volumetric evolution methods for determining hydrogen sulphide, namely, the uncertainty that attended the iodimetric titration of metallic sulphides. The authors' opinion that the reaction was not stoicheiometric was confirmed by his own experience of the method.

By applying the alkaline hypochlorite oxidation to this determination they had improved its scientific aspect, as distinct from manipulative technique, and so increased our knowledge of this attractive but hitherto disappointing method. With this improvement the method should become of service for many purposes other than those of the foundry chemist.

He asked if the all-glass apparatus described by the author was indispensable to the method and mentioned that S. G. Clarke (*Analyst*, 1931, **56**, 436) had used corks and black rubber tubing in an evolution method for hydrogen sulphide on samples weighing but 0·1 g and got good results.

Dr. Kitchener, in reply, thanked Mr. Okell for his remarks and said that, as an all-glass apparatus eliminated one possible source of trouble, they had not investigated the suitability of cork and rubber tubing. However, it was worth mentioning that in other work they had found polythene tubing satisfactory and had proved by direct test that, unlike rubber, it did not absorb hydrogen sulphide. Polyvinyl chloride tubing was also available and was superior to rubber for many purposes in the laboratory, *e.g.*, in the polarograph.

Mr. J. Haslam, although not necessarily implying that the test results would be affected, rather doubted the stoicheiometry of the reaction between hypochlorite, iodide and acid. That seemed to him to be taken account of in the best methods of standardisation of hypochlorite. In these methods what ultimately happened was that a slight deficiency of arsenite solution was added to a known amount of hypochlorite, and only at this stage, when a very small amount of hypochlorite was present in excess, was iodide added, the iodine then liberated being titrated by the addition of a further small volume of standard arsenite.

Mr. R. F. Milton said that in his experience titration of hypochlorites with potassium iodide and thiosulphate was unsatisfactory owing to the end-point being indefinite and variable. He had found that the arsenite and iodine titration was to be preferred.

Dr. Kitchener replied that although the arsenite method was no doubt preferable in some instances for determining hypochlorite, especially when chlorates were present (*e.g.*, in bleaching powder), they considered the direct potassium iodide reaction entirely satisfactory for pure solutions of hypochlorites. This opinion was supported by the literature (see reference 12, above) and by the reproducibility of their results (Table I). The end-point in their titrations was very sharp.

Dr. J. H. Hamence asked if the method could be used to estimate hydrogen sulphide in water directly, without distillation.

Mr. T. McLachlan said that he did not think it would be possible to apply this method to the determination of traces of hydrogen sulphide in waters on account of the large amount of dissolved air. Moreover, when they were present in traces, sulphur dioxide was frequently there in addition to hydrogen sulphide. With regard to the reliability of the methods of Dr. Kitchener and his co-workers, it should be remembered that in the determination of available chlorine in sodium hypochlorite solution, the analyst was concerned only with the amount of hypochlorite, whereas Dr. Kitchener was indifferent to the forms in which the chlorine was present, as long as he could eventually determine the total amount after liberation with acid and iodide.

Dr. Kitchener concurred.

The Determination of Germanium

The following three papers were presented at the Meeting of the Society on Wednesday, May 2nd, 1951.

Part I. Titration of Mannito-Germanic Acid*

By H. J. CLULEY

In aqueous solution germanium dioxide reacts with mannitol to form a strong, complex acid; germanium can be determined volumetrically by titration with sodium hydroxide solution of the mannito-germanic acid so formed. A method has been evolved in which the volumetric procedure is applied after a preliminary separation of the germanium by precipitation as sulphide. By a simple modification the interference of arsenic is readily obviated.

GERMANIUM, long a neglected element, has in recent years achieved prominence owing to its extensive application as a semi-conductor in crystal rectifiers and similar devices. To meet the consequent demand germanium is now being produced in this country by extraction from flue dusts occurring in the producer systems of gasworks using certain coals in which germanium is a trace constituent.[1] The analytical difficulties encountered during work on the extraction of germanium from flue dusts encouraged a search for new methods of determination of the element and the results of this analytical investigation are reported in this and the following two papers (pp. 523 and 530).

The majority of published procedures for the determination of germanium suffer from lack of selectivity and, in particular, from interference from arsenic, with which germanium is commonly associated. This is a serious objection, as a complete separation of germanium from arsenic is not readily achieved. The procedure most widely used for the determination of germanium is the tannin method, with which a separation from all elements other than tantalum, niobium and tungsten is stated to be theoretically possible.[2]

Initially, the tannin method was investigated, the conditions of precipitation recommended by Davies and Morgan[2] being used. It was found that at low concentrations the germanium - tannin precipitate tended to become colloidal, with consequent low results, and it was concluded from this investigation that the method was unlikely to be wholly satisfactory for the small amount of germanium it was required to determine. The method of precipitation employed by Davies and Morgan has subsequently been criticised by Holness,[3] who advocated precipitation from an oxalic acid solution in preference to sulphuric acid solution. A further disadvantage of the tannin method is the tedious preliminary ignition at 600° C, with repeated nitric acid oxidation, that is necessary to ensure the absence of the volatile germanous oxide before the final ignition at 900° C.

Attention was then directed to volumetric and absorptiometric methods. This paper describes a volumetric method applied to the determination of germanium; the development of an absorptiometric method and its subsequent application to the determination of germanium in flue dust, coal and coke are described in the following two papers.

EXISTING VOLUMETRIC METHODS

There appear to be only two published procedures for the volumetric determination of germanium. Willard and Zeuhkle[4] proposed a method based on the formation of a thiogermanate in an acetate-buffered solution. The thiogermanate was oxidised with standard iodine solution the excess of which was determined by titration with thiosulphate. The authors state that the method is of limited application.

The other method is due to Tchakirian,[5] who found that germanium dioxide in aqueous solution reacted with mannitol (and with other polyhydric alcohols) to form a strong complex acid. The mannito-germanic acid so formed could be titrated with sodium hydroxide solution

* Taken from a Thesis submitted to the University of London for the degree of M.Sc.

to the phenolphthalein end-point, one molecule of sodium hydroxide being equivalent to one atom of germanium. The mannito-germanic acid also reacted with an iodate - iodide mixture and the liberated iodine could be titrated with thiosulphate. Tchakirian stated that the alkalimetric procedure could not be applied to germanium solutions containing strong acids, and there is no evidence from his original[5] or subsequent[6,7] papers that either of his methods had been used except for pure germanium solutions.

The alkalimetric titration of boric acid in the presence of mannitol is a procedure universally used, and the corresponding method for the determination of germanium appeared worthy of investigation. In particular, the method for boron is applicable to solutions containing strong acids and this cast doubt on Tchakirian's statement that the method for germanium was not applicable under these conditions. An investigation was therefore made in an attempt to verify Tchakirian's observations and to extend the usefulness of his method.

The titration of mannito-germanic acid

Preliminary experiments were performed on the formation of mannito-germanic acid, and pH curves were drawn for the neutralisation of the acid with sodium hydroxide. These curves gave inflection points at about pH 7·8 and confirmed that 1 molecule of sodium hydroxide was equivalent to 1 atom of germanium. Three different titration procedures were then examined, germanium solutions containing free sulphuric acid being used.

(*a*) *Calcium carbonate neutralisation method*—The solution was neutralised with calcium carbonate and, after filtration, mannitol was added and the solution was titrated with sodium hydroxide solution to the phenolphthalein end-point. This procedure was based on the Wherry method for determination of boron.[8]

(*b*) *Double indicator method*—The solution was adjusted with the sodium hydroxide solution to the *p*-nitrophenol end-point; after addition of mannitol the solution was titrated to the phenolphthalein end-point. This type of procedure is commonly used for titration of boric acid.[9]

(*c*) *Fixed pH method*—This was a modification of the foregoing method (*b*) in which the pH value of the solution before addition of the mannitol and the pH value at the end of the titration are the same. This procedure has also been used for the titration of boric acid.[10,11]

It was found that all three methods were suitable for the determination of 1 to 10-mg quantities of germanium, approximately 0·02 *N* sodium hydroxide being used. Hence it was established that an alkalimetric procedure could be applied to the determination of germanium in solutions containing a strong acid. Methods (*b*) and (*c*) are more rapid as they avoid the necessity for filtration. The following theoretical comparison of methods (*b*) and (*c*) shows that the latter is likely to be less influenced by other substances present in solution.

In the double indicator method (*b*) the pH value at the *p*-nitrophenol end-point is about 6·0, falling to about 4·0 on addition of mannitol and increasing to about 8·4 at the phenolphthalein end-point. In this type of titration, therefore, the formation and subsequent neutralisation of the mannito-germanic acid are accompanied by a net change of pH value from 6·0 to 8·4. If substances exerting any buffering action are present the amount of sodium hydroxide required to effect this net pH change will be increased, with consequent error in the determination. In method (*c*) the neutralisation of the mannito-germanic acid is carried only to the stage where the pH value is the same as that before addition of mannitol, the net pH change is zero and the error due to any buffer effect vanishes. In practice, buffering agents can in fact be tolerated provided that they are not present in sufficient concentration to render the titration end-point indefinite. In this type of titration the neutralisation of the mannito-germanic acid is obviously incomplete and an empirical relationship exists between the germanium and the sodium hydroxide solution. This relationship must be established by titration of known amounts of germanium under closely standardised conditions.

The fixed pH method, (*c*), was therefore preferred as it appeared less susceptible to errors caused by the ionic environment of the germanium. A further advantage is the reduction in the size of the blank, a matter of some importance for the small titrations involved. The fixed pH value selected was 6·2, and the standardisation titrations were carried out in the following manner.

To the weakly acid germanium solutions, of volume about 80 ml, 7 drops of bromocresol purple indicator were added. Carbonate-free sodium hydroxide solution, 0·0185 *N*, was then added until a pH of 6·2 was reached, as indicated by colour comparison with pH 6·2 buffer solution containing indicator. Then 10 g of mannitol were added and the solutions were titrated with the sodium hydroxide solution until a pH of 6·2 was again attained. The usual correction for the blank was applied.

TABLE I

FIXED pH TITRATION METHOD: STANDARDISATION TITRATIONS

Germanium taken, mg	Corrected titre of 0·0185 *N* sodium hydroxide, ml	Weight of germanium equivalent to 1 ml of sodium hydroxide, mg
1	0·67, 0·70	1·461
3	2·19, 2·20	1·367
6	4·37, 4·42	1·366
10	7·35, 7·37	1·359
20	14·57, 14·59	1·371

NOTE—For complete neutralisation of mannito-germanic acid the theoretical relationship is 1 ml of 0·0185 *N* sodium hydroxide ≡ 1·343 mg of germanium.

From these titrations the germanium equivalence of the sodium hydroxide solution was calculated for each weight of germanium titrated, as shown in Table I. Theoretically the germanium equivalence of the titrant will vary slightly with the weight of germanium titrated, but it is clear that for the small amounts of germanium under consideration the use of a mean equivalence factor will occasion little error. It will be seen that the equivalence factor thus established empirically for this method of titration differs only slightly from the theoretical relationship obtaining for complete neutralisation of the mannito-germanic acid, so that the loss of sensitivity is very small.

To illustrate this type of titration a pH curve was prepared of the titration of 10 mg of germanium under the above conditions, and this is shown in Fig. 1. The dotted portion

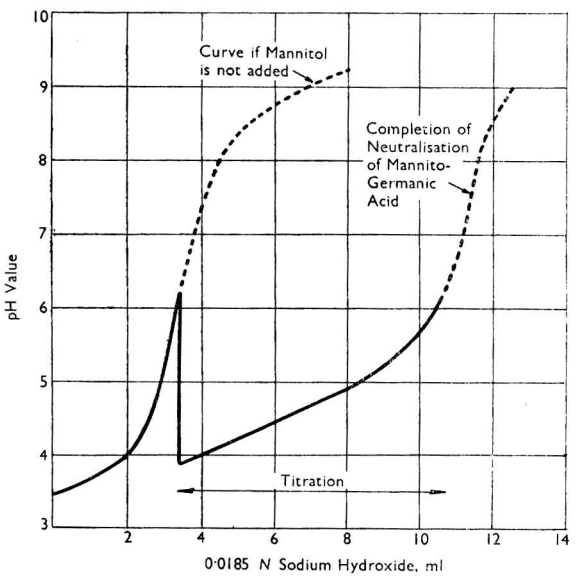

Fig. 1. Graph of a fixed pH titration

of the first curve shows how the titration continues if mannitol is not added; the dotted portion of the second curve shows the completion of the neutralisation of the mannito-germanic acid after pH 6·2.

Titration of germanium after separation as sulphide

Substances that would be expected to interfere with the titration of germanium fall into three classes—

(*a*) Buffering agents, if they are present in a sufficiently high concentration to render the titration end-points indefinite.

(*b*) Bases precipitated by sodium hydroxide within the pH range of the titration. (This is a special case of buffering.)

(*c*) Compounds which react similarly with mannitol, *e.g.*, boric acid.

For the general application of the volumetric method a preliminary separation of the germanium will normally be necessary. Precipitation as sulphide serves to separate germanium from boron and from the majority of bases of type (*b*). Experiments were therefore carried out on the application of the volumetric method after separation of germanium in this manner.

Known amounts of germanium were precipitated as sulphide and the filtered precipitates were dissolved in ammonium hydroxide solution and oxidised with hydrogen peroxide. After boiling with sodium hydroxide solution to eliminate ammonia and hydrogen peroxide the solutions were just acidified with sulphuric acid and the fixed pH titration method applied. At first difficulties were encountered owing to the effect on the indicator of oxidising substances still present in solution. This trouble was overcome by boiling with an excess of sulphuric acid after the elimination of ammonia and hydrogen peroxide, then making the solution just acid and applying the volumetric method. The results obtained in this manner are shown in Table II.

Table II

Titration of germanium after precipitation as sulphide

Germanium taken, mg	Germanium found, mg
1	1·00, 0·98
3	2·98, 3·04
6	6·03, 6·01
10	9·96, 9·90, 9·96, 9·85

These results show that the volumetric procedure can successfully be applied after separation of germanium as sulphide from pure solutions. However, in practice, other elements of the analytical sub-group IIB will accompany germanium in this separation. Of these elements arsenic is commonly associated with germanium and interferes with most methods for its determination. For these reasons the effect of arsenic on the volumetric method was examined in some detail.

Titration of germanium in the presence of arsenic

Titration by the fixed pH method of known amounts of germanium in the presence of arsenic established that as much as 100 mg of tervalent arsenic could be tolerated without detriment to the determination of 10 mg or less of germanium. Similar amounts of quinquevalent arsenic were found to interfere owing to pronounced buffering of the solution. If the sulphide separation procedure previously examined were used, the co-precipitated arsenic would be oxidised by the hydrogen peroxide to the quinquevalent state and would therefore interfere with the final titration. The non-interference of tervalent arsenic suggested that this difficulty could be resolved by reduction of the arsenic to the tervalent state before titration.

For this purpose reduction with sulphur dioxide was used because the excess of reducing agent could readily be removed by boiling. This reduction was applied to arsenate solutions containing germanium and in the subsequent titrations no interference from buffering occurred. Finally, this modification was applied to the volumetric determination of germanium after precipitation as sulphide from solutions containing arsenic. The results of these experiments are shown in Table III.

TABLE III

DETERMINATION OF GERMANIUM IN THE PRESENCE OF 100 mg OF ARSENIC

Quinquevalent arsenic reduced to the tervalent state before titration

Germanium added to 100 mg of arsenic, mg	1	3	5	5	6
Germanium found, mg	0·98	2·93	4·98	4·94	5·92

The results in Table III show that small amounts of germanium can be determined in the presence of substantial amounts of arsenic. The effect of other elements has not been extensively investigated. Traces of antimony and tin can be tolerated, but large amounts interfere owing to the formation of insoluble hydroxides or basic salts.

RECOMMENDED METHOD FOR THE VOLUMETRIC DETERMINATION OF GERMANIUM

APPARATUS—

It is necessary to use beakers or flasks made from boron-free glass or platinum apparatus where strongly alkaline solutions are employed, as any boron dissolved from borosilicate glassware will be titrated with the germanium.

SPECIAL REAGENTS—

Sodium germanate solution—Transfer to a platinum crucible 1·4408 g of pure ignited germanium dioxide, fuse with 5 g of sodium carbonate and dissolve the cold melt in hot water; just acidify the solution with dilute sulphuric acid, boil to eliminate carbon dioxide, cool and dilute to 1000 ml.

1 ml of solution ≡ 1 mg of germanium.

Buffer solution, pH 6·2—Mix 33·9 ml of 0·1 *M* citric acid with 66·1 ml of 0·2 *M* di-sodium hydrogen phosphate.

STANDARDISATION OF THE SODIUM HYDROXIDE SOLUTION—

Transfer volumes of standard sodium germanate solution, covering the range of 1 to 20 mg of germanium, to 250-ml flasks. Dilute each volume to about 50 ml, boil for 5 minutes to eliminate any carbon dioxide and then cool.

Add 7 drops of bromocresol purple indicator and add from a burette carbonate-free sodium hydroxide solution, approximately 0·02 *N*, until a pH value of 6·2 is reached as indicated by colour comparison with an equal volume of pH 6·2 buffer solution containing 7 drops of indicator. Add 10 g of mannitol and titrate with the sodium hydroxide solution until a pH value of 6·2 is again reached. Carry out a blank determination and correct the titration figures accordingly.

From the corrected titration figures calculate the equivalence of the sodium hydroxide solution, in terms of mg of germanium per ml, for each weight of germanium titrated. It will be observed that the germanium equivalence of the sodium hydroxide solution differs slightly for each weight of germanium titrated, but for the small weights of germanium used it may be permissible to use a mean equivalence factor.

PROCEDURE—

The sample solution, of volume about 100 ml and containing 1 to 20 mg of germanium, should not contain large amounts of antimony or tin, and sulphuric acid should be the only acid present.

Transfer the sample solution to a conical flask and add sufficient diluted sulphuric acid (1 + 1) to give a sulphuric acid concentration of about 5·5 *N*. Pass a rapid stream of hydrogen sulphide through the solution for 30 minutes, cork the flask and allow it to stand overnight.

Filter the solution through a close filter-paper and wash the precipitate well with 5·5 *N* sulphuric acid saturated with hydrogen sulphide. Dissolve the precipitate through the paper into a boron-free flask (or platinum dish) with three successive 8-ml portions of diluted ammonium hydroxide solution (2 + 1) and wash the paper first with dilute ammonium

hydroxide solution (1 + 9) and finally with hot water. Add 20 ml of 6 per cent. w/v hydrogen peroxide and allow to stand for 10 minutes in the cold to ensure complete oxidation of the sulphides.

Add 5 ml of freshly prepared 5 *N* sodium hydroxide and boil the solution vigorously until all ammonia and hydrogen peroxide has been evolved, adding hot water from time to time to maintain the volume of the solution. Add 8 ml of dilute sulphuric acid (1 + 6) and boil for 10 minutes to remove any oxides of nitrogen formed from oxidation of the ammonia. If desired, the solution may be transferred to ordinary glassware at this stage.

Pass a rapid stream of sulphur dioxide into the hot solution until it is cold; this takes about 30 minutes. Boil off the excess of sulphur dioxide, ensuring complete removal by continuing the boiling for 5 minutes after the smell of sulphur dioxide can no longer be detected. The treatment with sulphur dioxide may be omitted if arsenic is absent.

Add 2 drops of bromocresol purple indicator and then add freshly prepared 5 *N* sodium hydroxide until the solution is just alkaline. Add *N* sulphuric acid until the indicator just turns yellow, dilute to about 80 ml, boil for 5 minutes to eliminate any carbon dioxide and cool.

Add 5 more drops of bromocresol purple indicator, adjust to pH 6·2, add 10 g of mannitol and titrate to pH 6·2 as in the standardisation titrations. Perform a blank determination, correct the sample titration figure and deduce the germanium content of the sample solution.

NOTES—

The presence of germanium in reagents is unlikely, but it is necessary to carry out blank determinations because of the possibility of deriving traces of boron from reagents or glassware.

The titrations may be carried out with the aid of a pH meter if required, but it is advantageous to retain the use of the indicator so that readings need only be taken when the indicator colour shows proximity to pH 6·2.

The method is equally satisfactory for large amounts of germanium, for which a stronger solution of sodium hydroxide should be used.

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Part II. Absorptiometric Determination with Phenylfluorone*

By H. J. CLULEY

An absorptiometric method is described for the determination of germanium by means of 2:3:7-trihydroxy-9-phenyl-6-fluorone (phenylfluorone). The method is about four times as sensitive as the molybdenum blue method. By a simple distillation from hydrochloric acid solution the germanium is readily separated from the few elements that have been found to interfere with the phenylfluorone method.

Absorptiometric methods for the determination of germanium have been investigated to only a slight extent. The only methods at present available are those based on the formation of the yellow germano-molybdic acid[1] or on its subsequent reduction to molybdenum blue.[2,3] These procedures have the disadvantage that similar reactions are given by silicon, phosphorus and arsenic, and a complete separation of germanium from arsenic in particular is not readily achieved. A search of the literature was carried out in an endeavour to find a more selective colour reaction for germanium that might form the basis of an absorptiometric method.

Gillis, Hoste and Claeys,[4] who have examined a number of derivatives of fluorone for their potential value as analytical reagents, have stated that 2:3:7-trihydroxy-9-phenyl-6-fluorone (phenylfluorone, Fig. 1) is a specific reagent for the detection of germanium.

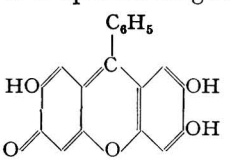

Fig. 1. Phenylfluorone

Their spot test is carried out on a test paper pre-treated with an alcoholic solution of the reagent acidified with hydrochloric acid; a drop of a test solution containing quadrivalent germanium gives a pink colouration that does not disappear on treatment with 6 *N* nitric acid.

The reaction of germanium with phenylfluorone has now been studied as the basis of an absorptiometric method.

Preliminary experiments

Phenylfluorone was prepared from tri-acetylhydroxy-hydroquinone and benzaldehyde (see Procedure) by the method employed by Gillis, Hoste and Claeys.[4] The reagent is only slightly soluble in alcohol and similar solvents, but its solubility in alcohol is increased by adding a small volume of dilute hydrochloric or sulphuric acid, which appears to convert phenylfluorone to the corresponding salt. For preliminary experiments a solution of 0·05 g of phenylfluorone in a mixture of 95 ml of alcohol and 5 ml of dilute sulphuric acid (1 + 6) was used, and this is subsequently referred to as a 0·05 per cent. solution.

This reagent solution, yellow in colour, gave a colour reaction with quadrivalent germanium in dilute hydrochloric or sulphuric acid solution. The colour so formed was orange, presumably due to a combination of the yellow colour of the reagent and the pink colour of the germanium complex. On standing, the germanium compound tended to precipitate, but the use of gum arabic as a protective colloid was effective in stabilising the colour, and this was used throughout the subsequent experiments.

Owing to the strong colour of phenylfluorone itself in acid solution, it was found necessary for absorptiometric measurements to use as a reference solution a blank containing the reagent but no germanium. Under these conditions of measurement the maximum absorption of

* Taken in part from a Thesis submitted to the University of London for the degree of M.Sc.

the germanium colour was in the blue - green region. The initial absorptiometric measurements showed that the intensity of the germanium colour tended to increase with time.

In their spot test, Gillis, Hoste and Claeys[4] had found it necessary to treat the colour spot with 6 *N* nitric acid to destroy the colours produced by certain interfering elements. Similar treatment of germanium colours developed in dilute sulphuric acid solution was found to result in destruction of the reagent. This was possibly due to the presence of nitrous acid, but the use of nitric acid was not pursued.

Colour development in sulphuric acid solution

Systematic experiments were carried out to establish conditions suitable for the rapid development of the germanium - phenylfluorone colour. For this work a sulphuric acid medium was used, as it was considered that in the preparation of a sample solution the use of hydrochloric acid should normally be avoided owing to the volatility of germanium tetrachloride.

The concentration of sulphuric acid, reagent and germanium were varied, but in all experiments 5 ml of 0·5 per cent. w/v gum arabic solution were added and the final volume was 50 ml. The absorptiometric measurements were carried out on a Spekker absorptiometer with 1-cm cells, the tungsten lamp and Ilford No. 603 blue - green filters, and as a reference solution a blank was used, prepared in the same manner as the test solution except that no germanium was added.

The rates of colour development were found to increase with increasing reagent concentrations and to decrease with increasing sulphuric acid concentrations. At low concentrations of acid precipitation of phenylfluorone occurred. The use of 15 ml of a 0·02 per cent. reagent solution, and of 10 ml of dilute sulphuric acid (1 + 6), giving an acid concentration of about 1·05 *N* in the final volume of 50 ml, proved satisfactory and ensured complete colour development within 30 minutes. The relationship between absorption and germanium concentration with these conditions of colour development is shown in Table I. There is a slight deviation from Beer's law that becomes more marked with increasing germanium concentration. The sensitivity of the reaction is apparent.

Table I

Effect of germanium concentration on colour development in sulphuric acid solution

Sulphuric acid concentration 1·05 *N*; 15 ml of 0·02 per cent. reagent solution used

Germanium, μg	10	20	30	40	50
Drum reading, measured after 30 minutes	0·200	0·405	0·608	0·800	0·945

With the conditions outlined above, absorptiometric determinations of 25-μg quantities of germanium were carried out in the presence of a large number of individual elements to assess their effect on the method. Initially the weight of each element added was 25 mg (1000 times the weight of germanium) except for calcium and boron, where low solubility necessitated the use of smaller weights, and for sodium, potassium and ammonium, where 1-g quantities were used.

This work showed that the following ions did not interfere: $NH_4^{\cdot}$, $Na^{\cdot}$, $K^{\cdot}$, $Li^{\cdot}$, $Cu^{\cdot\cdot}$, $Ag^{\cdot}$, $Be^{\cdot\cdot}$, $Mg^{\cdot\cdot}$, $Ca^{\cdot\cdot}$, $Zn^{\cdot\cdot}$, $Cd^{\cdot\cdot}$, $Hg^{\cdot\cdot}$, $Al^{\cdot\cdot\cdot}$, $Cr^{\cdot\cdot\cdot}$, $Mn^{\cdot\cdot}$, $Fe^{\cdot\cdot}$, $Co^{\cdot\cdot}$, $Ni^{\cdot\cdot}$, BO_3''', PO_4''' and Cl'.

The following ions were found to cause interference, the numbers in brackets giving the maximum permissible concentration of the element expressed as a multiple of the germanium concentration: $Ga^{\cdot\cdot\cdot}$ (< 1), $Ti^{\cdot\cdot\cdot\cdot}$ (2), $Sn^{\cdot\cdot}$ (< 1), $Sn^{\cdot\cdot\cdot\cdot}$ (< 1), $As^{\cdot\cdot\cdot}$ (50), AsO_4''' (100), $Sb^{\cdot\cdot\cdot}$ (< 1), $Bi^{\cdot\cdot\cdot}$ (10), MoO_4'' (< 1) and $Fe^{\cdot\cdot\cdot}$ (10). It will be observed that the interference of arsenic was very slight, but that that of gallium, tin, antimony and molybdenum was most marked. Strong oxidising agents such as dichromate and permanganate were also found to interfere by destroying the reagent.

Under the conditions used the absorptiometric procedure was, therefore, selective but not specific for germanium. Modification of the conditions of colour development showed little promise of eradicating the interferences observed and it was clear that a preliminary separation of the germanium would be necessary for the general application of the method.

The interference of arsenic, antimony, bismuth, molybdenum and tin precluded the

use of the sulphide method for separation of the germanium, but the alternative procedure of distillation of the tetrachloride appeared to be promising. Arsenic is the element that normally causes most difficulty in this separation, and the use of fractional distillation in a current of chlorine has been proposed as a method of avoiding the partial co-distillation of arsenic.[5] However, the relatively low sensitivity of the reagent to arsenic suggested that a simple distillation without any such precautions might effect an adequate separation.

The distillation is normally carried out from solutions containing at least half their volume of concentrated hydrochloric acid and similar concentrations of acid are present in the distillate. Before examining this method of separation it was therefore necessary to establish conditions suitable for the application of the absorptiometric procedure to hydrochloric acid solutions.

Colour development in hydrochloric acid solution

The work on colour development in hydrochloric acid solution was carried out by the same general procedure as was used for the study of the reaction in sulphuric acid medium. The results led directly to the conditions finally adopted for the absorptiometric determination of germanium, and it is considered useful to record these results in some detail.

Effect of phenylfluorone concentration—

With 25-μg quantities of germanium and a final hydrochloric acid concentration of 1·0 N, the effect of reagent concentration was studied for the range 2 to 10 ml of the 0·05 per cent. solution. In each experiment sufficient alcohol was added to bring the total volume of alcohol present to 15 ml. The results recorded in Table II again show the marked dependence of rate of colour development on reagent concentration and it will be seen that, for the acid concentration used, only the 8 and 10-ml quantities of the 0·05 per cent. phenylfluorone solution could effect complete development of the colour within 30 minutes. For subsequent work the intermediate quantity, 9 ml, was used, but to avoid separate addition of alcohol this was added as 15 ml of a 0·03 per cent. solution. Owing to the significant absorption of light by the reagent solution alone, it is a disadvantage to use quantities of reagent greatly in excess of the amount required to give an adequate rate of colour development.

Table II

Effect of phenylfluorone concentration on colour development in hydrochloric acid solution

25 μg of germanium used; final acid concentration 1·0 N

0·05% reagent, ml	Alcohol, ml	Drum readings, measured after			
		15 min.	30 min.	60 min.	90 min.
2	13	0·039	0·060	0·070	0·106
4	11	0·348	0·410	0·445	0·453
6	9	0·478	0·480	0·488	0·498
7	8	0·462	0·469	0·479	0·483
8	7	0·472	0·478	0·476	0·482
10	5	0·484	0·483	0·484	0·486

Effect of hydrochloric acid concentration—

With 25-μg quantities of germanium and 15 ml of 0·03 per cent. phenylfluorone solution the effect of hydrochloric acid concentration was studied for the range 0·25 to 5·0 N. The results, recorded in Table III, again show that the rate of colour development decreases with increasing acid concentration. For the quantity of reagent used, the acid range of 0·25 to 1·5 N is effective in producing rapid colour development and in giving almost identical drum readings for the fully developed colours. However, this does not mean that, within this range, the acid concentration need not be closely controlled. It was found that the absorption of the reagent solution itself varied somewhat with the acid concentration and in the acid range 0·25 to 1·5 N consistent drum readings are obtained only if the acid concentration in the blank or reference solution is essentially the same as that in the germanium solution.

For application of the absorptiometric method to highly acid distillates it was desirable to use the highest acid concentration consistent with the requirements of rapid colour

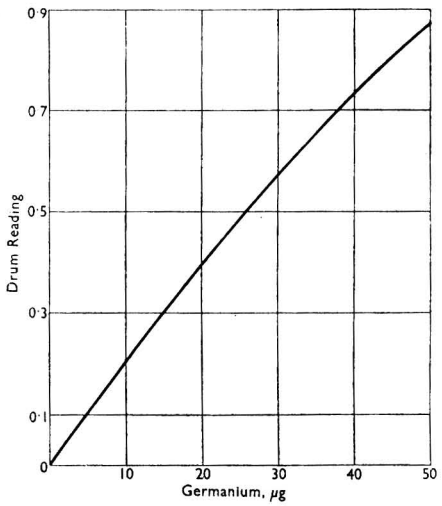

Fig. 2. Calibration graph for Determination of Germanium in Hydrochloric Acid Solution

development. For this purpose it was decided to use an acid concentration intermediate between 1·0 and 1·5 *N*.

TABLE III

EFFECT OF HYDROCHLORIC ACID CONCENTRATION ON COLOUR DEVELOPMENT IN HYDROCHLORIC ACID SOLUTION

25 μg of germanium and 15 ml of 0·03 per cent. reagent solution used

Hydrochloric acid concentration	Drum readings measured after			
	15 min.	30 min.	60 min.	90 min.
0·25 *N*	0·470	0·470	0·475	0·473
1·0 *N*	0·472	0·475	0·474	0·475
1·5 *N*	0·468	0·474	0·477	0·473
2·0 *N*	0·442	0·463	0·472	0·477
3·0 *N*	0·177	0·237	0·333	0·377
5·0 *N*	0·014	0·022	0·030	0·031

EFFECT OF GERMANIUM CONCENTRATION—

With the quantity of phenylfluorone previously decided upon, *viz.*, 15 ml of 0·03 per cent. solution, colours were developed and measured for amounts of 10 to 50 μg of germanium.

TABLE IV

EFFECT OF GERMANIUM CONCENTRATION ON COLOUR DEVELOPMENT IN HYDROCHLORIC ACID SOLUTION

Hydrochloric acid concentration 1·15 *N*; 15 ml of 0·03 per cent. reagent used

Germanium, μg	Drum reading				
	Series 1	Series 2	Series 3	Series 4	Mean
10	0·208	0·204	0·209	0·208	0·207
20	0·402	0·403	0·387	0·397	0·397
30	0·577	0·582	0·566	0·575	0·575
40	0·739	0·741	0·725	0·742	0·737
50	0·866	0·871	0·872	0·880	0·872

For convenience the volume of hydrochloric acid, sp.gr. 1·18, used was 5·0 ml, giving a concentration of about 1·15 *N* in the final volume of 50 ml. The results of four such series of measurements, made at intervals over a period of about four months, are shown in Table IV. A calibration graph, prepared from the mean values, is shown in Fig. 2. The deviation from Beer's law is more marked than was observed for the determination in sulphuric acid solution.

STABILITY OF THE DEVELOPED COLOURS—

The absorptiometric measurements recorded in Table IV were made after the solutions had stood in the cold for 30 minutes. Subsequent measurements confirmed that the colours were completely developed within this time and showed that their absorption remained constant for at least 14 hours. It was usually observed that after 2 or 3 days precipitation of the germanium - phenylfluorone compound commenced.

EFFECT OF OTHER ELEMENTS—

A hydrochloric acid distillation would clearly separate germanium from most elements and it was therefore considered unnecessary to examine the effect of a large number of elements on the absorptiometric determination in hydrochloric acid solution. The effect of arsenic, which would be expected partly to co-distil with the germanium, and of some of the other elements previously found to interfere, was investigated. Under the conditions selected for the absorptiometric determination in hydrochloric acid solution it was found that arsenic did not interfere, that the interference of tin and titanium was diminished but that molybdenum and antimony still interfered strongly.

DETERMINATION OF GERMANIUM AFTER SEPARATION BY HYDROCHLORIC ACID DISTILLATION

Initially the simple distillation of germanium from pure solutions containing 50 per cent. by volume of hydrochloric acid was examined. This acid concentration, being very nearly the constant boiling composition, was convenient in that the acid concentration of the distillate would approximate to the same known strength throughout the distillation.

With solutions of this acid concentration and of initial volume of 50 ml, the absorptiometric procedure was applied to successive fractions of the distillate. It was established that not less than 95 per cent. of the germanium distilled over in the first 10 ml, and that for complete recovery of the germanium it was necessary to distil only 20 ml. In this manner a number of determinations was made on pure germanium solutions, using an initial volume of 50 ml and applying the absorptiometric procedure to an appropriate aliquot of the 20 ml of distillate collected, with the results shown in Table V. A rate of distillation of about 2 ml per minute was used, so that a single distillation was complete in about 15 minutes.

TABLE V

ABSORPTIOMETRIC DETERMINATION OF GERMANIUM AFTER DISTILLATION FROM HYDROCHLORIC ACID SOLUTION

Germanium taken, μg	Aliquot of distillate used	Germanium found, μg
10	25/50	10
50	25/50	50
150	10/50	148
500	20/250	510
1000	20/500	1006

The same procedure was then applied to binary mixtures of germanium with other elements. These included arsenic, titanium and stannic tin, all of which form anhydrous chlorides with relatively low boiling-points, although it was considered unlikely that the last two elements would co-distil under the conditions used. The separations from molybdenum and antimony were also examined individually as these two elements were known to exert the greatest interference with the absorptiometric determination. The results in

Table VI show that none of these five elements interferes with the procedure even when initially present to the extent of 200 times the weight of germanium.

TABLE VI

ABSORPTIOMETRIC DETERMINATION OF GERMANIUM AFTER DISTILLATION FROM SOLUTIONS CONTAINING OTHER ELEMENTS

Element added	Weight of element, mg	Germanium taken, μg	Germanium found, μg
Arsenic, as As_2O_3	10	50	50
Tin, as $SnCl_4.5H_2O$	10	50	50
Titanium, as $Ti(SO_4)_2$	10	50	51
Antimony, as $SbCl_3$	10	50	49
Molybdenum, as $(NH_4)_6Mo_7O_{24}.4H_2O$	10	50	49

The separation of germanium from other individual elements by this method of distillation was not examined extensively as, apart from the elements discussed above, none of the common elements forms chlorides likely to distil under the conditions employed. However, as a final test, the procedure was applied to known amounts of germanium in the presence of a complex mixture prepared to simulate a flue dust and containing the following ions: $Na^{\cdot}$, $K^{\cdot}$, $Cu^{\cdot\cdot}$, $Ag^{\cdot}$, $Mg^{\cdot\cdot}$, $Zn^{\cdot\cdot}$, BO_3''', $Al^{\cdot\cdot\cdot}$, $Ga^{\cdot\cdot\cdot}$, SiO_3'', $Sn^{\cdot\cdot\cdot\cdot}$, $Pb^{\cdot\cdot}$, $Ti^{\cdot\cdot\cdot\cdot}$, PO_4''', $As^{\cdot\cdot}$, $Sb^{\cdot\cdot\cdot}$, VO_3', $Cr^{\cdot\cdot\cdot}$, MoO_4'', $Mn^{\cdot\cdot}$, $Fe^{\cdot\cdot\cdot}$ and $Ni^{\cdot\cdot}$. Each ion was present in amount equivalent to 10 mg of the corresponding oxide except sodium, for which the equivalent of 1 g of the carbonate was added. The results in Table VII show good recovery of the germanium and no evidence of interference. These experiments were designed to assess the possibilities of the method for the determination of germanium in flue dusts, but they serve to show that the method should be applicable to a wide range of materials.

TABLE VII

DETERMINATION OF GERMANIUM IN SYNTHETIC FLUE DUST MIXTURES

Elements present in the mixture	Germanium added, μg	Germanium found, μg
Na, K, Cu, Ag, Mg, Zn, B, Al, Ga, Si, Sn, Pb, Ti, P, As, Sb, V, Cr, Mo, Mn, Fe, Ni	20	20
	80	79
	300	293

RECOMMENDED METHOD FOR THE ABSORPTIOMETRIC DETERMINATION OF GERMANIUM WITH PHENYLFLUORONE

PREPARATION OF PHENYLFLUORONE—

Dissolve 25 g of tri-acetylhydroxyhydroquinone by warming with a mixture of 150 ml of alcohol, 130 ml of water and 40 ml of diluted sulphuric acid (1 + 1). Add 25 g (about 24 ml) of benzaldehyde and allow the mixture to stand for 8 days, with occasional stirring.

Filter by suction the yellow precipitate of phenylfluorone sulphate so obtained and wash the precipitate with the solution mixture. Suspend the precipitate in about 300 ml of water, add sufficient sodium hydroxide solution to give a pH value of about 4 to ensure complete hydrolysis to phenylfluorone, stir and allow the mixture to stand overnight.

Filter the precipitate of phenylfluorone by suction, and then wash first with water and finally three times with alcohol to remove the last traces of benzaldehyde. Dry the precipitate in a vacuum desiccator.

SPECIAL REAGENTS—

Phenylfluorone solution—Dissolve 0·030 g of phenylfluorone by warming with a mixture of 85 ml of alcohol and 5 ml of dilute sulphuric acid (1 + 6), cool and dilute to 100 ml with alcohol.

Standard sodium germanate solution (stock solution)—Transfer 1·4408 g of pure ignited germanium dioxide to a platinum crucible, fuse with 5 g of sodium carbonate and dissolve

the cold melt in hot water; just acidify the solution with dilute sulphuric acid, boil to eliminate carbon dioxide, cool and dilute to 1000 ml.

1 ml of stock solution ≡ 1 mg of germanium.

Standard sodium germanate solution (working solution)—Prepare freshly when required by 100-fold dilution of the stock solution.

1 ml of working solution ≡ 10 μg of germanium.

Gum arabic solution—Dissolve 1·0 g of gum arabic in 200 ml of hot water and cool.

APPARATUS—

The distillation apparatus consists essentially of a 100-ml distilling flask, a water-cooled condenser and a 25-ml measuring cylinder as receiver. The use of components with ground-glass joints facilitates the assembly and avoids trouble due to attack by hydrochloric acid on rubber bungs.

PREPARATION OF CALIBRATION GRAPH—

To 50-ml graduated flasks add amounts of 0 to 5 ml of the dilute (working) germanate solution, to cover the range 0 to 50 μg of germanium. Add sufficient water to give a volume of 20 ml, then add 5 ml of the gum arabic solution and 5·0 ml of hydrochloric acid, sp.gr. 1·18, and mix. Cool to about 20° C and add from a pipette 15 ml of the phenylfluorone solution, dilute to 50 ml and mix well. Allow the solutions to stand at about 20° C for 30 minutes.

Measure the germanium solutions on the Spekker absorptiometer or similar instrument with 1-cm cells, the tungsten lamp and Ilford No. 603 blue - green filters, using as a reference solution the solution containing no added germanium. Prepare a calibration graph from the results.

PROCEDURE—

The sample solution should contain 10 to 1000 μg of germanium and should be free from nitrates and other oxidants likely to liberate chlorine from strong hydrochloric acid solutions.

Transfer the neutral or alkaline sample solution, or a suitable aliquot, to the 100-ml distillation flask, neutralise if necessary with hydrochloric acid and dilute to 25 ml as indicated by an appropriate line previously marked on the flask.

Add 25 ml of hydrochloric acid, sp.gr. 1·18, swirl once and immediately connect the flask to the distillation apparatus. Heat the solution to boiling over a period of about 5 minutes and then distil at a rate of about 2 ml per minute. The distillate should be quite cold.

Collect exactly 20 ml of distillate, remove the receiver and stop the distillation. Dilute the distillate to a known volume and transfer to a 50-ml graduated flask an aliquot that should not exceed half the distillate and is expected to contain 5 to 50 μg of germanium.

With the same quantities of reagents carry out a blank distillation in the same manner and to a second 50-ml graduated flask transfer the corresponding aliquot of the blank distillate. Treat both the test and blank aliquots as follows, mixing after each addition.

Add 5 ml of the gum arabic solution followed by sufficient hydrochloric acid, sp.gr. 1·18, to make the total acid in the solution equivalent to 5·0 ml of hydrochloric acid. For this purpose it may be assumed that the 20 ml of distillate contained the equivalent of 10 ml of the concentrated acid. Add sufficient water to give a total volume of about 30 ml and adjust the temperature to about 20° C. Add with a pipette 15 ml of the phenylfluorone solution, dilute to 50 ml and allow to stand at about 20° C for 30 minutes.

With the blank as a reference solution, measure the absorption of the test solution under the conditions used in the preparation of the calibration graph. From the graph deduce the germanium content of the measured solution and calculate the germanium content of the sample.

DISCUSSION OF METHOD

The experimental work had established that the distillation provided a satisfactory separation of germanium from any common element likely to interfere by colour reaction

with phenylfluorone. The reagent is, however, destroyed by strong oxidising agents, and for this reason the sample solution should not contain any substance that might liberate chlorine from the hydrochloric acid during the distillation.

The practice of carrying out a blank distillation was adopted mainly to ensure that the acid concentrations in the blank and test aliquots were virtually identical. Any interfering elements present in reagents used prior to the distillation are also removed in this manner.

Hitherto the absorptiometric determination of germanium has been based on the formation of the yellow germano-molybdic acid or on its subsequent reduction to molybdenum blue, the latter method having the greater sensitivity and selectivity. Comparison with the published data on the molybdenum blue method[2,3] shows that the phenylfluorone procedure, as applied in hydrochloric acid solution, is of similar selectivity and is about four times as sensitive. The main advantages of the phenylfluorone procedure are that it can be applied directly to a hydrochloric acid distillate with the minimum of manipulation, and that the distillation can be simply performed without the precautions necessary to attain complete separation from arsenic.

After preparation of the sample solutions, the distillation and absorptiometric determination with phenylfluorone can be completed in 2 to 2½ hours for duplicate determinations. Hence this procedure should effect a considerable saving of time over such gravimetric methods as determination with tannin. This is illustrated in the following paper dealing with the application of the phenylfluorone procedure to the determination of germanium in flue dusts.

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Part III. Determination in Flue Dust, Coal and Coke

BY H. J. CLULEY

The author's absorptiometric method (p. 523) for the determination of germanium with phenylfluorone has been applied to the analysis of flue dusts. The high sensitivity of the absorptiometric procedure permits the use of samples weighing 0·1 g or less and this greatly facilitates the decomposition of the sample by sodium carbonate fusion. Duplicate determinations can be completed in 3 to 3½ hours.

The absorptiometric procedure has also been applied to the determination of germanium in coal and coke. In one method the sample is decomposed by a procedure similar to the Eschka method for the determination of sulphur in coal. In a second method the sample is decomposed by combustion in a bomb calorimeter. Samples of coal and coke examined by these methods were found to contain 7 to 12 parts of germanium per million.

THE available information on the determination of germanium in flue dusts and similar materials is somewhat meagre. One method for flue dusts that has been used at the Chemical Research Laboratory[1] consists in fusion of the sample with sodium hydroxide, separation of the germanium from the majority of constituents by precipitation as sulphide and finally gravimetric determination with tannin. Apart from being time-consuming, one apparent

disadvantage of this procedure appears to be that for low germanium contents it may be desirable to carry out the fusion on several grams of sample.

Alimarin and his co-workers have published a number of procedures for the similar problem of determining germanium in coal ash. These methods involve decomposition of the sample by treatment with hydrofluoric and sulphuric acids,[2,3] by fusion with sodium peroxide[2,4] or by fusion with sodium carbonate and sulphur[5]; the germanium is then either separated by distillation of the tetrachloride and determined by precipitation as sulphide,[2,3] or separated as sulphide and determined with tannin.[4,5] Of these methods those involving a hydrofluoric acid attack appear open to criticism owing to the risk of loss of volatile germanium tetrafluoride. The methods involving fusion with an alkaline flux and final determination with tannin are similar to the method outlined above. The fact that Alimarin and his colleagues have within a short time proposed four successive methods might suggest that the earlier procedures were not wholly satisfactory.

The previous work of the author on the absorptiometric determination of germanium with phenylfluorone (p. 523) indicated that this procedure might be applied with advantage to flue dusts. It had already been established that the procedure could readily be applied to germanium in the presence of substantial proportions of the large number of elements normally encountered in flue dusts. The high sensitivity of the method showed that a maximum sample weight of 0·1 g would be required and the use of such small weights should greatly facilitate the initial decomposition by fusion. The speed of the absorptiometric procedure also gave promise of a rapid method.

This paper describes the application of the absorptiometric determination of germanium with phenylfluorone to flue dusts and subsequently to coal and coke.

The determination of germanium in flue dust

Two flue dusts, samples 90 and 44, of different origins and of contrasting types, were selected for the initial experiments. In sample 44 most of the germanium was in a readily accessible form, as shown by the relatively high yield of germanium resulting from direct treatment with hydrochloric acid, whereas sample 90 had yielded only a small proportion of its germanium with this treatment. The germanium contents of these two samples were determined in the following manner.

A 0·1-g portion of the ground sample was fused with 1 g of sodium carbonate in a platinum crucible, the melt being finally heated at 1000° C for 15 minutes. The melt was disintegrated with water and the mixture was transferred to a 100-ml distillation flask, neutralised with hydrochloric acid and diluted to 25 ml. A volume of 25 ml of hydrochloric acid was then added, and the distillation and the subsequent absorptiometric determination on an aliquot of the distillate were carried out as previously described.[6] To investigate the possibility of loss of germanium by volatilisation during the fusion, further determinations were carried out in a similar manner but fusing at 1200° C, a treatment calculated to aggravate any such loss.

Table I

Determinations of germanium in flue dusts: comparison of phenylfluorone method with other methods

Sample	Method*	Germanium found, %	Mean, %	Spectrographic estimate of germanium, %
90	A_1	0·69, 0·72	0·71	0·8
	A_2	0·70, 0·70	0·70	
	B	0·65, 0·67	0·66	
44	A_1	0·92, 0·94	0·93	1·0
	A_2	0·93, 0·94	0·94	
	B	0·85, 0·87	0·86	

* The methods of determination were as follows—

Method A_1—Sodium carbonate fusion at 1000° C, separation of germanium by hydrochloric acid distillation, final absorptiometric determination with phenylfluorone.

Method A_2—As A_1, but fusion at 1200° C.

Method B—Sodium hydroxide fusion, separation of germanium as sulphide, final gravimetric determination with tannin.

Complete solution was always obtained prior to the distillation; this showed that the methods of carbonate fusion as used resulted in complete decomposition of the samples. The results, shown in Table I, methods A_1 and A_2, indicated good agreement between the individual determinations on each sample, and there was no evidence of loss of germanium during the fusion.

As an independent chemical check on the results obtained absorptiometrically, determinations were also made by a procedure similar to the Chemical Research Laboratory method previously outlined. The results so obtained are also shown in Table I, method B. In addition, spectrographic estimations, based on comparison with synthetically prepared standards, were made on the two samples.

Taking into consideration the difficulties of determining a minor constituent in such a complex material as flue dust, the agreement between the two chemical methods is reasonable. The results of both chemical methods are in approximate agreement with the spectrographic data, for which the expected accuracy would be $\pm 0{\cdot}1$ per cent. of germanium. The over-all mean of the chemical results for the two samples, 90 and 44, are 0·68 and 0·91 per cent. respectively, and when the completely independent chemical nature of the two chemical methods is borne in mind, it must be considered that these values are very near to the true germanium contents of the two samples.

Further trials of the method were made with samples of flue dust chosen to cover a range of germanium contents and to represent a number of different sources of the material. For these determinations fusion at the full heat of a Meker burner was used and the complete solution attained in all determinations proved that this method of decomposition was satisfactory. The results of the further trials are recorded in Table II, which, for completeness, also contains the results obtained earlier for samples 90 and 44. It will be seen that the precision of the method is of a high order and that for each sample the value for the germanium content is consistent with the spectrographic result based on comparison with synthetic standards.

TABLE II

ABSORPTIOMETRIC DETERMINATIONS OF GERMANIUM IN FLUE DUSTS

Sample	Germanium found, %	Mean, %	Spectrographic estimate of germanium, %
61	0·021, 0·021	0·021	negligible
38	0·14, 0·145	0·14	0·1
60	0·235, 0·23	0·23	0·2
65	0·30, 0·305	0·30	0·3
90	0·69, 0·72, 0·70, 0·70	0·70	0·8
55	0·825, 0·81	0·82	0·7
44	0·93, 0·94, 0·92, 0·94	0·93	1·0
43	1·15, 1·15	1·15	>1·0

Comparison with the tannin method shows the absorptiometric procedure to have a number of advantages. These include the ability to use small weights of sample, which greatly facilitates the decomposition by fusion and permits a more elegant procedure. The high sensitivity of the phenylfluorone reaction renders the absorptiometric method particularly suitable for samples containing small amounts of germanium; for example, 0·01 per cent. of germanium can readily be determined on 0·1 g of sample. The time required for duplicate determinations is 3 to $3\frac{1}{2}$ hours, compared with $1\frac{1}{2}$ to 2 days for the tannin method. The length of this latter method is largely due to the necessity for allowing the sulphide precipitate to stand overnight and to the lengthy low temperature ignition and nitric acid treatment of the tannin precipitate.

RECOMMENDED METHOD FOR THE DETERMINATION OF GERMANIUM IN FLUE DUST

APPARATUS AND REAGENTS—

The distillation apparatus and the special reagents required for the final absorptiometric determination are described on p. 528 of the preceding paper on the absorptiometric determination of germanium with phenylfluorone.

PROCEDURE—

Weigh 0·01 to 0·1 g of the ground sample, depending on expected germanium content, into a platinum crucible. Mix the sample intimately in the crucible with 0·5 g of sodium carbonate and cover the charge with a further 0·5 g of sodium carbonate.

Heat the crucible gently over a Meker burner, increasing the heat gradually over a period of about 5 minutes until a temperature of about 1000° C is reached. Continue heating in this manner, with occasional swirling of the melt, for a further 15 minutes.

To the cool melt add about 10 ml of hot water and allow the crucible to stand on the edge of a hot-plate until the melt is completely disintegrated. Cool the crucible in running water and then transfer the contents to the 100-ml distilling flask. Add to the crucible 4 ml of diluted hydrochloric acid (1 + 1), swirl to dissolve any solid particles still adhering to the crucible and then transfer the acid solution to the main solution contained in the flask.

Swirl the flask to promote the liberation of carbon dioxide and dilute the solution to 25 ml as indicated by an appropriate line previously marked on the flask. Add 25 ml of hydrochloric acid, sp.gr. 1·18, and carry out the distillation and the subsequent absorptiometric determination on a suitable aliquot of distillate as described on p. 529 of the preceding paper,[6] using a blank taken through the method as the reference solution.

NOTE—

Owing to the heterogeneous nature of flue dusts, precautions must be taken to ensure that the prepared sample is representative and homogeneous. This is particularly important owing to the small weight of the sample.

THE DETERMINATION OF GERMANIUM IN COAL AND COKE

The facility with which germanium in flue dust can be determined by the above method encouraged an attempt to apply the phenylfluorone procedure to the determination of germanium in coal and coke. The germanium content of so-called "germaniferous" coal appears to be of the order of 10 parts per million, and other workers[7,8] have ashed the coal to effect a concentration of the germanium before determination. It has been stated, however, that unless considerable care is taken to maintain strongly oxidising conditions during the ashing, substantial loss of germanium by volatilisation may result.[7,8] It was considered that the sensitivity of the phenylfluorone reaction might permit a direct determination on the coal or coke without recourse to preliminary ashing.

Initial experiments were carried out on a sample of Boldon coal and on a sample of coke from this coal. The procedure used for the decomposition of the sample was similar to that used in the Eschka method for determination of sulphur. Sodium carbonate alone was used in place of the usual admixture with magnesium oxide, as it was required to fuse the residue remaining after destruction of the carbonaceous matter. This destruction was carried out at 600° C instead of the customary 800° C in order to prevent loss by volatilisation of germanous oxide, which is stated to be volatile at 700° C. The determinations were completed by distillation and absorptiometric measurement as used for the determination of germanium in flue dusts.

As a check on the results of these determinations a different method of decomposition was used. Samples were decomposed in a bomb calorimeter by precisely the same method as used for sulphur determinations, and the contents of the bomb were washed into a platinum dish. Sodium carbonate was added to neutralise the acids formed during the combustion and the solution was evaporated to dryness. The residue was then fused with a further quantity of sodium carbonate and the determination completed by distillation and absorptiometric measurement.

The results of determinations by these two procedures are shown in Table III, which also includes results by the sodium carbonate - ignition method on further samples of coal and coke. Although the combustion in the bomb should reduce the risk of loss of germanium by volatilisation to a minimum, it will be observed that the results so obtained are slightly lower than those of the sodium carbonate - ignition method. This difference is attributed to some loss of ash incurred by fusion into the silica crucible used to contain the sample for combustion. It is likely that this loss could be avoided by using a platinum container

if work of higher accuracy were desired. It is interesting to note that spectrographic examination of the four samples, made before the chemical determinations, showed that the most sensitive lines of germanium could only just be detected, and from this it was concluded that the germanium content of the samples was approximately of the order of 10 parts per million.

TABLE III

DETERMINATIONS OF GERMANIUM IN COAL AND COKE

	Sodium carbonate - ignition method		Bomb method	
	Germanium found, p.p.m.	Mean, p.p.m.	Germanium found, p.p.m.	Mean, p.p.m.
Boldon coal	7·0, 8·0	7·5	6·6, 6·6	6·5
Coke from Boldon coal.. ..	7·0, 8·0	7·5	6·1, 6·4	6
Harton coal	7·2, 9·2	8	—	—
Coke from Harton coal ..	11·0, 12·5	12	—	—

A comparison of the two procedures shows that the ignition method requires considerably less manipulation and has the advantage that a number of samples can be decomposed simultaneously. However, the bomb method has a greater sensitivity as it uses a 1-g sample in contrast to a maximum of 0·5 g for the ignition method. The limitation of the size of the sample in the latter method is associated with the quantity of sodium carbonate required, as large concentrations of sodium salts result in precipitation of sodium chloride from the strong hydrochloric acid solution used for the distillation.

During these experiments it was found that the effective sensitivity of both methods could be enhanced by collecting a smaller volume of distillate and applying the absorptiometric procedure directly to this without subdivision. The loss of germanium resulting from this modification was found to be negligible for the very small amounts of germanium involved. In this manner it should be possible to determine germanium down to 4 p.p.m. with the ignition method and 2 p.p.m. with the bomb method.

Although complete data are not available for an accurate assessment of the efficiency with which germanium is deposited in flue dusts, it may be of interest to record the information resulting from the above determinations. This shows that on conversion of the Boldon coal to coke, some 60 per cent. of the germanium was retained by the coke; on use of this coke in producers, a much smaller proportion of germanium was deposited in the flue dusts in the waste heat system, although these dusts contained sufficient to justify extraction of the element.

THE TWO METHODS FOR THE DETERMINATION OF GERMANIUM IN COAL AND COKE

APPARATUS AND SPECIAL REAGENTS—

The distillation apparatus and the special reagents required for the final absorptiometric determination are described in the preceding paper on the absorptiometric determination of germanium with phenylfluorone.[6] The bomb calorimeter required for the bomb method is of the conventional type used for the determinations of calorific value and sulphur in coal and coke.

SODIUM CARBONATE - IGNITION METHOD—

Weigh into a platinum crucible 0·5 g of the sample previously ground to pass a 120 B.S. screen. Mix the sample intimately in the crucible with 1·5 g of sodium carbonate (do not tap down) and cover the charge with a further 0·5 g of sodium carbonate.

Transfer the uncovered crucible to a muffle furnace and, allowing access of air, raise the temperature to 600° C over a period of about an hour. Maintain this temperature for 1½ hours, or longer if carbonaceous matter is still visible. Remove the crucible from the furnace, stir the contents and return the crucible to the furnace for a further hour to destroy any last traces of carbonaceous matter.

Fuse the contents of the crucible, disintegrate the melt and prepare the solution for distillation in the same manner as used in the method for flue dusts, except that the volume of diluted hydrochloric acid (1 + 1) used should be 8 ml to neutralise the larger weight of sodium carbonate used.

Distil at the rate of about 2 ml per minute, collecting only the first 10 ml of distillate. Apply the absorptiometric procedure directly to this distillate without subdivision and to a blank distillate similarly prepared as described in the preceding paper.[6] The 10 ml of distillate contains the appropriate amount of hydrochloric acid for the absorptiometric determination and no further addition of acid is required.

BOMB METHOD—

Make about 1 g of the ground sample into a pellet and weigh it. Place 10 ml of water in the bomb and carry out the combustion of the pellet in the normal manner, with an oxygen pressure of 25 atmospheres.

After the combustion allow the bomb to stand for 30 minutes to permit the acid mist to settle and then slowly release the pressure. Wash the solution and ash into a platinum dish, add 0·2 g of sodium carbonate to make the solution alkaline and then evaporate to dryness.

Add 1 g of sodium carbonate, fuse, disintegrate the melt and prepare the solution for distillation exactly as in the method for flue dusts. Perform the distillation and the final absorptiometric determination exactly as in the ignition method.

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DISCUSSION ON THE ABOVE THREE PAPERS

MR. W. H. BENNETT asked whether fluorine interfered with the phenylfluorone colour reaction, and if it did, had the author considered the possibility of the interference of fluorine in the determination of germanium in coals, some of which contained small amounts of fluorine.

MR. CLULEY replied that he had no specific information on the effect of fluorine on the colour reaction. (Later work has shown that as much as 2·5 mg of fluorine may be present as fluoride in the final solution without effect on the colour reaction, although substantially greater amounts of fluorine cause interference by reducing the intensity of the germanium colour, presumably owing to partial complexing of the germanium. When the maximum sample weight of 1 g of coal was used, the amount of fluorine in the coal would not exceed 0·2 mg, so that no interference from this source would be expected.)

MR. R. F. MILTON drew attention to the author's statement that reduction of germanomolybdate to molybdenum blue was not satisfactory in the presence of arsenic, phosphorus or silicon. In point of fact, each one of this group of elements could be estimated in the presence of the others, if cognisance were taken of the conditions under which the complex molybdate formation occurred. Thus, silicomolybdate formed in 0·1 *N* sulphuric acid and the reduction occurred in 2 *N* sulphuric acid with stannous chloride. The molybdenum blue could be extracted with butyl solvent and estimated. If the residual solution were neutralised to 1·2 *N* sulphuric acid, phosphomolybdate would reduce and the colour could then be extracted and estimated. The reduction of acidity to 0·9 *N* would allow germanomolybdate to reduce and to be estimated. If arsenic, which had been maintained in the tervalent state, were then oxidised, it would form the arsenomolybdate, which could then be estimated after reduction with stannous chloride.

DR. J. H. HAMENCE said he had listened to the papers with great interest. He had two questions to put to the author. First, he asked if it was necessary in the determination of germanium in flue dust to make a fusion of the material before the hydrochloric acid distillation. In his experience, when arsenic and germanium were present together, the germanium usually distilled very readily with the arsenic when heated with hydrochloric acid. Was it possible to distil the flue dust directly with hydrochloric acid?

Secondly, he asked whether the author could give any idea of the sensitivity of the colour reaction

between phenylfluorone and molybdenum. Detection of traces of molybdenum had now assumed considerable importance in agricultural work, and new sensitive tests for this element were always welcome.

Finally, Dr. Hamence thanked the author for having shown in a most conclusive manner that the distillation process for the separation of arsenic from antimony and tin was in fact a most efficient method, and that neither of these elements distilled over with the arsenic. In the past this had always been assumed to be a fact, but to the best of his knowledge it had not previously been confirmed experimentally.

MR. CLULEY replied that although some flue dusts gave an almost quantitative yield of the germanium on direct distillation with hydrochloric acid, other dusts afforded only a small proportion of their germanium with this treatment. In the latter type of dust the germanium was presumably mainly present in a chemical form in which it was not readily soluble in hydrochloric acid. Decomposition of the sample by fusion was therefore necessary to ensure complete recovery of the germanium in the determination.

The sensitivity of the colour reaction of phenylfluorone with molybdenum was of the same high order as the germanium reaction, and phenylfluorone could probably be used for the detection of molybdenum if required. Gillis, Claeys and Hoste[1] have stated that the associated reagent *o*-hydroxyphenylfluorone is, under appropriate conditions, a specific reagent for the detection of molybdenum.

REFERENCE TO DISCUSSION

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Determination of the Grade Strength of Pectins

REPORT OF THE PECTIN SUB-COMMITTEE OF THE JAM PANEL, BRITISH FOOD MANUFACTURING INDUSTRIES RESEARCH ASSOCIATION

THE Jam Panel of the British Food Manufacturing Industries Research Association in May, 1948, appointed a Sub-Committee with the terms of reference—

> "To devise standard methods for the determination of the grade strength of pectins as commercially supplied for jam manufacture."

The members of the Sub-Committee were Miss M. Olliver (Chivers and Sons Ltd.), Messrs. L. M. Adams (Scott, Preserve Makers Ltd.), A. C. Francis (Rowntree and Co. Ltd.), H. Threadgold (Wm. P. Hartley Ltd.) and C. L. Hinton (B.F.M.I.R.A.), with the late Mr. T. Rendle (Chivers and Sons Ltd.) as Chairman. A considerable amount of experimental work on the subject was carried out by members of the Sub-Committee, which also had the benefit of hearing detailed results of work since published by Miss M. Olliver.[1,2] The following is the Report of the Sub-Committee that was formally adopted by the Jam Panel of the Research Association in May, 1950.

The system of expressing the jellying value of commercial pectins by means of a grade number was introduced in the U.S.A. So far as can be ascertained, no precise definition of "grade" has been laid down by any authoritative body, though a Committee of the American Institute of Food Technologists is at present studying the question. From statements that have appeared in the literature, however, it appears that the grade of a pectin is generally understood to be the weight of sugar with which one part by weight of the pectin will, under suitable conditions, form a satisfactory jelly. The conditions implied require a soluble solids content of 65 per cent. in the jelly, but apart from that, the conditions for the composition and preparation of the jelly are very imprecise. There has been a further lack of precision on what is to be regarded as a jelly of satisfactory firmness.

The Sub-Committee holds very strongly that the conditions under which the grading of pectins in the United Kingdom is determined should be related as closely as possible to the conditions under which the pectin is to be commercially used in jam manufacture in this country. In the proposed method of this Report an attempt has been made to implement this view as far as possible, though it is appreciated that the composition of different jams in so far as this affects the jellying behaviour of the pectin, is somewhat variable. The conditions of composition of the standard jelly have been chosen, after a survey of typical jams on the market, as conforming most closely with the composition of the majority of commercial jams in this respect.

At the same time, it is the opinion of the Sub-Committee that the recommended method should as far as possible, for commercial reasons, reproduce U.S. grade values, provided that

they can be brought into conformity with the conditions of use of pectins in British manufacture. It appears that if a rather weak jelly is accepted as the standard of jelly strength to satisfy the U.S. grade definition, then the results of grading by the method proposed by this Sub-Committee fall into line on the whole with U.S. grade figures (see Appendix, p. 539). For the purpose of the practical determination of jelly strength, however, a rather stronger jelly is preferred, prepared from a slightly larger proportion of pectin. The grade value found, however, is fundamentally referred to the definition of grade that was adopted by the Sub-Committee, as follows—

> "The grade of a pectin is defined as the ratio of total soluble solids to pectin in a jelly of standard strength prepared in a standard manner with total soluble solids between 70 and 71 per cent."

The Sub-Committee fully appreciates that there is much in the behaviour of pectins of various kinds that is not yet known or understood and that may affect their value in practical use in a sense not indicated by the proposed standard test. The Sub-Committee does not put forward the test as finally satisfactory in all its details, and in fact anticipates that it may be capable of improvement as further knowledge is gained.

For the measurement of jelly strength, the Sub-Committee recommends for the present two instruments as standard, *viz.*, the "B.A.R." Jelly Tester[3] and the California Exchange Ridgelimeter.[4] Measurements with other jelly-testing instruments could be accepted when data are available to relate their readings of the strength of the standard jelly to those obtained with the two instruments mentioned. It should be pointed out, however, that these two instruments essentially measure the elastic property of the jelly. Some other instruments, such as those that measure the breaking strain of the jelly, while perhaps giving valuable information on other properties of the jelly, may not be so suitable for use in measuring the jelly strength for the purpose of the proposed test.

This, and other aspects of the standardisation of pectin quality, require further and continued investigation, and it is the view of the Sub-Committee that it, or some similar body, should be kept in being by the Panel for the purpose of making any recommendations that may be desirable from time to time.

April 27th, 1950.

Provisional Standard Method for Determination of the Grade Strength of Pectins

1. PREPARATION OF A SOLUTION OF THE PECTIN—

(*a*) The pectin, if powdered, is made up with sugar syrup, on the basis of its alleged grade strength, to give a solution corresponding to a 5-grade liquid pectin, as follows—

(*b*) Into 75 g of cold 66 to 67 per cent. sugar syrup stir 15 g of 100-grade pectin, or an equivalent amount of any other grade, and distribute evenly. Then stir in 200 ml of hot distilled water, cool, and adjust the weight to 300 g, mixing well, and ensuring that no lumps remain undispersed. Allow to stand for at least 1 hour, and before using stir again to break up any lumps that are still visible. Cover the vessel during the standing period, otherwise a skin may form that is not easy to dissolve.

(*c*) Dispersion of the pectin must be complete and it is recommended that if possible examination under the microscope should be made to confirm that no particles remain. Should the measures recommended in 1 (*b*) be inadequate, more drastic methods may be adopted, such as the use of hot syrup or mechanical stirring or both for the initial dispersion. At no stage should the pectin solution be boiled.

(*d*) A larger or smaller quantity of pectin solution, with ingredients in the same proportions, may be prepared if desired, *e.g.*, to enable duplicate or repeat tests to be made.

(*e*) Liquid pectin usually has a grade strength of about 5 and should be used without dilution.

2. PREPARATION OF THE BUFFER SOLUTION—

(*a*) Dissolve 100 g of pure monohydrate citric acid in 600 ml of distilled water and add 220 ml of *N* potassium carbonate. Boil to remove carbon dioxide, cool, and dilute to 1 litre. This solution, undiluted, should have a pH of 2·82.

(*b*) In testing rapid-set pectins with a potassium buffer it is sometimes found that air entangled in the boiling jelly does not escape before setting occurs. In such circumstances it is permissible to use a buffer prepared as in 2 (*a*) but with *N* sodium carbonate in place of the potassium carbonate provided that the grade is ultimately determined from a calibration graph (see 6 (*b*)) obtained with jellies prepared with sodium buffers in the same way.

(*c*) If a stock of buffer solution is kept for any length of time, thymol may be added as a preservative unless the jellies are to be subsequently used for flavour tests.

3. SUGAR—

A high grade refined sugar (sucrose) should be used for the test.

4. PREPARATION OF THE JELLY—

(*a*) Weigh 92·5 g of the pectin solution or of the liquid pectin sample into a beaker and add 200 to 300 ml of distilled water, mixing well. Determine the pH by means of a glass electrode immersed in the liquid. If this is not in the range of 3·05 to 3·1 (or lower if sulphur dioxide is present), add with stirring 0·1 *N* or *N* hydrochloric acid or 0·1 *N* sodium hydroxide as required, so that the necessary pH range is reached. Remove the glass electrode and stirrer, washing them thoroughly with a jet of distilled water. Transfer the contents of the beaker to a weighed copper, aluminium or stainless steel boiling pan of about 8 litres capacity, washing out the beaker well with distilled water. Make up to such a weight with distilled water that the time taken to boil down to the required 600 g of jelly (see below) will be approximately 10 minutes. The weight after addition of this distilled water should not be less than 500 g and may be higher according to size and shape of pan and type of gas-ring used.

To the mixture then add 25 ml of the buffer solution, and 417 g of sugar (minus the solids in 92·5 g of pectin solution). Heat the mixture over an efficient gas-ring, with constant stirring by means of a wooden spoon or paddle or thick glass rod. Boil until the weight of the mixture is reduced to 600 g in approximately 10 minutes' actual boiling time. Check the finish of the boiling as this is approached by lifting the pan on to a scale and weighing to within 1 g. Pour the boiled mixture into a square box ($3 \times 3 \times 3\frac{1}{4}$ inches deep) if a B.A.R. Jelly Tester is to be used, or into two standard glasses fitted with paper collars for the Exchange Ridgelimeter test. Cover the surface of the jellies at once with waxed paper or, for jellies for the B.A.R. test, with a thin layer of high-grade liquid paraffin.

(*b*) A larger quantity of jelly may be prepared, *e.g.*, sufficient to fill three boxes for the B.A.R. Jelly Tester or the corresponding number of glasses for the Ridgelimeter, with amounts of pectin solution, buffer solution, and sugar in the same proportions as in 4 (*a*) with respect to the weight of finished jelly. The amount of water added should be adjusted so that the boiling time is approximately 10 minutes.

(*c*) The amount of sugar used is calculated to allow for the slight increase in weight from inversion. Should the percentage of soluble solids in the jelly when finally tested not fall within the range 70 to 71 per cent., the amount of sugar used in subsequent tests should be suitably adjusted.

5. COOLING, STORAGE AND TESTING OF THE JELLY—

(*a*) Allow the vessel containing the jelly to remain on the bench in air at room temperature (but as near to 20° C as possible) for 2 hours. Then place it in an incubator at 30° C for 22 hours. After removing the jelly from the incubator take off the wax paper, or remove the paraffin, and test the jelly strength at once without first cooling. After testing, determine the soluble solids content of the jelly by refractometer (this should be 70·5 per cent. $\pm$ 0·5 per cent.) and the pH of a 50 per cent. w/v solution of the jelly (this should be 3·10 $\pm$ 0·05).

(*b*) If the soluble solids content is not within the specified range, repeat the preparation of the jelly after suitably adjusting the amount of sugar as mentioned under 4 (*c*).

(*c*) If the pH is not within the specified limits, repeat the preparation of the jelly after suitably adjusting the pH of the buffer solution to the extent by which the pH of the jelly is either low or high.

(*d*) The B.A.R. Jelly Tester used in testing the jelly should have a pulley with a diameter (inside the grooves) of 4·32 cm ± 0·06 cm, and a square vane with a side of 2 cm ± 0·01 cm, and diagonals of 2·83 cm ± 0·02 cm.

(*e*) The Ridgelimeter glasses used in testing the jelly should conform with the specification given by Cox and Higby[4], *viz.*, Hazel Atlas No. 85 glasses with a depth of 3·125 inches at the centre.

6. CALCULATION OF THE GRADE STRENGTH—

(*a*) If the jelly strength obtained is 16 ml with the B.A.R. Jelly Tester (after deducting the blank), or 24·4 per cent. sag with the Exchange Ridgelimeter, calculate the grade strength of the pectin by the formula—

$$\text{Grade strength} = \frac{77}{\text{Percentage of pectin sample in jelly}}.$$

(*b*) If the jelly strength obtained is not as specified in 6 (*a*), but lies between 10 and 22 ml with the B.A.R. Jelly Tester or 28·6 per cent. and 21·5 per cent. sag with the Exchange Ridgelimeter, calculate the approximate grade strength from a calibration graph obtained as follows from jellies prepared from varying percentages of a standard pectin whose grade strength satisfies the conditions in 6 (*a*)—

Prepare a 5-grade solution of the standard pectin as directed in 1 (*b*). From this solution, using amounts from 80 to, say, 115 g, prepare a number of jellies as in 4 (*a*), and test as described in 5 (*a*). Plot the jelly strengths obtained with the B.A.R. Jelly Tester or the percentages of sag obtained with the Exchange Ridgelimeter against the percentages of 100-grade (or equivalent 100-grade) pectin in the jellies. To calculate the grade strength of the sample under test, take the reading obtained with it as under 5 (*a*) and from the graph find the percentages of 100-grade pectin corresponding to the reading. Suppose it is x per cent. and that the jelly prepared as in 4 contained p per cent. of the sample. Then the approximate grade strength of the latter is given by—

$$\text{Approx. grade strength} = \frac{100 \times x}{p}.$$

(*c*) To obtain a more accurate value for the grade strength, prepare a second jelly with the weight of pectin solution adjusted to correspond with the approximate grade strength found from the graph. If this second jelly has a strength equal to the required standard as in 6 (*a*), calculate the grade strength from the amount of pectin used, by the formula in 6 (*a*). If the jelly has a strength only slightly different from the required standard, calculate the grade strength from the calibration graph and the amount of pectin used, as in 6 (*b*).

APPENDIX

RELATION TO THE U.S. SYSTEM OF DEFINING GRADE STRENGTH

The grade strength of a pectin is defined as the ratio of total soluble solids to pectin in a jelly of standard composition and strength prepared by a standard procedure. The standard procedure and composition for the British standardisation have been defined in the foregoing description. For the purpose of the definition it is also necessary to define a standard strength for the jelly. It has been found that when commercial pectins of specific U.S. grade strength, as tested under the rather different conditions of accepted U.S. practice, are tested under the conditions laid down above, with the percentage of pectin in the jelly equal to—

$$\frac{70{\cdot}5}{\text{grade strength of pectin}'}$$

(*i.e.*, the jelly corresponds strictly to the grade strength definition given above), the strength of the jelly when tested with the B.A.R. Tester averages approximately 12 ml (blank deducted) or approximately 27 per cent. sag with the Ridgelimeter. Such a jelly, in the opinion of the Sub-Committee, is rather too weak to be taken as a standard jelly. It is, however, desirable to assume that it satisfies the "standard strength" of the definition in order not to conflict with accepted U.S. grading practice. For the proposed standard test, a rather firmer jelly is prescribed, having a strength of 16 ml with the B.A.R. Jelly Tester or 24·4 per cent. sag

with the Exchange Ridgelimeter. It has been found that to produce a jelly of this strength, the pectin must be used in approximately 12/11 times the concentration required for the "standard strength."

Hence the grade strength of a pectin is given by the proposed standard method as follows—

$$\text{Grade strength} = \frac{70{\cdot}5}{\frac{11}{12}\,(\text{percentage of pectin in jelly})}$$

$$= \frac{77}{(\text{percentage of pectin in jelly})'}$$

as in 6 (*a*).

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The Assay of Vitamin B_{12}

Part V

Some Substances that Interfere with the Response of an *Escherichia coli* Mutant to Vitamin B_{12}

BY W. F. J. CUTHBERTSON, H. F. PEGLER, C. QUADLING AND VALERIE HERBERT

(*Presented at the meeting of the Biological Methods Group on Tuesday, December 19th*, 1950)

The effects of various substances on the *Escherichia coli* plate assay of vitamin B_{12} have been investigated and permissible limits have been defined.

For satisfactory assay C must be less than KN, where C is the maximum permissible concentration of the interfering substance expressed as a percentage, K is a constant depending on the nature of the interfering substance and N is the concentration of vitamin B_{12} in μg per ml.

Some reagents increase while others decrease the apparent vitamin-B_{12} response.

IN a preliminary communication[1] from these laboratories, it was shown that a mutant of *Escherichia coli* could be used as an assay organism for the measurement of vitamin B_{12}. A more detailed description of the precise procedure recommended is in course of preparation; meanwhile it may be of assistance to others working in this field if we give an account of some essential preliminary work, for without its completion a routine method could not have been established. This work was concerned with the effect of various reagents on the response of the mutant (which we call variant M 200) when used in the cup-plate assay for vitamin B_{12}. The reagents tested were chosen for a number of reasons; (*a*) they might

TABLE I

EFFECT OF INTERFERING AGENTS ON RESPONSE TO VITAMIN B_{12}

Solutions containing 0·2 μg of vitamin B_{12} per ml

Response decreased		Response increased	
Added ethylene glycol, % v/v	Apparent vitamin B_{12} content, μg per ml	Added $CuSO_4.5H_2O$, % w/v	Apparent vitamin B_{12} content, μg per ml
0·0	0·2	0·0	0·2
25	0·158	0·01	0·226
50	0·145	0·05	0·236
75	0·133	1·0	0·258

be used as preservatives for vitamin B_{12} concentrates, (*b*) they might be used in the preparation of extracts, and (*c*) they might be expected to replace vitamin B_{12} in the nutrition of the test organism.

Different concentrations of the reagents studied were placed on the assay plates in absence or presence of the vitamin. When the reagents were tested in its presence the concentration of the vitamin used was always kept at 0·2 μg per ml, *i.e.*, the same as that

TABLE II

VALUES OF CONSTANT, K

	Substance	Qualitative action: In absence of vitamin B_{12}	Qualitative action: In presence of vitamin B_{12}	Values of K for: 2 per cent. error	Values of K for: 5 per cent. error
Response reduced	Alcohol	slight	reduces response	5	12·5
	Acetone	nil	"	0·2	0·5
	Ethylene glycol	nil	"	10	30
	Propylene glycol	nil	"	1	2·5
	Ascorbic acid	inhibits at 5%	"	0·002	0·005
	Formalin, 40%	inhibits at 0·2% v/v	"	0·5	1·0
Response unaltered at concentrations tested	Butanol	nil	nil	—	—
	Toluene	nil	nil	—	—
	Sodium formate	nil below 0·2%	nil below 0·2%	> 1	> 2
	$MnSO_4.4H_2O$	stimulates at 0·002 to 0·1%	nil up to 1·0%	> 5	> 10
	$FeSO_4.7H_2O$	nil up to 0·01%	nil up to 0·01%	> 0·05	> 0·1
	Choline	stimulates at 0·05%	nil up to 0·5%	> 2·5	> 5
	Betaine	stimulates at 0·5%; inhibits above 1%	nil up to 0·5%	> 2·5	> 5
	$(NH_4)_2SO_4$	nil up to 20%	nil up to 20%	>100	>100
Response increased	Methionine	stimulates	causes faint outer growth zones	very variable, ~ 0·007	very variable, ~ 0·015
	Thioglycollic acid	inhibits above 0·5%	increases response	0·5	1·7
	Phenol	inhibits above 5%	"	0·25	0·5
	$CuSO_4.5H_2O$	stimulates at 0·005%	"	0·005	0·015
	Sodium chloride	no effect below 10 to 20%	"	10	20 to 40
Effect variable	Homocystine	stimulates at 0·2%; inhibits at 1 to 2%	variable	0·2	0·5
	Potassium cyanide	inhibition above 0·1%	inhibition of growth	0·2 to 0·5	0·2 to 0·5

Cyanide in concentrations above 0·1 per cent. causes inhibition and prevents growth over the whole plate.

used in the provisional assay technique. In all experiments the growth zones were compared qualitatively with those caused by the vitamin at concentrations of 0·02 and 0·2 μg per ml on the same plate. If the test mixtures produced growth qualitatively similar to that obtained with the vitamin alone then the diameters of the growth zones were measured and the apparent concentrations of vitamin B_{12} in the test mixtures were calculated from them. In this way the effect of interfering agents on the apparent activity could be determined. In some instances the interfering agent caused an apparent decrease in response, in others an increase. Typical examples of each kind of effect are shown in Table I.

From these and similar results it is possible by graphical interpolation to determine the permissible concentration of the different substances allowable for a specified error.

In the assay procedure used all solutions are diluted to approximately 0·2 μg per ml before assay; clearly, the concentration of interfering agent producing a given error increases with the concentration of the original vitamin B_{12} solutions. Thus the allowable concentration of interfering substances may be expressed in the form $C = KN$ per cent. where C is the permitted concentration in the test solution expressed as a percentage, N is the vitamin B_{12} concentration (in μg per ml) and K is a constant depending on the interfering substance.

In Table II are given the values of K at levels leading to a 2 per cent. or a 5 per cent. error. This table shows whether the interfering agents cause an increase or a decrease in the apparent vitamin-B_{12} activity of the solution; the effects of the agents in the absence of the vitamin are also shown.

A number of amino-acids have been tested by Bessell and Lees[2] to determine whether they interfere in this assay. Histidine, arginine and isoleucine tested at a level of 5 mg per ml were found to promote growth corresponding to 0·002, 0·001 and less than 0·005 μg of vitamin B_{12} per ml, respectively; none of these substances at this concentration interfered with the assay of solutions containing more than 0·2 μg of vitamin B_{12} per ml.

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The Determination of Selenium and Tellurium in Copper

BY F. D. L. NOAKES

A description is given of a new method for determining selenium and tellurium in copper that is slightly more accurate and considerably more rapid than previously published methods. The main difference from other methods is the initial precipitation of selenium and tellurium from the nitric acid solution of the sample after complete removal of nitrous oxides by means of a solution of stannous chloride in strong hydrochloric acid. This avoids the tedious procedures hitherto used to remove nitric acid. After separation, the selenium and tellurium may be determined gravimetrically or, as preferred by the author, by the volumetric methods described.

In two appendices, the preparation of standard solutions of selenium and tellurium are described. In this connection, experiments showing the rate and degree of oxidation of quadrivalent selenium to hexavalent selenium by bromine and by nitric acid and the subsequent precipitation of the selenium are outlined.

SEVERAL methods for the determination of selenium and tellurium in copper have already been published. They involve rather lengthy procedures and do not always give concordant results. With the proposed volumetric method it is possible to determine selenium in $2\frac{1}{2}$ hours, and selenium and tellurium in $3\frac{1}{2}$ to 4 hours, or in slightly less time if the method described as "routine volumetric" is used.

In the previously published methods, the solution of the sample and the preliminary separation of the selenium and tellurium from the copper require considerable time. Challis[1] and Evans[2] used nitric acid, either alone or with the addition of bromine, for the solution of the sample, followed by evaporation to fumes with sulphuric acid. This evaporation must be carried out slowly to avoid loss by "bumping." Experiments described in Appendix I show that with certain methods of precipitation an apparent loss, due in fact to oxidation of part of the selenium to the hexavalent state, may occur when solutions containing bromine are used. Treatment with nitric acid and bromine has been used, and the long evaporation with sulphuric acid has been avoided by collecting the selenium and tellurium in an iron precipitate by a "basic acetate" method. The results for this method of precipitation were more consistent than those for direct precipitation with ammonia,[3,4,5] but they still tended to be slightly low.

The initial precipitate may be purified effectively by means of sodium hypophosphite,[1] but this does not seem to be necessary when volumetric methods are used. In general, the author found that the volumetric methods of Berg and Teitelbaum[6] and Evans[2] were more rapid and accurate than gravimetric methods. Although the end-point of the tellurium titration is not easy to distinguish, these methods were adopted. The sulphur dioxide separation of selenium from tellurium as described by Lenher and Kao[5] gave satisfactory results and was used with the addition of an improved collector for the selenium precipitate.

In the proposed method the sample is dissolved in nitric acid and the bulk of the nitrous oxides formed are removed by boiling. After neutralisation, the remaining nitrous oxides are decomposed by the addition of a reagent such as urea, sodium azide or hydrazine hydrochloride. The solution is then decolorised by the addition of a strong reducing agent and the selenium and tellurium are precipitated by adding an excess of the reagent. Many reducing agents were tried, but only stannous chloride in hydrochloric acid solution, as recommended by Schoeller,[7] proved wholly satisfactory. Sodium formaldehyde sulphoxylate was found to precipitate selenium completely in a form that is easily filtered, but under all the acid conditions tried tellurium was incompletely precipitated. The use of the sulphoxylate is therefore limited to the separation of selenium. The hydrochloric acid solution of stannous chloride, when added to the solution containing selenium, tellurium and copper, brings about the precipitation of the selenium and tellurium as copper selenide and copper telluride. The mixture should not be allowed to stand for more than 30 minutes before being filtered, nor should the temperature be allowed to rise over 70° C as, if so, a violent reaction takes place with intense frothing. The treatment of the precipitate follows closely the procedure described by Evans[2] and by Pollard.[8]

Recommended method

Reagents—

Hydrochloric acid (1 + 1)—Dilute hydrochloric acid, sp.gr. 1·18, with an equal volume of water.

Hydrazine hydrochloride—Dissolve 15 g in 100 ml of a 10 per cent. v/v solution of hydrochloric acid.

Stannous chloride—Add hydrochloric acid, sp.gr. 1·18, to an excess of solid stannous chloride and shake well. After settling, pour off the supernatant liquid and warm until clear.

Bromine - hydrobromic acid mixture—Add 10 ml of bromine to 100 ml of hydrobromic acid, sp.gr. 1·46 to 1·49.

Sulphur dioxide solution—Saturate hydrochloric acid, sp.gr. 1·18, with sulphur dioxide.

Sodium salicylate—Dissolve 12 g in 100 ml of water.

Potassium cobalticyanide—Dissolve 10 g in 100 ml of water.

Potassium iodide—Dissolve 4 g in 100 ml of water.

Gum arabic solution—Dissolve 1 g of gum arabic or gum acacia in 100 ml of water.

Standard sodium thiosulphate solution, 0·05 N—This strength is for use with more than 0·005 g of selenium. Dissolve 12·5 g of crystalline sodium thiosulphate per litre of water. 1 ml of solution ≡ 0·001 g of selenium.

Standard sodium thiosulphate solution, 0·01 N—This strength is for use with less than 0·005 g of selenium. Dissolve 2·5 g of crystalline sodium thiosulphate per litre of water. 1 ml of solution ≡ 0·0002 g of selenium.

Standardisation—The solutions of sodium thiosulphate are conveniently standardised against a solution containing 0·001 g of selenium per litre. Dissolve 0·25 g of pure selenium in 2 ml of fuming nitric acid and evaporate to dryness on the steam-bath. Dissolve the residue in water and dilute to 250 ml in a graduated flask. Five millilitres of this solution, after addition of potassium iodide solution, may be directly titrated with thiosulphate or, preferably, the selenium may be precipitated with sulphur dioxide after addition of 30 ml of hydrochloric acid, sp.gr. 1·18. The precipitate is then treated as described for the assay. (See also Appendix I, p. 546.)

Standard iodine solution, 0·01 N—Dissolve 3 g of potassium iodide and 2·55 g of iodine in 2 litres of water. 1 ml of solution ≡ 0·000319 g of tellurium. (See Appendix II, p. 547.)

Standardisation—This solution is conveniently standardised against 0·01 *N* arsenious oxide, starch being used as indicator. 1 ml of 0·01 *N* arsenious oxide ≡ 0·0012692 g of iodine.

PROCEDURE—

Dissolve 5 to 20 g of sample in 20 ml of water and 20 ml of nitric acid, if necessary adding more nitric acid. Boil the solution for 2 minutes to remove the bulk of the nitrous oxides and then add a solution of sodium hydroxide until a faint permanent precipitate is formed. Re-dissolve the precipitate by the dropwise addition of concentrated nitric acid and add an excess of 4 to 5 drops. Add 10 ml of hydrazine hydrochloride solution and 20 ml of hydrochloric acid, sp.gr. 1·18. Reduce immediately by adding stannous chloride soluton until the pale precipitate first formed is completely re-dissolved and then add 1 ml in excess. Warm the solution to about 60° C, but not above 70° C, and do not allow to stand longer than 30 minutes. The solution should now be clear, with the selenium and tellurium collected in a coagulated black precipitate.

Filter the precipitate with gentle suction on an asbestos pad in a Gooch crucible and wash the precipitate and the original beaker five times with hydrochloric acid (1 + 1). Dissolve the precipitate into the original beaker with two 5-ml portions of bromine - hydrobromic acid mixture, previously warmed to 30° C, washing with hydrochloric acid (1 + 1) between the additions. Finally, wash the pad with hydrochloric acid (1 + 1) until the washings are colourless.

Dilute the solution to 100 ml with hydrochloric acid (1 + 1), add crystals of sodium hypophosphite until the bromine is destroyed and warm gently until precipitation of the selenium and tellurium commences. Add a further 3 g of sodium hypophosphite and warm the solution to 50° C. Allow to stand at 50° C for 30 minutes to ensure complete coagulation of the precipitate. Filter, with suction, on an asbestos pad. Wash the pad and beaker five times with hydrochloric acid (1 + 1) and finally twice with hydrochloric acid, sp.gr. 1·18.

Dissolve the precipitate through the pad with 1 to 2 ml of bromine - hydrobromic acid mixture and wash with hydrochloric acid, sp.gr. 1·18, until the washings are colourless. Dilute the filtrate and washings to 50 ml with hydrochloric acid, sp.gr. 1·18, and add about half a gram of powdered kieselguhr to act as a collecting agent. Add hydrochloric acid, saturated with sulphur dioxide, slowly until the bromine colour is discharged and then rapidly add 20 ml or a suitable excess. Warm to about 30° C and agitate until the red precipitate of elemental selenium is coagulated. Filter on an asbestos pad with suction and wash four times with hydrochloric acid, sp.gr. 1·18, twice with hydrochloric acid (1 + 1) and twice with cold water, collecting all the washings in the same flask as the filtrate. Treat the precipitate as described below under "Selenium titration" or, if preferred, gravimetrically, after repeating the precipitation with sulphur dioxide.

Boil the filtrate to remove the bulk of the sulphur dioxide and then add bromine water until a faint colour persists for 2 minutes. Reduce the acidity to about 50 per cent. by the addition of water. Add crystals of sodium hypophosphite until precipitation commences and then add 3 g in excess. Bring the solution to the boil and allow to stand for 20 minutes to coagulate the tellurium. Filter, with suction, on an asbestos pad and wash five times with hydrochloric acid (1 + 1). Treat the precipitate as described below under "Tellurium titration" or, if preferred, gravimetrically, after repeating the sodium hypophosphite precipitation.

SELENIUM TITRATION—

Place a 100 or 150-ml glass-stoppered bottle under the Gooch crucible containing the selenium precipitate and pour 1 to 2 ml of bromine - hydrobromic acid mixture on to the

pad, covering the crucible at once with a watch glass. Allow to stand for 2 to 5 minutes, remove the watch glass, wash it with water and then wash the pad with water until the volume of solution and washings is 50 ml. (A mark on the receiving bottle facilitates this.) Add the asbestos pad to the contents of the bottle, insert the glass stopper and shake vigorously. Add 2 ml of hydrochloric acid, sp.gr. 1·18, and 2 to 4 ml of sodium salicylate solution and shake. Add more salicylate if the solution and suspension are not quite white. Then add 2 ml of potassium cobalticyanide solution, 5 ml of potassium iodide solution and 4 ml of carbon tetrachloride. Shake well and allow to stand for 1 minute. Titrate with the appropriate strength of thiosulphate solution until the iodine colour is reduced to a pale straw-yellow, shaking well between additions; then add 2 ml of a freshly boiled starch solution and continue the titration to a pale pink end-point.

TELLURIUM TITRATION—

Dissolve the precipitate from the pad with 1 to 2 ml of bromine - hydrobromic acid mixture and wash with the minimum amount of hydrochloric acid (1 + 1), collecting the solution and washings in a small beaker. Evaporate to dryness on the steam-bath until the hydrochloric acid is completely removed. Treat the residue with 5 ml of phosphoric acid, sp.gr. 1·75, and 5 ml of gum arabic solution. Shake well and allow to stand until clear. Transfer to a 250-ml conical flask with 50 ml of water, add 2 ml of potassium cobalticyanide solution and 2 g of sodium hypophosphite. Heat to brisk boiling and allow to cool. Dilute the cold solution with 150 ml of water and titrate with 0·01 *N* iodine solution until the colour changes to a pale straw. Add 10 ml of benzene and continue the titration until a pink colour appears in the benzene, taking care to shake vigorously after each addition of iodine.

ROUTINE VOLUMETRIC METHOD

The procedure may be simplified if a volumetric method is to be used for routine analyses. The precipitate from the stannous chloride separation is filtered, washed with hydrochloric acid, sp.gr. 1·18, and dissolved in bromine - hydrobromic acid mixture. The selenium and tellurium are then separated as previously described with sulphur dioxide but without the purification of the stannous chloride precipitate with sodium hypophosphite. The addition of potassium cobalticyanide solution during the titration prevents any interference from the small amounts of copper that may be present (Evans[2]).

DISCUSSION OF RESULTS

By the longer of the two methods described the author has made determinations of selenium alone in 2½ hours and of selenium and tellurium in 3½ to 4 hours. The range of samples studied was from about 0·001 to 0·1 per cent. of the elements determined. Lower amounts may be determined by taking several 20-g samples and combining the precipitates. The use of very dilute thiosulphate solutions for the selenium titration is not to be recommended, as the end-point becomes indistinct.

Numerous tests have been carried out with this method on synthetic solutions. Typical results are shown in Table I.

TABLE I

TYPICAL RESULTS FOR SYNTHETIC SOLUTIONS

Weight of copper taken, g	Selenium		Tellurium	
	added, %	found, %	added, %	found, %
20	0·001	0·0010	0·00095	0·0010
20	0·01	0·0098	0·0095	0·0089
10	0·03	0·0296	0·0190	0·0187
5	0·10	0·0980	0·095	0·0944

Both the "long" and "short" volumetric methods described have also been used for the analysis of numerous samples of copper with satisfactory results. It was found that results obtained by the slightly longer method did not differ appreciably from those obtained by the "routine" method and the author therefore adopted the shorter procedure for most of his work.

In conclusion, the author wishes to express his thanks for the advice and help he has received from the British Non-Ferrous Metals Research Association and their chief chemist, Mr. B. W. Drinkwater, from Dr. W. B. Pollard and from Mr. E. W. Yeoman, in whose laboratory at the Royal School of Mines this work was carried out.

APPENDIX I

STANDARD SOLUTIONS OF SELENIUM

During the early stages of the work it was found that if solutions of selenium in bromine - hydrobromic acid mixture were allowed to stand, the results of determinations of selenium content by direct titration with thiosulphate after the addition of potassium iodide solution became progressively lower with the lapse of time. This was not so if fuming nitric acid was used to dissolve the selenium initially. Hence it appeared that in the presence of bromine - hydrobromic acid mixture there is either a loss of selenium or selenium is converted into a form that does not liberate iodine from potassium iodide. To confirm these conclusions, experiments were made as follows—

(1) A solution was made by dissolving 0·25 g of selenium in bromine - hydrobromic acid mixture and diluting to 250 ml with water.
(2) A second solution was made by dissolving 0·25 g of selenium in fuming nitric acid and diluting to 250 ml with water.

Of each of these solutions, 5-ml samples were taken and the selenium was precipitated and titrated with thiosulphate as in the assay.

5 ml of solution (1) required 4·75 ml of thiosulphate.
5 ml of solution (2) required 4·70 ml of thiosulphate.

Portions of solutions (1) and (2) were then treated as follows—

(*a*) A 50-ml sample of solution (1) was placed in a 50-ml glass-stoppered bottle and the stopper sealed with paraffin wax.
(*b*) A 50-ml sample of solution (2) was treated as (*a*).
(*c*) A 50-ml sample of solution (1) was placed in a 100-ml bottle and the neck was left open but loosely plugged with glass wool to keep out dust.
(*d*) A 50-ml sample of solution (2) was treated as in (*c*).
(*e*) The remainder of solution (1) was allowed to stand in the original stoppered graduated flask.
(*f*) The remainder of solution (2) was treated as in (*e*).

These six solutions and the original sodium thiosulphate solution were kept in a dark cupboard for six weeks. At the end of that time, two 5-ml portions of each were taken, one being titrated direct with the thiosulphate and the other titrated after precipitation of the selenium with sulphur dioxide in hydrochloric acid of sp.gr. 1·18 as described in the paper. The following results were obtained—

Test solution	Sodium thiosulphate used	
	Direct titration, ml	After precipitation, ml
a	1·20	4·90
b	4·75	4·90
c	3·90	5·10
d	4·80	5·20
e	1·30	4·90
f	4·70	5·10

Two further portions of solutions (*e*) and (*f*) were treated with sulphur dioxide in hydrochloric acid (1 + 1), and the selenium precipitated from solution (*e*) required only 0·90 ml and that from solution (*f*) 4·10 ml of thiosulphate.

The slightly higher figures obtained after precipitation compared with the tests carried out before the solutions were stored are probably due to deterioration of the thiosulphate solution, although a check showed this to be slight. In experiments (*c*) and (*d*) evaporation of the solutions accounted for an apparent increase in the selenium content.

These tests indicate that there is no loss of selenium during solution but that selenium is gradually oxidised to the hexavalent state by bromine in hydrobromic acid solution and,

possibly, to a very slight extent, by the nitric acid. That the oxidation is chiefly due to the presence of bromine is confirmed by the very much lower state of oxidation found in solution (*c*) where the conditions of the experiment permitted evaporation of bromine, the solution becoming colourless after a few days. These experiments confirm the suggestion by Evans[2] that oxidation with bromine might in part go beyond the quadrivalent stage. Pollard[8] has shown that this oxidation is negligible if the solutions are treated soon after oxidation with the bromine, but the author found that oxidation can be detected if the solution is allowed to stand for even a few hours. For example, 5 ml of a fresh solution of pure selenium in bromine - hydrobromic acid mixture required 4·75 ml of sodium thiosulphate solution when titrated direct and gave a sharp end-point; 5 ml of the same solution titrated under the same conditions but 4 hours later required only 4·60 ml and gave a very unsatisfactory end-point.

As a further check, several tests were made with a solution of sodium selenate (Se^{VI}). These showed that—

1. Sulphur dioxide precipitates selenium completely from solutions of selenium^{VI} in hydrochloric acid, sp.gr. 1·18.
2. Sulphur dioxide does *not* precipitate selenium from solutions of selenium^{VI} in hydrochloric acid (1 + 1).
3. Sodium hypophosphite precipitates selenium rapidly from solutions of selenium^{VI} in hydrochloric acid, sp.gr. 1·18, but only *very* slowly from solutions in hydrochloric acid (1 + 1) unless the solution is boiled. From the boiling solution of the stronger acid the selenium is precipitated in the black modification.
4. Sodium formaldehyde sulphoxylate does *not* precipitate selenium from a solution of selenium^{VI} in dilute nitric acid and after the addition of urea, etc.
5. Selenium present in the hexavalent condition does *not* liberate iodine from potassium iodide under the conditions of the selenium titration.
6. Stannous chloride precipitates selenium from a solution of selenium^{VI} in hydrochloric acid, even in the presence of nitric acid, etc., as in the method described, if the solution is *warm*, but *not* if cold.

It is therefore necessary that selenium solutions containing bromine should be treated at once, unless precipitation by stannous chloride or by the method of Lenher and Kao[5] is to follow.

The dissolution of copper containing selenium and tellurium is usually complete without the addition of bromine.

APPENDIX II

Standard solutions of tellurium

Standard solutions of tellurium can be made by dissolving appropriate weights of tellurium oxide in hydrochloric acid, sp.gr. 1·18, but dilution *must not* be effected by adding water alone as tellurium hydroxide will be gradually precipitated by hydrolysis. Dilution with hydrochloric acid (1 + 1) may be used, but the presence of so much chloride in the standard solution is not always convenient. The author dissolved tellurium dioxide (TeO_2) in the *minimum* amount of a strong solution of sodium hydroxide and then diluted with water. Such a dilute solution is stable and has no appreciable effect on glassware.

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February, 1951

The Micro-Estimation of Iron with Triphenylmethylarsonium Thiocyanate

By F. P. DWYER and N. A. GIBSON

A new reagent, triphenylmethylarsonium thiocyanate, is recommended for the colorimetric estimation of iron in the presence of cobalt, nickel and chromium. The procedure is suitable for a range of 1 to 10 μg of iron per ml. The coloured iron complex has been isolated.

Many reagents have been suggested for the micro-colorimetric estimation of iron and they have been critically evaluated by Wenger and Duckert,[1] who recommend three for ferrous iron and five for ferric iron. Those recommended for ferrous iron, *viz.*, *o*-phenanthroline, α:α'-dipyridyl and dimethyl glyoxime, all suffer serious interference by cobalt and nickel. Of those recommended for ferric iron, *viz.*, pyramidone, salicylic acid - 5-sulphonic acid, acetyl acetone, barium *iso*nitrosothioglycolate and potassium thiocyanate, the last named is that most widely used.

Although the thiocyanate test for iron has been known for some time, and was suggested as a quantitative test in 1890 by Krüss and Moraht,[2] the composition of the substance responsible for the red colour in aqueous solution is still in doubt. The following formulae have been suggested: $Fe(CNS)_4'$ (Schlesinger[3]), $Fe(CNS)^{\cdot\cdot}$ and $Fe(CNS)_2^{\cdot}$ (Bent and French[4]) and $Fe(Fe(CNS)_6)$ (Schlesinger and van Valkenburgh[5]).

The red compound formed with triphenylmethylarsonium thiocyanate can be isolated easily, and has the formula $(Ph_3MeAs)_3(Fe(CNS)_6)$. This supports the work of Schlesinger and van Valkenburgh. The arsonium ferrithiocyanate complex has a low water solubility, but is soluble in chloroform, acetone, ethylene dichloride and *o*-dichlorobenzene. In the proposed method, an aqueous solution of the ferric salt is treated with triphenylmethylarsonium chloride and ammonium thiocyanate solutions. After extraction of the complex with *o*-dichlorobenzene, the iron is estimated colorimetrically. The absorption spectrum in *o*-dichlorobenzene closely resembles that obtained by Woods and Mellon[6] on treating a ferric salt with ammonium thiocyanate in 60 per cent. acetone, both having the absorption maximum at 478 mμ.

The chief disadvantages of the thiocyanate method as usually applied are the sensitivity of the colour to excess of thiocyanate ion, its dependence on the amount of sulphate ion and certain other ions present and the instability of the ferrithiocyanate complex.

As an extraction technique is used in the present work, the colour is virtually independent of the amount of thiocyanate ion added, provided the aqueous solution is saturated with respect to the quarternary arsonium thiocyanate. This is shown in Table I.

Table I

Effect of amount of thiocyanate ion used

Solution containing 100 μg of iron in 10 ml

Ammonium thiocyanate used	Iron found, μg
1 ml of 2 per cent. solution	91·2
1 ml of 5 per cent. solution	99·0
1 ml of 10 per cent. solution	99·6
1 ml of 25 per cent. solution	100·0
5 ml of 25 per cent. solution	102·0

For the same reason, as shown in Table III, the test solution can be normal with respect to sulphate ion without exceeding a 1 per cent. reduction in reading.

The most serious disadvantage of the thiocyanate method is the rapid fading of the colour, stated by Sandell[7] to be about 10 per cent. in 10 minutes. This is owing to the reduction of the ferric iron by thiocyanic acid or its decomposition products. Suggested methods for overcoming this disadvantage fall into three categories. The first is the addition of potassium

persulphate (Stokes and Cain[8]), which produces a yellow precipitate that has to be eliminated by the addition of mercuric thiocyanate. The second is extraction by solvents (Stokes and Cain[8], Bernhard and Dekter,[9] Vanossi[10]); Sandell[7] states that although extraction techniques increase the sensitivity, the stability is poor. The third method is addition of acetone (Woods and Mellon[6]), but the density of colour is very dependent on the amount of acetone present, and large errors can be introduced by evaporation of the solvent. Moreover, many salts are insoluble in aqueous acetone and their precipitation would cause further errors.

In the present work the solution rarely faded by 1 per cent. in less than 3 hours, and was often stable to this extent for 24 hours. The greatly increased stability of the ferrithiocyanate complex in *o*-dichlorobenzene is probably related to the low polarity of this solvent.

Beer's law is not obeyed perfectly, but the agreement is rather closer than that obtained by the usual thiocyanate method.

PREPARATION OF TRIPHENYLMETHYLARSONIUM FERRITHIOCYANATE, $(Ph_3MeAs)_3(Fe(CNS)_6)$—

Triphenylmethylarsonium chloride, 55 ml of a 5 per cent. solution, was treated with ammonium thiocyanate, 50 ml of a 20 per cent. solution, and sufficient water (800 ml) to keep the triphenylmethylarsonium thiocyanate in solution; to the mixture was added slowly a solution of ferric alum containing 1·06 g in 50 ml of 2 per cent. sulphuric acid. The fine red precipitate that separated was coagulated by stirring and, after filtration, washed with 0·01 *N* sulphuric acid.

The compound was recrystallised from 100 ml of acetone by the addition of 1600 ml of 0·01 *N* sulphuric acid and 40 ml of 20 per cent. ammonium thiocyanate solution.

Melting-point, 124° C.

Analysis—Calculated for $(Ph_3MeAs)_3(Fe(CNS)_6)$: Fe = 4·08%; As = 16·34%; C = 55·31%; H = 3·98%; N = 6·14%.

Found: Fe = 4·10%; As = 16·39%; C = 55·26; H = 4·06%; N = 6·22%.

PROCEDURE—

In a small separating funnel put 5 ml of *o*-dichlorobenzene, b.p. 180° to 183° C, and then from a pipette add 10 ml of test solution, which should contain between 1 and 10 μg of iron per ml and have a pH of 1 to 2. To this add 1 ml of 2 per cent. triphenylmethylarsonium chloride solution and 1 ml of 25 per cent. ammonium thiocyanate solution. Shake out the arsonium ferrithiocyanate with the *o*-dichlorobenzene and filter through a No. 2 sintered-glass filter. Extract the aqueous layer with a further 1 ml of *o*-dichlorobenzene. To the aqueous layer add 1 ml of 2 per cent. triphenylmethylarsonium chloride solution and extract twice with 1 ml of *o*-dichlorobenzene. Repeat this once more, then wash combined filtered extracts into a 25-ml graduated flask and compare with a blank solution in a Spekker absorptiometer, using 0·5-cm cells, heat absorption filters H503 and blue Spectrum filters No. 602. Calculate the amount of iron in the solution by reference to a calibration curve prepared by treating a series of known solutions of iron in the same way.

RESULTS—

Results covering the whole range suggested are shown in Table II, the average mean deviation being 0·5 per cent. or better, and the maximum deviation rarely exceeding 1 per cent.

TABLE II

RESULTS OF APPLYING THE PROCEDURE TO 10-ml SAMPLES CONTAINING 1 TO 10 μg OF IRON PER MILLILITRE

Four samples taken at each concentration

Iron taken, μg per ml	Iron found: Minimum, μg per ml	Iron found: Maximum, μg per ml	Mean deviation, μg per ml	Mean deviation, %
1·00	0·99	1·00	0·003	0·3
2·00	1·99	2·01	0·01	0·5
4·00	3·99	4·02	0·01	0·3
6·00	6·00	6·00	0·00	0·0
8·00	7·99	8·01	0·01	0·1
10·00	9·90	10·12	0·03	0·3

INTERFERENCE—

Anions—As has already been pointed out, sulphate in concentrations up to normal can be present without interference. Nitrate and chloride ions can be present in concentrations up to normal, but concentrations of nitric acid above 0·1 *N* oxidise the thiocyanate to give an opalescent solution and so lead to a high result, while similar concentrations of hydrochloric acid give a low result. The interference by hydrochloric acid was noted by Krüss and Moraht[2] and ascribed to the formation of the $FeCl_6'''$ ion by Schlesinger and van Valkenburgh.[5] It is more probably due to the formation of $FeCl_4'$, as $(Ph_3MeAs)(FeCl_4)$ can be isolated under similar conditions. Sulphuric acid up to a concentration of normal can be present, but above this concentration, the peak of the absorption spectrum becomes lower and moves towards longer wavelengths, being 486 mμ for 5 *N* sulphuric acid. These results are summarised in Table III.

TABLE III

INTERFERENCE BY IONS ADDED TO 100 μg OF IRON IN 0·01 *N* SULPHURIC ACID

Concentration of added reagent	Iron found after addition of—					
	H_2SO_4, μg	HCl, μg	HNO_3, μg	$(NH_4)_2SO_4$, μg	NH_4Cl, μg	NH_4NO_3, μg
2 *N*	97·8 (Colour change)	—	—	—	—	—
N	100·0	(Colour change)	(Rapid decomp.)	98·9	98·9	98·5
0·5 *N*	99·1	94·6	—	—	—	—
0·2 *N*	100·2	97·8	96·3 (Slight decomp.)	—	—	—
0·1 *N*	100·3	99·3	101·8	100·0	100·0	100·0
0·01 *N*	100·0	99·6	101·1	100·2	99·5	100·0

Fluoride, oxalate and pyrophosphate form colourless complexes with the iron and therefore must be absent. Iodide, nitrite, sulphite and thiosulphate act as reducing agents, while dichromate oxidises the thiocyanate ion.

Cations—A major advantage of this method is the fact that chromium and nickel salts do not form complexes and are not extractable. Test solutions of iron are unaffected by a thousandfold concentration of chromium and nickel.

Cobalt gives an analogous blue compound, but the absorption maximum is so far from that of iron that the concentration of cobalt can be 20 times that of iron without an increase of more than 1 per cent. in the absorptiometer reading for iron.

Silver and mercurous ions give insoluble thiocyanates and will be removed in the filtration.

Antimonous, cadmium, mercuric and zinc ions form colourless complexes, but this difficulty can be overcome by the use of greater amounts of the reagents.

Copper forms an analogous compound of similar colour and must be absent.

It is hoped to extend this work to the estimation of cobalt and copper.

The iron and cobalt complexes can be readily separated by chromatographic means, and this will be investigated later.

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CHEMISTRY DEPARTMENT
UNIVERSITY OF SYDNEY

March, 1951

Notes

ERRORS IN WEIGHING CAUSED BY ELECTRIC CHARGES DEVELOPED IN SILICA CRUCIBLES ON HEATING

THE possibility of the occurrence of error caused by an electrostatic charge on an object being weighed has long been recognised. On an ordinary balance the presence of such a charge is usually betrayed by erratic movements of the beam. We have recently observed that errors of this kind may pass undetected with an aperiodic balance.

A silica crucible, ignited in an electric muffle furnace at 1000° C and cooled over anhydrone, was weighed on an air-damped aperiodic balance with a "projected scale" reading to 100 mg. On adding the fractional weights necessary to bring the scale into use, a point was reached at which the highest scale reading was exceeded by a distance corresponding to an excess weight of about 2 mg. On addition of 100 mg to the weights already in the pan, however, the balance came to rest in a position well below the zero reading of the scale. That is to say, the addition of the 100-mg weight caused an abnormally large deflection of the beam. The balance was found to operate normally when the crucible was replaced by brass weights. On repeating the weighing of the crucible, the original effect was observed to have diminished but not to have disappeared.

The same effect could be observed to a greater or less degree in the weighing of all silica crucibles that had been ignited and cooled in the manner described above (see Table I, crucibles Nos. 1 and 2). Crucibles that had been handled, even momentarily, and replaced in the desiccator showed a much smaller effect when weighed after 10 or 15 minutes (see Table I, crucibles Nos. 3 and 4).

TABLE I

CONSECUTIVE WEIGHINGS OF FOUR IGNITED AND COOLED SILICA CRUCIBLES

	Crucible No. 1	Crucible No. 2	Crucible No. 3	Crucible No. 4
(*a*) Weighed by normal procedure ..	12·3286 g	12·1185 g	9·4154 g	14·2995 g
(*b*) Weighed under counterpoised nickel crucible (screened) ..	12·3354 g	12·1256 g	9·4161 g	14·2988 g
a − *b*	−6·8 mg	−7·1 mg	−0·7 mg	+0·7 mg
(*c*) Weighed by normal procedure after "earthing"	12·3340 g	12·1245 g	9·4158 g	14·2994 g
(*d*) Weighed, screened, after "earthing"	12·3356 g	12·1260 g	9·4163 g	14·2988 g
c − *d*	−1·6 mg	−1·5 mg	−0·5 mg	+0·6 mg
(*e*) Weighed by normal procedure after a second "earthing" ..	12·3339 g	12·1240 g	9·4158 g	14·2994 g
(*f*) Weighed, screened, after a second "earthing"	12·3356 g	12·1260 g	9·4160 g	14·2988 g
e − *f*	−1·7 mg	−2·0 mg	−0·2 mg	+0·6 mg

It was found that the error could apparently be completely eliminated by covering the crucibles, on the balance pan, with an inverted counterpoised nickel crucible. (Presumably, any other metal screening device would have served the purpose equally well.) By stroking the edges of the crucible with an earthed copper wire, the effect could be reduced, there being, usually, a slight persistence of the effect. Exposure of the crucibles to ultra-violet light for 1 minute was apparently without effect. Consistent weighings were, however, always obtained when the crucibles were screened as described.

That a charge existed on the crucibles could be demonstrated by bringing an earthed wire into the vicinity of a crucible on the balance pan in apparent equilibrium at the correct crucible weight, when it appeared lighter or heavier by as much as 50 to 100 mg, according as the wire was held above or below a certain point. The same effect was observed when other objects were brought near the crucible, the greatest response being elicited by another crucible that had been ignited and cooled in the manner described.

It is probable that the first-observed anomalous behaviour of the balance was due to the fact that when the crucible was near the base of the balance it was attracted towards it electrostatically and that, on adding 100 mg to the right-hand pan, the crucible, moving away from the base, experienced a rapidly decreasing attraction, according to the well-known inverse square law. It would therefore appear to be much lighter in its final than in its initial position.

The importance of the phenomenon, from the analyst's point of view, is that (*i*) the existence of a small electric charge on an object being weighed may pass unnoticed when an aperiodic balance is used and many introduce a considerable error into the weighing and that (*ii*) the charge developed on silica under the conditions described can be dissipated only with difficulty. It is suggested that a charged object can be weighed accurately by screening it, on the balance pan, with a suitable counterpoised metal cover.

Table I shows results of tests with four different "vitreosil" crucibles. Experience of the balance used suggests that the weighings recorded are correct to about $\pm 0{\cdot}2$ mg. The weights of the crucibles when screened (*b*, *d* and *f*) may, therefore, be regarded as constant.

The effect of handling the crucibles is to reduce the apparent error to about one-tenth of its initial magnitude. Even after earthing the crucibles (*c* and *d*) a small but real effect persists.

From the weighings of crucible No. 4 it appears that a positive error is possible, but no other example of this effect has been observed.

SCOTTISH CO-OPERATIVE WHOLESALE SOCIETY LIMITED
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A. W. ARMSTRONG
December, 1950

CONFIRMATORY TEST FOR ETHYL ALCOHOL IN BLOOD AND URINE

FOR the quantitative estimation of alcohol in blood and urine, the method due to Kozelka and Hine[1] has been found satisfactory over a period of years.

For legal purposes, however, it is desirable to confirm the presence of ethyl alcohol by chemical tests. The method devised by Gettler and Seigel[2] is ideal for this purpose in that the alcohol is isolated in the pure state and can be identified by the usual tests and produced in court if necessary.

The quantity of sample required for the foregoing method[2] is at least 100 ml, but as the majority of specimens examined in this laboratory, submitted by the Police under the Road Traffic Act, rarely exceed one fluid ounce, it was necessary to find a means of identification applicable to a small amount of sample.

Attempts were made to oxidise the alcohol to acetic acid for subsequent identification. This was found impracticable owing to the difficulty of obtaining the acetic acid in sufficient concentration to give a reliable test. However, when the alcohol was oxidised to acetaldehyde the aldehyde was easily separated and identified.

Procedure—Transfer 2 to 5 ml of urine or blood by means of a pipette into a 200-ml distillation flask and add 5 ml of *N* sulphuric acid and for blood samples 15 ml of 10 per cent. sodium tungstate solution and 2 drops of a 2 per cent. suspension of DC "Antifoam" A* in warm liquid paraffin. Distil and collect 5 ml of distillate in a 20-ml hard-glass Pyrex tube. Add 2 ml of 2 per cent. potassium dichromate solution to the distillate, followed by the careful addition of 5 ml of concentrated sulphuric acid. Close the tube with a rubber stopper, wrap in a cloth as a protection against breakage, and shake for approximately 1 minute. Cool, transfer to a distillation flask (at this stage the odour of acetaldehyde will be detected if alcohol was originally present), add a few millilitres of water, distil and collect 4 to 5 ml of distillate in a test tube.

Add to the distillate 2 drops of a 5 per cent. aqueous solution of phenol and carefully run 2 ml of concentrated sulphuric acid down the side of the tube. An orange - yellow colour or precipitate at the interface of the two liquids indicates the presence of acetaldehyde. Formaldehyde gives a bright crimson colour under the same conditions (Hehner).

The minimum amount of ethyl alcohol detectable by this method is 6 mg. As most of the specimens examined in this department have an alcohol content of between 120 and 400 mg. of ethyl alcohol per 100 ml, usually only 2 to 5 ml of sample are required for the test.

The method is not specific for ethyl alcohol, as *n*-propyl alcohol and its higher homologues also yield some acetaldehyde on oxidation. But where the quantity of specimen is insufficient for the isolation and subsequent identification of the alcohol, the above test confirms the presence of ethyl alcohol beyond all reasonable doubt, especially in cases of contravention of the Road Traffic Act.

It is of interest to note that with a specimen containing 8 mg of methylated spirits a *reddish* - orange band was produced, showing that the presence of a small quantity of methyl alcohol in ethyl alcohol can easily be detected.

* Obtainable from Albright and Wilson Ltd., London.

The author is indebted to Mr. A. R. Jamieson, City Analyst, for permission to publish this note.

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February, 1951

THE PRESERVATION OF AMINO-ACID PARTITION CHROMATOGRAMS

As suggested by Clegg,[1] the fading of amino-acid paper strip chromatograms developed with ninhydrin[2] can be inhibited by washing the dried papers with ether, drying, dipping them in a suitable preserving varnish, and re-drying.

The varnishes that have been used are—

(1) "Necol" label varnish adhesive 310–4326 (I.C.I.), diluted with an equal volume of ethyl acetate.
(2) Label varnish (B.D.H.) diluted with an equal volume of acetone.
(3) Collodion (transparent strips) as a 5 per cent. solution in acetone.

All three preserve the ninhydrin colours, but (2) and (3) leave a pale yellow wash over the paper. "Necol" varnish, which leaves no extraneous colour, has previously been found suitable for use as a varnish under tropical conditions.

The life of the colours is extended for at least several weeks, and this applies to the various abnormal colours[3] given by certain amino-acids. In particular, the yellow spot from proline loses its tendency to turn purple.

These results suggest that the fading is due mainly to atmospheric oxidation and that a similar technique might prolong the life of paper strip chromatograms of sugars.[4]

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THE BRITISH DRUG HOUSES LTD.
GRAHAM STREET
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H. W. ADAMS
R. G. STUART
February, 1951

Ministry of Food

STATUTORY INSTRUMENTS*

1951—No. 1135. The Feeding Stuffs (Manufacture) (Amendment) Order, 1951. Price 3d.

This Order, which came into operation on July 1st, 1951, amends the Feeding Stuffs (Manufacture) Order, 1950 (S.I., 1950, No. 1988; Analyst, 1951, 76, 119), by prescribing formulae for two compounds known as "National Cattle Food No. 6 (Dairy)" and "National Cattle Food No. 7 (Feeding)."

— **No. 1160. The Cream Order, 1951.** Price 3d.

This Order, which came into operation on July 1st, 1951, reimposes the prohibition on the manufacture and supply of cream except under licence and the obtaining of cream except from the holder of a licence.

It also restricts the serving of clotted cream by catering establishments to a specified area, namely, the counties of Cornwall, Devon, Dorset, Gloucester, Somerset and Wiltshire, and imposes a maximum price for clotted cream, except when supplied as part of a meal by a catering establishment in the specified area.

* Obtainable from H.M. Stationery Office. Italics indicate changed wording.

— **No. 1196. The Food Standards (Edible Gelatine (Order, 1951.** Price 3d.

This Order, which comes into operation

(*a*) as respects sales by the manufacturer of edible gelatine, on the 1st day of August, 1951;

(*b*) as respects sales by wholesale, on the 1st day of November, 1951;

(*c*) as respects sales by retail, on such date as the Minister of Food may by Order appoint

should be read with the Food Standards (General Provisions) Order, 1944, *as amended (S.R. & O.,* 1944, *Nos.* 42 *and* 654; *Analyst,* 1944, **69**, 49 *and* 247), *and prescribes a standard for gelatine for human consumption, as follows—*

STANDARD FOR EDIBLE GELATINE

The standard for edible gelatine shall be as follows:—

Edible gelatine shall be clean wholesome protein which-

(*a*) is obtained by extraction from collagenous material;

(*b*) is free from objectionable taste and offensive odour;

(*c*) dissolves completely in warm water to give a clear or translucent colloidal solution which sets to a jelly when cooled to and maintained at 60° F;

In the case of edible gelatine sold, offered or exposed for sale by retail, a 3 per cent. solution shall set as aforesaid:

Provided that where edible gelatine sold, offered or exposed for sale by retail is clearly and conspicuously described as being of low setting strength on a label (which includes directions for use) marked on or securely attached to the wrapper or container in which it is so sold, offered or exposed for sale, a solution made up in accordance with the directions for use shall set as aforesaid;

(*d*) yields not more than 3·25 per cent. by weight of ash;

(*e*) contains in each million parts by weight not more than two parts by weight of arsenic (expressed as arsenic), seven parts by weight of lead, thirty parts by weight of copper, one hundred parts by weight of zinc.

FOOD STANDARDS COMMITTEE

THE Minister of Food has approved for publication the following Report of the Food Standards Committee's Metallic Contamination Sub-Committee recommending that no exception need be taken to the presence of traces of copper in foods in amounts not greater than those set out below. The Report is as follows—

REPORT ON COPPER

The Food Standards Committee has considered and adopted a Report by its Metallic Contamination Sub-Committee in respect of the limits of copper in foods. The Report is in the following terms—

"1. In pursuance of their terms of reference, the Metallic Contamination Sub-Committee submit a Report on Copper in foods and beverages.

"2. Copper differs from the majority of trace elements in that it is a physiological constituent of plant and animal life. In plants it plays an important part in cell respiration; in the blood of vertebrate animals the formation of haemoglobin cannot take place unless traces of copper are present. On the other hand, copper is an active oxidation catalyst and if present in milk or butter to the extent of about 2 parts per million (p.p.m.) it activates some form of breakdown in the fat thereby imparting a tallowy flavour and impairing the keeping qualities of butter; copper accelerates the destruction of ascorbic acid; copper salts in quite small amounts inhibit the action of pepsin and trypsin; and inorganic copper compounds are highly toxic to unicellular organisms such as algae.

"3. It is not possible to say with any precision what is the amount of copper required daily by the human organism but the information at our disposal suggests that the daily amount required by an adult is from 1 to 2 mg, a quantity which would usually be supplied in a normal diet.

"4. At the other extreme when copper salts are present in sufficient concentration they are strongly astringent and irritating to the stomach. The medicinal dose of copper sulphate other than as an emetic is from 16 to 120 mg. The existence of chronic copper poisoning has not been established. In the light of present knowledge it is not possible to assess the risk to health which may arise from the consumption of foods with an unusually high copper content. The presence of quite harmless amounts of copper can, however, render many foods and beverages unpalatable.

"5. Our primary concern is to protect the consumer against the sale of foods with a copper content greatly in excess of the nutritional needs of the human organism. This hazard is not likely to arise unless food is processed under conditions conducive to copper contamination; verdigris could, for example, be a serious source of contamination. We are satisfied that the consumer will be adequately protected by proposing limits consistent with good commercial practice.

"6. We have received analytical data from the Government Chemist, the British Food Manufacturing Industries Research Association, the Port Medical Officers of Health, the National Association of Cider

Makers, the Institute of Brewing and food manufacturing firms, and after examination of the figures we recommend the classification of foods and beverages and the limits shown below—

(1)	Beverages other than wines, beer and cider	2 p.p.m.
(2)	Wines, beer and cider	7 p.p.m.
(3)	Foods not scheduled below	20 p.p.m.
(4)	Scheduled foods—	
	Chicory, dried or roasted	30 p.p.m.
	Cocoa powder	70 p.p.m. calculated on the fat free substance
	Coffee beans	30 p.p.m.
	Colourings	30 p.p.m. (on dry colouring matter)
	Flavourings	30 p.p.m.
	Edible gelatin	30 p.p.m. (already prescribed)
	Pectin, solid or liquid	30 p.p.m.
	Tea	150 p.p.m. (provisional)
	Tomato ketchup	50 p.p.m. on the dried total solids (already prescribed)
	Tomato purée	100 p.p.m. on the dried tomato solids
	Yeast and yeast extract	30 p.p.m.

"7. A provisional limit of 150 p.p.m. has been recommended for copper in tea in view of reports from the Tea Research Institute for Ceylon on the treatment of a disease known as blister blight, which is seriously affecting tea estates. Control measures involve the use of copper fungicides and at the experimental stage it is impossible to avoid a high degree of contamination. We understand that it may be possible to work to a lower limit when further experience of methods of control has been gained and the limit of 150 p.p.m. might be reviewed after one year. In the meantime we are satisfied that no exception need be taken to the importation of tea containing not more than 150 p.p.m. of copper. Only a fraction of the copper in the leaf is soluble and in view of the proportion which the weight of the infusion bears to the weight of the leaf, the copper content of the infusion would not substantially exceed the limit of 2 p.p.m. which we have recommended for beverages.

"8. Milk is outside our terms of reference, but in our view there is no reason to assume that copper would be present in liquid milk in excess of the limit of 2 p.p.m. proposed for other beverages.

"9. We have not attempted to prescribe limits for composite foods containing one or more of the scheduled foods in addition to foods not scheduled; and recommend instead that where a scheduled food is used in the preparation of any other article of food not being a beverage ready-to-drink, due allowance should be made for the additional amount of copper necessarily introduced by the use of the scheduled food.

"10. In view of the higher natural copper content of certain animal and vegetable products, *e.g.*, shell fish and crustacea, offals, etc., we have not proposed specific limits for these articles; but recommend that the sale of such articles containing copper in excess of 20 p.p.m. should be permitted if it can be shown that such copper is of natural occurrence.

"11. It will be apparent that, from the public health standpoint, copper is in a different category from arsenic and lead and we have felt justified in recommending limits of copper content which ought not to present any problem for the food manufacturer. We do not suggest that these limits should be embodied in a Statutory Order immediately. There is room for further enquiry into the physiological function served by copper. The spoilage of food by copper contamination also calls for further research. Finally, analytical tests for copper in foods have hitherto only been undertaken by a few food manufacturing concerns and there is need for more comprehensive data. For these reasons we suggest that whilst there may be occasion, as in the case of edible gelatin and tomato ketchup, to prescribe limits for a few foods which are particularly liable to copper contamination, the question of imposing comprehensive statutory limits might be reviewed in a year's time in the light of experience."

The members of the Metallic Contamination Sub-Committee are Mr. G. G. Barnes (Chairman), Professor G. R. Cameron, Dr. L. E. Campbell, Professor S. J. Cowell, Dr. J. M. Johnston, Dr. W. P. Kennedy, Dr. G. W. Monier-Williams, Dr. J. R. Nicholls, Dr. G. Roche-Lynch, Mr. G. Taylor and Mr. B. W. Smith (Secretary).

June 30th, 1951

British Standards Institution

DRAFT SPECIFICATIONS

A FEW copies of the following draft specifications, issued for comment only, are available to interested members of the Society, and may be obtained on application to the Secretary, Miss D. V. Wilson, 7–8, Idol Lane, London, E.C.3.

Draft Specifications prepared by Technical Committee LBC/3—Glassware for Pharmaceutical Purposes.
CN(LBC) 2444—Draft B.S. for Dispensing Measures for Pharmaceutical Purposes (Imperial Units).
CN(LBC) 2445—Draft B.S. for Dispensing Measures for Pharmaceutical Purposes (Metric Units).
Draft Specifications prepared by Technical Committee LBC/11—Microchemical Apparatus.
CN(LBC) 2421—Draft B.S. for Halogens and Sulphur Combustion Train (Grote Type).
CN(LBC) 2422—Draft B.S. for Micro-Kjeldahl Apparatus for the Determination of Nitrogen.
Draft Specifications prepared by Technical Committee LBC/1—Volumetric, Mouldblown and Lamp-blown Glassware.
CN(LBC) 2448—Draft B.S. for Glass Condensers.
Draft Specifications prepared by Technical Committee FCC/4—Solvents and Allied Products.
CN(FCC) 3031—Draft B.S. for *iso*-Propyl Acetate.
CN(FCC) 3033—Draft B.S. for 2-Ethylhexyl Alcohol (2-Ethylhexanol).

The Active Principles of Pyrethrum Flowers

Consultative Committee on Insecticide Materials of Vegetable Origin

REPORT OF THE STANDING SUB-COMMITTEE ON METHODS OF ANALYSIS OF VEGETABLE INSECTICIDES ON THE WORLD-WIDE COLLABORATIVE ANALYSIS OF PYRETHRUM FLOWERS, 1948–49*

A REPORT has been published recently by the Colonial Products Advisory Bureau (Plant and Animal) on a scheme of collaborative analyses designed to determine a number of factors concerned with the accuracy of the determination of the active principles of pyrethrum flowers.

Pyrethrum belongs to the genus *Chrysanthemum* of the family Compositae. A few species of the genus possess insecticidal properties and one, *Chrysanthemum cinerariifolium* Vis. (synonym, *Pyrethrum cinerariae folium*) is the most important commercially. The insecticidal properties of pyrethrum are due to two groups of esters, known collectively as "pyrethrins," which are concentrated in the flower heads with by far the greatest amount located in the achenes. In 1924, Staudinger and Ruzicka succeeded in isolating from pyrethrum flowers two principles exhibiting insecticidal activity. They showed these to be esters formed by the combination of a keto-alcohol, pyrethrolone, either with a mono-carboxylic acid or with the mono-methyl ester of a dicarboxylic acid. Both acids were shown to be *cyclo*propane derivatives. These esters were named pyrethrin I and pyrethrin II respectively. Subsequently, Staudinger and Harder proposed two methods for their determination in the flowers. One, which they designated the acid method, is of considerable importance, since it is the foundation on which most of the modern methods are based. Briefly, the acid method consisted in extracting the flowers with petroleum ether, liberating the carboxylic acids from the extract by hydrolysis and estimating the two acids after separating them by steam distillation (pyrethrin I acid being volatile and pyrethrin II acid non-volatile) and then calculating the pyrethrin I and pyrethrin II from the amounts of each acid obtained.

This method was further developed by Seil and has been widely used in this country and in the U.S.A. A variant of it, devised by Ripert, has been used mainly on the Continent. Later, the mercury reduction method was developed by Wilcoxon and by Holaday. This method depends on the fact that the monocarboxylic acid, after hydrolysis of the pyrethrins as in the Seil method, can be separated by extraction with light petroleum and estimated by its reducing action on Denigès reagent; the dicarboxylic acid is estimated as in the Seil method. The mercury reduction method has been adopted as an official A.O.A.C. method.

* Published by the Colonial Products Advisory Bureau (Plant and Animal), Imperial Institute Building, London, S.W.7. Price 4s.

It was soon recognised that exact determination of the pyrethrins was no simple matter on account of the difficulty of securing complete separation from inactive substances contained in the complex extract removed from the flowers by solvent extraction. It was evident that the chemical methods so far evolved yielded results that were indicative only of the relative richness of the samples tested and did not record the absolute values of pyrethrins I and II.

A great deal of practical experimental work was carried out by a number of workers in an effort to prevent, as far as possible, the inclusion of inactive substances as "pyrethrins." In the United Kingdom a study of the analytical methods for pyrethrum flowers had been conducted for a number of years by the Sub-Committee on Methods of Analysis of Vegetable Insecticides under the Imperial Institute Consultative Committee on Insecticide Materials of Vegetable Origin. As the result of their practical investigations, and those of other official bodies and of individual workers, it was considered that the existing methods should be modified in a number of respects. In 1946 it was decided that practical collaboration on an international basis was desirable if any universal agreement was to be reached on pyrethrum analysis.

As the result of the favourable response to an approach made to pyrethrum analysts throughout the world, the Sub-Committee were able to proceed to the practical application of their plans. It was decided that the scope of the investigation should be limited to the analysis of two samples of ground pyrethrum flowers by the Seil, Ripert and mercury reduction methods. The Sub-Committee considered that, if concordance of results was to be obtained, the methods to be used must be drawn up in great detail. To this end they asked Dr. Seil to revise his method with the co-operation of the Chemical Analysis Committee on Insecticides of the National Association of Insecticide and Disinfectant Manufacturers, U.S.A. At the same time, arrangements were made with Monsieur Ripert for the revision of his method by his chief assistant, Mlle. S. Gerhardstein, whilst the Sub-Committee undertook the revision of the mercury reduction method in conjunction with Mr. J. J. T. Graham of the Production and Marketing Administration, United States Department of Agriculture.

It was decided that the three revised methods should be employed for the analysis ot one sample of flowers that had been stored for four years, and another sample of flowers that had recently been harvested. It was also agreed that statistical help would be required in planning the experiment and in carrying out an analysis of the results, so that the maximum amount of information might be derived from the investigation.

By April, 1948, work on the modifications of the three methods had been completed. Details of the methods were despatched, with the two samples, with the request that analytical work should commence on June 15th, 1948, and that the experiments should be carried out in the random order indicated on an enclosure with each pair of samples. In all 42 reports were received. They were submitted to statistical analysis by the Department of Statistics, Rothamsted Experimental Station.

It is clear from the statistical examination of the results that the investigation failed to differentiate decisively between the Seil and mercury reduction methods as regards concordance of results. On the other hand, it showed that the Ripert method yielded less concordance than the other two methods. There is no doubt that the mercury reduction method gave consistently higher results for both pyrethrin I and pyrethrin II than the Seil method. The Ripert method yielded consistently lower values for pyrethrin I than either of the other two methods, while the values for pyrethrin II were too inconsistent for comparison with the results obtained by the other two methods. It was not possible to obtain definite evidence to show that there was any regular correlation between the values obtained by the Seil and mercury reduction methods either for pyrethrin I or pyrethrin II. To obtain this information, it would be necessary to carry out determinations on a greater number of samples.

The most important fact that emerged from the work was the magnitude of the inter-laboratory standard error obtained under the very strict conditions laid down for the test. While the standard error of a determination by any one individual was rather less than 5 per cent. by both the Seil and mercury reduction methods, the standard error for comparison between laboratories was of the order of 10 per cent. for pyrethrin I and pyrethrin II separately, the values being rather smaller for total pyrethrins. Repetition of the analyses by the same workers in the same laboratories did not reduce substantially the differences between laboratories.

The experiment has shown that neither the Seil nor the mercury reduction method is capable of giving the concordance with which they have been generally credited and that, even if the complete absence of sampling error can be assumed, a difference of 0·3 between Seil or mercury

reduction determinations as carried out by two laboratories cannot be regarded as significant for flowers containing between 1·0 and 2·0 per cent. total pyrethrins. The Ripert method was not considered to merit further consideration. There seems no doubt that the present chemical methods leave much to be desired both from the experimental aspect and also as a means of assessing the relative insecticidal effectiveness of pyrethrum flowers, extracts or preparations.

LaForge and his associates have shown that pyrethrolone is not a homogeneous compound, but is a mixture of two keto-alcohols, pyrethrolone and cinerolone. The esters of the latter keto-alcohol have been named cinerins. Recently, several homologues of pyrethrin I and cinerin I have been synthesised. Insecticidal evaluation of these esters has shown that toxicity is mainly dependent on the nature of the unsaturated side-chain of the keto-alcohols and to a lesser extent on the stereo-chemistry of the components. It has become necessary, therefore, to regard "pyrethrin I" and "pyrethrin II" as groups of esters characterised only by their acid component, which in each is esterified with more than one keto-alcohol. This later conception of the active principles of pyrethrum flowers as a mixture of esters that are not all of the same insecticidal value has increased the difficulties of obtaining a satisfactory evaluation of pyrethrum flowers. In order to obtain a true assessment of the insecticidal value of pyrethrum, it would therefore appear necessary to devise a method that will determine each of the biologically-active principles in the pure state by physical, chemical or biological means, or by a combination thereof. A large amount of work has been carried out in these three fields, but there is no evidence to show that a successful working method is yet in sight.

While the search for an absolute method will continue, the question arises whether the present empirical methods can be further improved. In this connection it must be noted that, since the collaborative work was begun, several papers on the analysis of pyrethrum have been published, notably by Mitchell and his co-workers,[1,2,3] giving practical evidence of errors in the present methods and suggesting means for improvement.

With effect from the 1st of April, 1949, the scientific and technical activities of the Imperial Institute were transferred to the Colonial Office, and the Plant and Animal Products Department of the Institute is now known as the Colonial Products Advisory Bureau (Plant and Animal). The Sub-Committee that published this report will continue to function under the new organisation. While there are other problems that the Sub-Committee will have to consider, attempts to improve methods of pyrethrum analysis will be continued. Suggestions by individual workers will be examined and adopted, if they prove acceptable; while at the same time original collaborative work will continue among the members and also, if possible, on a wider basis.

This Report deserves careful study by all those interested in the evaluation of pyrethrum flowers or extracts. The Sub-Committee responsible for it is to be congratulated on the planning of the experimental work and presentation of the results.

REFERENCES

1. Mitchell, W., Tresadern, F. H., and Wood, S. A., *Analyst*, 1948, **73**, 484.
2. Mitchell, W., and Tresadern, F. H., *J. Soc. Chem. Ind.*, 1949, **68**, 221.
3. Campbell, A., and Mitchell, W., *J. Sci. Food Agric.*, 1950, **1**, 137.

H. E. COOMBER
W. MITCHELL

Book Review

THE VITAMIN B COMPLEX. By F. A. ROBINSON, M.Sc., LL.B., F.R.I.C. Pp. xi + 688. London: Chapman and Hall Ltd. 1951. Price 60s.

During the last 20 years, few fields of nutritional research have been more fruitful than the study of the vitamin B complex. This study continues to produce results of great interest to chemists, biologists and clinicians. The members of the vitamin B complex show extreme chemical diversity, but most of them are known to exert a fundamental and highly specific influence on the life of all organisms, ranging from bacteria to men.

In the present volume, Mr. Robinson provides the most comprehensive survey of the subject that has yet appeared. The physical, chemical and biological properties of each factor, as well as methods for its extraction from natural sources, synthesis and estimation, are discussed. Bibliographies at the end of each section cover the literature up to the end of 1949. Altogether over 3300 references are listed.

The absence of references for 1950 is noticeable in the chapters on new factors, such as vitamin B_{12}, and several statements on the properties of this vitamin will need to be modified in the light of recent knowledge. Unfortunately the time taken at present to issue a book of this nature and the current spate of papers on vitamin B_{12} make such lapses unavoidable.

The vitamin B complex has presented the analyst with an exceptionally wide range of problems, some of which (*cf. Analyst*, 1951, **76**, 58) are still without a satisfactory solution. Various biological, microbiological, chemical and physical methods have been proposed for the estimation of each factor: they are here reviewed critically, but no practical details are given, so that it is necessary to consult the original literature for such information. The extensive references help one to do this.

This volume promises to become a standard reference book and can be recommended for the library of all persons interested in the B group of vitamins. J. E. PAGE

Publications Received

INDUSTRIAL OIL AND FAT PRODUCTS. By ALTON E. BAILEY. Second Edition. Pp. xxiv + 967. New York and London: Interscience Publishers Inc. 1951. Price $15.00; 120s.

MELLOR'S MODERN INORGANIC CHEMISTRY. Revised by G. D. PARKES, M.A., D.Phil. Tenth Edition. Pp. xxi + 967. London: Longmans, Green & Co. Ltd. 1951. Price 25s.

THE CARE AND BREEDING OF LABORATORY ANIMALS. Edited by EDMOND J. FARRIS. Pp. xvi + 515. New York: John Wiley & Sons Inc. London: Chapman & Hall Ltd. 1950. Price $8.00; 64s.

SIX-MEMBERED HETEROCYCLIC NITROGEN COMPOUNDS WITH FOUR CONDENSED RINGS. By C. F. H. ALLEN and five collaborators. Pp. xiii + 345. New York and London: Interscience Publishers Inc. 1951. Price $10.00; 80s. Subscription price: $9.00; 72s.

One of a series of monographs entitled "The Chemistry of Heterocyclic Compounds." Consulting Editor: Arnold Weissberger.

A SHORT GUIDE TO CHEMICAL LITERATURE. By G. MALCOLM DYSON, M.A., D.Sc., Ph.D., F.R.I.C., M.I.Chem.E. Pp. vii + 144. London: Longmans, Green & Co. Ltd. 1951. Price 8s. 6d.

AN ADVANCED TREATISE ON PHYSICAL CHEMISTRY. Volume II. THE PROPERTIES OF LIQUIDS. By J. R. PARTINGTON, M.B.E., D.Sc. Pp. xliv + 448. London: Longmans, Green & Co. Ltd. 1951. Price 50s.

DIE CHEMISCHE AFFINITÄT. EINE EINFÜHRUNG IN DIE LEHRE VON DER TRIEBKRAFT CHEMISCHER REACTIONEN. By EGON WIBERG, Dr.-Ing. Pp. xii + 254. Berlin: Walter de Gruyter & Co. 1951. Price DM 24.

MICROCHEMISTRY GROUP

A JOINT Meeting of the Group with the Liverpool and North-Western Section of the Royal Institute of Chemistry will be held at 7 p.m. on Thursday, October 18th, 1951, in the Chemistry Lecture Theatre of the University of Liverpool.

The following papers will be presented and discussed: "Some of the Principles of Quantitative Microscopical Analysis," by J. G. A. Griffiths, B.A., Ph.D., F.R.I.C.; "Some New and Simple Techniques for the Application of Fluorescence Microscopy," by J. King, O.B.E., F.R.I.C.; "Applications of Polarisation Microscopy in Chemical Practice," by N. H. Hartshorne, M.C., M.Sc., Ph.D., F.R.I.C.

The meeting will be preceded by visits to Messrs. Lever Brothers, Port Sunlight, Ltd., and to Messrs. J. Bibby & Sons, Ltd.

Members of the North of England Section of the Society are cordially invited to participate in both the meeting and the visits.

REPORT OF THE ANALYTICAL METHODS COMMITTEE

THE Report of the Meat Extract Sub-Committee, "Analysis of Meat Extract," reprinted from *The Analyst*, June, 1951, **76**, 329–333, is now available from the Secretary, Miss D. V. Wilson, 7–8, Idol Lane, London, E.C.3; price to members 1s. 6d. and to non-members 2s. 6d.

REPORTS OF THE ANALYTICAL METHODS COMMITTEE OBTAINABLE FROM THE SECRETARY

The Reports of the Analytical Methods Committee listed below may be obtained direct from the Secretary, Society of Public Analysts and Other Analytical Chemists, 7-8, Idol Lane, London, E.C.3 (not through Trade Agents), at the price of 1s. 6d. to members of the Society, and 2s. 6d. to non-members. Remittances must accompany orders and be made payable to "Society of Public Analysts."

Milk Products Sub-Committee:

Report No. 1. Analysis of Condensed Milks (1).
Report No. 2. Analysis of Condensed Milks (2).
Report No. 3. Analysis of Sweetened Condensed Milk in which the Sucrose has altered during Storage. *Out of print.*
Report No. 4. Determination of Water, of Total Solids and of Fat in Dried Milk.

Sub-Committee on Dirt in Milk. Report. Determination of Dirt in Milk.

Report on the Determination of Total Solids in Fresh Liquid Milk.

Essential Oil Sub-Committee:

Report No. 1. Estimation of Cineole in Essential Oils. (1) Cajuput and Eucalyptus Oils.
Report No. 2. Physical Constants (1).
Report No. 3. Physical Constants (2).
Report No. 4. Interim Report on the Determination of Acetylisable Constituents in Essential Oils.
Report No. 5. Determination of Phenols in Essential Oils.
Report No. 6. Determination of Citral in Lemon Oil. *Out of print.*
Report No. 7. Determination of Solubilities.
Report No. 8. Determination of Cineole in Essential Oils. (2) Camphor Oil. (3) Other Oils.
Report No. 9. Determination of Carvone and Menthone.
Report No. 10. Determination of Citronellal.
Report No. 11. Determination of Aldehydes other than Citronellal.
Report No. 12. Determination of Ascaridole.
Report No. 13. Determination of Esters. (Addendum to Report No. 13, Gratis.)
Report No. 14. Solubility Test for Ceylon Citronella Oil. (Gratis.)

Metallic Impurities in Foodstuffs Sub-Committee (formerly **Sub-Committee on the Determination of Arsenic, Lead, etc. in Food Colouring Materials**):

Report No. 4. Determination of Zinc.

Sub-Committee on the Determination of Unsaponifiable Matter in Oils and Fats and of Unsaponified Fat in Soaps:

Report No. 1. Determination of Unsaponifiable matter in Oils and Fats.
Report No. 2. Determination of Unsaponified Fat in Soap. *Out of print.*
Report No. 3. Determination of Free Alkali in Soaps.
Report No. 4. Determination of Free Alkali and Silica in Silicated Soaps.
Report No. 5. Determination of Rosin in Soaps.
Report No. 6. Determination of Phenols in Soaps.

Poisons Sub-Committee appointed to investigate Methods of Assay for Various Substances appearing in the Poisons Schedules of the Poisons Regulations, 1935:

Report No. 1. Assay of Lobelia (*Lobelia Inflata*).
Report No. 2. Assay of Gelsemium.
Report No. 3. Assay of Aconite.
Report No. 4. Assay of Yohimba.
Report No. 5. Assay of Jaborandi.
Report No. 6. Assay of Ephedra and of Ephidrine in Nasal Sprays.

Fluorine in Foods Sub-Committee:

Report on the Determination of Fluorine in Foods.
Addendum to above Report. (Gratis.)

Sub-Committee on Vitamin Estimations. Microbiological Panel:

Report on the Microbiological Assay of Riboflavine and Nicotinic Acid.

Sub-Committee on Vitamin Estimations. Carotene Panel:

The Determination of Carotene in Green-Leaf Material. Part 1. Fresh Grass.

Sub-Committee on Vitamin Estimations. Aneurine Panel:

The Chemical Assay of Aneurine in Foodstuffs.

Tragacanth Sub-Committee:

Report No. 1. Evaluation of Powdered Tragacanth.
Report No. 2. Evaluation of Flake Tragacanth.

Soapless Detergents Sub-Committee:

Examination of Detergent Preparations.

JOSEPH LUCAS LIMITED require Engineers to fill the following vacancies in the factory which they propose to open in Liverpool for the manufacture of Fuel Injection equipment.

METALLURGICAL ASSISTANTS and experience in at least one of the following:—

1. Production Heat Treatment Checking.
2. Physical Testing of Raw Materials.
3. Pyrometry and Radiography.

CHEMICAL LABORATORY ASSISTANTS and experience in one or more of the following:—

1. Process Control (Plating, Blacking, etc.).
2. Analysis of Raw Materials.
3. Analysis of Plating Solutions.

Selected applicants will be expected to spend a short period at the Company's Laboratories in Birmingham prior to taking up their positions in Liverpool. The Company will pay the expenses in connection with such visits.

Application, giving full details of previous experience, should be made in writing to the Personnel Manager, Joseph Lucas (Gas Turbine Equipment) Limited, Shaftmoor Lane, Hall Green, Birmingham, 28.

DUNLOP have two vacancies for assistant chemists at the Research Centre, Birmingham. Applicants should be aged 20—25, with a degree in chemistry. One post is concerned with the development of new analytical methods in the field of rubber and plastics, the other with microchemical analysis. Previous analytical experience, though desirable, is not essential. Salary according to age, qualifications and experience. Applications in writing, quoting ref. A.S 100, To: Personnel Manager, Dunlop Rubber Co. Ltd., Fort Dunlop, Erdington, Birmingham. 24.

ROCHE PRODUCTS LIMITED have an opening for assistants in their Analytical Department, age about twenty-five years, of B.Sc. or A.R.I.C. standard, who are trained or wish to do chemical analysis. Write stating qualifications, experience and salary required to the Secretary, Roche Products Limited, Welwyn Garden City, Herts.

THE Research and Development Dept. of the Distillers Co. Ltd. have a vacancy for a chemist to work on biochemical analysis connected with antibiotic fermentation research. Age under 28. Applicants must possess an Honours Degree in Chemistry or equivalent, and experience in biochemical analysis is essential. The vacancy affords good opportunities for any chemist wishing to enter the antibiotic research field, with good prospects of advancement. Salary will depend on qualifications and experience. Non-contributory pension scheme. Apply: Staff Manager, 21, St. James's Square, London, S.W.1.

HIS MAJESTY'S COLONIAL SERVICE
FEDERATION OF MALAYA

A VACANCY exists for a RESEARCH OFFICER (Forest Chemist) in the Forestry Department, Federation of Malaya. The duties of the post cover all aspects of wood chemistry with special reference to the manufacture of paper pulp and fibre-board from mixed tropical hardwoods. The post is pensionable after three years' probation. The salary scale, including pensionable expatriation allowance, is $500 per month to $1,180 per month (£700 per annum to £1,652 per annum, current sterling equivalent at one Malayan dollar to 2/4d.) plus a variable cost of living allowance subject to the following maxima: for a single officer, $240 per month (£336 per annum); for a married officer without children, $430 per month (£602 per annum); for a married officer with children, $505 per month (£707 per annum).

Government quarters, if available, are at a nominal rent and free first-class passages on first appointment and on leave are provided for the officer, his wife and children under 10 years, not exceeding four persons besides himself. A tour of service of three or four years earns approximately five months' paid leave in the United Kingdom. Income tax is at local rates, which are very much lower than those in the United Kingdom. Candidates should preferably be under 30 and must possess an Honours degree in Chemistry and Associate membership of the Royal Institute of Chemistry. Preference will be given to candidates who have done post-graduate research in wood-chemistry or who have some experience in wood-pulp and fibre-board manufacture and some knowledge of forest products research in general. The selected candidate will be required to pass examinations in Malay Standard I, General Orders and Identification of Timbers in due course.

Write giving brief details of age, qualifications and experience to the Director of Recruitment (Colonial Service), Sanctuary Buildings, Great Smith Street, London, S.W.1, quoting reference No. 27106/45.

COLONIAL PRODUCTS ADVISORY BUREAU (PLANT AND ANIMAL): SENIOR EXPERIMENTAL OFFICER. The Civil Service Commissioners invite applications for two permanent appointments as Senior Experimental Officer. Candidates must have been born on or before 1st August, 1916, and must have obtained the Higher School Certificate with Mathematics or a science subject as a principal subject, or an equivalent qualification. They must also have had considerable technical experience of the raw material resources of the Colonial territories which are of plant or animal origin.

Inclusive London salary scales:

MEN	WOMEN
£780–£1,000	£655–£900

Rates for posts outside London are somewhat lower.

Starting salary will be determined on an assessment of the successful candidates' qualifications and experience.

Further particulars and application forms from Civil Service Commission, Scientific Branch, Trinidad House, Old Burlington Street, London, W.1, quoting No. S.4067/51. Completed application forms must be returned by 4th October, 1951.

EXPERIENCED Senior ANALYTICAL CHEMIST with degree required for research laboratory in Northern Rhodesia specialising on non-ferrous metals. Excellent opportunity for advancement in chemical phases of research. Basic salary £1,050 per annum plus fluctuating cost of living allowance amounting now to about £7 per month. Life Assurance contributory Pension Scheme. In addition Cash Bonus equivalent at present rate to about 50 per cent. on basic salary. Reply by airmail to P.O. Box 172, Kitwe, Northern Rhodesia.

PROCESS CONTROL CHEMISTS required for rapidly expanding Chemical Works in East Anglia. The posts vacant are for men, not necessarily qualified, but of at least Inter. B.Sc. standard, with experience in Heavy Chemical and Allied Industry, and having a preference for plant control and management. Age preferably 25–35. Excellent prospects. Salary £450–£600 per annum according to qualifications and experience. Write (quoting Ad. 516), stating age and full particulars of experience to Box No. 3781, THE ANALYST, 47, Gresham Street, London, E.C.2.

SHIFT ANALYSTS required for routine process analysis. Some experience desirable. Five-day week. Lankro Chemicals Ltd., Eccles, Manchester.

LOVIBOND TINTOMETER. Type: White Light Cabinet, complete with all colour slides, etc. Nearly new condition, with full directions for use. Bargain, £50 or near offer. Can be seen at 19, Manor Way, Purley, Surrey, or 'phone Uplands 0887 for particulars.

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