THE ANALYST

Editorial

MORE and more original information is being offered for publication each year in all branches of science. Analytical chemistry is no exception. A publishing society such as ours is faced with steadily increasing printer's bills both because of this growth and because of the slow but inexorable increase in printing costs. The Society for Analytical Chemistry is unwilling to curtail its services by failing to publish papers that should appear in *The Analyst*, and is anxious that the coverage afforded by *Analytical Abstracts* should keep pace with the ever-increasing growth of scientific literature throughout the world.

The price of the joint subscription to *The Analyst* and *Analytical Abstracts* has been held at 8 guineas for the past 7 years, but this can continue no longer if the Society is to maintain the quality of its original publications and of its abstracting service, whose pre-eminence in the analytical field is recognised throughout the English-speaking world. The Council has reluctantly concluded that an increase of price is now inevitable, and the joint subscription to the Society's journals for 1965 will be raised to £10. Proportionate increases will be made in the subscription prices of *Analytical Abstracts* alone and to the prices of *Abstracts* printed on one side of the paper; details of these increases are given in the advertisement pages. Annual subscriptions for members of the Society for Analytical Chemistry are also being raised correspondingly.

The Absorptiometric Determination of Iron in Boiler Feed-water

Part III.* Method for Determining the Total Iron Content†

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A method has been developed for determining the total iron content of boiler feed-water. The sample is heated with thioglycollic acid to convert "non-reactive" iron to a "reactive" form that is then determined absorptiometrically by a modification of the method described in Part I of this Paper. The criterion of detection was approximately 0·7 μg of iron per litre for 200-ml samples, and the standard deviation of results varied from approximately 0·4 to 0·6 μg of iron per litre in the range 0 to 50 μg of iron per litre; the calibration curve was linear within this range. No appreciable interference was caused by other impurities likely to be present in feed-water, and a batch of 12 samples can be analysed in 3½ hours, of which approximately 2 hours is operator time.

BOILER feed-waters may contain insoluble oxides and hydroxides of iron that must be dissolved before they can be determined absorptiometrically. The efficiency of several different methods of dissolution has been investigated,[1] and it was concluded that heating the sample with thioglycollic acid was the most suitable technique. Measurement of the optical density of the iron^{II} - thioglycollic complex[2] is inadequately sensitive for our needs, but preliminary tests showed that the more sensitive method in which bathophenanthroline is used[3] could be applied in the presence of thioglycollic acid. This Part of the Paper describes the tests made of a method that uses thioglycollic acid to convert "non-reactive"‡ iron to a "reactive" form, and bathophenanthroline to determine the "reactive" iron.

EXPERIMENTAL

REAGENTS, APPARATUS AND TECHNIQUE—

Reagents—All chemicals were of analytical-reagent grade whenever possible, and all reagents were prepared as described under "Method," p. 446, except where stated otherwise. Doubly distilled water (the second distillation in an all-glass apparatus) was used throughout, and sufficient for each batch of experiments was collected in one polythene bottle to ensure homogeneity.

In preliminary tests, the thioglycollic acid was used as received, and the bathophenanthroline, acetate buffer and hydroxylammonium chloride solution were prepared as described in Part I.[3] However, under these conditions reagent blank determinations were rather unreproducible (standard deviation approximately 0·01 to 0·02 optical-density units). These variations were caused mainly by iron in the reagent solutions. Purification and preparation of these reagents, as described under "Method," allowed reagent blank solutions to be analysed with a standard deviation of approximately 0·004 optical-density units. Although hydroxylammonium chloride is not used in the method finally evolved, this reagent was used in some of the initial tests. It was purified essentially as described in Part I, except that 5 ml of thioglycollic acid were added to the solution, which was then heated at about 90° C for 1 hour

* For details of earlier parts of this series, see reference list, p. 452

† The efficiency of the method for determining all conceivable forms of iron has not been determined. Results in Part II of this Paper indicated that the method is satisfactory for determining oxides and hydroxides of iron; such compounds are thought to account for essentially all the "non-reactive" iron.

‡ In this series of Papers, "non-reactive" iron is defined (see Part II) as those forms of iron that are not determined by the method described in Part I.

before the acid was neutralised with ammonia and the iron was extracted. It is of interest that although the bathophenanthroline, buffer and hydroxylammonium solutions (prepared as in Part I) were quite satisfactory for determining "reactive" iron, all three solutions contained "non-reactive" iron that became "reactive" when thioglycollic acid was added. The method used for purifying the thioglycollic acid is essentially that described by Feigl and Caldas.[4]

Apparatus—The apparatus was as specified under "Method"; it was cleaned as described. Preliminary tests indicated that polythene bottles could be adequately cleaned more easily and rapidly than Pyrex-glass bottles; the former were accordingly used for all further work.

Technique—All measurements of optical density were made by using a Hilger & Watts Uvispek spectrophotometer with 4-cm glass cuvettes; the reference cuvette was filled with re-distilled isopentanol. During the work described in this Part of the Paper, the temperature of the laboratory varied between 18° and 23° C. Whenever appropriate, the analysis of a batch of samples was carried out in random order. All other experimental conditions and techniques were as described under "Method." Whenever confidence limits are quoted for experimental results, the limits refer to a 95 per cent. confidence level.

EFFECT OF THIOGLYCOLLIC ACID ON THE FORMATION AND EXTRACTION OF THE $IRON^{II}$ - BATHOPHENANTHROLINE COMPLEX—

The method for converting "non-reactive" to "reactive" forms of iron was chosen on the basis of the work in Part II,[1] and is described under "Method." Hydrochloric acid and the *m*-cresol purple solution (see Part I) were not used in this work, since samples for analysis contained a fixed amount of thioglycollic acid, which was neutralised to the desired pH by adding an acetate buffer containing a suitable amount of ammonia solution. As thioglycollic acid would be present during the formation, extraction and measurement of the $iron^{II}$ - bathophenanthroline complex, several tests were made to determine whether this acid caused any undesirable effects. Tests showed that the shape of the absorption spectrum of the $iron^{II}$ - bathophenanthroline complex was not affected by thioglycollic acid.

Effect of pH—Portions (200 ml) of solutions containing different concentrations of $iron^{III}$ were placed in separating funnels, and, in sequence, 2 ml of thioglycollic acid, 2 ml of 10 per cent. hydroxylammonium chloride solution, and 10 ml of an acetate buffer were added to each funnel. Each solution was then analysed exactly as described in Part I, beginning with the addition of bathophenanthroline. Different buffers (with identical concentrations of acetate) were used to give different pH values in the aqueous phase. For comparison, further solutions were analysed in the absence of thioglycollic acid. Duplicate determinations were made under each condition tested, and the mean results are given in Table I, which shows that the thioglycollic acid did not affect the pH at which the $iron^{II}$ - bathophenanthroline complex was extracted. In all further tests, an acetate buffer giving a final pH of 4·0 was used (see "Method").

TABLE I

EFFECT OF pH ON THE EXTRACTION OF THE $IRON^{II}$ - BATHOPHENANTHROLINE COMPLEX

Thioglycollic acid added, ml	Concentration of iron, μg per litre	Optical density at—		
		pH 3·5	pH 4·0	pH 4·5
2·0	0	0·045	0·046	0·041
2·0	50	0·687	0·692	0·686
0	0	—	0·030	—
0	50	—	0·669	—

Effect of amount of bathophenanthroline—Portions (200 ml) of solutions were analysed as in the previous section, but different volumes of the bathophenanthroline reagent were added. The volume of isopentanol added subsequently was adjusted so that the total volume added to all samples was identical. Duplicate determinations were made under each condition tested, and the mean results are given in Table II, which shows that the presence

of thioglycollic acid did not make the volume of the bathophenanthroline reagent more critical. Four millilitres of this reagent were used in all further work.

TABLE II

EFFECT OF THE VOLUME OF THE BATHOPHENANTHROLINE REAGENT

Concentration of iron, μg per litre	Optical density with— 2 ml of reagent	3 ml of reagent	4 ml of reagent
0	0·043	0·044	0·045
50	0·682	0·684	0·684

Effect of the duration of the preliminary equilibration with bathophenanthroline—Portions (200 ml) of solutions containing 0 or 50 μg of iron per litre were analysed as before, except that the duration of the preliminary equilibration with the bathophenanthroline reagent was varied. Triplicate determinations were made under each condition tested, and the mean optical densities due to 50 μg of iron per litre (after correction for the appropriate blank value) were 0·633 and 0·638 for shaking times of 0·5 and 1 minute, respectively. These results show that thioglycollic acid did not cause this time to become critical, and a period of 1 minute was used in all further work.

Effect of the duration of the final solvent-extraction—The effect of time of shaking on the extraction of the iron^{II} - bathophenanthroline complex was tested by using an experimental design identical to that in the previous section. The mean optical densities due to 50 μg of iron per litre were 0·624 and 0·636 for shaking times of 1 and 2 minutes, respectively. These results indicate that thioglycollic acid slightly decreases the rate of extraction of the iron^{II} - bathophenanthroline complex (see Part I), but that the results obtained after 2 minutes correspond to complete extraction. Accordingly, a shaking time of 2 minutes was used in all further work.

Linearity of calibration curve—Portions (200 ml) of solutions containing different concentrations of iron were analysed as above. Triplicate determinations were made at each concentration, and the mean results are given in Table III, which shows that thioglycollic acid did not affect the linearity of the calibration curve, whose slope is in good agreement with that found in the absence of thioglycollic acid (see Part I).

TABLE III

LINEARITY OF CALIBRATION CURVE

Concentration of iron, μg per litre	Optical density	Optical density* per 10 μg of iron per litre
0	0·041	—
2	0·066	$0·125_0$
5	0·105	$0·128_0$
25	0·360	$0·127_7$
50	0·680	$0·127_8$

* Corrected for the blank value.

Effect of hydroxylammonium chloride—Tests showed that, under the conditions used for the tests described above, omission of the hydroxylammonium chloride caused results to be low by about 2·5 per cent. when solutions containing 50 μg of iron per litre were analysed. This effect presumably arose from incomplete reduction of the iron^{III} initially present in the solutions. However, in the procedure described under "Method," any ferric ions present in the sample are in contact with thioglycollic acid for 1 hour at a high temperature; under these conditions it was thought that thioglycollic acid might reduce iron^{III} completely. To check this point, solutions containing 50 μg of iron per litre were analysed as in the tests above, and as described under "Method"; reagent blank determinations were also made for

both of these techniques. The results are given in Table IV, which shows that any difference between these two techniques was extremely small. Accordingly, in all further work hydroxylammonium chloride was not added to samples that had been heated with thioglycollic acid.

TABLE IV

EFFECT OF HEATING SAMPLES WITH THIOGLYCOLLIC ACID

Method of analysis	Mean optical density*— 0 μg of iron per litre	50 μg of iron per litre	Optical density due to 50 μg of iron per litre
As under "Method"	0·044	0·682	0·638 ± 0·002
Samples not heated with thioglycollic acid, and hydroxylammonium chloride added	0·044	0·684	0·640 ± 0·005

* Six reagent blank and twelve sample determinations were made for each method of analysis.

DETERMINATION OF THE CONCENTRATION OF IRON IN THE WATER USED FOR REAGENT BLANK SOLUTIONS—

A method has been described in Part I for determining the "reactive" iron content of the water used for reagent blank solutions. However, this water may contain "non-reactive" iron that is dissolved by thioglycollic acid. Thus, a procedure was required for determining the total concentration of iron that is determined by the method given in this Part of the Paper. The technique used in Part I was in essence to analyse 200- and 400-ml portions of the water, and on this basis the technique described under "Method" was devised. The distribution of bathophenanthroline between the aqueous and organic phases is not sufficiently different for 200- and 400-ml samples to cause errors greater than 0·001 optical-density units. However, it was not known whether the volume of the aqueous phase affected the distribution of thioglycollic acid, nor whether thioglycollic acid affected the extraction of small concentrations of iron from 400-ml samples.

The effect of the volume of the aqueous phase on the distribution of thioglycollic acid was checked by analysing 200- and 400-ml portions of water, to which were added either 1 or 2 ml of thioglycollic acid. For these tests, the heating stage was omitted, and other details were as given under "Method" for determining the iron content of the water used for reagent blank solutions. The mean differences between the optical densities of the final extracts (calculated for a final volume of 25 ml) with 1 and 2 ml of thioglycollic acid were $0{\cdot}002_6$ ($\pm 0{\cdot}002_5$) and $0{\cdot}002_8$ ($\pm 0{\cdot}001_7$) for 200- and 400-ml samples, respectively. These results indicate that the optical density due to 1 ml of thioglycollic acid was the same for 200- and 400-ml samples, and therefore that the distribution of thioglycollic acid was the same. The experimental uncertainties show that even if the distribution were different for 200- and 400-ml samples, the effect would not cause any systematic error greater than 0·004 optical-density units, *i.e.*, approximately 0·3 μg of iron per litre.

The efficiency of extracting small amounts of iron from 400-ml samples was determined by extracting duplicate 400-ml portions of solutions containing 0 and 2 μg of iron per litre; the mean recovery was 2·05 μg of iron per litre.

This method for determining the iron content of the water assumed that the same amount of iron in the water is dissolved when 2 or 4 ml of thioglycollic acid are present. The results in Part II indicate that this is a reasonable assumption when waters containing small concentrations of iron are analysed.

COLLECTION OF SAMPLES—

It was thought possible that prolonged contact of dilute aqueous solutions of thioglycollic acid with polythene might cause iron and other undesirable contaminants to be leached from the polythene. To check this, 1 per cent. solutions of thioglycollic acid in water were set aside in polythene bottles, and analysed after different times. The results showed that the optical densities of the extracts remained constant to within 0·002 optical-density units over a period of 4 weeks. Accordingly, no appreciable contamination effects are expected when samples are analysed within a day or so after collection.

METHOD

REAGENTS—

Thioglycollic acid—Purify general-purpose reagent grade thioglycollic acid by passing it through a column of a strongly acidic cation-exchange resin (Amberlite IR-120 is suitable) in the hydrogen form, to improve the reproducibility of blank values. (A column of 14- to 52-mesh resin, 40 cm long and 0·8 cm in internal diameter, is suitable for treating 1 litre of thioglycollic acid.) Pass the thioglycollic acid down through the column at a rate of 5 ml per minute. Allow an initial volume of effluent (equal to the volume of the resin column plus twice the volume of the space beneath the resin) to flow to waste. Then collect the effluent in the bottle, rinsed with water, that contained the original thioglycollic acid. This reagent was adequately stable for at least 2 months.

(In our work, fresh resin was used for purifying each batch of thioglycollic acid, but it is possible that the same resin may be used several times.)

Ammonia solution, approximately 9 N—Purify analytical-reagent grade ammonia solution by isopiestic distillation to decrease blank values. Place approximately 400 ml of ammonia solution in the base of a 10-inch desiccator, and approximately 400 ml of water in a polythene beaker supported in the desiccator above the ammonia solution. Replace the lid of the desiccator, store it in a cool place, and after about 2 weeks the polythene beaker will contain approximately 9 N ammonia solution. Store this ammonia solution in a polythene bottle previously soaked with analytical-reagent grade ammonia solution.

The concentration of the purified ammonia solution must be accurately determined. Titration with standardised acid is suitable.

Acetic acid—Use re-distilled, analytical-reagent grade acetic acid to decrease reagent blank values.

Ammonia solution - acetic acid buffer—Add 250 ml of re-distilled acetic acid to about 300 ml of water, and to this solution slowly add, with stirring, that volume of approximately 9 N ammonia solution that contains 2·8 equivalents of ammonia. Cool the mixture, and dilute it to 1 litre with water. Store the buffer in a Pyrex-glass bottle. This reagent was adequately stable for at least 2 months.

Isopentanol—Use re-distilled, analytical-reagent grade material.

4,7-*Diphenyl*-1,10-*phenanthroline solution*, 0·1 *per cent. w/v*—This reagent is also called bathophenanthroline. Dissolve 0·50 g of bathophenanthroline in 500 ml of isopentanol, and filter the solution into a Pyrex-glass bottle. (A Millipore HA filter was used in our work, but a Whatman No. 542 filter-paper, although slow, is also satisfactory.) This reagent was adequately stable for at least 2 months.

Ethanol—Industrial methylated spirit, 74° O.P. Filter the spirit through a Whatman No. 542 filter-paper, to reduce blank values. Store the filtered spirit in a Pyrex-glass bottle.

Water—Use water with a low iron content (preferably less than 0·5 μg of iron per litre) for preparing reagents and for reagent blank determinations. Water containing less than 0·5 μg of iron per litre has been consistently obtained by re-distilling distilled water (from a Manesty still) in an all-glass apparatus. Determine the iron content of the water used for reagent blank determinations as described under "Procedure." Shake the container of this water vigorously before withdrawing any of the water from it.

Acetone—Use analytical-reagent grade material.

Standard iron solutions—Prepare these as described in Part I.

APPARATUS—

Once the apparatus has been cleaned, reserve it solely for iron determinations. Detergents should not be used for cleaning apparatus, but unpurified thioglycollic acid is satisfactory for cleaning purposes. Care is also required to ensure minimum contamination of apparatus when it is not in use.

Polythene bottles, 16 fluid-ounce capacity—Wash the bottles well with water, and then fill them with water *plus* 10 ml of thioglycollic acid. Heat the bottles in a water-bath at more than 80° C for at least a day, and then store the bottle and the solution until required. Wash the bottles well with water before use. After use, keep the residual volume of sample in the bottles until they are required again, when they should be well washed with water, and thoroughly drained.

Pyrex-glass bottles—Soak the bottles overnight in a cleaning solution of chromic acid, and then clean them as for the 16-oz polythene bottles with a 2 per cent. v/v aqueous solution of thioglycollic acid.

Pyrex-glass separating funnels, 250-ml and 500-ml capacities—Soak the funnels overnight in a cleaning solution of chromic acid. Wash them with water, fill them with a 2 per cent. v/v aqueous solution of thioglycollic acid, and set them aside for at least a day. When they are required, wash them well with water. When they are used in the analysis of samples, rinse the funnels by shaking them with about 50 ml of water just before they are required. Wash the outside of the stems of the funnels thoroughly with water. Cover the stoppers and necks of the funnels with an inverted beaker whenever possible.

Pyrex-glass calibrated flasks, 25-ml capacity—Clean the flasks in the same way as the Pyrex-glass bottles. After treatment with the thioglycollic acid solution, wash each flask well with water, wash it with a little acetone and dry it in an oven. After use, leave the residual extract in the flask. When the flask is required again, discard the extract, wash the flask well with water, wash it with acetone and dry it.

Pyrex-glass, stoppered graduated cylinders, 25-ml capacity—Clean the cylinders in the same way as the 25-ml calibrated flasks.

PROCEDURE—

Testing cleanliness of apparatus—Some apparatus may give systematically high results. Before use in determinations, it is recommended that reagent blank solutions be analysed in all the apparatus to be used. The precision of the results obtained may then be used to judge whether the apparatus is adequately clean and other sources of contamination are adequately small. Results should also be examined from time to time to see whether any piece of apparatus tends to give systematically high results.

Sample collection—Add 4 ml ($\pm 0{\cdot}1$ ml) of purified thioglycollic acid to a 16-oz polythene bottle from a burette, and replace the cap of the bottle. Great care is required to prevent contamination during sampling. One arrangement is to drill a hole in the cap of the bottle and to insert a short length of plastic tubing that can be attached to the sampling point. Collect 400 ml (± 5 ml) of the sample. It is convenient to make a mark on the outside of the bottle at a level corresponding to a volume of 404 ml; tests have shown that the use of such a mark allows the sample volume to be controlled to within ± 2 ml.

It is important that every care is taken to avoid contamination at all stages (from sampling to final measurement) of the analysis. This is because thioglycollic acid can dissolve iron and its compounds even in the cold, and thus contamination of samples and extracts after the heating stage may lead to error.

Analysis of samples—Remove the cap of the bottle containing the sample, and cover the neck of the bottle (an inverted 20-ml Pyrex-glass beaker is suitable.) Place the bottle in a water-bath so that the temperature of the sample reaches at least 80° C, and then allow it to stand in the water-bath for a further hour. (It is possible that some samples of feed-water may contain materials for which a heating period greater than 1 hour is required (see "Discussion of the Method"); it is therefore desirable to check the effect of heating time for each particular application.) Cool the polythene bottles in running water, and then add a volume of the solution, equivalent to 200 ml of the original sample, to a separating funnel. Make allowance for the small loss by evaporation (approximately 0·5 per cent.) that occurs during the heating period. Care should be taken that small glass chippings from pipettes, stoppers, etc., do not enter the funnels or falsely high results may be obtained. Add 10 ml ($\pm 0{\cdot}5$ ml) of the acetate buffer solution, and swirl the contents of the funnel for a few seconds. From this point the formation, extraction and measurement of the iron^{II} - bathophenanthroline complex are carried out exactly as described in Part I, beginning with the addition of the bathophenanthroline reagent. The optical density of the extracts should be measured within 2 hours of the extraction. Let the measured optical density = A_S.

Reagent blank determinations—Repeat exactly the procedure given above for samples, but use 400-ml of water of low and known iron content in place of the sample. The thioglycollic acid must be from the same batch as that used for the samples. At least one reagent blank solution should be analysed with each batch of sample determinations. Let the measured optical density = A_B.

Determination of iron in the water used for the reagent blank solutions—Carry out a reagent blank determination exactly as described above, but use 2·0 ml of the bathophenanthroline reagent and 23 ml of isopentanol. Measure the optical density and volume of the alcoholic extract exactly as described in the corresponding section of "Procedure" in Part I. Let the optical density $= A_T$, and the volume of the extract $= V_T$.

Repeat this analysis exactly, but initially only add 2·0 ml of thioglycollic acid to the polythene bottle, whose entire contents are transferred to a separating funnel after the heating period. Let the optical density $= A_F$, and the volume $= V_F$.

The optical density, A_C, due to iron in the 200 ml of water used for the reagent blank solution, is given by the expression—

$$A_C = \left(\frac{V_F}{25} \times A_F\right) - \left(\frac{V_T}{25} \times A_T\right)$$

Calculation of results—The optical density, A_A, due to iron in 200 ml of the sample is given by the expression—

$$A_A = A_S - A_B + A_C$$

and the concentration of iron in the sample can then be determined from the calibration curve.

PREPARATION OF CALIBRATION CURVE—

To each of a series of polythene bottles add 4·0 ml of thioglycollic acid. Transfer 400, 395, 390, 380, 370, 360 and 350 ml of water of low iron content to the bottles, and then add 0·00, 5·00, 10·00, 20·00, 30·00, 40·00 and 50·00 ml, respectively, of a standard iron solution containing 0·4 p.p.m. of iron. Treat these solutions exactly as described above for reagent blank solutions, and repeat the determinations until the calibration curve is defined with the desired precision.

Subtract the mean optical density of the reagent blank solution from the mean optical density of each of the other solutions, and plot the corrected optical densities against the concentration of iron added to the solutions. The calibration curve should be linear to at least 50 μg of iron per litre in samples. The effect on sensitivity of using absorptiometers is given in Part I.

RESULTS

PRECISION—

Analysis of standard solutions—On each of 10 days, triplicate analyses were made in random order by the proposed method at concentrations of 0, 5, 25 and 50 μg of iron per litre. These samples were freshly prepared each day from one batch of water as described under "Preparation of Calibration Curve." The same reagent solutions were used throughout, and the analyses were made over a period of 2 weeks. The results were evaluated statistically in exactly the same way as for the corresponding experiment in Part I. The experimental design was chosen to obtain more degrees of freedom for the estimates of within-batch standard deviation, because it was thought that contamination was likely to be the major source of random errors. A summary of all the results is given in Table V.

TABLE V

PRECISION OF DETERMINATIONS OF IRON IN STANDARD SOLUTIONS

Concentration of iron added, μg per litre	Mean optical density— corrected for blank value*	Mean optical density— per 10 μg of iron per litre	Standard deviation, μg of iron per litre— within batches†	Standard deviation, μg of iron per litre— between batches†	Standard deviation, μg of iron per litre— Total
0·0	—	—	0·31	—	—
5·0	$0{\cdot}060_8$	$0{\cdot}121_6$	0·39	N.S.‡	0·40
25·0	$0{\cdot}314_2$	$0{\cdot}125_7$	0·43	N.S.‡	0·43
50·0	$0{\cdot}632_9$	$0{\cdot}126_6$	0·54	N.S.‡	0·59

* The mean optical density for reagent blank solutions was $0{\cdot}028_5$.

† The estimates of the within- and between-batch standard deviations have 20 and 9 degrees of freedom, respectively, except for the blank solution, which has 19 degrees of freedom because one abnormal result (2·5 μg of iron per litre higher than expected) was discarded.

‡ N.S. signifies that the between-batch variations were not significantly (at the level of the 95 per cent. confidence limits) larger than the within-batch variations.

A graph of the mean optical densities (corrected for the blank value) against concentration of iron, in μg per litre, is a straight line, of gradient 82·7, passing through the origin. The mean optical density of the solutions containing 5 μg of iron per litre appears to be slightly low, but the discrepancy is equivalent to only 0·15 μg of iron per litre.

Analysis of feed-waters—The precision attainable when feed-waters were analysed by the proposed method was also checked by analysing portions of samples taken at four different power stations. For this purpose, large volumes of samples were collected into 10-litre polythene bottles containing the appropriate amount of thioglycollic acid to give a final concentration of 1 per cent. by volume. The replicate analyses were made in a few large batches of samples rather than many small batches, since the results in Table V show that within-batch variations were the dominant source of random error. A summary of the results is given in Table VI. One result was rejected from each of power stations A and B because they were abnormally high by 1·6 and 3·0 μg of iron per litre, respectively.

TABLE VI

PRECISION OF DETERMINATION OF IRON IN FEED-WATERS

Power station	Concentration* of iron in samples, μg per litre	Standard deviation,† μg of iron per litre	Degrees of freedom
A	18 to 21	0·38	40
B	5 to 20	0·34	40
C	19	0·32	5
D	5	0·30	12

* A range is quoted when more than one sample was analysed; the standard deviations for different samples were not significantly different.

† This standard deviation refers to within-batch variations of the sample alone, and does not include allowance for the variability of the blank value.

ACCURACY—

Effect of other impurities—The effects of several other impurities were tested at each of two concentrations of iron, *viz.*, 0 and 25 μg per litre. Duplicate analyses were made by the proposed method under each of the conditions tested, and solutions containing only 0 and 25 μg of iron per litre were analysed with each batch of analyses. The results are shown in Table VII.

Efficiency of dissolution of "non-reactive" iron—The results in Part II show that both ferric and ferroso-ferric oxides are efficiently dissolved by the proposed method. However, it is conceivable that other forms of "non-reactive" iron may occur in feed-waters. Of these possible forms, it was considered that complex silicates and spinels of the general formula MFe_2O_4 (where M is a divalent metallic cation) would be most difficult to dissolve. Accordingly, the efficiency of dissolving such materials has been investigated. A standard sample (No. 98) of plastic clay was obtained from the National Bureau of Standards; it is reported to contain 2·05 per cent. of ferric oxide. A compound, approximately corresponding to $ZnFe_2O_4$, was used as an example of a spinel; this material had been prepared by calcining a mixture of zinc and ferric oxides at about 1200° C. The iron content of this spinel was determined by fusion with potassium hydrogen sulphate, and analysis of portions of a solution of the melt by the proposed method, but omitting the heating stage: duplicate analyses gave a mean result of 59·0 per cent. of iron.

Portions of each of these materials were weighed, and transferred to polythene bottles containing 400 ml of water and 4 ml of thioglycollic acid. The experimental technique was the same as for the similar experiments with iron oxides (see Part II). The resulting solutions were then analysed by the proposed method except that small portions of each of the solutions after the heating period were taken for analysis. Four separate determinations were made with the clay, about 20 mg being used for each. Visible amounts of clay remained after the heating period, and only about 17 per cent. of the reported iron content was recovered; further heating for 8 hours increased the recovery to about 21 per cent. The low recovery

is probably not caused by other constituents of the clay, because good recoveries were obtained for iron (as a standard iron solution) added to the solutions after the heating period.

For the spinel, again four determinations were made, about 2·5 mg being used for each. The mean recovery was about 40 per cent., but this increased to 105 per cent. after a further 8 hours' heating.

TABLE VII

EFFECT OF OTHER IMPURITIES

Impurity	Concentration of impurity, μg per litre	Iron recovered,* μg per litre— 0·0 μg per litre added	Iron recovered,* μg per litre— 25·0 μg per litre added
Tin^{II}	100	0·2	25·2
$Zinc^{II}$	1000	1·3	26·1
$Zinc^{II}$	100	0·5	25·6
$Nickel^{II}$	1000	2·0	27·9
$Nickel^{II}$	100	−0·1	25·2
$Copper^{II}$	100	1·9	27·0
$Copper^{II}$	10	0·3	25·7
$Chromium^{III}$	1000	2·8	26·9
$Chromium^{III}$	100	−0·3	25·3
$Aluminium^{III}$	1000	0·2	25·0
$Manganese^{II}$	100		
$Tungsten^{VI}$	100		
$Molybdenum^{VI}$	100		
$Titanium^{IV}$	100	2·4	27·6
$Cobalt^{II}$	100		
Tin^{IV}	100		
$Vanadium^{V}$	100		
$Vanadium^{IV}$	100		
$Sodium^{I}$	10,000		
$Potassium^{I}$	10,000		
Fluoride	1000	−0·2	25·2
Sulphate	20,000		
Nitrate	15,000		
Chloride	20,000	−0·1	25·3
$Calcium^{II}$	10,000		
$Magnesium^{II}$	10,000	0·6	25·8
Silicate	10,000		
Orthophosphate	10,000		
Octadecylamine	1000	0·6	25·0
Morpholine	10,000	0·1	24·6
Cyclohexylamine	10,000	0·2	25·2
Hydrazine	1000	0·3	24·6
Detergents†	1200	0·0	25·0

* The ranges of recoveries expected assuming no interference from impurities were calculated from the results in Table V; these ranges were—

0·0 ± 0·65 (when 0·0 μg of iron per litre was added);
25·0 ± 0·65 (when 25·0 μg of iron per litre were added).

† Omo, Daz, Surf, Dreft, Blue Tide and Quix (200 μg of each per litre) were used.

DISCUSSION OF THE METHOD

SENSITIVITY AND PRECISION—

The results in this Part and in Part I show that thioglycollic acid has no appreciable effect on the sensitivity of the method. Thus, in this Part and Part I, the slopes of the calibration curves were respectively equivalent to 0·128 and 0·126 optical-density units per 10 μg of iron per litre.

The results given in Table V show that random errors arose mainly from within-batch variations, and therefore the following discussion of errors refers only to within-batch errors. The standard deviation quoted for the blank value must be multiplied by $\sqrt{2}$ to make it directly comparable with the other standard deviations. When this is done, the result (0·44 μg of iron per litre) shows that the standard deviation was essentially independent of the concentration of iron in the range 0 to 25 μg per litre, and increased only slightly, though not significantly, at 50 μg per litre. Independent measurements showed that errors arising from the spectrophotometer and the use of calibrated apparatus were negligible. The

constancy of the standard deviation suggests that random contamination from the atmosphere, reagents, and apparatus was the major source of error. The within-batch standard deviations are larger than those obtained when the method described in Part I was used. The method described in this Part is more susceptible to errors from contamination because of the presence of thioglycollic acid. Contamination arising from reagents and the atmosphere is likely to be essentially independent of the volume of sample used for analysis. Thus, the precision could possibly be improved by using greater volumes of samples. However, the precision obtained was adequate for our purposes, and no attempt was made to improve it. During our work, a few abnormally high results have been obtained and were rejected because they were suspected to have arisen from excessive contamination. Such results were obtained with a frequency of about 1 in 70 determinations; the maximum difference between such a result and the expected value was about 3 μg of iron per litre.

The results given in Table VI show that the precision of results for samples of feed-water was similar to that obtained with standard solutions of iron. When allowance is made for the variability of the blank value, the within-batch standard deviations for these feed-waters becomes 0·49 to 0·43 μg of iron per litre.

The criterion of detection (for 95 per cent. confidence limits), defined by Roos[5] as 2·326 times the standard deviation, is thus equivalent to about 0·7 μg of iron per litre.

ACCURACY—

Dissolution of "non-reactive" iron—The method described in this Part was developed in an attempt to produce a convenient technique capable of determining any iron compound present in feed-water. Colloidal and particulate oxides and hydroxides of iron probably constitute the main components of "non-reactive" iron, and the results in Part II indicate that such compounds may be accurately determined. However, low results were obtained when suspensions of clay were analysed. Appreciable concentrations of clay should not be present in the feed-water of high-pressure boilers under normal operating conditions, but certain faults, *e.g.*, leakage in a condenser, may cause greater concentrations to be present. In the context of power stations, the inaccuracy of the method when clays are present is of minor importance, since the presence of "non-reactive" silicon[6] in the feed-water would then be the major operational problem.

The results above also show that the zinc spinel, $ZnFe_2O_4$, dissolved rather slowly, and the dissolution was not complete within the hour recommended in the proposed method. The tests with this spinel represent rather extreme conditions, in that the concentration of iron (about 4000 μg per litre) was much higher than the concentrations normally found in feed-water, and the particle size of the spinel was probably much greater than that likely to be found in feed-water. Further, the spinel had been prepared at a much higher temperature than could occur in feed-water, and this would probably decrease the rate of dissolution of the material. Nevertheless, on the basis of these results, it cannot be claimed that the method will permit all conceivable forms of "non-reactive" iron to be dissolved. Therefore, for each application, it is desirable to check the effect of using heating times longer than that of the proposed method. If this heating time has no effect, it is probably a reasonable assumption that results represent a close approximation to the total iron content, provided that the concentration of clays is inappreciable.

Effect of other impurities—With the exceptions of copper and octadecylamine, the concentrations of the impurities tested were much greater than those expected in feed-waters. On this basis, and assuming that the effect of an impurity is directly proportional to its concentration, the results given in Table VII show that interference effects should be unimportant. Further, octadecylamine caused no statistically significant effect. The effect of copper was much greater than for the method described in Part I. The results indicate that the effect of 10 μg of copper per litre was equivalent to about 0·3 μg of iron per litre. As the concentration of copper in the feed-water of high-pressure power stations is normally expected to be less than 10 μg per litre, the size of this interference is small.

The effect of copper is due to the formation of a brown complex that is extracted into the isopentanol. This complex requires the presence of both bathophenanthroline and thioglycollic acid, and has a well defined absorption spectrum with a peak at about 470 mμ. Unsuccessful attempts were made to decrease the interference by adding various amounts of potassium iodide, thiourea, potassium cyanide, ammonium citrate and EDTA, and by

backwashing the isopentanolic extracts with various concentrations of ammonia solution; the chemical added either had no effect or else it affected the formation or extraction of the iron^{II} - bathophenanthroline complex.

TIME OF ANALYSIS—

The method has been found convenient to use, and 12 determinations may be made in about $3\frac{1}{2}$ hours. Of this time, about $1\frac{1}{2}$ hours is taken up by the dissolution stage, but the analyst's attention is not then required.

This Paper is published by permission of the Central Electricity Generating Board. We thank Messrs. Bailey, Bowler, Settle and Wicks for providing the samples of feed-water.

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NOTE—References 1 and 3 are to Parts II and I of this series, respectively.

An Absorptiometric Method for Determining Ammonia in Boiler Feed-water

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A method has been developed for determining ammonia in boiler feed-water and similar high-purity waters. The method is based on the measurement of the optical density of the blue solution produced when ammonia reacts with sodium phenate and sodium hypochlorite in the presence of acetone. The criterion of detection was about 0·0013 p.p.m. of ammonia; the standard deviation of results varied from about ±0·001 to ±0·005 p.p.m. of ammonia at concentrations of 0·05 and 0·5 p.p.m. of ammonia, respectively. No appreciable interference was caused by other impurities likely to be present in feed-water, and a batch of 10 samples can be analysed in about 90 minutes, of which about 40 minutes is operator time.

The presence of ammonia in condensate and feed-water may affect the corrosion of metals in the pre-boiler system of power stations. Further, ammonia is also produced by the decomposition of compounds used in the chemical treatment of feed-water. The determination of small concentrations (less than 1 p.p.m.) of ammonia is, therefore, often required for plant-control purposes and studies of feed-water chemistry. The Central Electricity Research Laboratories were asked to provide a method that could be used in power stations for determining ammonia in feed-water; a limit of detection of about 0·02 p.p.m. and a coefficient of variation of about 10 per cent. at concentrations greater than 0·1 p.p.m. were desirable.

It was considered desirable to develop a method that did not require a preliminary concentration technique to obtain adequate sensitivity. The literature indicated that only absorptiometric techniques were sufficiently sensitive. Nessler's reagent is often used for determining small concentrations of ammonia, but the spectrophotometric sensitivity[1] of this reagent (0·038 μg of ammonia per sq. cm[2]) was considered inadequate. Non-linearity of the calibration curve at low concentrations of ammonia has also been reported.[3] The indophenol-blue method has adequate sensitivity (about 0·004 μg of ammonia per sq. cm[4,5]), and appeared to be straightforward and convenient. This method was therefore chosen for study.

Berthelot[6] first reported that a blue solution is produced when phenol and hypochlorite react with ammonia in alkaline solution; the blue colour is similar to that of the indophenol dyes. Since his work, many variants of this basic method have been reported.[4,7 to 11]

The sensitivities of three of the most recent methods were compared by analysing solutions of ammonia exactly as recommended by the authors; reagent blank solutions were also analysed at the same time. In all instances the absorption spectra of the solutions containing ammonia showed an absorption peak at 630 mμ; the optical density of reagent blank solutions was essentially constant in the range 600 to 650 mμ. The method of Crowther and Large[7] was about five times more sensitive than that of Datsko and Kaplin,[8] and about 15 per cent. more sensitive than that of Scheurer and Smith.[9] On this basis, the method of Crowther and Large was chosen for further detailed investigation.

Experimental

Reagents, apparatus and technique—

Analytical-reagent grade chemicals were used whenever possible. Some batches of phenol were slightly pink, but this had no significant effect on the precision or sensitivity of the method. The sodium hypochlorite reagents were stored in glass bottles in a dark cupboard. Water was obtained by passing distilled water through a cation-exchange resin (Amberlite IR-120, 16 to 50 mesh) column in the hydrogen form. Sufficient water for each batch of experiments was collected in one polythene bottle to ensure homogeneity.

Development of the colour was carried out in a thermostatically controlled water-bath at 25° C. Optical-density measurements were made by using a Hilger Uvispek spectrophotometer with 4-cm glass cuvettes; water prepared as described above was always used in the reference cuvette. All other experimental conditions and techniques were as given under "Method," p. 460. Whenever appropriate, the order of analysis of a batch of samples was randomised. The temperature of the laboratory varied between 18° and 25° C during the work.

RATE OF FORMATION OF INDOPHENOL BLUE—

Solutions containing different concentrations of ammonia were analysed as under "Method," except that three different temperatures were used during the colour-development stage. The optical densities of the solutions were measured at different times, and the results (see Fig. 1) show that the optical densities were essentially constant after 1 hour. It was

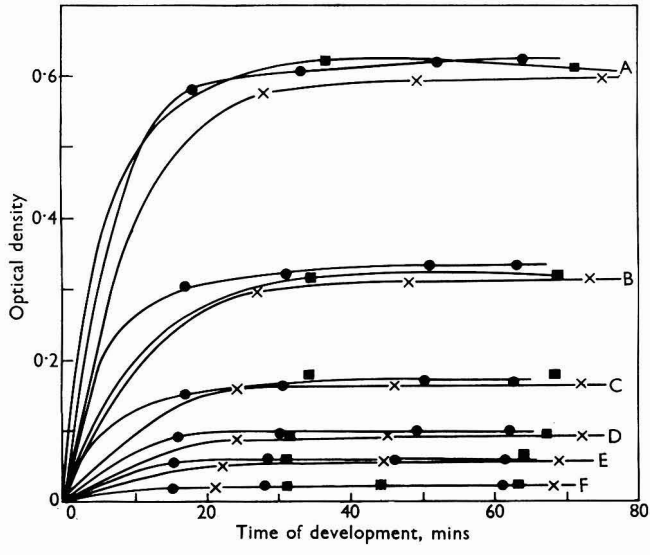

Fig. 1. Rate of formation of indophenol blue: ×, colour development at 14° C; ●, colour development at 20·5° C; ■, colour development at 30° C. Curves A, B, C, D, E and F refer to concentrations of ammonia of 0·8, 0·4, 0·2, 0·1, 0·05 and 0 p.p.m., respectively

concluded that most precise results would be obtained if measurements were made after 60 ± 5 minutes. The results indicate that appreciably shorter colour-development times, *e.g.*, 30 minutes, can be used if more rapid analyses are required; the precision may then be worsened slightly.

STABILITY OF THE SODIUM PHENATE REAGENT—

The sodium phenate reagent slowly darkens on standing, and simultaneously the sensitivity of the reagent decreases. Typical results showing this deterioration for reagents prepared as described under "Method" are given in Table I. Reagents prepared as described by Crowther and Large[7] also deteriorated at about the same rate, and the addition of thiosulphate to the reagent (as used by Datsko and Kaplin[8]) was without beneficial effect. It was decided, therefore, that the sodium phenate reagent should be freshly prepared as described under "Method," just before each batch of analyses. Other tests indicated that the rate of deterioration of the reagent was decreased, but not eliminated, if it was stored in a refrigerator.

EFFECT OF ACETONE—

Preliminary experiments to determine the optimum amount of acetone were made by using 4 ml of sodium phenate reagent, 2 ml of sodium hypochlorite reagent, and the optical densities were measured after 30 minutes; other conditions were as under "Method." The results in Table II show that small amounts of acetone caused a ten-fold increase in sensitivity. Other tests showed that acetone also produced a faster rate of colour development. Further experiments were then made exactly as described under "Method"; the results are given in Table III. For these experiments two different batches of acetone were compared. The results show that the effect of variations in the volume of acetone was slightly different for the two batches, but that the optimum amount of acetone was about 0·3 ml.

TABLE I

DECREASE IN SENSITIVITY OF SODIUM PHENATE REAGENT

Age of sodium phenate reagent	Mean optical density* for 0·5 p.p.m. of ammonia (corrected for reagent blank value)	Optical density of reagent blank solution
Freshly prepared ..	0·352	0·023
4·5 hours	0·343	0·026
1 day	0·335	0·030
5 days	0·328	0·034

* Single reagent blank and triplicate sample determinations were made on each occasion.

TABLE II

EFFECT OF AMOUNT OF ACETONE ON THE FORMATION OF INDOPHENOL BLUE

Acetone added, ml	Optical density—		Mean optical density due to ammonia
	blank solution	0·4 p.p.m. of ammonia	
0·00	0·007	0·035	0·028
0·05	Not done	0·160	—
0·15	0·010	0·267	0·257
0·40	0·012	0·294	0·282
1·25	0·013	0·290	0·277
1·50	0·014	0·290	0·276
3·00	0·016	0·270	0·254
4·00	0·017	0·256	0·239
12·15	Not done	0·145	—

TABLE III

EFFECT OF AMOUNT OF DIFFERENT BATCHES OF ACETONE ON THE FORMATION OF INDOPHENOL BLUE

Volume of acetone added, ml	Mean optical density* for 0·4 p.p.m. of ammonia (corrected for reagent blank value)	
	Acetone 1	Acetone 2
0·15	0·297	0·298
0·25	0·297	0·303
0·35	0·288	0·303
0·45	0·285	0·304

* Single reagent blank and duplicate sample determinations were made under each condition; the optical densities of the blank solutions varied between 0·016 and 0·017.

EFFECT OF THE AMOUNT OF EDTA REAGENT ON THE FORMATION OF INDOPHENOL BLUE—

Preliminary experimental work showed that copper caused low results to be obtained. For example, when solutions containing 0·4 p.p.m. of ammonia and 0·1 p.p.m. of copper (as cupric sulphate) were analysed, the optical densities were only about 50 per cent. of those obtained when copper was absent. As feed-waters contain copper it was necessary to eliminate

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this interference. It was found that the addition of 1 ml of a 6 per cent. w/v EDTA solution prevented this interference by copper. To check that the formation of indophenol blue was not critically dependent on the amount of EDTA, solutions containing 0 and 0·4 p.p.m. of added ammonia were analysed as under "Method," except that different amounts of the EDTA reagent were added. The results in Table IV show that the volume of EDTA reagent added was not critical.

TABLE IV

EFFECT OF AMOUNT OF EDTA ON THE FORMATION OF INDOPHENOL BLUE

Volume of EDTA reagent added, ml	Mean optical density* for 0·4 p.p.m. of ammonia (corrected for reagent blank value)	Optical density of reagent blank solution
0	0·284	0·012
0·5	0·286	0·014
1·0	0·285	0·018
2·0	0·278	0·028

* Single reagent blank and duplicate sample determinations were made under each condition.

PREPARATION AND STABILITY OF SODIUM HYPOCHLORITE REAGENT—

The sodium hypochlorite reagent (see "Method," p. 460) was prepared by diluting, with water, concentrated solutions of sodium hypochlorite containing 10 to 14 per cent. of available chlorine; the latter were standardised iodimetrically before dilution. Experiments showed that the optical density given by 0·4 p.p.m. of ammonia was unaffected as long as the concentrated hypochlorite solution contained at least 8 per cent. of available chlorine (even when that solution was six months old). However, the optical density was reduced by about 4 per cent. when the concentrated hypochlorite solution contained only 5·3 per cent. of available chlorine.

To check the stability of the sodium hypochlorite reagent solution, solutions containing 0 and 0·4 p.p.m. of added ammonia were analysed as under "Method," by using the same reagent when freshly prepared, and again after 4 weeks. The results are given in Table V and indicate that the reagent is adequately stable for at least 4 weeks.

TABLE V

EFFECT OF AGE OF THE SODIUM HYPOCHLORITE REAGENT

Age of reagent, weeks	Optical density—		Mean optical density due to ammonia
	blank solution	0·4 p.p.m. of ammonia	
0	0·029	0·311, 0·318, 0·321	0·288
4	0·024	0·310, 0·310	0·286

TABLE VI

EFFECT OF AMOUNT OF SODIUM PHENATE REAGENT ADDED TO SAMPLES

Volume of reagent added ml	Mean optical density* for 0·4 p.p.m. of ammonia (corrected for reagent blank value)	Optical density of reagent blank solution
8·0	0·277	0·019
10·0	0·292	0·021
12·0	0·295	0·023
15·0	0·301	0·024
18·0	0·313	0·026

* Single reagent blank and triplicate sample determinations were made under each condition.

EFFECT OF THE AMOUNT OF SODIUM PHENATE AND SODIUM HYPOCHLORITE REAGENTS ON THE FORMATION OF INDOPHENOL BLUE—

Solutions containing 0 and 0·4 p.p.m. of added ammonia were analysed as under "Method," except that different amounts of the sodium phenate reagent were used. The results are given in Table VI and show that the sensitivity for determining ammonia increased slowly,

but continuously, as the volume of sodium phenate reagent was increased. However, above 10 ml of reagent the increase was small; greater gains in sensitivity could be obtained, if required, by using greater volumes of sample rather than greater volumes of the phenate reagent. The addition of 10 ml of the reagent is convenient, and this amount was therefore chosen.

Several tests were also made in which both the volumes of the sodium phenate and the sodium hypochlorite reagents were varied simultaneously; all other conditions were as under "Method." Solutions containing 0 and 0·4 p.p.m. of ammonia were analysed under each condition, and the results, see Table VII, indicate that the volumes of these reagents were not critical when 5·0 ml of the sodium hypochlorite reagent and 10·0 ml of the sodium phenate reagent were used.

TABLE VII

EFFECT OF VARIATIONS IN THE VOLUMES OF SODIUM PHENATE AND HYPOCHLORITE REAGENTS ADDED TO SAMPLES

Volume of phenate reagent, ml	Mean optical density* for 0·4 p.p.m. of ammonia (corrected for reagent blank value) Volume of sodium hypochlorite reagent, ml			
	4·0	5·0	6·0	10·0
8·0	0·273 (0·022)	0·272 (0·022)	0·265 (0·022)	0·244 (0·022)
10·0	0·282 (0·023)	0·283 (0·023)	0·280 (0·023)	0·259 (0·023)
12·0	0·287 (0·028)	0·288 (0·028)	0·286 (0·028)	0·271 (0·028)

* Single reagent blank and duplicate sample determinations were made under each condition; the optical densities of the reagent blank solutions are given in brackets.

EFFECT OF LIGHT ON THE FORMATION OF INDOPHENOL BLUE—

Solutions containing 0 and 0·4 p.p.m. of added ammonia were analysed as under "Method," and were then set aside for 1 hour in different light conditions. The results obtained are given in Table VIII.

TABLE VIII

EFFECT OF LIGHT ON THE FORMATION OF INDOPHENOL BLUE

Light conditions during colour-development stage	Mean optical density* for 0·4 p.p.m. of ammonia (corrected for reagent blank value)	Mean optical density* of reagent blank solutions
Dark cupboard	0·300	0·019
Diffuse daylight	0·304	0·019
Fluorescent light	0·299	0·020
Direct sunlight	0·278	0·030

* Triplicate reagent blank and sample determinations were made under each condition.

In direct sunlight the reagent blank solutions were a pale yellow colour instead of colourless as with other light conditions. The solutions containing 0·4 p.p.m. of ammonia were a blue-green instead of a pure blue colour as with the other light conditions. In view of these results, it was considered desirable to allow the formation of indophenol blue to proceed under standard conditions of lighting; the absence of light was chosen because it was easily reproducible.

EFFECT OF TEMPERATURE—

Temperature may affect the analytical results either by affecting the optical density of solutions of indophenol blue or by affecting the amount of indophenol blue formed. The first possibility was investigated by analysing solutions containing 0 and 0·2 p.p.m. of added ammonia as under "Method." Before measurement the solutions were either rapidly cooled or warmed, and their optical densities measured as the solutions warmed or cooled to room temperature; the temperature range 14° to 33° C, was covered. The optical density of the reagent blank solution changed by only 0·001, and the optical density of the solutions with added ammonia increased by about 0·1 per cent. per degree Centigrade.

The effect of temperature on the formation of indophenol blue was investigated by analysing solutions containing 0 and 0·4 p.p.m. of added ammonia as under "Method,"

except that the temperature of the water-bath was varied during the colour-development stage. The results obtained, given in Table IX, indicate the desirability of controlling the temperature to minimise variations between batches of analyses.

TABLE IX

EFFECT OF TEMPERATURE ON THE FORMATION OF INDOPHENOL BLUE

Temperature during colour development, °C	Mean optical density* for 0·4 p.p.m. of ammonia (corrected for reagent blank value)	Optical density of reagent blank solutions
14	0·276	0·017
16	0·278	0·017
18	0·281	0·017
21·5	0·284	0·018
30	0·290	0·020

* Single reagent blank and triplicate sample determinations were made under each condition.

DETERMINATION OF AMMONIA IN THE WATER USED FOR REAGENT BLANK SOLUTIONS—

If the water used for reagent blank solutions contains ammonia, the reagent blank correction will be falsely high, and results for samples falsely low. It is essential, therefore, to be able to determine the concentration of ammonia in this water. Preliminary work showed that if the reagents were mixed, allowed to stand for several minutes, and then added to a sample containing ammonia, no indophenol blue was formed from the ammonia in the sample. Thus, it seemed probable that the ammonia concentration in the water could be estimated from the difference in optical density between two determinations made, firstly, by analysing a 25-ml portion of water in the normal way and, secondly, by adding the reagents to an empty 50-ml calibrated flask, setting the mixture aside for 5 minutes and then adding to it another 25-ml portion of water, and diluting to the mark with water. The difference between the optical densities of these two solutions should be that caused by the ammonia in the 25 ml of water *plus* the volume of water used for making each sample to the mark, *i.e.*, a total of 33·7 ml. Solutions containing 0 and 0·4 p.p.m. of added ammonia were analysed by both procedures to check the accuracy of this method. Further, to check that, in the reversed procedure, ammonia in the reagents reacted to the same extent as in normal determinations, a known amount of ammonia was added to a portion of the EDTA reagent which was then used for reversed blank tests. The results obtained are given in Table X.

TABLE X

EFFECT OF NORMAL AND REVERSE ORDER OF REAGENT ADDITION ON THE DETERMINATION OF AMMONIA

Order of reagents	Mean optical density*: 0·4 p.p.m. of ammonia in water (corrected for reagent blank value)	Mean optical density*: 10 p.p.m. of ammonia in EDTA reagent (corrected for reagent blank value)	Optical density of reagent blank solution
Normal	0·283	—	0·024
Reverse	0·000	0·294	0·023

* Triplicate reagent blank and sample determinations were made under each condition.

The results given in Table X show that ammonia in the water did not react in the reversed procedure. However, the tests in which ammonia was added to the EDTA reagent gave slightly greater results than those obtained for the same amount of ammonia in water by the normal procedure. If the optical density obtained for a given amount of ammonia in the reagents is different for the normal and reversed procedures, a bias in the determination of ammonia in the water will result. However, the above tests had been made with a much larger concentration of ammonia in the EDTA reagent than usual; therefore further tests were made with a more realistic concentration of ammonia in the reagent. The results

obtained (see Table XI) show that a given amount of ammonia produced a slightly greater optical density when present in the EDTA reagent than when present in the water. It can be seen from the results in Table XI that the effect was small (equivalent to a bias of about 0·003 p.p.m. of ammonia), and was not further investigated.

TABLE XI

REACTION OF AMMONIA IN REAGENTS IN REVERSED BLANK PROCEDURE

Order of reagents	Mean optical density*: 0·032 p.p.m. of ammonia in water (corrected for reagent blank value)	Mean optical density*: 0·8 p.p.m. of ammonia in EDTA reagent (corrected for reagent blank value)	Optical density of reagent blank solution
Normal	0·0213	—	0·0240
Reverse	—	0·0240	0·0227

* Triplicate reagent blank and sample determinations were made under each condition.

EFFECT OF TIMING IN ADDING REAGENTS—

It was thought possible that losses of ammonia from samples might occur while the strongly alkaline phenate reagent was being added. However, tests showed that any loss was less than 0·5 per cent. for solutions containing 0·4 p.p.m. of ammonia even when the phenate was added over a period of 1 minute; the normal time taken was only about 6 seconds.

Other tests were also made to determine if the time intervals between the addition of the phenate and hypochlorite reagents, and between the addition of the hypochlorite reagent and dilution to the mark with water had any effect. In both instances, solutions containing 0 and 0·4 p.p.m. of added ammonia were used. It was found that variation of the first time interval between 5 and 30 seconds had no significant effects; similarly, variation between 5 and 60 seconds for the second interval had no significant effects.

STABILITY OF SOLUTIONS OF AMMONIA AND AMMONIUM CHLORIDE—

Dilute solutions of ammonium chloride in water were found to be unstable. For example, after preparation and storage for 1 day in Pyrex-glass bottles, the concentration of ammonia in solutions initially containing 1, 0·2 and 0·02 p.p.m. of ammonia decreased by 0, 3·5 and 45 per cent., respectively. The corresponding figures after 3 days' storage were 3, 8 and 60 per cent. Solutions of ammonium chloride in this concentration range should, therefore, be freshly prepared as required. Solutions of ammonium chloride containing 1000 and 100 p.p.m. of ammonia were found to be stable (to within 1 per cent.) after storage in Pyrex-glass bottles for 20 and 4 weeks, respectively.

The stability of dilute solutions of ammonia was also investigated. For these tests, the solutions were prepared, stored in sealed polythene bottles, and analysed just after preparation and after 1 day's storage. For solutions containing between 0·05 and 1 p.p.m. of ammonia, the concentration of ammonia decreased by about 2 per cent. This indicates the desirability of analysing samples of feed-water as soon after collection as possible.

Finally, the effect of temperature on losses of ammonia from dilute solutions of ammonia was studied. This point is of possible importance in the collection of samples. Losses of less than 1 per cent. were found when solutions containing between 0·2 and 0·4 p.p.m. of ammonia were open to the atmosphere for 15 minutes at 30° C and 5 minutes at 40° C. A loss of about 20 per cent. occurred after exposure to the atmosphere for 5 minutes at 53° C. It appears, therefore, that loss of ammonia should be unimportant provided that the temperature of the sample being collected is no greater than 30° C.

EFFECT OF ALKALINITY OF PHENATE REAGENTS—

Russell[12] showed that the formation of indophenol blue was markedly affected by the pH of the solution, and thus the amount of sodium hydroxide used in preparing the phenate reagent was an important factor. She first recommended that equimolar amounts of phenol and sodium hydroxide be used, although her reagent was not exactly equimolar. Crowther and Large[7] used the amounts of phenol and sodium hydroxide recommended by Russell and these amounts were used by us in developing the method reported here. There appears to have been no detailed investigation of the effects caused by small variations (a few per cent.) in the ratio of phenol to sodium hydroxide. To investigate this point, solutions

containing 0 and 0·05 p.p.m. of ammonia were analysed as under "Method," by using phenate reagents prepared with different amounts of sodium hydroxide. The results are given in Table XII.

TABLE XII

EFFECT OF ALKALINITY OF SODIUM PHENATE REAGENT

Weight of sodium hydroxide used to prepare the phenate reagent, g	Mean optical density* for 0·5 p.p.m. of ammonia (corrected for reagent blank value)	Optical density of reagent blank solution
24·30	0·354	0·020
24·84	0·369	0·022
25·38	0·377	0·020
25·92	0·378	0·022
26·46	0·363	0·023
26·73	0·360	0·023
27·00	0·352	0·023
27·27	0·345	0·023
27·54	0·341	0·023

* Single reagent blank and triplicate sample determinations were made under each condition.

The results given in Table XII show that the amount of sodium hydroxide had a larger effect than was expected; optimum sensitivity was obtained when about 26 g of sodium hydroxide were used. Since the sensitivity was not critically dependent on the amount of sodium hydroxide, and as all the development and testing of the method had been made with 27·0 g of sodium hydroxide, it was decided to continue using this amount.

Other tests showed that the rate of deterioration of the phenate reagent was not appreciably affected by the amount of sodium hydroxide in the range tested above.

METHOD

The experimental investigation showed that many factors may produce small effects, and it is desirable, therefore, to maintain fairly strict control of experimental parameters. To help in this respect, a number of values specified in the method have tolerances placed after them. Provided the experimental parameters are not allowed to exceed the range given by these tolerances, reproducible results should be obtained.

REAGENTS—

Analytical-reagent grade chemicals should be used whenever possible.

Water—It is desirable that water of extremely low ammonia content should be available. Water containing less (usually much less) than 0·03 p.p.m. of ammonia may be consistently obtained by passing distilled water through a column of Amberlite IR-120 cation-exchange resin (16 to 50 mesh, 10 inches long, 1-inch internal diameter), previously converted to the hydrogen form. Store the effluent in a sealed glass aspirator. The resin column should be periodically regenerated with 50 per cent. hydrochloric acid. The concentration of ammonia in the water used for reagent blank solutions should be determined as described under "Procedure," p. 461.

Acetone—The calibration curve should be checked for each batch of acetone that is used.

EDTA solution, 6 per cent. w/v, aqueous—Dissolve 60 g (± 1g) in about 900 ml of water without heating it. Dilute to 1 litre with water, and store the solution in a glass bottle. This solution was adequately stable for 2 months.

Sodium hydroxide, 5 N—Dissolve 200 g (±2·0 g) of sodium hydroxide in water. Cool the solution to room temperature as rapidly as possible, and dilute to the mark with water in a 1-litre calibrated flask. Store the solution in a sealed polythene bottle. Determine the normality of the solution by titrating it with standardised hydrochloric acid. The solution was stable for at least 4 weeks.

Sodium phenate solution—Weigh 62·5 g (±0·1 g) of phenol in a glass beaker. Add 135·0 ml (±0·5 ml) of 5 N sodium hydroxide (or the equivalent amount of sodium hydroxide if the reagent concentration differs from 5 N), and when all the phenol has dissolved, dilute the solution to the mark with water in a 500-ml calibrated flask, and mix. Smaller volumes of the reagent may be prepared if desired; the tolerances should then be varied proportionally. The sodium phenate should not be allowed to stand in direct sunlight, and should be prepared for each batch of analyses immediately before it is required.

Sodium hypochlorite solution, 1 per cent. w/v available chlorine—Sodium hypochlorite solution (10 to 14 per cent. w/v available chlorine) is suitable for preparing this reagent. The concentration of available chlorine must first be determined; the iodimetric method described by Vogel[13] is suitable.

Dilute the concentrated hypochlorite solution to give a solution containing 1·00 per cent. (±0·05 per cent.) w/v available chlorine. Store the solution in a cool place in a glass bottle, and do not allow direct sunlight to fall on the solution. This solution was stable for at least 4 weeks.

The concentrated hypochlorite solution should not be used when its concentration of available chlorine is 8 per cent. w/v or less; at this stage a fresh solution should be used.

Standard ammonium chloride solution A—Dry ammonium chloride at 100° C. Dissolve 3·141 g of dried ammonium chloride in water in a 1-litre calibrated flask, dilute the solution to the mark with water, and mix. Store the solution in a glass bottle. This solution contains 1000 p.p.m. of ammonia, and was stable for at least 20 weeks.

Standard ammonium chloride solution B—Transfer 50 ml of standard solution A with a pipette to a 500-ml calibrated flask, dilute to the mark with water, and mix. This solution contains 100 p.p.m. of ammonia, and was stable for at least 4 weeks. Store the solution in a glass bottle.

Standard ammonium chloride solution C—Transfer 5·00 ml of standard solution B with a pipette to a 1-litre calibrated flask, dilute it to the mark with water, and mix. Store the solution in the calibrated flask or in a glass bottle. This solution contains 0·5 p.p.m. of ammonia, and must be prepared freshly each day as required.

APPARATUS—

Cleaning 50-ml calibrated flasks with chromic acid may cause low results, and this acid should not be used. Adequate cleaning may be obtained by filling the flasks with 5 N sodium hydroxide, setting them aside overnight, and then washing them well with water of low ammonia content. A reagent blank solution should be analysed in each flask before it is used for actual analyses.

PROCEDURE—

Sample collection—The temperature of the water leaving the sampling device should be less than 30° C. Polythene bottles are convenient sample containers; they should be completely filled with sample, and stoppered. Portions of the sample should be analysed as soon after sampling as possible.

Analysis of samples—Place 25 ml of the sample in a 50-ml calibrated flask, and add 1·0 ml (±0·1 ml) of EDTA solution. Stopper the flask, and mix by gently swirling the contents. Add 0·3 ml (±0·05 ml) of acetone, stopper the flask, and mix by swirling. Add 10 ml (±0·2 ml) of the sodium phenate solution by using a fast-running pipette, mix, and immediately add 5 ml (±0·2 ml) of the sodium hypochlorite solution again by using a fast-running pipette, and mix. Immediately dilute the solution to the mark with water, stopper the flask, and mix well by inversion. Place the flask in a water-bath at 25° C; the water-bath should be covered so that the inside is dark. After 60 minutes (±5 minutes) from the time of diluting to the mark, measure the optical density of the solution at 630 mμ in 4-cm cuvettes; the reference cuvette should be filled with water. Wash the flask with water after use, fill it with water, and set it aside until it is required again.

Reagent blank determinations—A reagent blank solution should be analysed with each batch of determinations. For this, place 25 ml of water of known ammonia content (see below) in a 50-ml calibrated flask, and proceed as described above, under "Analysis of Samples."

Subtract the optical density of the reagent blank solution from that of the sample, and by using this corrected result and the calibration curve, determine the apparent concentration of ammonia in the sample, C_A. The true concentration of ammonia in the sample, C_T, is given by $C_T = C_A + C_W$, where C_W is the concentration of ammonia in the water used for the reagent blank solutions.

For samples containing more than 0·5 p.p.m. of ammonia, the volume of sample used for analysis may be reduced appropriately.

Determination of ammonia in water used for reagent blank solutions—Place 1 ml (±0·1 ml) of EDTA solution in a 50-ml calibrated flask and then add 0·3 ml (±0·05 ml) of acetone. Add 10 ml (±0·2 ml) of the sodium phenate solution, mix them, and immediately add 5 ml (±0·2 ml) of the hypochlorite solution, and mix. Stopper the flask, and set it aside in darkness for 5 minutes. Add 25 ml of the water used for reagent blank solutions, and dilute to the mark with water. Stopper the flask, mix the contents well by inversion, and place the flask in the water-bath. Measure the optical density of the solution 60 minutes (±5 minutes) after the addition of the reagents. Subtract the optical density obtained by this determination from that of a reagent blank solution analysed at the same time. From this corrected result and the calibration curve, determine the apparent concentration of ammonia in the water, C_{AW}. The true concentration of ammonia in the water, C_W, is given by the equation—

$$C_W = \frac{25\,C_{AW}}{33{\cdot}7}$$

PREPARATION OF CALIBRATION CURVE—

To a series of 50-ml calibrated flasks, add 0-, 5-, 10-, 15-, 20- and 25-ml portions of the standard ammonium chloride solution C, and then dilute the contents of each flask to 25 ml with water. Analyse the solutions as under "Analysis of Samples." Subtract the optical density of the solution containing no added ammonia from that of the other solutions. Plot a graph of these corrected values *versus* the concentration of ammonia; the solutions correspond to 0, 0·1, 0·2, 0·3, 0·4 and 0·5 p.p.m. of ammonia. These determinations should be repeated until the calibration curve has been defined with the desired precision.

RESULTS

PRECISION AND CALIBRATION CURVE—

On each of 10 days, duplicate analyses were made by using the proposed method at concentrations of 0, 0·05, 0·25 and 0·5 p.p.m. of ammonia. These samples were prepared freshly each day in a similar way to that described under "Preparation of Calibration Curve."

The results of analytical interest are obtained by subtracting the optical density of the reagent blank solution from that of the sample; the precision of the corrected results was calculated by allowing for the variability of both blank solutions and samples. The results were analysed statistically to determine the standard deviations corresponding to variations occurring within and between days. The "between-days" standard deviation was never significantly greater (95 per cent. confidence limits) than the "within-days" standard deviation, and so all the results for each concentration of ammonia were pooled, and the overall standard deviation calculated. A statistical summary of the results is given in Table XIII.

TABLE XIII

SENSITIVITY AND PRECISION OF AMMONIA DETERMINATIONS

Concentration of ammonia, p.p.m.	Mean optical density— corrected for reagent blank value*	Mean optical density— per 0·1 p.p.m. of ammonia	Standard deviation, p.p.m. of ammonia	Degrees of freedom
0	—	—	±0·00054†	10
0·0499	0·0361	0·0724	±0·00088	19
0·2503	0·1807	0·0722	±0·00150	19
0·5000	0·3570	0·0714	±0·00500	19

* The optical densities of the blank solutions were about 0·023.
† This standard deviation is for "within-batch" variations only.

ACCURACY—

Analysis of samples of feed-water—Samples of feed-water from four power stations were analysed within 8 hours of sampling. Three portions of each sample and three portions of each sample to which the equivalent of 0·4 p.p.m. of ammonia had been added were analysed. The reproducibility of the triplicate determinations and the recoveries of ammonia were considered satisfactory, and the summarised results are given in Table XIV.

Interferences—The effect of several other impurities was tested at two concentrations of ammonia, *i.e.*, 0 and 0·5 p.p.m. The analyses were made in duplicate as under "Method," and solutions containing only ammonia were analysed with each batch of analyses. There was no indication that any of the impurities affected the precision of the determinations, and the mean results are given in Table XV.

TABLE XIV

RECOVERY OF AMMONIA ADDED TO DIFFERENT FEED-WATERS

Power station	Sample	Concentration of ammonia,* p.p.m. Sample	Sample *plus* 0·4 p.p.m. of ammonia	Recovery of added ammonia, per cent.
A	Condensate	0·534 (0·025)	0·934 (0)	100·0
	Feed-water	0·584 (0·035)	0·985 (0·018)	100·3
B	Feed-water	0·109 (0·004)	0·515 (0·006)	101·5
C	Condensate	0·237 (0·004)	0·628 (0·014)	97·8
	Feed-water	0·216 (0·010)	0·620 (0·027)	101·0
D	Condensate	0·042 (0·006)	0·464 (0)	105·5

* The ranges between maximum and minimum results for each sample are given in brackets.

TABLE XV

EFFECT OF OTHER IMPURITIES

Samples taken were 25 ml

Impurity	Concentration of impurity, p.p.m.	Ammonia recovered,* p.p.m. 0 p.p.m. added	0·5 p.p.m. added
$Iron^{III}$	0·1	0·000	0·501
$Iron^{III}$	1·0	0·006	0·508
$Iron^{II}$	1·0	0·004	0·506
$Copper^{II}$	0·1	0·000	0·497
$Copper^{II}$	1·0	0·013	0·504
$Copper^{I}$	1·0	0·002	0·505
$Chromium^{III}$	0·1	0·001	0·499
$Chromium^{III}$	1·0	0·007	0·524
$Nickel^{II}$	0·1	0·002	0·501
$Nickel^{II}$	1·0	0·003	0·493
$Aluminium^{III}$	1·0	0·005	0·496
$Manganese^{II}$	0·1		
$Tungsten^{VI}$	0·1		
$Molybdenum^{VI}$	0·1		
$Titanium^{IV}$	0·1	0·003	0·506
Tin^{IV}	0·1		
$Vanadium^{IV}$	0·1		
$Cobalt^{II}$	0·1		
Silicate	1·0		
$Calcium^{II}$	1·0		
$Magnesium^{II}$	1·0		
Nitrate	1·0	0·006	0·506
Sulphate	4·0		
$Zinc^{II}$	0·1		
Tin^{II}	0·1		
$Vanadium^{V}$	0·1	0·000	0·501
Sulphite	10·0	0·003	0·500
Phosphate	10·0	0·001	0·499
Hydrazine	2·0	−0·001	0·499
Morpholine	10·0	0·000	0·508
Cyclohexylamine	10·0	−0·001	0·506
n-Octadecylamine	0·5	0·001	0·501
n-Octadecylamine	2·0	0·010	0·511
Detergents†	10·0	0·004	0·496
Fulvic acids‡	0·1	0·001	0·503
Fulvic acids‡	1·0	0·002	0·507

* The ranges of recoveries expected (95 per cent. confidence limits), assuming no interference from impurities, were calculated from the results in Table XIII, and were—

0·000 ± 0·001 (when 0 p.p.m. of ammonia was added);

0·500 ± 0·010 (when 0·5 p.p.m. of ammonia was added).

† Omo, Daz, Surf, Dreft, Blue Tide and Quix (equal proportions by weight) were used.

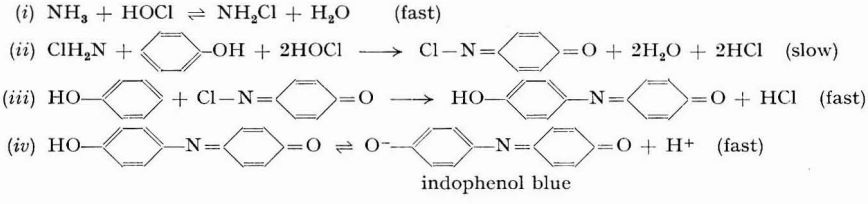
‡ Prepared as described previously.[14]

Discussion

Mechanism of formation of indophenol blue—

Crowther and Large[7] were the first to report the large increase in sensitivity obtained when acetone was added to the solutions, but they did not suggest any explanation for the effect. In view of the small amount of acetone required (0·6 per cent. by volume), it seems unlikely that the effect is caused by changes (*e.g.*, of the dielectric constant) in the reaction medium. The shape of the absorption spectrum of the blue product was unaffected by the presence of acetone, and it seems unlikely, therefore, that acetone causes a different final blue product to be formed. It is concluded that acetone causes greater amounts of indophenol blue to be formed. As acetone also increases the rate of formation of indophenol blue, it is possible that its effect is to increase the concentration of one or more of the reactants in the rate-determining reaction.

No rigorous proof of the mechanism involved in the formation of indophenol blue is known to us. However, Bolleter, Bushman and Tidwell[4] have suggested the reaction scheme below for a method developed by them which did not involve the use of acetone or EDTA.



indophenol blue

For the method developed by us, hypochlorite and phenoxide ions may be involved rather than hypochlorous acid and phenol, but the broad features of this reaction scheme are thought to apply to our conditions. It is apparent that many other reactions are possible, *e.g.*, oxidation of ammonia to nitrogen, chlorination of phenol with simultaneous loss of hypochlorite, and oxidation of phenol. From this reaction scheme and the considerations above on the effect of acetone, it seems possible that the latter hinders or prevents one or more side-reactions that would otherwise decrease the concentration of one or more of the reactants in reaction (*ii*). This interpretation is consistent with our experimental observations that (*a*) for equivalent amounts of nitrogen, *p*-aminophenol gives approximately three times greater optical densities than ammonia, (*b*) that the indophenol blue is formed very rapidly when *p*-aminophenol is used, and (*c*) that acetone has no effect on the sensitivity for *p*-aminophenol. It seems probable that a detailed study of the effect of acetone together with a systematic investigation of the effect of other compounds might allow still greater sensitivity to be achieved in the determination of ammonia.

Whatever the mechanism of formation of indophenol blue, the amount finally formed will, in general, be affected by many factors between which there may be several complex interactions. The present investigation has attempted to find those conditions for which variations in experimental conditions cause smallest changes in analytical results. However, to obtain results precise both within and between batches of analyses, it is desirable to control experimental conditions closely. The tolerances quoted under "Method" indicate the degree of control found to be suitable.

Precision and sensitivity—

As discussed above, many factors affect the results obtained by this method. However, when these factors were controlled within the limits given under "Method," the precision obtained (see Table XIII) was more than adequate for our purpose. The errors quoted in Table XIII were considered as arising from two sources, *i.e.*, variations in the amount of indophenol blue formed from a given amount of ammonia (chemical errors), and errors in measuring the optical densities of solutions (measuring errors). Volumetric errors in preparing solutions with a known concentration of ammonia were shown to be negligible, and errors from contamination must have been extremely small or the standard deviation would have been less dependent on the concentration of ammonia. Measuring errors were estimated from repeated measurement of the same solution; a standard deviation equivalent to approximately 0·0004 p.p.m. of ammonia was found at each of the concentrations given in Table XIII.

From this result and those in Table XIII, it follows that chemical errors were dominant for 0·25 and 0·5 p.p.m. of ammonia; for the reagent blank solution and 0·05 p.p.m. of ammonia, chemical and measuring errors were of approximately equal size.

Table XIII shows that the "within-batch" standard deviation of the reagent blank solution was approximately 0·0005 p.p.m. of ammonia. The criterion of detection (for 95 per cent. confidence limits), defined by Roos[15] as 2·326 times the standard deviation, is thus approximately 0·0013 p.p.m. of ammonia.

If required, it should be possible to improve the sensitivity, but not necessarily the precision, of this technique by acidifying the solution after formation of indophenol blue, and then extracting the red, un-ionised form into an organic solvent. The blue form may then be re-extracted into a small volume of aqueous sodium hydroxide so as to effect a concentration. Methods of this nature, in which sodium hypobromite is used, have already been reported by Chalupa and Vokourová-Gerovová[10] and Datsko and Kaplin.[16] Qualitative tests by us have indicated that such a technique should also be suitable when the indophenol blue is formed as described under "Method."

Calibration curve—

The results given in Table XIII show that the calibration curve was linear (to within 1 per cent.) in the range 0 to 0·5 p.p.m. of ammonia when 25-ml portions of sample were analysed. The equation of the curve was $C = 1{\cdot}389\,D$ where C is the concentration of ammonia in p.p.m. and D is the optical density due to ammonia in the sample. Greater concentrations may be determined simply by using smaller portions of sample. Once the conditions for the method had been finalised, tests over a period of $3\frac{1}{2}$ months showed that the slope of the calibration curve changed by no more than $\pm 1{\cdot}5$ per cent. When measurements were made with an absorptiometer and Ilford No. 607 filters, the calibration curve was linear but the optical densities were about 90 per cent. of those obtained when measurements were made at 630 mμ.

Speed of analysis—

When all the reagents, except the sodium phenate, and apparatus have been prepared, a batch of 10 samples may be analysed in approximately 90 minutes, of which approximately 40 minutes are operator-time.

This Paper is published by permission of the Central Electricity Generating Board. We also thank Messrs. Laing, Lee, Leighton, Matts and Wicks and the late Mr. Stanesby for their help in providing samples of condensate and feed-water.

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Automatic Procedures for the Colorimetric Determination of Phosphorus, Manganese and Silicon in Steel*

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The Technicon AutoAnalyzer has been successfully used for the automatic colorimetric determination of phosphorus, manganese and silicon in carbon steel and most types of alloy steel. Phosphorus and manganese are determined in one sample solution that is obtained by decomposing the sample in acid and removing silicon as metasilicic acid. For the determination of silicon, a second sample is decomposed in dilute acid and insoluble material removed by filtration. Silicon and phosphorus are determined as their molybdenum-blue complexes, and manganese is determined as permanganate. The procedures are largely free from interference, with the exception of niobium in the determination of phosphorus, and titanium in the determination of silicon.

An earlier publication[1] from this laboratory described spectrophotometric procedures for the analysis of steel-making slag with the Technicon AutoAnalyzer. This instrument performs colorimetric analysis automatically, and is particularly suited to the routine determination of 3 or 4 elements in large batches of samples. Recent study has been directed towards the development of automatic methods for determining the more common elements present in steel. This Paper describes the adaptation of established spectrophotometric procedures for determining phosphorus, manganese and silicon to include the use of the Technicon AutoAnalyzer.

DETERMINATION OF PHOSPHORUS

Experimental

The molybdophosphovanadate procedure described by us for determining phosphorus pentoxide in slag[1] proved to be of inadequate sensitivity for use with steel samples.

The reduced-molybdophosphate method was then considered as the alternative approach. This method is accepted as one of the most sensitive means of determining phosphorus, but it is rather unpopular in the United Kingdom, mainly because the conditions for colour development are critical. It is, therefore, more suitable for use with the AutoAnalyzer, in which conditions are rigidly controlled in a flowing system and where the coloured product need not necessarily be stable with respect to time.

A sample solution was prepared by dissolving 1 g of steel in an aqueous mixture of perchloric and nitric acids, "reflux fuming" the solution to convert the phosphorus present to orthophosphoric acid and then diluting the solution to 100 ml. (Reflux fuming is a technique whereby the sample is evaporated with a strong acid in a covered beaker until fumes appear. The solution is maintained at the boiling-point until the fumes are driven out of the beaker and the vapour phase within the beaker suddenly clears.)

It has been suggested that 5 or even 10 minutes' fuming is necessary to ensure complete oxidation of phosphorus, but tests established that a period of about 1 minute is sufficient. Erratic results were obtained if this period was exceeded owing to reduction of the acid concentration of the solution.

For colorimetric measurement, a manifold of plastic tubes and mixing coils was designed to follow the conventional manual technique.[2] The sample solution was first diluted with

* A summary of this paper was presented at the Pittsburgh Analytical Conference, March, 1964.

ammonium molybdate, then mixed with acid to give the correct acid concentration and finally the yellow molybdophosphate was reduced to molybdenum blue with aminonaphthol-sulphonic acid. In a private communication, Dr. J. F. Marten of Technicon Instruments Ltd., suggested that these three reagents could be combined to form a single reagent solution. Tests showed that this rather unconventional approach was most satisfactory, permitting the use of an extremely simple manifold (see Fig. 1).

The sample stream is divided into segments with air, mixed with the reagent solution and then passed through a double mixing coil to ensure thorough mixing. Colour development takes place in a heating-bath, consisting of a 40-foot length of glass tubing in the form of a coil, thermostatically controlled at 95° C in a bath of liquid paraffin.

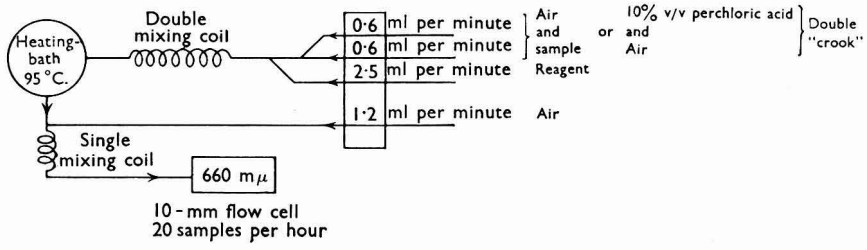

Fig. 1. Diagram of the manifold of the apparatus used for determining phosphorus in steel with 1-amino-2-naphthol-4-sulphonic acid and acidic molybdate solutions

Trials with the manifold indicated that it would be essential to maintain the acid concentration of the system at a constant level. When the normal type sampling "crook" is used for the AutoAnalyzer system, air is aspirated between successive samples. (The sampler-plate rotates, thus moving consecutive sample cups into position under the "crook.") In the flow system, there is thus a reduction in the acid concentration between samples, but this may be overcome by using a double sampling "crook" as shown in Fig. 2.

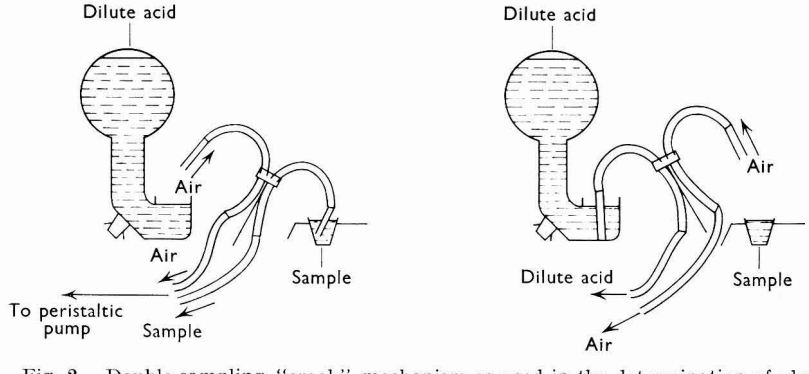

Fig. 2. Double sampling "crook" mechanism as used in the determination of phosphorus and silicon in steel

This system was suited to an operating speed of 20 samples per hour; attempts to increase the operating speed were unsuccessful. It was possible to cover a range of 0·001 to 0·10 per cent. of phosphorus by using a 1-g sample.

Initial tests with a wide range of steels indicated that arsenic, nickel, chromium and niobium would interfere with the procedure. Arsenic is eliminated in the conventional manner by heating the sample solution with hydrobromic acid and volatilising the arsenic as the tribromide before fuming the sample with perchloric acid to form orthophosphoric acid. Nickel and chromium form coloured ions that give a background colour to the molybdenum

blue complex. Allowance for this background colour may be made either by passing a compensation solution through the apparatus, omitting the ammonium molybdate reagent, or by subtracting a calculated optical density, equivalent to that supplied by the presence of nickel and chromium in the sample. In each instance, a deduction is made from the apparent phosphorus concentration to give the correct result. In the latter circumstance, it is necessary to determine the exact effect of nickel and chromium by adding various amounts of these elements to samples of phosphorus-free iron as shown in Table I.

TABLE I

INTERFERENCE EFFECTS OF NICKEL AND CHROMIUM IN THE DETERMINATION OF PHOSPHORUS

Composition of sample	Apparent phosphorus content, per cent.
High-purity iron *plus* 5 per cent. of nickel	0·0006
High-purity iron *plus* 10 per cent. of nickel	0·0015
High-purity iron *plus* 20 per cent. of nickel	0·0030
High-purity iron *plus* 5 per cent. of chromium	0·0030
High-purity iron *plus* 10 per cent. of chromium	0·0054
High-purity iron *plus* 20 per cent. of chromium	0·0140
High-purity iron *plus* 10 per cent. of nickel *plus* 20 per cent. of chromium	0·0136 (calculated 0·0145)
High-purity iron *plus* 20 per cent. of nickel *plus* 10 per cent. of chromium	0·0101 (calculated 0·0095)

This procedure is considered more time saving than passing a compensating solution for each sample through the apparatus. The apparent increase in phosphorus content due to nickel and chromium ions is proportional to the amounts of these elements present in the sample: it is equal to 0·00065 per cent. for each 1 per cent. of chromium present and 0·00015 per cent. for each 1 per cent. of nickel present. The validity of these corrections may be assessed by reference to results obtained for a series of low-alloy and high-alloy steels (see Tables II and III).

TABLE II

RESULTS OBTAINED FOR MILD AND LOW-ALLOY STEELS WITH THE AUTOMATIC PROCEDURES

British Chemical Standard number	Certificate value, per cent.			Mean of 3 results by proposed method, per cent.		
	Phosphorus	Manganese	Silicon	Phosphorus	Manganese	Silicon
291 Carbon steel	0·044	0·36	0·55	0·043	0·38	0·545
292 Carbon steel	0·019	1·46	0·06	0·0185	1·46	0·065
293 Carbon steel	0·067	0·62	0·25	0·065	0·62	0·25
294 Carbon steel	0·004	1·14	0·36	0·002	1·14	0·36
295 Carbon steel	0·036	0·72	0·54	0·035	0·735	0·54
215/1 Carbon steel	0·052	1·01	0·15	0·052	1·01	0·15
239/2 Carbon steel	0·030	0·85	0·27	0·030	0·85	0·275
159/2 Carbon steel	0·017	0·79	0·26	0·016	0·79	0·255
260/2 High-purity iron	0·004	0·013	0·003	0·004	0·01	0·005
149/1 High-purity iron	0·010	0·026	0·002	0·0095	0·02	<0·005
255/1 Low-alloy steel	0·025*	0·16	0·15	0·026	0·16	0·15
257/1 Low-alloy steel	0·020*	0·56	0·10	0·022	0·57	0·10
251 Low-alloy steel	—	—	0·013	—	—	0·015
254 Low-alloy steel	—	0·525	0·295	—	0·53	0·295
256 Low-alloy steel	—	1·21	0·13	—	1·21	0·125
258 Low-alloy steel	—	0·79	0·81	—	0·795	0·83
273 Mild steel + 0·07 per cent. of titanium	—	—	0·22	—	—	0·225
274 Mild steel + 0·09 per cent. of titanium	—	—	0·38	—	—	0·375

* Approximate value.

Niobium interferes seriously with the method, giving high and divergent results. For example, results obtained for British Chemical Standard No. 246 and 261, niobium-stabilised stainless steels containing 0·027 and 0·02 per cent. of phosphorus, respectively, varied from 0·032 to 0·049 per cent. and 0·033 to 0·048 per cent. Guyon, Wallace and Mellon[3] have recently shown that under certain carefully controlled conditions a complex molybdoniobate

may be formed in slightly acidic solutions, and that it can be reduced to form molybdenum blue. This would seem to account for the interference found by us, and, in view of this recent work, it must be concluded that the proposed method will not be suited to steels containing niobium.

TABLE III

RESULTS OBTAINED FOR ALLOY STEELS AND CAST IRONS WITH THE AUTOMATIC PROCEDURES

Sample No.	Certificate values, per cent.			Mean of 3 results by proposed methods, per cent.		
	Phosphorus	Manganese	Silicon	Phosphorus	Manganese	Silicon
B.C.S. 247/2 White cast iron ..	0·93*	—	—	0·95	—	—
B.C.S. 206/1 Cast iron	1·38	—	—	1·37	—	—
B.C.S. 220/1 High-speed steel ..	0·029	0·28	0·24	0·029	0·28	0·25
B.C.S. 241/1 High-speed steel ..	0·021	0·295	0·33	0·020	0·305	0·345
†D 1647 High-speed steel	0·026	—	—	0·026	—	—
†D 1648 High-speed steel	0·026	—	—	0·026	—	—
†D 1217 12 per cent. chromium stainless steel	0·029	—	—	0·027	—	—
B.C.S. 211/1 13 per cent. chromium steel	0·023	0·32	0·275	0·023	0·33	0·27
B.C.S. 261 18 - 12 + niobium steel	—	0·66	0·39	—	0·68	0·395
B.C.S. 235/1 18 - 8 + 4 per cent. of titanium	0·020	0·60	0·60	0·021	0·61	0·64
B.C.S. 233 P.M.A. + 0·8 per cent. titanium	—	0·235	0·57	—	0·215	0·61
B.C.S. 312 P.M.A. + 1·2 per cent. titanium	—	0·20	1·13	—	0·19	1·18
B.C.S. 232/1 Free-cutting steel ..	—	1·22*	—	—	1·225	—
B.C.S. 214/1 Mn - Mo steel ..	0·016	1·48	0·19	0·016	1·48	0·20
B.C.S. 224/1 Cr - V steel	0·022	0·66	0·30	0·022	0·67	0·31
B.C.S. 225/1 Ni - Cr - Mo steel ..	0·019	0·69	0·165	0·020	0·695	0·175
B.C.S. 219/1 Ni - Cr - Mo steel ..	0·028	0·64	—	0·027	0·65	—
B.C.S. 219/2 Ni - Cr - Mo steel ..	—	0·64	0·30	—	0·655	0·305
B.C.S. 213/1 Lead steel	0·027	—	—	0·0265	—	—
B.C.S. 290 13 per cent. manganese steel	0·075	—	0·16	0·075	—	0·165

* Approximate value.
† BISRA samples.

METHOD

REAGENTS—

Standard phosphorus solution—Dissolve 2·1960 g of potassium dihydrogen orthophosphate, KH_2PO_4, in water, and dilute the solution to 1 litre in a calibrated flask.

1 ml of solution ≡ 0·5 mg of phosphorus.

Solvent acid—Add 100 ml of nitric acid, sp.gr. 1·42, to 80 ml of water, cool the solution and add 800 ml of perchloric acid, sp.gr. 1·54, and dilute the mixture to 1 litre.

Acidic molybdate solution—Dissolve 7·8 g of ammonium molybdate in 800 ml of water, add 50 ml of sulphuric acid, sp.gr. 1·84, cool the solution and dilute it to 1 litre.

Solution of 1-amino-2-naphthol-4-sulphonic acid—Dissolve 150 g of sodium hydrogen sulphite, $NaHSO_3$, or 137 g of sodium disulphite (sodium metabisulphite; $Na_2S_2O_5$) and 5·0 g of hydrated sodium sulphite, $Na_2SO_3.7H_2O$, in about 800 ml of water. Heat the solution to 50° C, and then add 2·5 g of 1-amino-2-naphthol-4-sulphonic acid, with stirring, until the solid is almost dissolved. Dilute the mixture to 1250 ml, and filter it. For use, dilute 100 ml of the filtrate to 1 litre. The solution is stable for several weeks, provided that it is kept in a tightly stoppered amber-glass bottle and stored in a dark place.

Mixed reagent for phosphorus—Mix 500 ml of acidic molybdate solution with 500 ml of the sulphonic acid solution. Prepare a fresh solution daily.

PROCEDURE FOR DISSOLVING THE SAMPLE—

Dissolve 1 g of sample in 15 ml of acid solvent mixture. Add a few anti-bumping granules (either fused alumina or glass beads are suitable), then add 10 ml of hydrobromic acid, sp.gr. 1·5, and heat the solution to remove most of the bromine. Evaporate the solution until fumes of perchloric acid appear. Continue reflux fuming (see p. 466) for 1 minute, cool the mixture and dilute it to 100 ml.

ALTERNATIVE PROCEDURE FOR STEELS OF HIGH TUNGSTEN CONTENTS—

Dissolve 1 g of sample in a mixture of 10 ml of nitric acid, sp.gr. 1·42, and 10 ml of hydrochloric acid, sp.gr. 1·16. Evaporate the solution to dryness, but do not bake the residue. Re-dissolve the residue in 10 ml of hydrochloric acid, sp.gr. 1·16, add a few anti-bumping granules and 10 ml of hydrobromic acid, sp.gr. 1·5, and heat the solution to remove most of the bromine. Add 12 ml of perchloric acid, sp.gr. 1·54, evaporate the solution by the reflux-fuming technique and continue the fuming for 1 minute. Cool the mixture and dilute it to 100 ml. Filter the solution through a pad of paper pulp, discarding the first 40 to 50 ml of filtrate.

PROCEDURE FOR DETERMINING PHOSPHORUS—

Assemble the AutoAnalyzer as shown in Fig. 1. Set the sampler-plate at the rate of 20 samples per hour. Prepare a series of calibration solutions by adding varying amounts of standard phosphorus solution to 1-g portions of iron sponge (obtainable from Johnson Matthey & Co. Ltd.), and proceeding as described under "Procedure for Dissolving the Sample." Dilute these solutions to 100 ml in calibrated flasks, and store the solutions in tightly stoppered polythene bottles. Prepare a calibration graph by analysing the prepared calibration solutions in duplicate and deducting the optical density of a calibration blank solution, *i.e.*, a solution of iron sponge without added phosphorus. Having established that a linear calibration may be obtained, analyse three calibration solutions and the calibration blank solution each day to determine the slope of the calibration graph. At least one calibration solution should be measured with each sampler-plate of test samples as a check on calibration drift during the day.

Pass the test samples through the apparatus, deduct the reagent blank value obtained by subjecting a sample of iron sponge to the procedure, and calculate the percentage phosphorus by reference to the calibration graph.

For maximum sensitivity, expand the recorder range "$\times 2$" to give full-scale deflection equal to 0·30 optical-density units. This will cover a range of 0·001 to 0·06 per cent. of phosphorus. For samples containing nickel or chromium, or both, deduct 0·00015 per cent. for each 1 per cent. of nickel present and 0·00065 per cent. for each 1 per cent. of chromium present.

DETERMINATION OF MANGANESE

EXPERIMENTAL

It has been shown previously[1] that manganese can be determined automatically in steel-making slags by oxidising the slag with potassium periodate to form the pink permanganate ion. This method was applied to steel samples dissolved as described earlier for the phosphorus determination.

The sample solution is introduced into the flow system, divided into segments with air and mixed immediately with 25 per cent. v/v orthophosphoric acid. The diluted sample stream is then mixed with a solution of potassium periodate in 25 per cent. v/v orthophosphoric acid and passed through a heating-bath to oxidise the manganese. Optimum wavelengths for the measurement of the manganese colour are 525 and 545 mμ. Two interference filters, near to this range at 505 and 535 mμ, were available to us. The former was preferred, since this gave a linear relationship over a wider range of manganese contents.

Sexavalent chromium ions give rise to a background colour, allowance for which is made either by passing a compensating solution through the apparatus, or by subtracting a calculated optical density, equivalent to the percentage of chromium present in the sample. Owing to the size of the correction, the latter technique may only be used when the chromium content is relatively low. (For each 0·10 per cent. of chromium present, 0·005 per cent.

must be deducted from the apparent manganese content.) It is preferable to pass a compensating solution through the apparatus, omitting the periodate; in this instance, any slight colour due to partial oxidation of manganese during dissolution must be reduced by introducing dilute hydrochloric acid into the flow system (see Fig. 3).

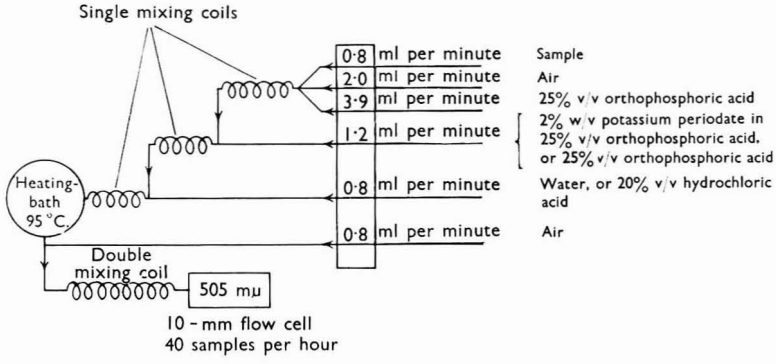

Fig. 3. Diagram of the manifold of the apparatus used for determining manganese in steel

Method

Reagents—

Standard manganese solution—Dissolve 5·752 g of potassium permanganate in water. Carefully add sulphurous acid dropwise until the pink colour has disappeared, and then boil the solution to remove excess of sulphur dioxide. Cool the solution, and dilute it to 1 litre.

1 ml of solution ≡ 2 mg of manganese.

Potassium periodate—Add 250 ml of orthophosphoric acid, sp.gr. 1·75, to 250 ml of water. Add 20 g of potassium periodate, heat the mixture until the solid has dissolved, cool the solution, and dilute it to 1 litre.

Procedure for dissolving the sample—

This is identical with the procedure described under the "Determination of Phosphorus," p. 470. When the determination of both these elements is required, it is carried out on the same sample solution.

Procedure for determining manganese—

Assemble the AutoAnalyzer as shown in Fig. 3. Set the sampler-plate at the rate of 40 samples per hour. Prepare a series of calibration solutions by adding varying amounts of standard manganese solution to 1-g portions of iron sponge and proceeding as described above, under "Procedure for Dissolving the Sample." Dilute these solutions to 100 ml in calibrated flasks and store the solutions in tightly stoppered polythene bottles. Prepare a calibration graph, and pass the test samples through the apparatus exactly as described for phosphorus, p. 470.

For samples containing chromium or other coloured ions, pass the samples through the apparatus for a second time, replacing the potassium periodate solution and water flow-lines in the flow system by solutions of 25 per cent. v/v orthophosphoric acid and 20 per cent. v/v hydrochloric acid, respectively. Deduct the optical density of the background colour to give net optical density due to manganese.

Alternatively, for samples containing small amounts of chromium, deduct 0·005 per cent. of manganese for each 0·1 per cent. of chromium present.

DETERMINATION OF SILICON

Experimental

The British Standard method[4,5] for determining silicon in steel was adapted to the AutoAnalyzer. To prepare a suitable sample solution, 0·5 g of steel was dissolved in an aqueous mixture of hydrochloric and nitric acids to give a solution of approximately 0·8 N

when diluted to 250 ml. A fine suspension of insoluble carbides remained after samples had been decomposed in this mixture, and efforts to dissolve the residue were unsuccessful. The residue was, therefore, removed either by filtration through a close-textured filter-paper or, when tungstic acid was present, through a pad of paper pulp to provide sufficient clear solution to fill a sample cup.

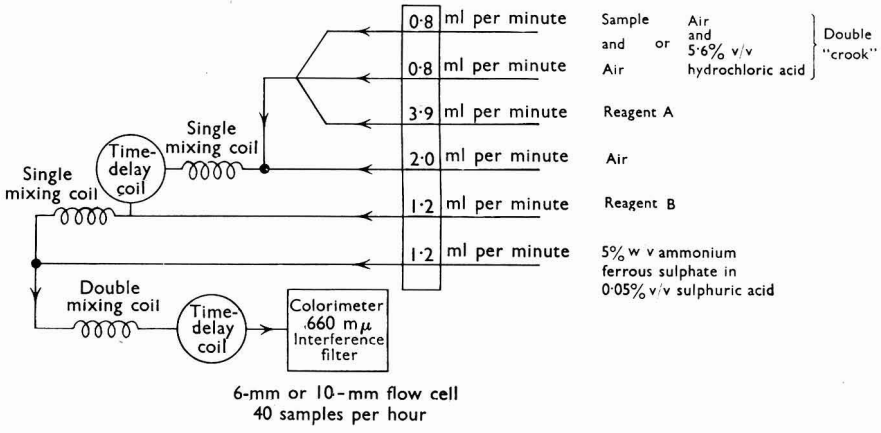

Fig. 4. Diagram of the manifold of the apparatus used for determining silicon in steel. Under usual conditions, reagent A is 0·75 per cent. w/v ammonium molybdate in 0·2 per cent. v/v sulphuric acid, and reagent B is 4 per cent. w/v oxalic acid; for the compensating solution, reagent A is 1·2 per cent. w/v oxalic acid, and reagent B is 2·4 per cent. w/v ammonium molybdate in 0·6 per cent. v/v sulphuric acid

A manifold of plastic tubes and mixing coils was designed to follow the manual technique[4] (see Fig. 4). Time-delay coils were used for providing time lapses of about 7 minutes each for the formation of the yellow molybdosilicate complex with ammonium molybdate and the subsequent reduction to molybdenum blue by ammonium ferrous sulphate. When aqueous ammonium molybdate was used, heavy precipitation of the molybdosilicate complex occurred in the first mixing coil, causing contamination between samples. The complex was kept in solution by adding dilute sulphuric acid to the ammonium molybdate reagent solution, but it was necessary to keep this addition minimal to obtain optimum formation of colour. Stringent tests showed that a reagent strength of 0·75 per cent. w/v ammonium molybdate in 0·2 per cent. v/v sulphuric acid gave well defined optical-density peaks on the chart recorder. Since colour development is sensitive to changes in acid concentration, a double sampling "crook" was used as described earlier under "Determination of Phosphorus," p. 466.

A linear calibration graph was obtained, based on additions of standard silicate solution to high-purity iron when 6-mm cells and 660 mμ interference filters were used with an operating speed of 40 samples per hour.

Allowance for background colour due to elements such as nickel and chromium was made either by passing a compensating solution through the apparatus, in which the order of addition of molybdate and oxalic acid was reversed to prevent colour development (see Fig. 4), or by subtracting a calculated optical density, equivalent to the percentage of coloured ions in the sample. In the latter instance, it was found that each 10 per cent. of nickel present was equivalent to an apparent silicon content of 0·002 per cent., and each 10 per cent. of chromium was equivalent to 0·003 per cent. of silicon.

Persistently high results were obtained for British Chemical Standard No. 235/1, an 18 - 8 stainless steel containing titanium; results ranged from 0·63 to 0·65 per cent., compared with a certified figure of 0·60 per cent. obtained by co-operating analysts, who all used the standard gravimetric method.[5] Other samples of highly alloyed steel were examined, and good agreement was obtained with Certificate values (see Tables II and III). Consideration of the composition of British Chemical Standard No. 235/1 suggested that the titanium present might be responsible for this interference, as it was known that titanate would form

a complex with molybdate under certain conditions. This was not confirmed, however, when solutions of titanium were added to samples of silicon-free high-purity iron and to mild steel of known silicon content; in both instances, there was no apparent interference. Subsequent tests on two Standard samples of magnet alloy containing about 1 per cent. of titanium also gave results in the order of 0·04 per cent. of silicon in excess of the certified gravimetric results.

Method

Reagents—

Acidified molybdate solution—(*a*) For the test solution, dissolve 7·5 g of ammonium molybdate in 800 ml of water. Add 2 ml of sulphuric acid, sp.gr. 1·84, cool the solution and dilute it to 1 litre. (*b*) For the compensating solution, dissolve 2·4 g of ammonium molybdate in 800 ml of water. Add 6 ml of sulphuric acid, sp.gr. 1·84, cool the solution and dilute it to 1 litre.

Oxalic acid solution—(*a*) For the test solution, dissolve 40 g of oxalic acid in 800 ml of water and dilute the solution to 1 litre. (*b*) For the compensating solution, dissolve 12 g of oxalic acid in 800 ml of water and dilute the solution to 1 litre.

Ammonium ferrous sulphate solution—Dissolve 50 g of ammonium ferrous sulphate in 800 ml of water, add 10 ml of 5 per cent. v/v sulphuric acid and dilute the solution to 1 litre.

Solvent acid—Add 200 ml of hydrochloric acid, sp.gr. 1·16, to 500 ml of water. Add 65 ml of nitric acid, sp.gr. 1·42, to the mixture, cool the solution and dilute it to 1 litre.

Primary silicate solution—Fuse 0·856 g of finely ground pure silica that has previously been ignited at 1000° C with 5 g of anhydrous sodium carbonate. Extract the melt with 100 ml of water in a nickel or polytetrafluoroethyelene beaker. Filter the extract through a pad of paper pulp, and wash the residue with 1 per cent. w/v sodium carbonate solution. Collect the filtrate and washings in a 1-litre flask, dilute the solution to the mark and mix it well.

1 ml of solution ≡ 0·4 mg of silicon.

The silicon content of this solution should be checked gravimetrically as a safeguard against extraneous errors during preparation.

Procedure for dissolving the sample—

Dissolve 0·5 g of sample in 70 ml of acid solvent mixture. Do not boil the solution. Cool the solution and dilute it to 250 ml in a calibrated flask. Filter it through a Whatman No. 544 filter-paper into a clean, dry beaker, and discard the first 10 ml of solution (see Note).

Solutions prepared from samples containing tungsten or niobium should be decanted through a pad of paper pulp, and the first 50 ml of filtrate should be discarded.

Procedure for determining silicon—

Assemble the AutoAnalyzer as shown in Fig. 4. Set the sampler-plate at the rate of 40 samples per hour. Prepare a series of calibration solutions by adding varying amounts of standard silicate solution to 0·5-g portions of iron sponge and proceeding as described above, under "Procedure for Dissolving the Sample." As an alternative, use a series of suitable British Chemical Standard steels, together with British Chemical Standard No. 149/1 as reagent blank sample, treated as described above, under "Procedure for Dissolving the Sample." Prepare a calibration graph, and pass the test samples through the apparatus exactly as described under "Procedure for Determining Phosphorus," p. 470.

For silicon contents up to 1·0 per cent., use a 1-cm flow-cell: this range may be extended to 1·6 per cent. by using a 6-mm flow-cell. For low silicon contents, *e.g.*, 0·005 to 0·10 per cent., expand the range of the recorder to "×4" to give a full-scale deflection with an optical-density reading of 0·12. If the compensating-solution method is used for determining the background colour of the sample solution, reverse the order of addition of ammonium molybdate and oxalic acid reagents as shown in Fig. 4. Alternatively, for samples containing nickel or chromium, or both, deduct 0·002 per cent. of silicon for each 10 per cent. of nickel present, and 0·003 per cent. of silicon for each 10 per cent. of chromium present.

Note—

If the samples under test do not normally include high-alloy steels, it is preferable to use the British Standard method[4] for dissolving and subsequently oxidising and reducing the sample. This procedure gives a clear sample solution that need not be filtered. It is, however, necessary to ensure that calibration tests are prepared under the same conditions. A solution of 1·4 per cent. v/v sulphuric acid must be substituted for the dilute hydrochloric acid solution specified in the AutoAnalyzer manifold (see Fig. 4).

Results

The proposed automatic procedures have been used for determining the phosphorus, manganese and silicon contents of many irons and steels; the results are given in Tables II and III.

For phosphorus, the results for carbon, low-alloy and high-alloy steel are within $\pm 0{\cdot}002$ per cent. of the mean certificate values; the standard deviation at the 0·02 per cent. phosphorus level is 0·001. Two samples of cast iron, in which the phosphorus was determined on a reduced sample weight diluted to 1 g with pure iron, also show good agreement with accepted values.

For manganese, the standard deviation is about 0·006 over the normal range of manganese contents present in steel. There is good agreement with manual procedures over the widest range of alloying composition.

With the exception of steels containing added amounts of titanium, the results obtained for silicon are within $\pm 0{\cdot}02$ per cent. of the mean certificate values. Standard deviations vary from 0·002 at the 0·25 per cent. level to 0·006 at the 0·8 per cent. silicon level.

Silicon may be determined in tungsten- and niobium-bearing steels by this method, as shown in Table II, provided that the absorption of silica by the earth-acid precipitate is negligible.

The lower limits of analytical determination for phosphorus, manganese and silicon are 0·001 per cent., 0·005 per cent. and 0·005 per cent., respectively.

Conclusions

The AutoAnalyzer system of automatic colorimetric analysis may be used for determining phosphorus, manganese and silicon in carbon, low-alloy and most types of highly alloyed steel. Sample solutions are prepared manually, but no great skill is required for this operation, provided that reasonable care is exercised in weighing the sample and in diluting it to the required volume. From this stage, analysis is automatic at the rate of either 20 samples per hour for phosphorus or 40 samples per hour for manganese and silicon. Time is saved in the preparation of sample solutions for phosphorus and manganese by determining both elements in the same solution. Optical-density values are recorded in the form of peaks on a moving chart; the operator is only required to record peak heights and to convert these into percentage concentration from a calibration factor. (In a working day, an operator could perform 60 determinations of phosphorus or 120 of silicon. Dove-tailing of operations is possible if two or three elements are to be determined on a routine basis; the operator prepares solutions while the previous batch is being analysed.

In our laboratory, these automatic methods have replaced British Standard manual procedures for the high-quality analysis of a wide range of commercial steels and experimental alloys. Errors due to the so-called "biological variable" are minimised, and the results obtained compare favourably with carefully operated Standard methods. On a routine basis, where accuracy of the highest order is not required, the methods provide a useful supplementary technique to spectrochemical analysis and are of particular value in laboratories with non-vacuum spectrographic instruments, which are unsuitable for phosphorus determinations in steel.

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The Determination of Some Triazine Herbicides by Gas-Liquid Chromatography with Particular Reference to Atraton in Soil

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Triazine herbicides may be chromatographed by using ethylene glycol adipate polyester as the liquid phase. With a Pye Argon chromatograph and a low loading of liquid phase on glass beads, residues of seven triazines have been determined in either crops or soils, or, in some instances, in both. A general extraction procedure for both crops and soils is described, together with a clean-up process applicable to both types of extract.

Full details are given for determining atraton in soil, with sufficient detail for the application of the method to other compounds. A feature of the method is the addition of a second, related triazine to the sample as an internal standard before extraction. This simplifies the procedure, since the final determination involves only the ratio between the amounts of the two compounds present.

Herbicides based on *sym*-triazine have been used in agriculture for several years; their chemical and biological properties have been described by Gysin.[1] They are all derived from 1,3,5-triazine substituted in the 4 and 6 positions with amino functions. (For details of those included in this investigation, see Table I.)

Residues in plants and in soil have been determined by using ultraviolet spectroscopy by Meyer and Delley,[2] and Major[3] has used paper chromatography for atrazine and simazine residues in maize. It has already been shown that a mixture of triazine herbicides can be resolved by gas - liquid chromatography on low-loaded columns of ethylene glycol adipate polyester on glass beads.[4] The application of this technique with a non-specific detector for determining residues in soil or in crops necessitates the development of suitable extraction and clean-up procedures to reduce interferences by compounds from the substrate. Such a procedure has been found by utilising the weakly basic properties of these compounds. For a quantitative determination, the use of an internal standard has certain advantages, and for this purpose another closely related triazine is chosen. The internal standard then has chemical and physical properties similar to the residues to be determined. The advantages of selecting a related compound as the internal standard have been discussed by Burchfield and Storrs.[5] A known amount of the internal standard can be added to the weighed sample before extraction and from then on the determination is based on the ratio of the residue present to the amount of the internal standard. The manipulations and transferences in the clean-up need not be quantitative, which saves a considerable amount of time during the analysis. Small losses in the extraction and clean-up are automatically compensated in the analysis.

The determination of atraton in soil

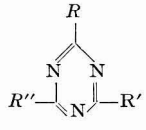
For the determination of atraton residues in soil, the internal standard selected is prometon. This has similar physical and chemical properties and, as will be shown later, responds to the clean-up procedure quantitatively to the same extent as atraton. Its relative retention on the column is 0·83, and the peaks due to prometon and atraton are distinctly separated. Although a small proportion of prometon may occur as an impurity in technical atraton, no interference has been encountered at residue level. Residues of prometon, simeton and atraton could be separately determined in a soil by making separate determinations of any one compound in which the other two were used successively as internal standards.

The dry soil, with a known amount of internal standard added, is extracted with a mixed solvent of methanol and dichloromethane in the presence of ammonia. This solvent can take up sufficient water to maintain a single liquid phase in the extraction. The solvent is removed from the extract and the residue is taken up in 10 ml of light petroleum - dichloromethane mixture (4 + 1) and shaken with 100 ml of N sulphuric acid. The partition coefficients for atraton and prometon are more than 100 to 1 in favour of the acid phase, so that with the volumes taken the loss of triazines in the solvent is less than 0·1 per cent. The acid extract is then made alkaline and the triazines extracted into chloroform. The solvent is removed and the residue is taken up in 0·1 ml of chloroform for the chromatography.

TABLE I

COMPARISON OF TRIAZINE HERBICIDES

General structure



Key to substituents

Me ≡ methyl
Et ≡ ethyl
*i*Pr ≡ isopropyl

Triazine (Common name)	Substituent *R*	Substituent *R'*	Substituent *R''*	Retention time relative to prometryne*	Partition coefficient†
Trietazine	Cl	NHEt	$N(Et)_2$	0·41	7 to 1
Simazine	Cl	NHEt	NHEt	1·14	1 to 5
Atrazine	Cl	NHEt	NH*i*Pr	0·92	1 to 1
Simeton	OMe	NHEt	NHEt	0·76	Not determined
Atraton	OMe	NHEt	NH*i*Pr	0·62	1 to 250
Prometon	OMe	NH*i*Pr	NH*i*Pr	0·50	1 to 100
Desmetryne	SMe	NHMe	NH*i*Pr	1·28	1 to 110
Simetryne	SMe	NHEt	NHEt	1·51	1 to 150
Prometryne	SMe	NH*i*Pr	NH*i*Pr	1·00	1 to 9

* Determined on a column of ethylene glycol adipate polyester.

† Partition coefficient between light petroleum - dichloromethane mixed solvent and N sulphuric acid.

The instrument used was a Pye Argon chromatograph with a 4-foot glass column packed with 0·1 per cent. liquid phase of ethylene glycol adipate polyester on glass beads. The pre-treatment of the glass beads was found to be extremely important for the preparation of columns of high resolving power. The glass beads obtained commercially were sieved and the 120- to 140-mesh size selected. The beads were cleaned by heating them with alkaline potassium permanganate, the excess of permanganate being removed with hydrogen peroxide and a minimum of hydrochloric acid. The beads were washed with water until they were no longer acid and then dried by washing them with ethanol and diethyl ether. Oven-drying the wet beads was found to increase surface adsorption and reduce the efficiency of the final column. The column temperature was controlled at 140° C, and the argon flow-rate at 50 ml per minute; these conditions were found previously to be the optimum for highest column efficiency, atraton and prometon peaks appearing between 20 and 25 minutes after injection. Voltage and sensitivity were adjusted to give maximum response without base-line instability. Two microlitres of the chloroform extract obtained from the soil were injected to obtain the chromatogram.

REAGENTS—

Dichloromethane.
Methanol.
Chloroform.
Light petroleum—Boiling-range 40° to 60° C.
The above solvents were distilled in all-glass apparatus.
Ammonia solution, sp.gr. 0·88.
Sulphuric acid, 1 ± 0·01 N.
Sodium hydroxide solution, 30 *per cent. w/v, aqueous.*

Standard prometon solution—Prepare a solution containing 5 μg of prometon per ml in dichloromethane.

Mixed solvent—Prepare by mixing 4 parts of light petroleum and 1 part of dichloromethane by volume.

The suitability of all reagents should be ascertained by carrying out a reagent blank determination; this should show no interfering peaks in the final chromatogram.

METHOD—

Dry the soil sample at room temperature and grind it to pass a 10-mesh sieve. Any stones should be removed and allowed for in the calculation. Mix the sample thoroughly and weigh 25 g into a 500-ml conical flask fitted with a B24 ground-glass joint. Add 5 ml of standard prometon solution (= 1 p.p.m. added to soil) to the flask and then 40 ml of methanol. Set the flask aside for 30 minutes, with occasional swirling, or overnight. Add 1 ml of ammonia solution and 75 ml of dichloromethane, stopper the flask securely and shake it mechanically for 1 hour. At this point a wet test-paper held above the liquid should show the presence of an excess of ammonia. Filter the mixture through a No. 1 Whatman filter-paper that has been thoroughly washed with chloroform, by using gentle suction on a Buchner funnel. (There is no need to wash the residue at this stage, nor at any filtration or transference stage.)

Transfer the filtrate to a 250-ml round-bottomed flask and evaporate it to 1 to 2 ml in a rotary evaporator at room temperature. Add 10 ml of mixed solvent and 100 ml of N sulphuric acid and shake the flask vigorously for 2 minutes. Pour the mixture into a 250-ml separating funnel and allow the layers to separate for 5 minutes. Run the acid layer into a second funnel and re-extract it with 10 ml of mixed solvent by shaking the funnel for 1 minute and allowing 5 minutes for the separation. Filter the acid through a cotton-wool plug (previously washed with N sulphuric acid), and run the filtrate into a 250-ml separating funnel. Add 100 ml of chloroform and 20 ml of 30 per cent. sodium hydroxide solution and shake the funnel for 1 minute. Allow the layers to separate, and filter the chloroform layer through a chloroform-washed cotton-wool plug. The analysis should not be interrupted during these extractions.

Evaporate the chloroform in a Kuderna - Danish evaporator fitted with a small test-tube as receiver. Finally, evaporate the chloroform just to dryness at 30° C. Dissolve the residue in 0·1 ml of chloroform and chromatograph it. This solution has been found stable for at least 5 days at 0° F (−18° C).

DISCUSSION

The internal standard (prometon) peak can readily be seen. The relative retention of prometon to atraton is known to be 0·83 by previous experiment, and the position of the atraton peak can be calculated. If no peak is present at this position atraton residues are absent in the soil sample. If a peak is found the amounts of prometon and atraton are proportional to peak areas, and the atraton content of the original sample is given by—

$$\text{Atraton residue in soil, p.p.m.} = \frac{\text{Area of atraton peak}}{\text{Area of prometon peak}} \times \text{p.p.m. of prometon added to sample.}$$

With local, medium-clay agricultural soil no interference was seen on the chromatogram from soil constituents (see Fig. 1) and the recovery of atraton added to the soil just before extraction was quantitative. It was also established that complete recovery of both atraton and prometon residues could be obtained after the fortified soil had been kept for 18 hours at room temperature. The method has also been found to give complete recovery with light sand, and medium and heavy clay soils from Malayan rubber estates; the ratio of the peak areas from a mixture of atraton and prometon added to and recovered from the soils was the same as that obtained for the separately injected mixture of the pure compounds.

As in all residue methods, a comparable sample of untreated soil is necessary for quantitative determinations to ensure the absence of interfering peaks on the chromatogram and to check recovery from the soil. Should this not be available, an upper limit may be set for the residue if a peak is found at the position at which atraton would appear, recovery being demonstrated by a proportional increase in peak area when a known amount of atraton is added.

APPLICATION TO OTHER TRIAZINES—

By suitable choice of internal standard, the method can be applied to the determination of other triazines. The requirements are that the standard and residue peaks should be separated, but fairly close to each other, on the chromatogram, and that the internal standard should be recovered as efficiently as the residue in the clean-up. In this instance the clean-up requires that the residue and standard should both be partitioned into normal acid from the mixed solvent. The relative retention and the approximate partition coefficients for several triazines are given in Table I. It should be noted that because of the volumes used in the clean-up, the partition is ten times more favourable to the acid phase than shown in the

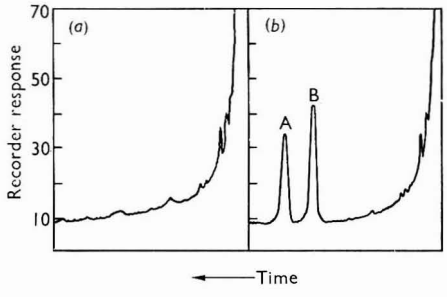


Fig. 1. Chromatogram showing recovery of atraton and prometon from soil: (*a*) unfortified soil; (*b*) 1 p.p.m. of atraton and prometon added. Peak A, atraton; peak B, prometon

Table. The Table shows that trietazine cannot be determined by using this procedure and this has been verified experimentally. Simazine can be used as internal standard for atrazine, and *vice versa*, but in this instance the recovery of atrazine in the clean-up, although not complete, is reproducible, and a factor can be used in the calculation. The ratio of the peak area of atrazine to the peak area of simazine has been found to be 0·79 after the clean-up process, both with and without the presence of soil extracts. Prometryne, desmetryne and simetryne can be determined in pairs; simetryne is the preferred internal standard for both desmetryne and prometryne residues. As mentioned previously, atraton, prometon or simeton can be determined as a residue by using another of the group as internal standard.

APPLICATION TO RESIDUES IN CROPS—

The method has been applied to the determination of residues in crops to prove that they are less than a given limit, in this instance taken at 0·1 p.p.m. Because of the difficulty of taking a small representative sample of some crops, particularly those containing both leaf and stalk, such as kale, it was found preferable to weigh out 200 g of a finely chopped sub-sample containing the correct proportion of leaf and stalk. This sample, with the internal standard added, is macerated with 250 ml of methanol and thoroughly blended. A portion containing approximately 10 g of the sample is taken, and extracted with methanol, dichloromethane and ammonia solution as in the method for atraton, and the chromatogram obtained. The ratio of the standard and residue peaks is unchanged by this procedure and the presence of a residue would be indicated by a peak at the correct retention. If no peak appears at this position there is no detectable residue in the sample. The limit of detection of the method is better than 0·1 p.p.m. of herbicide residue, and this should always be checked by making a recovery experiment with a fortified control sample. The residue in the sprayed sample is therefore less than 0·1 p.p.m. When the method was applied to some brassica crops, large interfering peaks were occasionally encountered. These could be removed by dissolving the residue after evaporation of the chloroform in a further 10 ml of mixed solvent and repeating the partition into N sulphuric acid.

Applications have been found in determining prometryne residues in harvest samples of wheat, potatoes, carrots, beans, peas, parsnips, leeks and green maize. Desmetryne residues have been determined in kale, and simazine in strawberries and wheat. In all the samples examined, the harvest residues were found to be less than 0·1 p.p.m.

We thank Messrs. J. R. Geigy for providing pure samples of the triazine herbicides, Miss D. A. Richardson and Mrs. M. Baker for technical assistance and the directors of Fisons Pest Control Limited for permission to publish this Paper.

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The Chromatographic Detection and Determination of Organo-chlorine Herbicides in Soil and Water

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A procedure is described for the paper-chromatographic determination of traces of MCPA, MCPB, 2,4-D, 2,4-DB and 2,4,5-T in soil and water, and dalapon in water only. A thin-layer chromatographic procedure for detecting, separating and identifying these six herbicides is also described.

In order to study the relative retention of herbicides by soils and their possible elution by percolating water, a method was required for determining traces of these materials in soil and water. Mitchell's[1] system for the paper-chromatographic separation of microgram amounts of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) has been investigated as applied to this problem, and as a result a method is proposed for quantitatively determining six organo-chlorine herbicides.

The system that has been devised for obtaining the type of chromatographic spot most suitable for quantitative determination, does not, however, give any effective separation of the spots one from another. A second paper-chromatographic system has therefore been devised that will separate the herbicides into four groups, and the use of thin-layer chromatographic techniques has yielded complete separation of all the herbicides, which may then be identified by their R_F values.

Experimental

The herbicides used in this study were 2,4-D, 2,4,5-T, 4-chloro-2-methylphenoxyacetic acid (MCPA), 4-(4-chloro-2-methylphenoxy)butyric acid (MCPB), 4-(2,4-dichlorophenoxy)-butyric acid (2,4-DB), and 2,2-dichloropropionic acid (dalapon). The extraction of the first five of these materials from water was readily achieved by a simple extraction with ether from dilute acidic solution. Dalapon is more water-soluble and can only be extracted with some difficulty from a strongly acidic solution.

The extraction of these compounds from soil was found to be more difficult. Tumbling with ether readily gave complete extraction of MCPA, but the recovery of 2,4-D by this process was only 80 per cent. after two extractions, and only about 75 per cent. recovery of 2,4,5-T was obtained after three successive tumbling extractions. Soxhlet extraction was found to be no better but satisfactory recoveries of 2,4-D, 2,4,5-T, MCPA, MCPB and 2,4-DB have been obtained by the extraction of a slurry of soil in dilute sulphuric acid with ether. The method has not been applied to dalapon in soil.

The paper chromatograms were run in a tank similar to that described by Evans[2]; the herbicide spots were developed by using the chromogenic reagent of Mitchell.[1] The use of Whatman No. 3 chromatographic paper, in place of the No. 1 grade used by Mitchell, gave spots more suited to quantitative measurement, either by measurement of areas or by using a reflectance densitometer.

The use of the mobile phase described by Mitchell[1] together with Whatman No. 3 chromatographic paper gave R_F values in the range 0·60 to 0·67 for all six herbicides. Clearly the presence of two or more of the herbicides in an unknown sample might not be detected. Also, since the relationship between reflectance and amount of herbicide present is dependent upon the identity of the herbicide, this must be known before quantitative results can be obtained. In attempts to obtain paper-chromatographic resolution of the herbicides, several combinations of mobile phase and grade of paper have been investigated. The solvent system proposed in this Paper, a mixture of liquid paraffin, benzene, acetic acid and cyclohexane, used with Whatman No. 1 chromatographic paper, has given the R_F values listed below for the herbicides: MCPB, 0·81; 2,4-DB, 0·79; MCPA, 0·68; 2,4,5-T, 0·66; 2,4-D, 0·58; dalapon, 0·45. A

paper chromatogram prepared by using this system is illustrated in Fig. 1; it is seen that MCPB and 2,4-DB are not separated from each other and that MCPA and 2,4,5-T are also not resolved. In instances in which two, or more, resolvable herbicides are present together, this sytem can also be used quantitatively provided that estimations are made visually or by measurement of areas. The use of the reflectance densitometer is not appropriate in this instance, owing to background density difficulties.

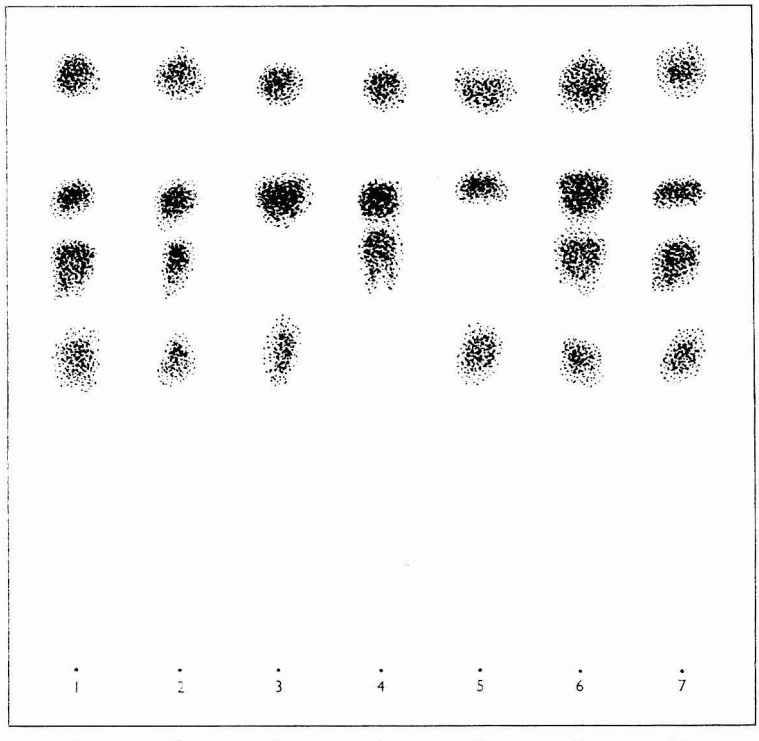

Fig. 1. Appearance of chromatogram on Whatman No. 1 chromatographic paper developed in mobile solvent B

In the hope of achieving complete resolution of all six herbicides, the technique of thin-layer chromatography has been applied. This technique has developed rapidly since Meinhard and Hall[3] produced starch-bound thin-layer plates. By using silica-gel plates, Petrowitz[4] was able to separate 11 organo-chlorine compounds, and Yamamura and Niwaguchi[5] have separated several organo-chlorine pesticides on silicic acid bound with potato starch. More recently, Walker and Beroza[6] have examined 62 pesticides by thin-layer chromatography with 19 different solvent systems on silica gel. In this Laboratory[7] the technique has also been applied to the detection of organo-chlorine and organo-phosphorus pesticides in various types of samples of vegetable and animal origin and to its use as a clean-up process before infrared examination.

Thin-layer plates of kieselguhr, silica gel and mixtures of these two materials in various proportions, have been used in the study. After they had been spotted with the herbicide in the usual way, the plates were developed with the liquid paraffin - benzene - acetic acid -

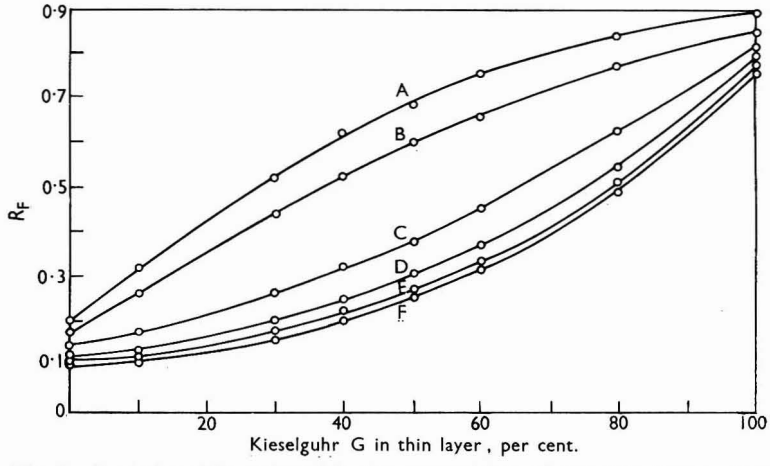

Fig. 2. Variation of R_F value with the composition of the thin layer. Curve *A*, MCPB; curve *B*, 2,4-DB; curve *C*, MCPA; curve *D*, 2,4,5-T; curve *E*, 2,4-D; curve *F*, dalapon

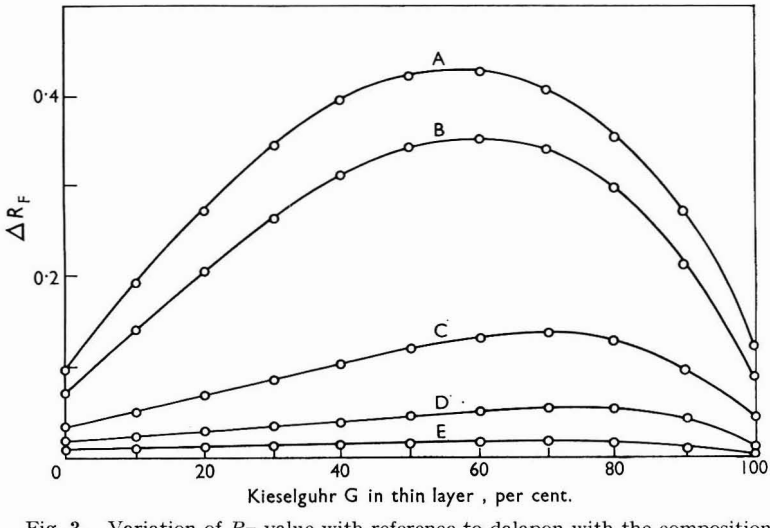

Fig. 3. Variation of R_F value with reference to dalapon with the composition of the thin layer. Curve *A*, MCPB; curve *B*, 2,4-DB; curve *C*, MCPA; curve *D*, 2,4,5-T; curve *E*, 2,4-D

cyclohexane mobile phase used for the separation of the herbicides on paper. The spots were marked by spraying the dried plate with alcoholic silver nitrate solution and irradiating it with ultraviolet light, whereupon the herbicides appeared as brown - black spots on an off-white background. Self-indicating plates containing "built-in" silver nitrate have also been used, but need care in preparation owing to gelation tendencies induced by the incorporated silver salt.

The degree of separation given by plates of various compositions is illustrated graphically in Fig. 2, and the relative adsorptions of the herbicides are represented in Fig. 3, dalapon being the slowest moving and used as reference. Fig. 3 clearly indicates the most suitable composition of the plate-coating mixture for maximum separation of the herbicides (as shown in Fig. 4), *i.e.*, 60 per cent. kieselguhr - 40 per cent. silica gel. Similarly, Fig. 2 shows that where the technique is required as a final clean-up stage also, then a high proportion of kieselguhr in the plate leads to high R_F values in general and consequently a good separation of the herbicides from the associated, generally slower-moving, impurities.

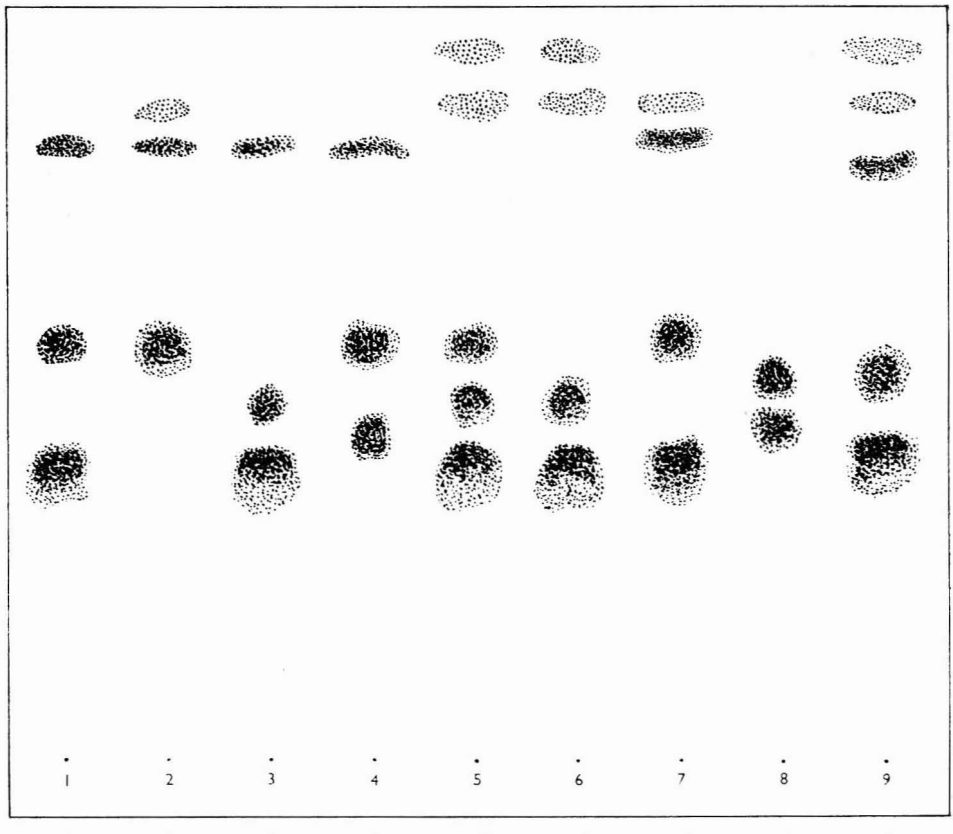

Fig. 4. Appearance of chromatogram on 60 per cent. kieselguhr G - 40 per cent. silica gel G developed in mobile solvent B

This thin-layer chromatographic system has also been extended to include 2-(1-methyl-n-propyl)4,6-dinitrophenol (dinoseb) and 2-methyl-4,6-dinitrophenol (DNOC). These materials are well separated from the organo-chlorine herbicides and appear on the dried plate as self-indicating yellow spots of R_F values 0·94 and 0·86, respectively (see Fig. 4).

Most of the thin-layer plates used in this work were prepared by means of a commercially available spreading apparatus. However, many successful plates were prepared quite simply and cheaply by pouring the mix on to a glass plate and smoothing out the layer by drawing a glass rod across the surface of the plate, a technique that was used by Davidek and Prochazka[8] for producing loose-layer plates.

In order to obtain reproducible R_F values, the plates must be layered and activated by a standardised procedure, and used within 36 hours. Activation of the plates at various temperatures and for varying times has been found to give rise to differing R_F values for each herbicide. Ageing of the plates for 2 to 3 days, even under desiccating conditions, also affects the R_F values, and in particular adversely affects the separation of 2,4-D and 2,4,5-T.

The spotting technique must also be standardised and over-spotting should be avoided as far as possible. When standards of mixed herbicides were run on the plates, the spots were better shaped if a solution of the mixed herbicides was applied as one spot, than if separate portions of the individual herbicide solutions were superimposed on one origin spot. Under the latter conditions some radial chromatography occurred, which affected both the final shape of the spots and their R_F value.

The angle at which the plate is supported in the tank has also been found to affect the shape of the spot. In general, the smaller the angle between the back of the glass plate and the horizontal surface of the mobile phase, the faster the rate of development of the chromatogram, but the more diffuse the spot appears. However, when the angle between the back of the glass plate and the horizontal is more than 90°, speed of development is again increased, but the spots are compacted; the ideal angle is about 135°.

Any suitable chromatographic tank, in which solvent - vapour phase equilibrium is attained, may be used; that described by Evans[2] was used for the thin-layer as well as the paper chromatography described in this Paper. Equally effective results have been obtained without the use of a tank and by using pieces of ordinary window glass as the plate support. A second glass plate was held on top of the layered plate by means of sleeves of opened poly(vinyl chloride) tubing along two sides. The plates were kept apart by a paper spacer at one end and a paper solvent feed at the other and could be developed by ascending-solvent, descending-solvent or horizontal chromatography. The ease and rapidity with which complete equilibration is achieved between the solvent and the vapour phase, owing to the low vapour volume, adds to the efficiency of the separations and gives extremely compact spots. Combination of this development technique with the use of hand-poured and spread plates gives a cheap way of carrying out thin-layer chromatography.

METHOD

APPARATUS—

Chromatographic tank—As described by Evans.[2]

Ultraviolet lamps—Two Phillips TUV ($\lambda_{max.} = 2537$ Å) 15-watt germicidal lamps mounted parallel with their centres 5 cm apart and 15 cm above the chromatograms, housed in a light-tight box.

Chromatographic drying oven—Set at 80° C.

Dipping trays—Glass trays approximately 20 × 8 × 4 cm, for the chromogenic reagent. Polythene trays 35 × 25 × 4 cm, for washing the papers.

Reflectance densitometer—The X.110 reflectance densitometer fitted with a Mark III head (obtainable from Evans Electroselenium Ltd.) has been found suitable.

Centrifuge—Capable of rotating two 250-ml containers at 2500 r.p.m.

Tumbling apparatus—A device for tumbling 500-ml flasks, end-over-end at 60 r.p.m.

Micropipettes—Made from drawn-out capillary tubing, having a capacity of 1 μl and calibrated to a tolerance of ± 1 per cent.

Thin-layer spreading apparatus—Apparatus suitable for the preparation of thin-layer plates, 20 × 20 cm, with a layer thickness of 250 μ. (The Desaga apparatus, available from Camlab Ltd., Cambridge, has been found suitable.)

Plate-drying oven—Air oven set at 120° C, suitable for activating and drying thin-layer plates.

Chromatographic spray.

REAGENTS—

Reagents should be of recognised analytical grade whenever possible. De-ionised water should be used.

Ethyl acetate.

Diethyl ether.

Sulphuric acid, sp.gr. 1·84, *and approximately* 6 N.

Sodium hydroxide, approximately 2·5 N.

Sodium sulphate, anhydrous.

Sodium sulphate solution, 8 *per cent. w/v, aqueous.*

Sodium thiosulphate solution, 0·5 *per cent. w/v, aqueous.*

Mobile solvent A—Add 2·0 ml of ammonia solution, sp.gr. 0·88, and 18 ml of t-butanol to 40 ml of water. Dilute to 200 ml with ethyl methyl ketone.

Mobile Solvent B—Add 10 ml. of liquid paraffin, 30 ml of benzene and 20 ml of glacial acetic acid to 200 ml of cyclohexane.

Chromogenic reagent—Dissolve 1·7 g of silver nitrate in 5 ml of water, add 20 ml of 2-phenoxyethanol and dilute the mixture to 200 ml with acetone. Add one drop of 30-volume hydrogen peroxide solution and mix. Set it aside overnight, and decant the solution before use.

Silica gel G—For thin-layer chromatography. (Available from E. Merck, Darmstadt.)

Kieselguhr G—For thin-layer chromatography. (Available from E. Merck, Darmstadt.)

Thin-layer mix—Mix 60 parts of kieselguhr G with 40 parts of silica-gel G as required.

Chromogenic spray reagent—Prepare a 0·5 per cent. v/v solution of silver nitrate in 95 per cent. v/v ethanol.

Standard herbicides—

(*i*) MCPA, m.p. 117° C.
(*ii*) MCPB, m.p. 101° C.
(*iii*) 2,4-D, m.p. 137° C.
(*iv*) 2,4-DB, m.p. 119° C.
(*v*) 2,4,5-T, m.p. 156° C.
(*vi*) Dalapon: 2,2-dichloropropionic acid prepared as required from the technical sodium salt (91 per cent. pure).

Prepare stock standard solutions each containing 20 mg of herbicide per ml of solution in ethyl acetate and dilute with ethyl acetate as required.

PROCEDURE FOR EXTRACTION AND CLEAN-UP—

(*a*) *Water samples*—MCPA, MCPB, 2,4-D, 2,4-DB and 2,4,5-T are readily extracted from acidified waters that are low in mineral and organic matter content. To 100 ml of the sample contained in a 500-ml separating funnel add 5 ml of 6 N sulphuric acid, shake the funnel for 1 minute and set it aside for 5 minutes. Add 100 ml of ether and shake the funnel for 1 minute, allow the phases to separate, transfer the aqueous phase to a second 500-ml separating funnel and extract it with a further 100-ml portion of ether for 1 minute. Allow the phases to separate, discard the aqueous phase and combine the ethereal extracts. Extract the ether solution with two portions each consisting of a mixture of 150 ml of sodium sulphate solution with 5 ml of sodium hydroxide solution. Combine the alkaline aqueous extracts, add 10 ml of 6 N sulphuric acid and extract with successive portions of 150 ml and 100 ml of ether. Combine the ethereal extracts and filter them through a column containing 50 g of anhydrous sodium sulphate into a Kuderna - Danish evaporator fitted with a 5-ml flask, washing the extracts through with 50 ml of ether. Reduce the volume of the solution to 2 to 3 ml on a warm-water bath and then remove the remaining solvent in a current of air. Dissolve the residual herbicide in 40 μl of ethyl acetate immediately before chromatographic spotting.

When dalapon is suspected, use the procedure given below: add 1 ml of sodium hydroxide solution to 100 ml of sample and evaporate the mixture to 30 ml on a water-bath. Cool the solution, and carefully add 40 ml of concentrated sulphuric acid with cooling. Extract the solution with successive 150-, 100- and 50-ml portions of ether, combine the extracts and proceed as above beginning at ". . . filter through a column containing. . . ." Special care is necessary in the final stages to avoid loss of the free acid by evaporation. Dissolve the residue in 40 μl of ethyl acetate when required.

(*b*) *Soil samples*—Grind 100 g of the soil into a paste with a little water in a 6-inch mortar. Add a further 100 ml of water, transfer the slurry to a 500-ml flask, and wash in the slurry with sufficient water to bring the total volume to 140 ml. Add 20 ml of 6 N sulphuric acid and 200 ml of ether. Stopper the flask securely and tumble it at 60 r.p.m. for 1 hour. Spin the slurry in a centrifuge for 15 minutes at 2500 r.p.m. at a radius of 30 cm and carefully decant the liquid phase into a 500-ml separating funnel, washing it in with ether as necessary. Discard the aqueous layer and proceed as described above for water samples, beginning at ". . . Extract the ether solution with two portions. . . ." This procedure will extract MCPA, MCPB, 2,4-D, 2,4-DB and 2,4,5-T.

PROCEDURE FOR THIN-LAYER CHROMATOGRAPHIC DETECTION AND IDENTIFICATION—

Prepare thin-layer plates, 250 μ thick, either by using a commercial spreading apparatus or by pouring the mix on to the plate and smoothing by means of a glass rod or Perspex strip with the aid of appropriate spacers. A mixture of 30 g of the kieselguhr G - silica-gel G (60 + 40) mix with 60 ml of water, if shaken for 1$\frac{3}{4}$ minutes before application, gives sufficient material for the preparation of 4 plates each 20 × 20 cm. Allow the plates to harden for 10 minutes, and then dry them, supported vertically, in an air oven at 120° C for 2 hours. Allow them to cool in a desiccator over silica gel, and use them within 36 hours. Mark the origin line 25 mm from one edge of the plate and carefully apply 1-μl portions of ethyl acetate solutions of the unknown sample and of the standard herbicides along this line at 20-mm intervals, commencing 10 mm from one edge. Place 200 ml of mobile solvent B into the tray of the chromatographic tank and allow to equilibrate for 1 hour. Place the plate in the tank so that the origin line is parallel to the solvent surface and about 15 mm above it. Support the plate either vertically or in such a way that the angle between the back of the plate and the solvent surface is 135°, and allow the plate to develop for 1 hour or until the solvent front has moved 150 mm. Remove the plate from the tank, allow the solvent to evaporate for about 3 minutes and dry the plate in an oven at 120° C for 10 minutes. Spray it with 0·5 per cent. alcoholic silver nitrate solution and again dry it at 120° C for 10 minutes. Irradiate the plate with germicidal ultraviolet light for 10 minutes, whereupon the positions of the herbicides are observed as black spots on an off-white background. Comparison of the R_F value of the spot due to the unknown sample with those given by the standard herbicides serves both to detect and identify the nature of the unknown compound.

PROCEDURE FOR PAPER-CHROMATOGRAPHIC DETERMINATION—

Cut Whatman No. 3 chromatographic paper into sheets 19 × 30 cm, with the machine direction of the paper parallel to the short edge. Lightly mark (graphite pencil) a paper with a line 20 mm from, and parallel to, one short edge; divide the line with marks at 25-mm intervals, commencing 20 mm from one side. Support the paper on a flat non-absorbent surface and transfer 1-μl portions of the prepared ethyl acetate solution of the herbicide to separate marked spots; two or three spots at different dilutions should be made for each unknown sample. The total time from dissolving the extracted residue in ethyl acetate, or the preparation of dilutions of this solution, to the transfer of the portion to the paper should not exceed 3 minutes. The flask containing the herbicide should remain stoppered both while the residue is being dissolved and, should a second portion be required, after the pipette has been filled. With these precautions it is possible, when necessary, for two portions to be transferred to a single spot, if about 20 seconds is allowed between applications for the evaporation of the solvent. Similarly prepare on the same paper a range of at least four standards of the herbicide sought, the unknown having been identified by the thin-layer procedure. A full range of standards is 2, 4, 6, 8, 10 and 12 μg; the sensitivity limit for each herbicide is about 0·5 μg.

Up to four papers may be run simultaneously in each chromatographic tank. Place 200 ml of mobile solvent A in the stainless-steel tray, set centrally and horizontally in the base of the tank, replace the lid and allow the tank to equilibrate for 1 hour. Clip the papers to the frame, remove the tank lid and carefully lower the frame on to its supports so that the bottom edges of the papers just enter the solvent and are parallel to its surface. Replace the lid and allow the chromatograms to develop for 100 minutes. Remove the papers from the tank and dry them in a current of air in a chromatographic oven at 80° C for 10 minutes. Draw the paper by continuous action from the solvent front to the origin line through the

chromogenic reagent contained in a narrow tray. Evaporate the solvent in a current of air and irradiate each side of the paper for 10 minutes with germicidal ultraviolet light. Wash the chromatogram 3 to 4 times in successive volumes of water by soaking and swirling it in a shallow tray. Immerse the paper in 0·5 per cent. sodium thiosulphate solution for 10 seconds and then immediately wash out the excess of sodium thiosulphate with several successive portions of water. Dry the paper in the chromatographic oven at 80° C and evaluate the chromatogram, either by measurement of areas as described by Evans[2] or by means of a reflectance densitometer as described below.

PROCEDURE FOR REFLECTANCE-DENSITOMETER EVALUATION—

The paper chromatograms prepared by the process described, consist of orange to brown coloured, nearly elliptical spots, "cometing" slightly in the direction of the solvent front and set on an off-white background, whose quality depends on the efficiency of the washing process. Adjust the sensitivity control of the densitometer galvanometer so that an average reading of 95 divisions on the linear scale is obtained when the reflectance head is made to scan two parallel paths on the paper chromatogram approximately 20 mm above and below the line of centres of the series of standard herbicide spots; for a satisfactory chromatogram the point-to-point variation should be smooth and within the range 90 to 100 linear scale

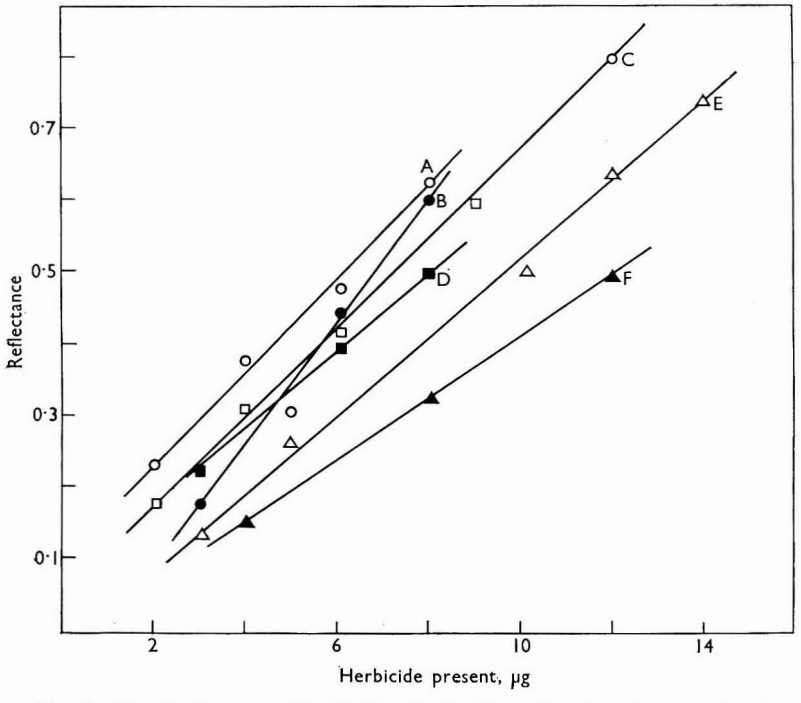

Fig. 5. Standard curves of herbicides obtained by using the reflectance densitometer. Curve *A*, MCPA; curve *B*, dalapon; curve *C*, 2,4-D; curve *D*, MCPB; curve *E*, 2,4,5-T; curve *F*, 2,4-DB

divisions, *i.e.*, ±5 per cent. Next adjust the galvanometer zero setting so that with the reflectance head located centrally over the chromatographic spot corresponding to the highest amount of standard herbicide, the reading obtained during scanning is as near to zero as possible. Repeat these two operations in turn until a maximum sensitivity is obtained. Scan the other standard spots, record the corresponding minimum deflection readings and plot a curve of the logarithm of the galvanometer reading (reflectance) *versus* micrograms

of herbicide. Determine the reflectance of the spots of the unknown sample and evaluate the amount of herbicide in the spot by reference to the standard curve. Express the result in terms of the amount of herbicide in the original sample as mg per litre or per kg, depending on the type of sample examined.

Owing to variation of the background reflectance a standard curve must be prepared for each chromatogram obtained. An approximately linear relationship exists between reflectance and amount of herbicide present over the range 2 to 12 μg. Standard curves of the six herbicides examined are illustrated in Fig. 5.

PROCEDURE FOR PAPER-CHROMATOGRAPHIC SEPARATION—

Prepare Whatman No. 1 chromatographic papers as described above, spot them with ethyl acetate solutions of the unknown sample and of the various herbicides, and develop them with mobile solvent B for 3 hours. Compare the R_F values of the spots of the unknown sample with those of the standard materials. Dalapon and 2,4-D may be determined by repeating the chromatogram of the unknown sample together with a series of standard amounts of the known herbicide, with Whatman No. 1 chromatographic paper, mobile solvent B and 3 hours development time, and by evaluating the spots by visual comparison or measurement of areas. If either MCPB and 2,4-DB or MCPA and 2,4,5-T appear to be present, these must be resolved by the thin-layer technique before any quantitative measurements are made.

RESULTS

The procedures described have been used to examine samples of clay, sandy and calcareous soils, waters that have percolated through these soils, and also some tap-waters. Results

TABLE I

RECOVERY OF HERBICIDES ADDED TO SOIL AND WATER

Herbicide	Sample	Herbicide added, mg per 100 g of sample	Recovery, per cent.
MCPA	water	25	100
	soil	2·0	90
	soil	0·2	100
MCPB	water	0·3	100
	soil	0·3	80
2,4-D	water	1·0	100
	soil	10	85
	soil	2·5	100
2,4-DB	water	0·1	95
	soil	1·0	90
2,4,5-T	water	2·5	90
	soil	2·5	80
Dalapon	water	30	90

of some recovery experiments are given in Table I. The infrared spectra of the final extracts from the soil samples were recorded and verified by comparison with the standard spectra.

Permission to publish this Paper has been given by the Government Chemist, Department of Scientific and Industrial Research.

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The Effect of Temperature on the Kjeldahl Digestion Process

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The sealed-tube method for the Kjeldahl digestion process has been examined critically. The effect of temperature on the digestion of a refractory test substance with concentrated sulphuric acid, in the presence and absence of either of two efficient catalysts, has been studied. The sealed-tube method has been used successfully, in conjunction with the indanetrione hydrate method, for determining micro amounts of nitrogen in various biological materials, particularly those containing a large excess of carbohydrate. It has been shown that the quantitative recovery of nitrogen is temperature dependent and that the presence of catalyst in the acid digestion mixture is not critical, provided that the period of digestion is adequate.

THERE have been numerous modifications of the digestion procedure in the Kjeldahl method since it was first described in 1883.[1] Sulphuric acid was used alone in the original method and decomposition of the organic substance was performed in the absence of a catalyst: the oxidation was completed by the addition of potassium permanganate crystals in powder form. Wilfarth[2] first used mercuric oxide as a catalyst, and Gunning[3] raised the temperature of the acid digestion mixture by introducing potassium sulphate. More recently, there have been attempts to standardise the digestion procedure[4,5] and to investigate the effects of adding salts and catalysts to the sulphuric acid.[5 to 10] There appear to be discrepancies in the temperatures recorded for the boiling-points of the digestion mixtures used by certain observers whose publications have been reviewed elsewhere.[5]

White and Long[11] described the use of a sealed-tube digestion method with a catalyst: the method has been modified by several workers.[12 to 17] In this laboratory, the sealed-tube digestion has been performed at 470° ± 10° C, either with or without the addition of a catalyst to the sulphuric acid. The method has been applied to various organic compounds,[15,17,18,19] including nicotinic acid, which is known to be a refractory substance.

This Paper describes further experiments performed to study the digestion of organic materials by sulphuric acid in sealed containers, and also to find a standardised procedure for examining substances that are difficult to analyse by the normal Kjeldahl digestion in open tubes.

METHOD

APPARATUS—

Small volumes of samples (0·010 to 0·500 ml) were delivered from an Agla micrometer syringe pipette (Burroughs Wellcome Ltd., London) or by means of the precision pipettes previously described[15]; larger volumes of solution were delivered from British Standard pipettes (grade A).

The sealed Pyrex-glass ampoules containing the sample and sulphuric acid were heated in an electric furnace type M supplied by Wild Barfield (see under "Procedure for Digesting the Sample").

The Beckman Spinco amino-acid analyser, model 120,[20] was used for examining the reaction products after the sealed-tube digestion with sulphuric acid.

The sealed ampoules were heated at 460° to 480° C for 30 minutes in a well lagged electric furnace, whose dimensions were 18 × 9 × 9 inches. The commercial thermocouple was situated at the top left-hand corner of the square back wall of the furnace, 1·5 inches from the top and the same distance from the side wall, and the chromel - alumel thermocouple used for

standardising the commercial thermocouple was placed in the centre of the oven door and extended into the centre of the furnace. It was not essential to enclose the sealed ampoules in protective sheaths,[15] provided that the ampoules were sealed correctly. The cold junction of the calibrating thermocouple was placed in a tube immersed in crushed ice, and the difference in potential between the hot and cold junctions of the calibrating thermocouple was measured with an accurate potentiometer reading to 0·01 mV. The temperatures recorded on the commercial thermocouple (or pyrometer) were compared with the temperatures calculated from the physical measurements made with the calibrating thermocouple. The results, which are given below, indicate that the commercial thermocouple was correct to ±2° C over the range 100° to 560° C—

Temperature indicated by commercial thermocouple—	100	450	460	470	480	490	500	520	540	550	560
Temperature indicated by calibration thermocouple—	100	449	459	468	478	489	500	518	539	550	562

REAGENTS—

All materials were of recognised analytical grade unless otherwise specified, and solutions were prepared with conductivity water obtained as described previously.[21] Reagents additional to those described in an earlier Paper on this subject[15] are listed below.

Sodium acetate, hydrated—This compound was purified by the method described previously.[20]

Sodium citrate buffer solution, 2·0 M, pH 5·0—The buffer was prepared by dissolving 80 g of sodium hydroxide and 180 g of citric acid in 1 litre of water.

Catalysts—Catalyst *A*: this was used with 0·15 ml of sulphuric acid for the trial digestions of nicotinic acid (see Table I), and contained 2 mg of mercuric oxide and 225 mg of potassium sulphate. Catalyst *B*: this was also used for digesting nicotinic acid, and consisted of 2 mg of a mixture of potassium sulphate, copper sulphate, mercuric oxide and selenium in the proportions 15 + 5 + 5 + 1.

TABLE I

EFFECT OF TEMPERATURE, PERIOD OF DIGESTION AND CATALYST ON THE DETERMINATION OF NITROGEN IN NICOTINIC ACID

Temperature, °C (±5° C)	Period of digestion, minutes	Nitrogen content,* per cent.— no catalyst	catalyst *A*	catalyst *B*
350	30	0·2	0·3	0·6
	60	0·3	1·2	1·3
	120	0·9	3·6	3·8
370	30	0·3	0·4	0·6
	60	0·3	2·8	1·9
	120	1·2	9·5	5·1
390	30	0·6	0·8	1·4
	60	3·2	8·2	7·9
	120	3·4	9·8	9·5
410	30	1·4	1·1	3·4
	60	3·3	8·3	8·5
	120	8·5	10·7	10·6
430	30	2·5	7·4	6·9
	60	8·0	11·3	11·5
	120	10·4	11·6	11·5
450	30	6·6	9·3	9·4
	60	9·2	11·4	11·4
	120	11·3	11·3	11·3
470	30	11·3	11·4	11·3
	60	11·3	11·4	11·4
	120	11·6	11·2	11·4
490	30	11·5	11·4	11·3
	60	11·1	11·5	11·1
	120	11·3	11·3	11·3

* Mean of triplicate determinations (theoretical value = 11·39 per cent. of nitrogen).

PROCEDURE FOR DIGESTING THE SAMPLE—

The solution of protein or food-diet hydrolysate (0·1 to 0·25 ml) was transferred to a 5-ml Pyrex-glass ampoule, dried in a vacuum desiccator over phosphorus pentoxide and the requisite amount of sulphuric acid (0·1 to 0·25 ml) was added to the dry residue. Samples of serum or food-diet hydrolysates were digested in sealed tubes with an equal volume of concentrated sulphuric acid as an alternative procedure.[22] In the latter instances, the digestion was performed without catalyst, and at the normal temperature (460° to 480° C).

Samples of tuberculin vaccine in glycerine were diluted (1 + 49) with water. Portions chosen to contain 20 to 30 μg of nitrogen, were dried in the ampoules before sulphuric acid was added and the digestion was completed in the absence of a catalyst.

The corticotrophin samples dispensed in ampoules with an excess of lactose in a freeze-dried form were digested without catalyst in the original ampoule after it had been re-sealed.

THE PHOTOMETRIC MEASUREMENT—

The digested sample was allowed to cool and the contents of the ampoule were diluted, neutralised and buffered to a pre-determined volume by the method previously described.[19] The digested sample was diluted to a convenient volume so that a portion of 2·0-ml final volume would contain 4 to 8 μg of nitrogen and an amount of sulphate ion not exceeding 105 mg (see Table II). The portion, diluted if necessary to 2·0 ml, was allowed to react

TABLE II

EFFECT OF SULPHATE IONS ON THE FORMATION OF THE AMMONIA - INDANETRIONE HYDRATE COMPLEX

Concentration of ammonia = 0·35 μM

Weight of sulphate ions, mg	Optical densities* of solutions— with ammonia absent	with ammonia present	corrected
0·0	0·069	0·771	0·702
17·5	0·070	0·769	0·699
35·1	0·075	0·769	0·694
52·5	0·088	0·779	0·691
70·2	0·084	0·788	0·704
87·7	0·088	0·794	0·706
105·2	0·088	0·791	0·703

* Mean optical densities at 570 mμ of triplicate samples in 10-mm cells, obtained after 0·35 μM ammonia solution had reacted with an excess of indanetrione hydrate solution and the reaction mixture had been diluted to 10 ml.

with 2·0 ml of the 2 per cent. indanetrione hydrate reagent in a water-bath at 100° C for 30 minutes. The reaction mixture was cooled and diluted to 10 ml by adding ethanol - water (1 + 1) solution. The optical density was measured by using a Hilger & Watts Uvispek within 1 hour of the dilution procedure.

RESULTS

The chromatogram, obtained as an elution-curve tracing by the recorder of the amino-acid analyser,[20] indicates the products of reaction between a sample of horse-serum protein and sulphuric acid after digestion by the sealed-tube technique previously described,[19] and shows the presence of only one amino compound (*viz.*, ammonia) with a positive reaction to indanetrione hydrate.

The effect of temperature on the digestion of nicotinic acid with sulphuric acid is illustrated in Table I. The recovery of nitrogen in the reaction mixture has been determined after the nicotinic acid had been digested for varying periods in the absence and also in the presence of either of the two catalysts.

The nitrogen contents of food diets examined by the open-tube method are compared with values obtained by the sealed-ampoule technique in Table III.

TABLE III

COMPARISON OF PERCENTAGE NITROGEN CONTENTS OF FOOD DIETS OBTAINED BY USING KJELDAHL OPEN-TUBE AND SEALED-TUBE DIGESTIONS

Figures given are the means of triplicate determinations, except for food-diet No. 1, for which the means of quadruplate determinations are given

Food diet	Method used— Kjeldahl open-tube, on whole diet	Method used— sealed-tube, on hydrolysate
No. 1	5·09, 4·67 4·87, 4·96	5·07, 5·12 5·00, 4·97
No. 2	3·22, 3·21 3·17	3·32, 3·29 3·32
No. 3	4·37, 3·89 4·45	4·26, 4·48 4·34
No. 4	3·03, 3·04 3·08	3·15, 3·19 3·11
No. 5	6·05, 6·06 6·12	6·15, 6·11 6·19

The results given in Table IV indicate the total nitrogen found in globin fractions of proteins and also of mucopolysaccharides by using the sealed-tube method. This Table shows the total nitrogen value calculated from the recoveries of the nitrogen in the eluate components after their separation on the ion-exchange column from the hydrolysate of the original protein material.

TABLE IV

NITROGEN CONTENTS OF PROTEINS AND MUCOPOLYSACCHARIDES

Sample	Nitrogen content, μg, found by using— sealed-tube method	Nitrogen content, μg, found by using— Beckman Spinco amino-acid analyser
Human foetal globin—		
Sample 1 ..	361	360
Sample 2 ..	362	370
Rabbit globin α-chains ..	232	225
Rabbit globin β-chains ..	237	229

Sample	Nitrogen content, per cent., found by using— sealed-tube method	Nitrogen content, per cent., found by using— Beckman Spinco amino-acid analyser
Chondroitin-4-sulphatepeptide—		
Sample 1 ..	3·15, 3·10	3·15, 3·09
Sample 2 ..	3·20, 3·14 3·18	3·22, 3·20 3·15
Heparan-hemi-sulphatepeptide	3·17, 3·15	—

TABLE V

NITROGEN CONTENTS OF TUBERCULIN VACCINES AND CORTICOTRIPHIN SAMPLES

Tuberculin (1·0-ml sample)	Nitrogen content, μg, found by using— micro-Kjeldahl open-tube method	Nitrogen content, μg, found by using— sealed-tube method
Batch 1	240, 232	234, 236
Batch 2	617, 596	613, 605
Batch 3	623, 611	626, 621

Corticotrophin	Nitrogen content, μg, found by using sealed-tube method
Batch 1	7·1, 6·8 6·9, 7·1, 6·8
Batch 2	7·4, 7·4 7·5, 7·4
Batch 3	7·3, 7·2 7·2, 7·3

Table V shows a comparison between the nitrogen contents of tuberculin vaccines obtained by the normal open-tube Kjeldahl method and the sealed-tube procedure; and also the nitrogen contents of corticotrophin samples.

Discussion

The reaction products obtained after the digestion of protein material in sulphuric acid have been shown to contain only one component that reacts with indanetrione hydrate, *viz.*, ammonia. The presence of only one peak on the elution curve (chromatogram) obtained when the neutralised and buffered reaction products are added to, and eluted from, the ion-exchange resin column of the Beckman Spinco amino-acid analyser indicates the absence of amino-acids and complete conversion of nitrogen to ammonia. This quantitative recovery of nitrogen from biological material has been obtained after digestion of the sample in 50 per cent. v/v aqueous sulphuric acid and also after digestion of the dried sample in concentrated sulphuric acid.

The potentiometric measurements recorded on p. 490 confirm the temperatures reported previously for the digestion of organic compounds by sulphuric acid in sealed tubes.[15] The results given in Table I show that the quantitative recovery of nitrogen from nicotinic acid is dependent upon temperature and that the presence of catalyst in the digestion mixture is not critical, provided that the period of digestion is adequate.

The total nitrogen contents of food diets have been determined by the normal micro-Kjeldahl digestion method and the sealed-tube technique. The latter method has been shown to compare favourably with the normal Kjeldahl procedure. The determination of nitrogen in food diets by the sealed-tube method has been shown to be quantitative for hydrolysates of the food diet. The direct digestion of the native food diet containing large amounts of carbohydrate with concentrated sulphuric acid produces a large volume of carbon dioxide, which creates an excessive pressure at high temperature. This may cause the ampoule to fracture, but the food diet, hydrolysed in 6 N hydrochloric acid and evaporated carefully to dryness under reduced pressure in the presence of solid sodium hydroxide pellets, may be analysed satisfactorily.

The total nitrogen contents of globins and mucopolysaccharides have been determined by the sealed-tube technique, and have been found to compare favourably with values determined on their respective hydrolysates after the constituent amino-acids and ammonia, resulting from hydrolysis in 6 N hydrochloric acid at 105° C for 24 hours, have been separated on ion-exchange resin columns by the method previously described.[20]

The sealed-tube technique has been used also for determining the total nitrogen in samples of tuberculin vaccine. The values obtained by that method are in close agreement with those obtained by the normal micro-Kjeldahl method, in which 8·0 ml of concentrated sulphuric acid 2 to 3 mg of catalyst *B* are used for each sample of tuberculin dissolved in 1·7 ml of 10 per cent. aqueous glycerine containing 0·5 per cent. of phenol. The digestion of the tuberculin in aqueous glycerine solution by the Kjeldahl method with the open-tube digestion required long periods of digestion with careful control of the heating. The addition of sulphuric acid to the aqueous glycerine solution of the tuberculin produced a dense mass of carbon that required 5·0 ml of 30 per cent. v/v hydrogen peroxide solution added at intervals after periods of digestion and cooling, to oxidise completely the carbon. On the other hand, analysis by the sealed-tube technique of small amounts of the sample after dilution (1 + 49) in water and evaporation to dryness over phosphorus pentoxide in the Pyrex-glass reaction ampoule, was relatively simple and more reliable. Attempts to digest with sulphuric acid in the sealed ampoules the native sample of tuberculin in the aqueous glycerine solution led to fractured ampoules. The conditions were then similar to those that obtain when food diets, containing a large proportion of carbohydrate, are digested with concentrated sulphuric acid in a closed container.

The 50-μg samples of corticotrophin were digested directly with 0·1 ml of concentrated sulphuric acid without catalyst in ampoules of 5·0- to 6·0-ml capacity in the presence of a large excess (5 mg) of freeze-dried lactose. In this instance, the digestion of the sample for 30 minutes at 470° $\pm$ 10° C was adequate, and no high pressure resulted from the formation of gaseous reaction products. The ampoules were cooled, the reaction products were diluted with water, buffered and neutralised before being diluted to 5·0 ml. The small amount of nitrogen in each ampoule allowed only duplicate portions of 2·0 ml to be analysed per sample. The normal procedure with samples containing amounts of nitrogen exceeding 10 μg allowed triplicate portions to be analysed from the 10 ml final volume of solution obtained by diluting the neutralised and buffered (to a final concentration of 0·2 M sodium citrate, pH 5·0) reaction products.

The sealed-tube digestion technique has been shown to be effective for the analysis of various biological materials.

A small amount of nitrogen, in the presence of a gross excess of carbohydrate, may be determined quantitatively provided that the sulphate ion content of the neutralised and buffered portion does not exceed 105 mg.

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SHORT PAPERS

The Determination of Parathion and Related Insecticides by Gas-Liquid Chromatography

With Special Reference to Fenitrothion Residues in Cocoa

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As part of a trial experiment it recently became necessary to determine residues of fenitrothion (dimethyl 3-methyl-4-nitrophenyl phosphorothionate) in cocoa beans, and it was thought that the colorimetric procedure of Averell and Norris[1] for determining residues of parathion (diethyl 4-nitrophenyl phosphorothionate) could be adapted for this purpose. The method involves reduction of the nitro group to form a primary amine, diazotisation and coupling with *N*-(1-naphthyl)-ethylenediamine to give an azo-dye whose concentration could be measured spectrophotometrically.

If solutions of pure fenitrothion were used it was possible to detect residues of the pesticide equivalent to 0·2 p.p.m., but when cocoa extracts were used, readings equivalent to fenitrothion residues of 2 p.p.m. were obtained, even from untreated samples. Further, the determination was made difficult by the development of a haze in the final coloured solution. The method was therefore abandoned.

During a study of the possible use of gas - liquid chromatography it was found that under suitable conditions fenitrothion gave a satisfactory response. The instrument used was a Pye Argon chromatograph with a β-ionisation detector fitted with a strontium-90 source, modified for electron-capture detection.

The column (125 × 0·4 cm) consisted of 0·25 per cent. w/w Epikote resin 1001 and 2·5 per cent. w/w silicone elastomer E.301 supported on 100- to 120-mesh Celite. With oxygen-free nitrogen as the carrier gas, satisfactory resolution was achieved with a column temperature of 200° C and a gas flow-rate of 6 litres per hour. The voltage applied to the detector was 13·5 V. Under these conditions the retention time for fenitrothion was 1·8 minutes, and there was a minimum of interference from the extraneous materials extracted from the cocoa beans.

Method

Reagents—

Benzene.
n-Hexane.
Sodium sulphate, anhydrous.
Sodium hydroxide, saturated solution.
Nitromethane.

Procedure—

Macerate a 50-g sample of cocoa beans and heat it under reflux with 150 ml of benzene for 1 hour. Filter the mixture and then wash the residue with two 25-ml portions of benzene. Combine the benzene extract and the washings, concentrate them to remove the solvent, and then extract the solution with two 10-ml portions of nitromethane. Wash the nitromethane extract with 10 ml of n-hexane, concentrate the extract to 10 ml and dissolve it in 100 ml of water. Add 30 ml of saturated sodium chloride solution to the solution and extract it with two 10-ml portions of n-hexane. Dry the hexane extract with anhydrous sodium sulphate and concentrate it to 5 ml. Examine 2-μl portions of this solution chromatographically.

Results

A check on the extraction of fenitrothion by the proposed method showed that the recovery was better than 95 per cent. The limit of sensitivity was approximately 0·1 p.p.m., *i.e.*, 2 nanograms (10^{-9} g) of fenitrothion in the portion of solution injected. The decreased sensitivity found

in the cocoa extract compared with that of a solution of pure fenitrothion is due to the presence of extraneous extractives that emerge from the chromatographic column at the same time as the fenitrothion.

At the conclusion of the work the opportunity was taken to study the gas - liquid chromatographic behaviour of other pesticides chemically similar to fenitrothion; these were parathion and chlorthion. Retention times at various temperatures for all three pesticides are given in Table I. Maximum sensitivity was achieved by using a column temperature of 175° C; the minimum detectable amount of each pesticide at this temperature is—

Fenitrothion, 0·2 nanogram; parathion, 0·2 ng; chlorthion, 0·2 ng; paraoxan, 5 ng.

The minimum detectable amount is defined, in this instance, as the amount of residue that gives a peak height of 1 per cent. full-scale deflection under normal operating conditions when the baseline noise value is less than 0·5 per cent. full-scale deflection.

Similar results are given for paraoxon, a highly toxic metabolite of parathion; when a mixture of all four compounds were examined, however, the paraoxon peak was satisfactorily resolved only at 160° C.

TABLE I

RETENTION TIME AT DIFFERENT COLUMN TEMPERATURES

Nitrogen flow-rate was 6 litres per hour

Temperature, °C	Retention time, minutes			
	Fenitrothion	Parathion	Chlorthion	Paraoxon
200	1·6	1·8	2·0	2·1
175	4·2	4·8	5·4	6·0
160	7·4	8·5	9·6	11·2

The quantitative behaviour of parathion was studied over the range 0·2 to 250 nanograms, with a column temperature of 175° C. The peak heights obtained were plotted against the weight of parathion and gave a smooth curve with a useful working range from about 0·2 to 25 nanograms of parathion (see Fig. 1).

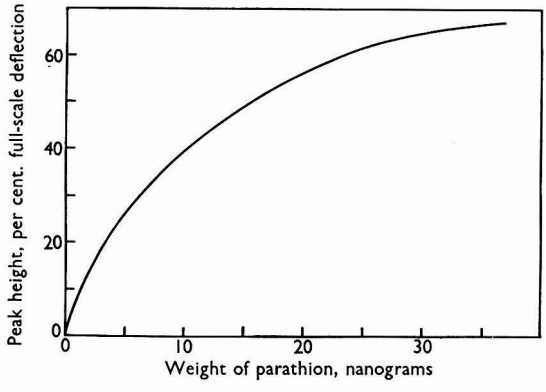

Fig. 1. Calibration curve for parathion

Chlorthion and fenitrothion were not examined in such detail as parathion; the results obtained, however, indicated a quantitative behaviour similar to that of parathion. Paraoxon behaved in a markedly different manner from the other compounds and the sensitivity of detection was only about one-twentieth of that of parathion, chlorthion and fenitrothion.

This investigation has formed part of a programme of research of the Tropical Products Institute and the results are published by permission of the Director.

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An Improved Procedure for the Extraction of Organo-chlorine Pesticides from Animal Tissue

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Although the method described by Taylor[1] for extracting organo-chlorine hydrocarbon pesticides from avian and mammalian tissues is quick, it results in poor recoveries, *e.g.*, 63 per cent. for γ-BHC and 69 per cent. for heptachlor epoxide. With a view to improving the recovery we have tried steam-distillation as suggested by Davidow and Sabatino,[2] and by Hartzell, Storrs and Burchfield.[3] The latter authors obtained 100 per cent. recovery of lindane, 50 per cent. recovery of aldrin and 40 per cent. recovery of methoxychlor. Davidow and Sabatino give no recovery figures and used the distillate obtained only for biological testing. We have carried out steam-distillation and subsequent gas - liquid chromatographic examination of cereals and avian tissues to which known amounts of γ-BHC or dieldrin had been added. In the instance of γ-BHC this was added either as a solution in acetone or as a mixture with lactose. In either instance it was not possible to recover more than 50 per cent. of the added insecticide by steam-distillation. All the γ-BHC that could be recovered was found in the first 75 ml of distillate. Steam-distillation of the sample immediately after the addition of an acetone solution of γ-BHC to whole grain gave complete recovery; as the interval between addition and distillation increased, the percentage recovery decreased, until after 6 days only about 45 per cent. of the added γ-BHC was recovered. Only some 50 per cent. of the γ-BHC was recovered from a γ-BHC - lactose mixture after 24 hours. The addition of 0·075 mg of γ-BHC in n-hexane solution to 15 g of pigeon-breast muscle and subsequent steam-distillation led to the recovery of 0·0167 mg, equivalent to 22 per cent. The addition of a similar amount of dieldrin also in solution in n-hexane resulted in 0·0052 mg being found, or some 7 per cent. A duplicate determination gave a similar result.

It was decided to re-examine the extraction method currently in use in this Laboratory. The filtration procedure was improved by the use of three-piece Buchner funnels as modified by Hartley.[4] Known amounts of various chlorinated pesticides were added to 15 g of pigeon-breast muscle at several levels, and the recoveries obtained are given in Table I. It can be expected

Table I

Recovery of added chlorinated pesticide from pigeon flesh

Insecticide	Added, μg	Found, μg	Recovery, per cent.
γ-BHC	150	145	97
	112·5	106	94
	75	57·5	77
	37·5	29	77
Endrin	150	147	98
	112·5	94·5	84
	75	62	82
	37·5	28·9	75
Dieldrin	150	113	76
	112·5	93	83
	75	63	84
	37·5	31	83
pp'-DDE	150	125	83
	112·5	95	86
	75	61	81
	37·5	37	99
Heptachlor epoxide	150	132	89
	75	63	85
	37·5	32·7	88

that similar recoveries should be obtained from other species. The recommended procedure for extracting organo-chlorine pesticides from animal tissue for subsequent gas - liquid chromatographic examination is given below.

* Present address: Midland Tar Distillers Co. Ltd., Four Ashes, Staffordshire.
† Present address: Union International Co. Ltd., West Smithfield, London, E.C.1.

METHOD

Macerate 15 g of breast muscle or liver (with small animals use all the liver) with half this weight of anhydrous sodium sulphate and about 40 to 45 ml of re-distilled acetone for 4 minutes in an MSE homogeniser (Cat. No. 7700) fitted with a 100-ml beaker. Pour the macerated tissue into a 10-ml Hartley funnel fitted with a Whatman No. 1 filter-paper, and filter it under slight suction. Replace the macerate in the MSE beaker, homogenise it with a further 25 ml of acetone, and then re-filter the macerate through the Hartley funnel after the funnel has been fitted with a second paper. Wash the beaker with about 20 ml of acetone, and filter the washings through the funnel. Transfer the combined filtrates to a separating funnel and then add 30 ml of water and 40 to 50 ml of n-hexane. Shake the funnel vigorously, allow the layers to separate, and run off the hexane layer into a 100-ml calibrated flask through a funnel containing a small amount of anhydrous sodium sulphate. Re-extract the acetone extract with two further 30-ml portions of n-hexane, separate the hexane layers, dry them and run them into the calibrated flask. Dilute the solution to the mark with n-hexane and examine this solution by gas - liquid chromatography as previously described.

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Recovery of Malathion Residues from Pimento

By D. G. ROWLANDS

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DURING an investigation into the recovery of malathion from various foodstuffs, erratic but consistently low figures were recorded for pimento, seemingly for reasons other than those applicable to other commodities studied.[1,2,3]

Pimento extracts were extremely dark, and some of the colouring matter passed into the aqueous layer with the dimethyl dithiophosphoric acid, thus masking the colour change at the stage where excess of alkali is neutralised and making it difficult to determine accurately the amount of alkali that remained after hydrolysis; this has previously been a useful criterion for indicating the presence of extraneous plant extractives, *e.g.*, free fatty acids.[2,3] The amount of free fatty acids present in pimento is negligible. When acidic ferric chloride was added to the extract in order to prevent possible reduction of cupric ions added later, the colour intensified and passed into the organic layer to give a yellow colour disconcertingly like that of the final chelate formed between cupric ions and the dimethyl dithiophosphoric acid. Several extra washes of the extract with organic solvent successfully removed this artifact, but high and variable blank values were obtained corresponding to 28 to 60 μg of "apparent" malathion. Moreover, the introduction of extra washes of the extract lengthens a regulated procedure and may mechanically remove dimethyl dithiophosphoric acid from the aqueous layer. Recoveries of malathion from pimento extracts analysed by this procedure ranged from 18 to 70 per cent.

Pimento is the dried, unripe fruit of *Pimenta officinalis* and contains from 3 to 5·2 per cent. of volatile oil,[4] of which up to 70 per cent. may be eugenol (4-allylguaiacol).

To ascertain whether this substance was the source of the yellow colour, about 100 μg of re-distilled eugenol were dissolved in 100 ml of carbon tetrachloride and gave a colourless solution. In accordance with the method of Norris, Easter, Fuller and Kuchar,[5] the solution was shaken with 1 ml of 6 N sodium hydroxide and a deep yellow colour was produced that was transferred to the aqueous layer when the solution was shaken with 75 ml of aqueous sodium sulphate solution. When the solution was neutralised with 6 N hydrochloric acid and acidic ferric chloride solution was added, the colour intensified and was successively extracted into the organic layers. The yellow artifact is, therefore, probably caused by eugenol extracted with the malathion.

Straightforward chromatography of the extracts on acid-washed alumina, silica gel or Fuller's earth did not improve the erratic nature of the recoveries. Run times often ranged from 20 minutes to 3 hours for identical extracts and absorbents, and many columns, notably those composed of Fullers' earth mixed with kieselguhr, had to be abandoned altogether. Centrifugation of the extract before its application to the columns, or partition with either acetonitrile[6] or aqueous methanol[7] proved of little value in assisting reproducibility of the flow-rate, though the neutralisation step was improved.

After a report had been published by Coffin and McKinley[8] on the use of polyethylene-coated alumina for the clean-up of plant extracts before chromatography on other absorbents, this material was tried, chiefly as a filtration device before the chromatography on acid-washed alumina.

By using this technique, reproducible recoveries of malathion added to pimento at both the 3 and 5 p.p.m. levels were obtained in the range 80 to 86 per cent., control blank values were low and the neutralisation stage satisfactory (see Table I).

TABLE I

RECOVERY OF MALATHION FROM EXTRACTS OF 50 g OF PIMENTO CHROMATOGRAPHED ON POLYETHYLENE-COATED ALUMINA AND ACID-WASHED ALUMINA*

Malathion added before extraction, μg	Malathion recovered, μg	Recovery, per cent. (corrected for blank values)
Nil	(6)	—
Nil	(9)	—
Nil	(9)	—
156	133	80
156	138	83
156	142	86
245	214	84
245	216	85
245	220	86

* No extra washes required.

METHOD

APPARATUS—

Soxhlet extraction apparatus.

Chromatographic tubes—Glass tubes 1 to 2 cm in diameter and 65 cm long, tapered at the lower end to retain a cotton-wool plug.

REAGENTS—

Polyethylene-coated alumina—As described by Hoskins, *et al.*[9]

Other reagents—As listed by Bates, Rowlands and Harris.[3]

PREPARATION OF EXTRACTS—

Extract 50-g samples of crushed pimento in a Soxhlet apparatus for 8 hours with n-hexane. For recovery experiments add a suitable amount of a solution of malathion in hexane to the Soxhlet thimble before extraction.

PROCEDURE—

Pass the extract (about 200 ml) under suction at 20 ml per minute through a column containing 15 g of polyethylene-coated alumina and an upper layer of 2 g of anhydrous sodium sulphate. Carefully wash out the flask with a further 50 ml of hexane and drain the column. Elute the malathion with 25 ml of acetonitrile to remove any malathion left on the column. Pass the hexane eluate through another column of 20 g of acid-washed alumina (Brockmann grade, II - III) and 2 g of sodium sulphate, at the same rate as before, and drain the column. Discard the hexane fraction, and elute the column with 25 ml of acetonitrile. Transfer the acetonitrile eluates from both columns to a separating funnel with 100 ml of carbon tetrachloride and shake them with 75 ml of 9 per cent. aqueous sodium sulphate solution, 75 ml of water and 5 ml of concentrated hydrochloric acid. After the organic layer has separated, run it through a fluted filter-paper into a second separating funnel and continue the hydrolysis and colour development according to the method of Norris, Easter, Fuller and Kuchar.[5]

DISCUSSION

It appears that satisfactory recoveries can be obtained for malathion residues on pimento by chromatography on acid-washed alumina, but that, to ensure effective running of columns, preliminary chromatography on polyethylene-coated alumina is essential.

I thank Mr. F. N. Wright for obtaining an amount of pimento, Dr. Coffin and Dr. McKinley of Ottawa for a sample of polyethylene-coated alumina and Mrs. M. B. Koszuta and Dr. E. A. Parkin for the interest they have shown in this work.

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Book Reviews

THE QUANTITATIVE ANALYSIS OF DRUGS. By D. C. GARRATT, D.Sc., Ph.D., Hon.M.P.S., F.R.I.C. Assisted by L. BREALEY, B.Sc., Ph.D., F.R.I.C., C. A. JOHNSON, B.Sc., B.Pharm., F.P.S., F.R.I.C., K. L. SMITH, M.P.S., and G. SYKES, M.Sc., F.R.I.C. Third Edition. Pp. xiv + 925. London: Chapman & Hall Ltd. 1964. Price 126s.

Even specialised subjects now cover so much ground and embrace so many scientific disciplines that it is becoming increasingly difficult for a single author to write an effective treatise. So it is with the subject of Drug Analysis. In preparing the third edition of his well known book, Dr. Garratt has wisely sought the help of four eminent experts who have described the application of their own special subjects to the examination of drugs. These contributors are Dr. L. Brealey (Physical Assays), Mr. C. A. Johnson (Chemical Assays), Mr. K. L. Smith (Bioassays) and Mr. G. Sykes (Microbiology). With such a team at work one expects a good result, and certainly we have it here.

The excellent plan of the original book, which was published in 1937, has been retained, and starting with Acetic Acid and ending with Zinc the assay of all substances of importance in pharmacy and all their pharmaceutical preparations are discussed. I am particularly pleased to note that the references to the old pioneers have been carefully preserved and the historical background maintained. At the same time there is nothing old fashioned about the descriptive matter, and relevant references even up to August, 1963, are included. This edition is larger than the last by 256 pages, and in considering the main body of the work, that is, the description of the quantitative analysis of substances in the alphabetical series, one notices that Steroids now occupy 23 pages compared with only 5 in the second edition. This, of course, is due to the substantial advances made during the last few years. There are now 8 pages on Digitalis as against a mere 2, but on the colorimetric side mention might have been made of the work of D. L. Keddie, J. M. Rowson and D. H. E. Kattje with *m*-dinitrobenzoic acid; however, the Keller - Kiliani *m*-dinitrobenzene method as given in the ninth edition of the Official Methods of Analysis of the A.O.A.C. and in the U.S. Pharmacopoeia XVI is described in full.

An important section new to this edition is that on Antibiotics: there are 25 pages in the main body of the book describing chemical and physical methods besides Appendix VII (10 pages by Mr. G. Sykes) giving an excellent account of microbiological methods as applied to antibiotics and vitamins. This Appendix embraces valuable tables giving the details of the organisms used and other conditions for the microbiological assay of the various antibiotics, a corresponding table for vitamins, and a third table showing the composition of culture media for the assay of the latter.

The Appendixes were always a feature of this book and their number has now been increased from 13 in the second edition to 22, embracing *inter alia* the former chapter on physical methods. Thus we now have, besides the microbiological methods, Appendixes on Sterility Tests, Pyrogen Testing, Electrometric Titrations, Flame Photometry, Gas Chromatography, Infra Red Spectrophotometry, Interpretation of Analytical Results, Oxygen-flask Combustion Technique and other new features. Most of these Appendixes are excellent and put the various subjects into a nutshell of actual practice, but one would have liked a somewhat more comprehensive treatment of the all-important new technique of gas chromatography. There are, of course, other books available devoted entirely to this subject; nevertheless, the busy consulting analyst turns almost every day for help from "Garratt." The Appendix on Interpretation of Analytical Results, which concerns statistical treatment, is appropriate for biological work, but I am pleased to read that "To what extent such application is necessary . . . to the treatment of chemical results . . . is a matter of opinion." So far as chemical analysis goes, we are only concerned with the *right* results. Nevertheless, it should be emphasised that this Appendix is a very good one indeed.

Referring again to the main body of the book, the general monographs arranged in alphabetical order of substances, it is important for a reviewer to mention that, apart from describing the usual procedures, there is everywhere the personal touch with discussion of methods developed by the author himself for overcoming difficulties. This is especially so in the accounts for the analysis of those preparations that contain several ingredients.

Although the work is necessarily based on the British Pharmacopoeia 1958 and the British Pharmaceutical Codex 1959, owing to the unique and eminent position held by the author in the analytical and pharmaceutical sciences, the modern methods of the British Pharmacopoeia 1963 and the British Pharmaceutical Codex 1963 are included and discussed.

Only the contribution of one of Dr. Garratt's collaborators has been specifically mentioned but, knowing them all, it is easy for me to recognise the high quality of the work achieved by the others. Dr. Garratt's aim in this edition has been to cover the lot, and without doubt his object has been achieved. In the Preface the help of many collaborators, additional to those whose names appear on the title page, is graciously acknowledged. Every analyst, no matter what his specialised subject may be, should possess a copy of "Garratt" because it is certain to serve him well.

NOEL L. ALLPORT

SECOND REPORT OF THE TOXICITY SUB-COMMITTEE OF THE MAIN TECHNICAL COMMITTEE OF THE BRITISH PLASTICS FEDERATION, WITH METHODS OF ANALYSIS OF REPRESENTATIVE EXTRACTANTS. Pp. 65 (loose leaf). London: The British Plastics Federation. 1962. Price 30s.

The increasing interest being taken in the migration of possibly toxic substances from packaging materials into foodstuffs and the prospect of legislation regulating the use of additives in such packaging materials makes this report of especial interest to all concerned with packaging at the present time, many of whom will be analytical chemists.

After discussing the toxicity problems associated with the use of plastics materials, the report proposes a method for the assessment of the toxic hazards that might arise from the use of foodstuffs either packaged in plastics materials or coming into contact with plastics surfaces in handling equipment. The method is based on the derivation of a toxicity quotient, Q, by the use of the expression $Q = \frac{E}{T} \times 1000$, where E is a measure of the amount of a particular additive extracted by a food-simulating extractant under controlled conditions, and T is a measure of the toxicity of the additive expressed as a toxicity factor. The food-simulating extractants used are distilled water, a 5 per cent. w/v aqueous solution of anhydrous sodium carbonate, a 5 per cent. w/v aqueous solution of citric acid, a 50 per cent. w/v aqueous solution of ethyl alcohol, and Olive Oil B.P.C. to which 2 per cent. w/w of oleic acid has been added (except in instances where analysis in this medium is impracticable, when the analytical method may specify the use of medicinal paraffin B.P. Lev. in its place). The extraction tests proposed vary in severity depending on the extent of a food's likely exposure to the plastics material and the form that that material may take, *i.e.*, thick or thin section. The toxicity factor approximates to the maximum daily oral dose expressed in mg per kg weight that is tolerated for 90 days by groups of animals without producing any detectable toxic effect. A preliminary list of toxic factors for some 230 individual

substances is given, and each factor has been classified into one of four categories of reliability; the data required by the Federation to permit such toxicity factors to be assessed is discussed in some detail.

To determine the quantity E above, it is necessary for p.p.m. amounts of an additive to be determined in the extracting liquids. Detailed methods, which have been critically examined by the Federation's Analytical Methods Sub-Committee, are included for the determination of barium (flame-photometric and polarographic methods), cadmium (colorimetric and polarographic methods), copper, lead, 2,2′-methylenebis-(4-methyl-6-t-butylphenol) (Calco **2246**) and *NN*′-di-β-naphthyl-*p*-phenylenediamine (Nonox CI). Since it is possible that those who carry out the extraction from the plastics materials may not be analysts, some elementary advice is appended so that the analyst may receive the extractants in a form suitable for his purpose.

The report is issued in loose-leaf form since it is envisaged that additions or amendments may be made from time to time, particularly new toxicity factors and new analytical methods.

A. G. JONES

KIRK-OTHMER ENCYCLOPEDIA OF CHEMICAL TECHNOLOGY. Volume 2. ALUMINUM COMPOUNDS TO AZO DYES. Edited by HERMAN F. MARK, JOHN J. MCKETTA, jun., DONALD F. OTHMER and ANTHONY STANDEN. Second Edition. Pp. xvi + 910. New York, London and Sydney: Interscience Publishers, a division of John Wiley & Sons. **1963**. Price £16 18s.; price per volume for subscribers to the complete set of 18 volumes £13.

Volume I of this impressive Encyclopedia was reviewed in *The Analyst* (**1963**, **88**, 899). The point was then made that it is extremely difficult to assess a work of this kind from the alphabetically first of 18 volumes. The second volume has now appeared and covers words, or rather subjects, from Aluminum Compounds to Azo Dyes, inclusive. It is surprising how many important subjects come within these alphabetical limits, *e.g.*, Amines and Ammonia and their derivatives and compounds, Anesthetics, Aniline, Antibiotics, Antimony, Antioxidants, Arsenic and its compounds, Ascorbic acid and Asphalt. In consequence, the general plan of the work and the method of treatment are now beginning to fall into some kind of recognisable perspective.

It is important to appreciate from the point of view of the analyst what this is likely to be. First of all there is no monograph or entry under the heading Analysis so, presumably, one must look for analytical details where required under individual headings. Secondly, if the system adopted in the first two volumes already published is followed subsequently, it would appear that the analytical aspects of purely chemical subjects (such as arsenic, aniline, antimony and ascorbic acid) will receive quite brief, even cursory, treatment. On the other hand, testing methods for classes of substances such as anti-freeze products, bitumens or asphalt, are being described in some detail; incidentally, American standard methods are being used where these exist.

On the other hand, there is an extremely comprehensive monograph of some 28 pages on Aquametry, and this is well done. As an indication of what may be expected from articles of this kind, its scope is as follows. It first deals with chemical methods such as use of the Karl Fischer reagent, and includes the dead-stop technique; less familiar chemical methods involve the use of acid chlorides and acid anhydrides. Then are described gravimetric methods, such as simple weighing, absorption or freezing; then separation techniques, such as distillation; and optical methods in which the ultraviolet and infrared regions are used. There are also sections on nuclear magnetic resonance spectroscopy as applied to the measurement of water, radiochemical methods, and methods depending on sonic phenomena, thermogravimetry and even electrolysis—the last use in particular for the determination of water in gases. This is the first really analytical subject to have been published in this series, and it augurs well for any of a similar nature that may come later.

As far as can be seen from these two volumes, the general arrangement of subject headings does give rise to some doubts as to the system of classification used. For instances, one wonders how many analysts interested in the determination of water would look for a dissertation on the subject under the letter A. Certainly few in this country. "Aromaticity" is another subject heading, under which the theory of the benzene ring and its related subjects is dealt with in general. Semantically the use of the word is logical enough, but it is certainly not in common use, and an article of this nature might pass unheeded because those likely to be interested would not know of its existence. Much the same applies to Automobile Exhaust Control, a subject of great topical

interest, but on the fringe of the range of an encyclopedia of chemical technology. In the present instance the 25 pages involved deal principally with the composition of the gases evolved under various conditions of combustion, atmospheric pollution and control (not analytical) methods. It would be a pity if the possessor of a book of this kind should fail to be able to make the maximum use of it merely because he did not know what was between its covers. It is therefore to be hoped that cross-referencing will be worked out in the greatest possible detail in the volumes that follow.

It is also to be hoped that information of topical interest will be dated. An example is the list of antioxidants with indications of those that are permitted for addition to foodstuffs in various countries of the world. These regulations are continually changing, and those cited for Great Britain are already out of date. This obviously is inevitable, but it would be of some help to know the date at which the list was compiled.

Quite apart from these questions of analytical methods and arrangement or classification, the same general comments apply to the second volume as to the first. This is without any doubt an invaluable series for those whose interests are in any way connected with chemical technology.

JULIUS GRANT

CHEMICAL INFRARED SPECTROSCOPY. Volume I. TECHNIQUES. By W. J. POTTS, jun. Pp. xvi + 322. New York, London and Sydney: John Wiley & Sons Inc. 1963. Price 64s.

For many years the most popular standard reference books used by infrared spectroscopists have been those written by Drs. L. J. Bellamy and A. D. Cross. More recently the coverage has been broadened by Bauman's Absorption Spectroscopy and Brugels' Introduction to Infrared Spectroscopy. These authors have largely devoted their activities to teaching and research at universities. The book under review has been written with a background of experience gained by the Chemical Physics Research Laboratories of the Dow Chemical Company. Two volumes are proposed: the first, under the sub-title Techniques, deals largely with the production of spectra for both quantitative and qualitative analysis. In this sense the present and proposed volume will undoubtedly bridge the substantial gap between the university and industrial research worker and, more particularly, the industrial analyst. Since the advantages of quantitative infrared analysis have become more widely recognised and comparatively low-cost instruments have become available, the technique finds potential application in even the smallest laboratory. It is for the chemist starting in this branch of analysis that the book under review is particularly suitable. It offers the additional facility that it describes more recent systems of instrumentation and technique which increasingly extend the type of materials capable of analysis.

The first 7 sections of Volume I deal with The Nature and Properties of Infrared Radiation, Elementary Theory, Absorption of Infrared Radiation by Molecules, Spectrometer Optics, The Basic Infrared Monochromator, The Performance and Operation of Infrared Spectrometers, Sample Preparation and Techniques, Quantitative Analysis, Auxiliary Devices and Special Techniques. All these topics have been dealt with in the traditional manner but with greater emphasis on practical manipulation, particularly in terms of obtaining the optimum performance from any one instrument. It is in this sense that the volume is thoroughly recommended.

Section 8 claims to be an outline of theory of infrared spectra of polyatomic molecules. The subject requires a knowledge of mathematics quite beyond the usual graduate chemistry course. Any attempt at explanation becomes largely a matter of what shall be assumed as understood by the reader. The author has attempted to condense the material into 54 pages, but the complexity of the subject has necessitated so many references to other sources that the presentation is difficult to follow. I feel that this section is out of context with the preceding subject matter and is largely an introduction to Volume II on spectral interpretation.

Apart from this criticism the subject matter is well presented with clear illustrative diagrams and spectra. About ten selected references are given at the end of each section and these have been very well chosen. The purist will criticise terminology used in some of the practical expositions, some confusion of script notation exists in Fig. 6–14, and little excuse can be offered for mispelling "spectra" in the title of Section 8. The book claims to be a practical guide to techniques by which infrared spectroscopy is applied to chemical problems. The author has undoubtedly succeeded in those aspects covered by Volume I, and I await with enthusiasm the appearance of Volume II.

W. L. SHEPPARD

METHODS IN IMMUNOLOGY: A LABORATORY TEXT FOR INSTRUCTION AND RESEARCH. By DAN H. CAMPBELL, JUSTINE S. GARVEY, NATALIE E. CREMER and DIETER H. SUSSDORF. Pp. xvi + 263. New York and Amsterdam: W. A. Benjamin Inc. 1963. Price $9.60.

This book is a manual of laboratory experiments for a course in immunology that is limited by the omission of any reference to viruses. The introductory paragraphs, covering the handling of laboratory animals and the preparation of antisera, are concise and form a convenient source of reference.

Macro methods have been used in the laboratory exercises; these are admirable for demonstrating immunological phenomena to the student, but are wasteful of materials and not the most suitable for general use. Apparatus that is found only in specialised laboratories is used unnecessarily, and end-points are calculated by methods unduly accurate and complex in relation to the sensitivity of the experimental procedures recommended. Some simpler experimental methods and experiments involving organisms non-pathogenic for man would have been advantageous to the elementary student, whereas the advanced student would have benefitted by the inclusion of some of the micro methods that conserve valuable biological reagents. G. FURNESS

THE DETECTION AND DETERMINATION OF ANTIOXIDANTS IN FOOD. Special Report No. 1. By The Association of Public Analysts. Pp. 27. London: The Association of Public Analysts. 1963. (Obtainable by post from the Hon. Treasurer, The Association of Public Analysts, County Hall, Maidstone, Kent.) Price 10s.

The history of food control in this country made a big step forward with the issue of "The Antioxidants in Food Regulations," by which certain antioxidants were permitted to be added in specified proportions to specified foods such as oils and fats, butter, and vitamin oils and concentrates, and also to essential oils. The Regulations are of obvious importance to manufacturers of fatty foods and to food authorities and public analysts. The Association of Public Analysts is to be congratulated on setting up a Committee to investigate methods for the detection and determination of these additives soon after the Regulations appeared, and on the success that has attended the efforts of the Committee. The Committee's conclusions are reported in this monograph. After a description of the properties of the various antioxidants, it gives directions for their extraction from oils and fats. Gallates are detected by a qualitative test, and it is shown how they may be separated chromatographically and determined. The butylated hydroxy compounds are similarly dealt with. A further section of the monograph makes observations on antioxidants not at present allowed in this country. K. A. WILLIAMS

FOOD CHEMICALS CODEX. Part I. Publication 1143. Prepared by the Committee on Specifications of the Food Chemicals Codex of the Food Protection Committee, National Academy of Sciences - National Research Council. Pp. xii + 118 (loose leaf). Washington, D.C.: National Academy of Sciences - National Research Council. 1963.

This loose-leaf book is the first part of the Food Chemicals Codex projected by the National Academy of Sciences and the National Research Council of the U.S.A. and intended to provide published, organised, national standards for food-additive chemicals comparable to those long available in the U.S.A. for drugs.

It is envisaged that the whole work will comprise 8 or 10 sections to be issued at 4-monthly intervals and that it will be published in book form in 1966. The standards included in this section are designed for chemicals of sufficiently high purity to be used in foodstuffs. There are 25 monographs dealing with such chemicals as acetic acid, ascorbic acid, sorbic acid, caffeine, potassium bromate, glycine, propyl gallate and thiamine hydrochloride. More than half the book is taken up with descriptions of methods of assay applicable to food chemicals, and the test methods given are for the most part familiar in character. K. A. WILLIAMS

Erratum

MAY (1964) ISSUE, p. 364, Legend to Fig. 1. *For* "V_1 = VA26T stabiliser" *read* "V_1 = VA26T photoelectric cell."