The ANALYST

The Journal of the Society for Analytical Chemistry

A monthly International Publication
dealing with all branches of
Analytical Chemistry

Published for the Society by
W. HEFFER & SONS LTD., CAMBRIDGE

Volume 93

No. 1113, Pages 773-836 **December 1968**

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

OFFICES OF THE SOCIETY: 9/10 SAVILE ROW, LONDON, W.1

Telephones: 01-734 6205 (General Enquiries and *Analytical Abstracts*)
01-734 3419 (*Analyst* Editorial only)

Advertisement Agent: J. Arthur Cook, 9 Lloyd Square, London, W.C.1
Telephone: 01-837 6315

Subscription Rates (other than for Members of the Society):
The Analyst, *Analytical Abstracts*, *Proceedings* and indexes, £25 ($60) per annum, post free

Subscriptions are NOT accepted for *The Analyst* alone

Single copies From January, 1969 £2 5s. ($5.50)

Volume 93, No. 1113 December, 1968

THE ANALYST

The Effect of Filtration and Centrifugation on Raw Sugar Polarisation Analysis

BY R. A. M. WILSON, C. G. SMITH, R. H. JAMES AND R. R. WALLACE
(The Colonial Sugar Refining Co. Ltd., Central Laboratory, Sydney, Australia)

In raw sugar analysis, the apparent sucrose content is determined as its polarisation value, a measure of the rotation of plane-polarised light passing through a solution of the sugar, which is directly proportional to sucrose concentration when the impurity level is low.

Before taking a reading with a polarimeter, a "normal" raw sugar solution (26·000 g per 100 ml) is clarified by addition of basic lead acetate solution to precipitate impurities, which are removed by simple filtration; about 50 ml of filtrate are needed for analysis. When the solution is filtered, the sucrose concentration increases because of preferential absorption of water by the filter-paper. Initially in the current study, the magnitude of the absorption has been examined, and it has been determined that the first 10 ml of filtrate should be discarded to minimise this effect.

Differences of 1 part in 40,000 in the polarisation value of sugar solutions, however, can now be detected by using modern photo-electric polarimeters and the refined analytical techniques described in this paper. This compares with 1 part in 2000 when using older visual instruments and standard techniques. Therefore, an insight into evaporation and humidity effects has also been gained, and a comparison of filtration and centrifugation, an alternative method of clarification, made.

Centrifugation has practical advantages over filtration in laboratories in which large numbers of analyses are performed. In addition, it is not subject to preferential absorption and evaporation errors, and currently appears to give the best estimate of polarisation value, as defined and specified by the International Commission for Uniform Methods of Sugar Analysis (ICUMSA).

THE polarisation value of a sugar is a measure, in units called International Sugar Degrees (° ISS), of the rotation of plane-polarised light passing through a solution of the sugar. The basis of the 100° point on the International Sugar Scale is defined by the International Commission for Uniform Methods of Sugar Analysis (ICUMSA)[1] as the optical rotation of the "normal" (26·000 g per 100 ml weighed in air) solution of pure sucrose in a 200·000-mm tube at 20·00° C at the wavelength of the green line of the mercury-198 isotope ($\lambda = 546{\cdot}227$ nm *in vacuo*).

The "normal" solution of a raw sugar is usually not clear enough to enable a reading to be taken with a polarimeter, and is, therefore, first clarified by the addition of basic lead acetate solution. This forms a flocculent precipitate that settles at the bottom of the flask, carrying with it suspended particles. The solution is separated from the precipitate by filtration, for which a 3-inch diameter stemless filter funnel (covered to minimise evaporation) resting in a 200-ml filter glass is used. The polarisation value of the filtrate is then determined as the optical rotation in ° ISS measured in a sugar polarimeter.

Hardin and Zerban[2] have reported that the first portions of sugar solution passing through a filter-paper undergo an increase in polarisation, which they attributed to preferential absorption of water from the solution by the filter-paper. To minimise this effect, they recommended that at least 25 ml of initial filtrate be discarded. The latest official ICUMSA method for raw sugar polarisation,[3] however, specifies that 10 ml be discarded.

At the time of the 14th Session of ICUMSA, in 1966, it was not known whether an air-dried filter-paper would reach equilibrium with the solution after only 10 ml had been filtered, or whether the concentration of the subsequent filtrate, and hence the polarisation value, would still be affected. It was, therefore, recommended by this Session[4] that "the optimum amount of filtrate discarded be investigated." This amount needs to be sufficient to ensure that the filtrate is unaffected by preferential absorption, but as small as possible to reduce the sample volume requirements, the time needed to complete the analysis and the consequent risk of concentration changes from solution evaporation.

The primary aim of the work presented in this paper, therefore, was to determine the optimum amount of filtrate to be discarded. During the course of the work, however, the development of improved analytical techniques, in conjunction with the use of a high-precision photo-electric polarimeter, enabled a detailed study of evaporation and humidity effects, and a comparison of filtration and centrifugation methods, to be undertaken.

In the usual method of centrifuging, the leaded raw sugar solution is sealed in a 50-ml capacity stainless-steel tube of about 2·7-cm diameter and centrifuged with either an MSE High Speed 17 or a Sorvall SS-3 Automatic Superspeed centrifuge. The speed is increased to 14,000 r.p.m. and then decreased to zero, under automatic braking. The cycle time is about 9 minutes for the MSE centrifuge and 5 minutes for the Sorvall machine. Under these conditions, the clarity of the final solution, measured as optical density at both 546 and 589 nm, is as good as, or better than, that of filtered solutions.

If a raw sugar solution is clarified by centrifugation there is a significant difference in its polarisation value, but centrifugation has practical advantages over filtration in a laboratory in which large numbers of samples are being analysed. In addition, centrifuged samples should not suffer from the effects of evaporation, preferential absorption or other possible sources of error occurring with filtration; therefore, centrifugation should give a better estimate of the polarisation value, as defined and specified by ICUMSA, than filtration.

The 14th Session of ICUMSA,[4] in 1966, also recommended "that the optimum moisture content in filter-paper . . . be investigated." The current study has been extended to include a determination of the equilibrium moisture content of filter-paper and a limited attempt to gauge the effect of filter-paper moisture content on the polarisation result.

Experimental technique

Use of high-precision polarimeter—

Before 1960, the polarisation value of a clarified raw sugar solution was determined with a visual polarimeter, calibrated in ° ISS, by measuring the rotation of plane-polarised light passing through the solution contained in a cell of 200-mm length. For a single, clear solution, two consecutive readings may differ by up to 0·10° ISS (1 part in 1000). The usual practice is to determine the average of five readings to the nearest 0·01° ISS, but the standard error of the mean is about 0·02° to 0·04° ISS, depending on the type and condition of the instrument, and the experience of the analyst.

In the early 1960's, the Bendix-NPL, Model 143A, automatic polarimeter was introduced into this Laboratory for raw sugar analysis. This instrument combines such features as short cell length (10 mm) and, therefore, small angular rotation, with the Faraday magnetic - optic effect for modulation of the plane of polarisation and compensation for the angle of rotation. These features made possible a considerable increase in the precision of results, so that consecutive readings seldom differ by more than 0·01° ISS. The use of a Solartron 1420·2 digital voltmeter to monitor the output enables each reading to be made to the nearest 0·0025° ISS (1 part in 40,000). The Bendix polarimeter can, therefore, be used to examine effects producing much smaller polarisation errors than could previously be detected with visual polarimeters.

Collection of small aliquots of filtrate—

To take full advantage of the high instrument precision, special analytical techniques required to be developed to minimise experimental errors in the preliminary solution preparation steps. In particular, special apparatus was designed to facilitate collection of filtrate in small aliquots without introducing sources of error, while at the same time preserving procedural and environmental consistency with normal filtration.

A thistle funnel of 20-ml capacity was attached with wax to the underside of a 3-inch diameter, stemless, glass filter funnel. To keep evaporation at an absolute minimum, 10 ml of filtrate were first collected in the thistle funnel and then, by loosening a spring-clip, released quickly through a short piece of small-bore plastic tubing into a 10-ml calibrated Quickfit test-tube (Fig. 1). The test-tubes were stoppered immediately after filling; the filter funnel was not kept full during filtration, and was, of course, always kept covered.

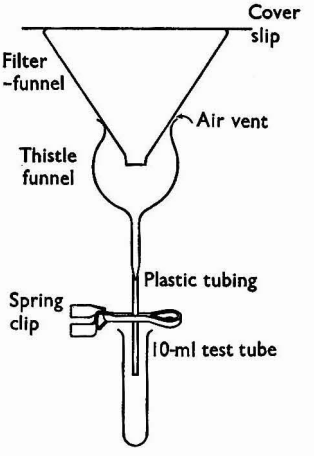


Fig. 1. Filtration apparatus

A "normal" solution (250 ml) of raw sugar (sucrose content between 96 and 100 per cent.) was prepared, defecated with basic lead acetate solution and filtered. For each such solution, 80 ml of filtrate were collected in 10-ml portions, as described above. Simultaneously, the usual filtration procedure was followed as a control, the first 10 ml of filtrate being discarded and about 70 ml collected. Carlson Ford, BIC quality, U4512 filter-papers were used throughout this work. Other papers of similar grade may be used, provided that they are quick filtering and yield a sparkling clear filtrate; preferential absorption characteristics may then be different.

POLARISATION OF SMALL ALIQUOTS ("MINI-POLS")—

At least 50 ml of solution are required to flush and fill a 200-mm visual polarimeter tube, and 30 ml to flush and fill the 10-mm long, 1-ml capacity, Bendix polarimeter flow-through cell by the usual gravity siphoning method. By using a vacuum, however, it was found that a minimum volume of 8 ml of solution was required with a Bendix polarimeter cell when two consecutive solutions differed by no more than 4° ISS; 8 ml of solution, with a small air bubble between samples to reduce sample mixing, was found to flush and fill the cell with no detectable contamination, that is, to within 1 part in 40,000. Without the small capacity of the Bendix cell, a detailed study of the effect of filtrate volume on polarisation would not have been possible.

All of the trials were carried out in a temperature-controlled laboratory at 20° ± 1° C. In addition, the polarimeter cell is provided with a water jacket, and strict temperature control was maintained at 20·0° ± 0·1° C.

RESULTS

TRIAL 1—

Polarisations of twenty-eight raw sugars were examined by the "mini-pol" procedure described above. By using the average polarisation value of the control over all twenty-eight samples as a base-line, the graph of average polarisation value against the progress of the filtration was plotted (Fig. 2).

Results show a decrease in polarisation as filtration progresses, until about 40 ml have been filtered. This presumably results from preferential absorption of water from the solution during the early stages of filtration. At this point the curve is about 0·001° ISS lower than

the control. The polarisation then increases steadily until, after 80 ml, it is about 0·009° ISS higher than the control. The increase is, presumably, caused by evaporation of water from the solution.

Filter-papers used in the trial were in equilibrium with ambient conditions and contained about 7 per cent. of moisture, determined by both Karl Fischer and oven-drying methods. The ambient relative humidity during the trial was 70 per cent.

TRIAL 2—

The alternative procedure of centrifugation was compared with filtration. Trials with thirty-eight raw sugar samples showed a highly significant (more than 99·9 per cent. significance) difference of 0·013° ISS between the polarisation values of solutions clarified by filtration and the same solutions clarified by centrifugation, the results for the former being higher than those for the latter. A horizontal line representing the result by centrifugation as 0·013° ISS lower than the filtered control is drawn in Fig. 2.

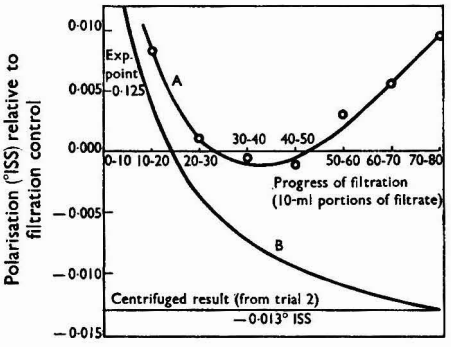


Fig. 2. Effect of filtrate volume on polarisation at 70 per cent. relative humidity (air-dry filter-papers used with about 7 per cent. of moisture). A, Experimental curve; and B, curve corrected for evaporation 0·85 × evaporation at 60 per cent. relative humidity subtracted (see Fig. 5)

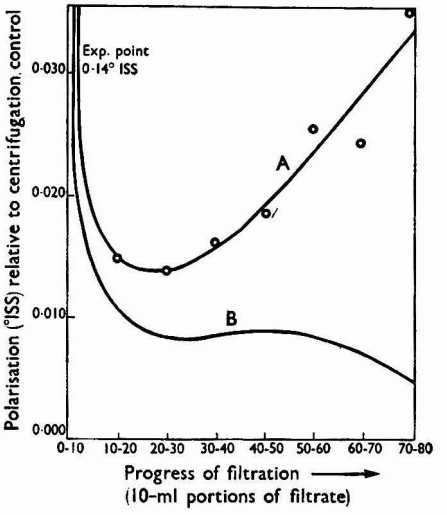


Fig. 3. Effect of filtrate volume on polarisation at 60 per cent. relative humidity (air-dry filter-papers used with about 7 per cent. of moisture). A, Experimental curve; and B, curve corrected for evaporation (see Fig. 5)

Earlier trials with 151 samples had shown a difference of 0·025° ISS, but 0·013° ISS of this was later attributed to insufficient rinsing of the centrifuge tubes and lids with the test solution. Tubes and lids are normally washed with water and, in the early trials, were rinsed only once with the test solution; three rinsings were subsequently found to be necessary, and this practice was adopted for the thirty-eight sugars mentioned above, and for later trials.

TRIAL 3—

The polarisations of seven raw sugars were examined by the "mini-pol" procedure as in Trial 1, except that the control on each sample was centrifuged. Filter-papers again contained about 7 per cent. of moisture, but the atmospheric humidity was lower (60 per cent.). The average polarisation value compared with the control is plotted against the progress of filtration in Fig. 3.

The form of the curve is similar to that for Trial 1, except that the right-hand portion (50 to 80 ml) is about 20 per cent. steeper than for Trial 1; this reflects a higher rate of evaporation at the lower prevailing ambient humidity.

The lowest value of the curve at about 30 ml is higher by about 0·015° ISS, with respect to the centrifuged control, than that in Trial 1, with respect to the filtered control. This agrees well with the result of Trial 2 for the difference in polarisation between filtered and centrifuged samples.

TRIAL 4—

To examine further the filter-paper preferential absorption effect, Trial 3 was repeated on eight raw sugars, except that the filter-papers were pre-conditioned to contain about 12 per cent. of moisture. The average polarisation value compared with the centrifuged control is plotted against the progress of filtration in Fig. 4.

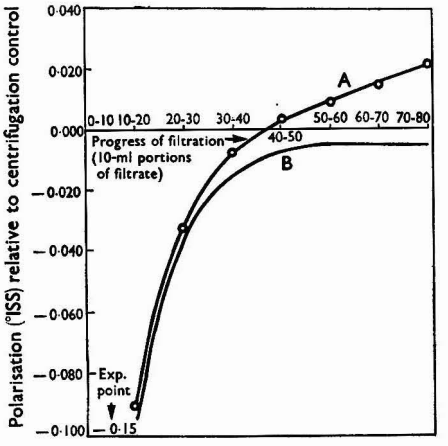


Fig. 4. Effect of filtrate volume on polarisation at 60 per cent. relative humidity (moisture-conditioned filter-papers used with about 12 per cent. of moisture). A, Experimental curve; and B, curve corrected for evaporation (see Fig. 5)

In the early stages, the polarisation increased as filtration progressed until the value of the curve at 40 ml was about the same as the control. The moisture content of the paper was presumably greater than the equilibrium value for the paper in contact with the "normal" sugar solution and, therefore, sucrose instead of water was preferentially absorbed from the solution during the early stages of filtration. From 50 to 80 ml, the curve is similar to that for Trial 3, and suggests that evaporation has become the dominant effect.

TRIAL 5—

To obtain further insight into the evaporation effect, the "mini-pol" procedure was carried out with six previously clarified raw sugar solutions. However, no filter-papers were used. To be consistent with the other trials, about 90 ml of clarified solution were added to the filter funnel (without a filter-paper) and 80 ml collected in 10-ml portions at the same rate as in Trials 1 and 3. The rate of flow was controlled by replacing the thistle funnel with a short length of small-bore plastic tubing and a screw-clip. In this way the preferential absorption effect by the filter-paper was eliminated. Average polarisation results are plotted against the progress of filtration in Fig. 5.

The polarisation of the 70 to 80-ml portion is 0·027° ISS higher than that of the 0 to 10-ml portion, indicating that evaporation causes a significant increase in polarisation during filtration, even when elaborate precautions are taken to prevent it. The prevailing relative humidity during Trial 5 was 60 per cent.

DISCUSSION

In earlier polarisation work, an accuracy of better than 0·01° ISS could not be expected. However, the use of modern photo-electric polarimeters and refined analytical techniques have enabled smooth filtration graphs to be drawn, in which the points are calculated and

plotted with a precision as high as 0·001° ISS, polarisation differences consequently detected with a range of precision far beyond that previously possible and factors causing small but consistent errors studied in detail for the first time.

The factor most likely to affect the polarisation of a sugar solution during filtration is preferential absorption of water or sugar by the filter-paper. The true polarisation value of the solution would then be approached as the volume of filtrate increases. This effect for the first 40 ml of filtrate is shown in Fig. 2. However, the sharp rise in polarisation over the last 30 ml of filtrate cannot be explained by preferential absorption, and is almost certainly caused by evaporation.

An alternative method for the removal of precipitate from a "leaded" raw sugar solution is centrifugation. Trial 2 shows that filtration gives a higher polarisation value than centrifugation, presumably as a result of preferential absorption and evaporation, and a comparison of Trials 1 and 3 (Figs. 2 and 3) confirms this finding. When the lead precipitate in a raw sugar solution is removed by centrifugation, the effects of preferential absorption and evaporation are eliminated.

Two questions remain to be answered—

(*i*) how much initial filtrate should be discarded to minimise the effect of preferential absorption and to obtain a filtered polarisation result as close as possible to the correct polarisation value; and

(*ii*) what is the correct value, and whether a centrifuged solution gives, on average, a more accurate estimate than the corresponding filter solution?

It is best first to consider the two effects, preferential absorption and evaporation, separately.

PREFERENTIAL ABSORPTION—

Karl Fischer moisture determinations have shown that filter-papers used for polarisation analysis have a moisture content of 6 to 8 per cent. when in equilibrium with ambient conditions of 20° C and 60 to 70 per cent. relative humidity. Oven-drying methods, although not precise, have confirmed a value of 7 per cent. for moisture. Papers with this moisture content preferentially absorb water from sugar solutions, as shown in Figs. 2 and 3. If the moisture content were increased, we would expect less preferential absorption in the early stages of filtration and, if sufficiently high, a preferential desorption, that is, the equivalent of an absorption of sucrose from the solution and a consequent underestimate of polarisation from the initial filtrate runnings. However, we would not expect the increase in polarisation from 40 to 80 ml of filtrate, presumably from evaporation effects, to be more than slightly affected by different initial filter-paper moisture contents.

These conclusions are confirmed by the results of Trial 4, shown in Fig. 4, in which papers pre-conditioned to 12 per cent. of moisture were used. Fig. 4 shows a reversed preferential absorption effect, followed by the characteristic increase in polarisation over the last 40 ml of filtrate collected. Therefore, to minimise the effect of preferential absorption, filter-papers should apparently have a moisture content of 9 to 10 per cent., when it should not be necessary, in theory, to discard any of the first runnings of filtrate. This is contrary to some beliefs that an optimum moisture content of 20 per cent. is required.

AMOUNT OF FILTRATE TO BE DISCARDED—

It is difficult to condition filter-papers to a pre-determined moisture content, and the first filtrate runnings need to be discarded, in any event, as the first few millilitres are sometimes slightly cloudy. It is, therefore, most convenient to use papers with the moisture content in equilibrium with ambient conditions (about 7 per cent.).

Under these conditions, sufficient of the first runnings must be discarded to ensure a subsequent sparkling, clear filtrate and that the effect of preferential absorption has become small enough to be insignificant. Figs. 1 and 3 indicate that at least 10 ml need to be discarded. However, the amount discarded should be kept to a minimum so that the time required for the analysis will be reduced to a minimum; there will be no need to replenish the solution in the filter funnel; and risk of evaporation will be minimised. For example, we see (Fig. 2) that if a 20 to 80-ml portion is collected, rather than a 10 to 70-ml portion, preferential absorption errors will be reduced, but evaporation errors will be increased with respect to the control.

At least 50 ml of filtrate need to be collected for flushing and filling a 200-mm visual polarimeter tube. As both the preferential absorption and evaporation effects result in an overestimate of polarisation, the 50, 60 or 70-ml portion that gives the lowest polarisation should be collected. In Table I, the average results for individual 10-ml portions over spans of 50, 60, 70 and 80 ml are compared for Trial 1 with the filtered control, and for Trial 3 with the centrifuged control *plus* 0·013° ISS. (0·013° ISS is the difference between filtered and centrifuged polarisations determined in Trial 2.)

TABLE I

COMPARISON OF AVERAGE POLARISATION RESULTS OF SEVERAL 10-ml FRACTIONS WITH CONTROL SAMPLE RESULTS

Polarisation difference (° ISS); solution average *minus* control

	Trial 1 (Fig. 2)	Trial 3 (Fig. 3)
Filtered control (10 to 80 ml)	0·000	—
Centrifuged control +0·013° ISS ..	—	0·000
0 to 50 ml	0·027	0·028
0 to 60 ml	0·023	0·025
0 to 70 ml	0·021	0·023
0 to 80 ml	0·019	0·023
10 to 60 ml	0·002	0·004
10 to 70 ml	0·003	0·006
10 to 80 ml	0·003	0·008
20 to 70 ml	0·001	0·007
20 to 80 ml	0·003	0·009
30 to 80 ml	0·003	0·011

The agreement between the 10 to 80-ml portion in Trial 1 and the filtered control is excellent and, in Trial 3 with the centrifuged control, satisfactory.

Clearly, if the first 10 ml are not discarded, the polarisation result will be seriously in error (by about 0·02° ISS). However, if additional filtrate is discarded, results will not differ significantly for most applications. We therefore recommend that the first 10 ml be discarded and the filtration continued until 50 to 60 ml have been collected.

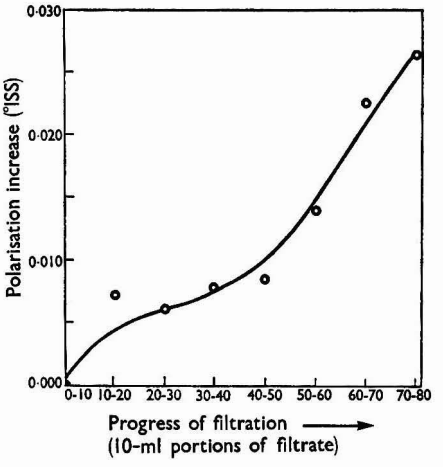


Fig. 5. Evaporation during filtration at 60 per cent. relative humidity (no filter-paper used)

EVAPORATION—

To study the evaporation effect independently of the preferential absorption effect, Trial 5 was conducted with solutions that were "filtered" under specially controlled conditions without filter-papers. There is a steady rise in polarisation throughout which, in the late

stages from 50 to 80 ml, is approximately linear in form, with a slope (Fig. 5) of 0·0060° ISS per ml. The slope of the 50 to 80-ml portion of the curve in Fig. 3 is 0·0045° ISS per ml; this is less steep than the evaporation curve of Fig. 5, presumably because of a small, gradually diminishing effect of preferential absorption. The slope of the 50 to 80-ml portion of the curve in Fig. 4 is, however, 0·0060° ISS per ml, the same as for the evaporation curve in Fig. 5. If the evaporation curve is now subtracted from the experimental curves in Figs. 3 and 4, the resultant curve, corrected for evaporation, represents a plot of preferential absorption alone. The best estimate of polarisation should now be that value which is approached asymptotically as preferential absorption diminishes.

For Trial 3 (Fig. 3), the corrected curve is relatively flat and approaches closer to the centrifuged control result in the final stages of the filtration. After 80 ml have been filtered, the corrected curve is only 0·005° ISS higher than the centrifuged control.

For Trial 4 (Fig. 4), there is a similar effect but, because the preferential absorption effect is reversed, the approach to the centrifuged control is from the low polarisation side; after 80 ml have been filtered, the corrected curve is 0·005° ISS lower than the centrifuged control. This indicates that clarification by centrifugation causes no significant errors in the determination of raw sugar solution polarisations. Centrifugation is, therefore, a more accurate method than filtration and gives a better estimate of the polarisation, as defined and specified by ICUMSA.

As mentioned earlier, centrifugation is preferred for large numbers of raw sugar analyses because it is less susceptible to the effects inherent in the filtration procedure; it is also rapid in comparison with filtration and requires less bench space.

Humidity effects—

Apart from the different controls, the curves of Figs. 2 and 3 represent similar trials differing only in humidity conditions; Trial 1 was carried out at 70 per cent. and Trial 3 at 60 per cent. relative humidity. Examination of the curves over the last 30 ml of filtrate shows that the experimental curve in Fig. 2 has a slope of 0·0038° ISS per ml compared with 0·0045° ISS per ml for that with 60 per cent. humidity in Fig. 3. Therefore, the evaporation effect seems to be only 0·85 times as large at 70 per cent. as at 60 per cent. relative humidity. If the evaporation curve in Fig. 5, with all values reduced by a factor of 0·85, is now subtracted from the experimental curve in Fig. 2, again the preferential absorption effect alone is illustrated. Again the corrected curve approaches the centrifuged result obtained in Trial 2, and plotted in Fig. 2, but here it seems that the effect of evaporation based on the above assumptions has been slightly overestimated. Evaporation at 70 per cent. relative humidity is probably only about 0·8 times that at 60 per cent. The amount of water required to saturate air of 70 per cent. humidity at 20° C is calculated to be about 0·8 times the amount required to saturate air of 60 per cent. humidity.

These trials were conducted in a laboratory at 20° C, considerable precautions being taken to prevent evaporation. The over-all effect in Trial 5 for 60 per cent. relative humidity was a polarisation increase of 0·027° ISS from the first 10-ml portion to the last. Under routine conditions, for lower ambient humidities and higher ambient temperatures, the effect is likely to be several times greater. The average effect over a 50 or 60-ml portion in a laboratory in the tropics could easily be 0·05° ISS for a raw sugar, or 0·05 per cent. of the polarisation value for cane juices and sugar products.

Conclusions

When a raw sugar solution is defecated with basic lead acetate and filtered through a single filter-paper that has a moisture content in equilibrium with the atmosphere, polarisation is significantly increased as a result of preferential absorption of water by the filter-paper. Although this effect continues until at least 50 ml of filtrate are collected, the effect is negligible for most practical purposes after the first 10 ml are filtered. In accordance, therefore, with the official method of the International Commission for Uniform Methods of Sugar Analysis for raw sugar polarisation, the initial 10 ml should be discarded.

The optimum moisture content of filter-papers to minimise the preferential absorption effect is 9 to 10 per cent. However, for convenience, papers in equilibrium with the atmosphere (containing 6 to 8 per cent. of moisture) should be used.

Even when great care is taken to prevent evaporation, there is a steady increase in the polarisation of small consecutive aliquots of filtrate, apart from the preferential absorption effect. The effect is about 20 per cent. higher for 60 per cent. relative humidity ambient conditions than for 70 per cent., with both at 20° C. For routine analysis under tropical conditions, errors as large as 0·05° ISS could be expected. To minimise this effect, not more than 10 ml of initial filtrate should be discarded, and only as much as is required for the polarisation measurement, usually about 50 to 60 ml, should be collected thereafter.

A raw sugar defecated with basic lead acetate solution and clarified by centrifugation, rather than by filtration, is not subject to preferential absorption and evaporation effects. Centrifuged solutions give the better estimate of polarisation value (as defined and specified by ICUMSA), whereas filtered solutions have polarisation values 0·013° ISS higher.

The authors thank the Colonial Sugar Refining Company Limited for permission to publish this paper.

REFERENCES

1. Saunier, R., *Editor*, "Proceedings of the XIVth Session of the International Commission for Uniform Methods of Sugar Analysis, 1966," ICUMSA, Subject 5, p. 16.
2. Hardin, G. H., and Zerban, F. W., *La Plr Sug. Mfr*, 1924, **73**, 388; *Ind. Engng Chem.*, 1924, **16**, 1175.
3. Gross, D., *Editor*, "Proceedings of the XIth Session of the International Commission for Uniform Methods of Sugar Analysis, 1958," ICUMSA, Subject 21, p. 86.
4. Saunier, R., *Editor*, "Proceedings of the XIVth Session of the International Commission for Uniform Methods of Sugar Analysis, 1966," ICUMSA, Subject 11, p. 61.

Received *July 11th*, 1968

Recent Methods for Determining Traces of Nitrogen in Mineral Oils

By P. GOUVERNEUR and F. VAN DE CRAATS
(*Koninklijke/Shell-Laboratorium, Amsterdam* (*Shell Research N.V.*))

Three methods for the determination of traces of nitrogen in oil are discussed, *viz.*, those based on extractive percolation, oxy-hydrogen combustion and hydrogenation - coulometry. Their scopes are compared with respect to their lower detection limit, the range of products to which they can be applied and their speed.

Although organic nitrogen compounds are only minor constituents of mineral oils, they can play a significant rôle, both during the manufacture and in the performance of oil products. The nitrogen concentrations involved are usually small, most often in the parts per million range, and sometimes below that level. Fortunately, there is a choice of analytical methods available today, thus enabling successful results to be achieved in this concentration range. Three of them are discussed in some detail and their scopes compared with respect to their lower detection limit, the range of products to which they can be applied and their speed.

In each of the following methods ammonia is the final reaction product, but the destructive treatment and the method of ammonia formation are different. The first method involves extractive percolation of the oil sample through sulphuric acid on a carrier, followed by Kjeldahl digestion of the nitrogen-containing concentrate; the second, oxy-hydrogen combustion, whereby the nitrogen compounds of the oil are initially converted into nitrogen oxides, and later into ammonia by Devarda reduction; and the third, catalytic hydrogenation, followed by microcoulometric titration of the ammonia formed.

Dumas' combustion method is not included in the present discussion because of the interference encountered in this method from dissolved molecular nitrogen, the amount of which often exceeds the combined nitrogen fraction by several times.

Extractive percolation through sulphuric acid on a carrier—

This is essentially a pre-concentration method and has been found a successful technique for concentrating all of the nitrogen compounds (basic and non-basic) contained in oil fractions. It is based on percolation of the sample through concentrated sulphuric acid distributed on an inert carrier, *e.g.*, fine-grade pumice, in a small column. After passage of the oil, the contents of the column are subjected to Kjeldahl treatment and the ammonia is determined titrimetrically[1] or, with small samples, spectrophotometrically.[2] Since its introduction in 1962, the technique has proved useful for a wide variety of petroleum distillate fractions ranging from low-boiling fractions to lubricating oils with nitrogen levels from 0 to 500 p.p.m.

Table I

Extractive percolation method: nitrogen content of refined kerosenes

2-litre samples; percolation rate 500 ml per hour

	Nitrogen content, parts per thousand million			
Sample	Natural	Added	Total present	Total found
A	19*	113†	132	122; 131
B		—		8; 14
C		—		31; 28
D		—		194; 199

* Average from multiple determinations by same method.
† Nitrogen-containing kerosene.

Paper presented at the Joint Symposium on Limits of Detection in Analysis, April 17th and 18th, 1968, Enschede.

It has been found more recently that this technique can be easily extended to the parts per thousand million range for highly refined oil fractions. Table I shows parts per thousand million amounts of nitrogen in kerosene samples, detected by percolation of 2-litre samples at a rate of 500 ml per hour.

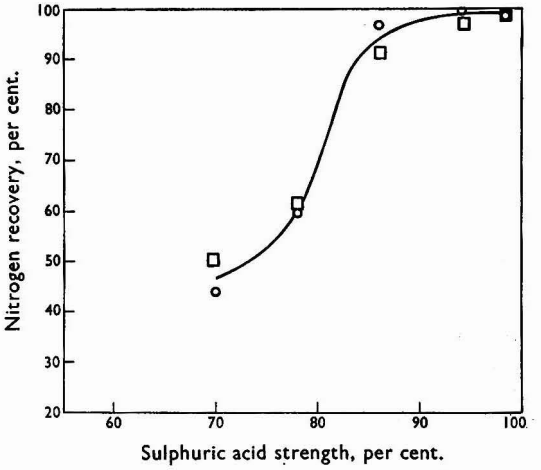
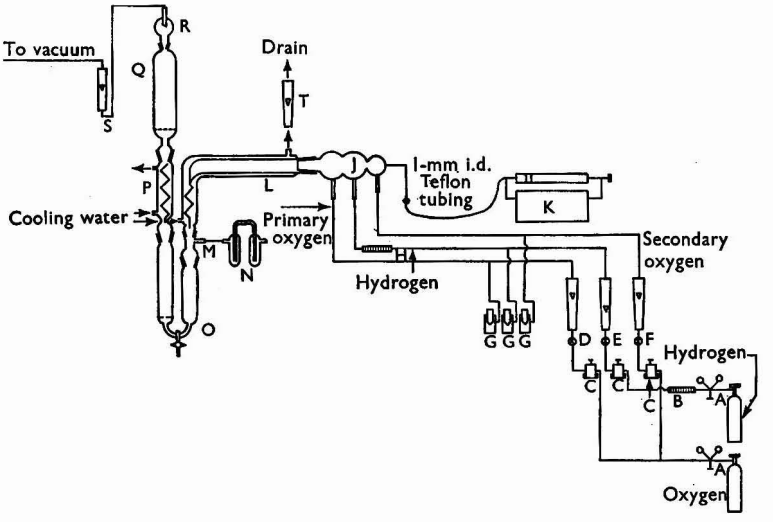


Fig. 1. Strength of sulphuric acid distributed on pumice *versus* nitrogen recovery for the extractive percolation method: ○ straight-run heavy gas oil, 200 p.p.m. of nitrogen; □ catalytically cracked light gas oil, 122 p.p.m. of nitrogen



A = Reducing valves
B = Flame arrester
C = Precision regulating valves
D = Control valve and rotameter for primary oxygen
E = Control valve and rotameter for hydrogen
F = Control valve and rotameter for secondary oxygen
G = Metal pressure safety valves
H = Flame arrester
J = Burner
K = Sample feed unit
L = Condenser
M = Adaptor
N = Flow indicator
O = Scrubber
P = Condenser
Q = Absorber
R = Splash bulb
S = Control valve and rotameter for suction
T = Water flow meter

Fig. 2. Diagram of apparatus for nitrogen determination by oxy-hydrogen combustion

The recommended concentration of the sulphuric acid is 98 per cent., although in later work this proved to be less critical than originally believed. Fig. 1 indicates that results are not greatly affected unless the concentration of the sulphuric acid drops below 94 per cent.

OXY-HYDROGEN COMBUSTION—

Oxy-hydrogen combustion is a well known method for decomposing organic material quickly and effectively, and the Wickbold apparatus developed for this purpose is widely used nowadays for determining traces of sulphur and halogen.[3] In this method the sample is fed into an oxy-hydrogen pilot flame and burned with a hot flame in an excess of oxygen. The combustion products are then drawn through a scrubber containing a suitable absorbent for trapping the component to be determined. Combustion rates can be high, *e.g.*, from 1 to 5 ml per minute, depending on the nature of the sample.

The determination of nitrogen in this way is a more recent development[4] and comprises the following steps: conversion of the organic nitrogen compounds in the hot flame, producing nitrogen oxides (mainly nitric oxide); trapping of the oxides formed on sodium chlorite on an alumina carrier, thereby producing a nitrite - nitrate mixture; and wet reduction of this mixture with Devarda alloy producing ammonia, which can be determined in the usual way.

The combustion apparatus used is shown in Fig. 2. A satisfactory sample feed-rate is about 2 ml per minute, and a useful upper limit for the amount of nitrogen to be converted in one run is 5 mg, preferably contained in 5 to 50 ml of sample. In this way any overloading of the sodium chlorite reagent is prevented.

The reduction of the nitrite - nitrate mixture to ammonia and the isolation of the latter is a one-step operation that proceeds simply and conveniently, so that the whole method is relatively quick in comparison with traditional methods for the detection of trace nitrogen. Fifteen determinations can be made in a single working day.

TABLE II

OXY-HYDROGEN METHOD: CONVERSION OF NITROGEN COMPOUNDS

All compounds dissolved in benzene - kerosene

Compound	Nitrogen theory, p.p.m.	Nitrogen average found, p.p.m.	Recovered as ammonia, per cent.
Pyridine	231	222	96
Nitrobenzene	1016	1028	101
Aniline	296	281	95
Azobenzene	246	99	40
Octylamine	204	204	100
Heptyl cyanide	171	169	99
Indole	203	200	99
Quinoline	215	207	96

In model experiments with blends of pure nitrogen compounds in nitrogen-free fuel, the conversions into ammonia were between 95 and 101 per cent., as Table II shows. Azobenzene, however, is an exception. This compound evidently splits off an appreciable fraction of its nitrogen as elemental nitrogen, which is only sparingly oxidised in the flame. However, this is of little consequence for the present purpose because the occurrence of compounds of this type in petroleum has not been reported.

TABLE III

OXY-HYDROGEN METHOD: NITROGEN CONTENT OF OIL FRACTIONS

Sample	Nitrogen by extractive percolation, p.p.m.	Nitrogen by oxy-hydrogen combustion, p.p.m.
Naphtha	97	94; 95
Light gas oil	15	16; 13; 16
Heavy gas oil*	267	261; 254
Gas oil concentrate*	577	542; 540
Luboil*	616	618; 613
Dark steam cylinder lubricant* ..	478	470; 496
Shale oil*	1·59 per cent.	1·57; 1·59 per cent.

* Diluted.

Fig. 4. Hydrogenation - coulometric apparatus

To face page 785]

Some results obtained on actual oil samples are presented in Table III, the oils having nitrogen contents from 15 p.p.m. upwards. In general, satisfactory agreement was found with results obtained by the extractive percolation method, both for low-boiling and for residual oil fractions, and also in the exceptional case of the shale oil sample. It may be concluded, therefore, that the nitrogen compounds of oils are generally converted into nitrogen oxides during the oxy-hydrogen combustion treatment. The conversion of molecular nitrogen, on the other hand, is poor and amounts to only a few per cent. under the flame conditions. In consequence, the effect of the portion of dissolved nitrogen present in the oils is not significant. Unfortunately the situation is quite different for the gases used for the combustion. Their molecular nitrogen impurity level often ranges from 100 to 1000 p.p.m. v/v and, as the gas consumption is relatively high, the blank contribution is significant. For this reason the method is no longer attractive for oils containing less than 15 p.p.m. of nitrogen.

CATALYTIC HYDROGENATION - COULOMETRIC METHOD—

This method was published by Martin[5] in 1966 and comprises catalytic hydrogenation of the oil sample with nickel catalyst, followed by microcoulometric acid - base titration, in a special cell, of the ammonia formed. An outstanding feature of this method is that it is capable of detecting nanogram amounts of nitrogen in milligram amounts of oil in a short time. Moreover, the use of such small samples and a relatively large excess of nickel catalyst appreciably reduces the effect of nickel de-activation by poisoning and coke deposits (a traditional drawback in Ter Meulen type hydrogenations).

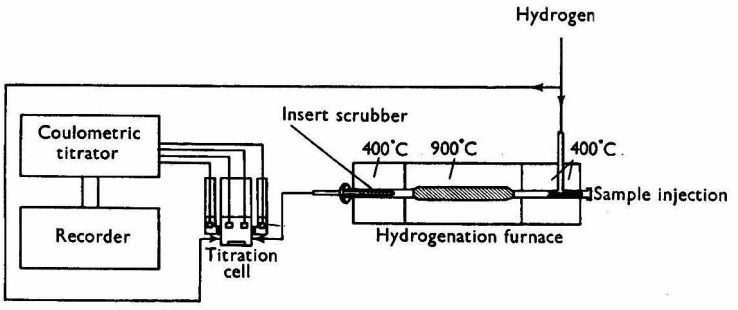


Fig. 3. Hydrogenation - coulometric nitrogen method

A schematic arrangement is given in Fig. 3. Fig. 4 shows the equipment, which is available from Dohrmann Instruments Company (Mountain View, California). The procedure is as follows: the 1 to 10-μl oil sample is injected into a 400° C zone and subjected to hydrogenation in a current of ultra-pure hydrogen (24 litres per hour) through nickel catalyst. The catalyst supplied by the manufacturer, *viz.*, granular nickel of high purity, is used at 900° C in contrast with Martin's catalyst, which was nickel on magnesium oxide used at 440° C. An insert scrubber tube containing, *e.g.*, magnesium oxide serves to remove interfering acidic products that originate from accompanying sulphur and halogens. The ammonia leaving the tube is fed into a titration cell designed to operate at a pre-determined constant hydrogen-ion concentration in the pH range 5 to 6. The cell contains 0·04 per cent. sodium sulphate solution as the electrolyte and has four electrodes, a sensing pair (hydrogen electrode *versus* lead - lead sulphate reference electrode) and a generating pair (two platinum electrodes). Any change in concentration (incoming ammonia) is detected by the sensing electrode pair as a potential difference, which leads through the coulometer amplifier to the generation of hydrogen ions at the generator electrode. The required current is recorded via a precision series resistance on a potentiometric recorder, the peak area representing the current integral with the chart speed as the time basis. The number of coulombs required for neutralisation is then known.

Initially, the application of this method to oil samples failed, although ammonia produced by heating weighed sub-micro amounts of ammonium chloride was completely recovered. This suggested that the trouble was in the catalytic hydrogenation step, and the experiences

of colleagues (E. M. Fredericks and E. D. Peters in a private communication) have confirmed it. They suggested a pre-treatment of the nickel catalyst and also ultra-purification of the hydrogen used. Fresh catalyst should be subjected *in situ* to an oxidative treatment (pure oxygen at 900° C) and subsequently to a reducing treatment (pure hydrogen at 400° C), with pure helium at the intermediate stages for safety reasons. This treatment was repeated as soon as the catalyst began to show signs of de-activation. The hydrogen used was purified by palladium diffusion with an A.5 Diffusion Unit manufactured by Johnson, Matthey and Co. Ltd.

TABLE IV

HYDROGENATION - COULOMETRY: OCTYLAMINE IN KEROSENE

Nitrogen theory, p.p.m.	Injected, μl	Nitrogen average found, p.p.m.	Recovery, per cent.
1000	0·5 to 1	667	67
500	0·5 to 2	374	75
250	1	237	95
125	1	130	104
50	2	48	96

Table IV illustrates the conversion of octylamine when blended with nitrogen-free kerosene. Best results were obtained for the more dilute blends, and for practical reasons the 50 p.p.m. nitrogen level was selected for further work. Results relating to the conversion of other nitrogen compounds at this level are shown in Table V. Satisfactory results were obtained for the compounds tested, except for azobenzene. Here again, the tendency of

TABLE V

HYDROGENATION - COULOMETRY: CONVERSION OF NITROGEN COMPOUNDS

All compounds dissolved in kerosene; 2-μl samples injected, inlet temperature 400° C

Compound	Nitrogen theory, p.p.m.	Nitrogen average found, p.p.m.	Recovered as ammonia, per cent.
Pyridine	65	61	94
Nitrobenzene	50	48	96
Aniline	50	50	100
Azobenzene	50	40	80
Octylamine	49	47	96
Heptyl cyanide	55	55	100
Indole	50	53	106
Quinoline	50	53	106

this compound to produce elemental nitrogen when decomposed obviously prevents complete conversion, as in the oxy-hydrogen method. Results for oil samples are shown in Table VI. In general they are in reasonably good agreement with known values, even those for the shale oil sample. The high-boiling dark steam cylinder lubricant gave a low value, however.

TABLE VI

HYDROGENATION - COULOMETRY: NITROGEN CONTENT OF OIL FRACTIONS

Inlet temperature 400° C, magnesium oxide scrubber 450° C

Sample	Nitrogen by extractive percolation, p.p.m.	Nitrogen by hydrogenation-coulometry, p.p.m.
Naphtha	97	94; 95
Kerosene	3·2	3; 4
Light gas oil*	187	165; 180
Heavy gas oil*	267	251; 283
Gas oil concentrate*	577	588; 623
Dark steam cylinder lubricant* ..	478	334; 392
Shale oil*	1·59 per cent.	1·43; 1·69 per cent.

* Diluted in kerosene to a 50 p.p.m. level.

These experiments led us to the conclusion that the hydrogenation - coulometric method is most effective for products that are sufficiently volatile below 400° C, and that it is rapid, the signal being available almost instantly and the calculated result well within 15 minutes, including the necessary dilution. The lower detection limit at present appears to be 1 p.p.m. Further work will be necessary to extend the method to products boiling well above 400° C.

Conclusion

In Table VII the salient features of the methods discussed have been summarised.

Table VII

Summary of characteristics of three methods for trace nitrogen determination in oils

	Extractive percolation	Oxy-hydrogen combustion	Hydrogenation - coulometry
Useful product range:			
low boiling	+	+	+
high boiling	+	+	(+)
residual	—	+	—
Useful nitrogen range, p.p.m.	0·01 to 500	15 to 1000	1 to 50
Sample requirements	10 ml to 2 litres	5 to 40 ml	2 to 10 μl
Repeatability, usual	5 per cent. amount present	5 per cent. amount present	5 per cent. amount present
Repeatability, best	0·01 p.p.m.	5 p.p.m.	1 p.p.m.
Analysis time:			
1 test elapsed	6 hours	45 minutes	15 minutes
Number of analyses per person per 8-hour day	3	15	30

The extractive percolation method, although of limited speed, is capable of determining parts per thousand million concentrations of nitrogen in certain refined oil fractions.

The oxy-hydrogen combustion method is more rapid, and is characterised by its general applicability to oil products with nitrogen contents of over 15 p.p.m.

The "Dohrmann" technique (hydrogenation - coulometry) appears to be the most rapid, and is useful for products that are sufficiently volatile below 400° C and have nitrogen contents of over 1 p.p.m.

References

1. Gouverneur, P., *Analytica Chim. Acta*, **1962**, **26**, 212.
2. Smith, A. J., Cooper, F. F., jun., Rice, J. O., and Shaner, W. C., jun., *Ibid.*, **1968**, **40**, 341.
3. "Proceedings of the Colloquium on Experiences with the Wickbold Combustion Apparatus for Organic Elementary Analysis, Hanau, February, 1966," Heraeus-Schott Quarzschmelze GmbH, Hanau, Germany.
4. Gouverneur, P., Snoek, O. I., and Heeringa-Kommer, M., *Analytica Chim. Acta*, **1967**, **39**, 413.
5. Martin, R. L., *Analyt. Chem.*, **1966**, **38**, 1209.

Received *May 7th*, 1968

Determination of Chloride in Chloride-containing Materials with a Chloride Membrane Electrode

By J. C. VAN LOON

(*Department of Geology, University of Toronto, Toronto 5, Ontario*)

The determination of chloride by using the membrane-type chloride electrode is described. Procedures are given for the analysis of chloride materials, including silver halides, both in the presence and absence of bromide and iodide.

Recently a solid-state silver chloride type membrane electrode has been developed commercially for the measurement of chloride activities. This electrode, together with an expanded-scale pH meter, can be used for the rapid determination of chloride concentration in solutions of constant ionic strength. Only the presence of OH^-, Br^-, I^-, S^{2-}, CN^-, NH_3 and $S_2O_3^{2-}$ in the solutions to be measured interfere directly with the results.[1] The availability of this system for rapid studies in the field and laboratory when large numbers of analyses are required makes it potentially useful.

Before the commercial development of the silver chloride membrane electrode the use of silver - silver chloride electrodes for the determination of chloride has been recorded for boiler waters,[2] chloride in ground water,[3] free chloride in the presence of many cations,[4] also sweat, urine and miscellaneous solutions.[5] This type of electrode developed redox potential errors in the presence of strong oxidising substances, a limitation not shared by the new silver chloride membrane electrode.

In this paper procedures are recorded for the rapid determination of chloride in soluble substances and insoluble silver halide materials, both when bromide and iodide are present and in their absence.

Experimental

Apparatus—

An Orion Specific Ion Chloride Electrode Model 94–17 was used, in conjunction with a Beckman Expandomatic pH meter. A scale expansion of 200-mV full scale was sufficient for these measurements.

Reagents—

All the water used was distilled and checked for the absence of chloride in amounts that would cause interference.

Chromic acid solution—Dissolve 40 g of analytical-reagent grade chromic oxide in 400 ml of sulphuric acid (1 + 2).

Standard sodium chloride solution, 0·1 M.

Dilute working standards for analysis of sample solutions were prepared by combining all the ingredients used to prepare the sample but substituting an aliquot of standard solution for the chloride sample in the graduated flask. All reagents were tested for the presence of chloride contamination and found to contain insignificant amounts. Solutions were stored in polythene bottles.

Procedures for soluble materials—

(*a*) *No bromide or iodide present*—Weigh an appropriate amount of sample (final chloride concentration 10^{-2} to 10^{-4} M) into a 250-ml beaker and dissolve it in water. Rinse it completely into a 1000-ml graduated flask and dilute to volume with water. Millivolt readings for these slowly stirred solutions were checked against the appropriate standards (meter was set to read +10·0 mV for 10^{-2} M chloride standard solutions), with the nearest standards being read before and after each sample.

(*b*) *Bromide and iodide present*—Weigh an appropriate amount of sample (final chloride concentration 10^{-2} to 10^{-4} M) into a 250-ml Erlenmeyer flask. Wash the sample into the bottom of the flask with 5 ml of water and tilt it to allow the sample to run into one edge. Add 20 ml of the chromic acid solution and bubble nitrogen into the mixture with a fritted bubbler for 15 minutes. Rinse the bubbler inside and out and remove it from the flask. Wash the solution in the flask carefully into a 500-ml graduated flask and dilute to volume with water. Millivolt readings for these slowly stirred samples were checked against the appropriate standards (meter was set to read +10·0 mV for 10^{-2} M chloride solutions), with the nearest standards being read before and after each sample.

PROCEDURES FOR SILVER HALIDE—

(*c*) *No bromide or iodide present*—Fuse a sample (final chloride concentration 10^{-2} to 10^{-4} M) gently in 0·7 g of potassium carbonate and a small amount of spectrographic carbon in a platinum crucible until all of the silver, as the metal, has formed a coherent coating on the bottom of the crucible. Place the crucible upright in a 250-ml beaker and cover with 200 ml of water. Place a small stirring rod in the crucible and stir gently to complete dissolution of the melt. Remove and rinse the crucible and stirring rod, then wash the solution from the beaker into a 500-ml graduated flask and dilute to volume with water. With standards prepared as indicated above and the meter set to read +10·0 mV for the 10^{-3} M chloride standard solution, the mV readings for the samples were obtained on slowly stirred solutions. The nearest standards are read before and after each sample.

(*d*) *Bromide and iodide present*—Fuse the sample gently as above, then cool the crucible and add 5 ml of water to the upright crucible on a magnetic stirrer. Stir with a small stirring rod until the dissolution is complete, then rinse the contents of the crucible with a minimum of water (4 to 5 ml) into a 250-ml Erlenmeyer flask. Add 30 ml of the chromic acid solution and support the flask in a tilted position so that the contents run to one side of the flask. Place a bubbler in the solution and bubble nitrogen through the solution for 15 minutes. Rinse the bubbler inside and out and wash the solution from the Erlenmeyer flask into a 500-ml graduated flask. Dilute to volume and measure the millivolt readings of the slowly stirred samples against appropriate standards with the meter set to read +10·0 mV for the 10^{-3} M chloride standards. The nearest standards are read before and after each sample.

It is important not to allow any metallic silver to contact the concentrated chromic acid solution as silver is easily oxidised to Ag^+ in this medium, with the resultant consumption of chloride. The most common causes of inconsistent values when the above methods are used can be summarised as follows: failure to allow sufficient time for the electrode reaction to reach a steady state; the presence of small particles of silver metal in the fusion, which subsequently are oxidised by the chromic acid (can be observed because of a resulting cloudy final solution); and failure to check higher and lower standards before and after each sample.

RESULTS AND DISCUSSION

METER NOISE AND DRIFT—

Meter readings were found to fluctuate by ± 5 mV, because of the movement of the hands around the chloride electrode. This was corrected by wrapping the electrode and two thirds of its body in three layers of aluminium foil and grounding the foil, somewhere on the lower body, to an outlet. The electrode response to changes in chloride concentration was not instantaneous and often drifted towards the correct value over an interval of 60 seconds. Response times became even longer after long exposure of the electrode to many samples containing chromic acid solution. This problem was easily rectified by a light buffing of the membrane with fine emery paper. Slow response in the concentration range below 10^{-3} M could be, to some degree, overcome by stirring slowly with a thin stirring rod.

REFERENCE ELECTRODE—

It was, of course, impossible to use the normal standard calomel electrode directly in contact with chloride sample solutions, hence a small diameter calomel electrode was inserted in a larger diameter glass casing from a broken reference electrode. The casing with its fibre removed was plugged with a small amount of agar-agar - 4 M ammonium nitrate and then filled with 4 M ammonium nitrate. This electrode was clamped to the side of the chloride electrode with the Orion Specific Ion Electrode Holder.

INTERFERENCES—

Serious interferences with the use of this electrode would be caused by OH^-, S^{2-}, CN^-, NH_3, $S_2O_3^{2-}$, Br^- and I^-. Of these, Br^- and I^- are the only ions often encountered in solutions resulting from chloride minerals. In addition, complexing cations, which remove free chloride from solution, could cause serious errors if not kept at an insignificantly low concentration by the use of dilute solutions. An agreement, within the level required by the accuracy of the method, of ionic strengths between samples and standards must be maintained in order to determine chloride concentration directly.

The bromide and iodide interference can be eliminated by anion exchange,[6] but this method was found to be too slow to be practical in this instance. Evans[7] recommended the use of chromic acid in 8 to 9 N sulphuric acid for the separation of bromide from chloride in wide ranges of bromide-to-chloride ratios. It was necessary to prove that the chromic acid system worked for the expected removal of iodide and that it did not cause serious problems with the operation of the chloride electrode.

Aliquots containing chloride (so that the final chloride content will be 10^{-4} M) and (or) bromide and (or) iodide, in 1 + 1 + 1 ratios, were treated according to the proposed procedure, to determine firstly if chloride was stable during the oxidation, secondly whether bromide and iodide were eliminated quantitatively by the proposed procedure, and thirdly if chromic acid solution interfered with the operation of the chloride electrode. Tests were also carried out to see if the resultant Cr^{3+}, produced during the oxidation, interfered in these solutions. Results for these experiments are listed in Table I, and from these it is obvious that the proposed procedure can be used without encountering an error of greater than 10 per cent. Although Cr^{3+} was shown to interfere, its molar concentration is usually less than chloride and at this level does not cause serious error.

TABLE I

TESTS WITH CHROMIC ACID OXIDATION

Test	Number of times performed	Maximum deviation from standard, mV	Maximum error (approximate), per cent.
Stability of chloride in presence of chromic acid mixture (no Br^- or I^- added)	4	−0·7	3
Removal of I^- by oxidation	3	±0·8	6
Removal of Br^- and I^- together by oxidation	5	±1·0	10
Possible interference by Cr^{3+}:			
MCl^- - MCr^{3+} (1 + 3)	1	+0·7	3
MCl^- - MCr^{3+} (1 + 10)	1	+3·1	15
MCl^- - MCr^{3+} (1 + 29)	1	+5·0	23

TEST OF PROPOSED PROCEDURE—

Many weighed samples of potassium chloride and sodium chloride, analytical-reagent grade salts, were subjected to the procedures (*a*) and (*b*), with aliquots of bromide and iodide solution being added to the sample to test the procedure (*b*). These results are given in Table II. Several naturally occurring chloride minerals were analysed by using procedures (*a*)

TABLE II

ANALYSIS OF "KNOWN" SALTS

Sample	Procedure used	Weight of samples taken, mg	Theoretical chloride, per cent.	Mean of chloride found, per cent.	Number of separate determinations	Standard deviation
KCl	*a*	100	47·7	46·5	6	1·18
	b	50·0	47·7	46·6	4	1·71
NaCl	*a*	100	60·7	60·6	6	0·56
	b	50·0	60·7	60·1	3	1·27
AgCl	*c*	18 to 21	24·7	24·0	4	0·22
	d	11 to 22	24·7	23·8	5	0·66

TABLE III

ANALYSIS OF CHLORIDE MINERALS

Sample	Procedure used	Weight of sample, mg	Theoretical amount of chloride, mg	Amount of chloride found, mg	Deviation, mg
Halite (NaCl)	*a*	49·0	29·7	30·5	+0·8
	a	47·0	28·5	29·8	+1·3
	b	49·0	29·7	28·7	−1·0
	b	48·5	29·4	28·4	−1·0
Sylvite (KCl)	*a*	50·4	24·0	23·0	−1·0
	a	51·8	24·6	23·2	−1·4
	b	49·1	23·4	24·1	+0·7
	b	48·7	23·2	21·6	−1·6
	b	51·3	24·5	25·7	+1·2
Carnallite ($KMgCl_3.6H_2O$)	*a*	50·7	19·4	18·4	−1·0
	b	50·6	19·2	18·8	−0·4
	b	49·0	18·8	18·6	−0·2

and (*b*) and the results are listed in Table III. Several analyses with precipitated silver chloride and silver chloride *plus* equal amounts of precipitated silver bromide or silver iodide, or both, were subjected to procedures (*c*) and (*d*) and the results are listed in Table II. From these results it is obvious that the proposed procedures can be used for the rapid determination of chloride in the presence of bromide and iodide when the greatest accuracy is not required. The time required for a single analysis is about 20 to 30 minutes with procedures (*b*) and (*d*), but this can be lowered to about 8 minutes for each when many samples are analysed by integrating the steps. Procedures (*a*) and (*c*) required 2 to 5 minutes for completion.

The author thanks the Department of University Affairs of the Province of Ontario for financial assistance for this work, and Dr. J. Mandarino of the Royal Ontario Museum for the samples.

REFERENCES

1. Instruction Manual, Halide Electrodes, Orion Research Corp., Cambridge, Mass. For mode 94–17.
2. Seidel, R. L., *U.S. Atomic Energy Corp.* KAPL-M-3MS-93, 1958, p. 35.
3. Black, W., *J. Amer. Wat. Wks Ass.*, 1960, **52**, 923.
4. Onashi, H., and Morozumi, I., *Hokkaido Doigaku Kogat ubu Kenkyu Hokoku*, 1967, **42**, 131.
5. Stern, M., Shwachman, H., Licht, T. S., and deBethune, A. J., *Analyt. Chem.*, 1958, **30**, 1506.
6. De Geiso, R. C., Rieman, W., and Lindenbaum, S., *Ibid.*, 1954, **26**, 1840.
7. Evans, B. S., *Analyst*, 1931, **56**, 590.

Received *May 7th*, 1968

Analyst, December, 1968, Vol. 93, *pp.* 792–796

Oxidimetric Determination of Thiocarbonate Sulphur with Chloramine-T, Potassium Ferricyanide and Potassium Permanganate

By K. N. JOHRI and N. K. KAUSHIK

(*Department of Chemistry, University of Delhi, Delhi 7, India*)

Oxidimetric methods for determining the concentration of aqueous solutions of potassium thiocarbonate by using chloramine-T, potassium ferricyanide and potassium permanganate are discussed. The chloramine-T method is based on the reaction of potassium thiocarbonate with a known excess of chloramine-T in alkaline medium at 60° C and back-titration of the unreacted chloramine-T against a standardised solution of sodium thiosulphate, with starch as indicator. Twenty-four equivalents of the oxidant per mole of potassium thiocarbonate are consumed, showing that the three sulphur atoms of thiocarbonate are oxidised to sulphate. When ferricyanide is used twenty-four equivalents of the oxidant per mole of potassium thiocarbonate are also consumed in an alkaline medium at 60° C. However, in acidic medium, potassium permanganate is found to oxidise the three sulphur atoms of thiocarbonate to elemental sulphur. The following molar relationships are established—

12 moles of chloramine-T ≡ 1 mole of K_2CS_3
6 moles of $KMnO_4$ ≡ 5 moles of K_2CS_3
24 moles of $K_3Fe(CN)_6$ ≡ 1 mole of K_2CS_3

Because of the important analytical applications of potassium thiocarbonate reported lately in the literature,[1,2,3,4,5] an investigation of efficient and rapid chemical procedures for the determination of thiocarbonate sulphur present in aqueous samples of the reagent was considered necessary. A gravimetric method[6] in which thallium(I) nitrate is used for the quantitative precipitation of thiocarbonate sulphur and titrimetric procedures involving iodimetric, as well as iodatometric, oxidation of the thiocarbonate contents of samples have been reported.[7] Iodic acid alone was found to react with the three sulphur atoms of the thiocarbonate; iodine and potassium iodate each oxidised one of the sulphur atoms, the other two forming carbon disulphide. The iodimetric and gravimetric methods[8,9,10] available relate only to the determination of sulphide sulphur and not to thiocarbonate sulphur. The results obtained by the present method are not only quantitative, but have confirmed that three moles of hydrogen sulphide per mole of potassium thiocarbonate are available in reacting solutions under optimum conditions.

As chloramine-T is able to break the C–S, N–S and S–S bonds in a variety of sulphur compounds,[11 to 17] oxidising all of the sulphur quantitatively to the sulphate form, it was of interest to investigate the reaction between chloramine-T and potassium thiocarbonate. The active constituent of chloramine-T is the hypochlorite ion, which is obtained by hydrolysis of chloramine-T. Chloramine-T is preferred to hypochlorite because of its relatively high stability. The oxidation of thiocarbonate sulphur was studied in both acidic and alkaline media, and a simple titrimetric procedure for the determination of potassium thiocarbonate has been developed by making use of the oxidation in alkaline medium.

Potassium ferricyanide has also been studied as an oxidant for thiocarbonate sulphur in hot alkaline medium. An investigation of the use of this oxidant was necessary because, being a weak oxidant, it is more selective than others. Moreover, oxidation of various organic and inorganic sulphur compounds in alkaline medium has been reviewed by Sant,[18] but no reference to thiocarbonate sulphur in this respect is found in the literature. However, recently Deshmukh[19] has standardised potassium thiocarbonate amperometrically by using ferricyanide as oxidant, with osmium tetroxide as catalyst, and has reported that only one third of the thiocarbonate sulphur reacted at room temperature.

Furthermore, evaluation of thiocarbonate sulphur in acidic medium has been carried out with potassium permanganate, a strong oxidant that reacts quantitatively with the hydrogen sulphide liberated by the thiocarbonic acid produced in acidic solutions of potassium thiocarbonate.

Chloramine-T method

Reagents—

Chloramine-T, 0·1 N—This solution was kept in amber-coloured bottles and standardised iodimetrically.[20]

Sodium thiosulphate, 0·1 N—This was prepared from analytical-reagent grade material and standardised against potassium iodate.

Starch solution, 1 *per cent., aqueous.*

Potassium thiocarbonate, 2 M—An aqueous solution was prepared by the direct method, and, after standardisation,[6] was used to prepare suitable dilutions. Other reagents used were of analytical-reagent grade.

Procedure—

Transfer 10 ml of 0·02 M potassium thiocarbonate into a 250-ml conical flask containing 50 ml of standard chloramine-T solution, made alkaline with 5 ml of M sodium hydroxide. Heat to 60° C for half an hour by immersing the flask in a hot water bath. Cool to room temperature, acidify with 20 ml of 5 N sulphuric acid and add 25 ml of 10 per cent. potassium iodide solution. Titrate the liberated iodine with standard sodium thiosulphate solution. The amount of chloramine-T consumed by potassium thiocarbonate is thus obtained from the titre value. Calculate the thiocarbonate sulphur content of the sample from the relationship—

$$1 \text{ ml of N chloramine-T} \equiv 7{\cdot}75 \times 10^{-3} \text{ g of } K_2CS_3 \equiv 4{\cdot}008 \times 10^{-3} \text{ g of S.}$$

Blank corrections were not necessary in these experiments. The titre values were found to be reproducible, and the results of a few representative experiments are given in Table I.

Table I

Results of the determination of sulphur in potassium thiocarbonate with chloramine-T in alkaline medium at 60° C

Volume of 0·02 M potassium thiocarbonate added, ml	Volume of 0·1 N chloramine-T consumed, ml	Number of equivalents of oxidant per mole of potassium thiocarbonate used	Sulphur present, mg	Sulphur found, mg	Difference, mg
10	47·98	23·99	19·20	19·23	+0·03
10	47·84	23·92	19·20	19·18	−0·02
5	24·02	24·02	9·60	9·63	+0·03
5	24·00	24·00	9·60	9·62	+0·02
3	14·38	23·96	5·76	5·76	0·0
3	14·40	24·00	5·76	5·77	+0·01

Discussion

It is evident from the results shown in Table I that the reaction of chloramine-T with alkaline potassium thiocarbonate at 60° C is such that the three sulphur atoms undergo oxidation. For the complete oxidation of sulphur in potassium thiocarbonate to the sulphate ion twenty-four equivalents of oxidant per mole of potassium thiocarbonate would be needed according to the following equations—

(*i*) $CH_3.C_6H_4.SO_2NCl - Na^+ + H_2O \longrightarrow CH_3.C_6H_4.SO_2NH_2 + Na^+ + OCl^-$.

(*ii*) $CS_3^{2-} + 12OCl^- + 4OH^- \longrightarrow 3SO_4^{2-} + 2H_2O + 12Cl^- + CO_2$.

Thus, 12 moles of chloramine-T ≡ 1 mole of K_2CS_3.

Experiments carried out in acidic medium revealed incomplete oxidation, even at a higher temperature. This was caused by the partial oxidation of potassium thiocarbonate to elemental sulphur which, once formed, resists further oxidation by chloramine-T. The

sulphur precipitated during these experiments in acidic media was observed to be suspended in solution.

Oxidation in alkaline medium at room temperature (20° C) also did not proceed to completion, and separation of elemental sulphur was clearly observed as a white turbidity. However, when the temperature was raised to 60° C this turbidity vanished and the oxidation of the entire sulphur was found to be quantitative. The finely divided sulphur reacts with hot alkali,[21] forming sulphide, sulphite and thiosulphate, all of which can be oxidised to sulphate by chloramine-T.

Ferricyanide method

Reagents—

Potassium ferricyanide, 0·1 N—This was prepared by dissolving analytical-reagent grade material in redistilled water and standardised by titrating against standard sodium thiosulphate solution.

Sodium thiosulphate, 0·1 N.

Starch solution, 1 *per cent., aqueous.*

Sulphuric acid, 5 N.

Zinc sulphate, 0·5 M—This was prepared by dissolving an analytical-reagent grade sample in redistilled water.

Potassium thiocarbonate, 2 M.

Procedure—

In a series of experiments to determine the optimum conditions for the complete oxidation of thiocarbonate sulphur with ferricyanide, carried out at room temperature, the time of reaction was varied and the amount of potassium thiocarbonate used kept constant. The excess of ferricyanide was determined by back-titrating against standardised thiosulphate solution. The reaction was slow at room temperature, the number of equivalents of oxidant per mole of potassium thiocarbonate increasing from 2·60 to 8·06 in 45 minutes at 5-minute intervals. However, in the second set of experiments the reactants were heated to 60° C and the reaction was found to be completed within 15 to 20 minutes.

Recommended procedure—

Introduce a measured aliquot of the test solution containing not more than 17 mg of sulphur into a measured excess volume of standard 0·1 N ferricyanide, previously made alkaline with 5 N sodium hydroxide so that its alkalinity is about 3 N. Heat to 60° C for 15 to 20 minutes by immersing the flask in a hot water bath. Cool to room temperature, and titrate the excess of ferricyanide against standardised thiosulphate after acidifying with 5 N sulphuric acid, adding 25 ml of 10 per cent. potassium iodide solution and an excess of zinc sulphate solution so that all of the resulting ferrocyanide can be precipitated as zinc ferrocyanide. Use starch as the indicator. Calculate the thiocarbonate sulphur content of the sample from the relationship—

$$1 \text{ ml of N ferricyanide} \equiv 7{\cdot}75 \times 10^{-3} \text{ g of } K_2CS_3 \equiv 4{\cdot}008 \times 10^{-3} \text{ g of S.}$$

The results of a few representative experiments are given in Table II.

Table II

Results of the determination of sulphur in potassium thiocarbonate with ferricyanide in alkaline medium at 60° C

Volume of 0·06 M potassium thiocarbonate added, ml	Volume of 0·1 N ferricyanide consumed, ml	Number of equivalents of oxidant per mole of potassium thiocarbonate used	Sulphur present, mg	Sulphur found, mg	Difference, mg
1	14·38	23·96	5·76	5·76	0·0
1	14·40	24·00	5·76	5·77	+0·01
2	28·76	23·96	11·52	11·52	0·0
2	28·78	23·98	11·52	11·53	+0·01
3	43·20	24·00	17·28	17·32	+0·04
3	43·22	24·01	17·28	17·33	+0·05

Discussion

It was seen from the results that the reaction between ferricyanide and potassium thiocarbonate is time consuming. It takes about 45 minutes to oxidise only one of the three sulphur atoms to sulphate at room temperature, after which the titre value remains constant. However, the reaction at 60° C is rapid, and the three sulphur atoms of the thiocarbonate are oxidised to sulphate, as seen in Table II. The reaction of ferricyanide with potassium thiocarbonate in alkaline medium at 60° C can be expressed—

$$CS_3^{2-} + 24Fe(CN)_6^{3-} + 26OH^- \longrightarrow 24Fe(CN)_6^{4-} + 3SO_4^{2-} + 13H_2O + CO$$

Thus, 24 moles of ferricyanide ≡ 1 mole of K_2CS_3.

Before titrating the excess of ferricyanide, sufficient zinc sulphate must be added to precipitate all the ferrocyanide as zinc ferrocyanide. Otherwise, the end-point would not be sharp because of the formation of Prussian blue, the presence of which has been verified by making the solution alkaline (colour fades).

Potassium permanganate method

Procedure—

Transfer 10 ml of 0·1 N potassium permanganate solution to a 250-ml conical flask and add 10 ml of 0·1 N sulphuric acid. Introduce, gradually, freshly prepared potassium thiocarbonate solution from a microburette with a bent nozzle, keeping the tip of the nozzle beneath the liquid surface. Continue adding until the colour of the potassium permanganate is discharged. Repeat the observations with different amounts of potassium permanganate. Calculate the potassium thiocarbonate sulphur content of the sample solution from the relationship—

$$1 \text{ ml of N } KMnO_4 \equiv 31{\cdot}006 \times 10^{-3} \text{ g of } K_2CS_3 \equiv 16{\cdot}03 \times 10^{-3} \text{ g of S.}$$

Discussion

The results in Table III show that the reaction of potassium thiocarbonate with acidified potassium permanganate is such that the three sulphur atoms of thiocarbonate undergo oxidation to elemental sulphur according to the following equations—

$$MnO_4^- + 8H^+ + 5e^- \longrightarrow Mn^{2+} + 4H_2O$$

$$CS_3^{2-} + 3(O) \longrightarrow CO_3^{2-} + 3S + 6e^-$$

i.e., $6KMnO_4 + 9H_2SO_4 + 5K_2CS_3 \longrightarrow 3K_2SO_4 + 6MnSO_4 + 9H_2O + 5K_2CO_3 + 15\ S$

Thus, 6 moles of $KMnO_4$ ≡ 5 moles of K_2CS_3.

The results of titrimetric evaluation with permanganate are accurate for 0·006 to 0·06 M concentrations of potassium thiocarbonate. The volume of permanganate taken should be such that not more than 8 mg of sulphur are precipitated out after the complete reaction.

Table III

Results of the determination of thiocarbonate sulphur with potassium permanganate in the presence of 0·05 to 0·1 N sulphuric acid

Volume of 0·05 N potassium permanganate taken, ml	Titre of 0·03 M potassium thiocarbonate, ml	Number of equivalents of oxidant per mole of potassium thiocarbonate used	Sulphur present, mg	Sulphur found, mg	Difference, mg
5	1·38	6·03	4·00	3·97	−0·03
5	1·40	5·95	4·00	4·03	+0·03
10	2·80	5·95	8·12	8·06	−0·06
10	2·82	5·91	8·12	8·12	0·0
15	4·46	5·60	12·18	12·84	+0·66
15	4·44	5·63	12·18	12·78	+0·60

The fact that the three sulphur atoms of thiocarbonate are oxidised by permanganate and no trace of carbon disulphide separated was confirmed by the negative result of a colorimetric test.[22] In this test a drop of the solution should produce a stable, pink colour if acetone

and elemental sulphur are present together with free carbon disulphide. The reaction of permanganate with potassium thiocarbonate in alkaline medium at room temperature was also studied, and it was found that only one sulphur atom of thiocarbonate had undergone oxidation to sulphate, while the other two formed carbon disulphide. Furthermore, results at 60° C are not reproducible.

To ensure that the liberated hydrogen sulphide undergoes oxidation, potassium thiocarbonate is added to the permanganate, dropwise, from a burette.

Conclusions

The results are quantitative, and suitable dilution of more concentrated solutions of potassium thiocarbonate is necessary to obtain accurate results. Under optimum conditions the three sulphur atoms of the thiocarbonate are oxidised, thus giving a rapid method for determining and identifying thiocarbonate from other sulphur compounds.

The authors thank Professor R. P. Mitra, F.N.I., Head of the Department of Chemistry, University of Delhi, for the facilities provided, and one of us (N.K.K.) is also grateful to the Council of Scientific and Industrial Research, New Delhi, India, for the award of a Research Fellowship.

References

1. Johri, K. N., *J. Scient. Ind. Res.*, 1969, **18B**, 430.
2. ——, *J. Indian Chem. Soc.*, 1961, **38**, 935.
3. ——, *Analyst*, 1961, **86**, 487.
4. ——, *Indian J. Appl. Chem.*, 1963, **26**, 114.
5. ——, "Chemical Analysis without Hydrogen Sulphide using Potassium Trithiocarbonate," Asia Publishing House, Bombay 1, 1963.
6. Johri, K. N., and Singh, K., *Indian J. Chem.*, 1965, **3**, 158.
7. ——, ——, *Analyst*, 1965, **90**, 745.
8. Jacobs, M. B., "The Analytical Chemistry of Industrial Poisons, Hazards and Solvents," Second Edition, Interscience Publishers Inc., New York and London, 1949, p. 323.
9. Scott, W. W., "Standard Methods of Chemical Analysis," Fifth Edition, D. Van Nostrand, New York, 1939, Volume 1, pp. 911 and 923.
10. Duval, C., "Inorganic Thermogravimetric Analysis," Elsevier Publishing Co. Ltd., Amsterdam, London and New York, 1953, p. 134.
11. Murthy, A. R. V., *Curr. Sci.*, 1953, **22**, 342.
12. Nair, C. G. R., and Murthy, A. R. V., *J. Scient. Ind. Res.*, 1962, **21B**, 146.
13. ——, ——, *Chem. & Ind.*, 1962, 1539.
14. ——, ——, *Mh. Chem.*, 1963, **94**, 134.
15. Rao, V. R. S., and Murthy, A. R. V., *Talanta*, 1960, **4**, 206.
16. Aravamudan, G., and Rao, V. R. S., *Ibid.*, 1964, **11**, 55.
17. Jose Jacob, T., and Nair, C. G. R., *Ibid.*, 1966, **13**, 154.
18. Sant, B. R., and Sant, S. B., *Ibid.*, 1960, **3**, 261.
19. Deshmukh, G. S., and Garde, P., *Bull. Chem. Soc. Japan*, 1967, **40**, 1543.
20. Bishop, E., and Jennings, V. J., *Talanta*, 1958, **1**, 197.
21. Nair, C. G. R., and Murthy, A. R. V., *Proc. Indian Acad. Sci.*, 1962, **55A**, 168.
22. Urbanski, T., *Talanta*, 1962, **9**, 799.

Received *March 3rd*, 1968

Analyst, December, 1968, Vol. 93, *pp.* 797–798

The Determination of Molybdenum in Mixtures Containing Molybdenum Disulphide by Atomic-absorption Spectrophotometry

By R. J. JULIETTI and J. A. E. WILKINSON

(*Morganite Research and Development Ltd., Battersea Church Road, Battersea, London, S.W.*11)

A rapid method is described for determining the total molybdenum content of mixtures containing molybdenum disulphide, graphite and a resin. The novel feature of the technique is the decomposition of the mixture by fusion with sodium hydroxide. Dissolution of the melt in sulphuric acid permits the determination of molybdenum by atomic-absorption spectrophotometry without interference. Results at the 8 per cent. molybdenum level are accurate to within $\pm 0{\cdot}1$ per cent.

Molybdenum disulphide is finding increasing use in industry as a solid lubricant. It is widely used as an additive to oils and greases, particularly for high temperature lubrication, in the form of a dispersion for "dry-film" impregnation, and in bearing components and electrical contacts. Rapid methods for its determination at the percentage level are, therefore, of value.

Experimental

Molybdenum disulphide is particularly difficult to decompose; the usual methods involve dissolution in strong acids, *e.g.*, as in Defence Specification DEF-2304,[1] in which fuming perchloric acid is used. In this laboratory we have also used mixtures of nitric and hydrobromic acids and of nitric and sulphuric acids for dissolution. A combustion method of decomposition has also been reported.[2]

The samples normally received for analysis contain molybdenum disulphide, graphite and a resin, and the determination of molybdenum is sufficient to define the molybdenum disulphide content. In earlier methods if the sample contained a resin, it was decomposed by heating at 450° C, at which temperature there is no loss of molybdenum. The material was then heated with a mixture of acids, usually containing nitric acid to accelerate the attack. After filtration and removal of all nitrate by fuming with concentrated sulphuric acid, the solution was passed through a Jones' reductor and titrated in the usual way. This method, although sufficiently accurate for process control analysis ($\pm 0{\cdot}1$ per cent.), was time consuming. The rapid finish afforded by atomic-absorption spectrophotometry suggested a means of shortening the procedure.

If an acid decomposition were used, the interference from sulphate and nitrate could be overcome as suggested by David.[3]

However, we found that decomposition by fusion with sodium hydroxide is much more rapid than by any other method. The molybdenum disulphide dissolves at just above the fusion temperature of the alkali, and further, by heating for a little longer at a higher temperature, the bulk of the graphite can also be decomposed.

The fusion was carried out in a nickel crucible and the melt dissolved in a standard amount of dilute sulphuric acid (1 + 1). No interference was found from either sodium or nickel. Interference from the variable amount of sulphate resulting from the oxidation of molybdenum disulphide is swamped by the presence of the large excess of sulphuric acid. The following procedure has been found satisfactory.

Procedure—

Weigh 5 g of sodium hydroxide pellets into a nickel crucible (about 40mm tall and 50mm diameter) and fuse over a bunsen burner. On cooling, swirl the crucible to coat the walls with a layer of flux.

Weigh 0·5 g of sample, containing about 8 per cent. of molybdenum, and spread it over the top of the melt by tapping. Fuse gently, without a lid, over a small bunsen flame until the effervescence subsides (about 15 minutes). Do not swirl.

Transfer the crucible to a muffle furnace at 650° C and leave for 30 minutes. Allow to cool, place the crucible upright in a 250-ml beaker containing 50 ml of distilled water and cover the beaker with a watch-glass. Cautiously add 20 ml of dilute sulphuric acid (1 + 1)* to the crucible, with the watch-glass slightly displaced. Heat, if necessary, to complete the dissolution of the melt. Remove and wash the crucible, collecting the washings in the beaker, and boil the solution for 5 minutes to remove any traces of hydrogen sulphide.

Allow the solution to cool and filter it through a No. 40 filter-paper into a 250-ml calibrated flask. Dilute to the mark and compare the absorbance with that of the standard solution by atomic-absorption spectrophotometry.

Standard solution of molybdenum—

Weigh accurately about 0·6 g of analytical-reagent grade molybdenum trioxide, previously dried for 1 hour at 150° C. Dissolve it in 50 ml of 10 per cent. w/v sodium hydroxide solution. Add 20 ml of dilute sulphuric acid (1 + 1). Cool and make up to 250 ml with distilled water. This solution is stable for at least 1 month.

Atomic-absorption measurements—

A Techtron A.A.4 atomic-absorption spectrophotometer was used, and the conditions were as follows: wavelength, 313·3 nm; slit width, 50 μm; flame, air - acetylene; burner, 50-mm slot A.B.40, high temperature burner, which was found to give much less variation in absorbance readings for molybdenum than the 100-mm, A.B.41, burner supplied as a standard fitting with the instrument; air pressure, 1 bar (15 p.s.i.); acetylene flow, approximately on position 4 of the flow meter, but adjusted to give maximum absorbance when a solution containing molybdenum is aspirated; height of light path above burner, about 10 mm, but also adjusted for maximum absorbance after the flame conditions have been set; and aspiration rate, about 2 ml per minute.

Results

Two samples were analysed for molybdenum by the above procedure. Fourteen results on the first sample, which contained a resin, had a mean value of 7·71 per cent. of molybdenum, compared with values of 7·79 and 7·84 per cent. by our previous chemical method.

The mean value of eight results on the second sample (not containing a resin) was 9·11 per cent. Values of 9·08 and 9·13 per cent. of molybdenum were obtained on this sample by the gravimetric benzoin α-oxime method.

The coefficient of variation on the two sets of results by the atomic-absorption procedure described was less than 1 per cent. in each instance.

Conclusion

This method has been used successfully in this laboratory and, compared with our previous method, has the following advantages.

(*i*) There is no need for a preliminary decomposition of the resin.
(*ii*) The dissolution of molybdenum disulphide is more rapid.
(*iii*) No nitrate ions are introduced.
(*iv*) The total working time is much shorter.

The authors thank the directors of Morganite Research and Development Limited for permission to publish this paper.

References

1. Defence Specification DEF-2304, January 1966, "Molybdenum Disulphide (Powdered)" ZX-35, H.M. Stationery Office, London, 1966.
2. Kalnin, I. L., *Analyt. Chem.*, 1964, **36**, 886.
3. David, D. J., *Analyst*, 1968, **93**, 79.

Received *July 4th*, 1968

* This reagent should be prepared and added as accurately as possible with a measuring cylinder, as the amount of sulphuric acid in the standard and sample solutions must be the same.

Analyst, December, 1968, Vol. 93, *pp.* 799–801

Determination of Antimony in Titanium Dioxide by Atomic-absorption Spectrophotometry

BY J. C. MÉRANGER AND E. SOMERS

(Research Laboratories, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada)

A method is described for the determination of the antimony content of titanium dioxide by atomic-absorption spectrophotometry, based on the extraction of antimony with isobutyl methyl ketone. This method is more sensitive than determination by emission-spectrographic and as sensitive as the colorimetric technique, and can detect 25 p.p.m. of antimony in a 0·1-g sample of titanium dioxide.

THE titanium dioxide used in pharmaceutical preparations, and as a food colour, may be contaminated with antimony, and there are several official specifications that give limits for the total, or acid-extractable, antimony. Spectrophotometric determination of the antimony in titanium dioxide with Brilliant green has been described by Galliford and Yardley,[1] and Ratcliffe and Stevens[2]; the latter workers showed that many of the pharmaceutical samples of titanium dioxide examined exceeded the British Pharmaceutical Codex limits for antimony. Atomic-absorption spectrophotometry was used by Mostyn and Cunningham[3] for the determination of antimony in non-ferrous alloys and, in view of the convenience and sensitivity of this technique, we have applied it to the determination of antimony in titanium dioxide. The atomic-absorption analysis has been compared with colorimetric and spectrographic methods, and we have determined the effectiveness of dilute hydrochloric acid in extracting antimony from titanium dioxide.

EXPERIMENTAL

SPECTROGRAPHIC ANALYSIS—

A Jarrell-Ash, Model 15000 Lpl, 1·5-m, wide-angle, grating emission spectrograph was used. Titanium dioxide samples (10 mg) were mixed with 30 mg of spectrographic-grade graphite and introduced into 3·2-mm electrodes with 2 × 15-mm craters, with a 3·2-mm counter electrode. The samples were arced for 25 seconds at 4 and 6A to completion; the resulting spectra were recorded on Kodak SA-1 film. The intensity of the antimony 259·8 nm line in the unknown samples was compared with that obtained from mixtures of spectroscopically pure antimony tetroxide and titanium dioxide.

FUSION OF TITANIUM DIOXIDE—

The method followed was that of the Food and Agricultural Organisation Specifications for Identity and Purity of Food Additives.[4] Titanium dioxide (0·1 g) was fused with 5 g of potassium hydrogen sulphate and 10 mg of glucose in 30-ml Kjeldahl flasks. The melt was dissolved by heating with 10 ml of sulphuric acid (96 to 98 per cent. w/v) then, when cool, made up to 100 ml with 30 per cent. w/v hydrochloric acid.

COLORIMETRIC DETERMINATION—

Aliquots of the above solution, containing from 1 to 10 μg of antimony, were oxidised with solid cerium(IV) sulphate, extracted with di-isopropyl ether, and the optical density of the complex formed with Rhodamine B measured at 555 nm.[4]

ATOMIC ABSORPTION—

A Perkin-Elmer, Model 303, atomic-absorption spectrophotometer, equipped with a Boling burner and a null recorder read-out accessory coupled to a Westronics, Model S11A/U, 11-inch strip-chart recorder, was used. The operating parameters were: acetylene flow-rate, 6; compressed air flow-rate, 13; solution uptake, 3·2 ml per minute; slit, position 4; wavelength, 217·6 nm; scale expansion, ×3; meter response, 4; and antimony hollow-cathode lamp, current 30 mA.

STANDARD SOLUTIONS—

Standards of 1, 10 and 100 μg of antimony per ml were freshly prepared by dissolving analytical-reagent grade potassium antimony tartrate, $KSbC_4H_4O_7.1/2H_2O$, in hydrochloric acid (30 per cent. w/v).

EXTRACTION WITH HYDROCHLORIC ACID—

The extraction method is that given in the U.S. Food and Drug Administration Color Additive Regulations.[5] Titanium dioxide (10 g) was boiled for 15 minutes in 50 ml of 0·5 N hydrochloric acid, then samples of the solution were filtered (Whatman No. 42) and made up to 100 ml.

RESULTS

SOLVENT EXTRACTION OF ANTIMONY—

When the acidic solutions from the potassium hydrogen sulphate fusion of titanium dioxide were nebulised in the atomic-absorption spectrophotometer high results were obtained, probably because of light-scattering by salt particles. In addition, this method was not sensitive enough to determine the low levels of antimony present in food-grade titanium dioxide. To overcome these inadequacies, an extraction procedure was devised. The extraction of the ammonium antimony pyrrolidinedithiocarbamate complex[3] was attempted with isobutyl methyl ketone. However, the chelate was not formed under the conditions of high acidity used in this work. Chelation was subsequently found to be unnecessary as isobutyl methyl ketone quantitatively extracted the antimony(III) or (V) directly from the aqueous acidic solutions. Consistent extraction of a standard antimony solution was obtained from solutions of hydrochloric and sulphuric acids in the acid concentration range of that of the dissolved, fused titanium dioxide (Table I).

TABLE I

EFFECT OF ACID CONCENTRATION ON THE EXTRACTION OF 50 μg OF ANTIMONY WITH ISOBUTYL METHYL KETONE (10 ml), BY ATOMIC ABSORPTION

	Hydrochloric acid, per cent. w/v			Sulphuric acid, per cent. w/v		
	10	12	14	6	10	13
Optical density*	0·042	0·041	0·041	0·041	0·042	0·043

* Average of duplicate determinations.

ATOMIC-ABSORPTION DETERMINATION OF EXTRACTED ANTIMONY—

Aliquots (10 ml) of the acidic solution from fused titanium dioxide were transferred, by pipette, into a 125-ml separating funnel, 5 ml of water and 10 ml of isobutyl methyl ketone added and the funnel shaken vigorously for 1 minute. After the layers had separated, the aqueous layer was drawn off and the organic solvent directly aspirated in the atomic-absorption spectrophotometer. For samples of titanium dioxide with less than 100 μg of antimony per g, the 10-ml aliquot can be increased to 40 ml, but 20 ml of water, instead of 5 ml, must then be added.

Two calibration graphs were prepared by following the above procedure with antimony standards over the range 10 to 100 μg and 0 to 10 μg. One millilitre of sulphuric acid (98 per cent. w/v) was added to the standards and they were diluted to 10 ml with 30 per cent. w/v hydrochloric acid. Blank values were obtained from the acidic solutions alone. A linear relationship between optical density and antimony concentration was found over the range 0·1 to 10 μg per ml, with a lower detection limit of 0·1 μg of antimony per ml.

When several samples are analysed it is desirable to have a stock solution of antimony (5 μg per ml) extracted into isobutyl methyl ketone. This solution, appropriately diluted, can be nebulised in the spectrophotometer at fixed intervals, and so slight variations in sensitivity can be normalised.

The analysis of a series of synthetic mixtures of titanium dioxide and standard antimony solutions (Table II) showed good recoveries of antimony over the range 250 to 10,000 p.p.m.

TABLE II

ANTIMONY RECOVERIES FROM 0·1 g OF TITANIUM DIOXIDE

Antimony added, μg	Antimony recovered, μg*	Percentage recovery
25	27	108
50	52	104
100	105	105
400	438	110
1000	905	91

* Average of duplicate determinations.

COMPARISON OF ANALYTICAL METHODS—

The antimony content of three commercial samples of titanium dioxide (anatase form) was determined by emission-spectrographic, colorimetric and solvent-extraction atomic-absorption methods. The samples were chosen to represent a range of antimony content, sample A being a high quality, food-additive grade. Table III shows that atomic-absorption, combined with extraction, provides a method of analysis that is both consistent with, and as sensitive as, the other two methods. The lower limit of detection of antimony in a 0·1-g sample of titanium dioxide is 25 p.p.m. Extraction with hydrochloric acid removed only a very small proportion of the total antimony.

TABLE III

COMPARISON OF METHODS OF ANALYSIS FOR THE DETERMINATION OF THE ANTIMONY CONTENT OF TITANIUM DIOXIDE, AS P.P.M.

				Extraction with 0·5 N hydrochloric acid	
Sample	Spectrographic	Colorimetric	Atomic absorption	Colorimetric	Atomic absorption*
A	<100	<25	<25	<0·1	<0·7
B	100†	100	120	<0·1	<0·7
C	12,500†	11,300	11,000	4·9	4·6

All values are averages of duplicate determinations.

* Direct aspiration of aqueous solution, *i.e.*, not extracted with isobutyl methyl ketone.

† ±10 per cent.

CONCLUSIONS

Until recently, the methods available for the determination of low concentrations of antimony in titanium dioxide were erratic and imprecise.[1,2] Atomic-absorption spectroscopy is a rapid, accurate and interference-free analytical technique, and the isobutyl methyl ketone extraction method we have adopted combines the advantages of atomic absorption, together with sensitivity equal to the existing colorimetric methods.

Acid extraction of antimony is used as the basis for official specifications of titanium dioxide.[4,5] Whatever the toxicological significance of the antimony extracted by hydrochloric acid may be, it is clear that this technique gives no useful information as to the total antimony content of the sample.

We are grateful to Mr. R. E. Horton and Mr. C. C. Durham of the Department of Energy, Mines and Resources, Ottawa, for the analyses by emission spectrography.

REFERENCES

1. Galliford, D. J. B., and Yardley, J. T., *Analyst*, 1963, **88**, 653.
2. Ratcliffe, R. J. M., and Stevens, S. G. E., *Mfg Chem. Aerosol News*, 1964, **35**, (9), 83.
3. Mostyn, R. A., and Cunningham, A. F., *Analyt. Chem.*, 1967, **39**, 433.
4. "Specifications for Identity and Purity of Food Additives," Food and Agricultural Organisation, Rome, 1963, Volume 2, p. 26.
5. *Fedl Register*, 1966, **31**, 1065.

Received *July 5th*, 1968

The Determination of Zirconium in Mineral Rutile with Alizarin Red S

By N. B. STANTON

(*Associated Minerals Consolidated Limited, Southport, Queensland, Australia*)

A colorimetric procedure has been developed for the determination of the zirconium oxide content of rutile. A fusion with potassium hydrogen difluoride enables the rutile to be brought into solution rapidly and eliminates the task of filtering off silica. The zirconium is separated from the titanium by a phosphate precipitation, but the interference of tin inherent in the method involving phosphate precipitation followed by ignition to oxide, is eliminated by dissolving the zirconium phosphate and applying a colorimetric finish, with Alizarin red S. Calibration is effected by making additions of zircon to titanium oxide and carrying each through the entire procedure, thus eliminating reagent-blank difficulties. It has been used in the range of 0 to 1 per cent. of zirconium oxide. Good agreement with independent determinations by X-ray fluorescence has been obtained.

The main constituents of the mineral sands of Australian east coast beaches are zircon, rutile and ilmenite. The guarantees under which these products are sold include the maximum amounts of each present as impurities in the others. A need, therefore, exists for a method for determining the zirconium oxide content of rutile. From this content the percentage of zircon, which is accepted as the sole contributor to the zirconium oxide content, can be inferred. The guarantee at the present time requires a maximum of 1 per cent. of zirconium oxide.

Methods previously used for this purpose were variants of two main procedures. The first was the precipitation of zirconium with diammonium hydrogen orthophosphate. As pointed out by Wood and McKenna,[1] tin interferes in this method. Rutile commonly contains about 0·1 per cent. of tin oxide, and it has been shown in this laboratory that tin oxide added to rutile can be recovered by phosphate precipitation. A cassiterite concentrate that had previously been analysed for tin oxide was used for additions to a rutile sample, which was then fused with potassium hydrogen difluoride and subjected to a phosphate precipitation technique to be described, followed by ignition of the phosphate precipitate. The weight of contained tin oxide added was 6·2 mg. The difference in weights between the oxide obtained from the rutile and from the "spiked" rutile was 5·2 mg. The method is, therefore, not satisfactory for this purpose.

The second method involves precipitation with mandelic acid. This approach is tedious and time consuming and, further, is not suited to the determination of amounts at the lower end of the range encountered, *e.g.*, 0·3 per cent. of zirconium oxide. It appears that a certain amount must be present before the precipitate can be collected.

Mayer and Bradshaw[2] first put forward the use of Alizarin red S as a reagent for the determination of zirconium, with reference to magnesium alloys.

Snell[3] states that titanium interferes in the determination of zirconium with Alizarin red S, and Wood and McKenna[1] correct for a small amount of interference by their method of calibration. An attempt by the author to determine zirconium in rutile directly was unsuccessful because of the difficulty of controlling the hydrochloric acid concentration of the dissolved melt, and the added disadvantage of the presence of sulphate ions. The approach adopted was that suggested by Vinogradov and Ryabchikov.[4] The titanium was first separated by precipitation of the zirconium with phosphate, the precipitate re-dissolved and a colorimetric finish applied. However, the technique used by these authors for re-dissolving the precipitate on the paper with 5 per cent. oxalic acid was not successful under the conditions experienced, and this approach was modified. The method used for colour formation was that recommended by Wood and McKenna[1] and the U.S. Atomic Energy Commission.[5]

Experimental

Calibration was carried out by weighing about 1·5, 4·5, 7·5, 10 and 15 mg of finely ground zircon consecutively into five platinum crucibles, each containing 6 g of potassium hydrogen difluoride and 1 g of Johnson, Matthey "Specpure" titanium dioxide. These additions correspond to 0·10, 0·30, 0·50, 0·675 and 1·0 per cent. of zirconium dioxide, respectively. Each was then taken through the full procedure to be described. This approach was adopted for two reasons. Firstly, Wood and McKenna[1] did not use zirconium nitrate solution because the reagent contains more than the theoretical amount of zirconium, resulting from gradual decomposition of the zirconium nitrate; instead, they used metallic zirconium. It was, however, possible to obtain a pure grade of zircon of known zirconium content for this work. Secondly, the problem of adjusting for the reagent blank is overcome as it is automatically incorporated in the calibration. Any approach in which an attempt is made to subtract a blank reading, obtained by taking the reagents alone through the procedure, from an observed reading is not considered valid. Anomalous enhancement and depression effects on colour development can occur. Wood and McKenna[1] refer to the enhancement effect of titanium on zirconium solutions, as compared with the effect on the reagent blank. The author has made a comparison in some instances of the colour development obtained by adding a solution of the element, and the colour reagent, to distilled water with that obtained by taking the element through the entire procedure and subtracting a reagent blank. An enhancement effect occurs with the former. For these reasons it is desirable to simulate the actual method in its entirety when carrying out the calibration.

Colour development—

Wood and McKenna heated the solution for 5 minutes at 70° to 80° C. Vinogradov and Ryabchikov advocate bringing it to the boil. It was found that neither approach, as applied to our problem, guaranteed full colour development, and prolonged boiling, as recommended by the U.S. Atomic Energy Commission,[5] was adopted successfully.

Double filtration of the Alizarin red S solution, as recommended by Wood and McKenna, is considered essential.

Method

Reagents—

Alizarin red S solution, 0·1 per cent.[1]—Dissolve 1·0 g of Alizarin red S in about 300 ml of hot water, boil and then filter the solution through a pad of paper pulp. Dilute the solution to 1 litre and again filter through a pad of paper pulp.

Procedure—

Fuse 1 g of previously ground sample with 6 g of potassium hydrogen difluoride in a platinum crucible. Cool the melt, transfer it to a platinum basin and add 60 ml of sulphuric acid (1 + 1). Heat gently until copious fumes of sulphuric acid are evolved. This removes the silica, and all of the titanium and zirconium is brought into solution. Cool the solution, dilute to 450 ml, and transfer it to a 600-ml beaker. Add 25 ml of 30 per cent. hydrogen peroxide and 25 ml of 20 per cent. dibasic ammonium hydrogen orthophosphate solution; allow to stand overnight in a warm place.

Filter the solution through a Whatman No. 31 paper and wash with 5 per cent. sulphuric acid, to which a little hydrogen peroxide has been added, until no colour remains on the paper. Continue washing (6 or 7 times) with 5 per cent. ammonium nitrate solution. Wash the precipitate from the paper into a 250-ml beaker with hot water, then add 10 ml of 10 per cent. sodium hydroxide solution. Bring the mixture to the boil and allow the precipitate to coagulate for 20 minutes in a steam-bath. Filter on a Whatman No. 41 filter-paper and wash the precipitate with 5 per cent. ammonium nitrate solution. Wash the precipitate from the paper into the same 250-ml beaker with hot distilled water and add 25 ml of 5 N hydrochloric acid; the volume should now be about 100 ml. Boil until the volume is reduced to 40 ml, cool, make up to 100 ml and filter through a Whatman No. 41 filter-paper. To a 10-ml aliquot of the filtrate, add 2·5 ml of 5 N hydrochloric acid. Add 5 ml of 0·1 per cent. Alizarin red S and make the solution up to 50 ml. After allowing it to stand for 1 hour, measure the absorption at 560 nm. The instrument used was a Bausch and Lomb Spectronic 20.

COMPARISON WITH X-RAY FLUORESCENCE DETERMINATION—

The accuracy of the method has been demonstrated by comparison of the results with those obtained by X-ray fluorescence determination, in which the method of additions, as described by Birks,[6] was used, background correction being allowed for. Results are shown in Table I.

TABLE I

DETERMINATION OF ZIRCONIUM OXIDE IN RUTILE BY CHEMICAL AND X-RAY FLUORESCENCE METHODS

	Zirconium oxide (ZrO_2), per cent., by—	
Sample No.	Chemical method	X-ray fluorescence method
1	0·27	0·28
2	0·36	0·37
3	0·38	0·40
4	0·46	0·43
5	0·51	0·50
6	1·10	1·06

CONCLUSION

With this method a single determination can be carried out in 24 hours. It requires a slightly longer time than the phosphate precipitation method, but eliminates the error caused by tin oxide inherent in the latter. The calibration method initially is time consuming, but no further calibration is required for a given set of reagents.

The assistance of Miss Rose Thomas in carrying out some of the chemical determinations is acknowledged with thanks. Acknowledgment is also accorded to the Directors and Management of Associated Minerals Consolidated Limited for permission to publish this paper.

REFERENCES

1. Wood, D. F., and McKenna, R. H., *Analyst*, **1962, 87**, 880.
2. Mayer, A., and Bradshaw, G., *Ibid.*, **1952, 77**, 476.
3. Snell, F. D., and Snell, C. T., "Colorimetric Methods of Analysis," Volume IV, Organic, II, Third Edition, D. Van Nostrand Co. Inc., New York; Macmillan & Co. Ltd., London, **1954**, p. **447**.
4. Vinogradov, A. P., and Ryabchikov, D. I., *Editors*, "Detection and Analysis of Rare Elements," National Science Foundation, Washington, **1962**, p. **366**.
5. "Manual of Special Materials Analytical Laboratory Procedures," *U.S. Atomic Energy Commission Report* ANL–5410, **1955**, p. **49**.
6. Birks, L. S., "X-Ray Spectrochemical Analysis," Interscience Publishers Inc., New York, **1959**, p. **67**.

Received *March 25th*, **1965**
Amended *July 10th*, **1967**

Analyst, December, 1968, Vol. 93, *pp.* 805–809

The Effect of Ethanol on the Colorimetric Determination of Formaldehyde and Glycollic Acid

By R. H. STILL, K. WILSON and B. W. J. LYNCH

(*Department of Chemistry and Biology, Hatfield College of Technology, Hatfield, Hertfordshire*)

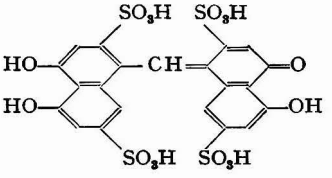
Contrary to previous reports ethanol has been found to interfere with the colorimetric determination of formaldehyde and glycollic acid with 1,8-dihydroxynaphthalene-3,6-disulphonic acid (chromotropic acid) or 2,7-dihydroxynaphthalene in concentrated sulphuric acid. The effect is greater for glycollic acid than for formaldehyde. The mechanism for these observations is given.

During the study of the rheological properties of some sodium carboxymethylcelluloses a routine method for the determination of the sodium glycollate impurity was required. The standard procedure, developed by Easterwood,[1] involves the preliminary removal of the salt impurities by washing with hot (50° to 60° C), 80 per cent. aqueous ethanol until the wash liquor no longer gives a positive test for chloride ions. The glycollic acid content of these ethanolic wash liquors is then determined by a colorimetric method based on the conversion of glycollic acid into formaldehyde, and its determination with chromotropic acid. Optical density measurements are made at 570 nm, and are related to concentration by using a calibration graph prepared for a series of standard aqueous glycollic acid solutions.

The use of standard aqueous solutions of glycollic acid is based on previous observations by Bricker and Johnson[2] that ethanol and methanol do not interfere with the colour reaction in the determination of formaldehyde. In a later paper, Bricker and Vail[3] modified their procedure to remove volatile organic impurities after adding chromotropic acid but before adding concentrated sulphuric acid. Even so, they were still of the opinion that these two specific alcohols did not, in fact, interfere with the colour development of the formaldehyde. This view is also supported by the later work of Ekberg and Silver,[4] and Beroza.[5]

A conductimetric method more suitable for the routine determination of sodium glycollate was developed,[6] but when it was applied to the ethanolic wash liquors from sodium carboxymethylcellulose it was found to give consistently higher values for the glycollate content than those obtained by the colorimetric method of Easterwood. Subsequent investigation revealed the discrepancies to be caused by the interference by ethanol in the colour development with chromotropic acid. This led to a re-appraisal of Easterwood's method and that of the standard colorimetric determination of formaldehyde in ethanolic solution with chromotropic acid.

The chemistry of the reaction ofchromotropic acid with form aldehyde is not known with certainty. Feigl[7] postulates that, as aromatic hydroxy compounds condense with formaldehyde to yield colourless hydroxyphenylmethanes, it is probable that the initial step consists of a condensation of the phenolic chromotropic acid with formaldehyde, followed by oxidation to a *p*-quinonoidal compound of the type shown below—



Concentrated sulphuric acid participates in the reaction both as a dehydrant and an oxidising agent.

Eyler, Klug and Diephus[8] have used 2,7-dihydroxynaphthalene in concentrated sulphuric acid to detect and determine formaldehyde colorimetrically. Several colour reagents have been investigated by West and Sen,[9] who concluded that chromotropic acid and 2,7-dihydroxynaphthalene were outstanding for determining formaldehyde.

Experimental

Reagents—

Glycollic acid—(Koch-Light crystalline), recrystallised from water, m.p. 79·5° to 80° C. A standard aqueous or ethanolic solution was prepared containing $1{\cdot}32 \times 10^{-3}$ moles per litre.

Chromotropic acid—Obtainable from British Drug Houses Ltd. for formaldehyde determination. A 5 per cent. aqueous solution of the disodium salt was prepared immediately prior to use.

2,7-Dihydroxynaphthalene—A solution in 95 per cent. sulphuric acid containing 0·1 g per litre was prepared.

Sulphuric acid, sp.gr. 1·84.

Ethanol—The procedure for the preparation of the various aqueous ethanol samples is similar to that for 80 per cent. aqueous ethanol. An 80-ml aliquot of absolute ethanol was introduced into a 100-ml calibrated flask and made up to the mark with distilled water.

Formaldehyde—An aqueous solution was obtained by the dry distillation of paraformaldehyde, and passage of the formaldehyde vapour formed (after rejection of the first 30 per cent. of the distillate to remove polymer) into ice-cold, continuously stirred, distilled water. This solution was standardised by using both sodium sulphite and iodimetric methods.[10,11]

Standard aqueous and ethanolic solutions containing $1{\cdot}66 \times 10^{-3}$ moles per litre of formaldehyde were prepared by dilution.

Ethyl glycollate—Standard aqueous and ethanolic solutions containing $1{\cdot}34 \times 10^{-3}$ moles per litre were prepared.

Diethyl formal—This was prepared by the method of Vogel[12] as a colourless liquid which, when fractionally distilled, yielded a fraction b.p. 87° to 89° C and n_D^{20} 1·3730. Standard aqueous or ethanolic solutions containing $1{\cdot}32 \times 10^{-3}$ moles per litre were prepared.

Apparatus—

Optical density measurements were made with 1-cm cells on a Unicam SP600 spectrophotometer and a Beckmann DB recording spectrophotometer.

Procedure

Beer - Lambert plots obtained by using aqueous and 80 per cent. aqueous ethanolic solutions of the substrates—

Chromotropic acid determinations—An aliquot of the standard solution of the substrate (glycollic acid, formaldehyde, diethyl formal or ethyl glycollate) dissolved in water or 80 per cent. aqueous ethanol was added, from a 2-ml microburette, to a 50-ml calibrated flask. Distilled water or 80 per cent. aqueous ethanol was then added to adjust the total volume to 2 ml, then 1 ml of chromotropic acid was added, followed by concentrated sulphuric acid to bring the total volume to about 40 ml. The reactants were mixed by shaking and the flask was immersed in a boiling water bath for exactly 30 minutes. The flask was removed and allowed to cool to room temperature and the volume adjusted to the mark with concentrated sulphuric acid so that, for the 80 per cent. aqueous ethanolic solution, the final concentration of ethanol in the reaction mixture was therefore 3·2 per cent. v/v.

A blank determination with either water or 2 ml of 80 per cent. aqueous ethanol was made by using the same procedure. When the blank solution was used as reference the optical density at 580 nm (the $\lambda_{max.}$ was found to be 580 nm, its position remaining constant, independent of the presence of ethanol) was measured.

2,7-Dihydroxynaphthalene determinations[8]—An aliquot of glycollic acid dissolved in water or 80 per cent. aqueous ethanol was added from a 2-ml microburette to a 50-ml flask. Distilled water or 80 per cent. aqueous ethanol was then added to adjust the volume to 2 ml. A 20-ml aliquot of 2,7-dihydroxynaphthalene was added, the contents of the flask well

mixed and the flask immersed in a boiling water bath for exactly 30 minutes. The flask was removed and allowed to cool to room temperature and the volume adjusted to the mark with distilled water.

EFFECT OF ETHANOL CONCENTRATION ON THE MOLAR EXTINCTION COEFFICIENTS WITH CHROMOTROPIC ACID—

Solutions of the four substrates in water, ethanol or aqueous ethanol were prepared, giving a range of ethanol concentration in the final reaction mixture of 0 to 4·0 per cent. and a substrate concentration of $3{\cdot}2 \times 10^{-5}$ moles per litre. The optical density was measured as previously by using an appropriate aqueous, ethanolic or aqueous ethanolic blank. The molar extinction coefficients were then calculated.

RESULTS AND DISCUSSION

TABLE I

Substrate	Colour reagent	Solution	Molar extinction coefficient, litres moles^{-1} cm^{-1} $\times 10^{-4}$
Glycollic acid	A	Aqueous	1·85
Glycollic acid	A	80 per cent. aqueous ethanol	0·64
Glycollic acid	B	Aqueous	2·32
Glycollic acid	B	80 per cent. aqueous ethanol	0·31
Formaldehyde	A	Aqueous	1·84
Formaldehyde	A	80 per cent. aqueous ethanol	1·45
Ethyl glycollate	A	Aqueous	1·68
Ethyl glycollate	A	80 per cent. aqueous ethanol	0·63
Diethyl formal	A	Aqueous	1·55
Diethyl formal	A	80 per cent. aqueous ethanol	1·19

Colour reagent A is chromotropic acid.
Colour reagent B is 2,7-dihydroxynaphthalene.

Beer - Lambert plots for all the substrates studied in both aqueous and ethanolic solution, were straight lines passing through the origin. The molar extinction coefficients calculated from the slopes of these lines are summarised in Table I.

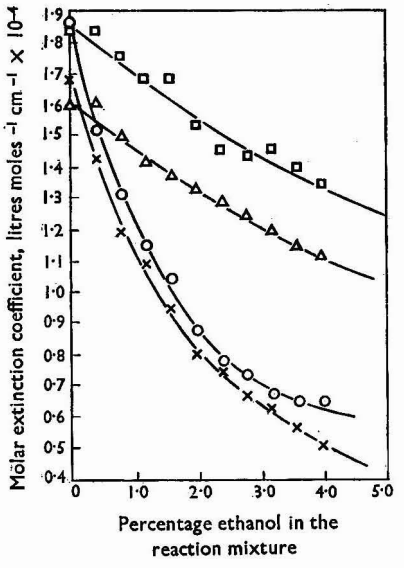
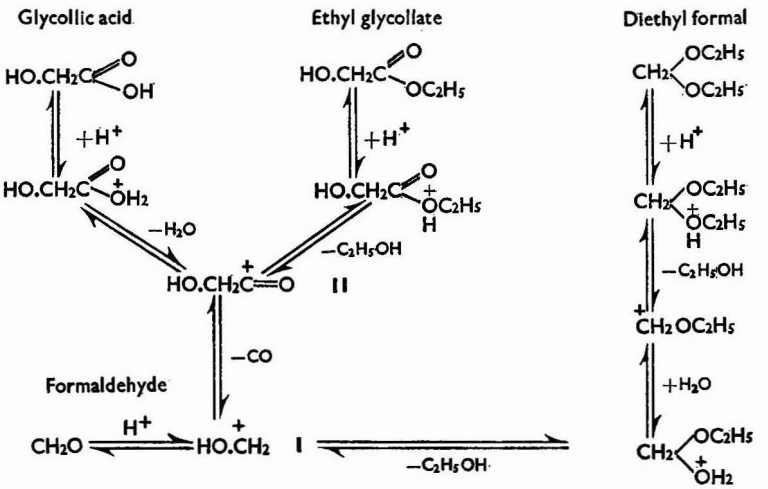


Fig. 1. Effect of ethanol concentration of molar extinction coefficient ϵ: □ formaldehyde; ○ glycollic acid; △ diethyl formal; × ethyl glycollate all with chromotropic acid

The influence of ethanol concentration on the molar extinction coefficients for the four substrates with chromotropic acid is shown in Fig. 1. These results show quite clearly that ethanol does interfere with the colorimetric determination of all four substrates with chromotropic acid and that the effect is greater for glycollic acid and ethyl glycollate than for formaldehyde and diethyl formal. The range of ethanol concentrations used in this work is considerably greater than that used by Ekberg and Silver[4] when they reported that ethanol had no effect. Our results confirm that with the ethanol concentration used by these workers, the effect would be far less than the experimental error. Significantly, the molar extinction coefficients of these compounds in the absence of ethanol are not the same.

In all of these reactions the species responsible for the production of the coloured complex with chromotropic acid is the protonated form of formaldehyde (**I**).



The generation of this reactive intermediate species from glycollic acid involves the intermediate formation of the acyl carbonium ion (**II**) and the subsequent loss from it of carbon monoxide. In the absence of ethanol these equilibrium reactions are displaced, in favour of the production of (**I**), as indicated by glycollic acid and formaldehyde which have the same molar extinction coefficients (1·85 and 1·84 $\times$ 10^4 litres moles^{-1} cm^{-1}). For ethyl glycollate and diethyl formal, however, the production of (**I**) involves the release of one and two molecules of ethanol, respectively. Ethanol, being a stronger nucleophile than water, will attack the intermediate carbonium ions with the resultant incomplete production of (**I**) and a lowering of their molar extinction coefficients (1·68 and 1·55 $\times$ 10^4 litres moles^{-1} cm^{-1}, respectively, for ethyl glycollate and diethyl formal).

The production of (**I**) from all four compounds in ethanolic solution involves equilibrium reactions that will be progressively reversed by ethanol, resulting in the observed lowering of the molar extinction coefficient.

The influence of ethanol on the molar extinction coefficients for ethyl glycollate and glycollic acid is greater than that observed for formaldehyde and diethyl formal, as both reactions involve the acyl carbonium ion intermediate (**II**). The attack on (**II**) by ethanol under these reaction conditions, in which ethanol is in considerable molar excess, will favour the formation of the ester.

In addition there is the reaction of (**I**) with ethanol which is common to all four substrates.

It is evident from these results that for the determination of glycollic acid in ethanolic solution, Easterwood's method must be amended to include an appropriate ethanolic blank. Alternatively, as the presence of ethanol greatly decreases the sensitivity of the colour reaction with chromotropic acid, the preliminary removal of it by evaporation to dryness is recommended.

The authors thank Mr. M. E. Shreeve and Mr. S. C. Elliston for some preliminary observations made in this study.

References

1. Easterwood, M., *Analyt. Chem.*, 1957, **29**, 981.
2. Bricker, C. E., and Johnson, H. R., *Ind. Engng Chem. Analyt. Edn*, 1945, **17**, 400.
3. Bricker, C. E., and Vail, W. A., *Analyt. Chem.*, 1950, **22**, 720.
4. Ekberg, D. R., and Silver, E. C., *Ibid.*, 1966, **38**, 1421.
5. Beroza, M., *Ibid.*, 1954, **26**, 1970.
6. Still, R. H., Wilson, K., Shreeve, M. E., and Boardman, W., in preparation.
7. Feigl, F., "Spot Tests in Organic Analysis," Seventh Edition, Elsevier Publishing Co., Amsterdam, London and New York, 1966, p. 434.
8. Eyler, R. W., Klug, E. D., and Diephus, F., *Analyt. Chem.*, 1947, **19**, 24.
9. West, P. W., and Sen, B., *Z. analyt. Chem.*, 1956, **153**, 177.
10. Walker, J. F., "Formaldehyde," American Chemical Society Monograph, No. 159, Third Edition, Reinhold Publishing Corporation, New York, Amsterdam and London, 1964.
11. Cumming, W. M., Hopper, I. V., and Wheeler, T. S., "Systematic Organic Chemistry," Fourth Edition, Constable and Co., London, 1950, p. 492.
12. Vogel, A. I., "A Textbook of Practical Organic Chemistry, including Qualitative Organic Analysis," Second Edition, Longmans, Green & Co. Ltd., London, New York and Toronto, 1954, p. 325.

Received *April 8th*, 1968

Analyst, December, 1968, Vol. 93, *pp.* 810–816

Simple, Rapid Quantitative Determination of Amino-acids by Thin-layer Chromatography

By MARY E. CLARK

(*Department of Organismic Biology, University of California, Irvine, California* 92664, *U.S.A.*)

A simple, rapid method for the quantitative determination of complex mixtures of amino-acids is described. After separation on thin layers of cellulose mounted on flexible plastic sheets, the chromatograms are sprayed with ninhydrin and developed under controlled conditions. The spots are cut out and eluted with 2 ml of 50 per cent. propyl alcohol and the optical density at 570 nm determined with a microspectrophotometer. From 4 to 6 chromatograms can be eluted and read in 1 day. An accuracy of about 5 nmoles is obtained over a range of 12·5 to 50 nmoles. At higher concentrations, accuracy is within ±10 per cent. Standard graphs are reproducible for at least 5 months.

During an investigation of the composition of the free amino-acid pools of marine invertebrates, a quick and reliable method for quantitative analysis was sought. Although mixtures of several amino-acids can be separated rapidly by thin-layer chromatography, its application has been severely limited because of the difficulties involved in making the method quantitative. Previously described methods involve scraping the coloured spots from glass plates, and either their subsequent elution for absorption spectrophotometry[1] or their analysis by reflectance spectrophotometry.[2,3] Both methods require considerable time in the handling of individual spots. The present paper describes a method in which commercially available thin layers of cellulose supported on a flexible plastic backing are used. After separation of the amino-acids and development of the coloured ninhydrin reaction products, the spots are cut out with scissors and eluted directly, in a few minutes, with a small volume of solvent. The optical density is then determined with a microspectrophotometer, the sensitivity and reproducibility of the method being of the same order as those previously described.

Experimental

Standard amino-acid mixture—

Throughout the development of this method a single batch of a standard mixture of 17 amino-acids, obtained from CalBiochem (Kit No. AA-5), was used. This contained 2·5 ± 0·05 μmoles per ml of each amino-acid. The graphs obtained by using this mixture were later compared with a known mixture of amino-acids, made up to resemble the free amino-acid pool of an experimental animal and with a concentration factor of 30 between the least and most concentrated amino-acids. As shown in Table II, amino-acids of the known mixture gave values within the error appropriate to their concentration levels. It would thus appear that the CalBiochem mixture provided a suitable standard.

Separation of the amino-acids—

Samples were spotted on to 20 × 20-cm sheets of MN-Polygram Cell 300 (MN 300), which is a cellulose powder produced by Machery-Nagel and Co. (Düren), and supported on inert sheets of poly(ethylene terephthalate). To increase the reproducibility of separation, spots were always placed at the same corner of each chromatogram, in relation to its position in the package as supplied. Spots were applied with Drummond "Microcap" capillary pipettes, which were rinsed once with distilled water to ensure complete transfer of the contents. A hot air stream was directed on to the spot during application.

* Present address: W. M. Keck Engineering Laboratories, California Institute of Technology, Pasadena, California 91109, U.S.A.

Chromatograms were run in Kontes Chromaflex developing tanks, with the solvent system described by Jones and Heathcote.[4] To increase further the separation of the larger spots, the times were slightly increased; thus, solvent I, which was isopropyl alcohol - formic acid - water (40 + 2 + 10), was run for 3½ hours and solvent II, t-butyl alcohol - ethyl methyl ketone - ammonia solution - water (25 + 15 + 5 + 5), was run for 3 to 3½ hours. Even so, with amounts of each amino-acid greater than about 25 nmoles, arginine and lysine overlap considerably. Between solvents I and II the chromatograms were dried for at least 5 minutes in a stream of cold air, and a line was then scored just below the zone of yellow impurities to separate them from the remainder of the chromatogram.[4] On removal from solvent II the chromatograms were subjected to a cold air stream for at least 20 minutes, and then stored between sheets of clean paper.

COLOUR DEVELOPMENT—

Before treating the chromatograms with ninhydrin they were once again subjected to a stream of cold air for 20 minutes to remove any ammonia absorbed from the atmosphere during storage. In our laboratory, where developing chromatograms could not be isolated from other chemical work, this proved the best insurance against high background colour.

Chromatograms were sprayed with 7 to 8 ml of freshly prepared 2 per cent. ninhydrin in absolute ethanol,[5] as this amount permits saturation without dripping.

As soon as the ethanol was evaporated in a cold air stream, the chromatograms were moved to a dark cupboard, under conditions of constant temperature and humidity. The importance of the physical conditions for reproducible colour development of ninhydrin-reaction products in paper chromatography has been demonstrated by Wellington, who found that, for most amino-acids, 20° C and 35 to 40 per cent. relative humidity gave maximum colour production.[5,6] Reproducible, though less intense, colour was produced under other conditions, provided they were constant. The effects of temperature and humidity on intensity of colour production on thin layers was not investigated here, reliance being placed on the air conditioning in the laboratory to maintain constant conditions. The temperature in the cupboard in which the chromatograms were developed remained between 23·3° and 26·1° C and the relative humidity between 60 and 64 per cent. over a period of several weeks.

The chromatograms were developed for 24 to 30 hours, as this length of time was found to yield a maximum reaction on paper.[5]

ELUTION OF SPOTS—

As elution entails destruction of the chromatogram, it is useful to have a record to indicate which spots were overlapping. Each spot was outlined with a blunt pencil and identified. In addition, background spots were drawn in at this time. The background varies across the chromatogram, especially in the direction of the second solvent. This may be caused either by impurities in the cellulose layer or by impurities in the solvents (or by both sources). It is convenient to divide the amino-acids into four groups, with a single background spot for each group. This spot was drawn the same size as the largest spot in the group, and when the other spots in this group were cut out, sufficient background was added so that all the spots were the same size as the background spot. The chromatograph was recorded with a Polaroid camera and Polaroid Land Projection film, Type 146-L.

Fine-tipped dissecting scissors were used to cut out individual spots, care being taken to avoid finger-prints. To prevent chipping of the cellulose layer and loss of coloured material it was necessary to cut in straight lines. Each spot was placed in a 1·2 × 10-cm test-tube. If the spots were large, they were cut in half so that they were small enough to be completely covered by 2 ml of eluting fluid, and the pieces placed in the tubes with the cellulose layers facing outwards for efficient elution.

Two millilitres of 50 per cent. propyl alcohol in distilled water were transferred by pipette into each tube. Elution required 20 minutes, after which each tube was shaken vigorously and left for 20 minutes to allow the cellulose particles to settle.

DETERMINATION OF OPTICAL DENSITY—

The optical density at 570 nm was determined for each spot, including the blanks, after setting the instrument at 100 per cent. transmission for 50 per cent. propyl alcohol. Micro-cuvettes and a conventional spectrophotometer can be used, or, for more rapid analysis,

a Gilford Microspectrophotometer 300 is convenient. Both have been used in obtaining the standard graphs in the present study. In the latter method, about 1·5 ml of the coloured eluate is carefully decanted into a small vial, avoiding transfer of cellulose particles, prior to taking a reading. The optical density of the appropriate blank was then subtracted from that of each spot.

Results

CalBiochem standard mixture—

As is well known, equimolar amounts of the various amino-acids do not produce the same amount of coloured reaction product with ninhydrin. Although proline appears to give a more purplish colour on thin layers of cellulose than on paper, the amount of colour is insufficiently reproducible to yield quantitative results, and no proline values are included here.

The results of analyses for three different amounts of the standard mixture are shown in Table I. The number of replicates (n) at each concentration is given at the top of each column, and for all three amounts, replicates were made over a period of 5 months.

As is to be expected, the percentage error decreases with increasing amounts of amino-acids. This is primarily caused by the uncertainties introduced by variable amounts of background colour. Thus, those amino-acids which produce less colour with ninhydrin, such as aspartic acid, glycine, cystine, histidine and tyrosine, tend to give a higher error than more chromogenic amino-acids. The variability of leucine, isoleucine and phenylalanine is also caused by background effects. These amino-acids lie in the upper right-hand quadrant of the chromatogram where the background is always deeper and more variable.

It will be seen that, at concentrations ranging from 12·5 to 50 nmoles, the absolute error for most amino-acids remains at about the same level, between 3 and 5 nmoles. At the highest concentration, the total load on the chromatogram was 0·85 μmoles of amino-acid.

When the mean optical density values for the individual amino-acids are plotted against concentration, rectilinearity (indicating agreement with Beer's law) is obtained for all amino-acids, except threonine, isoleucine and leucine (Fig. 1). Even for these three amino-acids, reproducible graphs are obtained, and thus such graphs can be used as standards for the determination of amino-acids in unknown samples.

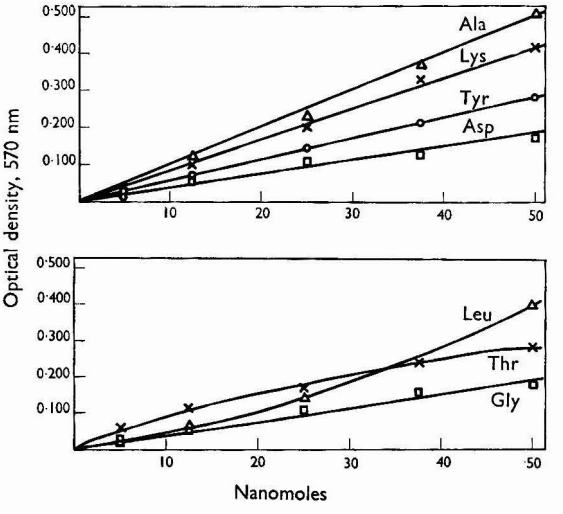


Fig. 1. Optical density *versus* concentration graphs of several amino-acids. Values at 12·5, 25 and 50 nmoles are taken from Table I. Values at 5 nmoles are based on 1 determination; those at 37·5 are the mean of 2 determinations. Threonine and leucine do not give straight-line graphs. (The graph for isoleucine resembles that of leucine.) All other amino-acids follow Beer's law

TABLE I

MEAN OPTICAL DENSITIES, STANDARD DEVIATIONS, PERCENTAGE ERROR AND ABSOLUTE ERROR FOR VARYING CONCENTRATIONS OF CALBIOCHEM AMINO-ACID MIXTURE

	12·5 nmoles $n = 5$				25·0 nmoles $n = 6$				50·0 nmoles $n = 4$			
	$\bar{X}$	Standard deviation	Error, per cent.	± nmoles	$\bar{X}$	Standard deviation	Error, per cent.	± nmoles	$\bar{X}$	Standard deviation	Error, per cent.	± nmoles
1/2 Cystine ..	0·054	± 0·034	63	±7·9	0·120	± 0·039	33	±8·3	0·212	± 0·017	8	± 4·0
Aspartic acid ..	0·051	± 0·007	14	±1·8	0·106	± 0·028	26	±6·5	0·177	± 0·036	20	±10·0
Glutamic acid ..	0·110	± 0·015	14	±1·8	0·201	± 0·050	25	±6·3	0·456	± 0·053	12	± 6·0
Arginine ..	0·093	± 0·025	27	±3·4	0·231	± 0·039	17	±4·3	0·477	± 0·076	16	± 8·0
Lysine	0·099	± 0·024	24	±3·0	0·200	± 0·023	12	±3·0	0·421	± 0·039	9	± 4·5
Glycine	0·053	± 0·015	28	±3·5	0·111	± 0·028	25	±6·3	0·184	± 0·021	11	± 5·5
Serine	0·102	± 0·022	22	±2·8	0·197	± 0·046	23	±5·8	0·418	± 0·022	5	± 2·5
Histidine ..	0·050	± 0·026	44	±5·5	0·146	± 0·025	17	±4·2	0·263	± 0·014	5	± 2·5
Alanine ..	0·121	± 0·013	11	±1·4	0·228	± 0·009	4	±1·0	0·512	± 0·019	4	± 2·0
Tyrosine.. ..	0·071	± 0·024	34	±4·3	0·148	± 0·034	23	±5·8	0·286	± 0·018	6	± 3·0
Valine	0·111	± 0·022	20	±2·5	0·211	± 0·034	16	±4·0	0·479	± 0·038	8	± 4·0
Methionine ..	0·106	± 0·012	11	±1·4	0·204	± 0·042	21	±5·3	0·455	± 0·017	4	± 2·0
Threonine ..	0·113	± 0·040	35	±4·4	0·204	± 0·017	13	±3·3	0·388	± 0·029	7	± 3·5
Isoleucine ..	0·083	± 0·025	30	±3·8	0·183	± 0·029	16	±4·0	0·421	± 0·063	15	± 7·5
Leucine ..	0·058	± 0·018	31	±3·9	0·148	± 0·032	22	±5·5	0·396	± 0·066	17	± 8·5
Phenylalanine ..	0·070	± 0·008	11	±1·4	0·140	± 0·026	19	±4·8	0·288	± 0·009	3	± 1·5

$\bar{X}$ = Mean optical density.
n = Number of determinations.

ARTIFICIAL MIXTURE RESEMBLING THE FREE AMINO-ACIDS OF A POLYCHAETE WORM—

In preliminary investigations, the composition of the free amino-acid pool of the polychaete worm *Stauronereis rudolphi* (Delle Chiaje) was determined on de-proteinised and desalted aliquots of whole-body homogenates. (Details of the preparative procedures will be described elsewhere.) Nineteen amino-acids were identified (excluding the amino-acid derivative taurine, which does not give reproducible colour reactions by the present method and must be determined by alternative procedures.) These ranged in concentration from 0·5 mmoles per kg of body water (histidine) to 15 mmoles per kg of body water (aspartic acid). An artificial mixture was then prepared (Table II) and analysed by the method described above. A chromatogram of this mixture is shown in Fig. 2.

The results of two individual chromatograms of this mixture are shown in Table II. The difference between the amount found and that expected falls, in most instances, near or within the range of error expected, as determined from the percentage error of the nearest amount used in preparing the standard graphs (Table I). Arginine and lysine spots overlap with each other and with asparagine, which gives a weak, brown colour with ninhydrin, but this latter error can be obviated by hydrolysing the mixture before it is used for a chromatogram. There were no standard graphs for asparagine nor tryptophane, and these were read on the lysine and threonine graphs, respectively. For tryptophane this resulted in a systematic error, as the colour for tryptophane under these conditions was about half that of threonine. It should also be noted that the CalBiochem standard mixture contained cystine (1·25 μmoles per ml), whereas the artificial mixture contained cysteine, which yields a much weaker colour with ninhydrin.

Aspartic acid consistently seems to give slightly high values, and alanine consistently slightly low ones. This may be a result of an interaction during migration of the amino-acids, when those present in disproportionately high concentration exert a salt-like effect on the movement of some of the other amino-acids.

The total recovery of amino-acids was within 10 per cent. of that expected.

DISCUSSION

The method described here provides a rapid means of quantitatively determining small amounts of amino-acids. It requires about 3 days to carry out the whole procedure, of which only the third day, when the spots are eluted and read, requires continuous attention from the researcher. It was found possible to elute and read as many as six chromatograms in 1 day.

It should be emphasised that during the development of this technique, every effort was made to treat replicate chromatograms on different days at each stage of the procedure, so that maximum variability of the conditions would be observed. In this way, it was possible to establish the true variation in reproducibility in our laboratory. In addition, all results were obtained on complex mixtures of at least 17 amino-acids; thus the range of error reported is conservative in all respects. It is probable that some of the error, especially at low concentrations, could be reduced by carrying out all the steps following the evaporation of the second solvent in a separate room kept scrupulously free from ammonia and other chemical fumes.

There appear to be only two earlier methods for the quantitative determination of amino-acids with thin-layer chromatography. Frodyma and Frei,[2,3] who use reflectance spectrophotometry, found that when silica gel on glass plates was used scanning did not give sufficiently reproducible results, and that it was therefore necessary to scrape the spots off the glass surface before determinations were made. By running simultaneous standards they achieved a 5 per cent. reproducibility, within a range of about 5 to 200 nmoles for various amino-acids. However, as the percentage reflectance is not linear with concentration nor, for several amino-acids, is it linear even with the square root of the concentration, analysis of complex mixtures necessitates running a complete set of standard graphs with each unknown.

The other method is that recently published by Bondivenne and Busch,[1] who used cellulose 300 MN spread on glass plates and after colour development scraped the individual spots off the glass and eluted the colour through sintered-glass filters. As this required 4 ml of eluant, the sensitivity of their method is about one half that of the present method. The smallest amount of the four amino-acids analysed by these workers was 50 nmoles, at which level the error ranged from 3·7 to 8·5 per cent. When a chromatogram was made of the

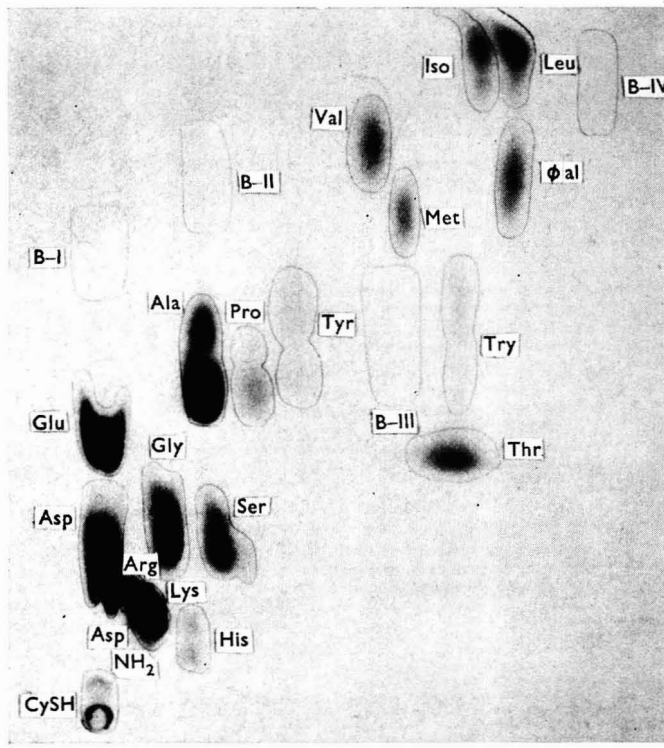


Fig. 2. The chromatogram of an artificial mixture of amino-acids resembling the composition of the free amino-acid content of the pool of the polychaete worm, *Stauronereis rudolphi*. Solvent I was run in the vertical direction, solvent II in the horizontal

To face page 814]

TABLE II

RECOVERY OF KNOWN MIXTURE OF AMINO-ACIDS FROM TWO THIN-LAYER CHROMATOGRAMS

	Artificial mixture of amino-acids, mmoles per litre	nmoles found	nmoles expected	Difference found, nmoles	Approximate difference expected, nmoles	nmoles found	nmoles expected	Difference found, nmoles	Approximate difference expected, nmoles
Cysteine	2·0	1·0	8·0	− 7·0*	±5·0	10·0	20·0	−10·0*	± 6·6
Aspartic acid	15·0	71·6	60·0	+11·6	±6·0	168·2	150·0	+18·2	±15·0
Glutamic acid	10·0	43·6	40·0	+ 3·6	±2·4	102·1	100·0	+ 2·1	± 6·0
Arginine	2·0	13·8	8·0	+ 5·8*	±2·2	24·8	20·0	+ 4·8*	± 3·4
Asparagine	5·0	5·6	20·0	−14·6	—	15·2	50·0	−34·8*	—
Lysine	5·0	12·8	20·0	− 7·2*	±2·4	35·0	50·0	−15·0*	± 4·5
Glycine	10·0	40·2	40·0	+ 0·2	±4·4	87·2	100·0	−12·8	±11·0
Serine	2·0	9·4	8·0	+ 1·4	±1·8	18·2	20·0	− 1·8	± 4·6
Histidine	0·5	1·8	2·0	− 0·2	±0·9	1·4	5·0	− 3·6	± 2·2
Alanine	10·0	37·0	40·0	− 3·0	±1·6	89·7	100·0	−10·3	± 4·0
Tyrosine	1·0	6·4	4·0	+ 2·4	±1·4	13·2	10·0	+ 3·2	± 3·4
Valine	1·0	4·4	4·0	+ 0·4	±0·8	7·6	10·0	− 2·4	± 2·0
Methionine	1·0	4·6	4·0	+ 0·6	±0·4	9·6	10·0	− 0·4	± 1·1
Tryptophane	1·0	1·8	4·0	− 2·2*	—	4·8	10·0	− 5·2*	—
Threonine	1·5	4·4	6·0	− 1·6	±2·1	11·4	15·0	− 3·6	± 5·3
Isoleucine	1·0	4·4	4·0	+ 0·4	±1·2	12·2	10·0	+ 2·2	± 3·0
Leucine	1·5	7·6	6·0	+ 1·6	±1·9	16·6	15·0	+ 1·6	± 4·7
Phenylalanine	1·5	5·8	6·0	− 0·2	±0·7	10·4	15·0	− 4·6	± 11·7
Total	71·0	276·2	284·0			637·6	710·0		
Recovery, per cent.		97·3				89·8			

* Spots where large errors were expected—see text for explanations.

four amino-acids together, in amounts exceeding 100 nmoles of each, the error increased, ranging from 7·8 to 13·5 per cent. for five replicate determinations. It would thus appear that the present method offers a more rapid analysis, with somewhat improved accuracy and sensitivity.

Recently Heathcote and Washington[7] have published a sensitive method based on paper chromatography, in which they state that, for most amino-acids, determination of amounts of from 1 to 250 nmoles is possible with an over-all accuracy of ± 10 per cent. It is not clear if this high degree of accuracy at lower concentrations could be obtained in complex mixtures, but it would appear that their method is more reproducible at lower concentrations than the present one. It entails, however, a rather elaborate, prolonged procedure for eluting the spots. The staining reagent used by these workers was chosen for its increased sensitivity, which is about five times that of ninhydrin alone. However, as the elution procedure required 10 ml for each spot, optical densities were equivalent to those obtained here. In addition, as with all methods in which paper chromatography is used, the time required in the solvent systems is about three times that necessary for separation on thin layers. When sufficient material is available to carry out several chromatograms on the same sample, better accuracy can be expected with the present method and this may prove less time-consuming than the more elaborate method of Heathcote and Washington, which would be the method of choice when sample size is limited.

This work was supported by USPHS Grant 12889, awarded to Dr. Grover C. Stephens, in whose laboratory the work was done. I am most grateful to Dr. Stevens for making this study possible, and for his encouragement throughout.

References

1. Bondivenne, R., and Busch, N., *J. Chromat.*, 1967, **29**, 349.
2. Frodyma, M. M., and Frei, R. W., *Ibid.*, 1964, **15**, 501.
3. ——, ——, *Ibid.*, 1965, **17**, 131.
4. Jones, K., and Heathcote, J. G., *Ibid.*, 1966, **24**, 106.
5. Wellington, E. F., *Can. J. Chem.*, 1952, **30**, 581.
6. ——, *Ibid.*, 1953, **31**, 484.
7. Heathcote, J. G., and Washington, R. J., *Analyst*, 1967, **92**, 627.

Received *May 5th*, 1968

Analyst, December, 1968, Vol. 93, *pp.* 817–820

The Continuous Polarographic Determination of Small Amounts of Nitroglycerine in Plant Effluent

BY A. R. HOLLAND AND A. G. S. BENHAM

(*Imperial Metal Industries Limited, Summerfield Research Station, Kidderminster*)

A fully automatic polarographic method is described for the continuous monitoring of nitroglycerine plant effluent, for which an arbitrary maximum level of 100 p.p.m. of nitroglycerine has been set.

Conventional polarographic principles are involved, but the polarograph used has been specially designed, and incorporates some novel features that make it admirably suitable for this and similar work.

Constructional details are given of the polarograph, and of an alarm system that operates when the permitted level of nitroglycerine in the effluent is exceeded.

IN the manufacture of nitroglycerine by the injection nitration process (Nitroglycerine Aktiebolaget, Gyttorp), an aqueous solution is produced, the nitroglycerine content of which, after neutralisation, is about 0·5 per cent. This solution also contains about 2·5 per cent. of sodium nitrate, 1·5 per cent. of sodium carbonate and 0·1 per cent. of sodium sulphate.

At this stage the nitroglycerine is in suspension, and most of it is recovered by a "settling out" process, the remainder (about 0·1 per cent.) being diluted with a 20-fold excess of water before it is discharged to waste. An arbitrary maximum level of nitroglycerine in this effluent has been set at 100 p.p.m., and the problem was how to provide a reliable analytical procedure so that in the event of this level being exceeded any failure at the dilution stage could be detected immediately and rectified.

It was therefore considered necessary to have an automatic sampling and analysis system for the continuous determination of levels of nitroglycerine normally present in this effluent, and some form of warning (alarm) that would alert plant operators when the amount of nitroglycerine in the effluent was in excess of the permitted level.

Small amounts of nitroglycerine can be determined by a spectrophotometric procedure in which *N*-1-naphthylethylenediamine is used, but the use of any colorimetric reagent is excluded because of the turbid nature of the effluent, especially during periods of heavy rainfall.

Nitroglycerine produces three distinct polarographic waves (—0·25, —0·45 and —0·75 volt *versus* mercury-pool anode) in a quaternary ammonium halide solution, and a procedure involving the use of this electrolyte, by Williams and Kenyon,[1] was used in the following experimental work.

EXPERIMENTAL

CALIBRATION GRAPH—

In preliminary experiments with a Cambridge photographic-recording polarograph, a linear calibration graph was obtained from the peak heights recorded at —0·75 volt when simulated effluent samples containing up to 150 p.p.m. of nitroglycerine were analysed; nitrogen was used to de-oxygenate the samples.

In further experiments, an alternative (cheaper) base electrolyte (ammonium chloride - potassium chloride) was used; sodium sulphite was added to the electrolyte to replace the use of nitrogen, because it was envisaged that a gaseous de-oxidant would lead to complications in any fully automatic system. Satisfactory results were obtained, and this electrolyte was used in subsequent experiments.

Design of the analytical system

The following automatic stages were planned.

(*i*) Sampling of effluent every 15 minutes.
(*ii*) Addition and mixing of base electrolyte.
(*iii*) Transfer of prepared sample to a polarographic cell.
(*iv*) Analysis of prepared sample.
(*v*) Removal of analysed sample from polarographic cell.

The sampled effluent is pumped through a filter into a header tank provided with an overflow. A week's supply of base electrolyte is also stored in another header tank, and this tank is provided with a floating lid to minimise evaporation losses.

Supplies of base electrolyte and sample enter the polarographic cell by means of a gravity feed via the solenoid-controlled valves, S1 and S2 (Fig. 1), and the chokes are adjusted so that equal volumes of both solutions can pass into the mixing chamber. In this way, the use of low-capacity pumps, which we have not found reliable in operation over long periods, is eliminated. A period of 30 seconds is allowed for the delivery of a 100-ml sample and also for the same volume of electrolyte; the system is self-purging.

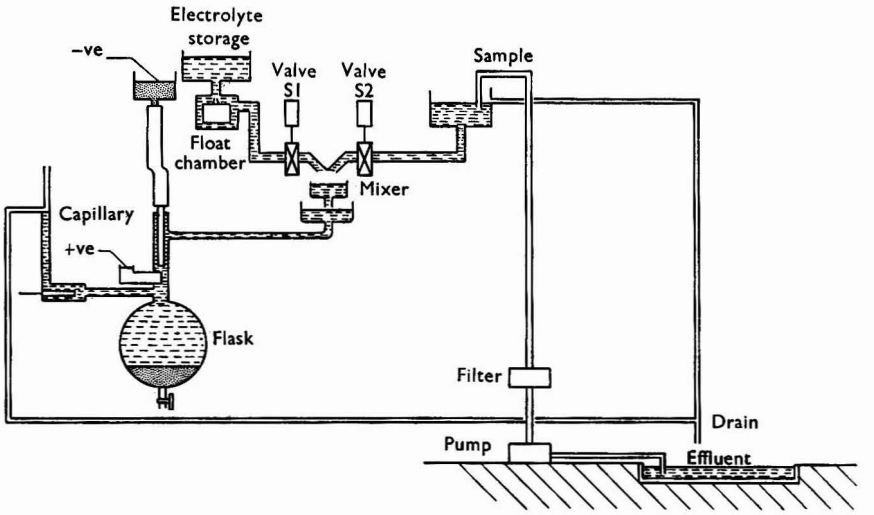


Fig. 1. Schematic layout

Electrical circuits (Fig 2)—

The two mercury cells are connected in series to provide the polarographic cell current via an adjustable resistor, R1, a potential divider, R3 (or R5), R4 and an adjustable resistor, R2.

The recorder is connected across R2, and the oscillating potential produced by the dropping-mercury electrode is damped by a condenser connected across R2, so that a constant current of 10 μA flows at full-scale deflection of the recorder.

The polarographic current flows through the cell via a platinum wire, which is in electrical contact with the (negative) mercury reservoir, and a second platinum wire in similar contact with the (positive) mercury pool in the neck of the flask (Fig. 1). The positive current is taken from the centre point of the potential divider via the microswitch, MS3, which makes contact for 5 minutes, four times per hour.

Operation—

The position of the valve voltmeter, V2, is shown in Fig. 2; the meter reading is adjusted to $-1{\cdot}00$ volt by means of R1. The reading on V1 is noted on the moving-coil meter, then V2 is disconnected. Any variation in voltage can be corrected by restoring V2 to the original reading.

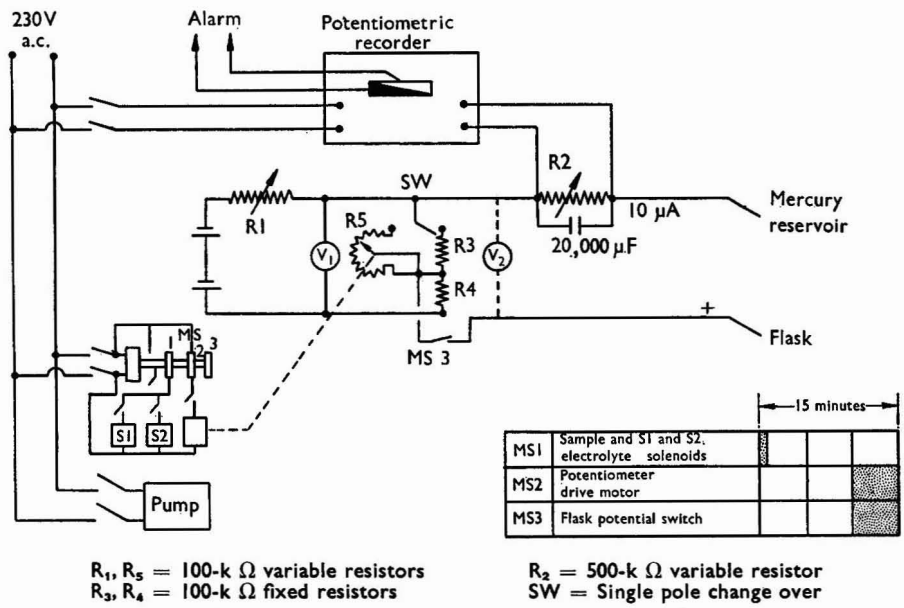
The sampling sequence is programmed by a 4 r.p.h. mains timer, which operates the microswitches, MS1, MS2 and MS3, by a camshaft. At zero time, the mains-operated solenoid valves, S1 and S2, are energised through MS1, thus permitting 100 ml of the sample and the same volume of the base electrolyte to pass into the mixing chamber. After a lapse of 9½ minutes to allow the solution to de-oxygenate, the microswitch, MS3, makes contact and allows a potential of −1·00 volt to be applied instantaneously to the cell. The concentration of nitroglycerine is shown on the recorder (Fig. 2).

After 5 minutes, MS3 breaks to stop the cell current and the sequence is repeated.

Switches are provided to enable manual operations to be made, and a second mains timer (12 r.p.h.) is operated by MS2 to permit the slide-wire, R5, to be used when required.

The nitroglycerine peak is displayed on a self-balancing potentiometer strip-chart recorder, which is fitted with a mercury switch that operates an alarm system when the nitroglycerine content of the sample exceeds 100 p.p.m.

Fig. 1 shows a schematic layout of the unit, and Fig. 2 the electrical circuit.



R_1, R_5 = 100-k Ω variable resistors
R_3, R_4 = 100-k Ω fixed resistors
R_2 = 500-k Ω variable resistor
SW = Single pole change over

Fig. 2. Electrical circuits

EFFECT OF VARIABLES—

Sodium carbonate—At the 50 p.p.m. nitroglycerine level, the presence of 4 per cent. of sodium carbonate gave an apparent nitroglycerine level of 47·5 p.p.m. Under plant operating conditions, this level of sodium carbonate is most unlikely to occur, so that a normal variation in sodium carbonate content is not significant.

Glycerol and sodium nitrate—The presence of 1 per cent. of glycerol in the effluent would indicate serious malfunctioning of the plant, but even at this level of glycerol the effect on the determined nitroglycerine content of the effluent is insignificant. The same observation was made in the presence of 1 per cent. of sodium nitrate.

Calcium—Because it is possible that calcium could enter the effluent stream between the plant and the sampling point, the effect of 2 per cent. of calcium oxide was investigated. This produced a lowering of the recorded nitroglycerine content by about 2 per cent.

Temperature—Wide fluctuations in the temperature of the effluent do not occur, and the small structure in which the polarograph is housed is controlled at the same temperature as that used to calibrate the instrument, *i.e.*, 20° C.

It was, however, established that a 1° C rise above this temperature increased the peak height of the polarographic wave by about 1·5 per cent. Temperature compensation could be arranged in the electrical circuits, if necessary.

Method

Reagents—

Base electrolyte—Dissolve 48·1 g of ammonium chloride, 7·5 g of potassium chloride, 0·5 g of sodium sulphite, $Na_2SO_3.7H_2O$, and 0·5 g of gelatine in warm distilled water and cool. Dilute the solution to 1 litre with distilled water and mix.

Standard nitroglycerine solution—Dissolve 100 mg of nitroglycerine in 15 ml of methanol, transfer the solution to a 1-litre calibrated flask, dilute to the calibration mark with distilled water and mix. This solution contains 100 p.p.m. of nitroglycerine.

Calibration—

Establish that 100 ml of sample and 100 ml of electrolyte are discharged into the mixing chamber during each 15-minute cycle. Variable chokes are fitted in each delivery pipe to the mixing chamber, which should be adjusted, if necessary.

Mix the base electrolyte and the standard nitroglycerine solution, adjust the temperature to 20° C, transfer the mixture to the polarographic cell via the mixing chamber and record the polarogram at a fixed potential of −1·00 volt.

Dilute the standard nitroglycerine solution with distilled water to provide standard solutions containing 20, 50 and 75 p.p.m. of nitroglycerine, and similarly record the polarograms obtained from these solutions; include a blank determination.

Procedure—

Proceed as outlined under Calibration, and calculate the nitroglycerine content of the sample by reference to the calibration graph.

The nitroglycerine referred to throughout this paper does not contain any ethylene glycol dinitrate and is usually known as "A-type nitroglycerine."

Conclusions

The proposed procedure can be satisfactorily used for the continuous determination of nitroglycerine in a typical effluent at the levels of nitroglycerine normally present in these samples, *i.e.*, less than 100 p.p.m., with an accuracy of 1 p.p.m. The polarogram can be recorded and interpreted visually, and the output of the recorder can be fitted with a mercury switch to operate an alarm system when the nitroglycerine content of the effluent exceeds 100 p.p.m.

There is no obvious reason why a similar unit should not be used for the continuous determination of certain other constituents in flowing streams, *e.g.*, metallic impurities.

The unit described has been in continuous operation for 12 months. No serious instrumental difficulties have been encountered, and periodic checks with solutions of known nitroglycerine content have confirmed the reliability of the method.

The authors acknowledge the assistance given by Mr. D. Facer of this laboratory, who carried out most of the preliminary experiments.

Reference

1. Williams, A. F., and Kenyon, D., *Talanta*, 1959, **3**, 160.

Received *August 16th*, 1968

Analyst, December, 1968, Vol. 93, *pp.* 821–826

A Simple Field Test for the Determination of Hydrogen Fluoride in Air

BY B. S. MARSHALL AND R. WOOD

(Ministry of Technology, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1)

A field method is described for determining hydrogen fluoride vapour in air at concentrations up to 20 μg of hydrogen fluoride per litre. The gas is collected in an acidic solution of zirconium - Solochrome cyanine R reagent and the observed bleaching of the colour compared with standards. The apparatus used is simple to operate and the time required for a determination is less than 5 minutes. A dynamic method for the generation of standard atmospheres of hydrogen fluoride is also described.

HYDROGEN fluoride is widely used in the chemical industry and in the petroleum industry in the synthesis of high octane spirit. It is also used in the manufacture of aluminium fluoride and synthetic cryolite for the production of aluminium. Considerable amounts of hydrogen fluoride are also used in the manufacture of refrigerants and fluorine-containing plastics. As hydrogen fluoride gas is highly toxic, with a threshold limit value of 3 p.p.m. v/v (2·5 μg per litre) in air,[1] there is a need for a simple, rapid field test for its detection in industrial atmospheres at this level. Any such test should preferably be capable of being used by non-scientific staff.

Several methods for the determination of hydrogen fluoride in air have been reviewed by Farrah.[2] Most of them involve the preliminary trapping and concentration of the gas in water or sodium hydroxide solution, or on filter-papers impregnated with calcium hydroxide, followed by a fluoride distillation and a final colorimetric determination of the fluoride ion. This general method, although normally precise and specific, is time consuming, requires considerable expertise with the necessary distillation apparatus and thus cannot be used to give a rapid check on the hydrogen fluoride concentration present in a factory atmosphere.

The main requirements of a field test are ease of manipulation, rapid colour development and a good visual colour discrimination between neighbouring standards. When possible, a positive increase in colour, rather than a bleaching effect, with increasing concentration of air-borne contamination is desirable. With these points in mind we selected for examination two of the methods in general use in fluoride determinations for possible development of a field test for hydrogen fluoride. These were the lanthanum - alizarin - fluorine blue method[3] and the zirconium - Solochrome cyanine R method,[4] as modified by Dixon.[5]

A preliminary assessment of these two methods was carried out with aqueous solutions containing a range of total fluoride from 0 to 2·5 μg. This would be equivalent to the range of hydrogen fluoride concentrations collected from 500-ml samples of industrial atmospheres containing up to twice the present threshold limit value of the gas. Although the zirconium - Solochrome cyanine R method involves bleaching of the colour, it was found to give better visual colour differentiation for this range of fluoride concentrations than the positive colours of the lanthanum - alizarin - fluorine blue method and, consequently, was chosen for further examination as a possible basis for a field test.

Experimental

Preparation and calibration of standard atmospheres of hydrogen fluoride—

As hydrogen fluoride gas attacks glass, the apparatus for the generation of standard atmospheres of it was constructed so that all of the surfaces in contact with the gas were made of non-reactive materials, *e.g.*, polythene.

Preparation—Hydrogen fluoride gas from a low-pressure cylinder (supplied by the Matheson Company Inc.) maintained at 22° C in a thermostatically controlled bath was passed through a polythene restrictor and then into a polythene mixing chamber, into which 10 litres of diluting air per minute were flowing. By using a second restrictor, a short length of PTFE tubing with an adjustable screw-clip, 10 ml of the dilute atmosphere per minute were passed into a second polythene mixing chamber, in which it was diluted with a further 10 litres of air per minute to give a working atmosphere of about 2·5 μg of hydrogen fluoride per litre. The 10-ml per minute flow-rate was achieved beforehand by connecting the output of the second restrictor to a flow meter and adjusting to the required flow by using hydrogen fluoride free air. Hydrogen fluoride atmospheres of other concentrations were prepared by suitably adjusting the flow-rate of the second diluting stream of air.

The polythene restrictor was made by removing the central wire, outer sheath and screening from a piece of co-axial cable, 70 mm long, closing down the bore of the remaining 5-mm o.d. polythene tubing, by heating, on to a piece of 42 s.w.g. wire and then removing this wire when cold. Adiabatic cooling and consequent liquefaction of the hydrogen fluoride vapour issuing from the polythene restrictor were overcome by inserting, between the cylinder and the restrictor, a 65-mm length of stainless-steel tubing of 6-mm i.d. wound with a low-output wire heater. Polythene screw-top bottles of 250 and 1000-ml capacity were easily adapted to serve as the first and second mixing chambers, respectively, and all of the pipework in contact with the hydrogen fluoride was made of polythene. No conditioning problems were experienced with this apparatus when used for the generation of hydrogen fluoride atmospheres.

Calibration—The output of the cylinder was checked by passing the gas issuing from the first restrictor into 0·1 N sodium hydroxide solution for a known length of time, and back-titrating the excess of alkali with standard acid. Under the above conditions, 31 ml of hydrogen fluoride gas were delivered per minute.

The working atmospheres were calibrated by passing samples at the rate of 1 litre per minute for 5 minutes through 15 ml of water contained in an absorber fitted with a polythene inlet tube. It had previously been found that virtually 100 per cent. of the hydrogen fluoride at the atmosphere concentrations used was collected in one absorber. Other workers[6] had shown that water was as efficient as an alkaline solution as a trapping agent for hydrogen fluoride from air samples. The fluoride collected was determined spectrophotometrically[3] with the lanthanum - alizarin - fluorine blue reagent at 625 nm and the concentration of the atmosphere was calculated by reference to a previously prepared standard graph.

Development of field test—

With the zirconium - Solochrome cyanine R reagent, Dixon[5] has devised an improved spectrophotometric method for the determination of fluoride in water in the range 0 to 2·5 μg. This procedure, and the range of fluoride it covered, appeared to suit our requirements. The development of a test involving the use of visual colour standards equivalent to 0, 1·25, 2·5 and 5 μg of hydrogen fluoride per litre of air was envisaged. A 500-ml sample would normally be taken in the field by using a hand aspirator of 125-ml capacity. However, by reducing the number of aspirations and using the same set of colour standards, the range of hydrogen fluoride concentrations in air capable of being determined could be extended.

Standard hydrogen fluoride atmospheres (500 ml), containing 1·25, 2·5 and 5·0 μg of the gas per litre, were sampled at the rate of 125 ml per minute through 5 ml of water; 1 ml of each of the Solochrome cyanine R and zirconium solutions[5] was added to the absorbing solutions after sampling. The respective colours obtained were compared with those of a similar set of solutions prepared from an aqueous fluoride solution and containing 0·6, 1·2 and 2·4 μg of fluoride. The visual colour matches obtained showed that the hydrogen fluoride was being quantitatively trapped and determined. Later, to minimise the number of operations required, it was found that 5 ml of diluted, mixed reagent containing 1 ml of each

of the chromogenic reagents could be used directly as trapping agent, and also gave colour matches with similarly prepared fluoride solutions over the range 0 to 2·4 μg of fluoride. The visual colour differentiation between the four fluoride levels, 0, 0·6, 1·2 and 2·4 μg, was considered to be entirely satisfactory for field test purposes.

PREPARATION OF COLOUR STANDARDS—

A set of colour standards for use with the proposed test was conveniently prepared by using a standard sodium fluoride solution and solutions of the chromogenic agents. Details of the preparation are given later. With these standards it was found possible to determine the fluoride content of a sample at least to the nearest ±0·625 μg per litre (*i.e.*, a quarter of the present threshold limit value) between 0 and 2·5 μg of hydrogen fluoride per litre and at least to the nearest 1·25 μg per litre between 2·5 and 5·0 μg of hydrogen fluoride per litre.

INTERFERENCES—

Aluminium, phosphate and sulphate are well known interferences in the zirconium - Solochrome cyanine R method for fluoride,[4] the first being a negative interference and the others positive interferences. The effects of these interferences on the proposed test were individually assessed by adding increasing amounts of each species in solution to a series of fluoride solutions equivalent to 0, 1·25, 2·5 and 5·0 μg of hydrogen fluoride per litre of air, and finally adding the mixed chromogenic reagent. The level of interference of each of the species was taken as the lowest concentration that would just produce a significant visual colour difference between the solution and an equivalent fluoride standard. The concentrations for aluminium, phosphate and sulphate were 1, 1·6 and 100, respectively, expressed as micrograms per litre of air.

Besides the above study, the effects on the proposed test of several substances, which might occur together with hydrogen fluoride in industrial atmospheres, were examined. When the threshold limit value of any gas known to interfere with the proposed field test is present in an industrial atmosphere, then such a concentration constitutes a hazard in its own right, irrespective of the hydrogen fluoride concentration. In view of this, experiments were conducted to ascertain the effects on the proposed test of possible interfering gases and vapours up to an arbitrarily chosen equivalent of twice their present threshold limit value. Atmospheres were prepared containing 1·25, 2·5 and 5·0 μg of hydrogen fluoride per litre by using, in the second dilution stage, air containing amounts of the interfering substances up to a concentration of twice their threshold limit value. These atmospheres were then sampled by the proposed test method. It was found that nitrogen dioxide and sulphur dioxide did not interfere at concentrations up to twice their present threshold limit values. *i.e.*, 18 and 26 μg per litre, respectively. Also, sulphur trioxide did not interfere at concentrations up to 1·6 μg per litre of air, *i.e.*, equivalent to twice the present threshold limit value for sulphuric acid.

Experiments were also performed to assess the effect on the test of various amounts of water vapour present in atmospheres containing hydrogen fluoride by setting up, as above, standard atmospheres of gas and using air containing known amounts of water vapour for the second dilution stage. Water vapour did not interfere at concentrations up to 18 mg per litre of air at 22° C. (This is equivalent to a relative humidity of 92·5 per cent.)

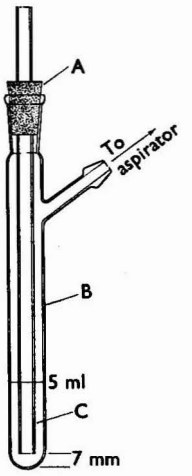
In view of the interfering effects of aluminium and phosphate, this field test is not recommended for the testing of industrial atmospheres polluted with fluoride-containing dusts. Several such materials used in industry, *e.g.*, cryolite, apatite and various other phosphate rocks, contain sufficient aluminium or phosphate to interfere with the proposed test.

Although water vapour does not appear to interfere with the test, provided that condensation does not occur in the sampling apparatus, it is recommended in certain instances that the inlet tube be washed with the mixed reagent after sampling. This is especially important when samples are taken of a damp atmosphere or one in which a dilute aqueous hydrogen fluoride spray has been used. In the sampling of such atmospheres, hydrogen fluoride may be trapped in water droplets on the inside of the inlet tube. The washing of this tube can be carried out by blowing carefully down the side-arm of the glass test-tube and thereby raising the level of liquid in the inlet tube to within 10 mm of its top.

Field test for the determination of hydrogen fluoride in air

Apparatus—

Absorbers—Several are required, each comprised of a glass test-tube with a polythene inlet tube (see Fig. 1). For ease of dispensing reagents, make a mark at the 5-ml level. Each test-tube should be fitted with its own inlet tube.



A=Rubber bung
B=Glass tube, 13mm i.d., 15·5-cm over-all length
C=Polythene inlet tube, 5mm i.d., 18·5-cm over-all length

Fig. 1. Absorber

Aspirator—A rubber-bulb hand aspirator (obtainable from Siebe Gorman and Co. Ltd., Davis Road, Chessington, Surrey) adjusted to deliver 125 ml of sample per minute.

Reagents—

All reagents should be of analytical-reagent quality unless otherwise stated.

Zirconium solution—Dissolve 36 mg of zirconium oxychloride octahydrate, $ZrOCl_2.8H_2O$, in water, add 120 ml of concentrated hydrochloric acid and dilute to 500 ml.

Solochrome cyanine R (*C.I.* 43820) *solution*—Dissolve 60 mg of the purified reagent in water, add 2·5 ml of N hydrochloric acid and dilute to 250 ml. This reagent, as normally supplied, contains variable amounts of sodium sulphate. The dye can be separated from it by extraction with methanol. The extract is evaporated to dryness under reduced pressure, and the resulting purified material used to prepare the reagent solution.

Mixed reagent—Combine 10 ml of each of the zirconium and Solochrome cyanine R solutions and dilute to 200 ml with water. This solution appears to be stable for a considerable time, but to obviate any possible contamination problems, it is recommended that fresh mixed reagent be prepared for each series of tests.

Standard fluoride solution—Dissolve 2·121 g of sodium fluoride in water, add 1 ml of 0·1 N sodium hydroxide solution and dilute to 1 litre with water. Dilute 2·5 ml of this solution to 1 litre to give a solution containing 2·4 μg of fluoride per ml, *i.e.*, equivalent to 2·5 μg of hydrogen fluoride per ml.

Procedure—

In an uncontaminated atmosphere, well away from the suspected source of hydrogen fluoride, place 5 ml of the mixed reagent in the absorbing tube, insert the inlet tube and connect the aspirator to the side-arm. Transfer the assembled apparatus to the sampling site, and draw a 500-ml sample (*i.e.*, four aspirations) of the atmosphere through the reagent. Remove the apparatus to an uncontaminated atmosphere. Disconnect the aspirator from

the side-arm of the glass test-tube. If the sample has been taken in a humid atmosphere, wash the inlet tube by gently blowing down the side-arm, and raise the level of the mixed reagent to within about 10 mm of the top of the inlet tube. Place a finger on the end of the inlet tube to hold the mixed reagent in that position for a few seconds. Allow the liquid to flow back into the glass test-tube and remove the inlet tube. Compare the colour of the sample solution, preferably in daylight, in turn with 5 ml of each of the fluoride colour standards contained in tubes of similar diameter to the sample test-tube. View through the depths of the respective liquids against a white (paper) background.

Should the level of hydrogen fluoride with a 500-ml sample of atmosphere be above 5 μg per litre, a more accurate determination of the true concentration can be made by using a 125-ml sample. The standard that gives a colour match with the sample solution is then multiplied by four to give the amount of hydrogen fluoride in micrograms per litre present in the atmosphere.

When a test is required on an atmosphere that is either not readily accessible or possibly contains high concentrations of hydrogen fluoride, the sampling should be carried out as follows.

Connect the site to be tested to the sampling apparatus with a length of polythene tubing. Start aspirating and continue until a colour change is obtained in the mixed reagent. Disconnect the absorber and rapidly replace it with another containing fresh mixed reagent. Carry out the test as described above. It is recommended that an operator sampling high concentrations of hydrogen fluoride in an atmosphere should have suitable respiratory protection.

NOTE—

To avoid cross-contamination between samples, each absorber, *i.e.*, test-tube and inlet tube, should be rinsed with a few millilitres of fresh reagent and the inlet tube dried before re-use.

PREPARATION OF FLUORIDE COLOUR STANDARDS—

To a set of four 100-ml graduated flasks add 0, 5, 10 and 20 ml of the dilute standard fluoride solution. Add 5 ml each of the zirconium and Solochrome cyanine R solutions to each flask and dilute to volume with water. These standards represent 0, 1·25, 2·5 and 5 μg of hydrogen fluoride per litre of air, respectively. The standards, if stoppered, are stable for at least 1 week.

APPLICATION OF METHOD—

The proposed test was assessed and checked under field conditions at various sites where hydrogen fluoride was being used. The check testing was normally carried out by taking three concurrent samples for each atmosphere analysed and using a 3-way manifold and a common sampling inlet. The three samples taken were as follows.

(*i*) A 500-ml sample by the proposed test over a period of 5 minutes.

(*ii*) A 5-litre sample taken at 1 litre per minute, with 50 ml of mixed reagent as absorbing solution. This was a ten times scaling-up of the field test, and the fluoride collected was determined visually as in (*i*) by using colour standards.

(*iii*) A 5-litre sample taken at 1 litre per minute, with 15 ml of water as absorbing solution to which lanthanum - alizarin - fluorine blue reagent was added after sampling. The fluoride was determined spectrophotometrically, with reference to a previously prepared calibration graph.

Table I gives the results obtained in one check-testing series carried out in and around a fume cupboard in a laboratory in which hydrogen fluoride was being used. Some of the samples were deliberately taken inside the fume cupboard to enable an assessment of the field test to be made at high levels of hydrogen fluoride contamination.

The results shown in Table I, and those obtained at other sites, appeared to confirm the validity of the proposed field test for the determination of hydrogen fluoride in air. It should, however, be mentioned that in some of the early check tests performed, high and erratic results were obtained when using the field method compared with the other two concurrently taken samples. This was traced to the contamination of the sampling absorber, the polythene inlet tube in particular, between the taking of successive samples. It appeared

TABLE I

COMPARISON OF THE RESULTS OF THE ANALYSIS OF HYDROGEN FLUORIDE CONTAMINATED ATMOSPHERES BY THE PROPOSED FIELD TEST AND TWO OTHER METHODS

Sample	Hydrogen fluoride concentration found, μg per litre, by—		
	Field test	Field test × 10	Lanthanum - alizarin - fluorine blue reagent, spectrophotometrically
1	0	0	0·25
2	0·625	0·625	1·0
3	0·625	0·625	0·625
4	0·625	0·625	0·85
5	1·25	1·25	1·75
6	1·25	1·25	1·12
7	2·5	2·5	2·5
8	3·75	3·75	2·5
9	10·0*	10·0†	9·3†
10	20·0*	20·0‡	24·5‡

* A 125-ml sample taken at the mid-point of time during which the sample for spectrophotometric determination was taken.
† A 2·5-litre sample.
‡ A 1·25-litre sample.

that as the volume of absorbing solution used in the field test was small compared with those used in the other two concurrent tests, it was relatively more susceptible to contamination from the inlet tube used. This effect was obviated either by using fresh absorbers for each test or washing the absorber components with mixed reagent between tests and drying the inlet tube. The test apparatus should always be assembled in an uncontaminated atmosphere.

This work was carried out on behalf of the Department of Employment and Productivity Committee on Tests for Toxic Substances in Air. We thank the Government Chemist for permission to publish this paper.

REFERENCES

1. "Ministry of Labour, Safety, Health and Welfare, New Series No. 8, Dusts and Fumes in Factory Atmospheres," Third Edition, H.M. Stationery Office, London, 1966.
2. Farrah, G. H., *J. Air Pollut. Control Ass.*, 1967, **17**, 738.
3. Greenhalgh, R., and Riley, J. P., *Analytica Chim. Acta*, 1961, **25**, 179.
4. Megregian, S., *Analyt. Chem.*, 1954, **26**, 1161.
5. Dixon, E. J., in preparation.
6. Pack, M. R., Hill, A. C., Thomas, M. D., and Transtrum, L. G., A.S.T.M. Special Technical Publication No. 281, 1958, p. 27.

Received *June 27th*, 1968

Analyst, December, 1968, Vol. 93, *pp.* 827–831

A Simple Apparatus for Separating Fluorine from Aluminosilicates by Pyrohydrolysis

By A. C. D. NEWMAN

(*Rothamsted Experimental Station, Harpenden, Herts.*)

Fluorine-bearing minerals are hydrolysed at 700° to 800° C in a gas-heated fused-silica tube. The hydrogen fluoride evolved is absorbed in alkali and determined by titration with thorium nitrate or absorptiometrically with cerium - alizarin complexone. Recoveries from sodium fluoride averaged 98·6 per cent., with a coefficient of variation of 3·2 per cent., and the determined fluorine contents of the silicate rock standards GSP–1 and G2 were 0·381 and 0·134 per cent. The method is simple and rapid, and is suitable for determining fluorine in micas and related aluminosilicates.

Fluorine is still most often separated from interfering elements by steam-distillation as fluorosilicic acid from sulphuric, phosphoric or perchloric acids (Willard - Winter distillation[1]); in silicate analysis, a preliminary fusion and precipitation of alumina and silica from the leached melt is usual. The procedure is long and its success depends on close attention being paid to detail.[2] Special stills have been designed to improve control of the distillation conditions, for recovery of fluorine is slow and often incomplete, so that a correction factor is sometimes applied.[3]

High-temperature hydrolysis ("pyrohydrolysis") quickly releases fluorine as hydrogen fluoride without preliminary fusion, and this reaction is the basis of an attractive alternative to Willard - Winter distillation,[4,5] yet recent literature suggests that pyrohydrolytic separation is still not widely known or applied, perhaps because the original workers used expensive, specially designed platinum apparatus.[6] The purpose of this paper is to show that aluminosilicates can be readily pyrohydrolysed in simple apparatus and that good results are obtained in a fraction of the time required for the Willard - Winter distillation.

The method is based on the following thermodynamic principle. The position of equilibrium in the general reaction

$$MF_{2n} + nH_2O \rightleftharpoons MO_n + 2nHF$$

depends on temperature and, because the standard entropy of two moles of hydrogen fluoride is about twice that of one mole of water, lies increasingly to the right as temperature increases. The rate of reaction is accelerated in the presence of some oxides, for instance, those of uranium, vanadium, aluminium and tungsten; vanadium pentoxide melts at about 670° C, so it also acts as a flux, and is preferred in this laboratory to tungsten trioxide. Warm carrier gas, saturated with water vapour, is passed over a heated mixture of sample and accelerator, and the hydrogen fluoride evolved is collected in dilute alkali. Fluoride in the condensate can be determined by many methods; a titrimetric and an absorptiometric finish have been used in this laboratory.

Experimental

Apparatus—

As shown in Fig. 1, compressed air is filtered through cotton-wool, A, and bubbled through distilled water heated to 90° C in a Pyrex flask, B, on an electric hot-plate. The warm, moist air passes through a water-trap, C, and immediately into the transparent fused-silica reaction tube, D, which is 45 cm long, with an outside diameter of 20 mm narrowing to 6-mm diameter, and bent at right angles a little beyond the constriction.* A small, detachable condenser, J, made from two Quickfit SQ13 screw-thread joints joined end-to-end with two side-tubes for water inlet and outlet, is fitted to the vertical section of the tube. The reaction tube is heated by two batswing burners, E and F, and a bunsen burner, G. The condensate is collected in a polythene bottle, H, containing sodium hydroxide solution.

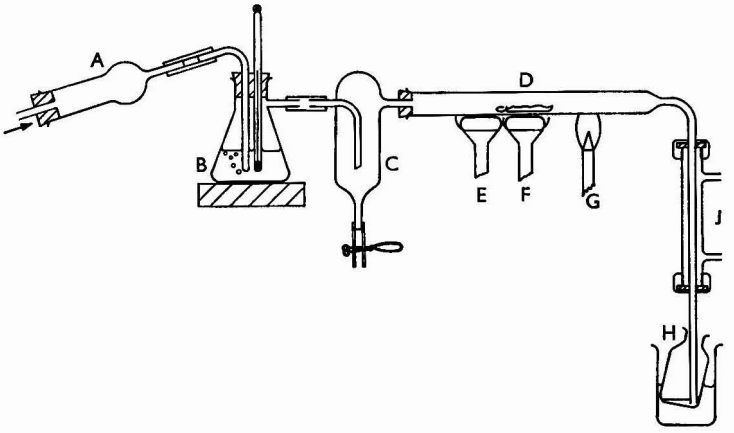


Fig. 1. Apparatus for separating fluorine by pyrohydrolysis (lettered parts of the apparatus are referred to in the text)

Reagents—

Except where specified, analytical-grade reagents are used throughout.

Vanadium pentoxide—Heat ammonium vanadate in a silica basin to between 500° and 550° C for 4 hours; should any black lumps remain, stir the powder and heat for a further period until the residue is uniformly dark red.

Thorium nitrate, 0·005 M—Dissolve 2·94 g of thorium nitrate, $Th(NO_3)_4.6H_2O$, in 1 litre of distilled water containing 0·4 ml of nitric acid.

Standard fluoride solution—Dissolve 1·105 g of sodium fluoride in 1 litre of distilled water; this solution contains 500 μg of fluorine per ml.

Acidimetric indicator—Dissolve 0·2 g of *p*-nitrophenol in 100 ml of distilled water containing a little sodium hydroxide to assist dissolution.

Alizarin red S indicator—Dissolve 100 mg of purified[7] Alizarin red S in 100 ml of distilled water. The normal-grade reagent can be used but the end-point is less clear.

Buffer solution, pH 2·9—Dissolve 22·7 g of monochloroacetic acid in 100 ml of distilled water. Titrate 50 ml with 25 per cent. sodium hydroxide solution, with phenolphthalein as indicator, mix with the remaining 50 ml of monochloroacetic acid solution and dilute to 500 ml.

Colloid stabiliser[5]—Mix 1 g of starch into a thin paste with a little water and pour it, with stirring, into 100 ml of boiling distilled water; boil for 1 minute, then cool.

Absorbent solution (0·1 M *sodium hydroxide*)—Dissolve 4 g of sodium hydroxide in 1 litre of distilled water.

Neutralising acid (0·1 M *hydrochloric acid*)—Dilute 8·6 ml of concentrated hydrochloric acid to 1 litre.

* A suitable tube was inexpensively made to order by T. W. Wingent, Ltd., Cambridge.

METHOD

PYROHYDROLYSIS—

Weigh 10 to 100 mg of sample (<300 mesh) containing 0·1 to 4 mg of fluorine, and about 250 mg of vanadium pentoxide accelerator. Grind the sample and one third of the vanadium pentoxide in an agate mortar and transfer the mixture to a silica boat (50 × 15 mm). Transfer the remainder of the mixture from the mortar by grinding with the remainder of the accelerator in two portions. Heat the water to 90° C, light the burners and pass moist air through the silica tube for 10 minutes. Separate the connection between the water-trap and the silica tube and turn off the burners. When cool, dry the mouth of the tube with absorbent tissue and push the boat into position above burner F. Place a polythene collecting vessel, containing 5 ml of alkaline absorbent solution and a few drops of *p*-nitrophenol indicator, under the exit of the reaction tube; the end of the tube should dip below the level of the liquid. Re-connect the water-trap to the reaction tube, light burners E and G, and pass air through the apparatus. When the vertical part of the silica tube below the condenser is slightly warm to the touch, light burner F, increasing the heat slowly until the vanadium pentoxide melts. Maintain this heat and collect the condensate for 30 minutes. Disconnect the air inlet from the silica tube, and turn off the burners. Remove the polythene collector, wash down the end of the silica tube with a little distilled water and add 0·1 M hydrochloric acid until the yellow colour of *p*-nitrophenol is just discharged. Dry the silica tube with absorbent tissue and remove the sample boat. The apparatus is now ready for the next determination.

TITRIMETRIC FINISH—

Transfer the whole condensate to a 150-ml conical flask, with a mark at 50-ml volume, and add 5 ml of starch solution, 2 ml of buffer and 3 drops of Alizarin red S indicator. Dilute nearly to the mark at 50 ml, and titrate the solution to a pink colour with thorium nitrate, so that the volume at the end-point is 50 ml. Prepare a calibration graph by titrating 1, 2, 3 and 4-ml aliquots of sodium fluoride standard containing 0·5, 1·0, 1·5 and 2·0 mg of fluorine, respectively, with exactly the same conditions as in the titration of the condensate.

ABSORPTIOMETRIC FINISH—

The colour reaction of fluoride with cerium(III) - alizarin complexone is a reliable spectrophotometric method; the preparation of the reagent solutions and the procedure have been described.[8] After neutralising the condensate, dilute to 100 ml and use a suitable aliquot containing 10 to 40 μg of fluorine, which is the range of the method.

BLANK DETERMINATION—

Occasionally repeat the determination without adding sample; the blank should be insignificant.

NOTE—

Water vapour in the air stream sometimes condenses to a fog and recovery of fluorine is then incomplete. Formation of fog can be prevented by decreasing the water flow in the condenser and increasing the air flow so that the air is still warm after passing through the condenser and thus does not become supersaturated; for additional control, the heat from burner G may be varied. An air flow of about 1 litre per minute was used, but accurate metering[4] seems unnecessary. If water collects near the constriction in the silica tube, it must be driven over into the condensate by gently heating, because it is likely to contain dissolved hydrofluoric acid.

RESULTS AND DISCUSSION

The procedure was evaluated by determining the recovery of fluorine added as sodium fluoride or as ground, clear, colourless fluorspar, which was assumed to contain the theoretical amount of fluorine (Table I), and by determining the fluorine contents of several standard samples. During the development of the method, the reaction tube was heated in an electric furnace but, later, gas heating was preferred, and Tables I and II include results obtained with both methods of heating. Recoveries were 98·6 per cent. from sodium fluoride (coefficient of variation 3·2 per cent.) and 98·9 per cent. from fluorspar (coefficient of variation 4·2 per cent.), both rather better than the 95 per cent. recovery often found after Willard - Winter

distillation[9]; the determined fluorine contents of the standard samples agreed well with those reported elsewhere. Results obtained by titration did not differ from those obtained by the absorptiometric method, so that the variance of the results can be attributed to slight variations in the experimental conditions during pyrohydrolysis.

TABLE I

RECOVERIES (PER CENT.) OF FLUORINE FROM SODIUM FLUORIDE AND FLUORSPAR

Method	Electric heating		Gas heating
	Titrimetric	Absorptiometric	Absorptiometric
Sodium fluoride (45·3 per cent. of fluorine)	100·2, 98·3 97·4, 101·7	99·3, 103·0	101·8, 100·7 94·9, 99·7
Biotite (B6)	99·5, 92·8	99·2, 97·7	98·2, 97·2
+ sodium fluoride	101·5, 99·9		97·3
Muscovite (M8)	—	95·7, 97·6	99·0, 104·5
+ sodium fluoride			90·0
Fluorspar (48·7 per cent. of fluorine)	97·2, 92·5 99·1, 98·3	99·6	107·1, 102·3 96·3, 100·0
Biotite (B6)	96·2, 100·7	98·3, 97·0	105·1, 95·4
+ fluorspar	96·9, 96·5 96·0, 99·8		103·0
Muscovite	—	91·8, 89·8	99·5, 97·3
+ fluorspar			

TABLE II

FLUORINE CONTENTS OF ROCK STANDARDS AND SOME MICAS

Method	Electric heating absorptiometric	Gas heating absorptiometric	Reported values
Phosphate rock, N.B.S. No. 56 ..	—	3·44, 3·53 3·54	3·49[1] 3·53[10] 3·58[11]
GSP-1 (69/19)	—	0·384, 0·380 0·380	0·38[12]
G2 (76/10)	—	0·134, 0·129 0·138	0·14[12]
Phlogopite (P1)	6·01	6·07, 6·05 5·99	—
Phlogopite (P6)	6·01, 5·96 5·94, 5·95	6·08, 5·87 6·02, 6·19	—
P6, not ground with accelerator ..	4·6, 5·1 3·9, 5·5	—	—
Biotite (B6)	0·263, 0·270 0·272	—	—
Muscovite (M8)	0·476, 0·465 0·439	—	—

Experience with the method shows that successful pyrohydrolysis depends mainly on two factors.

(*i*) Intimate mixing of the sample and vanadium pentoxide.

(*ii*) Condensation of hydrogen fluoride without formation of fog.

If the sample is not ground with an accelerator, erratic results may be obtained with refractory samples such as phlogopite (P6 in Table II). An extreme example was a micaceous mineral that had previously been ignited at 1000° C; when mixed with the accelerator without grinding, only one fiftieth of the fluorine was released, whereas after grinding together the full amount was recovered.

The reaction temperature is not critical, although below 600° C the reaction is slow,* and above 800° C vanadium pentoxide distils along the reaction tube, and small amounts may occasionally be detected in the distillate. Only when the temperature exceeds 850°

* I am grateful to Dr. P. G. Jeffery of Warren Spring Laboratory, Stevenage, for details of a procedure in which a temperature in the range 655° to 665° C is used; the full method is to be published by G. R. E. C. Gregory and G. O. Kerr.

to 900° C does sufficient vanadium pentoxide reach the condensate to cause slight interference in the absorptiometric determination, but it is not enough to interfere in the thorium nitrate titration.

As close temperature control is not necessary, gas heating is preferred to electric heating. The reaction tube cools rapidly after each pyrohydrolysis so that more determinations can be completed in 1 day. Also, because other parts of the reaction tube can be heated independently of the sample, it is much easier to prevent a fog forming when the sample is first heated and most of the fluorine has evolved.

The method is much quicker than any requiring a preliminary alkaline fusion, and very reliable, provided the sample and accelerator are well mixed and the hydrofluoric acid does not condense to a fog. One person can complete eight analyses in 1 day when using the absorptiometric finish.

I acknowledge with thanks the assistance of Mrs. Sylvia Shepherd, who carried out the analyses given in Tables I and II.

References

1. Willard, H. H., and Winter, O. B., *Ind. Engng Chem. Analyt. Edn*, 1933, **5**, 7.
2. Ingamells, C. O., *Talanta*, 1962, **9**, 507.
3. Evans, W. H., and Sergeant, G. A., *Analyst*, 1967, **92**, 690.
4. Nardozzi, M. J., and Lewis, L. L., *Analyt. Chem.*, 1961, **33**, 1261.
5. Bennett, H., and Hawley, W. G., "Methods of Silicate Analysis," Second Edition, Academic Press, London, 1965, p. 288.
6. Warf, J. C., Cline, W. D., and Tevebaugh, R. D., *Analyt. Chem.*, 1954, **26**, 342.
7. King, H. G. C., and Pruden, G., *Analyst*, 1968, **93**, 601.
8. Belcher, R., Leonard, M. A., and West, T. S., *J. Chem. Soc.*, 1959, 3577.
9. Peck, L. C., and Smith, V. C., *Talanta*, 1964, **11**, 1343.
10. Reynolds, D. S., *J. Ass. Off. Agric. Chem.*, 1934, **17**, 323.
11. Newman, A. C. D., *Analytica Chim. Acta*, 1958, **19**, 471.
12. Flanagan, F. J., *Geochim. Cosmochim. Acta*, 1967, **31**, 289.

Received *May 20th*, 1968

Analyst, December, 1968, Vol. 93, *pp.* 832–833

A Simple, Inexpensive, Pyrex to Aluminous Porcelain Vacuum Seal for Use in Thermal Analysis

By M. D. JUDD and M. I. POPE
(*College of Technology, Portsmouth, Hants.*)

A simple and inexpensive method of making a vacuum-tight seal between a B34 Pyrex glass socket and an aluminous porcelain 525 tube is described.

The need for a seal of this type is widely encountered in thermal analysis of compounds that evolve gases at temperatures within the range 1000° to 1350° C.

To carry out thermal analysis under vacuum at temperatures in the range 1000° to 1350° C, it is frequently necessary to connect an aluminous porcelain vessel to a Pyrex glass gas-handling system. This problem may be encountered in, for example, thermogravimetry, dilatometry, differential thermal and evolved gas analysis. Although it is possible to join Pyrex to aluminous porcelain directly, by means of a graded seal, joints of this type are both expensive and fragile. Furthermore, the life of aluminous porcelain tubes under vacuum at these temperatures is limited, so that the cost of seals becomes an important factor.

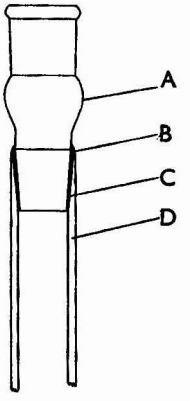


Fig. 1. Details of the seal used (lettered parts of the apparatus are referred to in the text)

The seal discussed here was used in conjunction with a McBain type, spiral silica spring-balance, details of the construction of which were recently described elsewhere.[1] The apparatus has been used to study the thermal decomposition of co-precipitated calcium, strontium and barium carbonates, at a minimum pressure of 2×10^{-4} torr, with a rate of rise of temperature of 200° C per hour up to 1250° C. As the carbonate decomposed it was therefore necessary for the seal to withstand the passage of hot carbon dioxide gas. The results obtained in this work were presented[2] at the Second International Conference on Thermal Analysis.

Details of the seal are illustrated in Fig. 1. The XA54 Pyrex glass adaptor, A, was ground to fit into a thermal aluminous porcelain 525 tube, of nominal bore 28·5 mm, D, with the aid of coarse valve grinding paste. After cleaning off the paste, the cone and tube were coated with Holts "Gun - Gum No. 1," C, pressed together, and then dried for 12 hours at room temperature, followed by 6 hours at 80° C. Finally, the junction between the cone adaptor and the tube was externally coated, B, with Epophen EL5 mixed with an equal volume of hardener EHR1 (marketed by the Borden Chemical Co.) and allowed to harden for 48 hours at room temperature.

In use, the seal was protected externally by a water-cooling coil, and has successfully withstood, over a period of several months, the conditions described.

References

1. Pope, M. I., *Educ. Chem.*, 1965, **2**, 246.
2. Judd, M. D., and Pope, M. I., Paper presented at the Second International Conference on Thermal Analysis, Worcester, Mass., 1968.

Received *May 22nd*, 1968

Thin-layer Chromatography of the Common Barbiturates

By A. S. CURRY AND R. H. FOX

(*Home Office Central Research Establishment, Aldermaston, Reading, Berks.*)

THIN-layer systems for barbiturates are of particular value to the clinical biochemist for the speedy differentiation of the different members of this class of hypnotics, and to the forensic chemist who has to detect therapeutic concentrations in small volumes of blood in "driving under the influence of drugs" cases.

We wish to report a system that is sensitive, rapid and has high resolving power. Merck-prepared cellulose plates with fluorescent indicator, or Kodak Chromatogram Sheet Type 6065 of 20 × 20 cm size, are cut to 4 × 10 cm size. By using smaller plates increased economy (about 1 shilling per plate) and decreased running time are obtained without significant loss of resolution. The plates are dipped in 10 per cent. w/v sodium orthosphate, $(Na_3PO_4).12H_2O$, aqueous solution which is drained off and the plates are then blotted. They are oven-dried at 100° C for half an hour. After cooling they are dipped in acetone - water (75 + 25) and the acetone allowed to evaporate by gently warming the plate for about 1 minute in air. Water remaining on the back of the plate is removed by blotting. The spots of barbiturate containing about 1 μg of compound are applied to the plate or sheet which is developed in n-amyl methyl ketone in a beaker (or, for the sheet, preferably in an Eastman Chromatogram Developing Apparatus Type 104). The time of running is about 10 minutes. The spots are detected by examination in 254-nm light, and by spraying with saturated aqueous mercury(I) nitrate solution. Both methods are very sensitive; on the Kodak sheet 0·4 μg of barbiturate can easily be detected. Typical R_F values are shown in Table I; it is emphasised that known control spots should be run on each occasion as, although resolution is always excellent, R_F values are not absolutely reproducible.

TABLE I

R_F VALUES

Compound	R_F
Barbitone	0·2
Phenobarbitone	0·3
Cyclobarbitone	0·4
Butobarbitone	0·6
Nealbarbitone	0·7
Amylobarbitone	0·75
Pentobarbitone	0·80
Quinalbarbitone	0·85

Received *June 28th*, 1968

Book Reviews

SCHWEIZERISCHES LEBENSMITTELBUCH. Volume I—Special Part. Loose leaf, Pp. x + 214. Fifth Edition. Bern: Eidg. Drucksachen-und Materialzentrale. 1967. Price (Part I) Sw.Fr. 60.

These pages are the first to be issued in the new format of the official Swiss Food Examination Manual which, when complete, will consist of two loose-leaf ring-binder volumes. In addition to introductory material, they comprise four of the chapters that ultimately form part of the second volume: *pasta*; diatetic foods; honey and honey substitutes; and wines. The complete volumes will contain 56 chapters in all, the majority of which will be based on individual food commodities. From the table of contents included with the introductory pages, the chapters on milk, cooking fats and margarine, coffee, tea and substitutes, and microbiological examination are due to appear in 1968. The whole is under the direction of the federal *Schweizerische Lebensmittelbuchkommission*, under the chairmanship of the *doyen* of Swiss food chemistry Professor O. Högl, who in making the publication generally available welcomes comments and suggestions.

This is clearly a comprehensive manual of practical food analysis, including, in many instances, both routine and reference methods for individual determinations and guidance for the interpretation of the analytical results obtained. The interpretations, though sometimes related to Swiss law in particular, add considerably to the value of the compilation.

Like other commodity chapters, that on *pasta* (pp. 18) opens with guidance to characteristic analytical data giving, for example, typical ether extract, total lipid, sterols, soluble protein and phosphate data for plain and "3-egg" *pasta*. While the analytical directions normally provide full working details, calculations and references to original publications are given, there are occasional references to other chapters of the "Lebensmittelbuch," for example for fibre determination or for the identification of added synthetic colouring matter. A separate index is provided for each chapter. Dietetic foods (pp. 81) are divided into separate sections on infants' foods; malt extracts; energy foods and slimming diet beverages (*diatetische Frühstucksgetranke*); diabetic foods; and low-sodium and sodium-free foods and adjuncts. The chapter contains a wealth of interpretative detail complete with statistical comment, *e.g.*, for the determination of dried-egg content of compound foods. Honey (with artificial honey, pp. 34) is a commodity to which the Swiss have devoted special attention: details are given for solids (three methods), ash, pH, hydroxymethylfurfural, dextrin, albumen precipitable by phosphotungstic acid, individual sugars (paper chromatography), apparent sucrose, saccharase and amylase (two methods) determination, together with general directions (including directions for further reading) for the microscopic examination of sediment. The predominant pollen grains contained may not be so characteristic of the origin of a honey as the particular combination of these with such other features as fungal spores, starch grains, yeast cells, root particles, insect hairs and plant fragments. This is indeed a subject for the expert as is also the interpretation of the various analytical criteria which attempt to distinguish honey predominantly of floral origin (*i.e.*, bee-honey) from substitutes and mixtures.

The chapter on wines (pp. 57) is supplemented by a specially compiled version of Osborne's tables (pp. 22) for the calculation of the alcohol content of alcohol - water mixtures from the relative density 20°/20° or the absolute density at 20° C. Various standards for wines are described, most of the basic analytical methods for arriving at which are generally similar to those used in Britain. Cellar-treatment agents will be considered in a separate chapter. It is refreshing to learn that wine tasting is best done in the morning, about 2 hours after breakfast; for the Swiss this puts the optimum time for the *dégustation* at about 0900 hours. H. EGAN

ADVANCES IN CHROMATOGRAPHY. Edited by J. CALVIN GIDDINGS and ROY A. KELLER. Volume 5. Pp. xviii + 317. London: Edward Arnold (Publishers) Ltd.; New York: Marcel Dekker, Inc. 1968. Price 135s.

This volume continues the series, several of which have been discussed by the reviewer. However, it must be said at the outset that, in the reviewer's opinion, this volume does not sustain the same standard as the previous volumes. Perhaps this is inevitable because one is conscious of the fact that chromatography could not expand at the explosive rate of, say, 10 years ago. The articles in the present volume appear to be more of the review article type, which in certain cases have been included to make up the volume.

As before, the book is divided into two sections, one dealing with general chromatography, the other with gas chromatography, each section containing three contributions.

The first section contains chapters on prediction and control of zone migration rates in ideal liquid - liquid chromatography; chromatographic advances in toxicology; and inorganic chromatography on columns of natural or substituted celluloses. Chapter 1 is important as it contains a relatively high proportion of references to the studies of Polish scientists who have worked in this field, most of which are not readily accessible to the majority of chromatographers. The problem facing the use of such models as are proposed, is that one is very rarely dealing with ideal models, and eventually complications arise that can only be surmounted by trial and error. The second chapter is one in which the emphasis is placed on problems facing the toxicologist, and how chromatography can be used to alleviate some of these problems. Although great progress has obviously been made, and the many references given show how useful the technique is, the outstanding feature of the chapter is really dealt with in the last 2 pages, where future trends are discussed. This gives an admirable key to where our society may lead, or in fact, drive us.

The third chapter in this section on inorganic chromatography is probably the field closest to the reviewer's heart. However, it is felt that the author has re-discovered columns of cellulose and found them useful. Indeed, in his closing remarks to the chapter he has very strong words to

say on the subject of why this field is neglected. This is appreciated, but some of his remarks are open to doubt or speculation and, as science is no less fashionable than fashion, he may be successful in obtaining popularity of this topic—but others have tried a long time ago.

Turning to the gas-chromatography section, the chapter on quantitative interpretation of gas-chromatographic data is a very good one. It puts clearly and succinctly the problems involved, and describes how various gadgets and devices are used to overcome these problems. The kernel of the problem is outlined on pages 222–225, where the required quality of the gas-chromatographic data required is listed. How many separations live up to all of these criteria—the concluding paragraph on page 226 possibly provides the clue.

The problem of pollution is one where argument abounds. On one hand are the people who say that pollution is intensifying and on the other those who say that maybe the case is overstated. Whichever school one follows, it is the analytical chemist who has cut through the morass of speculation and developed methods of analysis to combat the problem. The second chapter of this section discusses atmospheric analysis by gas chromatography, dealing only with "the atmosphere at or near ground level over urban and non-urban land masses." Diesel and internal-combustion engine pollution, etc., will be covered in a later volume of the series. Thus in the present chapter there is a discussion of the pollutants found as a result of man's activities. The most surprising comment that can be made is the lack of analyses that have been carried out, and the development work that will be necessary to rectify this situation, as the conclusions of the chapter are 2 pages of might-be, the need for, and have-not-yet-been applied.

The final chapter describes non-ionisation detectors in gas chromatography, and is a detailed survey review of the advances in the topic since 1964. Obviously, these detectors have a future, but how good it will be seems to depend almost entirely, not on their merits as detectors but on their cost, availability and, least tangible of all, on the whims and fashions of gas chromatographers. The chapter really says very little that is not already readily available in the literature.

In line with previous volumes of the series the book is well produced, both with regard to printing and binding, but is very expensive for the information it contains. It is a book for an organisation to buy.

G. NICKLESS

STANDARD METHODS FOR TESTING TAR AND ITS PRODUCTS. Edited by P. V. WATKINS. Sixth Edition. Pp. viii + 662. Gomersal, Cleckheaton and York: Standardization of Tar Products Tests Committee. 1967. Price 80s.

The first edition of this work was published in 1929 and fresh editions now appear to come every 5 years. This sixth edition represents a thorough revision of the previous one, and includes a list of the changes that have taken place. Among these may be noted the acceptance of a single expression for the weight/volume relationship, density at 20° C. This should be a great help, for the confusion in different parts of the world in respect of density is great, while even in a single country different industries have their own definitions.

It is to be noted that five gas-chromatographic tests appear, and these are accompanied by a 17-page dissertation on the general principles of the method. The five methods include determinations of benzene in toluene, toluene in benzene, *o*-cresol in cresylic acid and pyridine and homologues in tar bases.

Wherever possible, British Standard apparatus has been specified. It is interesting to find details of three standard methods of the U.S. Customs Laboratory—for creosote oil distillation, cresylic acid and other coal tar distillates, and for naphthalene.

K. A. WILLIAMS

Errata

OCTOBER (1968) ISSUE, p. 673. Replace diagram above legend 4 with diagram above legend 11 (p. 678).

IBID., p. 676. Replace key to Fig. 8 by

A = Stout	G = Light ale
B = Milk stout	H = Pale ale
C = Milk stout	J = Polish lager
D = Strong ale	K = Danish lager
E = Bitter ale	L = Danish lager
F = Brown ale	M = Continental beer

IBID., p. 677. 2nd line from the foot of the page. *For* "Fig. 10" *read* "Fig. 11."

IBID., p. 678, 13th line. *For* "Fig. 11" *read* "Fig. 10."

IBID., p. 678. Replace diagram above legend 11 with diagram above legend 4.

Summaries of Papers in this Issue

The Effect of Filtration and Centrifugation on Raw Sugar Polarisation Analysis

In raw sugar analysis, the apparent sucrose content is determined as its polarisation value, a measure of the rotation of plane-polarised light passing through a solution of the sugar, which is directly proportional to sucrose concentration when the impurity level is low.

Before taking a reading with a polarimeter, a "normal" raw sugar solution (26·000 g per 100 ml) is clarified by addition of basic lead acetate solution to precipitate impurities, which are removed by simple filtration; about 50 ml of filtrate are needed for analysis. When the solution is filtered, the sucrose concentration increases because of preferential absorption of water by the filter-paper. Initially in the current study, the magnitude of the absorption has been examined, and it has been determined that the first 10 ml of filtrate should be discarded to minimise this effect.

Differences of 1 part in 40,000 in the polarisation value of sugar solutions, however, can now be detected by using modern photo-electric polarimeters and the refined analytical techniques described in this paper. This compares with 1 part in 2000 when using older visual instruments and standard techniques. Therefore, an insight into evaporation and humidity effects has also been gained, and a comparison of filtration and centrifugation, an alternative method of clarification, made.

Centrifugation has practical advantages over filtration in laboratories in which large numbers of analyses are performed. In addition, it is not subject to preferential absorption and evaporation errors, and currently appears to give the best estimate of polarisation value, as defined and specified by the International Commission for Uniform Methods of Sugar Analysis (ICUMSA).

R. A. M. WILSON, C. G. SMITH, R. H. JAMES and R. R. WALLACE

The Colonial Sugar Refining Co. Ltd., Central Laboratory, Sydney, Australia.

Analyst, 1968, **93**, 773–781.

Recent Methods for Determining Traces of Nitrogen in Mineral Oils

Three methods for the determination of traces of nitrogen in oil are discussed, *viz.*, those based on extractive percolation, oxy-hydrogen combustion and hydrogenation - coulometry. Their scopes are compared with respect to their lower detection limit, the range of products to which they can be applied and their speed.

P. GOUVERNEUR and F. van de CRAATS

Koninklijke/Shell-Laboratorium, Amsterdam (Shell Research N.V.).

Analyst, 1968, **93**, 782–787.

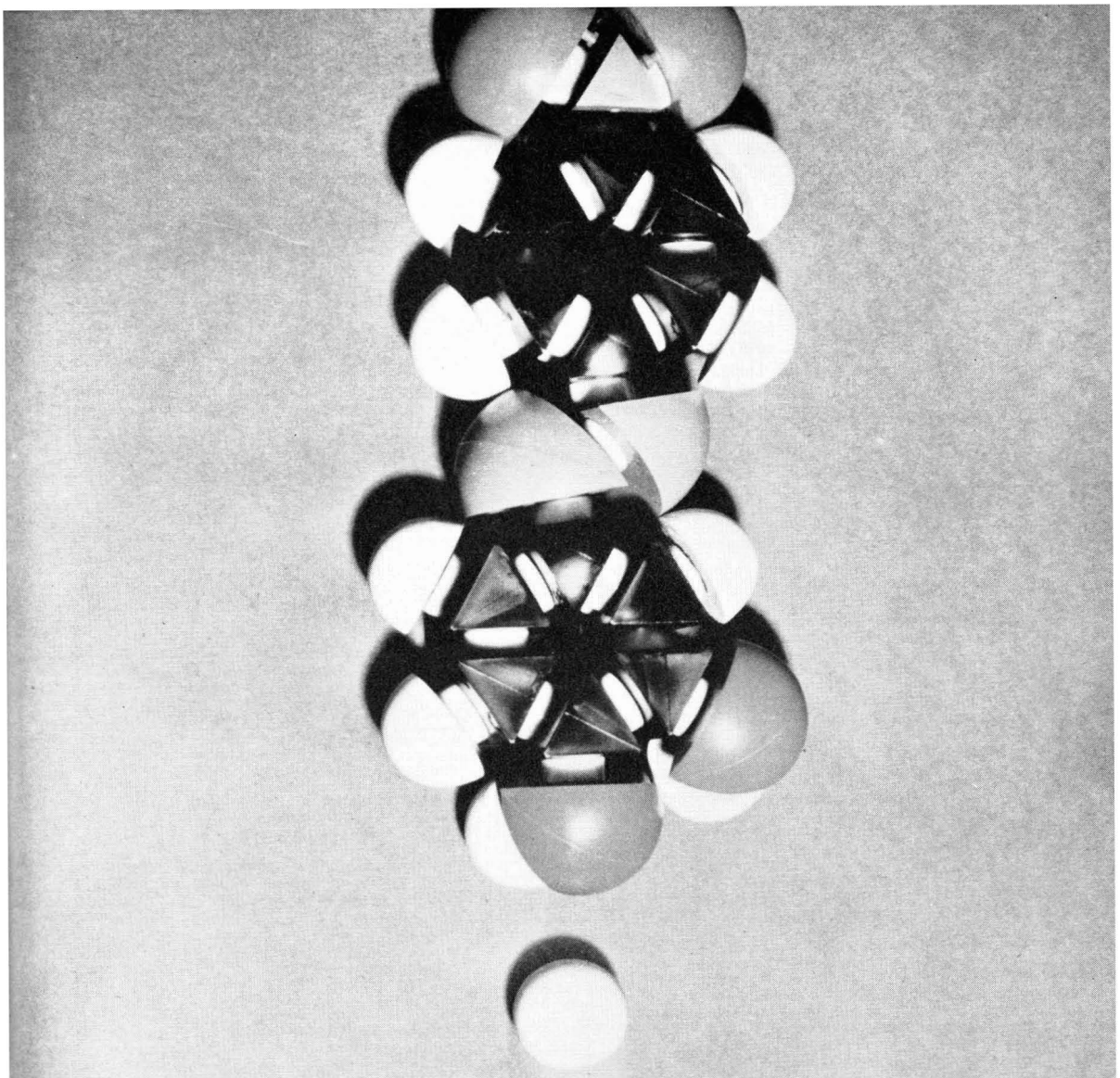
Determination of Chloride in Chloride-containing Materials with a Chloride Membrane Electrode

The determination of chloride by using the membrane-type chloride electrode is described. Procedures are given for the analysis of chloride materials, including silver halides, both in the presence and absence of bromide and iodide.

J. C. VAN LOON

Department of Geology, University of Toronto, Toronto 5, Ontario.

Analyst, 1968, **93**, 788–791.



TEST PAPERS FOR DETERMINATION OF IONS

CARLO ERBA

THE MODERN ANALYTICAL TECHNIQUES OFTEN EMPLOY ORGANIC REAGENTS WHICH FOR THEIR SENSITIVITY AND THEIR GIFTS OF SPECIFICITY ARE REPLACING THE CLASSICAL METHODS. THE TEST PAPERS FOR DETERMINATION OF IONS — DEVELOPED BY CARLO ERBA — ARE BASED ON THE USE OF ORGANIC REAGENTS AND COMBINE THE ADVANTAGES OF THESE, WITH RAPIDITY OF USE, AND A REMARKABLE STABILITY. THE TEST PAPERS ASSUME A DIFFERENT SHADE OF COLOUR FOR DIFFERENT CONCENTRATIONS OF A GIVEN ION SO THAT THE ANALYSIS CAN ASSUME A SEMI-QUANTITATIVE CHARACTER. THEY ARE SUPPLIED IN SINGLE PACKAGES FOR THE DETERMINATION OF THE FOLLOWING IONS:

Ag / Hg / Pb / Bi / Cu / Cd / As / Sb / Sn / Mo / Al / Fe / Cr / Zn / Co / Ni / Mn / Ca / Sr / K / Li / Mg / NH_4.

Oxidimetric Determination of Thiocarbonate Sulphur with Chloramine-T, Potassium Ferricyanide and Potassium Permanganate

Oxidimetric methods for determining the concentration of aqueous solutions of potassium thiocarbonate by using chloramine-T, potassium ferricyanide and potassium permanganate are discussed. The chloramine-T method is based on the reaction of potassium thiocarbonate with a known excess of chloramine-T in alkaline medium at 60° C and back-titration of the unreacted chloramine-T against a standardised solution of sodium thiosulphate, with starch as indicator. Twenty-four equivalents of the oxidant per mole of potassium thiocarbonate are consumed, showing that the three sulphur atoms of thiocarbonate are oxidised to sulphate. When ferricyanide is used twenty-four equivalents of the oxidant per mole of potassium thiocarbonate are also consumed in an alkaline medium at 60° C. However, in acidic medium, potassium permanganate is found to oxidise the three sulphur atoms of thiocarbonate to elemental sulphur. The following molar relationships are established—

12 moles of chloramine-T $\equiv$ 1 mole of K_2CS_3
6 moles of $KMnO_4$ $\equiv$ 5 moles of K_2CS_3
24 moles of $K_3Fe(CN)_6$ $\equiv$ 1 mole of K_2CS_3

K. N. JOHRI and N. K. KAUSHIK

Department of Chemistry, University of Delhi, Delhi 7, India.

Analyst, 1968, **93**, 792–796.

The Determination of Molybdenum in Mixtures Containing Molybdenum Disulphide by Atomic-absorption Spectrophotometry

A rapid method is described for determining the total molybdenum content of mixtures containing molybdenum disulphide, graphite and a resin. The novel feature of the technique is the decomposition of the mixture by fusion with sodium hydroxide. Dissolution of the melt in sulphuric acid permits the determination of molybdenum by atomic-absorption spectrophotometry without interference. Results at the 8 per cent. molybdenum level are accurate to within $\pm 0{\cdot}1$ per cent.

R. J. JULIETTI and J. A. E. WILKINSON

Morganite Research and Development Ltd., Battersea Church Road, Battersea, London, S.W.11.

Analyst, 1968, **93**, 797–798.

Determination of Antimony in Titanium Dioxide by Atomic-absorption Spectrophotometry

A method is described for the determination of the antimony content of titanium dioxide by atomic-absorption spectrophotometry, based on the extraction of antimony with isobutyl methyl ketone. This method is more sensitive than determination by emission-spectrographic and as sensitive as the colorimetric technique, and can detect 25 p.p.m. of antimony in a 0·1-g sample of titanium dioxide.

J. C. MÉRANGER and E. SOMERS

Research Laboratories, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada.

Analyst, 1968, **93**, 799–801.

The Determination of Zirconium in Mineral Rutile with Alizarin Red S

A colorimetric procedure has been developed for the determination of the zirconium oxide content of rutile. A fusion with potassium hydrogen difluoride enables the rutile to be brought into solution rapidly and eliminates the task of filtering off silica. The zirconium is separated from the titanium by a phosphate precipitation, but the interference of tin inherent in the method involving phosphate precipitation followed by ignition to oxide, is eliminated by dissolving the zirconium phosphate and applying a colorimetric finish, with Alizarin red S. Calibration is effected by making additions of zircon to titanium oxide and carrying each through the entire procedure, thus eliminating reagent-blank difficulties. It has been used in the range of 0 to 1 per cent. of zirconium oxide. Good agreement with independent determinations by X-ray fluorescence has been obtained.

N. B. STANTON

Associated Minerals Consolidated Limited, Southport, Queensland, Australia.

Analyst, 1968, **93**, 802–804.

The Effect of Ethanol on the Colorimetric Determination of Formaldehyde and Glycollic Acid

Contrary to previous reports ethanol has been found to interfere with the colorimetric determination of formaldehyde and glycollic acid with 1,8-dihydroxynaphthalene-3,6-disulphonic acid (chromotropic acid) or 2,7-dihydroxynaphthalene in concentrated sulphuric acid. The effect is greater for glycollic acid than for formaldehyde. The mechanism for these observations is given.

R. H. STILL, K. WILSON and B. W. J. LYNCH

Department of Chemistry and Biology, Hatfield College of Technology, Hatfield, Hertfordshire.

Analyst, 1968, **93**, 805–809.

Simple, Rapid Quantitative Determination of Amino-acids by Thin-layer Chromatography

A simple, rapid method for the quantitative determination of complex mixtures of amino-acids is described. After separation on thin layers of cellulose mounted on flexible plastic sheets, the chromatograms are sprayed with ninhydrin and developed under controlled conditions. The spots are cut out and eluted with 2 ml of 50 per cent. propyl alcohol and the optical density at 570 nm determined with a microspectrophotometer. From 4 to 6 chromatograms can be eluted and read in 1 day. An accuracy of about 5 nmoles is obtained over a range of 12·5 to 50 nmoles. At higher concentrations, accuracy is within ± 10 per cent. Standard graphs are reproducible for at least 5 months.

MARY E. CLARK

Department of Organismic Biology, University of California, Irvine, California 92664, U.S.A.

Analyst, 1968, **93**, 810–816.

The Continuous Polarographic Determination of Small Amounts of Nitroglycerine in Plant Effluent

A fully automatic polarographic method is described for the continuous monitoring of nitroglycerine plant effluent, for which an arbitrary maximum level of 100 p.p.m. of nitroglycerine has been set.

Conventional polarographic principles are involved, but the polarograph used has been specially designed, and incorporates some novel features that make it admirably suitable for this and similar work.

Constructional details are given of the polarograph, and of an alarm system that operates when the permitted level of nitroglycerine in the effluent is exceeded.

A. R. HOLLAND and A. G. S. BENHAM
Imperial Metal Industries Limited, Summerfield Research Station, Kidderminster.

Analyst, 1968, **93**, 817–820.

A Simple Field Test for the Determination of Hydrogen Fluoride in Air

A field method is described for determining hydrogen fluoride vapour in air at concentrations up to 20 μg of hydrogen fluoride per litre. The gas is collected in an acidic solution of zirconium - Solochrome cyanine R reagent and the observed bleaching of the colour compared with standards. The apparatus used is simple to operate and the time required for a determination is less than 5 minutes. A dynamic method for the generation of standard atmospheres of hydrogen fluoride is also described.

B. S. MARSHALL and R. WOOD
Ministry of Technology, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1.

Analyst, 1968, **93**, 821–826.

A Simple Apparatus for Separating Fluorine from Aluminosilicates by Pyrohydrolysis

Fluorine-bearing minerals are hydrolysed at 700° to 800° C in a gas-heated fused-silica tube. The hydrogen fluoride evolved is absorbed in alkali and determined by titration with thorium nitrate or absorptiometrically with cerium - alizarin complexone. Recoveries from sodium fluoride averaged 98·6 per cent., with a coefficient of variation of 3·2 per cent., and the determined fluorine contents of the silicate rock standards GSP-1 and G-2 were 0·381 and 0·134 per cent. The method is simple and rapid, and is suitable for determining fluorine in micas and related aluminosilicates.

A. C. D. NEWMAN
Rothamsted Experimental Station, Harpenden, Herts.

Analyst, 1968, **93**, 827–831.

A Simple, Inexpensive, Pyrex to Aluminous Porcelain Vacuum Seal for Use in Thermal Analysis

A simple and inexpensive method of making a vacuum-tight seal between a B34 Pyrex glass socket and an aluminous porcelain 525 tube is described. The need for a seal of this type is widely encountered in thermal analysis of compounds that evolve gases at temperatures within the range 1000° to 1350° C.

M. D. JUDD and M. I. POPE
College of Technology, Portsmouth, Hants. *Analyst*, 1968, **93**, 832–833.

Thin-layer Chromatography of the Common Barbiturates

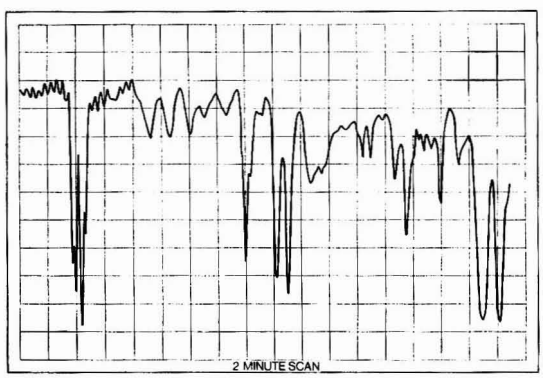
A thin-layer chromatographic system, in which cellulose plates or Kodak Chromatogram sheet is used, has been developed for the rapid separation of common barbiturates. The plates are prior treated with sodium orthophosphate and run in n-amyl methyl ketone. R_F values for eight barbiturates are given.

A. S. CURRY and R. H. FOX
Home Office Central Research Establishment, Aldermaston, Reading, Berks.

Analyst, 1968, **93**, 834.

APPOINTMENTS VACANT

£970



Our new Infragraph Spectrophotometer costs only £970.

Yet it's about the best medium-resolution infra-red spectrophotometer you can buy. And it's certainly the best value for money.

Take a look at this Infragraph trace and then check through these four vital value-for-money features and get the rest of the story.

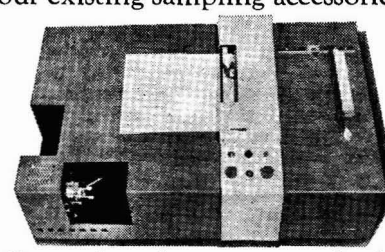
Wavelength range.
The Infragraph covers the whole of the most useful range from 4000-650 cm^{-1}. See trace for resolution achieved.

A4 graph paper or flow chart.
Our Infragraph uses either A4 or continuous paper equally well. Produces a clean, well-defined spectrogram at speeds ranging between two and ten minutes per scan.

Accessibility. The Infragraph opens up easily for inspection. It's just the job for lectures.

Easy to use. A first year student or lab assistant can be at home with the Infragraph in a matter of minutes.

But even that's not all.
The Infragraph is small, light and takes the minimum of bench space. And uses all your existing sampling accessories.



So send us the coupon and ask to be included on our demonstration tour.

Find out what a £970 Spectrophotometer can really do.

Please invite us to your demonstration tour.

Name_____

Address_____

M

HILGER & WATTS LTD.

Applied Optics Division
98 St. Pancras Way, Camden Road, London, N.W.1. Telephone: 01-485 5636 Telex: 23852
Grams: Sphericity London N.W.1.

THE QUEEN'S AWARD TO INDUSTRY

P. A. May 1874

Just one of the sophisticated automatic accessories designed for Pye Unicam's SP500 Series 2 ultraviolet and visible spectrophotometer system. Use it for kinetics or column effluent analyses. Lenses and apertures for microcells are standard fittings on all Pye Unicam instruments. Adding the SP505 programme controller, the SP506 automatic cell changer and the SP507 constant temperature cell holder to the basic instrument results in a system with up to eight different operational modes. Or plug in the SP40 automatic sample changer for analysis of up to 50 samples and record your results on the SP22 logarithmic recorder. Two other built-in facilities make the Series 2 one of the most versatile systems of its kind in the world. For speed you can use the built-in direct-reading meter, and for extra precision, use the null balance with photometric accuracy of ± 0.3%T. Pye Unicam house and tend samples with unrivalled care—call today and let us accommodate your samples.

PYE UNICAM

THE QUEEN'S AWARD TO INDUSTRY 1966

Precision Instruments

Pye Unicam Ltd
York Street, Cambridge, CB1 2PX, England
Telephone (0223) 58866 Telex 81215

Specify SPECPURE® and be sure of your analytical results

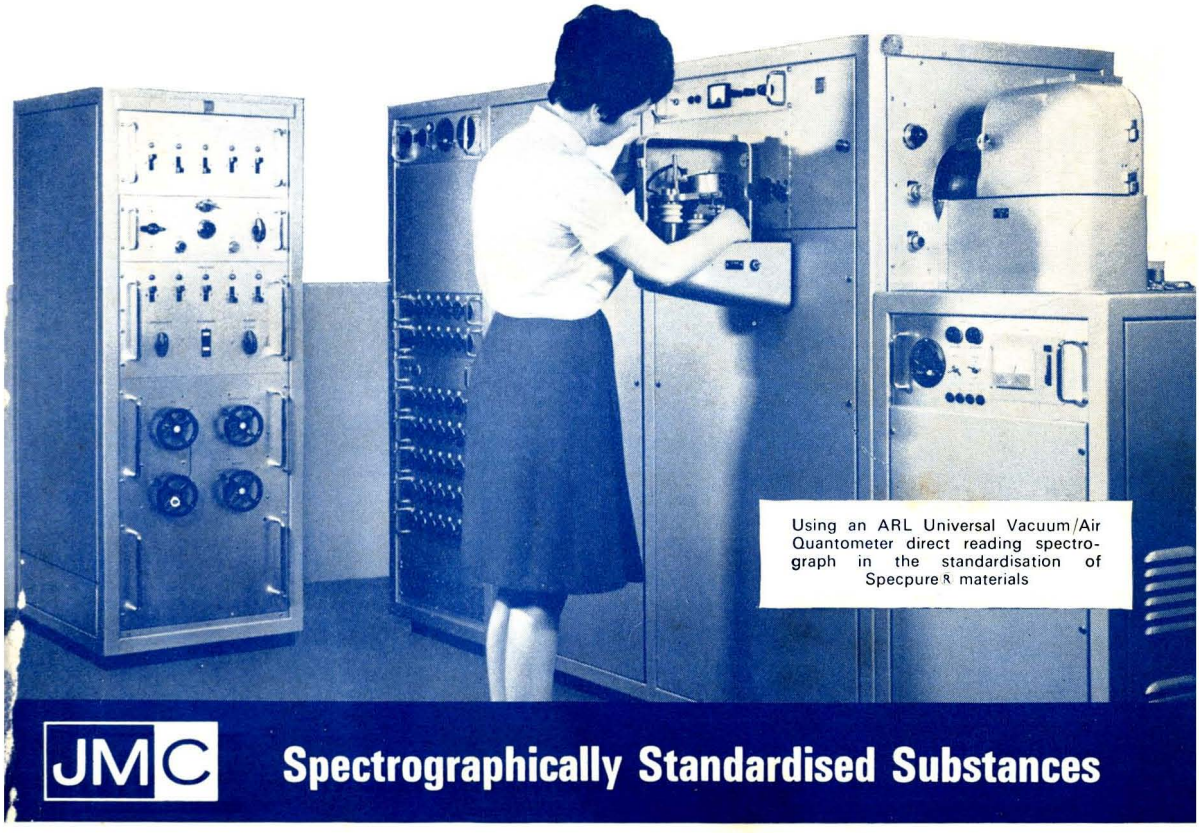
You know, like all experienced spectrographers the world over, that the accuracy and reliability of your results rest on the standards you use for comparison.

And you know that the only spectrographically standardised materials in which you can place absolute trust are the Specpure® range of metals and chemicals. You employ the substances as reference standards in qualitative spectrography and in making standard mixtures containing known amounts of certain elements for quantitative work.

So famous are Specpure® materials in analytical, process control and research laboratories throughout the world that the name has virtually gone into the language!

While that is a proud tribute to their quality and universal usefulness, this is a good time to remind you that there is only one Specpure® range and these materials are produced and marketed by Johnson Matthey Chemicals!

Write for full information on Specpure® materials and our price list today!



Using an ARL Universal Vacuum/Air Quantometer direct reading spectrograph in the standardisation of Specpure® materials

JMC **Spectrographically Standardised Substances**

Johnson Matthey Chemicals Limited
74 Hatton Garden, London, EC1 Telephone: 01-405 6989 Telex: 21465

Volume 93. No. 1113, Pages 773-836 December, 1968

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

CONTENTS

Printed and Published for the Society for Analytical Chemistry, by W. Heffer & Sons Ltd., Cambridge, England
Communications to be addressed to the Editor, J. B. Attrill, 9/10 Savile Row, London, W.1. Enquiries about advertisements should be addressed to J. Arthur Cook, 9 Lloyd Square, London, W.C.1.

Entered as Second Class at New York, U.S.A., Post Office.