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THE ANALYST

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The Use of the Plate Method for the Assay of Aneurine in Yeast and Yeast Products

BY A. JONES AND S. MORRIS

(*Read at the Annual General Meeting of the Biological Methods Group, on December 17th, 1948*)

THE assay of aneurine, both by the chemical and microbiological methods, has in the past yielded results of very doubtful accuracy. Of the chemical methods the thiochrome technique is, perhaps, the simplest and most frequently used. With pure aneurine solutions this method yields satisfactory results, but in presence of other materials, as in the assay of foodstuffs, great difficulty is found in the extraction of the aneurine uncontaminated with various coloured compounds which interfere markedly with the technique of assay.

Various microbiological methods have, at different times, been published, the yeast fermentation method of Schultz *et al.*,[1] the mould technique using *Phycomyces blakesleeanus*[2,3] and the bacteriological methods using *Streptococcus salivarius*[4] and *Lactobacillus fermentum*.[5] There are disadvantages in each of the methods, such as the special apparatus necessary for the yeast fermentation, the time required, two weeks, for the growth of the mould *Phycomyces* and the inherent difficulties in turbidimetric methods used with *L. fermentum*. Further, most microbiological techniques suffer from the disadvantage that the organisms respond to both the pyrimidine and the thiazole moieties of the aneurine molecule and allowance must be made for these factors.

Recently, a plate method of assay, using *L. fermentum*, has been published by Bacharach and Cuthbertson.[6] The method is not particularly sensitive but would appear to eliminate, at least in part, some of the difficulties encountered in the turbidimetric method. Further, the plate method is rapid and requires no special apparatus.

The present paper details a study of this method and gives a modified technique for the assay of aneurine in yeasts and yeast extracts. The final method adopted is given first, followed by experimental data on which the method is based. This has been done for ease of reading and of subsequent reference.

METHOD OF ASSAY

BASAL MEDIUM—

The basal medium used was that advocated by Sarett and Cheldelin[5] with addition of sodium chloride and 1·5 per cent. of agar. The composition of this medium is shown in the table overleaf.

This was prepared in lots of at least 2000 ml., tubed in 20-ml. amounts, autoclaved at 10-lb. pressure for 10 minutes, and stored at laboratory temperature until required. More accurate results with fewer irregularities in the readings were obtained when the medium was stored for at least 3 days after autoclaving, before being used. Normally, the medium was used within 2 weeks of preparation, but satisfactory results have been obtained with a medium stored as long as 5 weeks.

STOCK CULTURES—

The organism used, *Lactobacillus fermentum* P.36, was maintained on the medium of Cheldelin, Bennett and Kornberg,[8] and sub-cultured every 3 weeks. This medium contained 1 per cent. of Difco yeast extract, 1 per cent. of glucose and 2 per cent. of agar, with addition of 10 μg. of aneurine per tube.

BASAL MEDIUM

Glucose	20·0 g.
Sodium acetate (anhydrous)	6·0 "
Sodium chloride	5·0 "
Casein hydrolysate	2·5 "
Adenine	0·01 "
Guanine	0·01 "
Uracil	0·01 "
L-Cystine	0·1 "
Peptone (photolysed)	200 ml.
Inorganic salt solution A[7]	5·0 "
Inorganic salt solution B[7]	5·0 "
Riboflavine	100 μg.
Biotin	0·4 "
Pyridoxine	100 "
Calcium pantothenate	100 "
Nicotinic acid	100 "
p-Amino benzoic acid	100 "
Folic acid	1·0 "
Volume made up to	1000 ml. at pH 6·5

PREPARATION OF THE INOCULUM—

L. fermentum was grown for 18 hours at 37° C. in 10 ml. of basal medium minus agar, with the addition of 10 μg. of aneurine per ml. The medium was centrifuged, the supernatant liquor discarded and the organisms washed twice with saline. The washed organisms were finally suspended in 20 ml. of sterile saline and used as inoculum.

PREPARATION OF THE PLATES—

Five tubes of medium were used for the standard and for each test sample. The contents of the tubes were melted by immersing the tubes in a boiling water-bath. The tubes were cooled to a temperature of 48° to 50° C. and maintained at that temperature, and 1 ml. of the inoculum was added to each tube. Immediately after addition of the inoculum the contents of each tube were thoroughly mixed by rotation to distribute the bacterial suspension evenly throughout the medium. The contents of the tubes were poured severally into sterile Petri plates, 9 cm. diameter, on a flat, even surface, allowed to set, dried for 1·5 hours at 37° C. with the lids of the plates raised about half an inch from the bottom of the dishes on a specially constructed drying rack, and finally stored at laboratory temperature until used, usually 2 to 3 hours later.

Five holes were cut in each plate, each hole 10 mm. in diameter, the agar discs removed and the holes sealed with a drop of melted medium.

PREPARATION OF THE YEAST SAMPLES—

1 g. of the dried yeast and 0·05 g. of takadiastase were added to 15 ml. of 1 per cent. sodium acetate buffer at pH 4·5. The pH of the mixture was adjusted to 4·4 to 4·5 with *N* acetic acid or sodium hydroxide as necessary, 2 drops of benzene were added, and the whole was incubated at 37° C. for 16 to 18 hours.

It was then steamed for 10 minutes, cooled, adjusted to pH 6·5, diluted to 25 ml. with water and filtered. This filtrate was diluted 1 : 2, 1 : 4, 1 : 8 and 1 : 16. 0·1 ml. of the 1 : 16 dilution was measured into the first hole of each of the five plates, 0·1 ml. of the 1 : 8 dilution into the second hole of each of five plates and so on, 0·1 ml. of the undiluted filtrate being measured into the fifth holes.

PREPARATION OF THE STANDARD CURVE—

The assay range used for the preparation of the standard curve was 0·25, 0·50, 1·00, 2·00 and 4·00 μg. of aneurine hydrochloride per ml. 0·1 ml. of the aneurine standard solution

containing 0·25 μg. per ml. was measured into the first hole of each of five plates, 0·1 ml. containing 0·50 μg. per ml. into the second hole of each plate, and so on. The plates were incubated for 18 hours at 37° C. with the lids raised.

The diameters of the zones of growth were measured to the nearest 0·5 mm. and the mean values plotted against the logarithms of the concentrations of the standard aneurine hydrochloride in μg. per ml. or against the logarithms of the concentrations of the yeast samples.

Under the given test conditions, where the effect of doubling the aneurine concentration was to increase the zone diameters by 2·0 mm., the following equation expresses this relationship: $\log D = aZ - b$, where a and b are constants, D is the aneurine concentration in μg. per ml. and Z the zone diameter in mm. corresponding to a yeast concentration of M mg./ml. With the given test conditions, $a = 0{\cdot}1505$ and b is dependant upon the intercept of the standard curve for any given day. From this, the aneurine concentration in the yeast in μg. per g. is given by 1000 D/M.

Experimental

1. Effect of density of bacterial suspension on zone diameters—

The density of the inoculum used affected the zone diameters markedly. Typical diameters obtained with the bacterial suspension diluted to 20 ml., to 50 ml. and to 100 ml. are given in Table I as the mean of the five zones. The range of the five individual observations was never more than 1 mm.

The zones of growth obtained diminished in sharpness and increased in size as the dilution of the suspension increased.

Table I

Effect of diluting the bacterial suspension on the size of the zone diameters

Dilution of bacterial suspension	Mean zone diameter in mm.				
	0·25 μg. Aneurine	0·50 μg. Aneurine	1·0 μg. Aneurine	2·0 μg. Aneurine	4·0 μg. Aneurine
In 20 ml.	18·7	20·4	22·3	24·2	25·9
In 50 ml.	19·3	21·2	23·4	25·3	27·0
In 100 ml.	22·3	24·9	27·1	28·8	31·4

With a bacterial suspension diluted to 20 ml., the mean value of fifteen assays of a yeast was 92·7 μg. per g. of yeast; standard error of the mean, 1·59. With a bacterial suspension diluted to 50 ml., the mean value of fifteen assays was 83·7 μg. per g.; standard error of the mean, 2·93. Further reference is made to these figures at a later stage.

Because of the clarity of the zones and the ease of measurement, the bacterial suspension was in all cases diluted to 20 ml. as the most suitable volume.

2. Effect of preliminary drying of the plates—

A comparison was made of the zones of growth obtained when the plates were poured and used directly for assay and when the plates, after pouring, were given a preliminary drying of 1·5 hours at 37° C. When the plates were used directly the zones were 4 to 6 mm. larger in diameter, more diffuse and difficult to measure as compared with those that had been subject to a preliminary drying. The preliminary drying at 37° C. for 1·5 hours was therefore included in the standard procedure.

3. Effect of the pH of the standard aneurine solution on the zone diameters—

Standard aneurine solutions were prepared using distilled water at pH 6·5, buffer solution at pH 4·5 and buffer solution at pH 7·0. The average zone diameters obtained with these solutions are shown in Table II.

Table II

Effect of pH of the standard aneurine solution on the size of the zone diameters

Standard aneurine solution	Mean zone diameter in mm.				
	0·25 μg. Aneurine	0·50 μg. Aneurine	1·0 μg. Aneurine	2·0 μg. Aneurine	4·0 μg. Aneurine
Distilled water ..	19·1	21·5	24·1	26·5	28·6
Buffer solution, pH 4·5	19·2	21·5	24·3	26·4	28·5
Buffer solution, pH 7·0	19·0	21·4	23·7	25·4	27·5

In solutions with a pH not greater than 6·5 there would appear to be little or no difference, irrespective of the pH, in the zone diameters. In neutral solution, however, variations occurred, the zone diameters at the higher concentrations of aneurine being less than those found in acid solution. For this reason the aneurine solution was never maintained at a pH greater than 6·5.

4. EFFECT OF TAKADIASTASE AND PAPAIN ON ANEURINE—

Enzymatic digestion of foods with takadiastase and papain has been recommended for the complete extraction of aneurine and the conversion of the co-carboxylase to aneurine.[5,9] There appear to be no published results, however, on the effect of takadiastase and papain on aneurine itself. For this reason experiments were carried out on the effect of these enzymes on solutions of pure aneurine.

A solution containing 50 μg. of aneurine hydrochloride per ml., in acetate buffer at pH 4·5, was treated in the following manner:—

(*a*) 20 ml. were incubated at 37° C. for 18 hours with 0·05 g. of takadiastase.

(*b*) 20 ml. were incubated at 37° C. for 18 hours with 0·05 g. of takadiastase and 0·05 g. of papain.

To both tubes 2 drops of benzene were added before incubating. After incubation the tubes were steamed for 10 minutes and cooled, the pH was adjusted to 6·5 and both were diluted to 200 ml. with water. On assay, the results obtained were 5·2, 5·1 and 3·7, 3·2 μg. of aneurine per ml. respectively, the theoretical value being 5·0 μg. per ml.

It would appear that the presence of papain caused a loss of aneurine, whereas takadiastase had no effect. It was, therefore, decided to omit papain from the enzyme mixture added to extract the aneurine from yeast.

On purely theoretical grounds there would appear to be little reason for the addition of papain together with takadiastase for the extraction of aneurine, since the takadiastase contains a protease active in an acid medium and a protease would appear to be the only active enzyme in papain. The reason for the loss of aneurine in presence of papain is difficult to understand and calls for further work.

5. EXTRACTION OF ANEURINE FROM YEAST—

Although takadiastase had no effect on solutions of pure aneurine, it was necessary to ascertain if takadiastase digestion of a material such as yeast would be sufficient to extract all the aneurine present. A series of experiments was, therefore, carried out in which a direct digestion of yeast with takadiastase was compared with the effect of 0·1 *N* sulphuric acid and with that of sulphuric acid followed by takadiastase treatment.

To ascertain in the first place the amount of takadiastase necessary for maximum extraction of the aneurine, different amounts were added to a constant amount of dried yeast or yeast extract. The mixtures were incubated in acetate buffer of pH 4·5 at 37° C. for 18 hours.

The assay results are given in Table III.

TABLE III

EFFECT OF DIFFERENT AMOUNTS OF TAKADIASTASE ON THE EXTRACTION OF ANEURINE FROM YEAST

Amount of takadiastase added	μg. Aneurine per g. of material	
	Assay (i)	Assay (ii)
1 g. Dried yeast A + 0·01 g. takadiastase	94	59
1 g. " " + 0·05 g. "	89	97
0·1 g. Yeast extract A + 0·05 g. takadiastase	512	542
0·1 g. " " + 0·10 g. "	532	
0·1 g. " " + 0·15 g. "	450	
0·1 g. Yeast extract B + 0·01 g. takadiastase	210	
0·1 g. " " + 0·05 g. "	445	

The results indicate that 0·05 g. of takadiastase is sufficient to release the aneurine from 1 g. of dried yeast or 0·1 g. of the yeast extracts assayed.

For the comparative trials, using 0·1 *N* sulphuric acid and takadiastase, 0·05 g. of takadiastase per 1 g. of dried yeast was used. The treatment with the acid consisted of steaming 1·0 g. of dried yeast with 10 ml. of 0·1 *N* acid for 30 to 45 minutes. 10 ml. of acetate buffer of pH 4·5 were then added, the pH was finally adjusted to 6·5 and the volume brought to 25 ml. with water. In those experiments in which takadiastase digestion followed acid

treatment, the mixture, after steaming, was treated with 10 ml. of acetate buffer and 0·05 g. of takadiastase, and adjusted to pH 4·5, 2 drops of benzene were added, and the whole was incubated for 18 hours at 37° C. The results of the assays are given in Table IV.

TABLE IV

COMPARISON OF THE EFFECTS OF 0·1 *N* SULPHURIC ACID, 0·1 *N* SULPHURIC ACID FOLLOWED BY TAKADIASTASE, AND TAKADIASTASE ALONE ON THE EXTRACTION OF ANEURINE FROM YEAST

Treatment of yeast	μg. Aneurine per g. of yeast	
	Yeast A	Yeast B
0·05 g. Takadiastase alone	94, 99	69
0·1 *N* H_2SO_4—steamed 30 minutes	95	58
0·1 *N* H_2SO_4—steamed 30 minutes and followed by takadiastase	90	67
0·1 *N* H_2SO_4—steamed 45 minutes	67	—
0·1 *N* H_2SO_4—steamed 45 minutes and followed by takadiastase	96	—

Steaming in presence of acid does not always appear to extract all the aneurine from yeast, for subsequent treatment with takadiastase sometimes yielded a higher result. On the other hand, takadiastase treatment alone appeared to be as effective as steaming followed by enzyme treatment. Using the method of enzyme treatment alone, a series of assays was carried out on two samples of dried yeast. Fifteen assay values were obtained with one yeast and eighteen with the second. The results are given in Table V, which shows the mean values and the standard errors.

TABLE V

THE ASSAY OF TWO DRIED YEASTS GIVING THE MEAN VALUE AND THE STANDARD ERROR OF THE MEAN

	Yeast A μg./g.	Yeast B μg./g.
	97, 99, 94, 89, 93, 95, 82, 84, 89, 99, 99, 84, 89, 99, 99	67, 68, 71, 71, 70, 70, 70, 66, 69, 66, 67, 61, 61, 60, 62, 71, 71, 69
Mean value	92·7	67·2
Standard error S_n of the mean	1·59	0·90

6. EFFECT OF AN ANEURINE-FREE YEAST EXTRACT ON THE ASSAY OF PURE ANEURINE—

The possibility existed that the test samples, after treatment, contained some factor or factors affecting the assay. These would not be present in the standard aneurine solution and might affect the final results. This was examined by preparing an aneurine-free yeast extract, by autoclaving in presence of sodium hydroxide. The alkali-treated yeast extract was adjusted to pH 6·5 and, after filtration, diluted as for a normal yeast sample. Dilutions of the aneurine standard solution were prepared with this aneurine-free extract, by dissolving known amounts of aneurine in it. It is realised that this treatment is drastic and may possibly destroy some other unknown essential factor, but any method involving the destruction of aneurine must be similarly suspect.

Examination of the plates and measurement of the zone diameters showed clearly that yeast treated with sodium hydroxide to render it free from aneurine did not contain any factor that would affect the assay of aneurine. It was concluded that, for the present and until further work is carried out, yeast may be assumed to contain no substances which interfere with the assay of aneurine by the method adopted.

Since sending to the press, the effects of pyrimidine and thiazole, separately and together at levels equivalent to the aneurine content of yeast, have been examined. In no case was any growth of *L. fermentum* noted. It can, therefore, be assumed that, in the plate method, these compounds do not affect the assay results.

7. RECOVERY OF ANEURINE WHEN ADDED TO YEAST—

Proof of the validity of the method rested on the ability of the assay technique to give theoretical results for aneurine added to yeast. This was tested by adding different quantities of aneurine, 20, 40 and 100 μg. per g. of yeast, to a yeast sample or to a yeast extract, incubating with takadiastase for 18 hours at 37° C. and assaying as previously described.

The results are given in Table VI.

TABLE VI

RECOVERY OF ANEURINE ADDED TO YEAST AND YEAST EXTRACTS

μg. Aneurine per g. of yeast	μg. Aneurine added per g. of yeast	Total μg. aneurine present	μg. Aneurine found per g.	Per cent. found
82	20	102	108	106
82	40	122	110	90
108	100	208	204	98
μg. Aneurine per ml. yeast extract	**μg. Aneurine added per ml.**	**Total μg. aneurine present**	**μg. Aneurine found per ml.**	**Per cent. found**
3·2	2·0	5·2	5·1	98

The recovery of the added aneurine was satisfactory and it was considered that the validity of the method, at least for yeast and yeast extracts, had been proved.

DISCUSSION

The plate assay method appears to give more reproducible results than any of the previous methods, although its sensitivity is not particularly good. This one might expect from a plate assay method.

The dilution of the final inoculum is important; the greater the dilution the more vague and uncertain the final zone of growth. For this reason a dilution of 1 in 20 has been adopted in the final method. Although the zones of growth are small they are well defined and easily measured. If clearer and more well defined zones could be obtained at the higher dilutions of inoculum, a marked increase in sensitivity would be obtained. Clarity of zone may possibly be connected with the growth medium and further work in this connection will be carried out in the near future.

Occasionally a batch of medium is found to give invalid results for no apparent reason. Fortunately, this can be readily seen from the standard curve and in such circumstances the test and medium must be abandoned.

SUMMARY

1. A method for the assay of aneurine in yeast is given based on the plate technique of Bacharach and Cuthbertson.

2. A correct degree of dilution of the organism in the final inoculum is important to obtain a clear and well defined zone of growth.

3. Takadiastase appears to release all the aneurine from yeast and yeast products.

4. Steaming yeast in presence of 0·1 *N* sulphuric acid gives variable results and papain appears to have a destructive effect on even pure aneurine.

5. The method as advocated is not particularly sensitive, but has been shown to give valid and accurate results, and its precision is at least as high as that of any other method for the determination of aneurine in yeast.

The authors wish to thank Dr. R. H. Marriott and Dr. J. Farquharson for their interest in this work, and the Directors of Beecham Research Laboratories Limited for permission to publish.

They also wish to thank Dr. E. C. Wood and Mr. S. A. Price for helpful criticism.

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Discussion

The Chairman, Dr. A. J. Amos, congratulated the authors on what appeared to be a very useful assay method and referred to the difficulties hitherto encountered in determining the aneurine content of yeast. He would like to see comparisons between the results obtained by this and by other methods on the same samples.

Mr. J. S. Harrison quoted some of his results on dried yeast (*Analyst*, 1948, **73**, 539) by the *Neurospora crassa* (9 assays), thiochrome (6 assays) and yeast fermentation methods. The mean estimates, and their 95 per cent. limits of error, were respectively 15·7 ± 4·9, 14·6 ± 1·7 and 14·7 ± 7·0 μg./g.

Dr. E. C. Wood stated that in view of the difficulty, referred to by Dr. Morris, of checking the absolute accuracy of the cup-plate method on yeast, samples should be assayed both by this method and using rats. Dr. Morris's method of stating the precision of the technique, as the standard error of the results of successive assays on the same sample, was not so informative as the standard error of a single assay calculated from the internal evidence, *i.e.*, the difference between responses to the same dose. Microbiological assays of aneurine employing *Lactobacillus fermentum* in the usual "test tube" technique nearly always showed a certain number of quite anomalous responses in every dose group; had the authors encountered this phenomenon in the cup-plate technique?

Miss A. Jones said that they had not encountered such anomalies in their method.

Mr. S. A. Price asked whether the authors had investigated the effect of the pyrimidine and thiazole moieties of the aneurine molecule under their conditions. According to Sarett and Cheldelin these substances were inactive in the "test tube assay" with *L. fermentum* provided that the incubation time was no more than 18 hours. Was this also true of the cup-plate method?

Mr. Bacharach asked if there was any evidence that these compounds were inactive for mammals. Did their presence, in fact, vitiate an assay?

Dr. E. R. Dawson also emphasised the importance of ascertaining whether the organism responded to the pyrimidine and thiazole moieties under the conditions of the cup-plate test. In reply to Mr. Bacharach he pointed out that the Labelling of Foods Order specified "Aneurine hydrochloride," not "vitamin B_1."

Dr. W. F. J. Cuthbertson enquired whether the enzymic destruction of aneurine had been attempted in the preparation of basal media. This should show advantages in that aneurine may be removed specifically and the enzyme itself readily inactivated without altering any other constituents of the medium. He suggested also that *Streptococcus salivarius* might be tried as a test organism; it should have advantages over *L. fermentum* in being more sensitive to aneurine and less sensitive to the pyrimidine and thiazole breakdown products.

Dr. Morris replied that they had not so far investigated the influence on the assay of the pyrimidine and thiazole moieties,* neither had they attempted enzymic destruction of aneurine in the preparation of basal media.

* But see last four lines of p. 337. Ed.

The Microbiological Assay of Aneurine: An Improved Method Employing *Lactobacillus Fermenti* 36*

By E. E. FITZGERALD and E. B. HUGHES

The method is based on that described by Sarett and Cheldelin,[1] modified subsequently by Barton-Wright[2] and later by Cheldelin, Bennett and Kornberg.[3] The present improvement depends on the destruction of aneurine in an extract of the sample by a modification of the sulphite treatment of Schultz, Atkin and Frey,[4] and the use of this sulphite-treated extract to supplement the standard tubes. Thus all tubes used in an assay contain the same amount of the sample and a comparison is made between untreated extract and sulphite-treated extract plus standard aneurine. In this way inhibitory or stimulating effects due to substances other than aneurine in a sample are counteracted.

Method

The organism used is *Lactobacillus fermenti* 36; stab cultures are maintained by weekly sub-culture on a yeast-water glucose agar containing 10 μg. of aneurine per tube. The cultures are incubated for 30 to 48 hours at 37° C. and stored in a refrigerator (about 5° C.) when not in use.

The basal assay medium (Table I) is that described by Sarett and Cheldelin[1] with addition of 0·5 per cent. of sodium chloride and with a modified Salt Solution B as recommended by Barton-Wright.[2] The preparation and storage of stock solutions from which the medium is prepared are essentially those given in the Analytical Methods Committee's "Report on the Microbiological Assay of Riboflavine and Nicotinic Acid."[5]

The divergence from other methods is in the use of sulphite-treated sample extracts and in the design of the assay.

Table I

Basal medium

(sufficient for 100 tubes)

Photolysed peptone	200 ml.
Casein hydrolysate	2·5 g.
Glucose	20 "
Sodium acetate (cryst.)	10 "
Cystine	0·1 "
Adenine hydrochloride	0·01 "
Guanine "	0·01 "
Uracil	0·01 "
Sodium chloride	5 "
Salt solution A	5 ml.
" " B	5 "
Riboflavine	100 μg.
Ca D-pantothenate	100 "
Nicotinic acid	100 "
p-Aminobenzoic acid	100 "
Pyridoxine	100 "
Biotin	0·4 "
Folic acid	0·25 "
Glass-distilled water to	500 ml.
pH	6·5

Photolysed peptone—Dissolve 40 g. of Difco Bacto-peptone in 250 ml. of glass-distilled water and add 20 g. of sodium hydroxide dissolved in 250 ml. of water. Allow the mixture to stand for 24 hours in the light at room temperature, exposing it to strong light (*e.g.*, a 100-watt tungsten filament lamp at 18 inches) for at least 12 hours of this period. At the end of this time add 28 ml. of glacial acetic acid and 11·6 g. of sodium acetate (cryst.). Make up the volume to 800 ml. and preserve the solution under sulphur-free toluene in a refrigerator. This solution will normally keep for a fortnight, but should be discarded if a precipitate forms before this time.

* Also known as *Lactobacillus fermentum* 36. Ed.

Salt Solution A—Dissolve 25 g. of KH_2PO_4 and 25 g. of K_2HPO_4 in 250 ml. of water. The solution keeps indefinitely in the presence of toluene.

Salt Solution B—Dissolve 10 g. of $MgSO_4.7H_2O$, 0·5 g. of $MnSO_4.4H_2O$ and 0·1 g. of $FeCl_3$ (anhydrous) in 250 ml. of water and add 5 drops of concentrated hydrochloric acid. This solution keeps indefinitely.

PREPARATION OF EXTRACTS—

The sample extract should contain approximately 0·015 μg. of aneurine per ml. The weight of sample taken and the volume to which it is adjusted should be chosen to ensure this. With an unknown sample it may be necessary to make a preliminary test to obtain an approximate value for its aneurine content.

Heat a suitable weighed quantity of the finely divided sample (usually about 1 g.) with 25 ml. of 0·1 *N* sulphuric acid for 30 minutes at 100° C. in a steamer or an autoclave. Cool and adjust the pH to 4·5 with 2·5 *M* sodium acetate solution, using bromocresol green as external indicator. Add 20 mg. each of papain and takadiastase and incubate for 18 to 24 hours at 37° C. under a thin layer of toluene. Heat for 30 minutes at 100° C., cool, make up the volume to 100 ml. and filter through a Whatman No. 1 paper. Take two equal aliquots of the filtrate. Adjust the pH of the first to 6·5, using bromothymol blue as external indicator, and make up with water to such a volume as will give an estimated aneurine content of 0·015 μg. per ml. This is Sample Extract I. To the second aliquot add 4 ml. of a freshly prepared 10 per cent. solution of sodium sulphite crystals, adjust the pH to 5·4 with *N* sulphuric acid, using B.D.H. "4460" indicator externally, make up the volume to about 50 ml. with water and autoclave for 15 minutes at 15 lb. pressure. Cool, and add the exact amount of hydrogen peroxide (A.R. 10 vol.) required to oxidise the excess of sodium sulphite, using as external indicator a freshly prepared mixture of equal volumes of 5 per cent. potassium iodide solution, 1 per cent. starch solution and 50 per cent. sulphuric acid. Adjust the pH to 6·5 (indicator bromothymol blue) and dilute to the same concentration as Sample Extract I. This sulphite-treated extract will be referred to below as Sample Extract II.

PREPARATION OF INOCULUM—

Add 10 μg. of aneurine to 5 ml. of basal medium, dilute with distilled water to 10 ml. and sterilise by autoclaving for 15 minutes at 15 lb. pressure. Make a transfer from a fresh stock culture of the organism to a tube of this medium with a sterile platinum wire. Incubate for 16 to 18 hours at 37° C. and centrifuge aseptically. Pour off the supernatant liquid, suspend the bacterial cells in 10 ml. of sterile saline and dilute 1 drop of suspension in 10 to 25 ml. of sterile saline.

ASSAY PROCEDURE—

Into each of fifteen Pyrex test tubes (18 or 19 by 150 mm.) pipette 5-ml. aliquots of basal medium. To each of five tubes add 2 ml. of Sample Extract I and to each of the remaining ten add 2 ml. of Sample Extract II. One of these ten tubes serves as a blank. Add standard aneurine solution to the other nine at the levels 0·02, 0·03 and 0·04 μg. per tube, using three tubes at each level. Adjust the total volume in each of the fifteen tubes to 10 ml. with distilled water. Plug the tubes with cotton wool and sterilise by autoclaving for 10 minutes at 10 lb. pressure. After cooling, inoculate all tubes except the blank with 1 drop each of the diluted inoculum and incubate them all together in a constant-temperature water-bath at 37° C. for a period not exceeding 18 hours and preferably not less than 17 hours. After incubation remove the tubes, all together, cool for at least 15 minutes in a refrigerator at about 5° C. to arrest growth, and measure the turbidity in the tubes photo-electrically. We have found the Hilger Spekker absorptiometer, with neutral grey filters (H508), satisfactory for this purpose. With this instrument the difference between the optical density of each tube and the uninoculated blank is measured and the scale reading gives a measure of the turbidity produced by bacterial growth.

INTERPRETATION OF RESULTS—

Over the range 0·02 to 0·04 μg. the response of *L. fermenti* to aneurine is nearly linear. The aneurine content of the sample can therefore be deduced by interpolation from the turbidity readings. For example, the protocols of a typical assay were:—

Sample of malt extract—0·996 g. was digested and made up to 100 ml.; 40-ml. aliquots

were taken and finally diluted to 100 ml. 2 ml. of Sample Extracts I and II therefore contained 7·97 mg. of the sample.

	μg. Aneurine added	Spekker readings	Mean
Sample Extract I	nil	0·712; 0·713; 0·718; 0·710; 0·725	0·716
Sample Extract II	0·02	0·607; 0·602; 0·585	0·598
	0·03	0·698; 0·710; 0·683	0·697
	0·04	0·807; 0·783; 0·800	0·797

7·97 mg. of sample contain >0·03 μg. and <0·04 μg. of aneurine.
By interpolation 7·97 mg. of sample contain 0·0319 μg. of aneurine.
Hence the aneurine content of the sample is 4·00 μg. per g.

Discussion

The method of Sarett and Cheldelin was found unsatisfactory for some samples; drifts in the values obtained at different sample levels were obtained because of the stimulatory or inhibitory effect on *L. fermenti* of substances other than aneurine in the sample extracts. The present method was developed with two objects in view. First, to supplement the "standard" tubes with an extract similar to the sample extract except that its aneurine had been inactivated. Secondly, to simplify the design of the assay, for having overcome the stimulatory or inhibitory effects of substances other than aneurine it is no longer necessary to test the sample at more than one level.

Using pure aneurine solutions it was found that the sulphite treatment of Schultze *et al.* destroyed about 95 per cent. of the activity as determined by Sarett and Cheldelin's method but, when the solutions were autoclaved at 15 lb. pressure instead of being steamed for 30 minutes, 99 per cent. of the activity was destroyed in 15 minutes. When this modification of the sulphite treatment was applied to sample extracts supplemented with aneurine no residual activity was found.

If the sulphite treatment has a destructive effect on the stimulatory or inhibitory substances, assays carried out at different levels will show a drift in the results in the same way as in the Sarett and Cheldelin method. Recovery tests were carried out on some samples that had given drifts when assayed by Sarett and Cheldelin's method. In these tests aneurine solution was added in known amount to the weighed sample before extraction and the "fortified" sample was submitted to the full procedure as described above. The aneurine found was from 99 to 107 per cent. of the calculated amount, indicating that no drifts had occurred (Table II).

Table II

Sample	Weight of sample per tube mg.	Aneurine content: Calculated μg./g.	Aneurine content: Found μg./g.	Aneurine recovery %
Wheat germ	1·25	—	21·2	—
» » + aneurine 23 μg./g. ..	0·6	44·2	46·8	106
Dried yeast	0·8	—	35·3	—
» » + aneurine 35 μg./g. ..	0·4	70·3	70·3	100
Flour	8·0	—	3·77	—
» + aneurine 2 μg./g.	5·0	5·77	5·70	99
» + » 4 »	4·0	7·77	8·33	107
» + » 8 »	2·5	11·77	12·42	106
» + » 16 »	1·5	19·77	20·89	106

The sulphite treatment introduces into the treated extract (Sample Extract II) a small amount of sodium sulphate, amounting to about 5 to 8 mg. per tube. Tests showed that at some levels sodium sulphate has a slight stimulatory effect on the organism in the presence of aneurine but, by supplementing Sample Extract I with sodium sulphate prepared from sodium sulphite and hydrogen peroxide, the effect was shown to be negligible (Table III).

Table III

	Extract I	Extract I supplemented with sodium sulphate
Sample A	2·56 μg. per g.	2·56 μg. per g.
Sample B	2·89 »	3·00 »

Sarett and Cheldelin state that steaming at 100° C. for 15 minutes "is sufficient to keep the test free from contamination for the short time that it is run. Steaming, in contrast

to autoclaving at higher temperature, does not darken the medium and decreases the possibility of any thiamine destruction." When assay tubes were autoclaved for 10 minutes at 10 lb. pressure no destruction of aneurine occurred and there was only slight darkening of the medium. This treatment has been found to be satisfactory in all other microbiological assays carried out by us and was adopted throughout the present work.

L. fermenti shows very little growth in the first 20 to 24 hours of incubation in the basal medium to which the hydrolysis products of aneurine have been added (sulphite-treated yeast extract was actually used). After this period the organism grows rapidly, reaching full growth in a further 40 hours (Fig. 1). The growth in the first 20 hours, although small,

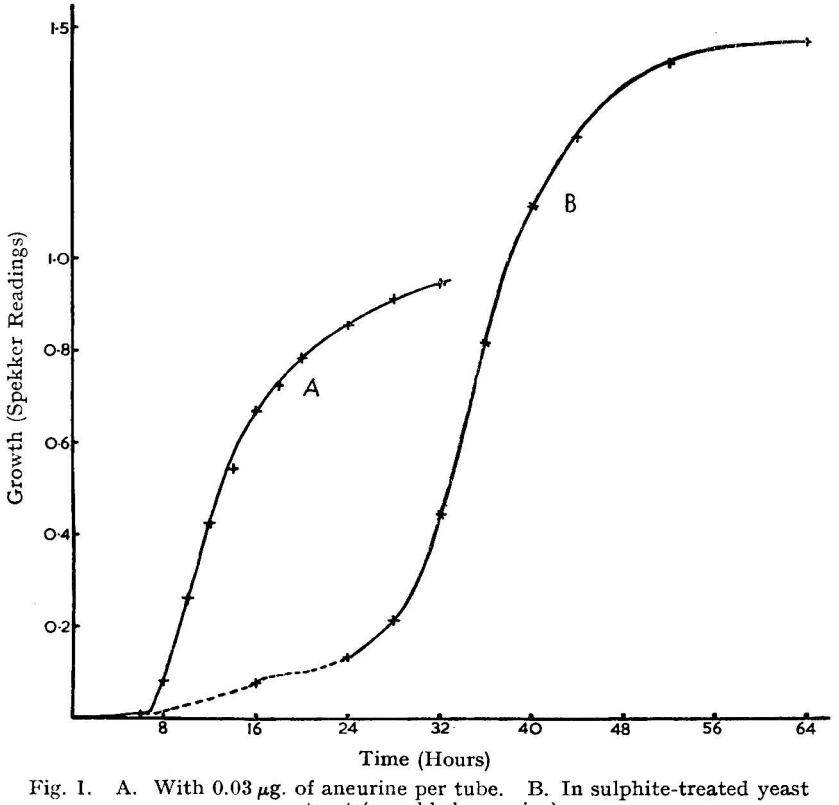

Fig. 1. A. With 0.03 μg. of aneurine per tube. B. In sulphite-treated yeast extract (no added aneurine)

was difficult to account for until it was found to be due to residual aneurine in the alkali-treated peptone in the medium. When the peptone was prepared by treatment with sulphite a very low "blank" was obtained in the first 20 hours of incubation. Under normal assay conditions, that is, in presence of about 0·03 μg. of aneurine per tube, the lag phase is about

TABLE IV

Sample	Separate assay results (μg. aneurine per g.)	Mean
Flour	3·45, 4·18, 3·73, 3·73, 3·62	3·74
Wheat germ	23·3, 23·7, 21·2	22·7
Dried yeast	37·6, 33·8, 37·5, 35·3	36·1
Malt extract	4·09, 4·01	4·05

6 hours, the logarithmic phase is passed in a further 10 hours, and after this growth continues more slowly. Thus, with an incubation time of 18 hours, growth is independent of aneurine degradation products, whilst the effect of intact aneurine is approaching its maximum.

This work indicated that by treating natural products such as peptone and yeast extract

with sulphite it should be possible to devise a simpler basal medium with a shorter list of synthetic supplements; this is now being investigated.

The method described in this paper has been applied to a wide range of food materials. The results have been satisfactorily reproducible (Table IV) and have agreed reasonably well with parallel chemical assays (Table V).

TABLE V

Sample	Microbiological method. Aneurine μg. per g.	Thiochrome method.* Aneurine μg. per g.
Flour	3·47	2·75
Wheat germ	22·7	18·9
Wheat germ preparation 1	24·0	20·5
" " " 2	17·7	14·1
" " " 3	19·7	15·8
Barley kernels	1·92	1·33
Rolled oats	6·4	6·1
Malt extract	4·05	3·37
Yeast extract	57·6	55·8
Dried yeast	36·1	29·8
Full cream milk powder	3·49	3·55
Skim milk powder	4·83	3·40
Dried egg	2·41	2·9
Lean pork	12·1	12·6
Ox kidney	4·2	4·4
Ox liver	2·9	3·1
Canned roast pork	1·46	1·46
Vitamin tablet	537	339

* Using Decalso for purification of the extracts.

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A Scheme for the Chromatographic Examination of Propellant Explosives

BY T. C. J. OVENSTON

THE application of the chromatographic method to the examination of some complex propellant compositions from foreign sources has demonstrated very clearly the value of this elegant technique in aiding the solution of the analytical problems involved. Used in conjunction with appropriate streak tests applied to the extruded column, the value of the method from a qualitative point of view lies in the fact that not only are colour tests employed on the separated ingredients, but the characteristic adsorption affinity of each ingredient is noted. The selectivity is thus greatly increased in comparison with the use of colour tests alone, particularly when the latter are applied to the mixture of ingredients to be found in the ether extract of the propellant.

The present paper describes a scheme for the chromatographic detection of any of twenty-four possible ether-soluble propellant ingredients in the presence of one another, and discusses briefly a few quantitative applications.

PROPELLANT INGREDIENTS STUDIED

The twenty-four ether-soluble propellant ingredients included in the present scheme are listed in Table I, together with the appropriate abbreviations. The list is arranged in

alphabetical order of abbreviation for easy reference as these abbreviations are used in other parts of this paper.

TABLE I

PROPELLANT INGREDIENTS AND THEIR ABBREVIATIONS

Chemical name	Abbreviation
N : N-Diphenylurea (Acardite)	AC
N : N'-Diphenylurea (Carbanilide)	CBAN
Carbazole	CBZ
Diamyl phthalate	DAP
Dibutyl phthalate	DBP
Diethylene glycol dinitrate	DEGN
Diethyl phthalate	DEP
Dimethyl phthalate	DMP
2 : 4-Dinitrotoluene	DNT
Diphenylamine	DPA
Diphenylformamide	DPF
N-Nitrosodiphenylamine (Diphenyl-nitrosoamine)	DPN
N : N-Diphenylurethane	DPU
N : N'-Diphenyl-N : N'-diethylurea (Carbamite or Ethyl Centralite)	EC
N : N'-Diphenyl-N : N'-dimethylurea (Methyl Centralite)	MC
Mineral jelly	MJ
1-Nitronaphthalene	MNN
Nitroglycerine	NG
2-Naphthyl-methyl-ether	NME
N-Phenyl-N-benzylurethane	PBU
N-Nitroso-N-ethylaniline (Phenyl-ethyl-nitrosoamine)	PEN
N-Phenyl-N-ethylurethane	PEU
2 : 4 : 6-Trinitrotoluene	TNT
N-(2-Tolyl)-urethane	TU

GENERAL EXPERIMENTAL TECHNIQUE

The comparative chromatographic characteristics of these compounds were investigated, and tables showing orders of adsorption with various binary developing solvents were constructed.[1] This information formed the basis of the present scheme of analysis. The following special points regarding materials and technique are noted.

APPARATUS—The chromatographic tubes are most conveniently made from ordinary uniform glass tubing, in lengths of 25 cm. No stricture is required, so that extrusion of the column is not impeded. The tube is held in an adaptor by a rubber ring, and rests on a filter plate in the adaptor. A receiving flask may be attached to the bottom of the adaptor (a ground glass joint is very convenient here), and a side-arm on the adaptor allows suction to be applied to the base of the column.

ADSORBENT—Except for the detection of mineral jelly, the same adsorbent is used throughout the scheme. This consists of a mixture of equal parts of silica gel and Celite 535, uniformly activated by heating at 120° C. The silica gel is a particularly fine grade, marketed by Messrs. Silica Gel, Ltd. The filter aid, Celite 535, is marketed by Messrs. Johns-Manville Co., Ltd. A closely similar adsorbent has been described by LeRosen.[2] The success of the present scheme of analysis depends, of course, on the reproducibility of this adsorbent, but experience suggests that there should be no difficulty in this matter.

SIZE OF COLUMN—The scheme is designed for use with columns 1 cm. wide by 15 cm. long. The actual length is not very critical (owing to the employment of "indicator zones"), but any appreciable difference in width from that stated above should be allowed for by suitable adjustment of the volumes of developing solvents prescribed. Thus, for a width of 1·1 cm., a prescribed volume of 100 ml. of developing solvent becomes 121 ml.

SOLVENTS—The developing solvents employed consist mainly of binary mixtures of light petroleum, benzene and ethyl ether. The light petroleum recommended is that fraction boiling between 40° and 50° C. Other than redistillation, special purification is not necessary here. The benzene should be of the pure "crystallisable" grade, and the ethyl ether should be dry and recently distilled over caustic alkali.

PACKING OF COLUMN—A plug of cotton wool is placed at the bottom of the glass tube, and sufficient adsorbent is poured in to give a column 2 to 3 cm. high. Suction is gently applied and then released again, and the tube is tapped to level the surface of the powder. Suction is then applied again and a further similar quantity of adsorbent is added in a thin

stream. The suction is released again and the tube tapped as before; then suction is reapplied and the tube again tapped to assist settlement. This procedure is repeated until the desired height is obtained.

Streak tests employed

Colour reactions are given by most propellant ingredients, and some of these are readily adaptable as chromatographic "streak" tests (the *Pinselmethode* of Zechmeister *et al.*[3]). Excluding mineral jelly, for which such a test is unnecessary, streak tests have been devised for all the compounds under consideration.

A method of application favoured by the author is to fill a small pipette (of about 4 inches over-all length) with the reagent and, starting at one end of the extruded column, draw the tip slowly along the column, allowing the reagent to flow freely through the tip, the size of which is chosen to suit the viscosity of the reagent. In this way narrow and uniform streaks can easily be made. By moving the column round, several different streaks may be made on the same column.

The *streak reagents* used in the present scheme are as follows:—

*S.R.*1. 0·5 per cent. solution of potassium dichromate in 60 per cent. w/w sulphuric acid.

*S.R.*2. 0·5 per cent. solution of potassium dichromate in water, followed by a superimposed streak of 90 per cent. w/w sulphuric acid.

*S.R.*3. 1 per cent. solution of diphenylamine in concentrated sulphuric acid.

*S.R.*4. 6 *N* aqueous sodium hydroxide.

*S.R.*5. bromocresol green solution (B.D.H.).

The first four of these reagents are more or less specific for certain groups of compounds. Descriptions of the streaks obtained with the compounds under consideration are given in Table II. The phthalic esters are not affected by any of these four reagents, and their detection by this method has proved to be difficult. Of many reagents tried, bromocresol

Table II

Streak tests of the propellant ingredients

(excluding phthalic esters and mineral jelly)

Propellant ingredient	Streak reagent			
	S.R.1	S.R.2	S.R.3	S.R.4
EC	Red (*a*)	Red (*a*)	No colour	No colour
MC	Red (*a*)	Red (*a*)	"	"
AC	Blue (*a*)	Blue (*a*)	"	"
CBAN	Olive-green	Olive-green	"	"
CBZ	Green (*a*)	Green (*a*)	"	"
DPA	Blue (*a*)	Blue (*a*)	"	"
DPU	No colour	Blue, fading (*a*)	"	"
PEU	Pink, fading	Magenta, fading (*a*)	"	"
PBU	No colour	Magenta, fading	"	"
TU	Pink, fading	Magenta, fading (*a*)	"	"
NME	Purple-brown (*a*)	Purple-brown	"	"
DPN	Blue (*a*) (*b*)	Blue (*a*) (*b*)	Blue (*b*) (*c*)	"
PEN	Golden yellow (*a*) (*d*)	Golden yellow (*d*)	Faint blue (*c*)	"
DPF	Faint blue-grey (*e*)	Faint blue-grey	No colour	"
DNT	No colour	No colour	"	Pale violet, fading to pale brown (*f*)
TNT	"	"	"	Brick red (*a*)
MNN	"	"	Orange-red (*a*) (*b*)	No colour
NG	"	"	Dark blue (*a*) (*g*)	"
DEGN	"	"	Dark blue (*g*)	"

Notes—(*a*) These tests are particularly sensitive.
(*b*) Given also by concentrated sulphuric acid alone.
(*c*) The test for aromatic N-nitrosoamines can be made very sensitive by diluting 2 parts of the reagent with 3 parts of water and using while still warm. (The DPA crystallises out on cooling.) Under these conditions nitric esters give no streak.
(*d*) Colour develops after a few seconds.
(*e*) This test is rendered very sensitive by first streaking with S.R.4 and then superimposing the streak of the dichromate reagent.
(*f*) This test is rendered more sensitive by saturating the streak reagent with acetone before application.
(*g*) The test can be made more sensitive, if necessary, by first streaking with S.R.4 and then superimposing the streak of S.R.3.

green solution has proved the most satisfactory. It can be applied to a silica gel - Celite column either immediately on extrusion (as normally), or 10 to 15 minutes after extrusion, when the excess of solvent has evaporated. The test behaves differently in the two cases, and it is advantageous to perform it in both ways. In each case a sky-blue streak is produced on the column by the indicator itself. This background colour remains throughout the test, and phthalic ester zones are indicated by a fading or a change in tint. This test will detect quantities of phthalic esters down to 1 mg. on silica gel - Celite columns of 1-cm. diameter. The reagent is by no means specific, and many other compounds listed in Table I will give some change of background with this indicator. Used in parallel with other streak tests, however, it has a definite value. Details of the test as applied to phthalic esters are given in Table III.

TABLE III

STREAK TESTS OF THE PHTHALIC ESTERS

Phthalic ester	S.R.5 applied immediately on extrusion	S.R.5 applied 10 minutes after extrusion
DAP	Sky blue background fades in a few minutes, particularly at leading and trailing edges of zone, latter edge showing widest fading. Optimum time for observation is 20 minutes after application, when main portion of zone is indicated by a greenish tint bordered by completely faded edges	Sky blue background darkens and assumes a greenish tint, at first without fading. Optimum time for observation is 10 minutes after application, when edges of main greenish zone are sharply defined by completely faded areas. These edges locate the actual borders of the DAP zone more accurately than the corresponding edges produced when S.R.5 is applied immediately on extrusion
DBP	As for DAP	As for DAP
DEP	As for DAP except that greenish tint is very faded	As for DAP but somewhat less sensitive
DMP	As for DEP	As for DEP except that original darkening is negligible, and final faded zone shows no obvious greenish tint

SCHEME FOR THE CHROMATOGRAPHIC DETECTION OF INGREDIENTS IN THE ETHER EXTRACT OF A PROPELLANT

This scheme has been developed in view of the possibility of any number of the compounds under consideration being present together. The tests to be described are made on suitable portions of the ether extract of the propellant after removal of the ether *in vacuo*. Each portion is taken up in the smallest practicable volume of the appropriate developing solvent and transferred to the column for chromatography.

Four simple chromatograms suffice to reveal the presence of any of the ingredients named in Table II, except in the presence of nitric esters, when a slight modification is necessary. For the more difficult separation of the phthalic esters a three-column procedure is employed. The approximate location of the various zones is shown in Fig. 1. Although these zones may be displaced somewhat by such factors as slight variations in activity of the adsorbent, interference with the developing rate due to interaction between certain solutes when present in excess, and so on, their relative positions will remain substantially as indicated.

A. PROCEDURE FOR INGREDIENTS OTHER THAN PHTHALIC ESTERS AND MINERAL JELLY—

Column 1—for 2-naphthyl-methyl-ether, 1-nitronaphthalene, diphenylamine, carbazole and nitroglycerine.

(i) Develop with 25 ml. of a mixture of 20 per cent. of benzene in light petroleum and post-wash with 20 ml. of pure light petroleum before extruding.

(ii) A faint yellow zone just more than half-way down the column indicates the possible presence of 1-nitronaphthalene.

(iii) S.R.1 detects 2-naphthyl-methyl-ether, diphenylamine and carbazole.

(iv) S.R.3 detects nitroglycerine and confirms the presence of 1-nitronaphthalene.

Column 2—for 2 : 4-dinitrotoluene, 2 : 4 : 6 trinitrotoluene, N-nitrosodiphenylamine, N-nitroso-N-ethylaniline and diethylene glycol dinitrate.

(i) Develop with 80 ml. of a mixture of 35 per cent. of benzene in light petroleum and post-wash with 20 ml. of pure light petroleum before extruding.

(ii) S.R.3 detects diethylene glycol dinitrate (near top of column). A blue streak at the bottom of the column indicates the trailing edge of the nitroglycerine zone. (This zone is normally entirely or almost entirely washed through.)

(iii) The presence of nitroglycerine or diethylene glycol dinitrate will affect the development of the other zones on this column. 2 : 4-Dinitrotoluene, for instance, if present, may remain undetected owing to displacement and spreading of the zone by the increased development produced locally on the column by nitroglycerine. Therefore, if nitric esters have been detected (on either Column 1 or Column 2), they should be removed as described in (iv) below. In absence of nitric esters the column prepared in (i) may be used for the detection of the remaining compounds, as described in (v) and (vi) below.

(iv) Place on a new column a fresh portion of propellant extract dissolved in 1 ml. of a mixture of 10 per cent. of ethyl ether in light petroleum, containing 0·2 mg. of 2 : 4 : 6-trinitro-N-ethylaniline as an indicator. Develop with further quantities of this solvent mixture until the leading edge of the yellow zone of the indicator is within 0·5 cm. of the bottom of the column. (About 50 ml. of solvent are required.) The nitric esters remain adsorbed on the column, which should be discarded. Add 0·5 ml. of B.P. paraffin to the eluate and evaporate *in vacuo* in dim light (the nitrosoamines are somewhat photo-sensitive). Re-chromatograph as described in (i) above, and then proceed as described in (v) and (vi).

(v) Streak with S.R.4 saturated with acetone to detect the di and trinitrotoluenes.

(vi) Streak with S.R.1 to detect N-nitrosodiphenylamine and N-nitroso-N-ethylaniline. Confirmation may be obtained by streaking with the modified S.R.3 described in Note (*c*) at foot of Table II.

Column 3—for ethyl Centralite, diphenylformamide, methyl Centralite, carbanilide and Acardite.

(i) Develop with 40 ml. of a mixture of 20 per cent. ethyl ether in light petroleum; then extrude the column.

(ii) Streak with S.R.1 to detect ethyl and methyl Centralite, carbanilide and Acardite. Diphenylformamide may give a faint colour.

(iii) Streak with S.R.4 and superimpose a streak of S.R.1 to detect diphenylformamide (see Note (*e*) at foot of Table II).

Column 4—for urethanes.

(i) Develop with 40 ml. of a mixture of 0·25 per cent. of ethyl ether in benzene and post-wash with 20 ml. of light petroleum before extruding.

(ii) Streak with S.R.2 to detect the urethanes. A faint blue streak near the top of the column may appear if diphenylformamide is present, but this should not be confused with the blue streak given by N : N-diphenylurethane which would normally appear just more than half-way down the column.

(iii) Streak with S.R.1 to confirm N-phenyl-N-ethylurethane and N-(2-tolyl)-urethane.

B. PROCEDURE FOR PHTHALIC ESTERS—

(i) Dissolve a small portion of the propellant extract in 1 ml. of benzene containing 0·5 mg. of picramide and 0·2 mg. of 4-nitroaniline (as indicators), and place on the column. Develop with benzene until the yellow zone of picramide (the lower of the two indicator zones) has just been washed through the column. (About 60 ml. of benzene should be required). At this stage the yellow zone of 4-nitroaniline should be not less than 7 cm. from the bottom of the column. The percolate contains the whole of the nitric esters and any of the more weakly adsorbed ingredients, and should be discarded.

(ii) Using a fresh receiving flask, continue development without delay, this time using a mixture of 2 per cent. of ethyl ether in benzene, until the leading edge of the yellow zone of 4-nitroaniline is within 1 cm. of the bottom of the column. (About 40 ml. of this developing solvent should be required.) The column retains the diphenylformamide and more strongly adsorbed ingredients, and may be discarded.

(iii) The percolate from (ii) above contains any phthalic esters and urethanes present. Evaporate off the solvent, dissolve in 1 ml. of a mixture of 10 per cent. of ethyl ether in light petroleum containing 0·2 mg. of 2 : 4 : 6-trinitro-N-ethylaniline, and place on a fresh column. Develop with a mixture of 10 per cent. of ethyl ether in light petroleum until the leading edge of the yellow zone of the indicator is about 2 cm. from the bottom of the column. (About 50 ml. of the developing solvent should be required.) The percolate contains any diamyl

phthalate or dibutyl phthalate completely separated from all the other ingredients under consideration. This should be treated as described in (vi) below.

(iv) Extrude the column from (iii) above and streak with S.R.5 to detect diethyl phthalate and dimethyl phthalate (Column 5, Fig. 1).

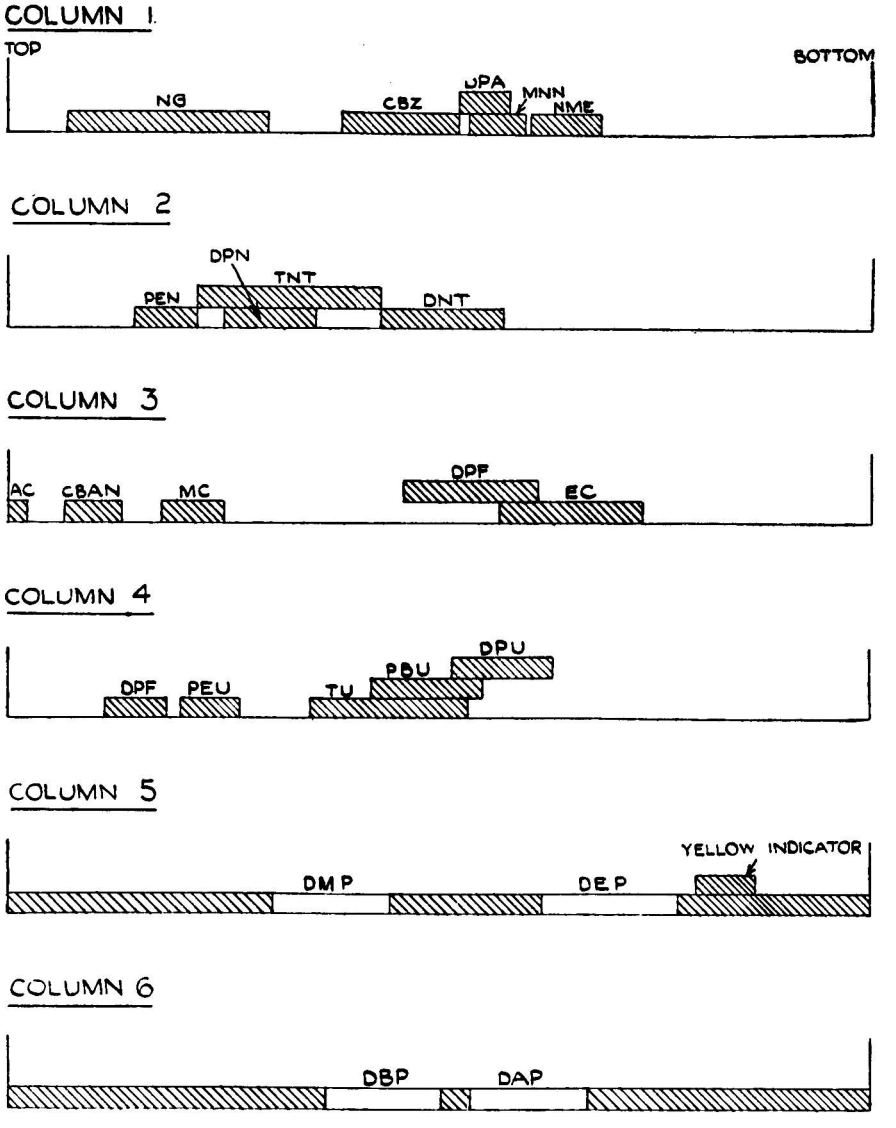

Fig. 1. Guide to the Location of Zones on the Developed Columns

(v) Streak with S.R.2 to detect any urethanes adsorbed near the bottom of the column. This will prevent confusion should a doubtful change in tint of the S.R.5 streak occur in this region, since urethanes may produce such an effect.

(vi) Evaporate the percolate from (iii) above, and take up the residue in 1 ml. of a mixture of 5 per cent. of ethyl ether in light petroleum. Place on a fresh column and develop with 35 ml. of this solvent mixture.

(vii) Extrude and streak with S.R.5 to detect diamyl phthalate and dibutyl phthalate (Column 6, Fig. 1).

C. PROCEDURE FOR MINERAL JELLY—

Mineral jelly can be detected, and at the same time estimated, by the aluminium oxide column method described in an earlier note.[4] No further description need be given here.

CONFIRMATORY TESTS

When the presence of a compound is indicated by a streak test, sufficient may be recovered by elution (after removal of the small portion penetrated by the streak reagent) to allow appropriate confirmatory tests to be made. The large number of existing colour "spot" tests are useful in this respect. When a nitric ester has been detected the refractive index will serve to confirm either nitroglycerine or diethylene glycol dinitrate, or will indicate perhaps that some other nitric ester should be suspected. In cases of doubt, a satisfactory method of checking identity is to mix the substance with a small amount of authentic material and re-chromatograph, when the development of a single zone showing no signs of separation (as indicated by the streak on the extruded column) confirms identity.

Most of the ingredients under consideration are best identified by their ultra-violet absorption spectra, which can readily be obtained on an alcoholic eluate. It is of advantage, therefore, to have available for purposes of reference a chart of absorption spectra of all likely ingredients. In applying the present scheme to the analysis of an unknown propellant the possibility of finding an ingredient not included in Table I should, of course, be borne in mind. The first indication of this may be the appearance of a positive streak at an unusual position on one of the columns. In such circumstances the plotting of an absorption spectrum may be all that is needed for final identification.

With the phthalic esters confirmatory tests are essential. However, the location on the column, coupled with the fluorescein test and measurement of refractive index, should suffice to identify the compound.

QUANTITATIVE APPLICATIONS

Having established the qualitative composition of the propellant extract, a method for the quantitative estimation of the components would normally be devised. If the ingredients are readily estimated by standard methods there seems to be no particular advantage in trying to use chromatographic methods. However, the analysis of the more complex mixtures will be greatly assisted by the use of chromatographic separations. The qualitative scheme already described and the "adsorption series" recorded in a separate paper[1] will suggest the appropriate means of chromatographic development to be employed for a given separation. The following examples will suffice to show the value of this method.

(*a*) Mixtures of nitroglycerine and diethylene glycol dinitrate can be separated quantitatively by development with a mixture of 35 per cent. of benzene in light petroleum.

(*b*) Acardite, methyl Centralite, ethyl Centralite and diphenylamine can be quantitatively resolved on a single column by development with a mixture of 10 to 20 per cent. of ethyl ether in light petroleum. The diphenylamine passes completely into the percolate leaving the other three compounds on the column in three clearly separate zones.

(*c*) N : N-Diphenylurethane is readily separated from the other urethanes considered by using 0·5 per cent. solution of ethyl ether in benzene. The separation of the remainder is difficult, but can be accomplished with the same solvent on a long column.

(*d*) Diphenylamine, 1-nitronaphthalene and naphthyl methyl ether cannot be separated satisfactorily on silica gel. However, quantitative separation of these three compounds is readily obtained (in this order—top to bottom) using a mixture of 20 per cent. of benzene in light petroleum on a column of activated aluminium oxide.

(*e*) The zones of diphenylformamide and ethyl Centralite overlap slightly when developed with ethyl ether - light petroleum mixtures. By using a 1 per cent. solution of ethyl ether in benzene, however, the relative positions of the zones are reversed, and complete separation is easily obtained.

It should be mentioned here that Schroeder[5] has recently published the results of work on the application of chromatography to the quantitative separation of some propellant ingredients. The separated compounds, after elution, were determined spectrophotometrically in alcoholic solution. Only one specific example is provided with a full experimental procedure, this being the separation and determination of diphenylamine and diethylphthalate in a powder containing also nitrocellulose and nitroglycerine, but many other interesting examples are briefly described. It is of interest that Schroeder states that

(referring to the specific example just mentioned) "usually, the results from duplicate aliquot portions of the same extract agreed to within 1 per cent. of the amount of the compound, and that the values from duplicate extracts of the same powder had a maximum spread of about 3 per cent. of the compound present," and adds later: "These results are representative of those which we obtained with quantitative chromatographic-spectrophotometric procedures for other compounds." In the present author's experience with the spectrophotometric finish to similar chromatographic separations this statement of the degree of precision obtainable is a fair one.

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A Combustion Method for the Estimation of Carbon Black in Compounded Rubber

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Introduction

Methods in general use for the estimation of carbon black in compounded rubber fall into three groups, *viz.*:—

(*a*) Those in which the rubber matrix, together with most of the compounding ingredients, are degraded or dissolved by hot nitric acid.

(*b*) Those that involve the removal of the rubber and some compounding ingredients by means of an inert solvent, usually at an elevated temperature.

(*c*) Combustion methods in which the organic ingredients of the mixing and the rubber itself are destroyed by dry distillation in an inert atmosphere.

In all three procedures the carbon black is left in association with some mineral matter, and its final assay is carried out either by collecting the black and other unattacked residue in a filter crucible and weighing before and after combustion in an oxidising atmosphere, or by combusting the carbon black to carbon dioxide which is collected and weighed in an absorption train of orthodox type.

Methods of the first group are very old-established in rubber analysis, some of the earliest work having been carried out prior to 1914 by H. Williams Jones.[1] The difficulty of retaining finely dispersed black on the filter pad has always been a troublesome feature, however, and as an improvement in this respect, Hammond[2] proposed the use of a filter pad of ignited kieselguhr supported on a layer of asbestos. This modification appears successful in a limited number of cases, but it is debatable whether the use of kieselguhr as a filtering medium provides any benefit over a properly compacted asbestos pad (*cf.* Curran[3]).

The strongly adsorptive nature of many grades of carbon black leads to retention of decomposition products of the rubber, which in turn gives rise to fictitiously high values for the final carbon black figure. The older method of dealing with this positive error was to employ a purely empirical factor of approximately 0·95, a correction which appears to be a mean value between those for the highly adsorptive channel blacks and the low volatile lamp blacks, these blacks being the only types then in common use.

Alternatively, it has been proposed to remove such adsorbed substances by washing the separated carbon black with powerful solvents such as hot acetone or chloroform.[4] The experience of this and other laboratories, however, is that the addition of such washing agents brings about immediate dispersion of the carbon black into the filtrate, necessitating troublesome refiltration with attendant inaccuracies. Even this modification, however, does not always solve the problem of adsorbed substances, for Oldham and Harrison[5] advocate the use of a correction factor of 0·95 *in addition to* washing the carbon black with acetone and chloroform.

In the present B.S.I. method,[6] interference from compounding ingredients that undergo a change in weight on heating (*e.g.*, china clay) is eliminated by oxidising the separated carbon black in a combustion tube to carbon dioxide which is collected and weighed in a soda-lime tube. Since the method estimates only "free" carbon, however, and in no way indicates the type of carbon black, the final adjustment of this figure to that for the carbon black originally present is entirely speculative.

The filtration difficulties encountered in nitric acid decomposition methods have led to proposals to use an inert solvent of high boiling-point for removal of the rubber matrix[7,8]; some improvement is gained thereby, but organic matter is almost always entrained by the insoluble residue, leading to high and variable results. A further disadvantage is that mineral ingredients of the mixing appear substantially in their original state, and their behaviour in subsequent steps of the assay is to a large extent unpredictable. Solvent methods, therefore, on the whole have not received general support.

Combustion methods, compared with the other types mentioned, have the merit of speed, and mainly for this reason have attracted the attention of analysts, particularly for control testing. Namita[9] recommends a simple procedure of heating the compounded rubber for a specified time in a Rose's crucible into which a stream of nitrogen is fed. The loss in weight after re-heating in an oxidising atmosphere, after suitable correction, is assumed to represent carbon black.

The work of Marty[10] gives the first published information on modifications to the above simple technique. He worked with a closed tube in which the sample was contained in a boat, and a current of nitrogen was passed through the tube. The weight of carbon was finally determined by the difference method.

The investigations reported here were carried out with the object of improving still further the basic process proposed by Marty, while retaining as far as possible the advantages of speed, and the use of a small sample. The increasing adoption of rubber-like synthetic materials on the one hand, and the variety of grades of carbon black now available to manufacturers on the other, have sensibly increased the task to the analyst, and have revealed some of the shortcomings of the older methods. It is hoped that this paper will provide a useful line of attack on a problem of rapidly increasing importance and complexity.

Experimental

1. Outline of method—

A stream of nitrogen from a cylinder is fed through a purifying train into the first combustion tube, where pyrolysis of the volatile organic constituents of the rubber compound takes place. The residue in the combustion boat consists substantially of carbon black and mineral matter. After weighing, the boat is transferred to the second combustion tube, where oxidation to carbon dioxide takes place. The carbon dioxide is trapped in an absorption train of orthodox type and weighed. The boat is again weighed after the oxidation stage and the carbon content obtained by difference. It will be shown later how a consideration of these two values for the "carbon" leads to (*a*) a figure for the total carbon black content of the compound, (*b*) an indication of the type of carbon black used and (*c*) in favourable instances, an estimate of the ratio of blacks in a mixture of two types.

A detailed account of the apparatus and procedure, together with a diagrammatic representation of the assembly is given on p. 359.

2. Inert atmospheres and gas purification—

The first requirement of the gas that provides the inert atmosphere in the pyrolysis train is complete freedom from oxygen, since preliminary investigation showed the extreme susceptibility of the carbon to oxidation at the temperature employed. Coal gas and hydrogen, although otherwise suitable, were not considered on account of the serious fire risk.

Carbon dioxide meets the requirements of inertness and non-inflammability, but there is no clear evidence that the reaction $CO_2 + C \rightarrow 2CO$, which takes place readily at 900° C. does not also proceed to some extent at the temperature of the initial pyrolysis (600° C.). Also there is the possibility that the carbon dioxide might combine with certain metal oxides normally used in rubber compounding and seriously affect the weighings.

Compressed nitrogen from a cylinder, after further purification was found satisfactory. Although the "pure" material was used, preliminary experiments with carbon black alone indicated considerable continuous losses due to traces of oxygen in the gas. Further purification was carried out by a two-stage process comprising (*a*) a pair of Drechsel washing bottles containing alkaline pyrogallol and (*b*) a 30-cm. column of reduced copper turnings maintained at approximately 500° C. A small wash-bottle containing concentrated sulphuric acid was interposed between the pyrogallol and the copper.

Some purification of the oxygen used in the second stage is also necessary; this was accomplished by a pair of U-tubes containing soda-asbestos and Anhydrone.

3. COMBUSTION TEMPERATURES—

For the initial dry distillation stage a temperature must be chosen that will ensure the removal of the organic constituents of the compound without bringing about their breakdown into carbon, which would, of course, produce high values for the "carbon" content of the rubber compound. Too low a temperature will entail an unduly long distillation time, and analytical errors may result from the failure to remove certain acid-forming substances such as sulphur. Nitrogen-containing compounds such as acrylo-nitrile polymers may give rise to oxides of nitrogen during the next (oxidation) stage, if not first removed by distillation; this also would lead to high results for the carbon content, unless the oxides of nitrogen are removed by a suitable reagent.

A temperature of 600° C. was found most suitable and it was concluded after investigation that a variation up to +30° was permissible. A total heating time of 10 minutes was found adequate to remove volatile substances at this temperature.

In the oxidation tube a temperature of 900° C. for 15 minutes was found suitable. Below 900° C. complete oxidation of the carbon becomes uncertain under the experimental conditions employed; higher temperatures tend to cause rapid deterioration of the furnace windings and to give rise to difficulties with fusion of the copper oxide tube filling.

The amount of inherent volatile matter of the carbon black that is evolved over the range 600° to 900° C. is used to indicate the identity of the type of carbon black used in the compound, and from this and the fixed carbon content as determined from the weight of carbon dioxide absorbed, the proportion of carbon black originally present in the compound may be calculated (see later).

4. ABSORPTION SYSTEMS—

(*a*) *Nitrogen tube*—The purpose of the absorption train at the exit here is merely to prevent the escape of unpleasant decomposition vapours from the rubber and compounding ingredients into the atmosphere of the laboratory. For this purpose Marty has suggested xylene as absorbent[10]; it was found necessary, however, with the high gas flow rates used, to draw off escaping vapours not trapped by the xylene by means of a filter pump.

(*b*) *Oxygen tube*—The main purpose of this absorption train is, of course, to absorb quantitatively the carbon dioxide formed by the combustion of the carbon black. Soda-asbestos ("Carbosorb" brand) was the absorbing agent used, contained in a stoppered U-tube. To prevent interference from oxides of nitrogen the U-tube is preceded by a small Arnold bubbler containing potassium permanganate solution and sulphuric acid, with an intermediate U-tube containing magnesium perchlorate for drying purposes. The absorption train is concluded by another Arnold bubbler containing concentrated sulphuric acid.

5. CALCULATION OF RESULTS—

(*a*) *Carbon residues from rubber and organic compounding ingredients*—For quantitative results to be obtained by the method outlined it is of obvious importance that organic constituents of the compound should leave either no residue at all apart from mineral matter after heating in nitrogen at 600° C., or should leave a constant proportion of carbonaceous residue whose influence on the final carbon figure can be calculated.

From none of the rubbers and rubber-like materials investigated were the non-mineral

constituents completely volatilised. A list of the polymers tested, and the proportion of residual carbon formed, is given in Table I.

TABLE I

Type of polymer	C, %*	
Natural rubber, plantation crepe	0·27,	0·27
Natural rubber, smoked sheet	0·24,	0·22
Butadiene - styrene copolymer (GR–S)	0·23,	0·22
*iso*Butene - isoprene copolymer (GR–I)	0·10,	0·10
Butadiene - acrylonitrile copolymer (Standard Perbunan)	1·5,	1·5
Butadiene - acrylonitrile copolymer (Perbunan 35)	1·9,	1·9
Butadiene - acrylonitrile copolymer (Hycar O.R.15)	3·6,	3·7
Thioplast (Thiokol F.A.)	2·6,	3·1
Polyvinyl acetate	2·7,	2·6
Polyvinyl chloride	5·7,	6·1
Chloro-butadiene (Neoprene)	15·4,	12·3

* This represents the proportion of residual carbon after heating the polymer in nitrogen at 600° C. for 10 minutes.

For most of the materials the amount of residual carbon produced is small and reasonably constant in amount, and for natural rubber, GR–S and GR–I, little error would be introduced by omitting this correction from the subsequent calculation.

The butadiene - acrylonitrile series show distinctly higher values and also some variation between different grades. It becomes imperative, therefore, when determining carbon black in compounds based on these materials to make a correction for the amount and type of rubber present. Experience with these compounds has shown that for this reason it is unwise to ascribe a higher degree of accuracy than ±2 per cent. of the carbon black content; with low loadings, for example about 5 per cent. of black, the experimental error may be even higher.

As would be expected, the chlorine-containing polymers offer considerable resistance to dry distillation, and with them the residual carbon is undesirably high and inconsistent in amount. It would seem, therefore, that the method is inapplicable to these polymers and further investigations with them were not pursued.

Most compounding ingredients of an organic nature are extractable with acetone or chloroform, and hence by determining the carbon black content on the extracted material, interference from these sources may be avoided. Factice (sulphur-vulcanised oil), however, will resist extraction with these solvents, but dry distillation of this material has shown it to be almost completely volatile, the residual carbon after combustion in nitrogen being constant at about 0·5 per cent. As factice is used in rubber compounding to only a minor extent, interference from this source will usually be negligible.

(*b*) *Influence of inorganic fillers*—The majority of inorganic compounding ingredients likely to be encountered in rubber compounded with carbon black will not change in weight between 600° and 900° C. Thus magnesium carbonate readily loses carbon dioxide below 600° C. and its presence therefore will not influence subsequent weighings.

China clay loses 10 per cent. of its weight at 600° C. and a further 2 per cent. at 900° C. In the method described, the loss in weight on heating in oxygen, therefore, will be increased by an amount corresponding to 2 per cent. of the clay present, and in making the final computation of the original carbon black content a correction must be applied based on the china clay content of the compound.

Calcium carbonate is not appreciably decomposed below 600° C., but loses all its combined carbon dioxide between 600° and 900° C. The carbon dioxide thus liberated will be absorbed in the soda-asbestos tube and must be corrected for in the final result by computing its value from the amount of calcium carbonate present.

(*c*) *Volatile matter in carbon black*—After making due allowance for the foregoing considerations it might be expected that the weight of carbon dioxide absorbed by the soda-asbestos would bear a strict stoichiometric relationship to the weight of carbon black after the initial heating in nitrogen, and be proportional to the loss in weight during the oxidation stage. It might therefore appear unnecessary to utilise both the loss of weight in the oxidation stage and the weight of carbon dioxide absorbed, in order to arrive at a figure for the carbon black content. The influence of the volatile matter in the carbon black on the relationship between these values is, however, a significant one, and must be considered in some detail.

Volatile matter, in this connection, is the term given to the gases adsorbed at the surface of the carbon particles, which can be successively removed at rising temperature levels. These gases range from oxides of carbon that begin to come off at about 500° C. to the hydrocarbons and hydrogen, for the elimination of which temperatures up to 1400° C. are considered necessary by some workers. With channel blacks the proportion of such adsorbed gases may be considerable, frequently exceeding 6 per cent. of the total weight, but lamp blacks, acetylene black and most blacks produced by the furnace process have a low volatile content. The amount of volatile matter attached to any particular type of black is one of its most characteristic features and provides a ready means of differentiation between (for example) channel blacks and furnace blacks.

The evolution of volatile matter for the purpose of the present investigation has been studied at 600° and 900° C. Volatile matter evolved below 600° C. is lost to the atmosphere (together with decomposition products of the organic materials in the rubber compound), but must be allowed for when computing the weight of carbon black originally present. Since, however, the extent of this volatile fraction is not actually determined, it plays no direct part in identifying the black used. Of the volatile matter evolved over the temperature range 600° to 900° C., which forms the larger proportion of the total volatile matter, some will be oxidised to carbon dioxide and will be weighed in the soda-asbestos tube, whilst the remainder is non-oxidisable, and is represented by the discrepancy between the carbon as determined by the difference in weight before and after oxidation, and the carbon as determined from the carbon dioxide formed. This non-oxidisable fraction, although only a part of the total volatile matter, is accurately determinable, is characteristic of the type of black, and forms the basis used in this investigation for the differentiation between types. Its amount is calculated as follows.

If the total weight of dry carbon black originally present in a rubber compound is W, and a, b, c, d and e have the following meanings:

a = weight of volatile matter evolved below 600° C.,

b = weight of volatile matter evolved over the temperature interval 600° to 900° C., and not oxidisable to carbon dioxide,

c = weight of volatile matter evolved over the temperature interval 600° to 900° C., and oxidisable to carbon dioxide,

d = weight of non-volatile carbon,

e = weight of carbon dioxide produced,

then $$W = a + b + c + d$$

and $$0{\cdot}2729 \times e = c + d;$$

hence $$W - a = b + 0{\cdot}2729\, e$$

or $$b = (W - a) - 0{\cdot}2729\, e$$

Since the term $(W - a)$ represents the difference in weight before and after oxidation, the value of b can be readily calculated.

Many of the commoner carbon blacks used in the industry have been examined in this way and the data are presented in Table II.

It will be seen that the values of $100\, b/(W - a)$ in column (1) fall into two clearly defined groups, the high-volatile channel blacks, ranging from about 5 to 8 per cent., and the low-volatile furnace and lamp blacks with figures usually below 3 per cent.

When the type of carbon black used has been determined by reference to Table II, it is a simple matter to make a correction for the total volatile matter. Column (2) of Table II shows the ratio of oxidisable carbon (*i.e.*, that which is weighed as carbon dioxide) to original carbon black for the same series of standard grades, and allowance has been made for the volatile matter lost below 600° C. Again, it will be seen that a greater correction is necessary with channel blacks than with other types, although the values do not follow a strict relationship with the figures in column (1).

With binary mixtures of carbon blacks a useful estimate of the ratio of the two components can be obtained. It is, of course, necessary with mixtures to know the types of black present, and the confidence to be placed in the final result is dependent on the difference in the value of b for the two components. The value of b for the mixture determined experimentally will lie between the values for the two blacks considered separately according to

the relative proportions of the blacks present. This ratio may be found from the following expression,

$$A = \frac{V - V_B}{V_A - V_B} \cdot 100,$$

in which

A = proportion of carbon black A calculated as a percentage of the total black present,
V = value of b for the mixture of blacks in the rubber as determined experimentally,
V_B = value of b for carbon black B
V_A = value of b for carbon black A
} as given in Table II, column (1).

TABLE II

Column (1) shows values for non-oxidisable volatile matter evolved over the temperature range 600° to 900° C., calculated as a percentage of the carbon black after heating in nitrogen at 600° C.

Column (2) shows the proportion (per cent.) of true carbon in the original black calculated from the yield of carbon dioxide.

Sample	Type	(1) $\frac{b}{W - a}$ 100	(2) $\frac{0{\cdot}2729\, e}{W}$ 100
Micronex	Channel	6·0	91·6
Kosmobile H.M.	"	5·6	91·3
Spheron 4	"	5·3	93·8
Spheron 6	"	7·9	90·7
Spheron 9	"	5·7	91·7
Dixiedensed H.M.	"	6·7	90·8
Dixie R.I	"	5·2	91·5
Spheron C	"	4·5	93·6
Magecol	Lamp	0·2	97·4
Champion	"	0·8	98·1
Statex 93	Furnace	0·0	99·3
Statex K	"	3·4	96·0
Furnex	"	2·3	93·4
P.33	Thermal	0·7	96·5
Seval	"	2·3	97·1
Shawinigan	Acetylene	0·0	

RESULTS

In the tables that follow, results are given for a series of unvulcanised and vulcanised rubber compounds. The behaviour of the main types of carbon black in association with the more commonly used polymers has been studied, and mixings have been chosen to illustrate the influence of whiting and china clay. All samples of carbon black were dried at 120° C. for 4 hours before mixing into rubber.

The results were calculated in the manner already outlined, but for the sake of further clarity intermediate stages in the calculation have been included in columnar form.

Although the black loadings are nominal, care was taken to reduce as far as possible the errors often associated with small-scale compounding.

With the unvulcanised compounds, somewhat greater variation than seemed desirable occurred in the pale crepe series. The carbon black content in these abnormal cases was checked by the nitric acid decomposition method and the results confirmed this variation, indicating that lack of uniformity in the compound was responsible. Thereafter, a stricter mixing technique was followed, and subsequent results showed a higher degree of precision.

The black loadings in the vulcanised compounds, nominally 15 per cent., were actually obtained by check-weighing, and the experimental results show a degree of accuracy, even in presence of china clay and calcium carbonate, that compares favourably with that given by the nitric acid decomposition method. Unfortunately the assessment of accuracy by direct comparison with laboratory mixed compounds is not valid beyond a certain point, owing to the difficulty of preparing compounded rubber whose actual composition is itself beyond all doubt.

TABLE III

UNVULCANISED CHANNEL BLACK COMPOUNDS

(Micronex; $\frac{0{\cdot}2729\,e}{W}100 = 91{\cdot}6$)

Polymer	(a)	(b)	(c)	(d)	(e)
Pale crepe	30	0·1026	0·1050	0·0002	30·2
	30	0·1014	0·1035	0·0002	30·2
	15	0·1186	0·0607	0·0002	15·0
	15	0·1048	0·0532	0·0002	14·9
	7·5	0·1134	0·0278	0·0003	7·0
	7·5	0·1040	0·0254	0·0003	7·0
	3·75	0·1168	0·0148	0·0003	3·5
GR–S	15	0·1250	0·0640	0·0003	15·0
	15	0·1774	0·0907	0·0004	15·0
	7·5	0·1263	0·0326	0·0003	7·4
	7·5	0·1190	0·0309	0·0003	7·5
GR–I	15	0·1212	0·0615	0·0001	15·0
	15	0·1447	0·0734	0·0001	15·0
	7·5	0·1094	0·0279	0·0001	7·5
	7·5	0·1170	0·0297	0·0001	7·5
Perbunan	15	0·1121	0·0616	0·0014	15·0
	15	0·1008	0·0555	0·0013	15·0
	7·5	0·1303	0·0394	0·0018	7·5

TABLE IV

UNVULCANISED LAMP BLACK COMPOUNDS

(Magecol; $\frac{0{\cdot}2729\,e}{W}100 = 97{\cdot}4$)

Polymer	(a)	(b)	(c)	(d)	(e)
Pale crepe	30	0·1071	0·1140	0·0002	29·6
	30	0·1018	0·1086	0·0002	29·7
	15	0·0777	0·0417	0·0002	14·8
	15	0·0893	0·0478	0·0002	14·8
	7·5	0·0840	0·0231	0·0002	7·4
	7·5	0·0920	0·0249	0·0002	7·3
	3·75	0·0744	0·0107	0·0002	3·83
	3·75	0·0592	0·0085	0·0002	3·75
Smoked sheet ..	15	0·1258	0·0676	0·0002	14·9
	15	0·0954	0·0514	0·0002	15·0
	7·5	0·1260	0·0341	0·0003	7·4
	7·5	0·1321	0·0359	0·0003	7·4
GR–S	15	0·1136	0·0609	0·0002	14·8
	15	0·2038	0·1100	0·0004	14·9
	7·5	0·1042	0·0284	0·0002	7·4
	7·5	0·1026	0·0279	0·0002	7·4
GR–I	15	0·1326	0·0709	0·0001	14·9
	15	0·1269	0·0680	0·0001	14·9
	7·5	0·1130	0·0303	0·0001	7·4
	7·5	0·1200	0·0321	0·0001	7·4
Perbunan	15	0·1040	0·0603	0·0014	14·9
	15	0·1108	0·0643	0·0014	14·9
	7·5	0·1115	0·0354	0·0016	7·5
	7·5	0·1200	0·0381	0·0017	7·5

KEY TO TABLES III AND IV—

Column (*a*) Nominal carbon black loading, per cent.
" (*b*) Weight in grams of the sample taken for test.
" (*c*) Weight of CO_2 obtained.
" (*d*) Correction for carbonaceous residue from the polymer as determined by consulting Table I.
" (*e*) Carbon black found, per cent.

With binary mixtures of carbon black, substitution of the appropriate values for V_A and V_B in the formula $A = 100\,(V - V_B)/(V_A - V_B)$ requires a knowledge of the type and brand of the two separate constituents, and since such data are unlikely to be available for compounded rubbers of unknown origin, the method of evaluating mixtures can usually be applied with confidence only to routine control analysis where the qualitative nature of the carbon black is not in doubt. The calculated values of A are very sensitive to the substituted values of V_A and B_B, and experience has shown that it is unwise to substitute even the mean values for each class of black, as values of A obtained in this way may vary up to about 20 per cent.

TABLE V

VULCANISED COMPOUNDS

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Smoked sheet	67·5	77·5	77·5	67·5	—	—	—
GR-S	—	—	—	—	77·5	—	—
Perbunan	—	—	—	—	—	74·0	74·0
Sulphur	2·5	2·5	2·5	2·5	2·5	2·5	2·5
Stearic acid	1·0	1·0	1·0	1·0	1·0	2·0	2·0
Mercapto-benzthiazole	1·0	1·0	1·0	1·0	1·0	1·5	1·5
Zinc oxide	3·0	3·0	3·0	3·0	3·0	5·0	5·0
China clay	10·0	—	—	—	—	—	—
Calcium carbonate	—	—	—	10·0	—	—	—
Furnace black (Statex 93)	14·5	—	—	—	—	—	—
Lamp black (Champion)	—	—	15·2	—	—	—	—
Acetylene black	—	14·5	—	—	—	—	—
Channel black (Spheron 9)	—	—	—	15·8	—	—	—
Channel black (Kosmos H.M.)	—	—	—	—	15·8	—	—
Furnace black (Statex K)	—	—	—	—	—	16·2	—
Channel black (Spheron C)	—	—	—	—	—	—	16·5
Carbon black by experiment	14·3	14·0	15·3	15·5	16·0	16·6	16·9
Value of $\frac{b}{W-a} \cdot 100$	0	0	0·23	5·8	5·6	3·2	4·4

When the precise grades of carbon black are known, however, and the appropriate values of V_A and V_B can be substituted, a useful estimate of the ratio can be obtained. Thus, for a mixing containing Micronex ($V = 6{\cdot}0$) and Magecol lamp black ($V = 0{\cdot}2$), the value of V determined experimentally for the mixture was 3·7. Substituting these values in the formula, the value of A becomes 40 per cent., indicating a 40 : 60 lamp black - channel black mixture. Such good agreement, however, is regarded as fortuitous and a degree of accuracy within ± 5 per cent. even in favourable circumstances is not claimed.

In a second example it was known that a mixture of Micronex and Statex K furnace black had been incorporated in a rubber mixing; it was required to find the ratio of these constituents. The following values were substituted in the formula:

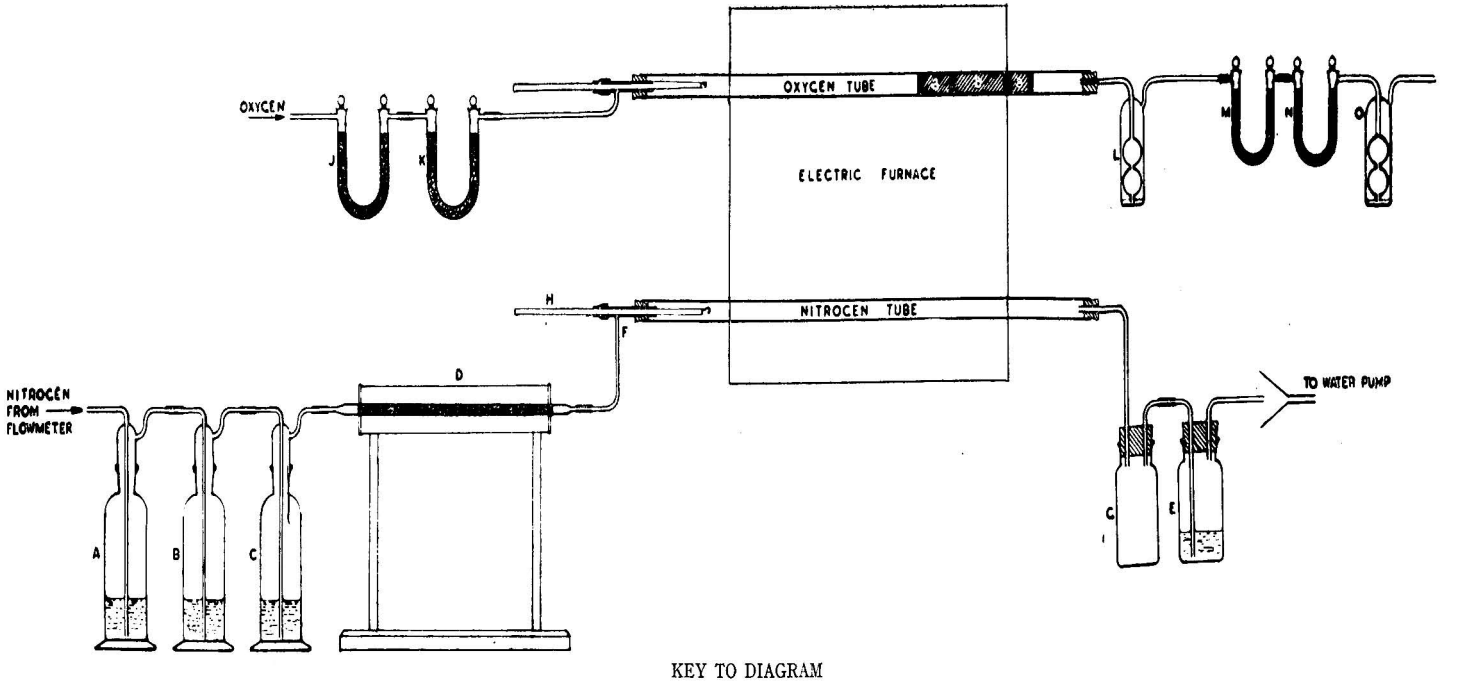
V_A	3·4
V_B	6·0
V	4·6 (determined experimentally)

The value of A is calculated as 46 per cent., which suggests a mixture of approximately equal parts of Micronex and Statex K. For reasons stated previously, however, the limits of variability of the ratio might reasonably be expected to range from 40 to 50 per cent. of Statex K.

DESCRIPTION OF METHOD

REAGENTS REQUIRED—

(1) "Oxygen-free" or "pure" nitrogen.
(2) Oxygen.
(3) Alkaline pyrogallol solution—Dissolve 50 g. of pelletised caustic potash in 100 ml. of water and dissolve 30 g. of pyrogallol in the solution.
(4) Xylene.
(5) Concentrated sulphuric acid.
(6) Magnesium perchlorate (Anhydrone).
(7) Soda-asbestos, Carbosorb, 14 to 20 mesh.



KEY TO DIAGRAM

A, B	Alkaline pyrogallol wash-bottles	J	U-tube containing anhydrone
C	Concentrated sulphuric acid wash-bottle	K	U-tube containing soda asbestos
D	Heater for reduced copper column	L	Acidified permanganate wash-bottles
E	Absorption bottle containing xylene	M	U-tube containing anhydrone
F	T-piece entry tube	N	U-tube containing soda-asbestos
G	Bottle for trapping effluent gases	O	Sulphuric acid bubbler
H	Silica rod for manipulating boat		

(8) Acidified potassium permanganate solution—Dissolve potassium permanganate crystals in the minimum volume of water and dilute with concentrated sulphuric acid to give a 0·02 *N* solution.

(9) Cupric oxide
(10) Platinised asbestos } for combustion tube packing.
(11) Ignited Gooch asbestos
(12) Copper turnings reduced *in situ* with hydrogen.

APPARATUS—

Nitrogen supply—"Oxygen-free" nitrogen is fed from a cylinder, through a flowmeter (not shown on diagram) at the rate of 500 ml. per minute. It is passed then through two Drechsel gas-washing bottles, A and B, containing alkaline pyrogallol solution, a third Drechsel bottle, C, containing concentrated sulphuric acid and a 30-cm. column of reduced copper turnings heated by a small furnace, D. The purified gas is admitted to the combustion tube via a T-piece, F, through which passes a silica rod, H, with a hooked end of nickel-chromium wire for manipulating the combustion boat. G is a trap for collecting condensible vapours formed during the pyrolysis and E is the final absorption vessel containing xylene. Residual gases are drawn away through a small funnel attached to a filter pump.

For the oxidation train oxygen is taken from a cylinder and purified by passing through two U-tubes, J and K, containing Anhydrone and soda-asbestos respectively. As in the first tube, the boat is manipulated by means of a silica rod sliding in a glass T-piece. The combustion tube is packed in the positions shown on the diagram with (*a*) platinised asbestos and (*b*) cupric oxide, with intermediate and supporting packings of Gooch asbestos.

L is an Arnold bubbler containing acidified potassium permanganate, and after passing through this and a U-tube, M, containing Anhydrone, the effluent gases are passed through a U-tube, N, containing soda-asbestos, in which the carbon dioxide is absorbed. The train is terminated by an Arnold bubbler, O, containing concentrated sulphuric acid which serves to indicate the rate of oxygen flow.

PROCEDURE—

Samples are given a prior extraction with acetone and, if mineral rubber is thought to be present, chloroform extraction is necessary also, these operations being carried out by standard methods.[11]

Weigh accurately an amount of sample equivalent to approximately 0·1 g. of the original sample into a silica combustion boat (5 cm. by 1 cm.) and place just inside the cool end of the nitrogen combustion tube which is then closed with the entry fitting. Pass nitrogen into the train at the rate of 500 ml. per minute, switch on the preheater furnace, D, and the main electric furnace and allow them to reach temperatures of 500° and 600° C. respectively. When these temperatures have been attained push the combustion boat slowly forward by means of the silica rod, until in 5 minutes it reaches the hot zone of the furnace, where it is heated for a further 5 min. Then return the boat to its original position at the end of the tube, cool for 10 minutes with nitrogen still passing, and weigh if it is required to determine the type of black or the proportions of black present in a binary mixture.

Now transfer the boat and contents to the oxygen tube, to which oxygen is admitted at the rate of 25 ml. per minute, and when the temperature of the furnace has reached 900° C., push the boat into the hot zone for 15 minutes. Disconnect from the train the soda-asbestos absorption tube, which has previously been weighed, and re-weigh, and calculate the corrected figure for carbon dioxide as already described under "Calculation of Results."

After the absorption tube has been disconnected remove the entry fitting, take out the boat, cool in a desiccator and weigh. The loss of weight of the boat and its contents on oxidation is equal to $(W - a)$ in the expression $0{\cdot}2729\, x/(W - a)$.

SUMMARY

The method described in this paper is based on the removal of volatile organic ingredients of the compound by heating in a current of purified nitrogen and, after reweighing if desired, subsequent combustion of the carbon black to carbon dioxide, which is collected and weighed. The effect of the adsorptive nature of the carbon black has been studied, and the volatile constituents of many of the standard grades of carbon black used in the industry have been determined under the experimental conditions used.

The replacement of the arbitrary factor used in earlier methods by the determination of the volatile matter in the carbon black not only allows a more accurate correction to be applied to the quantitative determination of carbon, but also provides an indication of the type of carbon black used in a rubber compound. Where a mixture of high- and low-volatile carbon blacks has been used the method gives a useful estimate of the ratio of the two types.

The authors wish to express thanks to Dr. Davey for helpful suggestions in connection with the work, and to the Technical Director of the Dunlop Rubber Company Limited for permission to publish this paper.

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THE DUNLOP RUBBER COMPANY
ERDINGTON, BIRMINGHAM

August, 1948

Rapid Determination of Carbon Dioxide in Absorbent Liquors or other Carbonate Solutions

BY B. E. DIXON AND R. A. WILLIAMS

IN the course of experimental work on alkaline absorbent liquors a requirement arose for a robust, self-contained and simple apparatus for use in the rapid and accurate determination of carbon dioxide. Several methods for the estimation of carbon dioxide have been described that depend on the measurement of the gas pressure developed on treatment of carbonate by acid,[1,2,3] etc. Most of these methods, however, involve the use of one or more instruments or laboratory services such as thermostats, balances, barometers, vacuum pumps, a supply of carbon dioxide or a source of heat. The ease of operation of the method in its simplest form is so attractive that an attempt was made to improve the accuracy by a study of the inherent sources of error. The apparatus now described can be used for the estimation of carbon dioxide in absence of the usual laboratory facilities.

Apparatus

The reaction vessel (Fig. 1) was constructed in stainless steel, which permitted the rapid attainment of a constant temperature in the gaseous phase by quick dissipation of the heat of reaction. The alloy used, "Staybrite FDP," is completely inert towards both phosphoric acid (20 per cent. v/v) and the bases used, under the conditions of operation. The vessel is a squat cylinder having a flat lid fitted with an exit tube and a thermometer. The lid is secured to the vessel by means of six swing bolts and lugs, and the apparatus made gas-tight by means of a smooth rubber washer between lid and cylinder. The base of the vessel is divided into two compartments by two planes inclined at a gentle slope. The exit tube is connected to a Y-piece, one end of which is attached to a tap, the other end leading to a manometer. The cubic content of the reaction vessel and of the connection as far as the level of mercury in the manometer is measured.

Procedure

The reaction vessel may be used (A) with an open manometer or (B), if a vacuum pump is available, with a closed manometer. An accurate mechanical pressure gauge can replace the manometer in certain circumstances (see below).

(A) WITH AN OPEN MANOMETER—

Five ml. of phosphoric acid (20 per cent. v/v) are measured into one compartment and 5 ml. of the sample liquor (containing not more than 1 g. of base or 0·4 g. of combined CO_2) into the other compartment. The lid is carefully secured by means of the bolts and the tap on the Y-piece closed. The initial temperature is taken. The vessel is now tilted gradually

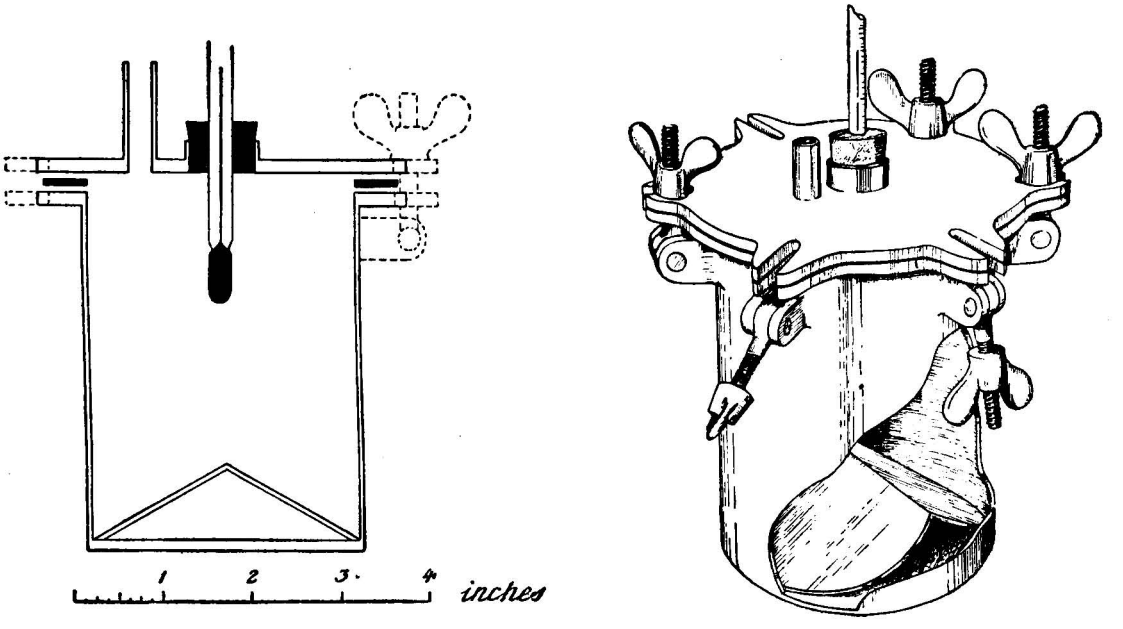

Fig. 1

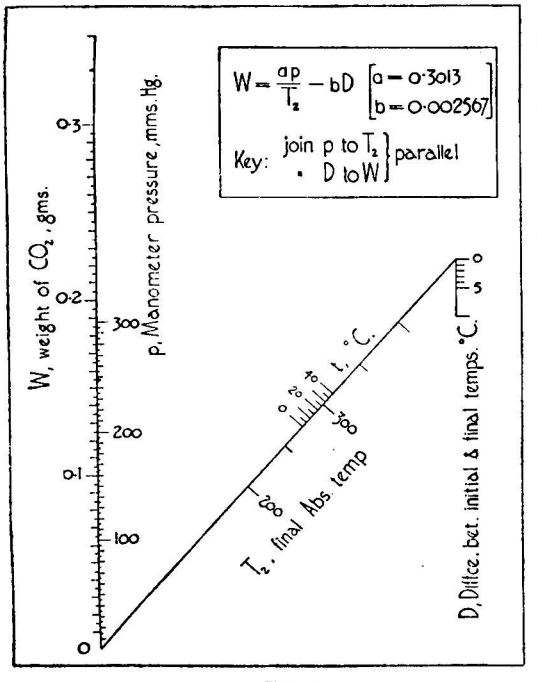

Fig. 2

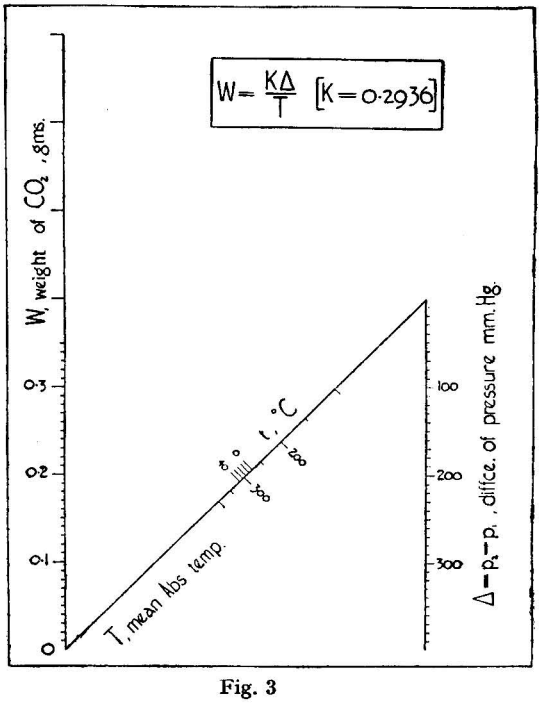

Fig. 3

to mix the acid and base and the mixture finally thoroughly roused by vigorous shaking. After about 1 minute the column of mercury becomes steady and the manometer pressure and final temperature are taken.

(B) WITH A CLOSED MANOMETER—

The open manometer in (A) is replaced by a closed manometer and a vacuum pump is attached to the tap on the Y-piece. The acid and base are added, the lid secured and the initial temperature taken as in (A). The vessel is then evacuated to a pressure of about 10 mm. of mercury as shown by the difference in level of the manometer columns. The pump is then cut out of circuit by means of the tap and the liquids mixed as before. The difference in manometer levels and the final temperature are noted.

The weight of carbon dioxide is then read directly from the graphs (Fig. 2 for Procedure (A), or Fig. 3 for Procedure (B)).

EQUATIONS AND GRAPHS

(A) WITH AN OPEN MANOMETER—

The weight in grams of carbon dioxide obtained in Procedure (A) on the assumption that no change occurs in the barometric pressure or in the volume of the gases is given by the expression:

$$W = \left(\frac{V \times 0{\cdot}001965 \times 273}{760}\right) \times \left(\frac{P + p}{T_2} - \frac{P}{T_1}\right) \quad .. \quad .. \quad (i)$$

which can be expressed in the form

$$W = a_1\left(\frac{p}{T_2} - \frac{PD}{T_1T_2}\right) \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (ii)$$

Equation (ii) can with no appreciable loss of accuracy be simplified to

$$W = \frac{a_1p}{T_2} - bD \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (iii)$$

where a_1 and b are constants.

By substituting in equations (i) and (iii) imaginary values for pressures and temperatures within the working range, it was found that the deviation never exceeded a few tenths of a milligram. For practical purposes, therefore, equation (iii) can be substituted for equation (i).

There are two small sources of error for which correction must be made in the above equations, *viz.* (*a*) the amount of carbon dioxide dissolved in the mixed liquids, and (*b*) the volume of gas displacing the mercury in the manometer at the end of the reaction. Both these quantities are functions of p, and hence proper correction factors may be incorporated in the constant a_1 in equation (iii) to give the working equation,

$$W = \quad - bD \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (iv)$$

the nomograph corresponding to (iv) is shown in Fig. 2.

(B) WITH A CLOSED MANOMETER—

In this case the weight of carbon dioxide is given by

$$W = \left(\frac{V \times 0{\cdot}001965 \times 273}{760}\right) \times \left(\frac{p_2}{T_2} - \frac{p_1}{T_1}\right) \quad .. \quad .. \quad .. \quad (v)$$

which can be simplified to

$$W = \frac{K_1\Delta}{T} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (vi)$$

Here again, for values of T, T_1, p_1, etc. within the working range, equations (v) and (vi) give practically identical values for W.

The slight changes in temperature and volume of the gas during the reaction are insignificant as sources of error in Procedure (B), but a correction must be made as in Procedure (A) to offset the solubility of carbon dioxide in the mixed liquids.

The nomograph corresponding to the working equation (vii) is shown in Fig. 3.

$$W = \frac{K\Delta}{T} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (vii)$$

If a mechanical pressure gauge is used in place of a manometer in Procedure (B), the method is unchanged and the results can be read off on Fig. 3. In Procedure (A), however, the manometer cannot be replaced by a pressure gauge if the gauge is of such a type that the gases enclosed in the instrument change appreciably in volume.

Symbols

W = Weight of carbon dioxide in g.
V = Volume of gases (ml.) in reaction vessel and connection.
T_1 = Temperature (abs.) of gases before reaction.
T_2 = Temperature (abs.) of gases after reaction.
D = $T_2 - T_1$.
p = Manometric pressure (mm. of mercury).
P = Barometric pressure (mm.), which in practice is unchanged during the determination.
$a_1 = \dfrac{V \times 0{\cdot}001965 \times 273}{760}$
b = $760 \times a_1/(293)^2$. The second term in equation (ii) is relatively small and P can be equated to 760 and T_1T_2 to $(293)^2$, the average temperature of operation being taken as 20° C.
a = $F_1F_2a_1$, where F_1 is the volume correction factor and F_2 the solubility correction factor. F_1 is calculated from the dimensions of the manometer tube, which cannot conveniently be much less than 5 mm. in diameter. The solubility of carbon dioxide in the mixed liquids (*viz.*, 5 per cent. phosphoric acid partially neutralised with up to 3 per cent. of base) was found to be approximately 0·01 g. CO_2 per 10 ml. at 760 mm. partial pressure of CO_2.
p_1 = Manometric pressure (mm.) before reaction.
p_2 = Manometric pressure (mm.) after reaction.
Δ = $p_2 - p_1$.
T = Mean temperature (abs.) of gases during reaction.
K_1 = a_1.
K = F_2a_1.
a, b and K are apparatus constants.

Results

Results obtained with this apparatus, using Procedures (A) and (B), are shown in the table.

Absorbent solution, 5 ml.	CO_2 theoretical, mg.	CO_2 determined by other methods mg.	CO_2 found, mg. Procedure (A)	CO_2 found, mg. Procedure (B)
Sodium carbonate	320·5	318·5 (1)	318	
	194		195	
	172·5			172·5
	124			125·5
	86		84	85
	43		41	43
Monoethanolamine		122 (1)		122·5
		112 (1)	112·5	
Triethanolamine		71 (1)	71	71
		35·5 (1)	34	36
Ammonium carbonate		197·5 (2)	200	198
Ethylenediamine		66·5 (1)	66	69

(1) By addition of barium chloride followed by separation of the precipitated barium carbonate, which is dissolved in acid and titrated back with alkali.
(2) By Schrötter apparatus.

The method has been described for use with liquid samples. Solid water-soluble or acid-soluble carbonates can also be determined provided that water is added to give a total bulk of 5 ml. in the compartment containing the base.

Summary

A simple and robust apparatus of stainless steel is described for the rapid determination of carbon dioxide in liquid absorbents or other carbonate solutions. The carbon dioxide is

generated by the action of cold dilute acid and from the pressure and temperatures recorded the weight of gas is read off directly from a graph. By the use of simplified expressions for the weight of carbon dioxide embodying corrections for significant sources of error it has been possible to retain a simple technique and apparatus design without sacrifice of accuracy.

We wish to thank the Government Chemist for permission to publish this work.

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December, 1948

Notes

THE COLORIMETIRIC DETERMINATION OF SMALL QUANTITIES OF ALUMINIUM

A RECENT abstract[1] describes a method by Mervel for the extraction of the aluminium 8-hydroxyquinoline complex with benzene and implies that iron and certain other interfering elements may be removed by precipitation with sodium hydroxide.

In these laboratories we have observed that the removal of iron by precipitation is invariably incomplete, even in the absence of tungsten. Gentry and Sherrington,[2] in their very comprehensive treatment of the subject, do not recommend precipitation for the separation of iron and their method, which employs conversion of iron to ferrocyanide, successfully eliminates interference. They also suggest[2] that mercury cathode electrolysis may be used for the removal of many of the heavy metals before determination of aluminium. However, we have observed that if this latter technique is adopted sufficient iron still remains in solution after electrolysis to interfere with the aluminium determination, and this iron must be removed by a preliminary extraction at pH 2·0 to 2·5 before the pH is raised to 5·0 for extraction of the aluminium complex.

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July, 1948

THE SEPARATION OF BERYLLIUM, ALUMINIUM, URANIUM AND VANADIUM BY CUPFERRON IN SYSTEMATIC QUALITATIVE ANALYSIS

IN a modified scheme of analysis, based on that of Noyes and Bray,[1] embracing the commoner of the "rarer' elements, it is necessary to separate in Group III the ions Fe^{***}, Cr^{***}, Al^{***}, WO_4'', VO_4''', PO_4''', Ti^{****} Zr^{****}, Ga^{***}, Mn^{**}, Zn^{**}, Co^{**}, Ni^{**}, UO_2^{**}, Be^{**}, Ce^{***} and Th^{****}. Following the usual scheme, this group is subjected to a basic acetate separation followed by treatment with sodium peroxide, yielding a filtrate containing CrO_4'', AlO_2', WO_4'', PO_4''', VO_4''', $HZnO_2'$, BeO_2'' and peruranate, *e.g.*, UO_8''''. This filtrate is treated under pressure with sodium bicarbonate, leaving a residue that may contain beryllium, aluminium, uranium, vanadium and zinc. The zinc is removed by an ammonia precipitation.

The separation of the remaining elements is tedious and involves evaporation to basic acetates, chloroform extraction and the use of several reagents. It was thought that the process could be simplified by the use of cupferron.

The following scheme was developed for use on the semi-micro scale.

The residue from removal of zinc will contain $Al(OH)_3$,$Be(OH)_2$, $(UO_2)_3(VO_4)_2$,$AlVO_4$ and uranium occluded by the alumina.

Residue: Dissolve in 0·5 ml. of conc. HCl, add 2·5 ml. of water, excess of 6% aqueous cupferron and macerated filter paper, shake well, centrifuge, wash with 10% HCl containing a few drops of cupferron solution

Residue: V and Fe. Dark red ppt. shows V. Evaporate to dryness with conc. HNO_3 to destroy organic matter. Dissolve in 10% H_2SO_4, add 1% $KMnO_4$ solution until faint pink, then 2 drops of H_2O_2. Red colour confirms **V**	*Filtrate*: Add 0·5 g. of sodium acetate, boil, centrifuge and wash		
	Residue: **Al.** Destroy organic matter with HNO_3; confirm Al by alizarin or morin test	*Filtrate*: Destroy organic matter with HNO_3, take up in few drops of dil. HCl, add slight excess of $(NH_4)_2CO_3$ solution, boil and centrifuge	
		Residue: **Be.** Confirm by quinalizarin	*Filtrate*: Add H_2O_2—orange-yellow colour; or acidify and add $K_4Fe(CN)_6$ solution—dark brown ppt. shows **U**

This process has been tested on the macro, semi-micro and micro scales by proportional alteration of quantities and found satisfactory in all combinations of ions.

The degree of separation has been tested by classical methods and by microchemical tests and would appear to be quantitative within the limits of these tests. Each precipitate, after washing, appears to be free from contamination.

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A. B. CRAWFORD
September, 1948

THE PREPARATION OF N-PHENYLANTHRANILIC ACID INDICATOR SOLUTIONS

THE generally recommended method of preparing N-phenylanthranilic acid indicator solutions with sodium carbonate[1] is somewhat tedious. Furthermore, the solution quickly darkens in colour, and the sensitivity decreases slightly on keeping. More objectionable is the rapid growth of a fungoid organism, possibly owing to the relationship of the indicator molecule to the well known plant growth factors. The metabolic products of this organism do not seem to affect the properties of the solution, but may contribute in some way to decreasing sensitivity. In six instances examined, the organism was *Aspergillus niger*, probably air-borne, or carried in the distilled water.

It was thought that it would be simpler and more convenient to prepare the solution with the theoretical amount of sodium hydroxide, and that mould growth might be inhibited by a suitable preservative.

Of the solutions studied, the following were worthy of note.

(*a*) 0·005 *M* Indicator in 0·0125 *M* sodium carbonate[1]; 0·535 g. of the indicator was boiled with 30 ml. of water containing 0·50 g. of sodium carbonate and diluted to 500 ml. The substance dissolves with difficulty.

(*b*) For this solution, 0·25 g. of the indicator was warmed with 12·0 ml. of 0·1 *N* sodium hydroxide and diluted to 250 ml. (0·1 per cent. solution). The substance dissolves readily.

(*c*) As in (*b*) but with addition of 0·10 g. of thymol to the sodium hydroxide solution.

(*d*) As in (*b*) but with 1 ml. of chloroform added.

These solutions have been in use for 18 months, stored out of contact with direct sunlight, but intermittently exposed during use. Solution (*a*) rapidly turned brown in colour and in a few weeks had a heavy mycelial growth of *Asp. niger*. Solutions (*b*), (*c*) and (*d*) remained water-white for 5 or 6 months and then gradually became very slightly discoloured, the order of increasing intensity being (*b*), (*c*), (*d*). Mould growth did not begin until 5 or 6 months had elapsed, and after 18 months (*c*) was practically clear, (*b*) had a very slight growth, and in (*d*) the growth was about one-eighth of that in (*a*). In each the mould was *Aspergillus niger*. All solutions suffered to a varying degree an initial slow decrease in sensitivity, or in speed and quality of response, and maintained an order of increasing sensitivity of (*a*), (*c*), (*b*), (*d*) throughout. The indicator correction remained constant for each solution during the period of test, and may be shown by the following figures obtained in ferrous iron titrations by 0·1 *N* potassium dichromate at an acidity of 4 *N* sulphuric acid, using 1·0 ml. of indicator 18 months old:

Indicator solution	(*a*)	(*b*)	(*c*)	(*d*)	Theory
Mean titration, ml.	24·18	24·19	24·21	24·19	24·11

It appears that the use of sodium hydroxide as a solvent is advantageous, and that whilst chloroform appears in some way to catalyse the indicator response, it is ineffective in reducing mould growth and increases colour formation in comparison with that in the simple sodium hydroxide solution. Thymol is effective in preventing mould growth, but introduces a small error by consuming oxidant, and has a slightly adverse effect on the quality of the end-point.

Figures obtained have shown that the indicator correction with N-phenylanthranilic acid is not so negligible as was formerly assumed. If the titration is taken to the first permanent colour change, the correction amounts to 0·04 ml. of 0·1 *N* oxidant per 0·5 ml. of 0·005 *M* or 0·1 per cent. indicator solution; or if the titration is continued to the first development of the purple colour (requiring a waiting time of 5 minutes) the correction is 0·06 ml. of 0·1 *N* oxidant per 0·5 ml. of indicator solution. Addition of phosphoric acid reduces the correction slightly, but renders the end-point more sluggish.

It is recommended that the indicator be prepared by method (*b*) above and that, in titrations, 5 minutes be allowed after each of the last 3 drops of the titration. The indicator solutions appear to be stable and active for long periods.

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September, 1948

THE HYDROLYTIC EVALUATION OF CELLULOSE

THE methods for the quantitative estimation of cellulose are divisible into two groups. The first regards the cellulose as the main structural material of plants after protein, starch, fat, lignin, etc., have been removed. The second group dates from the demonstration by Monier-Williams[1] and others, that cellulose could be almost completely converted to glucose by hydrolysis with strong sulphuric acid.

In the hydrolytic methods, the "cellulose" is calculated from the reducing value of the hydrolysate as "glucose anhydride." The reaction is represented, $(C_6H_{10}O_5)_n + nH_2O \rightarrow nC_6H_{12}O_6$, and as practically quantitative conversion to the monosaccharide has usually been assumed, the theoretical factor of 0·90 is used to convert the estimated dextrose to cellulose.

During a recent investigation on the isolation of plant celluloses, the hydrolytic method of Waksman and Stevens[2] has been examined for use in evaluating "purified" celluloses. For the sake of convenience, half the quantities recommended by them were employed. Between 0·3 and 0·4 g. of cellulose was weighed directly into a light 250-ml. conical flask with a standard joint. Five ml. of 80 per cent. sulphuric acid were added, and the mixture allowed to stand for 2½ hours with frequent stirring, and then diluted with 75 ml. of cold water, added rapidly to prevent charring, and boiled under reflux for 5 hours. The liquid was cooled, neutralised with caustic soda solution (using Johnson's Universal indicator paper), filtered through a small Buchner funnel and made up to 200 ml.

The dextrose of the hydrolysate was determined by the Jackson[3] modification of the Lane and Eynon method,[4] using 10 ml. of the mixed Fehling - Soxhlet solution.

The effect of slight variations in concentration and volume of acid employed in the hydrolytic procedure was studied, using Whatman Filter Paper, Grade 42 (α-cellulose 98·8 per cent., copper number 0·15, ash 0·012 per cent., pentosan content 0·54 per cent.), which had been cut into small pieces and dried over sulphuric acid *in vacuo*.

The results are shown in Table I.

TABLE I

Paper taken, g.	Strong acid composition: 80% H_2SO_4 ml.	Strong acid composition: H_2O ml.	Hydrolysate titre ml.	Factor (Lane and Eynon)	Corrected factor (Jackson modification)	mg. Dextrose per 100 ml. mg.	Cellulose found g.	Cellulose recovery %
0·5130	5·0	0·0	19·5	49·4	50·39	258·4	0·4651	90·7
0·3582	4·8	0·2	28·0	50·0	51·00	182·1	0·3278	91·5
0·4150	4·8	0·2	23·9	49·8	50·80	212·6	0·3827	92·2
0·4490	5·0	0·1	21·8	49·6	50·59	232·1	0·4178	93·1
0·4060	10·0	0·0	28·7	50·0	51·00	177·7	0·3199	78·8
0·3390	10·0	0·0	34·8	50·4	51·41	147·7	0·2659	78·2

The recovery of 90·7 per cent. by the Waksman technique shows that the hydrolytic procedure does not result in the complete conversion of the polysaccharide to the monosaccharide, and that the theoretical factor of 0·90 for the conversion of the dextrose found to cellulose is unsuitable. The low recovery may be due either to incomplete hydrolysis, or to degradation of the products of hydrolysis by the strong acid.

With 10 ml. of 80 per cent. sulphuric acid, the recovery is about 11 per cent. lower still, suggesting that hydrolysis has proceeded as far as possible, but that the degradation of the resulting products is increased. The same conclusion is inferred from the effect of dilution of the strong acid, which is seen to favour the greater recovery of dextrose.

The procedure described above has been standardised, using a purified cotton cellulose, to obtain an arbitrary factor for assessing the recovery of cellulose from the hydrolysate (*cf.* determination of pentosans).

Purified Texas cotton was thoroughly extracted with chloroform, shaken with 0·1 *N* hydrochloric acid for 2 hours, washed with distilled water until the washings were neutral to bromocresol purple, and partially dried over concentrated sulphuric acid *in vacuo*. It was stored in a stoppered bottle. Its moisture content was 1·51 per cent. and it contained no ash.

The results obtained with this pure cellulose are presented in Table II.

TABLE II

Cotton taken, g.	Moisture-free cotton, g.	Titre, ml.	Corrected factor	Dextrose per 100 ml. mg.	Cellulose found, g.	Cellulose recovery, %
0·3653	0·3598	28·4	51·00	179·6	0·3233	89·86
0·5130	0·5052	19·6	50·39	257·1	0·4628	91·62
0·4158	0·4095	24·6	50·80	206·5	0·3717	90·77
0·4754	0·4682	21·4	50·49	235·9	0·4246	90·69

The mean recovery is 90·73 ± 0·45 per cent. (standard error of mean).

The factor to convert dextrose to cellulose is therefore,

Cellulose = Dextrose × 0·99.

The factor 0·99 is suggested for use with the technique given above, provided the limitations of the use of an arbitrary factor, and of the method itself generally, are clearly understood.

On application to Whatman filter paper, Grade 54 (α-cellulose 99·3 per cent., copper number 0·23, ash 0·025 per cent., pentosan content 0·59 per cent.), the method gave a recovery of 100·25 ± 0·25 per cent., indicating the applicability of the suggested factor to the evaluation of purified celluloses.

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DEPARTMENT OF AGRICULTURE
THE UNIVERSITY
LEEDS, 2

EDWARD DRUCE
J. S. WILLCOX
October, 1948

AN INTERNAL INDICATOR FOR ARSENITE - HYPOCHLORITE TITRATIONS

WE have carried out some tests by the modified Penot method described by Sinn[1] which uses quinoline yellow as an indicator. We have not been able to obtain satisfactory results; the indicator did not change colour at all during the titration. This may be due to differences in the dyestuffs used as indicators.

We have, however, found that our quinoline yellow is a very satisfactory internal indicator for the classical Penot method for the titration of hypochlorite with sodium arsenite. It is much more convenient than the use of starch iodide paper as an external indicator. We have used it for the analysis of hypochlorite bleach liquors for some time past and the results always agree well with check tests using starch iodide indicator paper. There is only one small precaution to be observed—the quinoline yellow indicator must be added shortly, say 2 or 3 ml., before the end-point. The colour change is sharp, colourless to yellow-green.

By means of this internal indicator the great disadvantage of the Penot method—the use of an external indicator—is overcome and the method becomes as convenient as the iodide - thiosulphate method and, of course, very much more economical in reagents.

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REFERENCE

1. *Analyst*, 1948, **73**, 236.

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TITAGHUR
WEST BENGAL, INDIA

J. H. YOUNG
R. N. DAS GUPTA
December, 1948

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Analysis of Sulphadiazine and Sulphathiazole Mixtures. D. Barnes (*J. Assoc. Off. Agric. Chem.*, 1948, **31**, 653–655)—This simple, rapid spectrophotometric method for the analysis of mixtures containing sulphadiazine and sulphathiazole is based on the observation that, in strongly acid solution, sulphadiazine shows a peak absorption at 239 mμ., which diminishes to a low flat region at 270 to 300 mμ., whilst sulphathiazole has a maximum at 278 mμ. and a minimum at 240 mμ.

Procedure—Dissolve a convenient quantity of the sample by thorough shaking in 200 ml. of 3 *N* hydrochloric acid and dilute the solution to 500 ml. with 3 *N* hydrochloric acid. For ointments, transfer the sample to a separating funnel, dissolve in 50 ml. of ether, extract the solution with successive portions of 50 ml. of 3 *N* hydrochloric acid, collect the aqueous extracts, and dilute the mixture to 500 ml. with 3 *N* hydrochloric acid. Dilute an aliquot, containing 1 to 2 mg. of total sulphonamides, to 100 ml. with 3 *N* hydrochloric acid and determine the optical density of this solution at 239 and 280 mμ., using distilled water in the reference cell.

At the same time prepare solutions containing 1·5 mg. of sulphadiazine and 1·5 mg. of sulphathiazole in 100 ml. of 3 *N* hydrochloric acid and measure the optical density of these solutions and of 3 *N* hydrochloric acid as a correction blank. From the data obtained, compute the extinction coefficients and calculate the concentrations of sulphadiazine and sulphathiazole in the unknown solution from the equations

$$C_N = 1000\,(K_4E_{239} - K_2E_{280})/(K_1K_4 - K_2K_3)$$
$$C_L = 1000\,(K_1E_{280} - K_3E_{239})/(K_1K_4 - K_2K_3)$$

where K_1 = extinction coefficient for sulphadiazine at 239 mμ., K_2 = extinction coefficient for sulphathiazole at 239 mμ., K_3 = extinction coefficient for sulphadiazine at 280 mμ., K_4 = extinction coefficient for sulphathiazole at 280 mμ., C_N = concentration of sulphadiazine in unknown, C_L = concentration of sulphathiazole in unknown, E_{239} = optical density of unknown solution at 239 mμ., E_{280} = optical density of unknown solution at 280 mμ.

Values obtained for K_1, K_2, K_3, and K_4 (at 1·5 mg. per 100 ml. of 3 *N* acid) were 555, 106, 103, and 469, respectively. A. H. A. ABBOTT

Composition of Seed Fats of West Indian Citrus Fruits. H. C. Dunn, T. P. Hilditch, and J. P. Riley (*J. Soc. Chem. Ind.*, 1948, **67**, 199–203)—The dried seeds of West Indian grapefruits, oranges, and limes contain approximately

40 per cent. of fatty oils. Their constituent glycerides resemble those of cottonseed oil, except for the presence of small amounts of linolenic glycerides.

TABLE I

OIL CONTENTS OF CITRUS SEEDS AND CHARACTERISTICS OF THE OILS

	Grapefruit			
	"Foster"	"Marsh"	Sweet orange	Lime
(i) Seeds:				
Average wt. (50 seeds) ..	8·7 g.	8·0 g.	5·0 g.	3·0 g.
Shell, %	24	25	35·5	25
Kernel, %	76	75	64·5	75
Oil in whole seed, %	43	41	40	39
(ii) Oils:				
Saponification value	197·2	196·5	195·5	195·2
" equivalent ..	284·0	285·0	286·5	287·0
Iodine value	100·2	100·8	97·6	111·1
Free fatty acid (as oleic), % ..	5·7	4·6	22·5	3·3
Unsaponifiable matter, % ..	0·3	0·3	0·6	0·6
Refractive index at 25° C. ..	1·4687	1·4706	1·4686	1·4676

TABLE II

PROBABLE COMPONENT GLYCERIDES OF SWEET ORANGE, LIME, AND COTTONSEED OILS

	Sweet orange per cent. (mol.)	Lime per cent. (mol.)	Cottonseed per cent. (mol.)
Tri-saturated:			
Tripalmitin	—	2	—
Mono-unsaturated di-saturated:			
Dipalmito-oleins or -linoleins	19	10	8
Palmitostearo-oleins or -linoleins	8	15	5
Di-unsaturated mono-saturated:			
Palmito-oleo-linoleins	33	26	41
Palmito-dilinoleins	1	2	18
Palmito-linoleo-linolenins	6	20	—
Stearo-oleo-linoleins	10	4	—
Stearo-linoleo-linolenins	—	3	—
Tri-unsaturated:			
Dioleo-linoleins	1	—	—
Oleo-dilinoleins	15	8	28
Oleo-linoleo-linolenins	7	5	—
Dilinoleo-linolenins	—	5	—

The composition of the component acids, + unsaponifiable matter, of citrus seed oils was:—

TABLE III

	Grapefruit seed oil		Sweet orange seed oil (neutralised)	Lime seed oil
Acid (per cent.) wt.	"Foster"	"Marsh"		
Myristic	0·8	1·2	—	0·3
Palmitic	28·8	27·5	23·7	26·0
Stearic	2·1	2·9	8·3	9·5
As arachidic	0·6	2·1	0·7	0·5
Oleic	24·9	21·0	24·6	11·1
Linoleic	36·4	39·2	36·8	39·0
Linolenic	5·9	5·8	5·2	13·0
Unsaponifiable matter	0·5	0·3	0·7	0·6

E. B. DAW

Biochemical

N-Dimethyl-leucyl-glycine as a Buffer. J. Leonis (*C. r. Lab. Carlsberg Ser. Chim.*, 1948, **26**, 357–360)—N-Dimethyl-leucyl-glycine is recommended as a chemically inert buffer for the physiologically important pH range between 7 and 8·5.

Procedure—Recrystallisation of N-dimethyl-leucyl-glycine—Suspend 20 g. of the powder in 100 ml. of chloroform and dissolve by dropwise addition of alcohol; shake after each addition. Add light petroleum (b.p. 40° to 60° C.) slowly until a faint turbidity is obtained and complete the crystallisation by storage for a few hours in a refrigerator. Filter, wash the residue with light petroleum, and dry *in vacuo* over phosphorus pentoxide. The recrystallised product has the formula

$$C_{10}H_{20}O_3N_2.1\tfrac{1}{2}H_2O.$$

Stock buffer solution—Dissolve 2·433 g. of N-dimethyl-leucyl-glycine and 1·159 g. of sodium

chloride in water free from carbon dioxide and dilute to 50 ml.

Buffer solution A—Dilute 20 ml. of stock solution with 20 ml. of water free from carbon dioxide.

Buffer solution B—Mix 20 ml. of stock solution with 20 ml. of 0·2 *N* sodium hydroxide.

To prepare buffer solutions of known pH—Mix aliquots of buffer solutions A and B in accordance with Table I.

TABLE I

Mixture				
Soln. A ml.	Soln. B ml.	pH (observed)	pK	pH (calc. from pK)
0·50	4·50	8·77	7·82	8·76
1·00	4·00	8·42	7·82	8·41
1·50	3·50	8·20	7·83	8·17
1·75	3·25	8·09	7·83	8·07
2·00	3·00	7·98	7·80	7·98
2·25	2·75	7·89	7·81	7·89
2·50	2·50	7·80	7·80	7·80
2·75	2·25	7·70	7·79	7·72
3·00	2·00	7·61	7·79	7·63
3·25	1·75	7·53	7·80	7·54
3·50	1·50	7·41	7·78	7·43
4·00	1·00	7·19	7·79	7·20
4·50	0·50	6·79	7·75	6·85

The buffer does not react with carbonyl-containing compounds or carbon disulphide and, as it is not decomposed by ordinary peptidases, it can be used in proteolytic experiments.

The buffer has a relatively low temperature coefficient; Table II shows the values of pK over the temperature range 20° to 40° C.

TABLE II

T °C. ..	20°	22°	24°	26°	28°
pK (calc.)	7·86	7·82	7·79	7·76	7·72
T °C. ..	30°	32°	34°	37°	40°
pK (calc.)	7·69	7·66	7·63	7·58	7·53

A. H. A. ABBOTT

Method for the Quantitative Estimation of Theophylline in Blood and Urine: Application to the Dog. A. J. Plummer (*J. Pharmacol. Exp. Therap.*, 1948, **93**, 142–146)—The method described is based upon a reaction reported by Plummer and Mendenhall (*Ibid.*, 1938, **63**, 31) and is sensitive, technically simple, and applicable to both blood and urine, and the reagents are stable. Caffeine, theobromine, uric acid, ethylenediamine, sodium acetate, and normal blood constituents do not interfere.

Procedure—Add 5 ml. of a saturated solution of copper acetate in methyl alcohol to 4 ml. of a methyl alcohol solution of theophylline in a 15-ml. centrifuge tube and allow to stand in the tightly stoppered tube for 4 hr. to precipitate the theophylline copper compound quantitatively. Centrifuge for 15 min. at 1000 r.p.m. Decant the supernatant liquid, and drain, and wash the precipitate with 5 ml. of methyl alcohol. Centrifuge as before and again decant and drain. Dissolve the precipitate in 4 ml. of 0·2 *N* sulphuric acid and add 0·5 ml. of an aqueous solution containing 1 g. of potassium iodide per ml. Titrate the liberated iodine with 0·02 *N* sodium thiosulphate, using soluble starch as indicator. 1 mg. of theophylline is equivalent to 0·57 ml. of 0·02 *N* sodium thiosulphate.

Blood—De-proteinise by adding 25 parts of blood to 40 parts of 13 per cent. trichloroacetic acid solution. Allow to stand for 20 min.; filter or centrifuge. Make the solution just alkaline to litmus with 2·5 *N* sodium hydroxide and then add 10 ml. of a phosphate buffer (pH 8·0). The final pH must lie between 7·3 and 8·2. Extract the theophylline by shaking the buffered filtrate with three 20-ml. portions of a mixture of 3 volumes of chloroform and 1 volume of *iso*propyl alcohol for 5 min. in each extraction. Evaporate the combined extracts just to dryness on a water-bath and dissolve the residue in methyl alcohol by warming on the water-bath. Transfer the solution to a 15-ml. graduated centrifuge tube keeping the final volume of methyl alcohol between 0·3 and 0·5 ml., and evaporating if necessary. Complete the determination as previously described, using 0·005 *N* sodium thiosulphate, 2·28 ml. of which are equivalent to 1 mg. of theophylline.

Urine—Adjust the pH to between 7·3 and 8·2 by adding 2·5 *N* sodium hydroxide and one-fifth the volume of phosphate buffer of pH 8·0. Finish the estimation in a manner similar to that used for theophylline in blood. The volume of methyl alcohol used to dissolve the drug should be from 1 to 2 ml. Use 0·01 *N* sodium thiosulphate for the final titration; 1·14 ml. are equivalent to 1 mg. of theophylline.

Between concentrations of 6 and 100 mg. per cent. of theophylline in methyl alcohol, the volumes of thiosulphate used are directly proportional to the drug concentration. In dogs, an excretion of 1·8 per cent. in 90 min., after the injection of 8 mg. of theophylline per kg. of body weight, is reported. The method can be applied to human blood and urine. G. A. STEWART

Agricultural

Colorimetric Method for the Estimation of 2 : 2-bis-(p-Chlorophenyl)-1 : 1 : 1-trichloroethane (DDT). F. R. Bradbury, D. J. Higgons, and J. P. Stoneman (*J. Soc. Chem. Ind.*, 1947, **66**, 65–68)—When *pp'*-DDT is warmed with a solution of hydroquinone in pure concentrated sulphuric acid a wine-red colour, characterised by strong absorption in the 480 to 500 mμ. band, is produced, and the reaction serves as a simple and rapid method for analysing commercial samples of DDT. The reagent is a 0·5 per cent. solution of pure re-crystallised hydroquinone in pure, concentrated, nitrogen-free sulphuric acid (*infra*). Purity of all the reagents used is essential.

Procedure—Heat an aliquot of the sample solution containing 0·2 to 1 mg. of DDT in a boiling-tube in a water-bath to remove the solvent, finally passing in a current of air to remove the last traces.

Remove the tube from the bath, add 0·5 ml. of pure, recently distilled diethyl sulphate, rotate the tube so as to dissolve all the DDT adhering to the sides, add 5·5 ml. of the hydroquinone reagent, and mix by shaking. Heat in boiling water for exactly 3 min. with occasional shaking and then cool the tube rapidly in running water. Make a blank determination, omitting the DDT. Compare the light absorption of the coloured solution and that of the blank in a Spekker absorptiometer, using No. 5 green filters and 1-cm. cells of capacity 6 ml. Within 20 min. of the formation of the colour ascertain the number of milligrams of DDT corresponding to the Spekker reading from a calibration curve. Under the conditions described, 0·1 mg. of DDT is just detectable.

The major impurities of commercial DDT, *viz.*, *op'*-DDT and *pp'*-DDD do not react sufficiently to interfere significantly with the method.

Since many DDT products are in coarse lumps and contain particles of extraneous matter, it is necessary to take large samples for analysis. The following method is recommended. Homogenise about 10 g. of the material by powdering in a mortar, dissolve 1 g. in 100 ml. of alcohol, and dilute 10 ml. of this solution to 500 ml. with alcohol. Take 5 ml. of this solution (*i.e.*, 1 mg. of the original material) for each determination. With amounts greater than 1 ml. an opalescence forms when the reaction tube is cooled after the colour development. This prevents accurate measurement of the light absorption in the absorptiometer.

The 98 per cent. sulphuric acid may contain enough nitrogen compounds to interfere seriously with the colour reaction by forming a brownish-red colour of lower intensity than the true colour given by the nitrogen-free reagent. To free analytical quality sulphuric acid from interfering nitrogen compounds, heat 1 litre with 2 g. of ammonium carbonate at 150° C. for 4 hr. A. O. JONES

Rapid Conductometric Method for Estimating Gypsum in Soils. C. A. Bower and R. B. Huss (*Soil Science*, 1948, **66**, 199–204)—The gypsum is extracted from the soil with water and then precipitated by adding acetone. The solid is re-dissolved in water and the calcium sulphate content determined from a measurement of the conductivity of the solution.

Procedure—Weigh 10 to 20 g. of air-dried soil into a bottle, add sufficient water to dissolve all the gypsum present, stopper the bottle, and shake the mixture mechanically for 30 min. (To avoid conversion of the gypsum, $CaSO_4.2H_2O$ into $CaSO_4.0{\cdot}5H_2O$, the soil should not be over-heated.) After filtering off the undissolved material, transfer 20 ml. of the extract, containing 0·1 to 0·6 mg. equivalents of calcium sulphate, to a centrifuge tube, add 20 ml. of acetone, and allow the precipitate to flocculate. Centrifuge the mixture, wash the solid residue with 10 ml. of acetone, and again centrifuge the solution. After draining off all the acetone, add 40 ml. of water to dissolve the solid, and measure the conductivity of the resulting solution. Calculate calcium sulphate content from a calibration curve constructed from the following figures:

Mg.-equivalents of calcium sulphate per litre	Electrical conductivity in milli-ohms per cm. at 25° C.
1	0·121
2	0·226
5	0·500
10	0·900
20	1·584
30·5	2·205

The results obtained by this method are in good agreement with those obtained by the usual method involving the separate determination of calcium and sulphate. None of the common ions found in soil interferes with the determination.

J. G. WALLER

Organic

Determination of Glycerol in Fermentation Residues. P. J. Elving, B. Warshowsky, E. Shoemaker, and J. Margolit (*Anal. Chem.*, 1948, **20**, 25–29)—In complex solutions such as fermentation residues glycerol is determined by measuring the formaldehyde produced on oxidation by periodic acid. Substances that interfere by themselves forming formaldehyde with periodic acid are removed by treatment with an alcoholic solution of lime. The formaldehyde can be determined by any convenient standard method; the polarographic method and the sulphite method with electrometric titration are the most suitable for routine work.

Procedure—Weigh sufficient of the sample to contain approximately 150 mg. of glycerol, dilute to 50 ml. with water, transfer 10 ml. of the solution to a small conical flask containing 1 g. of lime, and heat at 50° C. on a water-bath for 30 min. Add 50 ml. of 95 per cent. ethyl alcohol, stir, and filter by decantation through a Buchner funnel, using gentle suction and a fine filter paper. Wash the residue five times with 5-ml. portions of 95 per cent. ethyl alcohol saturated with lime. Mix the filtrate and washings, add 20 ml. of water and 1 ml. of 30 per cent. sodium hydroxide solution. Evaporate until about 20 ml. of solution remain, neutralise to methyl red with 2 *N* sulphuric acid, transfer the mixture to a Kjeldahl flask, and dilute to approximately 50 ml. with water.

Add 5 ml. of 0·5 *N* periodic acid and a few glass beads. Attach the Kjeldahl flask to a spray-trap and thence to a vertical, water-cooled condenser, the end of which is covered to a depth of 2 cm. by 50 ml. of water contained in a beaker. Heat the contents of the flask gently at first and then distil the solution at the rate of 3 to 4 ml. per min. until about 5 ml. remain in the flask. Near the end of the distillation lower the receiver so that the end of the condenser is free from the surface of the distillate.

Determine the formaldehyde in the distillate by any convenient method. A. H. A. ABBOTT

Potentiometric Titration of Weak Acids in Anhydrous Ethylenediamine. M. L. Moss, J. H. Elliott, and R. T. Hall (*Anal. Chem.*, 1948,

20, 784–788)—Phenols and other acids that are too weak to be titrated in aqueous solution give sharp end-points if the titration is carried out with anhydrous ethylenediamine, or a similar suitable basic material, as solvent. The titration is carried out with a solution of sodium aminoethoxide in anhydrous ethylenediamine as the base, and the end-point is detected by means of a pair of antimony electrodes.

The apparatus used excludes all atmospheric moisture and carbon dioxide, and the tip of the burette reaches below the surface of the liquid to be titrated. One antimony electrode is immersed in the solution, which is stirred by means of a magnetic stirrer. The second antimony electrode is mounted in the burette below the stopcock.

Reagents—The anhydrous ethylenediamine is prepared by drying the commercial material over sodium hydroxide and distilling it over sodium. To prepare the solution of sodium aminoethoxide, dissolve 2·5 g. of sodium in 100 ml. of anhydrous ethanolamine, which has been three times fractionated, and dilute the solution to 500 ml. with ethylenediamine. The sodium should first be washed with ethyl alcohol and ethanolamine.

Procedure—Dissolve 0·1 to 1·0 g. of material in 75 ml. of ethylenediamine and titrate the solution with the sodium aminoethoxide solution. The titrant solution is standardised by titrating a weighed sample of benzoic acid in the same way.

The method has been used for the determination of weakly acidic groupings in resins and for the titration of amino acids. J. G. WALLER

Constitution and Analytical Applications of Methylene Blue Periodide. M. J. A. Gautier (*Bull. Soc. Chim.*, 1948, 836–838 M)—Different formulae have been attributed to the compound that precipitates when solutions of methylene blue and iodine are mixed. These formulae were based upon experiments on the volumetric applications of the reaction. The constitution of the compound has now been established by its isolation and analysis. The periodide was precipitated from a solution of 0·5 g. of methylene blue in 500 ml. of water by addition of 100 ml. of 0·1 *N* iodine. The washed and dried precipitate is a brown powder melting with decomposition at 126° to 127° C. (block). Its iodine content (determined by reduction with zinc and sodium hydroxide and precipitation as silver iodide) corresponds to the formula $C_{16}H_{18}N_3S.HI.2I_2$. Confirmation is provided by the fact that dilute solutions of methylene blue and iodine decolorise one another when mixed in proportions corresponding to this formula.

The periodide yields practically no iodine to water or to solutions of the alkali iodides, but is decomposed by a number of other reagents such as sodium thiosulphate, alkaline reducing agents, and unsaturated compounds, with regeneration of the blue colour of the dye. Aqueous solutions also turn blue in contact with many organic solvents such as chloroform, ether, carbon disulphide, alcohols, and pyridine.

Detection of stannous ions—Using a plate, add 1 drop of 0·01 *N* iodine to 1 drop of 0·01 per cent. methylene blue solution. To the colourless mixture add a drop of a dilute solution of a stannous salt. An immediate blue colour results: sensitivity 1 μg.; dilution limit 10^{-5}. The reaction can also be carried out on filter paper. A large excess of stannous ions discharges the colour by reducing the dye to the leuco base.

Detection of mercuric ions—The procedure is the same as for stannous ions, but an excess of mercuric salt does not discharge the blue colour.

Use as an indicator in iodimetry—This application was recommended by Sturdy and Sinnatt (*Analyst*, 1910, **35**, 309). An aqueous suspension of the periodide or a drop of 0·1 per cent. methylene blue solution is added to the iodine solution. On titration with thiosulphate a change to blue occurs at the equivalence point.

The compound also behaves as an indicator in acidimetry, but has no advantage over the indicators commonly employed. W. C. JOHNSON

Determination of Substances Containing an Alkyl Pyridinium Group. E. Kahane and O. Sackur (*Bull. Soc. Chim.*, 1947, 239–242 M)—On oxidation of *N*-alkyl pyridinium compounds in alkaline solution the following have been observed: (1) formation of an α-pyridone, (2) migration of the alkyl group to the α- or γ-position, and (3) rupture of the nucleus with formation of glutaconic aldehyde and an alkyl amine. Conditions under which most compounds of this type react quantitatively according to (3) have been devised.

Procedure—The determination is best carried out on a micro-analytical scale. Take 5 to 25 mg. of the substance for analysis, 5 to 20 ml. of concentrated sodium hydroxide solution, and 5 ml. of a saturated solution of potassium permanganate, and distil from a Parnas - Wagner apparatus, titrating the distillate progressively with 0·02 *N* sulphuric acid until no more base distils. The time required is generally 10 to 20 min. A mixed indicator of methylene blue and methyl red is suitable.

The limits of error were −1·7 and +1·6 per cent. when the method was applied to the following substances: methyl pyridinium iodide, ethyl pyridinium bromide, hydroxyethyl pyridinium chloride, nicotinic acid methiodide, methyl nicotinate methiodide, nicotinamide methiodide, picolinic acid methiodide, α-picoline methiodide, and γ-picoline methiodide.

The probable general course of the decomposition is discussed and the distilled bases are shown, by analysis of their phosphotungstates, to be variable mixtures of alkylamines and ammonia.

W. C. JOHNSON

Colorimetric Method for the Determination of Alginic Acid in Seaweed Specimens. E. G. V. Percival and A. G. Ross (*J. Soc. Chem. Ind.*, 1948, **67**, 420–421)—Seaweed (0·1 g.) of known moisture content is left standing overnight, in a 30-ml. beaker with 10 ml. of 0·2 *N* sulphuric acid, filtered through a 1-inch funnel and washed with water and the filter paper transferred to a 100-ml. beaker containing 20 ml. of 3 per cent. sodium carbonate

solution. The paper is disintegrated by means of two glass rods. After the solution has been kept at 50° C. for 2 hr., it is stirred occasionally at room temperature and finally kept overnight, then filtered through a 9-cm. fluted filter into a 100-ml. flask, washed with warm water, and diluted to the mark. Three ml. are put in a test tube (8 in. × 1 in.) fitted with a high-speed stirrer, driven by a "Quickfit" chuck. The bottom end of this tubular stirrer is perforated for about 1·5 in. with a small hole through which compressed air is blown during stirring. To the tube, in a bath of ice and salt, 18 ml. of concentrated sulphuric acid (A.R.) are added from a burette—approximately 9 ml. at a rate of 1 drop per sec. and the rest at a faster rate.

After addition of the acid, the tube is kept for exactly 20 min. in vigorously boiling water, after which half the contents are used as a blank, and the rest is kept in a bath at 80° C. for 5 min. and 0·3 ml. of a 0·2 per cent. carbazole solution in ethanol is then added. (The carbazole is re-crystallised twice from glacial acetic acid.) After 45 min., the blank and sample are compared in a Spekker absorptionmeter, model H560, a green filter being used. The percentage of alginic acid is found from a calibration curve. Quantitative details for micro-determinations are also given.

E. B. DAW

Anthrone for Estimating Low Concentrations of Sucrose. E. E. Morse (*Anal. Chem.*, 1947, **19**, 1012–1013)—The Molisch α-naphthol test for sucrose, being a ring test, cannot be adapted readily to transmittancy measurements. Dreywood (*Ind. Eng. Chem., Anal. Ed.*, 1946, **8**, 499; *Analyst*, 1946, **71**, 544) suggested anthrone dissolved in concentrated sulphuric acid as a qualitative reagent for carbohydrates, and the extreme sensitivity of this test and the fact that it is not a ring test suggested its development for the quantitative determination of sucrose in low concentrations such as 50 to 250 p.p.m., *e.g.*, in the condensed vapours and solutions of sugar processing factories.

Procedure—To 2 ml. of the solution to be tested contained in a clean, dry Pyrex test tube (15 × 125 mm.) add 3 ml. of a 0·05 per cent. solution of anthrone in concentrated sulphuric acid (prepared afresh every 3 or 4 days) so as to form a lower layer, and then shake the tube to effect complete mixing. Measure the transmittancy of the solution for white light in a photo-electric colorimeter against distilled water as standard with a transmittancy of 1·00. By reference to a previously determined calibration curve or table, ascertain the concentration of sucrose in the original sample.

In mixing the sample and reagent, the tube should be shaken as one shakes a clinical thermometer. Alternatively, a small glass stirrer can be used. Since the reaction is highly sensitive to temperature, it is important that the test be performed in a standardised manner. Variations in the procedure will alter the temperature rise caused by the heat of dilution of the acid, and hence change the amount of blue-green colour produced by a given concentration of sucrose.

The anthrone used was prepared by the method in *Organic Syntheses* (Collective Vol. I, p. 60). Since the presence of an impurity was suspected, the anthrone was re-crystallised from glacial acetic acid and yielded material, the colour of which slowly changed to orange-green on long standing. A second re-crystallisation from the same solvent was of benefit, but not a third. A. O. JONES

Colour Test for Identification of Glucose. S. Hestrin and J. Mager (*Anal. Chem.*, 1947, **19**, 1032–1035)—The test is based on the fact that glucose, unlike many other sugars examined, including the aldohexoses, galactose, and mannose, forms no colour when heated with phosphoric acid alone under the conditions described but forms a characteristic lilac colour when pyrocatechol is subsequently added to the reaction system.

Procedure—To an amount of the powdered sample containing 0·2 to 1·0 mg. of carbohydrate in a dry test tube, preferably with a ground-glass stopper, add 1 ml. of 85 per cent. orthophosphoric acid. (If the sample is in solution add 0·05 ml. of the sugar solution to 1 ml. of 90 per cent. phosphoric acid.) Heat the tube in boiling water for 15 min., and shake after 1 min. to hasten dissolution. If no colour forms, heat for a further 15 min. If still no colour forms, add to the warmed solution 4 ml. of a freshly prepared 0·2 per cent. solution of pyrocatechol in 85 per cent. phosphoric acid, heat the mixture for 15 min., and compare the colour with that of a control made simultaneously with glucose.

Group (*a*)—Glucose, glucosamine, diglucoses (maltose, trehalose), polyglucoses (starch, glycogen, cellulose), and glucuronic acid form no colour when heated for 15 min. with phosphoric acid alone.

Group (*b*)—Mannose, galactose, pentoses (xylose, arabinose, lyxose, ribose), methyl pentose (rhamnose), ketoses (sorbose, fructose), oligosaccharides containing a non-glucose unit (sucrose, melibose, raffinose), and polysaccharides containing a non-glucose unit (inulin, mannan) form a greenish-yellow to amber-brown colour when heated for 15 min. in phosphoric acid. (Galactose, lactose, and melibose give a relatively weak colour. If galactose is present in very small amount, the weak amber colour may be difficult to see. However, the red colour formed with pyrocatechol distinguishes galactose from glucose.)

Absence of colour after heating with phosphoric acid alone shows that none of the sugars in group (*b*) is present. If the amount of carbohydrate added is large enough (more than 2 mg. per ml.), the different glucoses (glucose, maltose, glycogen, starch, and cellulose) yield a pink colour when heated with phosphoric acid for 30 min.

The relative amounts of colour formed with phosphoric acid by these common aldohexoses were plotted against the sugar concentration. The extinction - concentration curves were all straight lines and there was a particularly large difference between glucose and mannose in colour-forming activity. In a mixture of equal parts of glucose and mannose the amount of colour produced is within 3 per cent. of the colour produced by the

mannose constituent alone. A study of the application of this reaction to the determination of mannose in presence of glucose seems desirable.

Differentiation of glucose, galactose, and mannose by the reaction with pyrocatechol was studied in greater detail. Transmission was measured by means of a Pulfrich Step-photometer, usually with a solution layer of 1 cm. A characteristic feature of the glucose product is its marked absorption peak in the region of 500 mμ. (filter S50). The ratio $\epsilon_{843}/\epsilon_{850}$ is different enough to provide a clear-cut distinction between these three substances. The ratio, though sensitive to differences of configuration within the sugar unit, is independent of the nature of the interglucosidic linkage, the polymerisation degree of the sugar unit, and the carbohydrate concentration. Although it was difficult to obtain good reproduction of the absolute amount of colour formed by a given sugar, the ratio $\epsilon_{843}/\epsilon_{850}$ remained constant to within ±7 per cent.

The method is valid as a means of identifying glucose only if no other colour-forming substance is present in the test material. Common pentoses (D- and L-arabinose, ribose, xylose, lyxose, and rhamnose) when heated in the pyrocatechol reagent produce colours that are intermediate in tone between the red-brown given by galactose and the lilac given by glucose. Moreover, two ketoses (fructose and sorbose) and one pentose (xylose) yield colours that are not certainly distinguishable in terms of $\epsilon_{843}/\epsilon_{850}$ from the colour given by glucose. It is particularly fortunate that the presence of non-glucose sugars (hexoses and pentoses) is revealed by their colour in the first step of the standard procedure, *i.e.*, on heating with phosphoric acid alone, and specific testing for these interfering sugars is therefore unnecessary. Glucuronic acid, on heating with pyrocatechol, forms a red-brown colour different from that given by glucose. Like glucose, however, it forms no colour when heated with phosphoric acid alone. It is desirable therefore to demonstrate the absence of glucuronic acid by independent means.

Identification of glucose by this test is necessarily presumptive. It is always possible that units different from glucose but simulating glucose in the test can occur in biological material.

Tryptophan and nitrites interfere with the test. The absence of interfering colour formation in the presence of any amino acid, with the exception of tryptophan, is an important merit of the method. As may be expected, tryptophan-free proteins such as gelatin, do not interfere. The reaction can be applied safely in presence of certain proteins containing tryptophan, *e.g.*, edestin, yet, clearly, caution is necessary where the test is being applied to an unknown protein material containing tryptophan. Plasma albumin and β-lactoglobulin interfere with the test. A. O. JONES

Inorganic

Accuracy of Estimation of Hydrogen Peroxide with Potassium Permanganate Titration. C. E. Huckaba and F. G. Keyes (*J. Amer. Chem. Soc.*, 1948, **70**, 1640–1644)—The trustworthiness of this method has been questioned. Decomposition of hydrogen peroxide by a catalyst and measurement of the volume of oxygen evolved is chosen as the only trustworthy method with which the titration methods could be compared. Results of analyses by the volumeter method are compared with those obtained with potassium permanganate titration under various conditions.

The decomposition method is too inconvenient for general use. The sample of 2 to 3 per cent. hydrogen peroxide was weighed in a tube which was then attached to the cooled decomposition vessel. The liquid was run into the vessel, which was cooled in "dry ice" and the tube was rinsed with water. The rinsing water was run into the decomposition vessel under vacuum. Osmic acid solution or lead oxide suspension as catalyst was added in a similar manner to the frozen hydrogen peroxide solution. The gas evolved as the liquid warmed was pumped into a volumeter by a Toepler pump.

The glass apparatus used in the potassium permanganate method was made from glass previously treated with hot fuming sulphuric acid. A 10-g. sample of 2 to 3 per cent. hydrogen peroxide was weighed in a glass-stoppered flask and rinsed with about 50 ml. of conductivity water into a beaker containing 150 ml. of conductivity water and 7 ml. of 95 per cent. sulphuric acid. Approximately 0·16 *N* potassium permanganate, standardised by the method of Fowler and Bright (*J. Res. Nat. Bur. Standards*, 1935, **15**, 493), was run in from a 500-ml. weight burette at 30 to 40 ml. per min. with moderate stirring.

The deviation between the results obtained by the two methods for the same sample on the same day was with one exception not greater than 1 in 300. The average deviation for nine comparisons was 1 in 2500. The comparison between the two methods was equally favourable with osmic acid and with lead oxide as catalyst. The results obtained by the permanganate method were still concordant with those by the decomposition method when double the acid concentration was used, and when the titration rate was 10 ml. per min., and 50 ml. per min. To avoid the formation of manganese dioxide it is advisable to perform the titration at a rate of less than 40 ml. per min. and with a ratio of sulphuric acid to hydrogen peroxide of at least 60 to 1 by weight.

B. ATKINSON

Action of Mercury on Various Compounds. E. Montignie (*Bull. Soc. Chim.*, 1947, M 377–378)—The interaction of mercury with solutions of various oxidising agents is described as follows. Each experiment is conducted at room temperature with continuous agitation. *Sodium perborate*—Oxygen evolution begins after 1 hr. and continues until the perborate is completely decomposed. The mercury is not oxidised. *Potassium permanganate*—The products obtained with a 0·5 *N* solution are hydrated manganese dioxide, potassium manganate, and mercurous oxide. *Ammoniacal silver oxide*—Silver is deposited and the mercury is partially oxidised to mercurous oxide. *Sodium nitrite*—In 5 hr., a small amount of ammonia is

formed and some mercurous oxide. *Sodium arsenate*—Sodium arsenite and mercurous oxide are formed. *Potassium ferricyanide*—A greenish-blue precipitate, consisting of Turnbull's Blue and mercuric oxide, is slowly produced. *Sodium persulphate*—The products obtained after 6 hr. are sodium sulphate, sulphuric acid, and basic mercuric sulphate. *Potassium cyanate*—Soluble mercuric oxycyanide and potassium hydroxide are formed (3 hr.). *Potassium percarbonate*—Oxygen is evolved and the mercury remains unchanged.

W. C. JOHNSON

Automatic Potentiometric Titration of Iron and Titanium with Chromous Ion. J. J. Lingane (*Anal. Chem.*, 1948, **20**, 797–801)—The conditions for the potentiometric titration of titanic and ferric ions with chromous ions have been investigated with an automatic titration apparatus (*cf. Ibid.*, 1948, **20**, 285; *Analyst*, 1949, **74**, 219). The same conditions apply to electrometric titrations carried out with manual apparatus.

Titration of ferrous ions—A platinum indicator electrode and a saturated calomel reference electrode are used, and the titration is carried out in solutions that are 1·8 *M* with repsect to sulphuric acid.

Titration of titanic ions—In this case a mercury indicator electrode is used in conjunction with a saturated calomel reference electrode, and the solution should be approximately 4·0 *N* with respect to sulphuric acid. Chloride solutions are less suitable.

Titration of mixtures of ferric and titanic ions—The standard potentials of the ferrous-ferric and titanous-titanic couples are sufficiently separated for $Fe^{'''}$ and $Ti^{''''}$ to be titrated in the same solution. The solution is made 4·0 *N* with respect to sulphuric acid, and a platinum reference electrode is used until the ferrous-ferric end-point has been slightly passed. The mercury electrode is used for completing the titration.

In addition, small amounts of iron can be determined in presence of large amounts of titanium. For the determination of small amounts of titanium in presence of excess of iron, the ferric ions are first reduced to the ferrous state with sulphur dioxide, and after boiling off the excess of sulphur dioxide, the titanium is titrated.

J. G. WALLER

Separation of Nickel and Cobalt in the Micro-Estimation of Copper with Diethyldithiocarbamate. H. Cheftel, J. Bail, R. Fouasson, and P. Clavié (*Bull. Soc. Chim.*, 1947, M 311–313)—The authors are concerned with estimating traces of copper in fresh vegetables and in canned vegetables and in avoiding interference from metals derived from machinery and from the packages. The metals considered are iron, tin, nickel, chromium, manganese, and cobalt. Addition of sodium pyrophosphate in sufficient quantity prevents interference by iron (MacFarlane, *Biochem. J.*, 1932, **26**, 1022) and by manganese. Neither tin nor chromium interferes with the determination of 10 to 50 μg. of copper when present in 100-fold excess. Nickel yields a yellow colour with diethyldithiocarbamate and interferes when present in 3-fold excess; it is removed by precipitation with dimethylglyoxime and extraction of the nickel complex with chloroform. Cobalt gives a green colour with the copper reagent but this effect is also eliminated by the addition of dimethylglyoxime, although the cobalt - glyoxime compound is not extracted by the chloroform.

Procedure—Destroy the organic matter by heating with mixed nitric, sulphuric, and perchloric acids, evaporate until white fumes appear, cool, dilute, and neutralise to litmus paper with concentrated aqueous ammonia solution. Transfer to a volumetric flask and dilute to the mark. The solution at this stage should contain 1 to 10 mg. of copper in 1 litre. Test 2 to 3 ml. of this solution for nickel by adding concentrated aqueous ammonia solution until the pH is at least 9·0, followed by 2 to 4 drops of a 1 per cent. solution of dimethylglyoxime in 95 per cent. alcohol, and heating in a water-bath at 80° C. for 10 min. If no rose colour appears proceed directly to the estimation of copper. If a positive reaction for nickel is obtained proceed as follows. Take an aliquot containing 10 to 25 μg. of copper, add 5 to 10 ml. of a 6 per cent. w/v solution of tetrasodium pyrophosphate, the greater quantity if the solution contains as much as 500 mg. of iron plus manganese, per litre. Heat in a water-bath at 80° C. for 15 min. To the hot solution add an excess of dimethylglyoxime solution and concentrated aqueous ammonia dropwise until the nickel is precipitated. A large excess of ammonia renders difficult the subsequent extraction of the precipitate. Cool to 50 to 55° C. and shake for 1 to 2 min. in a glass-stoppered tube with 2 ml. of chloroform. Transfer to a separating funnel, rinsing the tube with two 2-ml. portions of chloroform and then twice with 1 to 2 ml. of water. Separate the chloroform layer and wash the aqueous layer with two 2-ml. portions of chloroform. Wash the mixed chloroform extracts with two 3-ml. quantities of water and add the washings to the original aqueous layer. To the aqueous liquid, contained in a separating funnel, add 0·5 ml. of 2 per cent. sodium diethyldithiocarbamate solution and 5 ml. of *iso*amyl alcohol, shake for 1 min., separate and pass the alcoholic layer through a dry filter. Compare the colour of the filtrate with a suitable series of standards similarly prepared or, preferably, use a photo-electric absorptiometer calibrated for the method.

The procedure permits the estimation of quantities of copper of the order of 25 μg. to within ±1 μg.

W. C. JOHNSON

Estimation of Quartz in Presence of Silicates. S. S. Gurvits and V. V. Podgayts (*Zavod. Lab.*, 1948, **14**, 935–938)—The determination of quartz in siliceous dust is important for assessing silicosis risk. The Soviet specification GOST 1324–47 limits the concentration of dust containing 50 per cent. of quartz to 2 mg. per cu. metre, but permits 10 mg. per cu. metre of other siliceous dusts.

Tests were carried out on various silicate powders containing 95 per cent. of particles, of diameters up to 2 mμ., using hydrofluoboric and hydrofluosilicic acids as selective solvents of non-quartz silica.

Tests with hydrofluoboric acid—To 0·2 g. of the material in a platinum crucible were added 5 ml. of hydrofluoboric acid solution (approximately 27 per cent.) and, to remove the effect of the fluoride ion, 2 ml. of 2 per cent. ferric chloride solution. The insoluble matter was filtered off, washed twice with 5 ml. of *N* hydrochloric acid and three times with 5 ml. of hot water, and ignited to constant weight. Tests were carried out at room temperature and at 45° to 50° C. The duration of the reaction ranged from 24 hr. to 5 days.

Quartz was practically unchanged after 5 days. Dissolution of the silicates took place but incompletely. At 45° C., 67 to 70 per cent. of the kaolin and hornblende, 50 to 60 per cent. of the felspar and talc, and 80 to 90 per cent. of the biotite and serpentine were dissolved.

Tests with hydrofluosilicic acid—To 0·2 g. of the powder moistened with water, were added 5 ml. of hydrofluosilicic acid and the undissolved matter was collected, washed, and ignited after 1 to 10 days. After 4 to 5 days the residues of the silicates were between 1 and 10 per cent. but in most cases contained no silica. In the first day quartz lost 2·5 to 3 per cent. and, at the end of 10 days, 11 per cent. Further experiments showed that the dissolution of the silicates was hastened by preliminary treatment with hydrochloric or sulphuric acid.

Procedure—Place 0·1 to 0·2 g. of the finely ground material (particle size 2 to 3 mμ.) in a platinum crucible, add 5 to 10 ml. of concentrated hydrochloric acid, heat carefully on a sand-bath for 10 to 15 min., add 5 ml. of water, boil, filter through a close paper, wash twice with 5-ml. portions of hot, diluted hydrochloric acid (1 + 5) and three times with 5-ml. portions of hot water, and return the filter and insoluble matter to the platinum crucible. Ash the paper at a low heat and then ignite for 20 to 30 min. at a white heat. Cool, add 5 ml. of 48 per cent. hydrofluosilicic acid, cover with a platinum lid, and leave at room temperature for 24 to 30 hr. Filter through a close paper, wash three times with 5-ml. portions of hot water, ash, ignite to constant weight at white heat, treat with sulphuric acid and hydrofluoric acid, evaporate, ignite, and weigh again. The difference in weight gives the quartz content.

With equal parts of quartz, biotite, talc, and kaolin and with equal parts of quartz, felspar, serpentine, and garnet, theoretical results were obtained. Quartz was under-estimated by about 5 parts in 100 in presence of serpentine only, and over-estimated by a few parts in 100 in presence of felspar only, kaolin only, or hornblende only.

G. S. SMITH

Colorimetry of Aluminium Oxinate. M. M. Raynes and Yu. A. Larionov (*Zavod. Lab.*, **1948, 14**, 1000).—For extracting the aluminium salt of 8-hydroxyquinoline from aqueous solutions, Kuskova (*J. Anal. Chem. Russ.*, **1947, 2**, 7; *Analyst*, **1948, 73**, 114) recommended *iso*amyl alcohol and rejected benzene, and Mervel (*J. Anal. Chem. Russ.*, **1947, 2, 103**; *Analyst*, **1948, 73**, 172) recommended benzene, finding *iso*amyl alcohol to be unsuitable. Gentry and Sherrington (*Analyst*, **1946, 71**, 432) used chloroform.

Mervel's objections to *iso*amyl alcohol have now been confirmed. Tests were carried out with the solvents mentioned above and also with carbon tetrachloride, light petroleum, and butyl acetate. The limits of visibility of the colour, in mg. of aluminium per 10 ml., are 0·002 for carbon tetrachloride, chloroform, and benzene, 0·005 for butyl acetate, 0·01 for light petroleum, and 0·02 for *iso*amyl alcohol. Carbon tetrachloride has certain advantages over the other solvents: colours are better defined and the solutions are more transparent.

G. S. SMITH

Rapid Electrometric Method of Determining Aluminium in Ores. S. K. Chirkov (*Zavod. Lab.*, 1948, **14**, 783–787)—Well-polished aluminium and nichrome wires are suitable as electrodes in the non-compensated electrometric titration of aluminium with sodium fluoride in acetic acid solutions.

The quantitative relation between aluminium and fluoride ions, as indicated by the titration curves for dilute aluminium solutions ($\geqslant$ 0·005 *M*) from the points of intersection of tangents, depends on the pH. With pH 3·5 to 4·5, the ratio Al : F at the equivalence point is 1 : 3, with pH 6·0 it is 1 : 6, with pH $<$ 3·5 it becomes $>$ 1 : 3, but with pH $<$ 3 no interaction occurs, *i.e.*, the galvanometer reading rises steadily from the beginning of the addition of fluoride and then flattens out, whilst in the less acid solutions the reading is almost constant until the equivalence point is approached, and then it rises rapidly.

With more concentrated aluminium solutions titrated with fluoride solutions of concentration greater than 0·2 *M*, *e.g.*, 0·01 *M* aluminium chloride titrated with 0·5 *M* sodium fluoride, there are two equivalence points, the complete curve consisting of an initial horizontal portion, a rise to a maximum, followed by a fall, and then a second rise followed by a flattening-off. The intersections of the tangents to the rising portions with the tangent to the initial part of the curve correspond to the ratios Al : F of 1 : 3 and 1 : 6. This effect is shown only when the aluminium concentration exceeds the solubility of the cryolite that is formed. Addition of about 2 to 3 g. of sodium chloride per 50 ml. eliminates the maximum and the minimum, and only one equivalence point, corresponding to the ratio 1 : 6, is obtained. This point is shown by a definite break in the curve and hence it can be found, without the drawing of a curve, merely by observation of the galvanometer.

Apparatus—Wires of aluminium and nichrome, 3 mm. thick and 16 cm. long, are carefully polished with fine glass-paper and filter paper, and the lower ends are filed round. The cleaning and polishing must be carried out before each titration. The upper ends of the electrodes are securely clamped to flexible well-insulated copper wires. A small glass stirrer is joined to the electrode pair, which should be separated as far as the beaker will allow. They are immersed to a depth of 1 to 1·5 cm. in the solution to be titrated. The electrodes

are connected in series with a 20,000- to 30,000-ohm fixed resistance and a pointer galvanometer with a 17-mv. scale having 0·1- or 0·2-mv. divisions. Stirring is carried out by rotation of the platform on which the beaker rests.

Sodium fluoride solution—Prepare 0·3 to 0·4 *M* sodium fluoride, neutralise the excess of alkali with hydrochloric acid, using methyl orange, and keep the solution in a paraffined bottle. Standardise on approximately 0·05 *M* aluminium potassium sulphate, the aluminium content of which has been determined gravimetrically or by other means. To 10 ml. of the aluminium solution add 2 ml. of diluted hydrochloric acid (1 + 1), 10 ml. of 20 per cent. sodium chloride solution, and 10 ml. of 20 per cent. sodium acetate solution, dilute to 45 to 50 ml., make just alkaline to methyl orange with 8 to 10 per cent. sodium hydroxide solution, and titrate electrometrically with the sodium fluoride solution, obtaining the full titration curve.

Procedure—Fuse 3 g. of sodium hydroxide in an iron crucible, add 0·2 to 0·5 g. of the ore to the cooled melt, fuse again, cool, extract with water, warming slightly, transfer the solution to a 200-ml. graduated flask, cool, make up to the mark, allow the insoluble matter to settle, and filter through a dry filter, rejecting the first 10 to 20 ml. of the filtrate. Transfer 100 ml. to a beaker, neutralise to methyl orange with diluted hydrochloric acid (1 + 1), and add an excess of 2 to 3 ml. of this acid. Evaporate to 25 to 30 ml., cool, add 10 ml. of 20 per cent. sodium acetate solution or 2 g. of the pure salt, neutralise to methyl orange, and titrate with the sodium fluoride solution.

If a maximum appears or if the galvanometer needle becomes erratic as the equivalence point is approached, repeat the titration after adding 5 to 10 ml. of 20 per cent. sodium chloride solution.

Bauxites with aluminium oxide contents of from 17·5 to 41·4 per cent., and iron, and titanium ores containing about 5 per cent. of aluminium oxide gave results correct to within 1 or 2 parts in 100.

G. S. SMITH

Physical Methods, Apparatus, etc.

Preparation of Aluminium Oxide for Chromatography. G. Dupont, R. Dulou, and M. Vilkas (*Bull. Soc. Chim.*, 1948, 785–786 M)—Aluminium hydroxide of commerce is used as the starting material in a study of the conditions for ignition and activation. At 200° to 350° C., a monohydrate of low activity is obtained; at 350° to 450° C., a highly absorbent amorphous powder results, suitable for use as a desiccant but too fine for chromatography. At 450° to 500° C., the oxide changes to the granular, crystalline γ-form which, after further treatment, has the desired characteristics. The α-modification, a non-absorbent variety, begins to form at 800° C.

Procedure—Heat aluminium hydroxide for 1 hr. at 500° C. Cool, and mix the oxide with sufficient concentrated hydrochloric acid to cover it. Allow to stand for 2 to 3 hr., with occasional stirring. Then stir with a large volume of water and decant after 5 min. so as to remove the finer powder still in suspension. Repeat the treatment with water until the oxide settles completely in 2 to 3 min. (5 to 6 treatments). Filter off the product, wash it free from acid, and place it in an oven at 105° C. Control the drying by measurement of the absorptive power according to the standardised procedure of Brockmann (Ber., 1941, **74**, 73) and store the product in a bottle with a ground-glass stopper.

To fill a chromatographic column the following method is recommended. Close the bottom end of the column and fill it two-thirds full with an appropriate solvent. Add the dry aluminium oxide in a thin stream from a funnel with a constricted stem, revolving the column slowly on its own axis during the process. W. C. JOHNSON

Removal of Metals at Mercury Cathode. Separation of Interfering Metals in the Determination of Aluminium, Alkaline Earth and Alkali Metals. T. D. Parks, H. O. Johnson, and L. Lykken (*Anal. Chem.*, 1948, **20**, 148–151)—Operating conditions for the self-contained electrolysis cell previously described (Johnson *et al.*, *Ibid.*, 1947, **19**, 481; *Analyst*, 1948, **73**, 476) have been investigated.

Operating conditions—Observations were made of the effect of current and distance between electrodes on the efficiency of removal of metals from solution. The most complete deposition after electrolysis for 30 min. is obtained by using a current of 5 amp. from the 5-volt supply and a distance between the electrodes of 8 to 10 mm. The efficiency of deposition in a fixed time rises with an increase in area of the mercury cathode, but the original cathode of area 10·4 sq. cm. is the most convenient for general use.

Procedure for the electrolysis—Concentrate the neutral or slightly acid solution to 25 to 30 ml. and transfer to a 250-ml., tall-form beaker. Clamp the electrode assembly in place, fill the cathode with mercury to within 1 or 2 mm. of the top and raise the beaker until the electrode almost touches the bottom of the beaker. Add concentrated sulphuric acid until a current of 5 amp. is passing, cover the beaker with a split, notched watch glass, and electrolyse for 15 or 30 min. Add water to maintain constant volume. With the current on, lower the beaker and rinse the electrode with distilled water. Replace the amalgam with clean mercury, electrolyse for 15 or 30 min., and rinse. Repeat the cycle one or more times.

Examples of the use of the technique—A series of samples containing 1 mg. of sodium and from 0·5 to 3 g. of other metal was electrolysed and the resulting solutions were analysed for sodium by the polarographic method (Weaver and Lykken, *Ibid.*, 1947, **19**, 372–376; *Analyst*, 1948, **73**, 169). A blank determination for sodium was performed on the comparable amount of each metal and appropriate corrections were applied. After removal of copper, iron, cadmium, mercury, nickel, and zinc, 1 mg. of sodium was determined with an accuracy of ±0·04 mg. After removal of cobalt, the polarographic method could not be used even though the amount of cobalt remaining in solution was small. The flame-photometer method (Barnes

et al., Ind. Eng. Chem., Anal. Ed., 1945, **17**, 605–611) was used successfully.

Other separations for which the mercury cathode procedure has been used are, removal of iron before the colorimetric determination of aluminium, removal of iron, nickel, and chromium before the determination of calcium by micro-titration with ammonium hexanitratocerate solution, and removal of interfering metals before the determination of thorium as oxide.

Metals requiring special techniques—Chromium—See (Johnson *et al., Anal. Chem.*, 1947, **19**, 481; *Analyst*, 1948, **73**, 476).

Tin—When a sulphuric acid solution is used insoluble tin salts are precipitated and prevent deposition of tin at the cathode.

Procedure—Dissolve the sample in 20 ml. of concentrated hydrochloric acid, add 2 g. of hydroxylamine hydrochloride, and dilute to 50 ml. Use an electrode assembly fitted at the top of the electrode holder with a fume hood in the form of a wide-angle inverted funnel attached by a side-tube to suction. Electrolyse the solution at 2·5 amp. or less, for 30 min. With the current on, lower the beaker, rinse the electrode, and replace the amalgam with clean mercury. Electrolyse for 30 min., rinse, and repeat the cycle.

The amount of tin left in a solution originally containing 0·25 g. is not less than 3 mg. This amount is not sufficient to interfere in the determination of 1 mg. of sodium.

Lead—The formation of lead peroxide on the anode short-circuits the cell.

Use a procedure similar to that given for tin. Use 2 g. of hydroxylamine hydrochloride, 0·3 *N* hydrochloric acid, and a current of 5 amp. from the 5-volt supply. The procedure is satisfactory for removing 0·5 g. of lead before determining 1 mg. of sodium.

Molybdenum—With the standard procedure the solution becomes dark and gelatinous. Use a 1·2 *N* sulphuric acid solution and a current of approximately 5 amp. Electrolyse for a total of 90 min., changing the mercury twice. A molybdenum content of 0·1 g. is reduced to less than 1 mg.

Bismuth, antimony, and arsenic—No procedure has been found by which amounts of these metals of the order of 0·1 g. can be quantitatively removed from solution by deposition at the mercury cathode.

B. ATKINSON

Rapid Precipitate Dryer and Solvent Evaporator. I. R. Hunter (*Anal. Chem.*, 1948, **20**, 186)—The dryer is made from two Pyrex funnels of Büchner type with fritted discs. To each of the funnels is sealed a ground-glass joint provided with glass hooks. Funnels with fritted discs 30 to 90 mm. in diameter have been used. Place or filter the sample into the lower funnel,

attached to a socket, then attach the upper, inverted funnel, and put rubber bands over the hooks. Pass a slow stream of air upwards through the precipitate for a few minutes. Break up any lumps that may form. Most of the common solvents are completely removed in 5 to 15 min.

To evaporate a solution pour it into the lower funnel while a slow stream of air is passing upwards through it. Attach the upper funnel and increase the air stream.

B. ATKINSON

Review

TECHNICAL DICTIONARY, ENGLISH - ITALIAN, ITALIAN - ENGLISH. By Dr. ING. G. MAROLLI. Second Edition. Pp. 630 + 16 Plates. Florence: Felice de Monnier. 1949. Price L.2700.

It is rather surprising that while there are a number of technical dictionaries in other languages no work of this kind in English and Italian has hitherto been available. The present volume by an engineer of the Fiat company and published in Florence should do much to supply this need.

It comprises terms used in the chemical, engineering, shipbuilding, aeronautical, textile, radio and other industries. The chemical terms, with which the writer is more familiar, seem to be well chosen and correctly translated. That a second edition has been called for within a few months shows that the work has been well received in Italy and the opportunity has been taken to add a considerable number of fresh words in the form of supplements to the two parts of the dictionary.

The printing is clear and free from errors and the volume is of handy size and can be recommended to chemists interested in this subject.

A. H. BENNETT

REPORTS OF THE ANALYTICAL METHODS COMMITTEE OBTAINABLE THROUGH THE EDITOR

The Reports of the Analytical Methods Committee listed below may be obtained direct from the Editor of THE ANALYST, 7-8, Idol Lane, London, E.C.3 (not through Trade Agents), at the price of 1s. 6d. to Members of the Society, and 2s. 0d. to non-Members. Remittances must accompany orders and be made payable to "Society of Public Analysts."

The Reichert-Polenske-Kirschner Process. (Test for Butter Fat.) *To be reprinted.*

Milk Products Sub-Committee:

Reports Nos. 1 and 2. Analysis of Condensed Milks.
Report No. 3. Analysis of Sweetened Condensed Milk in which the Sucrose has altered during Storage. *To be reprinted.*
Report No. 4. Determination of Water, of Total Solids and of Fat in Dried Milk.

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

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

Essential Oil Sub-Committee:

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

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

Report No. 1. Determination of Arsenic. *Out of print.*
Report No. 2. Determination of Lead.
Report No. 3. Determination of Copper.
Report No. 4. Determination of Zinc.

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

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

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

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

Fluorine in Foods Sub-Committee:

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

Sub-Committee on Vitamin Estimations. Microbiological Panel:

Report on the Microbiological Assay of Riboflavine and Nicotinic Acid.

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