THE ANALYST

The Journal of The Society of Public Analysts and Other Analytical Chemists

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

Volume 73 Price 3s. 6d. August, 1948
No. 869, Pages 423—478

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

The Freezing-Point of Bulk Milk

BY F. J. MACDONALD

(*Read at the Meeting of the Society on April 7th*, 1948)

THE employment of the freezing-point test over a number of years has made it increasingly evident that the presumptive standard of 8·5 per cent. of solids not fat (S.N.F.) is of somewhat limited use in deciding whether a milk is genuine or not. Many cases are known of milks of much lower S.N.F. that have proved to be genuine when submitted to cryoscopic examination, and this test is consequently of much value in preventing unjust conclusions as to the cause of such deficiencies. Most of the published work of this nature has been concerned with samples drawn from individual cows or from herds, where considerable variation both of S.N.F. and of freezing-point depression is encountered, the point at issue usually being whether a milk deficient in S.N.F. is genuine or not.

There is, however, another side to this picture, namely, whether a milk that satisfies the presumptive standard for S.N.F. is genuine or contains added water. When bulked milks of from one to three thousand gallons in volume are considered, it is evident that the individual variation of S.N.F. at both ends of the scale will tend to cancel out to give a milk of "average composition." This will naturally vary with the time of year and the predominant breed of cow in the area, but this variation will be comparatively small. If an average value of 8·75 per cent. of S.N.F. be taken for bulk milk it will not be far from the truth, and in general it can be accepted that a bulk milk with S.N.F. below the presumptive standard of 8·5 per cent. is not genuine. Furthermore, it is apparent that such milk could contain some 2 per cent. of added water and yet be comfortably above the presumptive standard with a S.N.F. of 8·57 per cent. Such an addition would decrease the freezing-point depression by about 0·011° C., a difference very easily measured by the Hortvet cryoscope, but only a third as great as the published figures for the natural range of variation of this value for genuine individual cow and herd milk (0·530° to 0·562° C.). The application of the freezing-point test to estimate the presence of small proportions of added water in bulk milk would thus seem to be of limited value unless a sample of the original genuine milk were at hand for purposes of direct comparison, or unless the natural variation of the freezing-point depression of bulk milk were much smaller than that of samples from individual cows and herds.

VARIATIONS IN THE FREEZING-POINT OF MILK FROM INDIVIDUAL COWS AND FROM HERDS

A large number of results for the freezing-point of genuine milk have now been published. The figures that have been obtained by applying the Hortvet method to fresh milk are in good general agreement, both in the range of variation and the average value, and are shown in Table I.

Hortvet's[1] figures relate to 60 individual cow samples and 15 from herds, Bailey's[2] to 179 healthy individual cows and 61 herds, and Stubbs and Elsdon's[3] to 330 "appeal-to-cow" samples.

TABLE I

FREEZING-POINT DEPRESSION OF MILK IN °C.

Observer	Individual cows			Herds		
	Max.	Min.	Average	Max.	Min.	Average
Hortvet	0·562	0·534	0·547	0·562	0·545	0·551
Bailey *et al.*	0·566	0·530	0·545	0·562	0·530	0·544
Stubbs and Elsdon	0·563	0·530	0·545	—	—	—

The results published by Stubbs and Elsdon[3] on 1000 samples of genuine milk are of considerable interest, these samples including the 330 results cited in Table I. Their frequency distribution is shown in Table II.

TABLE II

FREQUENCY DISTRIBUTION OF FREEZING-POINT DEPRESSIONS

Δ° C.		No. of samples
0·529	to 0·530	6
0·531	2	4
3	4	20
5	6	36
7	8	68
9	0·540	121
0·541	2	125
3	4	157
5	6	139
7	8	109
9	0·550	83
0·551	2	51
3	4	34
5	6	23
7	8	8
9	0·560	10
0·561	3	6
Mean	0·544	1000

The above 1315 results indicate that genuine milk may have a freezing-point depression that lies between 0·530° and 0·562° C., with a mean value of 0·544° C.

From these data it may be safely concluded that any sample of milk from individual cows or from herds with a depression smaller than 0·530° C. must contain added water. The precise amount of adulteration must be a matter of conjecture, unless direct comparison with "appeal-to-cow" samples is possible, since the difference between the smallest and the greatest observed depression for genuine milk is equivalent to more than 5 per cent. of added water. In the absence of comparison samples, it would be unfair to condemn a sample with a depression greater than 0·530° C., and Stubbs and Elsdon[4] recommend that the average depression for genuine milk be taken as 0·540° C., but that no sample be considered as watered on the evidence of the freezing point alone unless the depression falls below 0·530° C. An alternative method is to calculate the proportion of added water on such samples on a basis of 0·530° C. and to use the expression "minimum percentage of added water."

VARIATION IN THE FREEZING POINT OF BULK MILK

It will have been noted that the mean value for the freezing-point depression of genuine milk is 0·544° C. and that some 90 per cent. of the depressions recorded in Table II fall between the limits 0·537° C. and 0·554° C. It is reasonable to assume that volumes of milk of the order of 1000 to 3000 gallons, *i.e.*, volumes that are the result of mixing the produce from 500 to 1500 cows, should exhibit the properties of a sample of "average composition." The freezing-point depression of any such bulk might be expected to approach the average value, and the range of variation to be appreciably narrowed.

This assumption was tested over the period of a year by examining a total of 480 samples drawn from rail-tankers of 3000 gallons capacity. Each month 10 samples were taken from the tanks arriving from four different creameries in three different counties (Derbyshire, Westmorland, and two in Sussex). All the milk was brine-cooled before despatch and was examined on the day of arrival in London before any increase in acidity had taken place, the acidities being of the order of 0·14 per cent. (calculated as lactic acid). It may be argued that these results are open to criticism on the score that there was no guarantee that every churn of milk that went into every tank was free from added water, so that too small a value might have been obtained for the depression in those instances. This point is not disputed, since it was obviously an impossible task to examine each individual churn, and the figures must therefore be regarded as the normal depressions associated with good quality commercial bulk milk. On the other hand, all our general experience of the supplies coming into the creameries concerned indicated that the proportion of producers habitually adding water was extremely small and would not affect the bulked supply. There was, moreover, no evidence that water was gaining admittance to the milk during its passage through the

creameries. The alternative contention that the observed depressions were greater than would be obtained on genuine milk is a most unlikely one. Abnormally large depressions would only be observed if souring had begun, if the greater part of the milk in each tank had come from cows suffering badly from mastitis or if a large proportion of the producers or of the creameries themselves had concentrated the milk before it was run into the tankers. Acidity tests proved that the first of these objections did not exist, there was no evidence of the second, and the third can safely be ruled out.

All freezing-point tests were made on a Hortvet cryoscope, and the A.O.A.C.[5] technique was employed throughout the work.

The results obtained are shown in the form of a frequency distribution in Table III.

TABLE III

FREQUENCY DISTRIBUTION OF FREEZING-POINT DEPRESSIONS OF BULK MILK; 480 SAMPLES, MARCH 1938 TO FEBRUARY 1939

	Δ° C.	No. of samples
	0·542	2
	0·543	79
	0·544	229
	0·545	95
	0·546	51
	0·547	16
	0·548	8
Mean	0·544	480

The variation thus proved to be small, 0·542° to 0·548° C., and the mean of all observations was 0·544° C., a figure in complete agreement with the results obtained on individual cow and herd samples.

No significant differences were found between milk from one county and another, and no seasonal variations were observed, as will be seen by reference to Tables IV and V.

TABLE IV

FREEZING-POINT DEPRESSIONS OF BULK MILK FROM THREE COUNTIES IN ° C., 1938 TO 1939

County	Maximum	Minimum	Mean	No. of samples
Derbyshire	0·547	0·543	0·545	120
Westmorland	0·547	0·543	0·545	120
Sussex	0·548	0·542	0·544	240

TABLE V

MONTHLY FREEZING-POINT DEPRESSIONS OF BULK MILK IN ° C., 1938 TO 1939

Month	Maximum	Minimum	Mean	No. of samples
March, 1938	0·548	0·542	0·544	40
April	0·547	0·542	0·544	40
May	0·548	0·543	0·545	40
June	0·548	0·543	0·544	40
July	0·547	0·543	0·544	40
August	0·548	0·543	0·544	40
September	0·548	0·543	0·545	40
October	0·547	0·543	0·544	40
November	0·548	0·543	0·544	40
December	0·548	0·543	0·544	40
January, 1939	0·547	0·543	0·544	40
February	0·548	0·543	0·544	40

On the basis of the above results it seemed justifiable to accept the value of 0·544° C. as the freezing-point depression of genuine bulk milk. In order to make practical use of this figure it was thought advisable to extend a tolerance approximating to 1 per cent. of added water by classifying as genuine all milks that had a depression of 0·539° C. or more, but to calculate any added water on a basis of 0·544° C. in those cases where the depression was smaller than 0·539° C.

Thus added water per cent. $= 100\,\frac{(0{\cdot}544-\Delta)}{0{\cdot}544}$

where Δ, the observed depression, is smaller than 0·539° C.

Practical application of the tentative standard

It was decided to test the above conclusions by the systematic examination of bulk milk coming into London and to draw the attention of creamery managers to any supplies that fell below the tentative standard. Not only would many additional data be obtained, but frank discussion with the suppliers concerned would help to connect our freezing-point results with the information in their hands. It was anticipated that some reluctance to accept a higher standard than 0·530° C. might be expected, since this had been for so long the generally accepted figure for unbulked milk. Under modern conditions there is no deliberate addition of water to bulk supplies, but the possibility of small quantities of water gaining access cannot be ruled out.

Before the systematic examination could be put into operation, however, the war had started, and it was not until nearly the end of 1941 that it was possible to begin this work on milk received from a large number of suppliers.

As was anticipated, a certain amount of opposition to our conclusions was encountered, the two principal objections being—

(*a*) that milk could not contain added water if the S.N.F. were above 8·5 per cent.,
(*b*) that milk could not contain added water unless the freezing-point depression was smaller than 0·530° C.

Usually a discussion of the evidence on which we based our claims served the purpose of demonstrating that they were not made without reason, and an improvement in the quality of subsequent supplies was observed. In one or two instances our conclusions were not accepted, but an improvement in the quality took place after a time in spite of this.

Some examples of the results of these discussions may serve to illustrate the point.

Creamery A—On raising the question of presumed added water it was at first maintained that milk must be genuine if the freezing-point depression is not smaller than 0·530° C. After we had advanced our reasons and the creamery in question had made cryoscopic examinations of a number of their filled tankers, they were prepared to take 0·542° C. as the mean figure for genuine bulk milk, with a tolerance to 0·538° C.

We obtained the following results:

	Before discussion	After discussion
Maximum Δ° C.	0·544	0·549
Minimum Δ° C.	0·529	0·539
Mean Δ° C.	0·537	0·544
No. of samples	16	16

Creamery B—Here our suggestion that added water might be present was flatly denied, and our conclusions were not accepted. As this creamery did not possess a cryoscope, samples were submitted by them to a consulting chemist who stated that they conformed to the requirements of the Food and Drugs Act for genuine milk. Subsequently, however, the freezing-point of this supply improved and became satisfactory.

We obtained the following results:

	Before discussion	After discussion
Maximum Δ° C.	0·545	0·546
Minimum Δ° C.	0·526	0·539
Mean Δ° C.	0·535	0·543
No. of samples	13	13

Creamery C—The supplies from this creamery had been consistently good until on one occasion a tanker of milk containing some 3 per cent. of added water was received. In the reply to our enquiry it was stated that on the day in question they had been puzzled by what appeared to be a surplus of milk, which could not be explained by the most careful checking of the quantities handled. They admitted that our results explained this matter.

Creamery D—After supplies from this creamery had long consistently satisfied our standard, they suddenly became erratic and on occasions appeared to contain between 1 and 2 per cent. of added water. In reply to our note concerning this alteration in quality we received a letter which admitted the fault and explained that it had been traced to the careless use of a hose-pipe by a new member of the dairy staff.

An interesting check on the conclusions drawn from the cryoscopic examination is often possible when one of a number of tankers received on the same day from a particular creamery contains milk that appears to contain added water. By calculating the original composition of such milk it can be seen that the resultant figures are of the same order as those of the satisfactory samples. This point is illustrated by Table VI.

TABLE VI

EFFECT OF ADDED WATER ON THE FAT AND S.N.F. OF BULK MILK

Sample	Fat %	S.N.F. %	Δ° C.	A.W. %	Calculated original composition: Fat %	Calculated original composition: S.N.F. %
Creamery E. Tank 1 . ..	4·10	8·91	0·547	nil	—	—
" 2	4·15	8·89	0·546	"	—	—
" 3	3·95	8·83	0·538	1·1	3·99	8·93
" 4	4·20	8·93	0·547	nil	—	—
Creamery F. Tank 1 (Front) ..	3·70	8·60	0·529	2·8	3·81	8·85
" (Rear) ..	4·10	8·86	0·541	nil	—	—
Creamery G. Tank 1	3·70	8·75	0·535	1·7	3·76	8·90
" 2	3·80	8·85	0·540	nil	—	—
Creamery H. Tank 1	3·70	8·85	0·544	nil	—	—
" 2	3·50	8·76	0·546	"	—	—
" 3	3·50	8·61	0·534	1·8	3·56	8·77
Creamery J. Tank 1 (Front) ..	3·20	8·55	0·530	2·6	3·29	8·78
" (Rear) ..	3·30	8·72	0·542	nil	—	—
Creamery K. Tank 1	3·80	8·80	0·544	nil	—	—
" 2	3·95	8·63	0·534	1·8	4·02	8·79
" 3	3·90	8·84	0·544	nil	—	—

TABLE VII

FREQUENCY DISTRIBUTION OF FREEZING-POINT DEPRESSIONS OF BULK MILK, 1942 TO 1947

Δ° C.	Number of samples: 1942	1943	1944	1945	1946	1947
0·498						1
0·516	1					—
8	—				1	—
9	—				1	—
0·520	—				1	—
3	1				1	—
4	2				—	—
5	2				1	—
6	2			1	—	—
7	4	2	3	—	—	—
8	4	1	—	2	—	1
9	6	1	2	—	1	1
0·530	12	2	4	1	2	2
1	10	2	—	—	1	2
2	8	2	4	5	2	—
3	13	3	2	1	5	2
4	17	9	2	2	10	2
5	23	7	2	4	10	4
6	31	15	3	4	20	1
7	31	18	—	1	10	4
8	37	18	1	3	4	—
9	53	33	17	15	63	50
·540	77	70	123	155	187	166
1	59	51	109	167	172	139
2	66	85	270	366	304	252
3	67	152	558	524	463	337
4	67	167	547	438	440	412
5	48	142	99	45	162	267
6	50	153	45	21	80	134
7	43	110	43	11	23	50
8	46	112	37	8	10	6
9	12	113	16	3	1	6
0·550	—	—	—	—	4	1
Total	792	1268	1887	1777	1979	1840
Mean Δ:						
All samples ..	0·541	0·543	0·543	0·543	0·543	0·543
Genuine samples	0·543	0·544	0·543	0·543	0·543	0·543

SUMMARY OF RESULTS OF SIX YEARS' APPLICATION OF THE TENTATIVE STANDARD

During 1942 a number of tank samples from all sources proved to contain small amounts of added water. This state of affairs was doubtless due to the difficulties under which the receiving depots were operating because of the war, not the least of which was that of staff. By the end of the year a considerable general improvement in the quality of incoming supplies was noticeable, and the position was greatly improved during 1943. The proportion of unsatisfactory samples has remained small during 1944, 1945, 1946 and 1947, and no supply has been consistently below our tentative standard during these last four years.

In Table VII is shown the frequency distribution of the results obtained in the six years, and it will be observed that the proportion of samples with depressions less than 0·539° C., *i.e.*, above the ruled line in the Table, has become much smaller since the first year of systematic examination.

Table VIII gives the percentage of samples falling in the following three groups during the period 1942 to 1947; below 0·530° C., 0·530° to 0·538° C., and 0·539° to 0·550° C.

TABLE VIII

PERCENTAGE DISTRIBUTION OF FREEZING-POINT DEPRESSIONS OF BULK MILK, 1942 TO 1947

	Percentage of samples					
Δ° C.	1942	1943	1944	1945	1946	1947
0·529 and below	2·78	0·32	0·26	0·17	0·30	0·16
0·530 to 0·538	22·98	6·00	0·95	1·18	3·23	0·92
0·539 to 0·550	74·24	93·68	98·79	98·65	96·47	98·92

In Table IX is shown the proportion of samples classified as unsatisfactory over the six-year period. Although the number of samples examined yearly has considerably increased since 1942, the number falling below the tentative standard has declined sharply. This decline is regarded as a confirmation of the ideas set forth in this paper.

TABLE IX

PROPORTION OF BULK MILK SAMPLES FALLING BELOW THE TENTATIVE STANDARD OF 0·539° C., 1942 TO 1947

Year	Total number of samples	Number below standard	Per cent. below standard
1942	792	204	25·8
1943	1268	80	6·3
1944	1887	23	1·2
1945	1777	24	1·4
1946	1979	70	3·5
1947	1840	20	1·1

It is of interest to note that during 1946 the procedure previously used of notifying suppliers immediately of suspected added water was temporarily discontinued. The results of this are shown very strikingly by the fact that in that year the percentage of samples falling below the tentative standard increased. Resumption of the former procedure in 1947 was accompanied by a decrease in the number of unsatisfactory samples.

SUMMARY

A tentative standard for the freezing point of bulk milk is proposed, and the effect of the application of this standard to bulk milk supplies received in London is discussed.

I wish to thank the Chairman of the Express Dairy Co. Ltd. for permission to publish this work.

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Discussion

Mr. J. King congratulated the author on what was the most authoritative account he had seen of the examination of the freezing-point depression of bulk milks over an extended period. He enquired if the author could say whether in bulk milks there was a smoothing out of the small differences noted by the workers at the National Institute for Research in Dairying between morning and evening milk, and the smaller depression of the freezing-point noticed when cows are given access to a lush type of grass in the spring.

Dr. R. C. Wright said that United Dairies were very interested in the subject of the paper and had carried out a similar examination of rail and road tank milks coming into their London depots. The results were very similar to those reported by Mr. Macdonald: for instance, the mean depression for tank milks giving depressions over 0·539° was 0·544° in 1946 and 0·543° in 1947.

The question of sampling was most important, and he wondered whether Mr. Macdonald's samples were all taken from the road or rail tanks before discharge.

Concerning the absence of seasonal variations in Mr. Macdonald's results, the speaker had found indications of a minimum depression in each of the past three years. It occurred in May or June—similar findings had been reported by the National Institute.

Finally, Dr. Wright drew attention to the variation in frequency distribution shown in the author's tables, and wondered why the broad distribution of depressions between 0·540° and 0·550° noted in 1942 and 1943 changed to a peak distribution around 0·543° to 0·544° in the subsequent years. Was this connected with acidity?

Mr. G. Taylor said he was interested in the influence of acidity on the freezing-point. He gathered that Mr. Macdonald's figures showed no variation of depression due to acidity. Was there no difference between hot and cold weather in this respect? Had Mr. Macdonald any information as to the effect on the depression of variations of acidity within the limits of, say, 0·16 per cent. of lactic acid?

Mr. N. Heron said he considered 0·545° a reasonable average depression for fresh milk, but he had the impression that the value was tending to drop, possibly owing to war conditions. The limit of 0·539 adopted by Mr. Macdonald was considerably higher than that now insisted upon by Public Analysts, 0·530. The average depression of the milks analysed by him was 0·535 and those from large creameries had an amazing habit of being just above the lower limit.

Dr. J. R. Nicholls noted that the frequency distribution data shown by the author did not quite correspond to a "normal" curve. He considered that results corresponding to quantities of excess water of the order of 1 per cent. could easily be due to lack of care in seeing that no water is left in the equipment rather than to deliberate addition of water.

Dr. J. H. Hamence, returning to the subject of acidity, asked if the author could indicate what was the normal acidity of bulk milk; in view of the consistency of his data it would be a useful figure to know.

Mr. Macdonald, in reply to Mr. King and Dr. Wright, said that he had not observed any obvious seasonal variation in the freezing-point depression of bulk milk. In answer to Dr. Wright's further questions, all of the samples were taken from the rail or road tanks before discharge, and he could give no reason for the variation in frequency distribution beyond stating that it was unlikely to be connected with acidity. Replying to Mr. Taylor's and Dr. Hamence's questions on acidity, the normal acidity of bulk milk fell between 0·14 and 0·16 expressed as a percentage of lactic acid. Brine-cooled milk in properly insulated tankers did not normally develop acidity during transit, and if rejection of unsatisfactory supplies was carried out efficiently at the creamery the acidity was unlikely to rise above the maximum value stated above. It could be taken that an increase of 0·01 per cent. of lactic acid would increase the depression by about 0·003° C. at this level.

The Determination of Linoleic Acid in Edible Fats

By W. J. Stainsby

(*Read at the Meeting of the Society on April 7th*, 1948)

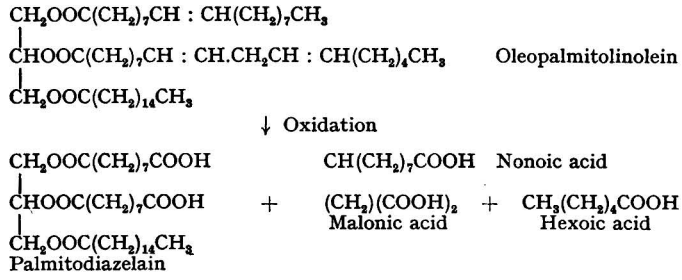
Two methods are in use at the present time for the determination of the fatty acid composition of edible fats containing linoleic acid. They are the thiocyanogen method of Kaufmann,[1] which has been modified by several investigators[2,3,4] and the more recent spectrophotometric method which depends upon alkali isomerisation of the unsaturated linoleic acid to form a conjugated acid and measurement of the latter by absorption in the ultra-violet region of the spectrum.[5,6]

Both these methods have been the subject of much research and have been standardised so that reliable results can be obtained, but an alternative procedure, which involves no special apparatus or elaborate technique may, it is considered, find useful application. The thiocyanogen method is difficult to use and in order to obtain reliable results strict precautions

must be taken to exclude all traces of moisture from the reagents and during the estimation. The spectrophotometric method employs special apparatus and technique and is therefore not available in many laboratories.

These considerations have led to the development of a simple method for the determination of linoleic acid in edible fats, which involves no special reagents or precautions and utilises apparatus available in all food laboratories.

To calculate the composition of a fat containing saturated acids and oleic and linoleic acids, three simultaneous equations are necessary. These are the total acid equation, another involving the use of the iodine values of oleic and linoleic acid, and a third which usually makes use of the thiocyanogen values of the unsaturated acids. The new method is based on the quantitative determination of the acidic glycerides produced by oxidation of the fat, and enables the equation involving the thiocyanogen values to be replaced by one using the titration of the acid groups produced by rupture of the unsaturated linkages. For example, a typical glyceride of cottonseed oil, oleopalmitolinolein, can be oxidised in acetone solution by means of potassium permanganate to give rise to the following oxidation products:



It will be seen that both linoleic acid and oleic acid on oxidation give rise to one free acid group attached to the glyceride residue and, providing the other acid fission products can be removed, determination of the acid value of the azelaoglycerides can be used to formulate a third equation.

Investigation has shown that the acids of lower molecular weight produced on oxidation can be quantitatively removed, and a method for the determination of linoleic acid in edible fats based on determination of the acidic glycerides has been developed.

Oxidation of the fat—Oxidation of fats by potassium permanganate in anhydrous acetone has been studied extensively by Hilditch and his co-workers, and the ease with which almost quantitative recovery of the acidic glycerides from the more saturated fats can be obtained has been known for some time. It has been firmly established that, under the conditions employed, cleavage of the unsaturated linkage occurs with production of two carboxyl groups, one attached to each of the oxidation products. The method therefore has for its initial stage the oxidation of the fat in anhydrous acetone with powdered permanganate.

Extraction of oxidation products—In preliminary experiments the oxidised fat, after removal of the acetone by distillation, was freed from manganese oxides and unchanged permanganate by means of sulphur dioxide and the fat was taken up in ether and washed free from salts and mineral acid. When sulphur dioxide was used, however, it was found extremely difficult to remove the excess from the ether solution and it remained even after the free fatty acids had been distilled off. The excess of sulphur dioxide could be first removed by titration with permanganate, but this proved tedious and the manganese oxides were finally brought into solution by using a mixture of sulphuric acid and hydrogen peroxide.

Removal of free acid—The most convenient method for removing the free acids is steam distillation. Preliminary experiments indicated that this was fairly satisfactory, but owing to the vigorous action of the steam a small quantity of fat tended to be carried over as fine droplets. To standardise the distillation procedure the Polenske apparatus was used, and by distilling known volumes of water and determining the volatile acidity distilling in various times, a standard procedure was evolved which ensured that the volatile acidity had been effectively removed.

Titration of the acidic glycerides—After removal of the volatile free acids the remaining acidic glycerides were dissolved in neutral alcohol - ether mixture and titrated with standard alkali in presence of phenolphthalein.

METHOD

REAGENTS—

Anhydrous acetone—Obtained by allowing acetone to stand over calcium chloride for 24 hours and, after filtration, distilling over barium hydrate.

Potassium permanganate—Ground and sifted through a No. 60 sieve.

Standard alkali—0·1 *N* sodium or potassium hydroxide.

Sulphuric acid—0·1 *N*.

Hydrogen peroxide solution (20 vols.).

PROCEDURE—

Weigh 1 g. of the fat into a 250-ml. round-bottomed flask and dissolve it in 100 ml. of anhydrous acetone. Attach the flask to a reflux condenser and stand it on a hot plate. Add in three equal portions, at intervals of 5 minutes, a total of 5 g. of the powdered permanganate and heat the mixture under reflux for 1 hour. Then gently distil off the acetone, removing the last traces under suction by a water pump.

Add 50 ml. of *N* sulphuric acid, followed by hydrogen peroxide solution until all the manganese oxides are converted into manganese sulphate; this usually requires about 25 ml. of hydrogen peroxide solution. Transfer the contents of the flask to a 250-ml. separating funnel and extract the fat with 100 ml. of redistilled ether. After separation, re-extract the lower aqueous layer in another funnel with a further 75 ml. of ether. Shake the first ether extract with 40 ml. of distilled water, run the water washing, after separation, into the second funnel and shake it with the second ether extract. Wash the ether layers in this way four times with successive 40-ml. quantities of distilled water and then combine the ether layers and wash once more with a fifth 40-ml. quantity of distilled water.

Transfer the ether solution to a 300-ml. Pyrex Polenske flask and remove the ether by distillation. Add 210 ml. of recently boiled distilled water and distil off 200 ml.; then add a further 100 ml. of distilled water and distil this off, thus obtaining a total of 300 ml. of distillate. Cool the flask and take up the fat - water residue in a neutral mixture of 50 ml. of alcohol and 25 ml. of ether. (The solution should be clear or only slightly opalescent in analysis of the more saturated fats, otherwise the titration will not be complete.) Finally, titrate the acidic glycerides with 0·1 *N* sodium or potassium hydroxide.

CALCULATION—

To calculate the percentage of linoleic acid the three equations used are

$$O + L + S = 100$$

$$(86{\cdot}2 \times O) + (173{\cdot}6 \times L) = 100 \times \text{I.V.}$$

$$\frac{56{\cdot}1}{294{\cdot}7} O + \frac{56{\cdot}1}{292{\cdot}7} L = \frac{5{\cdot}61 \times T}{10 \times W}$$

where O = percentage of oleoglycerides

L = percentage of linoleoglycerides

S = percentage of saturated acid glycerides

I.V. = iodine value of fat

T = ml. of standard alkali (corrected to 0·1 *N*) used in determination

W = weight of fat, in g., taken for oxidation.

From these three equations

$$\text{Linoleic acid, per cent.} = \frac{100 \times \text{I.V.}}{86{\cdot}82} - \frac{2{\cdot}927 \times T}{W}$$

Example—

Cottonseed oil.	Iodine value	104·7
	Weight taken	1·025 g.
	Titration	25·55 ml.

$$\text{Linoleic acid, per cent.} = \frac{100 \times 104{\cdot}7}{86{\cdot}82} - \frac{2{\cdot}927 \times 25{\cdot}55}{1{\cdot}025}$$

$$= 120{\cdot}6 - 73{\cdot}0 = 47{\cdot}6$$

EFFECT OF FREE ACIDITY—The method is intended for use with edible fats of low acid value, that is, below the B.P. limit. For fats of higher acid value there will arise errors due to uncertainty as to the exact nature of the free acids and the correction to be applied. Such fats are best neutralised before the determination is carried out.

COMPARISON WITH THIOCYANOGEN AND SPECTROPHOTOMETRIC METHODS—

For several hydrogenated cottonseed oils, three estimations of the thiocyanogen values were carried out on each and the corresponding linoleic acid contents are tabulated below.

TABLE I

	Thiocyanogen value			Linoleic acid percentage		
Iodine value	1	2	3	1	2	3
104·7	63·5	62·9	62·0	50·4	51·2	52·1
92·6	63·4	63·2	61·9	35·7	35·9	36·4
74·1	62·8	62·2	61·1	13·8	14·5	15·7
59·2	57·0	57·0	56·0	2·7	2·7	3·5
45·8	44·3	44·4	43·3	1·8	1·7	3·0

These cottonseed oils had been hydrogenated by the batch process with nickel - kieselguhr catalyst at 180° C., conditions that usually ensure a high degree of selectivity in the hydrogenation of linoleoglycerides. It is most probable that the amounts of linoleic acid shown as present in the fats of lower iodine value are not correct and are due to low thiocyanogen values given by this set of oils. The percentages of linoleic acid were calculated with use of the empirical value 90·8 for the thiocyanogen value of linoleoglycerides, as recommended by Hilditch and Murti.[4]

The linoleic acid contents of these hydrogenated cottonseed oils were determined by the oxidation method and the results are given in Table II.

TABLE II

	Oxidation value*		Linoleic acid percentage	
Iodine value	1	2	1	2
104·7	72·6	71·7	48·0	48·8
92·6	71·7	71·2	35·0	35·5
74·1	72·9	72·5	12·4	12·8
59·2	67·7	68·0	0·5	0·2
45·8	53·0	52·8	0·0	0·0

$$* \text{ Oxidation value} = \frac{2{\cdot}927 \times T}{W}$$

The results are lower than those obtained by the thiocyanogen method and it seemed desirable to establish the accuracy of the method by determinations on samples of oils the composition of which had been obtained by the fractionation method.

Through the courtesy of Professor T. P. Hilditch, three small samples of sesame, sunflower-seed and palm oils were procured and the results of the oxidation method were compared with those obtained by the fractionation procedure. It was considered that sunflower-seed oil was the most unsaturated oil likely to be met and that if any hydrolysis of the azaleoglycerides were to occur it would most probably take place in such a fat. The following results show that very good agreement was obtained with all three oils.

LINOLEIC ACID CONTENT

Type of oil	From oxidation procedure	From lead salt method*	From low temperature crystallisation*
Sesame oil	39·5	40·4	41·2
Sunflower-seed oil	68·0	66·2	67·5
Palm oil	9·6	8·2	9·6

* Communicated by Prof. Hilditch.

A further set of hydrogenated cottonseed oils that had been analysed for linoleic acid by the spectrophotometric method after alkali isomerisation of the fatty acids were subjected

to the thiocyanogen and oxidation methods and the linoleic acid contents of the oils found by all three methods are tabulated below.

LINOLEIC ACID CONTENTS OF HYDROGENATED COTTONSEED OILS

Iodine value of oil	Isomerisation method†	Thiocyanogen method	Oxidation method
102·0	42·1*	46·0	44·6
88·5	25·9	32·5	29·6
75·3	12·5	16·0	15·9
64·9	2·8	4·0	4·5
58·5	0·4	2·5	1·6
44·5	0·4	0·5	0

* Plus 2·0 per cent. of linolenic acid. † Communicated by Prof. Hilditch.

The results by the thiocyanogen and oxidation methods are in very good agreement, but the linoleic acid content of the hydrogenated oils obtained by the isomerisation process appear to be somewhat low; this may be due to the fact that the *iso*-acids produced by hydrogenation may not all be conjugated by the isomerisation process[7] used.

DETERMINATION OF THE TOTAL UNSATURATION OF A FAT CONTAINING LINOLENIC ACID

Linolenic acid on oxidation gives rise to one carboxyl group attached to the glyceryl residue, and for a fat containing oleic, linoleic and linolenic acids the oxidation equation becomes

$$\frac{56{\cdot}1}{294{\cdot}7}\,O + \frac{56{\cdot}1}{242{\cdot}7}\,L + \frac{56{\cdot}1}{290{\cdot}7}\,Le = \frac{5{\cdot}61 \times T}{10 \times W}$$

This reduces to

$$O + 1{\cdot}007\,L + 1{\cdot}014\,Le = \frac{T \times S \times 294{\cdot}7}{100 \times W}$$

The left-hand side of this equation is unfortunately not very different from that of the equation

$$O + L + Le = \text{Total unsaturated acids}$$

The differences are therefore extremely small and the accuracy of the determination of oxidation titration is insufficient to give a method for the determination of the linolenic acid in drying oils.

The first equation can, however, be reduced with very little loss of accuracy to

$$O + L + Le = \frac{2{\cdot}927 \times T}{W}$$

and this equation can be used to obtain the total unsaturated acids or the total saturated acids of oils containing linolenic acid in combination with two other unsaturated acids. This method appears to have certain advantages over the more complex Bertram oxidation usually employed.

SUMMARY—

A simple and time-saving method has been described for the determination of the composition of fats containing two unsaturated acids. The method is based on oxidation of the fat in anhydrous acetone with potassium permanganate followed by titration of the acidic glycerides after removal of the steam-volatile acid products. Results obtained with several types of oils compare very favourably with those obtained by the thiocyanogen and isomerisation methods. The method can be extended with very little loss of accuracy to determine the total unsaturated acids of oils containing more than two unsaturated acids.

The author is indebted to Prof. T. P. Hilditch, F.R.S., for advice and samples, to the Council of the Research Association and to the Department of Scientific and Industrial Research for permission to publish this paper.

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DISCUSSION

Mr. J. KING asked if the method was applicable to all other oils and fats likely to be met with. Petroselenic acid, present to the extent of 25 to 75 per cent. in seed oils of the *Umbelliferae* and *Arabaceae*, would when present in a glyceride oxidised under the conditions described give rise to lauric acid, the low volatility of which in the Reichert - Polenske apparatus used at 100° C. might require a subsequent longer steam distillation for the complete volatilisation essential for the accuracy of the method.

Dr. S. PAUL asked if the method could be applied to fats containing fatty acid chains in which the first double bond occurred in other positions than the normal 9 : 10 position as in oleic and linoleic acid. He also enquired what was the smallest percentage of linoleic acid that could be detected by the method.

Dr. STAINSBY, in reply, stated that, during hydrogenation, movement of the unsaturated linkages occurred and in view of the fact that the method had been quite successful when applied to hydrogenated fats it was reasonable to assume that it could be used for oils containing acids, the unsaturated linkages of which were not in the usual 9 : 10 position, but further work would obviously be necessary. The smallest amount of linoleic acid that could be safely detected was 1 per cent.

Determination of Fluorine in Organic Compounds

BY N. B. CHAPMAN, R. HEAP AND B. C. SAUNDERS

DURING the war we carried out extensive investigations on organic compounds containing fluorine with a view to the production of toxic materials (McCombie, H., and Saunders, B. C., Reports to the Ministry of Supply[1]). A survey of this work has recently been given by McCombie and Saunders.[2]

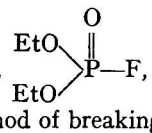
For these investigations it was of vital importance to have trustworthy methods of determining the fluorine contents of the compounds. In a report to the Ministry of Supply[3] we described the methods that we had found satisfactory for the types of compounds under investigation. Permission has now been granted to publish these methods, and details of them are given in the present communication.

The two types of fluorine compounds with which this communication deals are

(*a*) compounds containing the P–F link and known as fluorophosphonates.
(*b*) compounds containing the C–F link and known collectively as fluoroacetates.

In each class the problem may be resolved into two essential parts: (1) the breakdown of the organic compound under appropriate conditions to give a quantitative yield of fluoride ions in aqueous solution, and (2) the determination of the concentration of these fluoride ions. Methods of breaking down the organic compounds were examined and the procedure adopted for the fluorophosphonates was different from that used for the fluoroacetate series. From both, however, sodium fluoride was obtained as the breakdown product containing all the fluorine present. After numerous preliminary experiments we came to the conclusion that the best method of determining the quantity of fluoride ions in the products was by precipitation as lead chlorofluoride,[4] PbClF, which was then dissolved in dilute nitric acid and the chloride was determined by the Volhard method and calculated to the equivalent amount of fluorine. Conditions for the quantitative precipitation of lead chlorofluoride have been determined.

DETERMINATION OF FLUORINE IN FLUOROPHOSPHONATES

Diethyl fluorophosphonate,  , is a typical fluorophosphonate. The problem was to find (*a*) an efficient method of breaking down the fluorophosphonate so as to liberate fluoride ions quantitatively, and (*b*) a method of determining the fluoride ions in the presence of phosphorus-containing compounds.

BREAKDOWN OF THE FLUOROPHOSPHONATE—

The equation for the hydrolysis of alkyl fluorophosphonates by dilute aqueous sodium hydroxide was given in Report No. 5 on Fluorophosphonates[5] as

$$(RO)_2\overset{\overset{O}{\|}}{P}\text{–F} + 2NaOH \rightarrow (RO)_2\overset{\overset{O}{\|}}{P}\text{–ONa} + NaF + H_2O$$

Although this hydrolysis is usually fairly rapid it was desirable to obtain conditions of a general nature that would be likely (*a*) to effect the complete hydrolysis of the more resistant fluorophosphonates, (*b*) to ensure homogeneity of the reaction mixture so as to avoid possible mechanical losses of oily material and (*c*) to ensure that the hydrolysis does not proceed so far as to cause an accumulation of phosphate ions, which might interfere with the subsequent determination.

These conditions have been realised by treating the fluorophosphonic ester, dissolved in alcohol, with five times the quantity of sodium required by the equation:

$$2(RO)_2\overset{\overset{O}{\|}}{P}\text{–F} + 2Na + 2C_2H_5OH \rightarrow 2(RO)_2\overset{\overset{O}{\|}}{P}\text{–OC}_2H_5 + 2NaF + H_2$$

Further action of sodium ethoxide would convert the phosphate ester into a compound of the type $OP(OEt)_2ONa$, but this is known not to be attacked by sodium ethoxide in anhydrous alcohol and so the production of PO_4''' is unlikely to occur to any extent.[6]

It is not impossible that the sodium in the initial reaction may have a reducing action:

$$(RO)_2POF + 2Na + C_2H_5OH \rightarrow (RO)_2POH + NaF + C_2H_5ONa$$

A hydrogen phosphite might therefore be produced, but here again this compound is not likely to be broken down to any extent by the sodium ethoxide. The concentration of phosphate ions will therefore presumably be small.

The above method (of which details are given in the example on p. 439) requires at most 45 minutes' heating; with pure di-*iso*propyl fluorophosphonate 5 minutes' heating was found sufficient.

After the treatment with sodium, the reaction mixture was washed with water into a beaker and brought to the correct *p*H for the determination of fluoride.

DETERMINATION OF FLUORIDE IN THE DECOMPOSITION PRODUCTS—

After an investigation, described below, to ascertain the best conditions, the method adopted for the determination of the fluoride in the decomposition products was to precipitate it as lead chlorofluoride, PbClF, the chloride in which was titrated by the Volhard method and calculated as the equivalent amount of fluorine.

Sodium fluoride standard—To serve as a material of known fluoride content for studying and checking the method we prepared a specimen of sodium fluoride as follows. A.R. anhydrous sodium carbonate was slowly added to an excess of A.R. 40 per cent. hydrofluoric acid (containing not more than 0·2 per cent. of silica) in a platinum basin, and after standing for 4 hours the excess of acid was removed by heating. The mixture was then cooled, more acid was added, the whole thoroughly mixed and the acid again removed by heating. The mass was then fused in a muffle furnace, with the basin covered by a silica plate. After cooling, it was pulverised in an agate mortar and dried in a platinum crucible at 110° C.

Its fluorine content was determined gravimetrically as calcium fluoride by the Rose-Berzelius method. Briefly, this involved precipitating a weighed sample (about 0·4 g.) with 5 per cent. calcium chloride solution at the boiling point in presence of sodium carbonate. The precipitate, after standing overnight, was filtered—a slow process—and dried and ignited in a platinum crucible. The residue, which then became easily filterable, was digested with hot dilute acetic acid and evaporated to dryness. It was then transferred to a filter paper and washed with hot water, ignited and weighed.

Duplicate determinations gave 42·73 and 42·63 per cent. of fluorine in the product; mean, 42·68 per cent. (Calculated for NaF, 45·24 per cent.)

The same specimen of sodium fluoride (F = 42·7 per cent.) was used throughout the series of experiments described below.

THE LEAD CHLOROFLUORIDE METHOD—The estimation of fluoride as lead chlorofluoride, PbClF,* was first described by Starck[4] and developed by Hoffman and Lundell,[7] Hawley,[8] Fischer and Peisker[9] and Kapfenberger.[10] Hoffman and Lundell determined the correct *p*H and chloride ion concentration for the precipitation. The time for complete precipitation and the effect of varying amounts of fluoride on the composition of the precipitate were examined by Hawley, and these factors have now been re-examined by us in greater detail.

The particular variable factors investigated were:

(*a*) the concentration of fluoride at the time of precipitation,

(*b*) the lengths of time during which the precipitate is heated (t) and then allowed to remain in contact with the supernatant liquid before filtration (T).

Using quantities of the standard sodium fluoride (F = 42·7 per cent.) ranging from 0·1 to 0·2 g. and adopting definite times for t and T, we carried out determinations by the following general procedure.

To the sample of sodium fluoride, W g., dissolved in 100 ml. of distilled water in a 500-ml. squat beaker, were added 5 drops of bromophenol blue indicator and 3 ml. of 10 per cent. sodium chloride solution. The liquid was then diluted with 200 ml. of distilled water, and 40 per cent. nitric acid was added from a burette until the indicator turned yellow. The solution was rendered faintly blue by addition of 10 per cent. sodium hydroxide solution. One ml. of concentrated hydrochloric acid was then added from a burette and the solution heated for 10 minutes on a water-bath, a temperature of about 80° C. being reached. Finely powdered solid lead nitrate (5 ± 0·025 g.) was then added and the mixture gently stirred for 3 minutes. At the end of this time the lead nitrate had dissolved and a precipitate of lead chlorofluoride had formed. Pure crystalline sodium acetate, finely powdered (5 ± 0·025 g.), was then added and the mixture stirred gently until it had dissolved. The addition of the sodium acetate caused the colour of the indicator to change to blue. The sides of the beaker were washed with about 5 ml. of distilled water and the heating was continued, with occasional stirring, for a time t. The mixture was then removed from the water-bath and cooled and allowed to stand for a total time T.

The precipitate was then filtered off on a Swedish filter paper and washed once with water, four times with saturated PbClF solution and again once with water. This involved a total volume of washing liquids between 100 and 150 ml., giving a total volume of filtrate between 400 and 450 ml. The precipitate together with the paper was replaced in the original 500-ml. squat beaker together with 100 ml. of distilled water and heated to about 60° C. Fifteen ml. of 40 per cent. nitric acid solution were then added and the mixture was stirred at about 60° C. for 10 minutes. By this time the precipitate had completely dissolved.

A measured volume of standard silver nitrate solution, in excess of that required to precipitate all the chloride present, was run in from a burette. The precipitate was coagulated by heating on a water-bath and, after cooling in ice to room temperature, was filtered through a sintered glass crucible. The precipitate and filter paper were then stirred well with water and filtered again and pressed to remove adhering silver nitrate. The residual silver in the combined filtrates was then determined by titration with standard potassium thiocyanate solution, using as indicator 10 ml. of ferric alum solution (120 g. of ferric alum in 250 ml. of water, decolorised by about 50 ml. of nitric acid).

In the experiments where the lead, as well as the chloride, was determined, the two determinations were made on separate aliquots of the filtrate from the silver chloride and a slight modification of the procedure described above was necessary—

(*a*) Only the slightest excess of silver nitrate was used to precipitate the chloride, in order to avoid contamination of the lead sulphate precipitate with silver sulphate. It was found that, if the excess of silver nitrate used amounted to only about 0·75 ml. of 0·1 N solution in the whole solution, this excess of silver need not be removed previous to precipitation of the lead as sulphate. It could be removed by addition of a slight excess of 0·1 N hydrochloric acid and filtration, but comparison of results of lead determinations, with and without previous removal of silver in this manner, showed no difference, provided that the final lead sulphate precipitate was well washed.

(*b*) When the final solution containing the lead and silver had been filtered it was not titrated as a whole for silver, but was made up to 500 ml.; 200 ml. were used for the thiocyanate titration and two 100-ml. aliquots for the lead determination.

* Fluoride has been estimated as lead bromofluoride, PbBrF, by Vasil'ev[11]; this method seems to us to offer no special advantages.

Particulars of the experiments in which the fluorine content of the sodium fluoride standard was determined by the Volhard silver titration of the PbClF precipitate, as described in this section, are given in Table I and represented by curves A, B, C, D of Figs. 1 and 2.

TABLE I

RESULTS OF SILVER TITRATIONS

Weight, W, of NaF taken g.	Time, t, of heating	Time, T, of cooling and standing	Apparent fluorine content %	See curve
0·1050	½ hr.	cooled by standing in air; 40 hr.	42·39	A, Fig. 1
0·1640	"	" "	43·68	"
0·1948	"	" "	45·53	"
0·1100	½ hr.	" "	43·63	B, Fig. 1
0·1518	"	" "	43·91	"
0·1739	"	" "	44·08	"
0·1720	"	cooled in water; 1½ hr.	43·77	* Fig. 1
0·1067	¼ hr.	cooled in ice; 10 min.	42·07	C, Fig. 2
0·1586	"	" "	43·66	"
0·1300	¼ hr.	" "	42·97	D, Fig. 2
0·1657	"	" "	43·56	"
0·1853	"	" "	43·62	"

Lead determinations—The Volhard chloride determinations represented on curve B, Fig. 1, and on curve D, Fig. 2, were accompanied by lead determinations, represented by curves B′ and D′, in Figs. 1 and 2. These were carried out according to the following method. The precipitate of lead chlorofluoride having been dissolved, the chloride precipitated by a very slight excess of silver nitrate, and the filtrate therefrom made up to 500 ml., 100 ml. of this solution were taken and the lead was precipitated as sulphate. (It was found unnecessary to precipitate the slight amount of silver present as AgCl.) To the 100 ml. of solution taken, 120 ml. of "lead acid" (20 per cent. sulphuric acid saturated with lead sulphate) were added. This mixture was then evaporated until it fumed strongly. The cooled residue was then diluted with 105 ml. of water, and boiled well to free the lead sulphate from soluble salts. After standing for 24 hours the precipitate was filtered through a weighed Gooch crucible. The precipitate was washed with "lead acid," 50 per cent. alcohol, and then with pure alcohol. The crucibles were dried to constant weight by heating in a muffle furnace raised to dull redness. The estimations were duplicated, and the fluorine was calculated on the assumption that 1 atom of Pb corresponds to 1 atom of fluorine. The results are shown in Table II.

TABLE II

RESULTS OF LEAD ESTIMATIONS

	Weight of NaF taken g.	Apparent fluorine found %
Corresponding to curve B′	0·1100	44·55, 44·57
	0·1518	45·18, 45·37
	0·1739	46·14, 46·34
Corresponding to curve D′	0·1300	43·60, 43·64
	0·1657	43·58, 43·83
	0·1853	44·03, 43·73

DISCUSSION OF RESULTS—All the fluorine contents found in this series of experiments, whether the ending was by Volhard titration or lead determination, were calculated on the assumption that the whole of the fluorine in the sample was precipitated as pure PbClF, and they are here referred to as *apparent* fluorine contents. The true fluorine content of the sodium fluoride sample was taken to be that determined by the calcium fluoride method (p. 435) and is represented in Figs. 1 and 2 by the horizontal line Z.

From Figs. 1 and 2 it is clear that the deviation of the apparent fluorine from the true value varies with the amount of fluorine determined and also with the values of t and T, *i.e.*, the times during which the lead chlorofluoride precipitate is heated with the reaction mixture and subsequently remains in contact with it. For the determinations with Volhard ending, the effect of t and T is not very great but exceeds the probable experimental error.

The conditions giving the best results are those of curve D, Fig. 2, corresponding to a small value of T, 10 minutes. This is shown by

(*a*) the relatively small gradient of the curve;
(*b*) the close correspondence of the lead curve, D′, indicating that less co-precipitation has occurred;
(*c*) the closer approximation of the apparent fluorine contents on curve D than those on curve B to the true value.

The conditions corresponding to curve D were accordingly adopted for subsequent determinations.

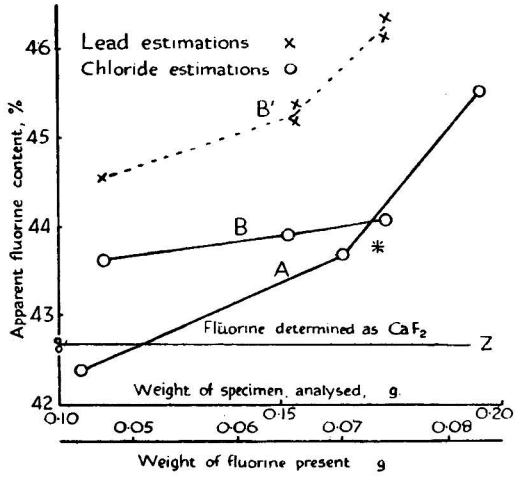


Fig. 1

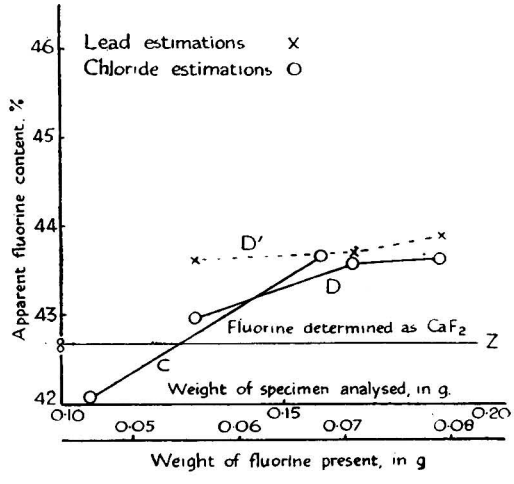


Fig. 2.

CORRECTION OF APPARENT FLUORINE CONTENTS—In Figs. 1 and 2 the error of a determination of apparent fluorine content corresponding to any point is represented by the vertical distance of the point above the horizontal line Z. If, therefore, through curve D a straight line is drawn fitting the points on the curve as closely as possible, its inclination to and distance from line Z will show how the average error in the *apparent* fluorine content (and therefore the correction necessary to obtain the true fluorine content from it) varies with the weight of fluorine determined. Corrections obtained from Fig. 2 in this way are in terms of percentage of fluorine in the sample of sodium fluoride analysed. But for general purposes, in the analysis of other materials, they have been converted into "correction factors" (= true quantity of fluorine/apparent quantity of fluorine).

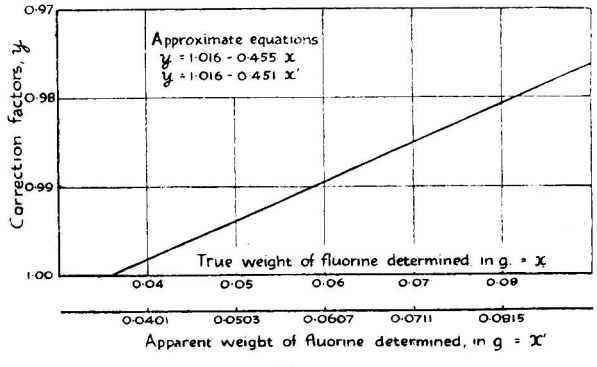


Fig. 3

Fig. 3 shows the correction factors, y, plotted against the weights of fluorine actually determined, x (in grams). When the apparent percentage of fluorine is multiplied by the

corresponding value of y, the true percentage of fluorine is obtained. For convenience, the weight of fluorine, x', apparently contained in the analysed sample is also marked.

These values of y may be represented approximately by the equations—

$$y = 1{\cdot}016 - 0{\cdot}455\,x; \qquad y = 1{\cdot}016 - 0{\cdot}451\,x'$$

If desired, the correction, instead of being made on the percentage of apparent fluorine finally calculated, may be made on the volume of 0·1 *N* silver nitrate consumed in the Volhard titration. For this purpose a graph equivalent to Fig. 3, but with ml. of 0·1 *N* silver nitrate plotted in place of the corresponding weights of fluorine, is constructed. The correction factors, ϕ, range, as a straight line, from 0·9988 for 20 ml., to 0·9825 for 40 ml., of 0·1 *N* silver nitrate consumed.

These corrections, being based on curve D, are valid only for determinations carried out under conditions to which that curve applies.

With the aid of the correction factors the silver titration method is estimated to give results having a maximum error of 1 part in 100 parts of the correct value.

It is recommended that the weight of fluorine determined be between 0·05 and 0·06 g. in order to keep the correction small.

Note—Owing to the diminished solubility of lead chloride in aqueous alcohol, not more than 12 ml. of alcohol should be present per 250 ml. of solution when precipitation of lead chlorofluoride takes place.

Analysis of sodium fluoride—As an additional cross-check a second specimen (B) of standard sodium fluoride was analysed by (*a*) the lead chlorofluoride method with Volhard ending and the appropriate correction and (*b*) the Berzelius - Rose calcium chloride method. The specimen B was finely powdered in an agate mortar and dried in a platinum crucible at 110° C. The results are given below.

(*a*) *PbClF method*

Standard $AgNO_3$ solution was 0·05063 *N*.

(1) 0·1347 g. NaF ≡ 60·9 ml. $AgNO_3$ solution.
Per cent. F (uncorr.) = 43·5
Per cent. F (corr.) = 43·06

(2) 0·1440 g. NaF ≡ 65·36 ml. $AgNO_3$ solution.
Per cent. F (uncorr.) = 43·66
Per cent. F (corr.) = 43·22
Mean: 43·14 ± 0·15 per cent.

(*b*) *CaF_2 method*

(1) 0·4345 g. NaF ≡ 0·3845 g. CaF_2
(2) 0·3971 g. NaF ≡ 0·3533 g. CaF_2
(3) 0·4402 g. NaF ≡ 0·3888 g. CaF_2
Per cent. F: (1) 43·30, (2) 43·06, (3) 42·97
Mean: 43·11 ± 0·15 per cent.

The close agreement between the results obtained by the two different methods confirms the validity of the empirical correction factor used.

SPECIMEN DETERMINATION OF FLUORINE IN DI-*iso*PROPYL FLUOROPHOSPHONATE—

A weight of fluorophosphonate, containing about 0·05 g. of fluorine, was dissolved in 10 ml. of dry alcohol and metallic sodium (about 0·5 g., *i.e.*, at least 5 equivalents) was added. After the sodium had dissolved, the mixture was gently heated under reflux for 5 minutes* and then washed out with about 100 ml. of water into a beaker, made acid to bromophenol blue with dilute nitric acid and then just alkaline with 10 per cent. sodium hydroxide solution. Three ml. of 10 per cent. sodium chloride solution were added and the solution was diluted to 250 ml. One ml. of concentrated hydrochloric acid was added, and the solution heated on a water-bath to about 80° C. Then 5·0 g. of finely powdered A.R. lead nitrate were added with stirring (still at 80° C.). As soon as all the lead nitrate had dissolved, 5·0 g. of crystalline sodium acetate were added, with vigorous stirring. The product was then heated on the water-bath for 15 minutes and cooled in ice, and the precipitate was filtered off on a

* In general, with a fluorophosphonate of unknown composition, 45 min. should be allowed.

Swedish filter paper. It was washed once with water, four times with saturated PbClF solution and finally once with water. It was then transferred to the beaker in which the precipitation had been carried out, 100 ml. of 5 per cent. nitric acid were added and the paper was macerated. A measured excess of 0·1 *N* silver nitrate solution was added, and the mixture was heated on the water-bath for 30 minutes and cooled in the dark. It was then filtered through a No. 4 sintered Gooch crucible and the filtrate titrated with 0·1 *N* potassium thiocyanate in presence of 10 ml. of a saturated ferric alum solution as indicator (the indicator solution being rendered clear by addition of nitric acid).

Weights of sample taken: 0·8459 and 0·8166 g.
Fluorine found, uncorrected: 10·43 and 10·58 per cent.
Mean: 10·5 ± 0·1 per cent.
Corrected mean: 10·3 ± 0·15 per cent.
Calculated for $(C_3H_7O)_2POF$: F = 10·32 per cent.

Determination of fluorine in fluoroacetates

Here again the fluorine was determined ultimately by precipitation as lead chlorofluoride, but the breakdown of the organic compound is more difficult than with the fluorophosphonates. The two methods recommended are:

(*a*) Fusion with metallic sodium in an evacuated tube at 400° (*cf.* Elving and Ligett[12]).
(*b*) Fusion in a stainless steel bomb with sodium peroxide as recommended by Briscoe and Eméleus,[13] and described earlier by Finger and Meed.[14]

Details of both these methods as used by us are given below in specimen analyses. With regard to the bomb method, the lead washer supplied by the manufacturers was useless; the lead melted and disintegrated at the temperature of the reaction. Copper was also found to be attacked and was otherwise unsatisfactory. Gold, on the other hand, was found to be most suitable. It is soft, will withstand the required temperature without melting or disintegrating and is not attacked by fluorine, fluoride or alkali.

Fluoroacetamide standard—For standardising the estimation of fluorine in fluoroacetates a compound of undisputed purity containing the CH_2F group is required. It is important to select a stable compound that has some other element capable of independent determination. Fluoroacetamide was found to satisfy these conditions. It could be obtained pure, and ammonia determination provided a rigid cross-check.

A pure specimen was prepared as follows (*cf.* McCombie and Saunders[15]). Methyl fluoroacetate (39 g.) was shaken with 30 ml. of ammonia solution (sp.gr. = 0·89). Heat was evolved, and on standing overnight the first crop of almost pure crystals was deposited. These were filtered off and dried (yield, 16 g.). By concentrating the filtrate *in vacuo* a second crop of crystals was obtained (yield, 5 g.). The two crops were united and could be recrystallised from chloroform (fine needles) or acetone (prisms). We used dry chloroform and each recrystallisation gave about 75 per cent. yield. After recrystallising three times, the final yield of pure substance was 8·7 g., m.p. 108° C.

The determination of nitrogen in the pure product was performed by the usual method of slow distillation with excess of dilute sodium hydroxide solution, the evolved ammonia being collected, during 4 hours' distillation, in standard acid, and determined by titration as usual, with methyl orange as indicator. The yield of ammonia corresponded to 18·19 per cent. of nitrogen in this sample. Calculated for FCH_2CONH_2: N = 18·18 per cent. The standard fluoroacetamide was therefore 100 per cent. pure.

Specimen determination of fluorine in methyl fluoroacetate by fusion with sodium—

A specimen of methyl fluoroacetate, b.p. 104·5° C., was analysed for fluorine as follows (*cf.* Elving and Ligett[12]). The liquid (0·25 g.) was weighed in a small closed glass ampoule which was then introduced into a thick-walled glass tube containing 0·4 g. of sodium in 5 ml. of ether. The tube was then evacuated and, after the ether had been removed, sealed off while still connected to the water pump. It was then heated to 400° C. for 1 hour in a Carius oven. After cooling, the tube was opened and the contents treated with alcohol and washed out with water. After removal of the excess of alcohol by evaporation to small bulk, the liquid was diluted to about 100 ml., filtered and made up to 250 ml. The fluorine in it was then determined by the PbClF method given above.

Found: F = 20·80 per cent. (uncorr.) = 20·65 per cent. (corrected).
Calculated for $CH_2F.COOCH_3$: F = 20·64 per cent.

STANDARDISATION OF THE SODIUM PEROXIDE BOMB-METHOD WITH PURE FLUOROACETAMIDE—

For this determination a quantity of the fluorine-containing sample containing approximately 0·06 g. of fluorine was in general used. For fluoroacetamide, therefore, the quantity weighed out was 0·24 g. This was enclosed in the bomb with about 2·5 g. of sodium peroxide and 0·05 g. of cane-sugar (which may sometimes be omitted). The bomb was heated gently to start the combustion. The reaction products (after cooling) were dissolved out in about 200 ml. of hot water and boiled to destroy hydrogen peroxide. The fluorine was then determined by the PbClF method with Volhard finish, using the correction curve obtained previously. The PbClF precipitate was allowed to stand for ½ hour and cooled immediately in ice.

Weight of sample taken = 0·2380 g.
Volume of 0·1 *N* silver nitrate required = 31·20 ml.
Apparent fluorine content = 24·91 per cent.
Correction factor = 0·991.
Hence corrected fluorine content = 24·69 per cent.
Calculated for FCH_2CONH_2: F = 24·68 per cent.

SUMMARY

Efficient methods are described for the determination of fluorine in (*a*) fluorophosphonates and (*b*) fluoroacetates and related compounds. After the organic compound has been broken down to sodium fluoride, the actual estimation can be carried out well within 90 minutes.

A very convenient reference standard for estimations in the fluoroacetate series is fluoroacetamide, FCH_2CONH_2. This is a stable highly-crystalline solid and can be obtained in a high state of purity.

During the course of the work several hundred organic compounds containing fluorine have been analysed successfully by the methods described in this communication.

We are greatly indebted to the Chief Scientist, Ministry of Supply, who has given permission for the publication of the work. We are also grateful to F. J. Buckle, H. G. Cook, F. L. M. Pattison, F. E. Smith, G. J. Stacey and I. G. E. Wilding, all of whom have carried out numerous analyses by the methods described.

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March, 1947

The Ultra-violet Absorption of Riboflavine

By D. C. M. ADAMSON

ALTHOUGH the characteristic absorption exhibited by riboflavine was used to a great extent in elucidating its structure, little work has been reported on the values of the extinctions at various wavelengths. Kuhn *et al.*[1] quote the following values for aqueous solutions:

TABLE I

λ	X	$E^{1\%}_{1cm.} = \frac{X}{2{\cdot}3 \times 36{\cdot}4}$
445 mμ.	$2{\cdot}40 \times 10^4$	286
365 "	$2{\cdot}15 \times 10^4$	257
265 "	$7{\cdot}8 \times 10^4$	931
220 "	$7{\cdot}1 \times 10^4$	847

where $X = \frac{2{\cdot}30}{c.d} \log \frac{I_o}{I}$, c being expressed in g.-mols. per litre and d in cm. These authors took the molecular weight of riboflavine as 364, instead of the true value 376.

Elvidge[2] has given the value 287 for $E^{1cm.}_{1\%}$ at 445 mμ. on the Medical Research Council standard and further states that samples of B.P. riboflavine have given values for $E^{1\%}_{1cm.}$ at this wavelength ranging from 275 to 305, average 290. Unfortunately, neither the number of samples nor the medium was stated.

In contrast to the wavelengths quoted by Kuhn *et al.*,[1] Rosenberg[3] states that riboflavine shows absorption maxima at 445, 372, 269 and 225 mμ., agreeing closely with those stated by Warburg and Christian,[4] *viz.*, 454, 370 and 269 mμ. It was thought that it would be of value to re-examine this property of riboflavine with a view to utilising the data for analytical control work. Of the four absorption maxima, that at about 370 mμ. is, under optimum conditions, resolvable into two peaks very close together. With a discontinuous light source and fast plates, this maximum is seldom resolved and in the following work the peak has been treated as if it were a single absorption band. The absorption maximum at 220 to 225 mμ. can be recorded only by the use of the thinnest cells and is unsuitable for routine work. Attention has therefore been focussed on the three peaks at 445, 371 and 266 mμ., the first of which is in the visible region.

EXPERIMENTAL—

The solubility of riboflavine in pure water is small and two methods exist for effecting its solution, the first that prescribed in the B.P. for determining its specific rotation and the second that employed by the Microbiological Panel of the Society's Sub-Committee on Vitamin Estimations, which was found to be capable of producing a solution ten times as strong as that used for microbiological work.[5]

Preliminary work showed that less satisfactory agreement between replicate results was obtained with solutions prepared by means of alkali, and that increasing amounts of alkali increase the value for the extinction of the 266 and 371 mμ. peaks, the visible peak remaining unaffected. The acetic acid solution[5] gave more constant results and has been used exclusively in obtaining the values given below.

Preparation of solutions—18 to 20 mg. of sample, accurately weighed, were transferred with distilled water to a boiling tube containing 1 ml. of glacial acetic acid and the volume was made up to 20 to 25 ml.

The boiling tube was then immersed in boiling water until a clear solution resulted and, after cooling, the contents were transferred to a 100-ml. graduated flask and made up to volume. The absorption spectra were recorded with a Hilger medium quartz spectrograph fitted with Spekker photometer, on Ilford 1S0-Zenith plates, match points being observed visually. With a 0·33-cm. cell, the 371 and 445 mμ. peaks were recorded and with a 0·1-cm. cell the 266 mμ. peak.

Results—Twenty samples of riboflavine have been examined, each in duplicate, and each plate being independently spotted, with repeats on the few occasions where agreement was not reached. The observed wavelengths at which maximal absorption occurred did not

vary more than 2 mμ. for any sample; their average values were 445, 371 and 266 mμ., and the variation in the last was only 265 to 266 mμ.

Table II shows the observed values of the extinction at each of the three wavelengths.

TABLE II

Sample	$E^{1\%}_{1\,cm.}$ 266 mμ.	$E^{1\%}_{1\,cm.}$ 371 mμ.	$E^{1\%}_{1\,cm.}$ 445 mμ.
1	764	267	301
2	792	264	299
3	755	260	275
4	777	269	312
5	780	266	291
6	793	267	306
7	799	268	309
8	792	268	306
9	803	269	303
10	754	276	301
11	759	260	304
12	741	266	298
13	759	260	298
14	771	259	305
15	772	257	300
16	790	261	301
17	783	266	302
18	785	264	306
19	763	260	302
20	803	267	307
Average	776·7	264·7	301·3
Standard deviation	18·1	4·6	7·7

Comparison of these average results with those calculated from the original data of Kuhn *et al.* given in the third column of Table I, shows good agreement for wavelengths 445 and 371 mμ., but for 265 mμ. agreement is not good.

It was possible that some destruction of riboflavine would occur owing to irradiation with ultra-violet light during the recording of the spectra at different apertures, and re-examination of the solutions after a prolonged irradiation was carried out in order to observe the effect, if any, on the extinctions. In obtaining the results given in the table, each solution was exposed for about 60 seconds before the peak absorption was recorded, and, since each solution was exposed for a further 60 seconds in recording extinctions greater than that of the peak, an irradiation of 7 minutes, followed by a second exposure in the same manner as the first, gave a tenfold increase of irradiation between the first and second plate. This showed that any change in ultra-violet light absorption was too small to be detected. This of course does not prove that degradation to lumichrome does not take place, but it shows that the characteristic absorption is unchanged.

The results in Table II were obtained on samples of production material; Table III gives a few results for samples of riboflavine from other sources.

TABLE III

Sample	$E^{1\%}_{1\,cm.}$ 266 mμ.	$E^{1\%}_{1\,cm.}$ 371 mμ.	$E^{1\%}_{1\,cm.}$ 445 mμ.
A	768	254	277
B	768	260	279
C	769	267	302
D	747	248	272
E	772	262	295
F	734	250	274
G	747	259	299
H	741	264	306
I	781	274	301
J	770	260	306
K	784	265	296
L	771	270	299
M	791	269	314

Samples A to E were alleged to be pure riboflavine and, with the exception of A, all were of American origin. F and G were samples of crude material obtained in the laboratory

by a process different from that used in the manufacture of the production samples cited in Table II, and samples H to M were pure samples prepared by this process.

SUMMARY

The extinction values for the absorption bands exhibited by riboflavine in dilute acetic acid solution at 266, 371 and 445 mμ. have been determined and found to be $E^{1\%}_{1cm.} = 777$, 265 and 301, with standard deviations of 18·1, 4·6 and 7·7 respectively. Since these absorption bands are associated with the *iso*-alloxazine portion of the molecule they can no more be taken as proof of identity than can a determination of total fluorescence or total nitrogen (*cf.* U.S.P. XIII and B.P. 1932, 6th Add.), but such constants have value in control work on pharmaceutical preparations, which prompts the presentation of these figures.

Acknowledgements are made to J. P. Gordon, B.Sc., for the practical work, and to Roche Products Ltd. for permission to publish these data.

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January, 1948

Micro-Estimation of Zinc in Teeth

BY D. B. CRUICKSHANK

THIS work was undertaken as part of a research programme into certain problems relating to calcification phenomena in tuberculosis—a known mechanism of defence against the disease. The present communication deals only with certain difficulties encountered during the course of the analyses; the general results of the investigation will be reported in full elsewhere.

In earlier phases of the investigation a modified Sylvester and Hughes method[1] was used (range 0·1 to 1·0 mg.) and this proved most satisfactory. Later, when it became necessary to estimate zinc in the 0 to 20 μg. range, all attempts to reduce the above method to a micro-scale (by using 1/5th volumes and a Rheburg or Conway burette) failed on account of an unexpected difficulty, *viz.*, that instead of obtaining a simple constant linear relation between zinc and iodine (Lang's reaction,[2] $3Zn \equiv I_2$) the ratio *varied as a function of the zinc concentration*, so precluding all possibility of accurate estimations. A typical titration curve is shown in Fig. 1.

Extensive investigation into the cause of these anomalies points to the formation of a series of complex ferrocyanides of zinc, which will form the subject of a separate note (*Research*, in press).

TABLE I

ZINC RECOVERED AFTER FULL EXTRACTION PROCESS

Zinc added		Zinc recovered	
Batch 1	Batch 2	Individual titrations	Mean
μg.	μg.	μg.	μg.
0	—	—	11·1
5	—	4·3, 4·6, 3·8	4·3
10	—	10·4, 10·4, 9·1	9·9
—	15	15·2, 15·4, 14·1	14·9
20	—	19·3, 20·6, 20·6	20·1
—	20	20·0, 20·7, 18·7	19·7
—	25	25·7, 24·1, 24·9	24·9
—	35	34·2, 35·5, 35·5	35·0

Control was achieved eventually by introducing a combination of features used by other workers, a change that resulted in the constant formation of $Zn_2Fe(CN)_6$ with a concomitantly constant relation, $2Zn \equiv I$. This modified technique proved most satisfactory and gave steady and reliable results over many hundreds of analyses. (Fig. 2 and Table I.)

The chief modifications of the Sylvester and Hughes method were:—ammonium acetate was replaced first by tartrate (Keilin and Mann[3]) and finally by citrate (Hibbard[4]) as the last prevented precipitation of calcium and phosphate ions at the dithizone extraction (*p*H 7 to 8) required for satisfactory full recovery. One per cent. of gelatin was used to give colloidal suspensions of precipitated $Zn_2Fe(CN)_6$ (Milton[5]), and a mixture of phosphate and potassium hydroxide to inhibit possible interaction of iron with potassium ferricyanide and potassium iodide (Sahyun and Feldkamp[8]).

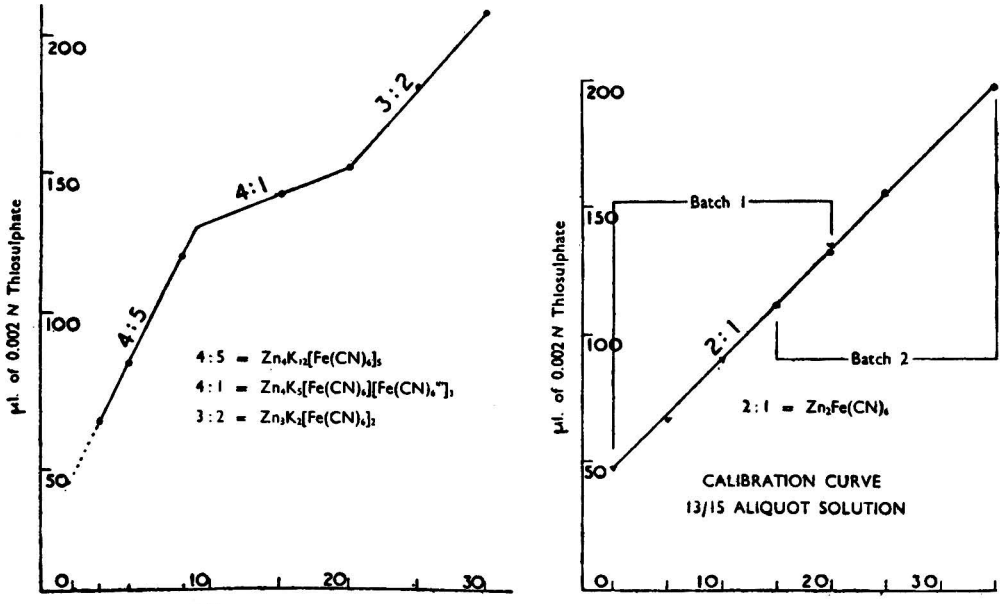

Fig. 1. Typical titration curve obtained with the unmodified method over zinc molarities 0·0001 to 0·001 *N*. In these preliminary experiments no dithizone extraction process was used—added zinc was directly titrated.

The Zn : I ratios were determined from the "gradients" of the linear runs. The *physical* characteristics of the precipitate frequently altered visibly at the inflection points.

Many of the precipitates contained chemically combined and chemically adsorbed ferricyanide.

Fig. 2. The modified method stabilised the reaction and gave a constant precipitate of $Zn_2Fe(CN)_6$ over the whole zinc range 0·0001 to 0·001 *N*.

Inter-batch variations were practically eliminated (note the satisfactory congruence of independent batches covering different molar ranges). The burette readings of 60 consecutive 20-μg. standards put through 20 consecutive batches, each subjected to the full dithizone extraction process, averaged 97 $\pm$ 2·58 μl. of 0·002 *N* thiosulphate, a coefficient of variation of only $\pm$ 2·77 per cent.

This variation can be still further reduced by using the calculation recommended under "Method."

The extraction process was also simplified by using an aliquot of a single timed dithizone extraction; this enhanced the accuracy. Results were always calculated in terms of parallel standards and blanks. Analyses were run in batches of twelve (this being the capacity of the mechanical shaker), and all titrations were made in triplicate.

Method

Reagents—

20% *Ammonium citrate solution*—Dissolve 16 g. of A.R. citric acid in about 70 ml. of distilled water. Add a few drops of bromothymol-blue indicator and run in A.R. aqueous

ammonia (sp.gr. 0·88) from a burette until the solution is distinctly blue (about 16·5 ml.). Cool and make up to 100 ml. with distilled water.

Potassium iodide - gelatin solution—Take 2 ml. of A.R. glacial acetic acid, 5 ml. of 0·5 per cent. starch solution, 13 ml. of 1 per cent. gelatin solution and 4 ml. of special phosphate solution. Mix, and in the mixture dissolve 0·5 g. of A.R. potassium iodide. Decolorise if necessary with 0·002 *N* thiosulphate.

Special phosphate solution—To 5 ml. of glacial phosphoric acid (H_3PO_4) add sufficient 10 per cent. potassium hydroxide solution (60 ml.) to bring to *p*H 3 and distilled water to make up to 100 ml.

Potassium ferricyanide solution—One per cent. in freshly boiled and cooled distilled water.

Sodium thiosulphate solution—Dilute 2 ml. of 0·1 *N* solution to 100 ml. with boiled and cooled distilled water.

Dithizone solution—0·1 per cent. in chloroform purified by the method of Sylvester and Lampitt.[7]

All glassware should be Pyrex, and all chemicals of AnalaR purity.

PROCEDURE—

Ash 0·1 g. of dried dentine or 0·07 g. of enamel at a temperature not exceeding 500° C. and dissolve the ashed material, containing 5 to 20 μg. of zinc, in 1 ml. of 5 *N* hydrochloric acid in a Pyrex centrifuge tube. For the standard, add the required amount of zinc to 1 ml. of 5 *N* hydrochloric acid in a centrifuge tube.

Add 4 ml. of 20 per cent. ammonium citrate solution and 4 drops of 0·04 per cent. bromothymol blue solution and bring to a distinct blue (*p*H 7 to 8) with diluted aqueous ammonia (1 + 3).

Add 3 ml. of dithizone solution, cork securely and shake for 20 minutes. Pipette off the whole of the dithizone layer and transfer it to distilled water in a clean Pyrex centrifuge tube. Withdraw a 2·6-ml. aliquot, transfer it to a clean Pyrex 5-ml. test tube containing 2 ml. of 0·5 *N* hydrochloric acid, cork, and shake for 5 minutes. Pipette off all dithizone with a fine bulb-pipette and evaporate the aqueous layer to dryness on the water bath. Transfer to a sand-bath, add 1 drop of 20 per cent. perchloric acid and heat until fumes of perchloric acid are no longer driven off.

Cool and add 0·5 ml. of potassium iodide - gelatin mixture and wash down the sides of the tube by tilting and revolving. Then add 0·1 ml. of 1 per cent. potassium ferricyanide solution and allow to stand for exactly 5 minutes. Titrate with 0·002 *N* thiosulphate from a micro-burette until the contents of the tube are clear yellow, without a trace of orange colour.

Calculation—

Standard: for a μg. of Zn taken, burette reading $= B_a$.
Blank: for no Zn taken, burette reading $= B_o$.
Unknown: for x μg. of Zn present, burette reading $= B_x$.

$$\text{Then } x = \frac{a\,(B_x - B_o)}{B_a - B_o}.$$

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December, 1947

A Colorimetric Method for the Determination of Small Quantities of Glycerol*

By V. HELWEG MIKKELSEN

It has been demonstrated by Denigès[1] that characteristic colours are produced by oxidising glycerol with bromine water and adding concentrated sulphuric acid and various hydroxy-compounds, of which the most suitable were found to be codeine, resorcinol, thymol and β-naphthol.

This reaction was subsequently tested by Délaby[2] and especially by De Coquet,[3] who modified the technique, particularly the heating time, and brought the reaction into such a form that it may be employed for the colorimetric determination of glycerol, *e.g.*, in wine.

The reaction is due to the fact that the glycerol is oxidised to 1:3-dihydroxyacetone $CO(CH_2OH)_2$ and probably also to glyceraldehyde, $OHC.CHOH.CH_2OH$, as these two compounds are interconvertible by movement of hydrogen atoms.

On addition of concentrated sulphuric acid 1:3-dihydroxyacetone is dehydrated to methyl glyoxal, $H_3C.CO.CHO$, which reacts with codeine, thymol, resorcinol, β-naphthol and other substances to form coloured compounds.

For the purpose of examining whether it was possible to work out a quantitative colorimetric method of determining glycerol in galenical preparations, especially solutions for injection containing glycerol, Denigès' report was investigated.

I was able to verify all his colour reactions. That with codeine was best suited to the purpose, because the blue colour obtained, when measured by the step photometer, gave a characteristic absorption curve with a pronounced maximum at 6600 A. (see Fig. 1).

The colour curves for the reactions with thymol and resorcinol showed no characteristic absorption bands. β-Naphthol gave an emerald green colour with maximum absorption at about 6100 A., but this colour is less intense than the one given by codeine, which has proved to be stable for at least 4 hours.

However, it was proved that the Denigès method in its original form could not be applied directly to quantitative determinations: with the step photometer there was no strict proportionality between the extinction coefficient and the glycerol concentration, and the method had other defects. By systematically varying (1) the quantity of bromine, (2) the glycerol concentration and (3) the heating time both for the oxidation and for the reaction with codeine, I arrived at the following procedure which gave reproducible results and proportionality between the extinction coefficient, k, and the glycerol concentration—

To 0·75—1·00 g. of glycerol dissolved in 50 ml. of water add 50·0 ml. of saturated bromine water. Heat the mixture in a boiling water bath for 20 minutes and then immediately boil in order to expel the excess of bromine completely (about 5 minutes). After cooling, make up to 100·0 ml. with distilled water. Of this solution make a dilution of 1 in 10 to be used for the colour test.

This is carried out as follows.

To 1·00 ml. of reagent (a solution of 0·5 g. of codeine in 10·0 ml. of 96 per cent. alcohol) plus from 1·00 to 4·00 ml. of the above ten-fold diluted solution, plus water if necessary to make up to 5·00 ml., add 20·0 ml. of concentrated sulphuric acid (of at least 95 per cent. concentration), cooling rapidly in running water. Then heat the mixture in a boiling water bath for exactly 120 seconds, cool immediately with running water to room temperature and make up to 25·0 ml. with sulphuric acid (at least 95 per cent.). After the solution has been left for 15 to 30 minutes, measure its blue colour in the step photometer, using filter S66, and layer thickness normally 1·00 cm., though with values of k above 0·5 a thickness of 0·50 cm. is preferable. For comparison, use a cooled mixture of 5·0 ml. of water and 20·0 ml. of sulphuric acid (at least 95 per cent.).

Construct a standard curve for known quantities of glycerol and use the resulting straight line for verification.

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* Preliminary Communication from the Control Laboratory of the Danish Society of Dispensing Chemists.

Further details of these experiments will be published later.

Instead of saturated bromine water (about 3 per cent. of bromine by weight), it is an advantage to use an aqueous solution of potassium bromate and bromide to which an equal volume of 2 *N* hydrochloric acid is added before use. A solution that in 100·0 ml. contains 2·10 g. of potassium bromate and 9·35 g. of potassium bromide will, after addition of an equal volume of 2 *N* hydrochloric acid, give a solution corresponding to saturated bromine water.

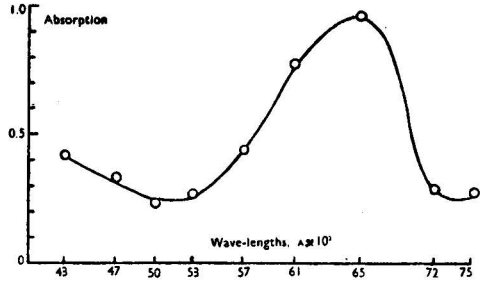

Fig. 1

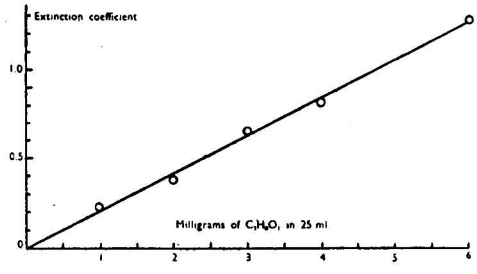

Fig. 2

Fig. 2 is a standard curve plotted in the manner described. The ordinate is the extinction coefficient, *k*, and the abscissa mg. of $C_3H_8O_3$.

Colorimetric determination of the quantity of glycerol in morphine solution for injection

The following is an example of the application of the above method to the determination of glycerol in morphine solution for injection (1 ml. contains 0·50 g. of glycerol and 0·05 g. of morphine hydrochloride).

Fifty ml. of an injection solution were prepared, with a glycerin content of 25·00 g. Titration with dichromate showed that the glycerol used had a content of 85·13 per cent. of $C_3H_8O_3$. For the analysis 5·00 ml. of the injection solution were taken, corresponding to a content of 2·50 g. of glycerin or 2·128 g. $C_3H_8O_3$. The 5·00 ml. were diluted with 25 ml. of water and then the morphine base was precipitated by addition of a solution containing 0·25 g. of $Na_2CO_3,10H_2O$ and 0·75 g. of $NaHCO_3$ in 25 ml. of water (*i.e.*, a buffer solution of about *p*H 9). After the liquid had been left for about 2 hours the crystals were filtered off and washed on the filter with water, 25 ml. in all. After addition of three drops of phenolphthalein indicator to the filtrate this was decolorised by addition of *N* hydrochloric acid and made up with water to 100·0 ml. To 50·0 ml. of this solution were added 5·0 ml. of N hydrochloric acid and then a mixture of 25 ml. of bromide – bromate solution (2·10 g. of $KBrO_3$ and 9·35 g. of KBr in 100 ml.) and 25 ml. of 2 *N* hydrochloric acid. Subsequent procedure was as described above. For producing the colour the quantities used were 1·00 ml. of reagent, 2·00 ml. of the ten-fold diluted test solution, 2·00 ml. of water and sufficient concentrated sulphuric acid to make up 25·0 ml. The extinction coefficient was 0·435, which according to the standard curve (Fig. 2) corresponds to 2·06 mg. of $C_3H_8O_3$ in the 2·00 ml. of diluted test solution taken, this represents 96·9 per cent. of the calculated amount, and a duplicate determination gave 97·9 per cent.

Quantitative methods for determining glycerol in other galenical preparations are being elaborated and will be published later.

These experiments were begun at Apotekens Kontrollaboratorium in Stockholm and I would take this opportunity to thank the principal, Mr. T. Canbäck, for valuable help in the work.

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May, 1947

The Composition of Concentrated Tomato Purée and the Estimation of the Tomato Content of Tomato Ketchup

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(*Read at the Meeting of the Society on May 5th, 1948*)

Introduction

The need for a quantitative standard for tomato ketchup to augment the present standard (S.R. & O., 1944, No. 865; and 1946, No. 2216), which states that the product shall contain no fruit or vegetable other than tomatoes (except onions, garlic and spices added for flavouring purposes), has been felt for some time. The work reported here was carried out in order to find if it is possible to estimate the tomato content of a ketchup and thus enable such a standard to be enforced.

The greater part of the tomato ketchup sold in this country is prepared from imported concentrated tomato purée. It was therefore thought desirable to analyse such purées from various sources in order to see if any analytical characteristics could be used in estimating tomato content in ketchups. The most commonly available tomato purée contains 27 to 30 per cent. of dry tomato solids, but other purées offered to manufacturers have been found to contain as little as 9 and as much as 44 per cent. It was decided to express the tomato content of ketchups as dry tomato solids rather than as the raw fruit, which is liable to some variation in solids content.

The problem of determining the tomato content of ketchup is very similar to that of the determination of the fruit content of jam. The tomato purées were therefore analysed in the first instance for those constituents that have been found helpful in jam analysis.[1,2] In addition, the potash contents of the purées were determined and, in order to relate the various constituents with the tomato solids contents, these also were determined. The methods used are described below.

Analysis of concentrated tomato purée

Total solids—Dry to constant weight at 70° C. under vacuum, using the A.M.C. Tomato Products Sub-Committee's method,[3] or alternatively, in a vacuum drying oven. In either method the amount of sample taken must be such as to give only a thin film of material over the surface of the drying vessel. A convenient amount is from 2 to 3 g.

Insoluble solids—Boil 5 g. of the purée with 100 ml. of distilled water gently for 30 minutes, stirring occasionally. Decant off the supernatant liquid through a No. 1 filter paper, wash back into the original beaker any material on the filter, add 50 ml. of water, and again boil for 15 minutes. Decant again, using a fresh filter paper, and repeat the boiling process. Finally, bring the whole of the insoluble material on to a tared No. 1 filter paper, wash very thoroughly with hot water, drain, and dry overnight at about 105° C.

Preparation of the aqueous extract—Boil gently 100 g. of the purée with 400 ml. of water for 1 hour in a covered beaker. (For purée of 10 per cent. solids content, take 250 g., and 250 ml. of water.) Stir frequently. Cool, and make up the mixture to 500 g. with water. Filter through a 24-cm. No. 1 paper, taking precautions to prevent loss by evaporation.

Determination of the extract factor—The aqueous extract is made up by weight, but subsequent determinations carried out on it are most conveniently done on volume aliquots. In order to calculate the results obtained per 100 ml. of extract to 100 g. of purée, the following factor, F, is used (when 100 g. of the purée sample were used to make the extract).

$$F = \frac{500 - \text{percentage of insoluble solids in purée sample}}{100 \times \text{sp.gr. of extract}}$$

Preparation of pectin-free filtrate—Pipette 250 ml. of the aqueous extract into a 500-ml. volumetric flask. While gently swirling round the flask, slowly add acetone (not below 97 per cent.), avoiding as much as possible the entanglement of air bubbles in the precipitate.

Make up to volume with acetone and mix. Filter through a 24-cm. No. 1 paper, keeping the filter covered and the neck of the receiving flask plugged with cotton wool in order to minimise evaporation. With some purées of low pectin content it is not easy to obtain a clear filtrate, a small amount of precipitate passing through the filter; this can be ignored.

Free acid—Take 20 ml. of the aqueous extract and determine the free acidity in the manner described by Hinton.[2]

Combined acid determination—Determine the combined acidity by Hinton's method[2] on 50 ml. of the pectin-free filtrate, taking 25 ml. of 0·1 *N* hydrochloric acid for dissolving the ash, and using 5 ml. of strong neutral calcium chloride solution instead of the 10 ml. stipulated. (Prepare the calcium chloride solution by adding 200 g. of washed calcium carbonate to 350 ml. of concentrated hydrochloric acid, boiling and filtering.) Preserve the titrated solution for sulphate determination.

Total fruit acid—The sum of the free and combined acidities as determined above gives the total fruit acid expressed as hydrated citric acid.

Determination of sulphate—Acidify the solution obtained in the combined acid determination, with a little dilute hydrochloric acid, and determine sulphate by precipitation with barium chloride in the usual manner. Carry out a blank determination at the same time.

Aqueous lead number—Take an amount of pectin-free filtrate (to the nearest 5 or 10 ml.) containing 0·5 g. of total fruit acids, and carry out the determination by Hinton's method,[2] treating the solution as for fruits in group (i). In addition, before calculating the aqueous lead number (number of ml. of 2 per cent. lead acetate solution completely precipitated by 10 g. of original sample) apply a correction for the sulphate present, in accordance with Table I.

TABLE I

CORRECTION FOR SULPHATE

Sulphate as K_2SO_4 in the amount of pectin-free filtrate taken	Deduction for titration difference
g.	ml.
0·01	0·1
0·02	0·2
0·03	0·3
0·05	0·4
0·07	0·5
0·10	0·6

Also calculate the "lead number" per 1 g. of dry tomato solids.

Acetone lead number—Take an amount of pectin-free filtrate containing 0·5 g. of total fruit acid (to the nearest 5 or 10 ml.), and carry out the determination by Hinton's method, again treating the solution as for group (i) fruits. Also apply a correction for sulphate, deducting 0·25 ml. from the titration difference for each 0·01 g. of sulphate as K_2SO_4 in the amount of pectin-free filtrate taken. Calculate the acetone lead number originally defined, and also per 1 g. of dry tomato solids.

Total sugars—Determine total sugars as invert sugar on 10 ml. of the aqueous extract after inversion with hydrochloric acid, using Lane and Eynon's method.[4] With 10 ml. of aqueous extract finally diluted to 200 ml., 10 ml. of mixed Fehling's solution is convenient for use.

Chlorides—To 5 g. of the original purée in a platinum dish, add 20 ml. of approximately 0·1 *N* potassium carbonate. Mix, evaporate to dryness, char over an Argand burner and finally ash at dull red heat in the electric furnace. Determine chlorides in the ash by the Volhard method, calculate as percentage of sodium chloride after allowing for any blank due to the carbonate solution.

Ash and potash—Ash 20 g. of the original purée in a tared platinum dish in the electric furnace at dull red heat, after first drying on the water-bath and charring over an Argand burner. Record the percentage of ash. The method for potash determination is essentially that of S.R. & O., 1918, No. 659. Moisten the ash with 2 to 3 ml. of water and cautiously add 2 ml. of concentrated hydrochloric acid. Warm to dissolve, and evaporate to dryness on the water-bath. Take up the residue in 2 ml. of 2 *N* hydrochloric acid plus a small volume of water, warming to dissolve. Filter into a 250-ml. beaker and wash dish and filter with hot water. Bring the filtrate nearly to boiling and add cautiously small portions of solid

barium hydroxide until the solution is just alkaline to litmus paper. Filter into a second 250-ml. beaker and wash the precipitate and filter with hot water. Add 1 ml. of aqueous ammonia (sp.gr. 0·880) to the filtrate, and then a slight excess of saturated ammonium carbonate solution to precipitate all the barium. Add a few crystals of ammonium oxalate and boil. Filter, and wash with hot water. The total volume of filtrate and washings should now be not more than 200 ml. Evaporate the solution to dryness in an evaporating dish on the sand-bath, taking special care to prevent loss by spurting during the final stages. When the residue is dry, cautiously heat the dish over a small flame to drive off ammonium salts. The dish must not be allowed to become red hot nor the salts allowed to fuse. When fuming ceases, cool and moisten the residue with concentrated hydrochloric acid. Again evaporate on the sand-bath and heat over a small flame to remove any residual ammonium salts. Cool, take up in 5 ml. of dilute hydrochloric acid plus a small volume of water. Filter through a small paper into a glass evaporating basin. Wash the original dish and filter thoroughly with water. Add 10 ml. of 20 per cent. perchloric acid and evaporate on the sand-bath until copious fumes of perchloric acid are evolved. Add sufficient hot water to dissolve the precipitated salts and a few drops of 20 per cent. perchloric acid and again evaporate to fuming. Cool, and add 20 ml. of 95 per cent. ethyl alcohol (S.V.R.). Filter cold through a tared Gooch crucible, with asbestos as the filter pad. When preparing the crucible wash finally with alcohol saturated with potassium perchlorate and filtered just before use. Use alcohol saturated with potassium perchlorate for transferring and washing the precipitate. Dry at 100° C. Calculate the percentage of potash as K_2O, using the factor $KClO_4 \times 0{\cdot}34 = K_2O$.

*p*H *value*—Determine *p*H on the aqueous extract, using the quinhydrone or glass electrode. (The *p*H values were obtained purely as a matter of interest.)

Analysis of tomato ketchup

The presence of starch and/or gums causes the filtration of simple aqueous extracts of ketchup to be extremely difficult. For this reason the determination of insoluble solids was abandoned, and a modified aqueous-acetone extract used instead of the aqueous extract and pectin-free filtrate used with tomato purées.

Total solids—Proceed as for purée after adding water and twice evaporating almost to dryness on the water-bath in order to remove acetic acid. Take care not to evaporate to dryness. Acetic acid is tenaciously held in the dry material during vacuum drying unless it is previously removed by evaporation with water.

Total free acid—This determination is not required for estimating the tomato content, and need only be carried out if the acetic acid content is wanted. Dilute 10 g. of ketchup with 500 ml. of freshly boiled and cooled distilled water. Titrate with 0·5 *N* sodium hydroxide, using phenolphthalein as indicator. The end-point is not easy to see, especially if artificial colouring matter is present in the ketchup. Spotting with the indicator on a white tile is helpful.

Aqueous-acetone extract—Transfer 150 g. of the ketchup to a 500-ml. volumetric flask, using 130 ml. of water. Heat in a boiling water-bath for 1 hour, shaking frequently. Cool, and make up any loss in weight by addition of water. While gently swirling the flask, slowly add acetone and finally make up to the mark with acetone. Mix well, stopper the flask and set it aside until the precipitated material has settled somewhat. Filter, taking precautions against loss by evaporation. It was proposed to correct for the volume of the precipitate in this extract in subsequent determinations, by obtaining the ratio of the percentage of chloride determined from the extract and from analysis of the original ketchup. The factor thus obtained, for the samples examined so far, was so nearly unity as to be negligible.

Non-volatile free acid—Evaporate 40 ml. of the extract to dryness in an evaporating dish on the water-bath, take up the residue in about 10 ml. of water and again evaporate. Carry out three further evaporations. Determine the residual acid as for purée.

Combined acid—Carry out the determination on 50 ml. of extract in the same manner as for purée.

Lead numbers—As for purée, using the requisite quantities of extract.

Total sugars—Determine as invert sugar on a suitable aliquot of the extract or on a 50 per cent. aqueous acetone extract of a 5 g. sample of the original ketchup. Remove the acetone by evaporation before carrying out the inversion.

Ash and potash—Proceed as directed for purée, taking 25 to 30 g. of ketchup.

Chlorides—Determine chlorides as sodium chloride as for purée. A suitable aliquot of the extract or 5 g. of the original sample should be taken. Although not essential for the estimation of the tomato content, a knowledge of the salt content is of value when comparing the sum of the constituents found by analysis with the total solids.

Other components—It may be necessary to detect and estimate other components of the ketchup such as starch and gum, in order to check the total solids content as mentioned above. Starch may be determined by the iodine precipitation method of Edwards, Nanji and Chinoy.[5,6] By carrying out a "starch" determination by precipitation with alcohol, after removal of sugars, digestion with alcoholic potash and solution in 0·7 per cent. potassium hydroxide solution, a value for starch plus gums, etc., may be obtained and an estimation of gums, etc., made by difference.

Results of analyses of concentrated tomato purées

Through the co-operation of trade users and importers of tomato purée, thirty-one samples were obtained for analysis during the period 1944 to 1946. These samples were the produce of twenty-one different packers, and not more than three were from any one source.

The results of the analyses by the described methods, together with simple statistical data, are given in Table II, and a survey of the frequency distribution of the samples in relation to the analytical characteristics is shown in Table III.

Table III

Frequency distribution of samples in relation to the analytical characteristics

Free acid per cent. of the tomato solids (Average 6·48, Maximum 9·71, Minimum 4·19)

	4·0 to 4·5	4·5 to 5·0	5·0 to 5·5	5·5 to 6·0	6·0 to 6·5	6·5 to 7·0	7·0 to 7·5	7·5 to 8·0	8·0 to 8·5	8·5 to 9·0	9·0 to 9·5	9·5 to 10·0
Free acid per cent. of tomato solids												
Number of samples ..	1	1	8	4	2	4	3	5	1	1	0	1

Combined acid per cent. of the tomato solids (Average 6·19, Maximum 10·32, Minimum 4·19)

	4·0 to 4·5	4·5 to 5·0	5·0 to 5·5	5·5 to 6·0	6·0 to 6·5	6·5 to 7·0	7·0 to 7·5	7·5 to 8·0	8·0 to 8·5	8·5 to 9·0	9·0 to 9·5	9·5 to 10·0	10·0 to 10·5
Combined acid per cent. of tomato solids													
Number of samples ..	2	2	2	8	8	3	4	1	0	0	0	0	1

Total acid per cent. of the tomato solids (Average 12·68, Maximum 17·51, Minimum 8·46)

	8·0 to 8·5	8·5 to 9·0	9·0 to 9·5	9·5 to 10·0	10·0 to 10·5	10·5 to 11·0	11·0 to 11·5	11·5 to 12·0	12·0 to 12·5	12·5 to 13·0	13·0 to 13·5	13·5 to 14·0	14·0 to 14·5
Total acid per cent. of tomato solids													
Number of samples ..	1	0	1	0	2	2	6	2	3	2	2	1	1

	14·5 to 15·0	15·0 to 15·5	15·5 to 16·0	16·0 to 16·5	16·5 to 17·0	17·0 to 17·5	17·5 to 18·0
Total acid per cent. of tomato solids							
Number of samples ..	2	3	0	1	0	1	1

Potash (K_2O) per cent. of the tomato solids (Average 4·79, Maximum 6·35, Minimum 3·59)

	3·5 to 4·0	4·0 to 4·5	4·5 to 5·0	5·0 to 5·5	5·5 to 6·0	6·0 to 6·5
Potash per cent. of tomato solids						
Number of samples ..	3	8	13	3	3	1

Aqueous lead number per 1 g. of the tomato solids (Average 12·7, Maximum 21·1, Minimum 7·4)

	7·0 to 8·0	8·0 to 9·0	9·0 to 10·0	10·0 to 11·0	11·0 to 12·0	12·0 to 13·0	13·0 to 14·0	14·0 to 15·0	15·0 to 16·0	16·0 to 17·0	17·0 to 18·0	18·0 to 19·0	19·0 to 20·0	20·0 to 21·0	21·0 to 22·0
Aqueous lead number per 1 g. tomato solids															
Number of samples ..	1	0	2	7	3	6	2	1	3	2	1	0	0	0	1

Acetone lead number per 1 g. of the tomato solids (Average 16·7, Maximum 24·8, Minimum 10·4)

	10·0 to 11·0	11·0 to 12·0	12·0 to 13·0	13·0 to 14·0	14·0 to 15·0	15·0 to 16·0	16·0 to 17·0	17·0 to 18·0	18·0 to 19·0	19·0 to 20·0	20·0 to 21·0	21·0 to 22·0	22·0 to 23·0	23·0 to 24·0	24·0 to 25·0
Acetone lead number per 1 g. tomato solids															
Number of samples ..	1	0	0	4	6	4	4	2	1	3	2	0	0	0	2

TABLE II—COMPOSITION OF CONCENTRATED TOMATO PURÉE

No.	Origin	Total* solids %	Insoluble solids, % on total solids	Combined acid (as citric), % on total solids	Free acid (as citric), % on total solids	Total acid (as citric), % on total solids	Aqueous lead number: Per 1 g. of total solids	Aqueous lead number: Per 1% of total acid	Acetone lead number: Per 1 g. of total solids	Acetone lead number: Per 1% of total acid	Sugars (as invert), % on total solids	Sodium chloride % on total solids†	Potash (K_2O), % on total solids	Ash, % on total solids	Potash in ash, %	*p*H
1	California ..	35·90	8·50	6·81	7·49	14·30	15·5	10·8	19·2	13·4	55·16	—	4·29	7·58	56·6	4·11
2	„ ..	31·00	9·32	5·91	5·10	11·01	10·2	9·3	14·5	13·1	51·93	—	4·32	7·32	59·0	4·27
3	„ ..	27·15	12·67	6·07	5·35	11·42	10·9	9·5	15·3	13·4	59·03	—	4·50	7·71	58·4	4·25
4	„ ..	27·07	9·42	4·19	5·25	9·44	9·2	9·8	13·2	13·9	59·89	0·61	3·65	6·29	57·9	4·20
5	„ ..	27·30	16·30	5·94	5·75	11·69	11·3	9·7	16·0	13·7	50·32	1·07	4·86	8·46	57·5	4·31
6	„ ..	25·95‡	11·99	4·96	5·40	10·36	10·3	9·9	14·9	14·4	56·69	4·17	4·20	7·33	39·3	4·22
7	„ ..	26·95	12·80	5·61	4·93	10·54	10·3	9·8	13·8	13·1	57·42	1·11	4·64	7·94	58·4	4·26
8	„ ..	26·00	9·50	5·81	6·23	12·04	12·8	10·6	16·2	13·5	59·46	0·88	4·46	7·88	56·6	4·11
9	„ ..	26·80	14·42	5·79	5·52	11·31	11·4	10;0	15·0	13·3	52·83	1·23	4·93	8·40	58·7	4·25
10	„ ..	28·10	11·60	6·43	8·51	14·94	15·7	10·5	20·6	13·8	52·71	1·14	4·95	8·47	58·4	3·97
11	„ ..	27·10	10·22	5·89	5·44	11·33	11·3	10·0	14·8	13·1	57·15	0·66	4·72	8·08	58·4	4·23
12	„ ..	33·52	7·73	6·95	7·60	14·55	15·6	10·7	19·4	13·3	57·37	0·54	4·60	7·88	58·3	4·07
13	„ ..	35·30	8·61	7·19	7·91	15·10	16·3	10·8	20·1	13·3	55·49	0·68	4·93	8·48	58·4	4·14
14	„ ..	26·25	8·76	6·11	5·53	11·64	12·8	10·9	15·9	13·7	61·59	0·72	4·34	7·31	59·4	4·17
15	Canada ..	39·34‡	10·68	6·79	6·70	13·49	14·1	10·5	18·0	13·4	53·47	2·36	5·28	9·23	47·9	4·12
16	Argentina ..	31·00	11·26	6·31	7·67	13·98	13·7	9·8	18·6	13·3	54·83	1·42	5·22	9·13	57·2	3·97
17	California ..	27·86	12·35	5·21	5·33	10·54	10·6	10·1	14·5	13·8	56·99	1·36	4·45	7·83	57·1	4·16
18	„ ..	26·13‡	12·17	5·02	5·02	10·04	9·9	9·8	13·6	13·5	57·53	3·90	4·32	7·30	42·0	4·12
19	Maryland ..	9·76	10·24	6·06	7·36	13·42	13·1	9·7	17·6	13·1	53·08	1·09	4·85	8·46	57·2	4·13
20	Ohio	9·27‡	10·47	7·33	9·71	17·04	18·0	10·6	25·0	14·7	45·09	13·81	5·87	10·14	23·6	3·99
21	Utah	25·53	9·44	7·21	7·89	15·10	16·1	10·6	19·7	13·0	56·51	1·06	5·37	9·32	57·8	4·21
22	California ..	26·00	8·35	6·04	6·92	12·96	12·8	9·9	16·8	13·0	58·68	1·00	4·65	8·15	56·8	4·21
23	South America	38·10‡	13·49	7·77	8·41	16·18	—	—	—	—	—	—	6·35	11·00	32·5	3·98
24	„	41·46‡	12·27	7·45	7·85	15·30	—	—	—	—	—	—	5·89	10·21	34·5	4·02
25	France ..	19·25	10·50	5·61	6·80	12·41	12·4	9·9	16·8	13·5	61·61	0·91	4·91	7·84	63·0	—
26	„ ..	31·61	12·05	5·82	6·80	12·62	12·8	10·2	15·7	12·4	59·80	0·89	4·94	8·32	59·3	—
27	„ ..	35·09	10·97	4·93	6·30	11·23	10·8	9·6	14·0	12·5	63·92	0·78	3·64	8·09	45·1‖	—
28	California ..	25·87	10·43	6·22	5·80	12·02	10·9	9·1	14·4	12·0	56·63	1·04	4·83	9·77	49·4	4·14
29	„ ..	26·71‡	11·01	4·27	4·19	8·46	7·4	8·7	10·4	12·3	61·84	3·50	3·59	6·66	38·9	4·14
30	New Zealand	8·33‡	12·00	10·32	7·19	17·51	21·1	12·1	24·8	14·2	55·85	—	6·00	10·32	30·5	—
31	U.S.A. ..	24·84	12·84	6·08	5·11	11·19	12·7	11·3	16·1	14·4	59·41	1·41	4·91	8·74	56·2	—
Averages		11·04	6·19	6·48	12·68	12·7	10·1	16·7	13·4	56·63	0·98§	4·79	8·37	57·7§	4·14
Standard deviation		—	±1·13	±1·30	±2·25	±2·8	±0·7	±3·2	±0·6	—	—	±0·64	—	—	—
Standard error of mean ..		—	±0·20	±0·23	±0·40	±0·5	±0·1	±0·6	±0·1	—	—	±0·12	—	—	—

* Other than in column 13 (sodium chloride % on total solids), total solids are corrected for *added* salt, where necessary

† Total solids as determined, including any added salt.

‡ Sample contained added salt.

§ Average for samples without added salt.

‖ Sand in ash.

Several of the samples had on the labels the information that added salt was present, and from the analysis of some unlabelled samples it was deduced that these also contained added salt. It would appear that where the percentage of salt in total solids exceeds 1·5, salt has been added. If the percentage of potash in the ash is appreciably less than 56, this is further indication of the presence of added salt, provided that, as would seem to be usual, the sample does not contain excessive siliceous matter. Fairly good agreement for the amount of added salt present was obtained by calculating on the average figures of 0·98 per cent. of salt in total solids and 57·7 per cent. of K_2O in ash. As stated in the footnotes to Table II, the total solids were corrected for added salt where present and thus become synonymous with "tomato solids." The calculated amounts of added salt in the samples were: No. 6, 0·86; No. 15, 0·56; No. 18, 0·79; No. 20, 1·37; No. 23, 3·26; No. 24, 2·85; No. 29, 0·69; No. 30, 0·78 per cent. In some samples the amount of added salt in relation to the total solids would seem to be excessive.

One sample, No. 10, was stated to be "made in part from residual tomato material from canning." This residual material was probably that resulting from the extraction of the tomato juice. This sample does not, however, show any marked differences in composition from the other samples, the insoluble solids being only slightly above the average and much less than in some samples prepared from whole fruit.

As a matter of interest, and for comparison with imported concentrated tomato purées, a sample of English tomatoes was also analysed. These were grown under glass in the Lea Valley district during 1944. The whole fruits were steamed in a covered beaker for 1½ hours and, after cooling, the slight loss in weight was made up with water. The resulting pulp was rubbed through a fine sieve in order to remove skins, seeds and cores (together amounting to 6·7 per cent. of the raw fruit) and the purée was analysed, with the following results: total solids, 6·66 per cent.; insoluble solids, 0·84 per cent. (12·61 per cent. on total solids); combined acid, 0·42 per cent. (6·30 per cent. T.S.); free acid, 0·56 per cent. (8·41 per cent. T.S.); total acid, 0·96 per cent. (14·71 per cent. T.S.); aqueous lead number per 1 g. of total solids, 15·6; aqueous lead number per 1 per cent. of total acid, 10·6; acetone lead number per 1 g. of total solids, 20·3; acetone lead number per 1 per cent. of total acid, 13·8; total sugars (as invert), 3·18 per cent. (47·74 per cent. T.S.); K_2O, 0·36 per cent. (5·49 per cent. T.S.); ash, 0·63 per cent. (9·46 per cent. T.S.); K_2O in ash, 58·0 per cent.; pH, 4·18.

When these results (on the dry basis) are compared with those in Table II, it is seen that the composition of the purée prepared from these tomatoes is much the same as that of imported purées.

The estimation of the tomato content of ketchup

Bigelow, Smith and Greenleaf[7] described admittedly inadequate methods for estimating tomato solids in ketchups free from added thickeners. The first is based on the assumption that the non-sugar solids in whole-tomato products are exactly 50 per cent. of the total solids. From the determination of total solids, sugar and salt content of a ketchup, an estimate of tomato solids can therefore be made. The second method is based on the assumption that the insoluble solids of tomato purée are always 12·5 per cent. of the total solids. A determination of the insoluble solids of ketchup enables an estimation of tomato content to be made.

As would be expected, the analytical results show that there are considerable variations in any one characteristic of tomatoes. Variety, degree of ripeness, climatic conditions, soil composition, etc., all contribute to variation in composition.[8] Of the characteristics studied, the potash content appears to be the most satisfactory for the estimation of tomato in ketchup. The combined acid value is next in order of importance, a fact to be expected, since a high proportion of the alkalinity of the ash is due to potassium carbonate. The use of the average value for free acid is less satisfactory since, with the samples analysed so far, the values obtained fall into two groups, above and below the mean, very few being close to the average. This suggests that there are two groups of tomatoes, of high and low free acid content respectively, in relation to the total solids. In the United States a very distinct variety, San Marzano, which has pear-shaped fruits, is commonly grown, in addition to the more familiar round-fruited varieties. Lo Coco[8] has shown that the ratio of total solids to free acid is higher in the San Marzano than in the round tomatoes, the latter being lower in total solids and higher in free acid content on the average. From the values presented graphically in Lo Coco's paper it has been calculated that the average free acid on total solids

for round tomatoes is 7·5 per cent. (maximum 10·7, minimum 6·2), while for the pear-shaped fruits it is 5·7 per cent. (maximum 7·5, minimum 4·6). The original values for free acid and total solids were presented as average weekly values throughout the growing seasons during the years 1941, 1942 and 1943. There were 46 such weekly averages for round fruits, and 40 for pear-shaped fruits. All the tomatoes analysed were grown in Northern California. Although there is such a marked difference between the average values for the percentage of acid on total solids in the two classes of fruit, the ranges of values do show a certain amount of overlapping. It is therefore necessary to take the average value for both classes without attempting to distinguish between them. This would in any event be impossible in analysing ketchups; although it was at first considered that, by using the tomato solids calculated from the potash content, in conjunction with the non-volatile free acid of a ketchup, it might be possible to decide which type of tomato had been used.

Of about equal value for estimating tomato content is the total acid figure. The values obtained for this are spread fairly evenly over the range, with few samples close to the average. Both aqueous and acetone lead numbers per 1 g. of solids are somewhat less satisfactory, but the lead numbers per 1 per cent. of total acid, although not usable for estimating tomato solids, are of value in determining the nature of the acids in the product.

In view of the extra work entailed, and the quantity of sample required for the lead number determinations, it would seem hardly worth while carrying out these determinations, except where the presence of fruit other than tomato is suspected. In such cases the lead numbers per 1 per cent. of total acid would be helpful.

Owing to the difficulty of filtering an aqueous extract of ketchup, due to the presence of starch or gums or both, it is not possible to make use of the values for insoluble solids.

Nine samples of tomato ketchup and one sample of tomato sauce were analysed by the methods previously described. The total tomato solids calculated from these analyses are presented in Table IV. Ketchup D and sauce I, which were purchased, are typical of certain products offered for sale, containing little, if any, tomato and consisting in the main of starch paste, artificial colouring, sugar, acetic acid, spices and flavouring. The other samples were supplied by the manufacturers, and the actual tomato contents were not divulged until the analyses were completed. In some cases a sample of the concentrated tomato purée used in preparing the ketchup was also supplied. When the tomato contents of ketchups were calculated from the analyses of the purées used, excellent agreement with the theoretical tomato contents were obtained, indicating that the method of analysis was sound. All results in Table IV, however, have been calculated from the average values of Table II.

TABLE IV

TOMATO SOLIDS CONTENT OF TOMATO KETCHUPS

		Per cent. of tomato solids calculated from average values						
Ketchup	Tomato solids present per cent.	Free acid	Combined acid	Total acid	Potash	Aqueous lead number	Acetone lead number	Average of all values, per cent.
A	9·6	7·7	8·9	9·0	8·8	8·9	9·0	8·7
B*	12·8	13·9	14·4	15·4	13·2	15·7	15·3	14·6
C	8·5	6·7	8·3	8·1	7·7	7·9	8·0	7·8
D	unknown	1·0	1·6	1·4	0·8	—	—	1·2
E	13 (approx.)	10·6	11·9	12·2	11·9	—	—	11·6
F	10·9	12·9	10·5	12·8	11·3	12·8	12·1	12·1
G	11·6	10·2	9·0	10·6	10·9	11·9	11·0	10·6
H	11·6	10·5	12·4	12·4	12·1	14·2	15·8	12·9
I†	unknown	0·6	1·9	1·3	1·5	—	—	1·3
J	5·6	4·5	5·7	5·4	5·4	—	—	5·3

* Ketchup prepared from tomato purée No. 13 (Table II), a sample with characteristics higher than average.

† Sample labelled tomato sauce.

Table IV shows that by taking the averages of the six separate estimations it should be possible to obtain an estimate of the tomato solids in a ketchup to within something like ±10 per cent. of the true value, provided that purée of average composition has been used. On the potash value alone a slightly better estimation can be made. It must b remembered that these results were obtained by using average values for the concentrate

tomato purées. In deciding whether a particular sample of ketchup should be reported against as being deficient in tomato content, it would be necessary to calculate the tomato content by using the minimum values for the concentrated purées.

As previously mentioned, a rough check on the estimated tomato solids can be made by comparing the calculated total solids in a ketchup with the total solids found by analysis. Thus with ketchup J, the total solids were found to be 21·09 per cent., the sugar content was 18·40 per cent. as invert sugar and the ash 0·45 per cent. The calculated total solids are obtained as follows:

	Per cent.
Estimated tomato solids	5·3
Added sugar $\left(18{\cdot}40 - \frac{5{\cdot}3 \times 56{\cdot}6}{100}\right)$	15·4
Added salt $\left(0{\cdot}45 - \frac{5{\cdot}3 \times 8{\cdot}37}{100}\right)$	nil
Unaccounted for (gums, etc.)	0·4
Total	21·1

Similarly with tomato sauce I, found by analysis to contain total solids 14·73, ash 1·82, sugars 4·61 and starch (partially gelatinised wheat starch) 6·86 per cent., the total solids may be computed thus:

	Per cent.
Estimated tomato solids	1·3
Added sugar $\left(4{\cdot}61 - \frac{1{\cdot}3 \times 56{\cdot}6}{100}\right)$	3·87
Added salt $\left(1{\cdot}82 - \frac{1{\cdot}3 \times 8{\cdot}37}{100}\right)$	1·70
Starch	6·86
Protein equivalent to 6·86 per cent. of wheat starch ..	1·30
Total	15·03

An estimated tomato solids content of 1·3 per cent. in this sauce is probably generous, because the wheat flour present would contribute both to the combined acid, through the alkalinity of its ash, and to the potash content, as potash is a natural constituent of wheat.

Summary and conclusions

Thirty-one samples of imported concentrated tomato purée, as well as purée prepared from English tomatoes, have been analysed. The results obtained have been used as a basis for estimating the tomato content of tomato ketchups containing no other fruits or vegetables except onions, garlic and spices added for flavouring purposes. From the analyses of ketchups of known tomato content it is concluded that an accuracy to within about ±10 per cent. is possible in the estimation, provided that purées of average composition have been employed.

The author wishes to record his thanks to Mr. P. D. Barnet for assistance in carrying out the large number of analyses, and to the Council of the British Food Manufacturing Industries Research Association, and the Department of Scientific and Industrial Research, for permission to publish the results of this investigation.

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DISCUSSION

Mr. O. B. DARBYSHIRE asked if the author had considered using oil-soluble colour as a measure of tomato content. He had measured the colour of petroleum extracts of a number of purées; there was a variation of about ±40 per cent., but the method might have its uses.

Mr. MORPETH said the possibility of using extractive colour had been considered but the variability was thought to be too great. The degree of ripeness of the tomatoes would affect the colour very much.

Dr. J. H. HAMENCE asked if there was any means of estimating apple pulp in tomato ketchup. Would the lead number be of use?

Mr. MORPETH pointed out that at present the addition of apple pulp was barred by statutory orders. It would lead to differences in the lead numbers per 1 per cent. of total acid. The presence of swedes would cause abnormal values for free acids.

Mr. W. LEE asked if the purées used were of any particular type and if the method had been applied to the estimation of the tomato content of tomato soup powders.

Mr. MORPETH replied that the purées were representative of those imported into this country during 1944 to 1946 and covered all types of tomatoes. The method had been applied to one dried tomato product and with satisfactory results.

Mr. G. TAYLOR asked whether immaturity of the tomatoes used would affect the results seriously. What would be the effect of green tomatoes?

Mr. MORPETH said he had not been concerned with green tomatoes.

Mr. A. TAYLOR asked if any correlation between the amount of added dye and the copper content of ketchup had been noted.

Mr. MORPETH said it is customary to use colouring matters when unripe tomatoes with insufficient colour were used, but he thought unripe tomatoes were used for tomato chutney rather than tomato ketchup. He had not been concerned with the presence of metals, but a purée of very high concentration might be expected to contain more copper than usual owing to prolonged evaporation.

Mr. D. D. MOIR asked if the author could give any information as to what acids were present in tomatoes. If the amount of citric acid present was substantial and fairly constant, its estimation might provide another valuable figure for assessing the tomato content of ketchup, particularly in admixture with other pulps.

Mr. MORPETH said the main acids present were citric and malic, with traces of others. Their determination would be helpful but lengthy.

Mr. DARBYSHIRE said that the citric acid content of tomatoes appeared to vary between 6 and 8 per cent. of the tomato solids. He asked whether the free acid determined in the method described would not be largely due to vinegar acid.

Mr. MORPETH pointed out that the volatile acid was removed before the determination of the "free acid."

Note

A COLORIMETRIC METHOD FOR THE APPROXIMATE ESTIMATION OF TOMATO SOLIDS

THE natural colour of ripe tomatoes can be extracted with light petroleum, and it was thought that the intensity might be used to give some indication of tomato content of foods.

When a petroleum extract of tomatoes was examined in a Lovibond Tintometer fitted with the Rothamsted device, it was found that the red and yellow values varied widely for different operators. When, however, these values were converted to visual density, the results were independent of personal idiosyncrasy, and between the limits of about 6 to 18 yellow units they were reasonably proportional to concentration.

The visual density was obtained from the graph No. 2 supplied with the tintometer. Theoretically, the obscuration reading should be added to this figure but in actual practice more consistent values were obtained when obscuration was ignored. I can only assume that while the yellow and red components are measures of the characteristic colour of the tomatoes, the obscuration may be influenced by irrelevant absorption effects, such as slight variations in the clarity of the extract.

The method finally adopted was as follows—Wash 5 g. of the sample into a 500-ml. measuring flask with water. Shake well to disperse and dilute to the mark. Mix well and immediately measure out a suitable volume (2 to 5 ml. for a purée, 5 to 10 ml. for a ketchup, 10 ml. or more for soups, etc.) into a small separator, using a pipette with a jet wide enough to pass the suspended fibrous matter. Extract with two 15-ml. portions of light petroleum of b.p. 100° to 120° C. Pour the extracts in succession through a dry filter, dilute if necessary to exactly 30 ml. and match the colour in the Lovibond - Schofield tintometer, using a 3-inch cell.

From the graph, read off the visual density (v) due to the yellow and red components. Calculate from this the visual density V for a 1 per cent. extract in a 1-inch cell, i.e.

$$V = v \times \frac{\text{ml. of petroleum extract}}{\text{ml. of 1 per cent. water extract used} \times \text{cell depth in inches}}$$

If the tomato solids content is known, as in a tomato purée, it is possible to calculate the V value for dry tomato solids (V_S).

An example of this calculation is given herewith for a tomato purée.

Solids by refractometer	25·7 per cent.
Volume of 1 per cent. dispersion used for extraction	2 ml.
y (= yellow component)	8·2
r (= red component)	0·65
v (= visual density from graph)	0·087
V (calculated as above)	0·435
V_S (=100 × 0·435/25·7)	1·69

The table below gives V_S values obtained on a number of tomato purées. In dealing with an unknown tomato product an estimate of tomato solids content can thus be made by comparing the determined V value with the V_S figures in the table.

	Purée	V_S		Purée	V_S
1	S. American	1·69	10	U.S.	1·10
2	" "	1·05	11	"	0·91
3	" "	1·25	12	"	1·67
4	" "	2·27	13	"	1·24
5	" "	1·39	14	"	1·42
6	" "	1·56	15	"	1·60
7	" "	1·23	16	"	2·19
8	Italian	1·14	17	Canadian	0·95
9	"	1·37			

Maximum 2·27. Minimum 0·91. Average 1·41.

The preliminary results obtained by this method suggest that it might be of use as an adjunct to analytical procedures such as the determination of potassium and citrate. Moreover, except in the rather unlikely event of there being added oil-soluble colours present, it is fairly specific and suitable for dealing with low tomato concentrations.

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O. B. DARBISHIRE
April, 1948

Official Appointments

PUBLIC ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Food since the last record in THE ANALYST (1948, **73**, 161). These appointments have been approved by the Minister of Food in accordance with the Transfer of Functions (Food and Drugs) Order, 1948 (Statutory Instrument, 1948, No. 107; ANALYST, 1948, **73**, 100).

Public Analyst	*Appointments*
ALCOCK, Arthur (Deputy)	County Borough of Leeds.
CHILDS, Hugh	Nottinghamshire County Council.
COX, Henry Edward (Deputy)	Middlesex County Council.
DEMBREY, Ivor (Deputy)	City of Bristol.
HAMENCE, Jack Hubert (Additional)	County Borough of East Ham.
" " " (Deputy)	Hertford County Council.
" " " (Joint)	Borough of Wanstead and Woodford, Leicester County Council, Wiltshire County Council.
JAMES, George Vaughton (Deputy)	City of Bath.
MORRIS, Fred	Borough of Bolton.
MORRIS, Fred (Deputy)	East Riding of Yorkshire.
PARK, John (Deputy)	Warwickshire County Council.
PEDEN, Miss Joan Davena (Deputy)	City of Stoke-on-Trent and Urban District of Brierley Hill.
TAYLOR, George	Hertfordshire County Council.

OFFICIAL AGRICULTURAL ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Agriculture and Fisheries since the last record in THE ANALYST (1947, **72**, 543).

Official Agricultural Analyst	*Appointments*
ALCOCK, Arthur (Deputy)	County Borough of Leeds.
DALLEY, Richard Arthur (Deputy) ..	County of Somerset.
DEMBREY, Ivor (Deputy)	County Borough of Bristol.
HAMENCE, Jack Hubert (Deputy) ..	Counties of Bedford, Cornwall, Dorset, Essex, Hertford, and Leicester. Administrative Counties of Southampton and West Suffolk. County Borough of East Ham.
ILLING, Edward Thomas	County of Somerset.
TAYLOR, George	Counties of Bedford, Cornwall, Dorset, Essex, Hertford, and Leicester. Administrative Counties of Southampton and West Suffolk. County Borough of East Ham.
PARK, John (Deputy)	County of Warwick.

Ministry of Food

STATUTORY INSTRUMENTS*

1948—No. 1962. The Chocolate, Sugar Confectionery and Cocoa Products (Control and Maximum Prices) Order, 1944 (Amendment No. 8) Order, 1948. Dated August 24th, 1948. Price 1d.

This Order, which came into force on August 29th, 1948, adds Candy (or Fairy) floss to the list of specified products that are deemed not to be sugar confectionery, chocolate or chocolate confectionery.

The Tin Research Institute

THE SPECTROSCOPIC ANALYSIS OF TIN AND TIN - LEAD SOLDERS†

THIS new booklet by D. M. Smith is a critical summary, in part not previously published, of present knowledge of the quantitative spectroscopy of tin and tin - lead alloys. It is designed to supply a reader with sufficient information on optical apparatus, electrical equipment and methods of analysis to enable him to select the procedure best suited to a particular analytical problem. The subject-matter includes descriptions of the D.C. and intermittent A.C. arc, and the condensed spark methods of excitation, plate processing and a full discussion of the various methods that have been proposed and used for the quantitative interpretation of spectra. The procedures described for the analysis of tin and solder are detailed and complete, with analytical tables and three plates illustrating the spectra obtained. A classified bibliography covers the period from 1922 to 1947. F. L. OKELL

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Contribution to the Chemical Investigation of Digitalis Preparations. D. L. Kedde (*Pharm. Weekblad*, 1947, **82**, 741–757)—Pharmacological standardisation of *Digitalis* preparations is unsatisfactory because, by this method, the aglycones have merely a somewhat smaller lethal dose than the glycosides, whereas by oral administration their effect is very small owing to rapid elimination. The digitoxin content is also important as this glycoside has a particular action on the heart and is mainly responsible for the cumulative effect. The author gives new methods of assay for *Digitalis* leaf or tincture, and for Digisol (Liquor Digitalis).

Assay of Digitalis—Prepare a tincture (1 : 10) in the usual way with dilute alcohol. Treat 10 ml. of the tincture with 0·5 ml. of *N* ferric chloride, dilute to 12·5 ml. with water, and filter the mixture through a G.4 filter crucible. To 10 ml. of the filtrate add 5 ml. of 0·1 *N* sodium hydroxide, and dilute to 20 ml. with water. Without filtering, treat 12·5 ml. of this mixture with 1 ml. of basic lead acetate solution, and dilute to 20 ml. Filter through a filter paper, and treat 10 ml. of the filtrate with 1 ml. of 0·5 *N* sodium phosphate, making up the volume to 12·5 ml. Filter through a hardened paper and treat 5 ml. of the filtrate (≡ 1 ml. of tincture) with 5 ml. of a 2 per cent. solution of 3 : 5-dinitrobenzoic acid in 95 per cent.

* Italics signify changed wording.

† Gratis from the Tin Research Institute, Fraser Road, Greenford, Middlesex.

alcohol, 9 ml. of 70 per cent. alcohol, 2 ml. of *N* sodium hydroxide, and dilute to 25 ml. with water. Compare the colour with that obtained from a standard of *k*-strophanthidine, using 5 ml. of the filtrate as compensation for the residual colour of the liquid. Colour filter S.53 should be used. This determination gives the total of aglycones and glycosides. Aglycones are then determined as follows.

Determination of aglycones—Treat 4 ml. of the tincture with 2 ml. of a solution containing 6·2 per cent. of crystalline cupric chloride in 95 per cent. alcohol and 4·5 ml. of 95 per cent. alcohol, and pass the mixture through a column containing 3 g. of barium hydroxide in fairly fine powder and previously moistened with 1 ml. of alcohol. The column is formed in an Allihn filter tube of 20-mm. cross-section, and with a G.3 sintered-glass filter plate. Wash the column through with 5 ml. of 95 per cent. alcohol, add to the filtrate 2 ml. more of copper chloride solution, and pass the mixture through another baryta column, washing with 4 ml. of alcohol, and collecting the filtrate in 1 ml. of 0·1 *N* sulphuric acid. Add 1 ml. of 0·1 *N* sodium sulphate and check that the reaction is acid. Evaporate the solution to 1·5 ml., dilute with water to 5 ml., and filter, washing the filter with water. Make up the volume of the filtrate to 20 ml. Take 10 ml. of this liquid (≡ 2 ml. of tincture), add 9 ml. of alcohol, 5 ml. of dinitrobenzoic acid reagent, and 1 ml. of 2 *N* sodium hydroxide, and dilute to 25 ml. Determine the colour as before, using as compensation liquid 5 ml. of the filtrate made up to 12·5 ml. with alcohol and alkali in the same concentration as above. A similar method is given for Digisol.

Determination of digitoxin content of Digisol—Shake 12·5 ml. of Digisol for 1 min. with 5 ml. of a freshly prepared suspension of bismuth hydroxide (see below), and dilute to 25 ml. Filter through a G.4 crucible and take 10 ml. of the filtrate for the colorimetric determination, and another 10 ml. for the compensation liquor. The reagent used in this case is sodium nitroprusside at a *p*H of 11·0. To the 10 ml. of filtrate add 2·1 ml. of 20 per cent. alcohol and 10 ml. of Ringer buffer solution (50 ml. of 0·1 *M* sodium phosphate and 16·0 ml. of 0·1 *N* sodium hydroxide), and dilute to 25 ml. Add 0·2 ml. of 4 per cent. solution of sodium nitroprusside, and measure the colour within 20 to 30 min. The digitoxin (which is absorbed by the bismuth hydroxide) is determined from the difference in extinction before and after this treatment. The bismuth hydroxide suspension is prepared as follows. Dissolve 3 g. of basic bismuth hydroxide by warming with 18 ml. of 4 *N* nitric acid and 75 ml. of water. After cooling, allow the solution to drop into a well-stirred mixture of 20 ml. of 25 per cent. aqueous ammonia (carbonate-free) in 200 ml. of water. Filter immediately by suction through a double filter paper and wash with 100 ml. of water in 10-ml. portions, adding each portion before the filter cake cracks. Finally, suck as dry as possible. The filtrate should give only a slight Nessler reaction. Rub down the precipitate in a mortar with water to a volume of 26 ml. Five ml. of this suspension correspond to 0·5 g. of bismuth hydroxide; it is neutral.

G. MIDDLETON

Estimation of Aloe-Emodin and the Aloins in Curaçao Aloes. K. G. Stone and N. H. Furman (*Anal. Chem.*, 1947, **19**, 105–107)—A polarographic method has been developed for the determination of aloins in Curaçao aloes. The same half-wave potential and current - concentration ratio was obtained for barbaloin and *iso*barbaloin, and for aloin known to contain both isomerides, hence only the sum of the two isomerides can be determined polarographically. None of the known constituents of aloes (see Viehoever, *Amer. J. Pharm.*, 1935, **107**, 47) was found to interfere with the determination of aloin, and a mixture of aloin and aloes gave only one wave, the height of which was equal to the sum of those given by the individual components of the mixture.

Method for aloin—Extract 0·2 g. of Curaçao aloes by heating with 20 ml. of 95 per cent. alcohol and 25 ml. of water for 15 min. to a temperature just below the boiling-point of the mixture. Cool to room temperature, filter into a 100-ml. volumetric flask, wash the residue on the filter with three 10-ml. portions of distilled water, add the washings to the original filtrate, and dilute to the mark with water. Mix 10 ml. of the extract with 10 ml. of a buffer solution prepared by neutralising 0·25 g.-mol. of acetic acid, diluted with 600 ml. of water, with concentrated sodium hydroxide to *p*H 4, using a glass electrode, and diluting the mixture to 1 litre. Dilute to 25 ml. De-aerate with nitrogen, transfer to the cell of a polarograph and take a polarogram from 1·0 v. applied potential. Compare the wave-height with the calibration curve. *Calibration curve*—Mix suitable volumes of a standard solution containing 1·00 g. of pure aloin in 1 litre of 20 volume per cent. ethyl alcohol with 10 ml. of the acetate buffer solution, add sufficient ethyl alcohol to give a concentration of 8 per cent. by volume in the final solution, and dilute to 25 ml. with water. Take a polarogram as for the sample and construct a calibration curve relating wave-height to the amount of aloin. This is usually a straight line for quantities of aloin up to 10 mg. per 25 ml. of the test solution. This procedure appears to give satisfactory results when applied to pharmaceutical preparations of aloes.

Since aloe-emodin present in aloes may be responsible for some of the undesirable physiological effects of preparations of the drug, a colorimetric method for its determination has been evolved. Tutin and Naunton (*Pharm. J.*, 1913, **91**, 836) have stated that aloe-emodin can be extracted from aloes by means of chloroform, but this is true only when the solvent is free from alcohol. Commercial chloroform, containing alcohol as a stabiliser, extracts some aloin as well, but if the extract is filtered, the small amount of aloin soluble in the stabilised solvent does not affect the determination of aloe-emodin. The change in colour of solutions of aloe-emodin in chloroform with change in concentration has been shown to be due not to chemical change, but probably to association of the

molecules. For yellow solutions, *i.e.*, containing 0·5 mg. per ml. or less, Beer's law is followed at a wavelength of 450 mμ. Orange or red solutions, *i.e.*, those containing more than 0·5 mg. per ml., follow Beer's law at 490 to 530 mμ., the greatest sensitivity being at 490 mμ.

Method for aloe-emodin—Transfer about 5 g. of aloes, accurately weighed, to a Soxhlet thimble and extract with 75 ml. of chloroform for 1 hr. If the solvent is not free from alcohol, filter into a 100-ml. volumetric flask, wash the residue with 10 ml. of chloroform, collecting the washings in the flask, and dilute to the mark with chloroform. If the solution is yellow, measure the extinction at 450 mμ.; otherwise, use a wavelength in the region 490 to 530 mμ. Calculate the amount of aloe-emodin corresponding to the value obtained by reference to a calibration curve constructed by applying the method to a standard solution containing 0·50 mg. of aloe-emodin per litre of alcohol suitably diluted.

Satisfactory results are obtained by applying this method to samples of aloes and to pharmaceutical preparations of the drug. J. ALLEN

Biochemical

Volumetric Semimicro- and Micro-Determination of Vitamin B_1. H. Wachsmuth (*Bull. Soc. Chim. Belg.*, 1947, **56**, 261–267)—Vitamin B_1 can be determined volumetrically by adding to an alkaline solution an excess of ferricyanide, and titrating the excess, for which three methods are given. The process is accurate, and takes only 5 min. Aldehydes and reducing sugars do not interfere.

Procedure—Iodimetric method—To a solution of 0·1 to 1 mg. of the vitamin in 10 ml. of water add 5 ml. of 0·005 *N* potassium ferricyanide and 4 drops of 20 per cent. sodium carbonate solution. After 2 min., add 3 ml. of a solution containing, in 100 ml., 5 g. of zinc sulphate, 25 g. of sodium chloride, and 2·5 g. of potassium iodide; add 3 ml. of 30 per cent. acetic acid. Titrate the iodine liberated by the residual ferricyanide with 0·002 *N* sodium thiosulphate. *Micro-titration*—To 0·01 to 0·1 mg. of the vitamin in 5 ml. of water, add 1 ml. of 0·0025 *N* potassium ferricyanide. After 2 min., add 2 ml. of *N* acetic acid and 1 ml. of the zinc iodide solution as above. Titrate the iodine with 0·001 *N* sodium thiosulphate. In both determinations subtract the number of millilitres of thiosulphate solution from that used in a standardising titration without the vitamin.

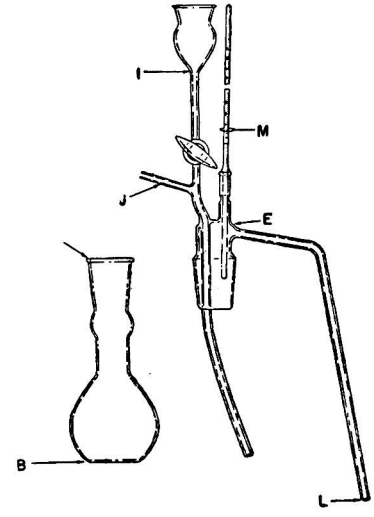
Cerimetric method—To a solution of 0·1 to 1 mg. of the vitamin in 10 ml. of water, add 5 ml. of 0·005 *N* potassium ferricyanide and 4 drops of 20 per cent. solution of sodium carbonate. After 2 min., add 1 ml. of 10 *N* hydrochloric acid and 2 drops of indicator (0·0347 g. of crystalline ferrous sulphate and 0·0812 g. of *o*-phenanthroline hydrochloride in 5 ml.). Titrate the ferrocyanide in the solution with 0·002 *N* ceric sulphate, the end-point being shown by a change in colour from orange-red to green. Allow for a blank determination on the ferricyanide. *Micro-method*—To a quantity of 0·01 to 0·1 mg. of vitamin in 5 ml. of water, add 1 ml. of 0·0025 *N* potassium ferricyanide and 3 drops of 20 per cent. sodium carbonate solution. After 2 min., add 0·5 ml. of 10 *N* hydrochloric acid and 1 drop of indicator, and titrate with 0·001 *N* ceric sulphate. The two methods given above are considered preferable to a third one in which the excess of ferricyanide is determined by titration with indigo carmine in alkaline solution.

G. MIDDLETON

Agricultural

Routine Method for Determining Selenium in Horticultural Materials. J. S. McNulty (*Anal. Chem.*, 1947, **19**, 809–810)—Perchloric acid and a vanadium catalyst with nitric and sulphuric acids destroy selenium-containing organic matter. The selenium is distilled with hydrobromic acid and the distillate titrated by a modified Norris - Fay procedure. Recovery is improved and the working time reduced. The procedure is as follows.

DIGESTION—*Tissues*—Quickly moisten 10 g. of the prepared sample with 30 ml. of "starting solution" and 5 ml. of water in a digestion flask. (Prepare the starting solution by dissolving 1·6 g. of ammonium metavanadate in 300 ml. of water and 1500 ml. of concentrated nitric acid. When seething stops, add 75 ml. of concentrated nitric



acid, 5 ml. of 60 per cent. perchloric acid, and 50 ml. of concentrated sulphuric acid.) Heat the flask slowly to 140° to 150° C. and when nitrogen peroxide ceases to be evolved raise the temperature slowly to 210° C.; cool, and rinse the thermometer with 10 ml. of water. If the solution becomes green during digestion, add 1 ml. of perchloric acid and 10 ml. of nitric acid, and heat less strongly.

Soils—Heat 50 g. of soil with 30 ml. of starting solution. When the foam breaks add 75 ml. of concentrated nitric acid, 7 ml. of perchloric acid, and 100 ml. of sulphuric acid. Incline the flask in a 600-ml. Moroney anti-bumping cup and digest as for tissues.

Nutrient solutions—Evaporate an appropriate volume to 30 or 40 ml. with 0·5 g. of sodium peroxide in a digestion flask. Add 50 ml. of sulphuric acid, 1 ml. of 60 per cent. perchloric acid, and 5 to 10 mg. of ammonium metavanadate. Add nitric acid only if the colour of the solution turns green. Heat to 210° C.

DISTILLATION—*Tissues and solutions*—Apply silicone grease to the joints and assemble on a ring stand the apparatus shown in the figure. Pass air in J so that 2 or 3 bubbles per sec. emerge from L, which is immersed in 50 ml. of 0·1 per cent. aqueous hydrazine sulphate solution in a cooled 250-ml. Berzelius beaker. Add 5 ml. of 48 per cent. hydrobromic acid solution to the digested sample through I. Heat the flask until most of the bromine is driven off, and allow 10 ml. of hydrobromic acid to be drawn into the flask at a rate of 1 ml. per min.; maintain a vapour temperature of 125° to 135° C. Heat E when necessary to remove the condensate.

Soils—Distil as above, after mixing with 10 ml. of hydrobromic acid, and add only 5 ml., while heating, at a rate of 0·5 ml. per min.

TITRATION—Add 3 g. of urea and 2·5 ml. of 90 per cent. formic acid solution to the receiver and heat to reduce the bromine: add 45 per cent. sodium hydroxide solution until the solution is neutral to phenolphthalein. Add 13 ml. of 18 *N* sulphuric acid and cool, then add 5 ml. of a 1 per cent. solution of potassium iodide in 0·1 per cent. wheat starch paste, and immediately titrate with 0·005 to 0·01 *N* sodium thiosulphate. The end-point is taken when the purple to pink colour change is stable for more than 7 sec. Standardise the thiosulphate against a pure selenite or selenium dioxide by the above titration method.

The employment of the vanadium-catalysed digestion is necessary to effect complete selenium recovery in the presence of high concentrations of organic material as in pot soils. The use of a small amount of potassium iodide favours a sharp end-point, as it avoids a high concentration of free selenium and iodine. Elimination of transfer between distillation and titration, hydrolysis of selenium tetrabromide before contact with air, and more effective oxidation all contribute to greater recovery.

Results—Of six values for selenium added to alfalfa meal, ranging from 50 to 330 p.p.m., five were correct to within 1 p.p.m. Of seven values for selenium added to pot soil, ranging from 2 to 50 p.p.m., five were correct to within 0·1 p.p.m.

M. E. DALZIEL

New *p*H Indicators for Determination of Total Alkalinity in Water. Disodium 4 : 4′-bis(p-dimethylaminophenylazo)-2 : 2′-stilbenedisulphonate and Disodium 4 : 4′-bis(o-tolyltriazeno)-2 : 2′-stilbenedisulphonate. M. Taras (*Anal. Chem.*, 1947, **19**, 339–341)—In an investigation of the indicator properties of the disazostilbeneamine-disulphonate series of dyes, amines of the aniline and naphthylamine series were coupled with 4 : 4′diaminostilbene-2 : 2′-disulphonic acid. Some of the indicators formed have pronounced colour changes that make them suitable for use in coloured solutions. Two of these compounds are discussed.

Disodium 4 : 4′-*bis*(p-*dimethylaminophenylazo*)-2 : 2′-*stilbenedisulphonate*—The colour change of this indicator is from orange-red at *p*H 5·5 to blue at *p*H 4·0. Because the indicator becomes progressively less soluble as the colour change proceeds, the titration is best carried out in a dish, the blue rings of dye deposited on the sides assisting in the detection of the end-point. In the range *p*H 4·0 to *p*H 5·0 the change of colour is sufficiently pronounced for titration to be performed to intermediate *p*H values. Because the colour change begins at *p*H 5·0 as against *p*H 4·5 for methyl orange, the indicator gives a more correct end-point in the titration of dilute carbonate solutions. It may be used in yellow carbonate solutions, *e.g.*, marshy water, in which methyl orange does not give a good end-point. Use 2 drops of a 0·1 per cent. solution of the indicator in each 100 ml. of liquid.

Disodium 4 : 4′-*bis*(o-*tolyltriazeno*)-2 : 2′-*stilbenedisulphonate*—The indicator changes from deep yellow at *p*H 5·0 to muddy at *p*H 4·0. It is more soluble than the above compound and more suited for use in colourless solutions. The colour change is marked only if titrations are performed in a porcelain dish. Use 5 drops of a 0·5 per cent. solution of the indicator in each 100 ml. of liquid.

Preparation of the indicators—Dissolve a paste of 9·25 g. of 4 : 4′-diaminostilbene-2 : 2′-disulphonic acid and 50 ml. of distilled water by adding a solution of 2·5 g. of sodium hydroxide in 100 ml. of water. Add, with stirring, a solution of 3·45 g. of sodium nitrite in 10 ml. of water. Run the liquid slowly, with stirring, into a mixture of 25 ml. of crushed ice and 25 ml. of concentrated hydrochloric acid. Allow the beaker to stand in a refrigerator for 1 hr., remove the supernatant liquid, and add the slurry to a solution of 7·3 ml. of dimethylaniline or, for the other indicator, to 6·0 ml. of *o*-toluidine in 25 ml. of glacial acetic acid. Stir for 1 hr., filter, and dry the precipitate. Add the calculated volume of 0·5 *N* sodium hydroxide and dilute the solution to the required concentration.

B. ATKINSON

Colorimetric Determination of Free Chlorine with Methyl Orange. M. Taras (*Anal. Chem.*, 1947, **19**, 342–343)—The method described is for the determination of chlorine in tap water. It makes use of the reaction by which two molecules of chlorine oxidise one of methyl orange.

Procedure—Fill a 100-ml. Nessler cylinder to the mark with the sample containing not more than 0·65 p.p.m. of chlorine. Add 2 drops of 5 *N* hydrochloric acid and exactly 3 ml. of 0·005 per cent. methyl orange solution and mix. Compare immediately against permanent standards prepared as follows. Calculate the volume of methyl orange solution in excess when portions of 3 ml. of 0·005 per cent. methyl orange solution are added to samples containing 0·1, 0·15, 0·20 . . . 0·65 p.p.m. of chlorine. Add these amounts of 0·005 per cent. methyl orange solution to 100 ml. of water acidified with 2 drops of 5 *N* hydrochloric acid. Keep the standards in the dark.

The method is sensitive to 0·1 p.p.m. of chlorine. By using more methyl orange, up to 1·3 p.p.m. of chlorine can be estimated. The solutions compared should be at *p*H 3, at which point the colour of methyl orange does not vary with *p*H, and the amount of acid added may have to be increased if the water is alkaline. The bleaching reaction is slow if sulphuric or acetic acid is used for the acidification. Up to 1·25 p.p.m. of chloramine does not interfere if the comparison is made quickly, and substantial amounts of ferric chloride can be present. As the methyl orange is equally sensitive to manganic ion and elementary chlorine, only 0·05 p.p.m. of manganic ion can be tolerated.

B. ATKINSON

Organic

Determination of the Alcoholic Hydroxyl Group in Organic Compounds. P. J. Elving and B. Warshowsky (*Anal. Chem.*, 1947, **19**, 1006–1010)—The authors have investigated the esterification of alcoholic hydroxyl groups with phthalic anhydride; by modifying the procedure of Sabetay and Naves (*Ann. Chim. anal.*, 1937, **19**, 35) and standardising the conditions, satisfactory results were obtained from a variety of compounds containing alcoholic hydroxyl groups. Phenolic hydroxyl groups do not react with the reagent, and work with synthetic mixtures and a complex condensate from a vapour-phase catalytic reaction showed that the presence of water and several types of organic compounds does not interfere with the determination. Amines generally show a satisfactory reaction, but sometimes the abnormally high results obtained may be due to phthalimide formation. Tertiary hydroxyl groups do not give satisfactory results. Any material present in the sample capable of reacting with phthalic anhydride will cause erroneous results.

Reagent—Dissolve 20 g. of reagent-grade phthalic anhydride in 200 ml. of purified anhydrous pyridine prepared by distilling reagent-grade pyridine over barium oxide and collecting the portion distilling at 115° C. This solution must be freshly prepared. Pyridine, purified by distillation over barium oxide, is also required as a solvent.

Procedure—Weigh accurately about 1·0 to 1·5 g. of samples containing a high percentage of ethyl alcohol, and correspondingly larger weights of samples of monohydric alcohols of high molecular weight or of dilute solutions of ethyl alcohol, into a 50-ml. volumetric flask containing 30 to 40 ml. of anhydrous pyridine, and dilute to volume with anhydrous pyridine. If the sample is extremely volatile, it should be weighed directly into thin-walled glass ampoules, which are transferred to the volumetric flask and crushed below the surface of the pyridine. Transfer 25 ml. of phthalic anhydride reagent to a dry pressure bottle by means of an automatic pipette and add 10 ml. of the diluted sample. Place the sealed bottle in an air-oven set at 100° C. ± 2° C., and heat at that temperature for 1 hr.; carefully release the pressure and add 50 ml. of distilled water. Mix, cool, and immediately titrate with 0·35 *N* sodium hydroxide, using phenolphthalein as indicator. Conduct a blank determination on the reagents employed.

Percentage of hydroxyl $= V \times N \times 1{\cdot}70/W$, where W = weight in grams of the sample in the aliquot taken, V = difference in volumes of the standard sodium hydroxide required by the blank and sample titrations, and N = normality of the standard sodium hydroxide solution.

To avoid low results, all reaction equipment must be dry.

A. H. A. ABBOTT

Estimation of Glycerol in Presence of Propylene and Ethylene Glycols. S. H. Newburger and C. F. Bruening (*J. Assoc. Off. Agric. Chem.*, 1947, **30**, 651–655)—When glycerol, propylene glycol, and ethylene glycol are oxidised by potassium periodate the only acid oxidation product, formic acid, is yielded by the glycerol. Shupe (*J. Assoc. Off. Agric. Chem.*, 1943, **26**, 229) proposed a method for determining glycerol based on the alkali titration of the formic acid so produced; the present authors have introduced modifications, including the reduction of the excess of iodate by propylene glycol, which render the method capable of recoveries of 100 ± 0·9 per cent. when glycerol is determined alone or in solutions containing propylene and ethylene glycols. [*Cf.* Pohle and Mehlenbacher, this vol. p. 42.]

Reagents—(*a*) *Potassium periodate solution*, 0·02 *M*—Dissolve 4·6 g. of potassium periodate in about 500 ml. of hot water, dilute to 900 ml. with water, cool to room temperature, and dilute to 1 litre. (*b*) *Commercial propylene glycol*, which must comply with the following specification. Dilute 0·5 g. to 25 ml. with water, add 25 ml. of 0·02 *M* potassium periodate and 3 drops of 0·1 per cent. bromocresol purple indicator solution, and set aside for 10 min. For titration to a light purple end-point, the solution should require not more than 0·05 ml. of 0·02 *N* sodium hydroxide.

Procedure—Add 1 drop of 0·1 per cent. bromocresol purple indicator solution to an aliquot of the sample containing not more than 45 mg. of glycerol and neutralise to a light purple colour with 0·02 *N* sodium hydroxide. Add 50 ml. of 0·02 *M* potassium iodate, dilute to 110 ml. with water, and allow to stand for 1 hr. Transfer 50 ml. to a flask, add about 10 drops of propylene glycol, mix well, and allow to stand for 10 min. Add 3 drops of bromocresol purple indicator and titrate with 0·02 *N* sodium hydroxide to a light purple end-point. 1 ml. of 0·02 *N* sodium hydroxide ≡ 0·00184 g. of glycerol.

A. H. A. ABBOTT

***p*-Triphenylmethylphenyl and 2-Fluorenyl Isocyanates as Reagents for Alcohols. B. Witten and E. E. Reid** (*J. Amer. Chem. Soc.*, 1947, **69**, 2470–2472)—When only small quantities of alcohol are available *p*-triphenylmethylphenyl *iso*cyanate has the advantage of high molecular weight, but high-melting derivatives are obtained with *n*-alcohols only up to butyl. For the *n*-alcohols C_1 to C_{18}, 2-fluorenyl *iso*cyanate gives a melting-point series similar to that afforded by *p*-xenyl *iso*cyanate but with greater differences for adjacent alcohols. Of all the carbamates prepared by the

authors the fluorenyl compounds are the most easily recrystallised to constant melting-point.

Procedure—The *iso*cyanates are prepared from the corresponding amines by the method of Hardy (*J. Chem. Soc.*, 1934, 2011). To prepare the derivatives heat a 50 per cent. excess of the *n*-alcohol with a solution of 0·5 g. of the *iso*cyanate in 5 ml. of toluene in boiling water for 3 hr. With methyl and ethyl alcohols use a five-fold molecular excess of the reagent and, with methyl alcohol, a reflux condenser. Evaporate to dryness under reduced pressure and recrystallise the residue twice from alcohol, in which substituted urea, which is always formed, is insoluble.

The following are the melting-points in ° C. of the derivatives prepared from the alcohols named and *p*-triphenylmethylphenyl and 2-fluorenyl *iso*cyanate, respectively. Methyl, 214°, 120°; ethyl, 216°, 120°; *n*-propyl, 177°, 114°; *n*-butyl, 140°, 112°; *n*-amyl, 85°, 93°; *n*-hexyl, 81°, 98°; *n*-heptyl, 55°, 97°; *n*-octyl, 61°, 119°; *n*-nonyl, 62°, 110°; *n*-decyl, 64°, 100°; *n*-undecyl, 68°, 109°; *n*-dodecyl, 70°, 112°; *n*-tridecyl, 74°, 107°; *n*-tetradecyl, 76°, 106°; *n*-pentadecyl, 77°, 108°; cetyl, 79°, 108°; *n*-heptadecyl, 79°, 108°; *n*-octadecyl, 79°, 107° C.

New derivatives from p-*xenyl* iso*cyanate*, with melting-points, are *n*-tridecyl, 114°; *n*-tetradecyl, 113°; *n*-pentadecyl, 113°; cetyl, 113°; *n*-heptadecyl, 113°; *n*-octadecyl, 114°; from *phenyl* iso*cyanate*—*n*-tridecyl, 70°; *n*-heptadecyl, 79°; from α-*naphthyl* iso*cyanate*—*n*-undecyl, 73°; *n*-tridecyl, 80°; *n*-tetradecyl, 81°; *n*-octadecyl, 89° C. W. C. JOHNSON

Quantitative Determination of Galactose, Mannose, Arabinose, and Rhamnose. E. L. Hirst, J. K. N. Jones, and E. A. Woods (*J. Chem. Soc.*, 1947, 1048–1051)—In methods for determining galactose in the mixture of sugars obtained by hydrolysis of plant gums, by weighing its insoluble phenylmethylhydrazone (Neuberg, *Biochem. Z.*, 1907, **3**, 519; Ludtke, *Ibid.*, 1919, **212**, 419; Neuberg and Schweitzer, *Monatsh.*, 1937, **71**, 46; Freeman *et al.*, *Biochem. J.*, 1940, **34**, 316), the galactose is converted into the hydrazone under standard conditions and a factor is used to convert the weight of hydrazone into the weight of galactose originally present. This method is accurate only if the amounts of galactose in the control solution and in the unknown solution are identical. To avoid the necessity for control estimations on each occasion the yields, under standard conditions, of the phenylmethylhydrazone from various amounts of galactose have been determined. A graph constructed from these results was linear even when glucose, xylose, rhamnose, and glucuronic acid were present. Mannose and arabinose interfere, but mannose can be estimated by the phenylhydrazine method (Bourquelot and Herissey, *Compt. rend.*, 1899, **129**, 399, *vide infra*) or by fermentation, and arabinose, although not removable by fermentation, can be estimated by means of diphenylhydrazine (Neuberg, *Ber.*, 1900, **33**, 2243; Wise and Paterson, *Ind. Eng. Chem.*, 1930, **22**, 365), or by boiling with 12 per cent. hydrochloric acid and estimating the furfural evolved. Since the precipitation of arabinose by phenylmethylhydrazine appears to be quantitative in presence of galactose, an estimation of the galactose present can be made after deducting the weight of the phenylmethylhydrazone formed from the arabinose, the amount of which has been determined by another method. Quantitative estimation of arabinose in presence of galactose is somewhat difficult, as most reagents react similarly with both sugars. An exception is benzoylhydrazine, which has the advantage of ease of preparation and stability (Fischer and Paulus, *Arch. Pharm.*, 1935, 83; Militzer, *J. Chem. Educ.*, 1941, 25). Of many sugars examined, the only one interfering with the estimation by means of this reagent (*vide infra*) was rhamnose when present in amounts greater than 300 mg.

Galactose—The reagent is 1-phenyl-1-methylhydrazine (25 g.) mixed with 100 ml. of absolute alcohol containing 3 ml. of glacial acetic acid and stored in a stoppered, brown-glass bottle at 0° C. To the sugar sample containing not more than 1·4 g. of sugars, dissolved in 10 ml. of water, add 10 ml. of the reagent and keep the mixture in a tightly stoppered flask at 33° C. for 12 hr. with occasional shaking, and then at 0° C. for 9 hr. Collect the crystals (m.p. 186° C.) in a tared Gooch crucible, wash them with ice-cold alcohol (10 ml.), dry at 100° C., and weigh. The weights of phenylmethylhydrazone obtained with various weights of galactose either with or without other sugars satisfy the equation $y = 0{\cdot}673\,x + 0{\cdot}013$, where y is the weight of galactose giving a weight x of the hydrazone.

Arabinose—To prepare the reagent dissolve 25 g. of benzoylhydrazine, m.p. 112° to 113° C., when recrystallised from water, in 500 ml. of 95 per cent. alcohol. Dissolve the sugar sample, containing 50 to 500 mg. of arabinose, in 2 ml. of water in a 50-ml. stoppered flask, add 10 ml. of the reagent and keep the mixture at 20° C. for 24 hr. with occasional shaking, and then at −3° C. for 22 hr. Collect the crystalline residue (m.p. 186° to 190° C. with decomposition) in a tared Gooch crucible, wash with 10 ml. of ice-cold alcohol, dry at 100° C. for 30 min., and weigh. Of the sugars glucose, mannose, xylose, galactose, glucurone, and rhamnose, only the last-named interferes, and then only when present in amounts exceeding 300 mg. The relationship between the weight of arabinose and the yield of derivative is linear and satisfies the equation $y = 0{\cdot}555\,x + 0{\cdot}022$, where y is the weight of arabinose giving a weight x of derivative. In a second method the reagent is a saturated aqueous solution of benzoylhydrazine (about 4·3 g. per 100 ml.) in which the derivative is more soluble but from which it separates in a form better for filtration. Since the reagent contains no alcohol, alcohol-insoluble material such as oligosaccharides and the barium salts of uronic acids remain in solution and do not contaminate the precipitate. Dissolve the dry, water-soluble sample containing from 50 to 150 mg. of arabinose in 5 ml. of the reagent, keep the solution at 30° C. for 48 hr. and then at 0° C. for 2 hr. Collect the crystalline precipitate, wash with 10 ml. of 95 per cent. alcohol and dry at 100° C. to constant weight. If the material contains from 150 to 400 mg. of arabinose

10 ml. of the reagent should be used. The results obtained satisfy the equation $y = 0{\cdot}67x + 0{\cdot}010$, where y is the weight of arabinose yielding a weight x of derivative.

Rhamnose—Rhamnose cannot be so successfully estimated as arabinose by the use of benzoylhydrazine owing to the greater solubility of the rhamnose derivative and the interfering effect of small amounts of arabinose. Mix the sugar sample (250 to 600 mg. in 2 ml. of water) with 10 ml. of a saturated alcoholic benzoylhydrazine solution and keep the mixture at 30° C. for 41 hr., seeding it with a trace of rhamnose benzoylhydrazone after 17 hr. At the end of the 41-hr. period, cool the liquid to −3° C., collect the precipitate, wash it with 30 ml. of 95 per cent. alcohol and, to avoid charring, dry it first in a desiccator and then at 90° C. for 1 hr. The results satisfy the equation $y = 0{\cdot}482x + 0{\cdot}217$, where y is the weight of rhamnose hydrate yielding a weight x of rhamnose benzoylhydrazone. The derivative separates as fine white crystals, which can be recrystallised from methyl alcohol (m.p. 180° C., with decomposition).

Mannose—To prepare the reagent mix 25 ml. of re-distilled· phenylhydrazine with 100 ml. of absolute alcohol containing 3 ml. of glacial acetic acid and store it in a stoppered dark bottle. Dissolve the sugar in 10 ml. of water, add 10 ml. of the reagent, and allow the mixture to stand in a stoppered flask for 15 hr. at 32° C., with occasional shaking, and at 0° C. for 12 hr. Collect the phenylhydrazone, wash it with 10 ml. of ice-cold alcohol, dry at 100° C. for 30 min., and weigh. The results obtained satisfy the equation $y = 0{\cdot}652x + 0{\cdot}031$, where y is the weight of mannose giving a weight x of mannose phenylhydrazone. Glucose, arabinose, galactose, rhamnose, and glucuronic acid do not interfere with the estimation of mannose, which can be made to within ± 3 mg. on amounts exceeding 100 mg. A. O. JONES

Rapid Determination of Nitroglycerin and Ethyl Centralite in Rocket Propellant Powder. I. S. Hirschhorn (*Anal. Chem.*, 1947, **19**, 880–882)—Standard analytical procedures for determining nitroglycerin and ethyl centralite in rocket propellant powders are based upon ether extraction for as long as 72 hr. Both substances can be extracted, however, by heating them under refluxing conditions with 84 per cent. acetic acid for 20 min. The two constituents can then be determined in the acetic acid solution by the ferrous chloride and titanous chloride method of Becker (*Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 152) or the bromination method of Waugh *et al.* (*Ibid.*, 1946, **18**, 636; ANALYST, 1947, **72**, 73). A blank determination corrects for the slight solubility of nitrocellulose in 84 per cent. acetic acid.

Procedures—To prepare the sample, slice a wafer of rocket powder into thin slices and then into small chips (about 10 × 5 × 2 mm.). Mix the chips and grind one chip at a time in a clean Wiley mill, vented at both top and bottom and working at medium speed, until enough powder has been collected for analysis.

To prepare the solvent mix 5 parts of pure glacial acetic acid with 1 part of water, using carbon dioxide as the mixing gas. If necessary, adjust the concentration of the acid to 84 ± 0·5 per cent. after titration with a standard base.

To 1 g. of sample accurately weighed in a 250-ml. Erlenmeyer flask provided with a ground-glass joint add 50 ml. of the acetic acid previously warmed to 100° C., and mix by swirling. Connect the flask to a reflux condenser and heat in boiling water for 20 min. Filter the cooled extract through a No. 4 Whatman paper into a 750-ml. reduction flask, rinsing the extraction flask first with 20 ml. and then with two 10-ml. portions of the acetic acid. For the ethyl centralite determination, filter the extract directly into a 250-ml. iodine flask.

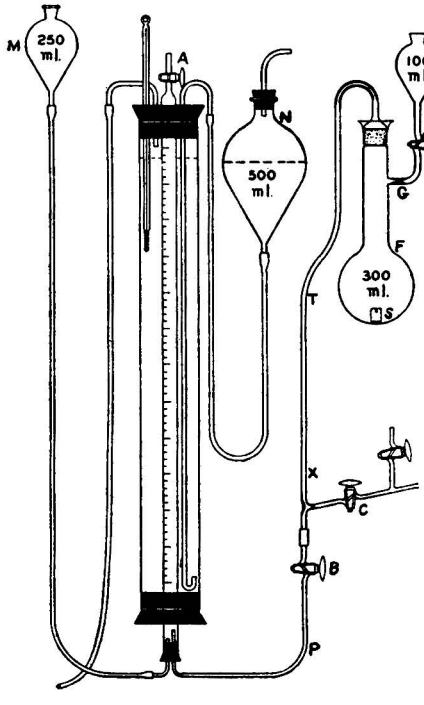
For the nitroglycerin determination, place glass beads in the flask and pass in carbon dioxide at a rate sufficient to maintain a ripple on the surface of the liquid throughout the titration. Add 30 ml. of 0·07 *N* ferrous chloride and 50 ml. of diluted hydrochloric acid (1 + 1), and swirl. Connect the reduction flask to an Allihn condenser and boil the solution on the hot-plate for 10 min. If brown fumes appear in the condenser, discard the determination, which will be erroneous. Cool the liquid, rinse the condenser with water and disconnect the flask, rinsing the ground-glass joint. Titrate the solution with 0·25 *N* titanous chloride and, after about 50 ml. have been added, add 5 ml. of 40 per cent. ammonium thiocyanate solution and continue the titration until the colour changes sharply from red to peach. Correct the result by means of a blank determination. The nitroglycerin content (per cent.) is given by $2{\cdot}523N(A - B)/WR$ where N is the normality of the titanous chloride solution, A is the number of millilitres required by the sample solution and B the number required by the blank solution, W is the dry weight (g.) of the sample, and R is the recovery expressed as a decimal fraction. To find R, determine the nitrogen content of a nitroglycerin sample by the Du Pont nitrometer method (T. L. Davies, "*Chemistry of Powder and Explosives*," pp. 271–3, New York, John Wiley & Sons, 1943; Pitman, *J. Soc. Chem. Ind.*, 1900, **19**, 982) and the moisture content by vacuum desiccation for 24 hr. Prepare a synthetic powder sample by weighing 0·42 to 0·43 g. of the nitroglycerin into the extraction flask containing 50 ml. of 84 per cent. acetic acid, then adding 0·5 g. of smokeless grade nitrocellulose, 0·03 g. of diethyl phthalate, and 0·01 g. of ethyl centralite. Mix by swirling and determine the nitroglycerin as already described. The recovery per cent. is given by $2{\cdot}523N(A - B)/W$ where W is the weight in grams of pure nitroglycerin in the sample.

For the determination of ethyl centralite, add 25 ml. of 0·04 *N* potassium bromate - bromide solution to the acetic acid extract, add 5 ml. of diluted hydrochloric acid (1 + 5), stopper the flask, swirl for 1 min., add 5 ml. of 10 per cent. potassium iodide solution, and titrate the liberated iodine with 0·02 *N* sodium thiosulphate, with starch as indicator. Make a blank determination on the reagents. The ethyl centralite content is given by $6{\cdot}704N(C - D)/W$, where N is the normality of

the sodium thiosulphate solution, C is the number of millilitres required by the blank solution, D is the number required by the sample solution, and W is the dry weight of the sample in grams.

A. O. JONES

Analysis of Diazo Compounds, particularly Diazodinitrophenol. W. E. Shaefer and W. W. Becker (*Anal. Chem.*, 1947, **19**, 307–310)—The diazo-nitrogen in diazodinitrophenol is determined by treating the sample with a large excess of titanous chloride and measuring the volume of nitrogen evolved. The nitrogen present as the nitro-group plus that present in impurities as the amino-group is determined by a modification of the sodium dithionite ($Na_2S_2O_4$) method of Simek (*Chem. Listy*, 1931, **25**, 322–325). When the amount of diazo-nitrogen is known, the nitro-nitrogen is determined by a modification of the titanous chloride method of Becker (*Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 152).



DETERMINATION OF DIAZO-NITROGEN—A diagram of the apparatus is given in the figure.

Reagent—Mix 40 ml. of 15 per cent. titanous chloride solution, 25 ml. of concentrated hydrochloric acid, and 25 ml. of distilled water and heat the mixture under a reflux condenser for 15 min. Then heat it in an open flask for 3 min.

Procedure—Fill with distilled water the bulb, M, the 50-ml. gas burette, and the connecting tube P to beyond the tap B, and close the tap A. Lower into the flask, F, a small cylinder, S, containing about 0·3 g. of the diazo-compound, attach the flask to the apparatus, placing a small amount of glycerol in the cup of the joint, and wrap black cloth round the flask. Evacuate the flask, add 10 ml. of ether through the tap D, and evacuate the flask again. After closing D always place a small amount of glycerol in the bulb to make the tap air-tight. Open tap B to admit water to the point X. Add 100 ml. of boiling water to the flask through D or, if the sample is insoluble in water, add 100 ml. of a 1 : 1 water - alcohol mixture. While still protecting it from the light, heat the flask in boiling water for 10 to 20 min. Add through D the titanous chloride solution prepared as described above, and immerse the flask in boiling water for 5 min., shaking it occasionally. Place 100 ml. of boiling water in the bulb above D, cool the flask a little, and admit water until the nitrogen in the flask is at atmospheric pressure. By opening tap B, admitting water through D, and adjusting the level of the bulb M, transfer the nitrogen to the burette. Cool the burette to room temperature and measure the volume of nitrogen. If alcohol has been used to dissolve the sample, wash alcohol vapour from the nitrogen by running 100 ml. of nitrogen-saturated water into the burette slowly through tap A. Perform a blank determination following the above procedure but adding no sample. The procedure was tested using pure diazodinitrophenol and the results obtained were within 5 parts in 1000 of the theoretical values.

DETERMINATION OF COMBINED NITRO- AND AMINO-NITROGEN—*Procedure*—Weigh 0·25 g. of the sample into a 200-ml. Kjeldahl flask, add 25 ml. of alcohol, and warm on a steam-bath until dissolution appears to be complete. Add 25 ml. of hot water and heat for a few minutes. If the dark coloured solution is not clear, add more alcohol. Add rapidly a freshly prepared solution of 5 g. of sodium dithionite ($Na_2S_2O_4$) in 25 ml. of hot water and heat the mixture on a steam-bath for a few minutes, shaking occasionally. Cool, decompose the excess of sodium dithionite by adding 5 ml. of 50 per cent. sulphuric acid, and add cautiously 20 ml. of concentrated sulphuric acid. Add a few particles of carborundum to prevent bumping, heat the flask over a low flame until the alcohol and water have evaporated, and then heat it at a higher temperature until the solution becomes colourless or a very pale straw colour. Remove any sulphur from the neck of the flask by heating with a free flame and then heat the flask for 30 min. more. Cool, add some water cautiously, transfer the solution to a 500-ml. Kjeldahl distilling flask, add a few pieces of granular zinc and a little magnesium oxide, and through a funnel add an aqueous solution of 75 g. of potassium hydroxide. Distil the ammonia into 40 ml. of 0·1 N hydrochloric acid and titrate the excess of acid with standard alkali. For pure diazodinitrophenol the method gave a percentage of nitro-nitrogen close to the theoretical value.

DETERMINATION OF NITRO-NITROGEN—Use the method of Becker with the following modification. Add an acetic acid solution of the sample slowly, with constant swirling, to a measured volume, about 100 per cent. excess, of titanous chloride solution. The percentage of nitro-nitrogen can be

calculated if the percentage of diazo-nitrogen is known: 1 ml. of N titanous chloride ≡ 0·02802 g. of diazo-nitrogen or 0·002335 g. of nitro-nitrogen. The method gives closely reproducible results when used on diazodinitrophenol samples.

B. ATKINSON

Acid-base Reactions in Benzene and Other Organic Solvents: Behaviour of Bromophthalein Magenta with Different Classes of Organic Bases. M. M. Davis and P. J. Schuhmann (*J. Res. Nat. Bur. Stand.*, 1947, **39**, 221–263)—The reactions of a number of aliphatic amines and of di- and triaryl derivatives of guanidine with bromophthalein magenta in benzene and other organic solvents have been studied. Visual observations are recorded and transmittancy curves and "molar absorbancy curves" are determined with the Beckman quartz photo-electric spectrophotometer. Bromophthalein magenta is an ester of tetrabromophenolphthalein, bromophthalein magenta E being the ethyl ester and bromophthalein magenta B the *n*-butyl ester. As indicators the two are indistinguishable, but the *n*-butyl ester is five times more soluble in benzene on a molar basis and can be used in *cyclo*hexane, in which the ethyl compound is too insoluble. Benzene is used for much of the work on account of its aprotic character, *i.e.*, its inability to add or release protons. A 5×10^{-5} M solution of the indicator in benzene is yellow and shows no measurable absorption at wavelengths greater than 530 mμ. Maxima appear at 285 mμ. and 405 mμ. An almost identical curve is obtained if either *cyclo*hexane or ethylene dichloride replaces benzene as solvent.

Tertiary aliphatic amines and symmetrical di- or triaryl guanidines—The addition of a base of one of these types to a benzene solution of bromophthalein magenta brings about a colour change from yellow to vivid magenta through orange and red. By plotting transmittancy curves for benzene solutions of bromophthalein magenta E, each containing a 1·25 molar equivalent of one of these bases, it has been possible to arrange the bases in increasing order of the extent to which they react with the indicator. When the bases are also arranged in increasing order of their ionisation constants (pK$_b$) in water, there is a direct parallel for five out of eight bases. The lack of agreement for three bases is considered as evidence that pK$_b$ determinations in water for these bases are not valid. Transmittancy curves for varying proportions of indicator and base make it possible to determine the limiting curve, *i.e.*, that corresponding to the maximum reaction of base with indicator, and curves for three bases, when present in excess of this proportion, are almost identical.

Secondary aliphatic amines—These yield a purple-blue colour with bromophthalein magenta in benzene, with green intermediate colours. Curves illustrate the transmittancies but the small variation of pK$_b$ (water) amongst secondary amines and the comparatively large variations in literature values for individual members of the class do not allow of comparison with transmittancy characteristics.

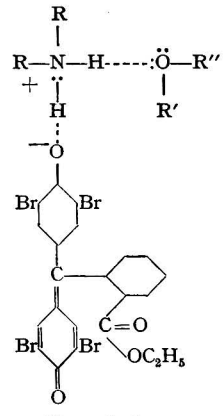
Primary aliphatic amines—Primary amines give a red-purple colour and the transmittancy curves resemble those of the secondary amines, except that they produce a smaller effect at a given concentration. Contrary to the behaviour of the tertiary amines, reactivity with the indicator in benzene decreases with increasing pK$_b$ for water. The main absorption band obtained with an excess of one of these amines shows two maxima corresponding respectively to the maxima for tertiary and secondary amines. There is also some evidence of dimerisation.

Tetra-alkylammonium salts—The tetra-ethyl- and tetra-*n*-butyl-salts of bromophthalein magenta E and bromophthalein magenta B, respectively, are prepared in the solid state by reaction of the indicators with solutions of the quaternary ammonium hydroxides. The salts yield blue solutions in benzene; the absorption curves are closely similar and show marked and irregular deviation from Beer's law. This deviation might be accounted for on theoretical grounds, but is also possibly due to reaction with the glass of the vessels employed. Absorption characteristics for solutions in benzene, ethyl acetate, and ethylene dichloride are qualitatively similar and divergencies in intensity may possibly be due to incomplete solution of the salts in benzene.

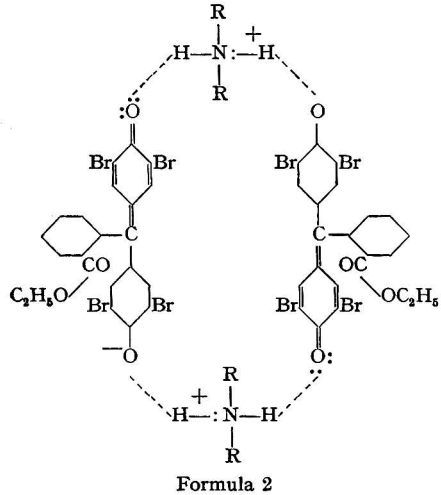
The stability of benzene solutions of substituted ammonium salts of bromophthalein magenta increases with an increase in number of substituent groups and decreases with an excess of amine.

From the absorption data values of K$_b$, the basicity or equilibrium constant in benzene, are calculated for five bases, using the equation $K_b = [S]/\{[B] \times [A]\}$, where [S], [B], and [A] represent the concentrations of salt, base, and indicator, respectively, in g. molecules per litre. The curves relating $\log\{[S]/[A]\}$ to $-\log[B]$ are linear with approximately unit negative slope, and thus accord with simple equilibrium theory. Also $\log K_b$ in benzene plotted against pK$_b$ in water shows a constant relationship in respect of triethylamine and 1 : 3-diphenyl- and 1 : 3-ditolylguanidine.

To account for the different colour reactions of the four types of amines the following theories are suggested. The quaternary ammonium salts exist predominantly as individual ion pairs. In the tertiary ammonium salts there is closer approach of the positive and negative ions to each other and the formation of an oxygen bridge between the nitrogen of the base and the oxygen of the acid. The purple-blue colour produced by secondary amines is modified to a magenta, similar to that produced by tertiary amines, by addition of various oxygen compounds such as dioxan and acetone. This is attributed to the formation of a hydrogen bridge as in formula 1, and in absence of an oxygen compound the hydrogen bridge links two molecules of the indicator acid to form a dimer stabilised by resonance, formula 2. The intermediate behaviour of primary amines is based on their ability to form dimers and to participate in bridge formation like tertiary amines.



Formula 1



Formula 2

other basic drugs give the colour reactions to be anticipated from the character of their nitrogen atoms. Aromatic amines are too weakly basic to show measurable reactions at the concentrations employed for aliphatic amines. Decahydroquinoline gives the usual reaction of a secondary amine, but tetrahydroquinoline is only weakly basic and shows some similarity to primary amines in its reaction with the indicator. Di*cyclo*hexylamine is more basic than diphenyl- or di-*o*-tolylguanidine and differs from them in giving a blue colour. The reaction of trimethylamine oxide is also studied.

Other solvents—In *cyclo*hexane and other aprotic solvents the same colours are observed for the different bases as in benzene. In ethylene dichloride primary, secondary, and tertiary bases all give a magenta colour, attributed to a hydrogen bridge between N and Cl. The colours produced in a large number of other solvents, ethers, esters, ketones, alcohols and others, are recorded. Marked solvent effects are evident in most cases, except with respect to the quaternary ammonium salts. These effects are discussed with particular reference to the Brönsted - Lowry theory and the theory of the hydrogen bridge.

Other indicators—Similar phenomena have been observed with other indicators whose structure is related to bromophthalein magenta, *e.g.*, the halogenated sulphonphthaleins, but bromophthalein magenta has the advantage of greater solubility in inert solvents; as a monobasic acid its reactions are more readily studied and its colour changes are brilliant and sensitive.

Acid - base titrations—The literature on titrations in non-aqueous solvents is reviewed. As bromophthalein magenta is a relatively strong acid it cannot be used in the titration of acids as weak as benzoic or acetic acids. The following table is a summary of a series of titrations in benzene solution using a 0·1 per cent. solution of bromophthalein magenta in benzene as indicator.

Reference base	*Acid*	*Molarity found for acid*
Diphenylguanidine, 0·0100 *M*	*d*-Camphorsulphonic acid, approx. 0·005 *M*	0·00527, 0·00529, 0·00528
Reference acid	*Base and approximate molarity*	*Molarity found for base*
d-Camphorsulphonic acid, 0·00528 *M*	Di*cyclo*hexylguanidine, 0·0025 *M*	0·00238, 0·00237
Trichloroacetic acid, 0·106 *M*	Di-*o*-tolylguanidine, 0·05 *M*	0·0484, 0·0484, 0·0487
Picric acid, 0·0100 *M*	Di-*o*-tolylguanidine, 0·01 *M*	0·00970, 0·00972, 0·00972
Trichloroacetic acid, 0·106 *M*	Triethylamine, 0·1 *M*	*0·0942, 0·0944
Trichloroacetic acid, 0·0106 *M*	Triethylamine, 0·01 *M*	0·00935, 0·00935, 0·00936
Picric acid, 0·0100 *M*	"	0·00962, 0·00962, 0·00960, 0·00962
Trichloroacetic acid, 0·106 *M*	Piperidine, 0·1 *M*	*0·0957, 0·0953
Picric acid, 0·100 *M*	"	†0·0982, 0·0990, 0·0977

* A cloud formed during the titration. † Picrate began to precipitate.

W. C. JOHNSON

Other organic bases—Secondary organic bases containing oxygen, *e.g.*, morpholine and methylethanolamine, added in small amount to a benzene solution of the indicator, give a blue colour changing to magenta on further addition. Alkaloids and

Determination of Methylol Groups and Dibenzyl Ether Linkages in Phenol Alcohols and Derived Phenolic Resins. Part I. H. S. Lilley and D. W. J. Osmond (*J. Soc. Chem. Ind.*, 1947, **66**, 340–341)—The method uses the oxidation

of the methylol (CH_2OH) group by iodine, resulting in its elimination as formic acid, the reaction being represented by the scheme $R.CH_2OH + I_2 + H_2O \rightarrow RH + H.COOH + 2HI$. Allowance is made for the substitution, $RH + I_2 \rightarrow RI + HI$, that also occurs. This is done by the addition of iodate and a second iodine titration.

Procedure—If the resin is of low methylol content (*e.g.*, ammonia-catalysed resin, etc.) make a solution of about 20 g. of resin in 1 litre of carbon tetrachloride. Pipette 10 ml. of this solution into a 250-ml. bolt-head flask fitted with a mechanical stirrer and off-set burette. Add 20 ml. of approximately 0·5 *N* sodium hydroxide and 25 ml. of approximately 0·2 *N* iodine. Stir at moderate speed for 20 to 30 min. Add 20 ml. of approximately 0·5 *N* sulphuric acid. Stop stirring and add 50 ml. of distilled water to prevent emulsification. Titrate the liberated iodine with 0·2 *N* sodium thiosulphate and then add 20 ml. of approximately 0·5 *N* potassium iodate and follow by a second titration. For pure phenolic alcohols or resins of high methylol content, *e.g.*, soda-catalysed resins, the carbon tetrachloride can be omitted.

Calculation—The percentage of methylol as CH_2O equals $\{3f(\frac{1}{2}t_1 + t_2) - F\}$/weight of sample in milligrams, where f = the factor of thiosulphate, t_1 and t_2 are the two titrations, and F is derived from a blank experiment in which $F = f(\frac{1}{2}t_1 - t_2)$.

Results—The method decreases in accuracy with decreasing methylol content, since the scatter of the results is constant. The value obtained will also include free formaldehyde or any other oxidisable material present. If the base is stronger than the acid it is necessary to add in the blank determination a small amount of an unmethylolated compound such as *p*-cresol, liberating hydriodic acid to neutralise the excess of base and give a blank reading. The amount of hydriodic acid so formed is automatically compensated for by the method of calculation. An accuracy of to within 0·5 to 3·0 per cent. is claimed over the range 30 to 10 per cent. methylol content, but the accuracy is considerably less for lower methylol contents.

W. C. WAKE

linkage is estimated indirectly by determination of the water eliminated during its fission by hydrobromic acid. The water is titrated with the Karl Fischer reagent. This method is more trustworthy than analysis of the bromomethyl compounds that also result in this fission of the ether linkage. A correction must be applied for the methylol groups present. No details are given for the preparation and use of the Fischer reagents, which are assumed to be well-known. [Cf. ANALYST, 1946, **71**, 483.]

Apparatus—The following apparatus-train is required. (*a*) A generator for gaseous hydrobromic acid from potassium bromide and *ortho*-phosphoric acid. (*b*) A large Buchner flask immersed in cold water to trap water of reaction. (*c*) A red-phosphorus trap for bromine. (*d*) Two towers of 14-mesh calcium chloride. (*e*) Two towers of anhydrone. (*f*) The reaction vessel, which should be a 75-ml. flask. (*g*) Two further flasks as (*f*) each containing dry chloroform to entrap any water of reaction carried over.

Procedure—The chloroform to be used is dried specially and should not show a water content corresponding to more than 0·2 ml. of the Fischer reagent per 40 ml. of chloroform. Cool the vessels (*f*) and (*g*) in acetone to which solid carbon dioxide is added, dissolve about 300 mg. of the substance to be analysed in 40 ml. of dry chloroform in the vessel (*f*), and draw by means of a vacuum pump a slow stream of hydrobromic acid through the apparatus for about 30 min. Disconnect the reaction vessel (*f*) and the trapping vessels (*g*). Allow the former to stand for 12 to 24 hr. at room temperature before titrating with the Fischer reagent, but the contents of the trapping vessels (*g*) may be titrated immediately. In calculating the ethers present, a correction must be made for methylol groups determined by the method given in Part I of this paper. (See preceding Abstract.)

Results—Results are given for seven pure complex ethers and for five resin samples. With the latter, comparison is given in the table between the results obtained by direct bromine determination after fission, by ultimate analysis, and by the present method using the Fischer reagent.

Resin	—CH_2OCH_2— per cent. From bromine determination	From ultimate analysis	From present method
Ammonia-catalysed (1) ..	2·4	2·20	2·4, 2·0
(2) ..	0·55	0·62	0·60, 0·46
(3) ..	2·7	2·60	2·80, 2·84
Soda-catalysed (1) ..	very low, scattered results	9·8	9·3, 10·0
(2) ..		1·1	1·2, 0·7

W. C. WAKE

Determination of Methylol Groups and Dibenzyl Ether Linkages in Phenol Alcohols and Derived Phenolic Resins. Part II. Ethyl Linkages. H. S. Lilley and D. W. J. Osmond (*J. Soc. Chem. Ind.*, 1947, **66**, 425–427)—The ether

Determination of Nicotine with Picric Acid. J. F. van Elteren (*Chem. Weekblad*, 1947, **43**, 819–820)—In the usual method for the determination of nicotine by picric acid, it is specified that the mixture should either be cooled in ice or allowed

to stand for 24 hr. Actually, ice cooling is always essential to reduce the error due to the solubility of the picrate, and it is also necessary to cool in ice for 12 hr. The amount of picric acid should be increased, as an excess reduces the solubility of the picrate. If the amount of nicotine is small, 1 g. of picric acid should be added to the distillate, or for larger amounts of nicotine, 1·5 g. The picric acid is dissolved in 50 ml. of warm water. The correction to be added for solubility is then 2, 3, 4, or 5 mg. at 15°, 18°, 21°, or 25° C., respectively.

G. MIDDLETON

Inorganic

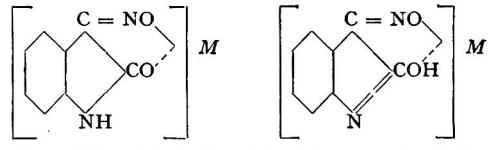
Some Properties of Lead, Copper, and Antimony Applicable to the Analysis of their Alloys. A. Lassieur and L. Martelli (*Chimie Analyt.*, 1948, **30**, 9–11)—When lead sulphate is precipitated by the usual procedure, filtered on a Gooch crucible and washed with alcohol, drying to constant weight at 100° C. leads to high results. Heating at 500° to 700° C. is necessary for satisfactory accuracy. If the precipitate is collected on a filter paper, losses up to about 1·5 per cent. of the lead occur during ignition, even with the usual precaution of igniting the paper after separation from the bulk of the precipitate and treating the ash from the paper with nitric and sulphuric acids.

Determination of lead in presence of tin and antimony—Lead may be determined as sulphate in presence of at least half its weight of tin, but an alloy containing 12·7 per cent. of antimony and 87·3 per cent. of lead gave a figure of 92·5 per cent. for lead. The method in which lead is precipitated as chloride with addition of alcohol (*cf.* Treadwell and Hall, "*Analytical Chemistry*," Vol. 2, 7th Ed., p. 233) also yields high results when antimony is present. An alloy containing 78·87 per cent. of lead, 10·91 per cent. of tin and 10·09 per cent. of antimony gave about 84 per cent. for lead by this method. The lead chloride was then extracted with sodium sulphide solution and a quantity of antimony recovered from it which, when calculated to $SbCl_5$ and deducted from the weight of lead chloride, reduced the latter to approximately the correct value.

Separation of antimony from lead and copper—Although cupric sulphide is said to be insoluble in sodium sulphide solution (*Ibid.*, Vol. 1, 6th Ed., p. 231) this is shown to depend upon the concentration of the solution. Fifty ml. of a 50 per cent. solution (sp.gr. 1·17) dissolved cupric sulphide equivalent to 7 to 8 mg. of copper when sulphide equivalent to 25 mg. of copper was treated with it for 30 min. in the cold. It was necessary to dilute 25 ml. of the sodium sulphide solution with 30 ml. of water to reduce the quantity of copper dissolved to 0·5 mg. in 10 min. When this method is employed for the separation of antimony (28 mg.) and copper (25 mg.) as sulphides the concentration of sodium sulphide may vary between that of a mixture of 25 ml. of 50 per cent. solution with 30 ml. of water and that of a mixture of 2 ml. of 50 per cent. solution with 48 ml. of water. Weaker mixtures than the latter will dissolve only part of the antimony sulphide. When lead is also present in similar amount, extraction with 10 ml. of sodium sulphide solution diluted with 40 ml. of water gives complete separation of the antimony, but when the proportion of lead is high (200 mg.), 20 ml. of sodium sulphide solution diluted with 30 ml. of water must be employed, the mixtures must be heated for 30 min. on a water-bath, and the undissolved sulphides must be washed with the same solution.

W. C. JOHNSON

Isatin β-oxime and its Use in Analytical Chemistry [Determination of Uranium]. V. Hovorka, V. Sykora, and J. Vorisek (*Chimie Analyt.*, 1947, **29**, 268–275)—Isatin β-oxime in either of its possible tautomeric forms provides the conditions necessary for the formation of cyclic complexes:



where *M* is a bivalent metal. A 1 per cent. solution of the oxime in 50 per cent. alcohol yields precipitates with a number of metals from solutions of their acetates, or from solutions buffered with sodium acetate, as follows:—silver (orange-red precipitate), mercury^{II} (orange-yellow), mercury^{I} (orange-yellow), copper^{II} (olive-green), copper^{I} (orange-red), lead (yellow), iron^{II} (dull green), uranyl (yellow). Nickel and cobalt yield precipitates only on boiling. Precipitates formed in the cold are amorphous and voluminous and entrain the electrolytes and the precipitant, the latter separating in crystalline form later. From hot solutions crystalline precipitates are obtained, particularly with silver, mercury,^{II} and uranium. Cerium^{IV}, cerium^{III}, thorium, and zirconium are not precipitated from acid or from acetate buffered solutions, but ceric salts oxidise the oxime in acid solution to isatin. With an ammoniacal solution of the oxime the ceric salt is precipitated quantitatively as an amorphous, red-brown substance; the thorium precipitate is light yellow and dissolves on washing with water; the zirconium and cerous precipitates are mixtures of basic salts and hydroxides.

The mercuric salt has the composition $C_8H_4O_2N_2Hg$ and the oxime here behaves as a dibasic acid. The composition of the silver precipitate varies with the conditions of precipitation. The uranyl compound has the formula $UO_2(C_8H_5O_2N_2)_2$ and is a cyclic complex; it is liable to contain entrained precipitant, but may be employed in the quantitative separation and determination of uranium.

Preparation of the reagent—Boil a suspension of 15 g. of isatin in a solution of 8 g. of hydroxylamine hydrochloride in 200 ml. of water until the red isatin changes to the yellow oxime (about 15 min.), breaking up any lumps of isatin. Cool, filter, and crystallise the oxime from 50 per cent. alcohol. Yield 80 to 85 per cent.; melting-point 214° C.; decomposes at 217° C.

Gravimetric determination of uranium—To the boiling solution containing 0·001 to 0·24 g. of uranium as nitrate, acetate, chloride, sulphate, or perchlorate in 50 to 100 ml. add an excess of a 1 per cent. solution of isatin β-oxime in 50 per cent. alcohol, followed by hot 10 per cent. sodium acetate solution previously acidified to phenolphthalein with acetic acid. The acetate solution is added slowly with constant stirring. Allow to cool, and to stand for 3 hr., filter, and wash with 25 to 150 ml. of a mixture of 25 ml. of the reagent solution with 500 ml. of water. Ignite in a platinum crucible and weigh as U_3O_8.

No modification is required in presence of manganese, zinc, cadmium, or magnesium. The presence of calcium, strontium, or barium leads to slightly high results and, when barium is present, 10 to 15 drops of 0·5 *N* acetic acid should be added per 150 ml. When nickel or cobalt is present (up to 0·3 g.), add 10 to 25 ml. of 10 per cent. sodium acetate solution acidified to phenolphthalein, then 5 to 15 ml. of a 2 per cent. solution of ammonium thiocyanate or 2·5 to 15 ml. of a 2 per cent. solution of sodium potassium tartrate, and precipitate the uranium in the cold by gradual addition of the reagent solution. Stir, allow to settle for 15 min., and proceed as before. The precipitate obtained in the cold is voluminous and not more than about 0·1 g. of uranium should be taken. W. C. JOHNSON

Sensitive Reaction for Thallium and Alkaloids. H. Wachsmuth (*Chimie Analyt.*, 1947, **29**, 276–278)—A dilute solution of thallous sulphate to which has been added potassium iodide - iodine solution yields a precipitate with a few drops of a solution of an alkaloidal salt. When produced from sufficiently dilute solutions such precipitates often consist of characteristic crystals with definite melting-points. Using cocaine hydrochloride, the reaction will serve to detect less than 1 part of thallium in 6×10^6 if sufficient iodine is employed. Alternatively, the method may be used as a reaction for alkaloids, such as aconitine, atropine, brucine, cocaine, codeine, morphine, papaverine, pilocarpine, quinine, sparteine, and strychnine. The following also yield precipitates:—phenazone, procaine, amidopyrine, hexamine, and similar basic substances. Caffeine does not give the reaction. Sensitivities are quoted for brucine (1 in 7×10^5), cocaine (1 in 2×10^6) and strychnine (1 in $2·5 \times 10^6$). Precipitates that are ordinarily obtained not in crystalline form can be so obtained by precipitation from alcohol or acetone solution. The alkaloids can be recovered by decomposition with an alcoholic alkali solution and extraction with a suitable solvent.

Procedure—To 10 ml. of the solution under test add 1 ml. of a solution containing 4·5 per cent. of iodine and 8·3 per cent. of potassium iodide, and 0·6 to 0·7 ml. of a 0·2 per cent. solution of thallous sulphate. In a blank experiment the precipitate of thallous iodide that tends to form disappears after a few seconds.

The precipitates obtained with five alkaloids were found to have the composition $TlI_3.A.HI$, where A = 1 mol. of alkaloid, and the following are the melting-points of the iodothallates of the respective alkaloids:—cocaine, 179° C.; codeine, 155° to 156·5° C.; pilocarpine (recrystallised from a mixture of acetone, alcohol, and water), 157° to 158·5° C.; atropine (recrystallised from dilute alcohol), about 206° C. The strychnine compound was also prepared and analysed, but no melting-point is stated. W. C. JOHNSON

Determination of Perchlorate in Dilute Solution. A. Husken and F. Gaty (*Chimie Analyt.*, 1948, **30**, 12–14)—The determination of perchlorate by means of titanous chloride in solutions acidified with sulphuric acid is unsatisfactory for concentrations of perchlorate corresponding to 1 to 500 mg. of chlorine per litre, errors in both directions being observed. Satisfactory results are obtained if the reduction takes place in a solution that contains half its volume of concentrated hydrochloric acid.

Procedure—Dilute 100 ml. of 15 per cent. titanous chloride solution with 1 litre of concentrated hydrochloric acid and 1 litre of water and keep under an atmosphere of carbon dioxide. Determine the equivalent of this solution with respect to a solution of 1 g. of potassium perchlorate in 1 litre of water. Prepare also a 0·1 *N* solution of ferric sulphate containing 60 ml. of sulphuric acid per litre, and a saturated solution of sodium thiocyanate. Fit a 1-litre conical flask with a reflux condenser and an inlet tube for carbon dioxide. Measure into the flask 50 ml. of concentrated hydrochloric acid and 50 ml. of boiled water, heat to boiling, and allow to cool, maintaining a current of carbon dioxide during this and subsequent stages of the procedure. Raise the cork and introduce a volume of titanous chloride solution in excess of that required to reduce the perchlorate solution, which is next added. Re-stopper and boil gently for 1 hr. Allow to cool for a few minutes, remove the reflux condenser, add 100 ml. of boiled and cooled water, and 20 ml. of sodium thiocyanate solution, and titrate the excess of titanous chloride with 0·1 *N* ferric sulphate. W. C. JOHNSON

Determination of Thorium and its Separation from Uranium by Ferron (7-Iodo-8-hydroxyquinoline-5-sulphonic acid). D. E. Ryan, W. J. McDonnell, and F. E. Beamish (*Anal. Chem.*, 1947, **19**, 416–417)—The separation of thorium from uranium by precipitation with ferron is quicker than separation by the oxalate method in which the occlusion of uranium by thorium oxalate introduces a complication. Lanthanum, cerium, titanium, nickel, and cobalt do not interfere in the precipitation of thorium by ferron, but silver, mercury, and copper are precipitated by ferron at the *p*H used.

The method was tested by using samples containing about 10 mg. of thorium and gave accurate results in presence of up to 20 mg. of uranium. In presence of larger amounts of uranium up to 100 mg., a double precipitation is necessary. Some thorium is lost if the first precipitate is simply dissolved in diluted hydrochloric acid, and the

precipitate cannot be treated with the reagents usually used for destroying organic matter, as interfering substances are formed: in particular, sulphate prevents the precipitation of the thorium - ferron complex. The procedure found to give correct results is to heat the first precipitate at 550° to 600° C. for 1 hr. only, volatilising the sulphur and destroying organic matter, but not rendering the residue difficult to dissolve.

Procedure for samples containing little uranium—Place in a 100-ml. beaker about 10 mg. of thorium as nitrate, and add 10 per cent. ammonium acetate solution and hydrochloric acid to buffer the solution to *p*H 2 to 3·5. Place on a steam-bath and, after 10 min., add 25 ml. of 0·2 per cent. aqueous ferron solution, or approximately 2 ml. of the ferron solution for each 1 mg. of thorium present. Digest on the steam-bath for 30 min., filter through a No. 42, 9-cm. Whatman filter paper, char the paper, ignite the residue at 900° C. in a muffle, and weigh as thorium oxide.

Procedure for samples containing larger amounts of uranium—After the first precipitation as described above, filter through a filter crucible and heat the crucible for 1 hr. at 550° to 600° C. Wash the residue into the original beaker by means of dilute nitric acid, and evaporate to dryness. Evaporate to dryness with 10 ml. of concentrated hydrochloric acid and 5 ml. of concentrated nitric acid, then with 5 ml. of concentrated nitric acid, and finally with water. Dissolve the residue in 10 ml. of water and precipitate the thorium with ferron as described above.

B. ATKINSON

Zirconium Determination in the Presence of Interfering Elements. C. A. Kumins (*Anal. Chem.*, 1947, **19**, 376–377)—On addition of mandelic acid to solutions of zirconium sulphate or zirconium chloride in diluted hydrochloric acid the zirconium is precipitated quantitatively as white zirconium mandelate. As few elements interfere in the precipitation and the precipitate is easy to handle, this method of precipitation compares favourably with the phosphate method and published methods in which organic reagents are used. The precipitate has a composition nearly corresponding to zirconium tetramandelate.

Procedure—Dilute a solution of from 0·05 to 0·3 g. of zirconium oxide to 20 ml. by adding concentrated hydrochloric acid. Add 50 ml. of 16 per cent. mandelic acid, dilute with water to 100 ml., heat slowly to 85° C., and maintain the liquid at this temperature for 20 min. Filter, wash the precipitate with a hot solution containing 2 per cent. of hydrochloric acid and 5 per cent. of mandelic acid, and ignite the precipitate to the oxide.

The results of analyses of zirconium sulphate and chloride by the cupferron and ammonia methods are close to those obtained by the procedure described. Low results are obtained if the zirconium mandelate is precipitated in presence of more than 5 per cent. of free sulphuric acid. Free acid may, with some loss of accuracy, be neutralised with sodium hydroxide, but addition of ammonia must be avoided.

The method was tested in presence of various elements added in quantities such that the molar ratio of the oxide of the added element to zirconia was from 6 : 1 to 15 : 1. Titanium, iron, vanadium, aluminium, chromium, cerium, barium, calcium, copper, and cadmium do not interfere. Slightly high results are obtained in presence of thorium, antimony, tin, or bismuth, perhaps because of the precipitation of basic chlorides. A sample of zirkite [ZrO_2] was attacked by fusion with borax and dissolved in sulphuric acid, and the solution was analysed for zirconium by the above method and by the cupferron method. The results obtained by the two methods differed by 1 part in 500.

B. ATKINSON

Applications of the Polarograph to Metallurgical Analysis. III. G. W. C. Milner (*Metallurgia*, 1947, **36**, 287–289)—The method described in a previous paper (ANALYST, 1945, **70**, 250–253) for the polarographic determination of lead in brass or bronze is complicated when the alloy contains more than 1·0 per cent. of manganese. Removal of the manganese by precipitation always results in loss of some of the lead, but addition of triethanolamine to the solution gives a manganese complex salt that is soluble in the alkaline cyanide base electrolyte used, and is not reduced at a potential close to that of the biplumbate ion.

Procedure for determining lead in copper base alloys—Dissolve 2·0 g. of the sample in 25 ml. of nitric acid (sp.gr. 1·20), boil off the nitrous fumes, cool, and dilute to 50 ml. Filter through a dry Whatman No. 40 filter paper into a dry beaker and pipette 10 ml. of the filtrate into a second dry beaker. Add 10 ml. of a 50 per cent. sodium sulphite solution followed by 16 ml. of a 13 per cent. potassium cyanide solution, agitating the solution so that the cuprous cyanide redissolves as the stable cuprocyanide ion. To the resulting solution add 5 ml. of a 25 per cent. triethanolamine solution, 10 ml. of 10 *N* sodium hydroxide, and 2 ml. of 0·2 per cent. gelatin solution, mix well, and pour a quantity of the solution into a polarographic cell. Place the cell in a thermostat at 25° C. for 10 min. and record a polarogram from 0 to −1·0 v. *versus* the mercury pool as anode.

The lead content of the alloy can be determined with an accuracy to within ±5 per cent. of the mean for samples containing not less than 0·1 per cent. of lead. If the lead content is less than 0·1 per cent., iron interferes with the determination.

J. G. WALLER

Colorimetric Determination of Nitric and Nitroso-sulphuric Acids in Nitration Spent Acids. F. L. English (*Anal. Chem.*, 1947, **19**, 850–852)—This method depends on the colour formed by nitric and nitrous acids with ferrous sulphate and on the destruction by sulphamic acid of the nitrous acid formed by hydrolysis of the nitroso-sulphuric acid.

METHOD—*Reagents*—Dissolve 40 g. of ferrous ammonium sulphate in 100 ml. of water acidified with 20 ml. of concentrated sulphuric acid, and filter. Dissolve 10 g. of sulphamic acid in water, dilute to 100 ml., and filter.

Procedure—Sum of nitric and nitroso-sulphuric acids—Pipette 25 ml. of the sample into a weighing bottle and weigh to the nearest 0·1 g. Using the same pipette, transfer another 25-ml. portion to a 250-ml. graduated flask containing about 200 ml. of water, and dilute to the mark. Keep the tip of the pipette just submerged in the liquid until all but the last few drops have been discharged. Pipette 1 ml. of water into a 50-ml. beaker, add 2 ml. of the solution of the sample, cool in an ice-bath, add 1 ml. of the ferrous ammonium sulphate solution and, while swirling the liquid, add slowly 25 ml. of diluted sulphuric acid (5 + 1). Determine the colour intensity of the liquid by means of a photo-electric colorimeter, using a green filter, and with diluted sulphuric acid (5 + 1) in the comparison cell. If bubbles are present in the liquid allow it to stand for about 10 min. before taking the reading. The reading obtained is a measure of the total of nitric acid and nitroso-sulphuric acid.

Nitric acid—Pipette 1 ml. of the 10 per cent. sulphamic acid reagent into a 50-ml. beaker, add 2 ml. of the solution of the sample, swirl until effervescence has ceased, cool in an ice-bath, and proceed as above to obtain the intensity of absorption equivalent to the nitric acid present. To obtain the intensity of absorption equivalent to the nitroso-sulphuric acid subtract this reading from the figure for the total acids.

If the absorption of light is too high, take 1-ml. portions for the determinations and add 2 ml. of water or 1 ml. of water and 1 ml. of sulphamic acid solution.

Calibrate the instrument used by analysing a series of solutions containing known amounts of sodium nitrite and a series containing known amounts of potassium nitrate. Straight-line graphs may be obtained by plotting log (I_0/I) against concentration.

The colour intensity is a maximum at the concentration of sulphuric acid used and is stable for about 30 min. To avoid slight oxidation of the nitrous acid by dissolved air, air-free water must be used in diluting the sample to 250 ml. The solution should be used within 30 min. The method as described is suitable for the analysis of samples containing 0·01 to 1·2 per cent. of nitric acid or up to 3 per cent. of nitroso-sulphuric acid. The method of determining the nitric acid is more trustworthy than the widely used evaporation method. Determination of nitrous acid by titration with sulphanilic acid usually gives results slightly higher than those obtained by the method described here. Iron, lead, nickel, copper, chloride, acetic and formic acids, and formaldehyde do not interfere.

B. ATKINSON

Colorimetric Determination of Uranium with Thiocyanate. J. E. Currah and F. E. Beamish (*Anal. Chem.*, 1947, **19**, 609–612)—The method described has been successfully used in the rapid colorimetric determination of from 0·05 to 0·80 mg. of uranium in presence of at least 1·25 g. of thorium and small amounts of iron and copper; it depends on the development of an intense yellow colour with uranium and thiocyanate ions.

Procedure—To 2 ml. of 5 *N* hydrochloric acid add an aliquot of thorium nitrate solution containing approximately twice the amount of thorium (within 0·5 g. of thorium) expected in the test solutions, titrate to *p*H 1·0 with *N* potassium hydroxide, and dilute to 50 ml. with water; use this solution as a blank. Prepare a series of solutions of uranyl nitrate containing from 125 μg. to 2 mg.; to each add 1 ml. of 5 *N* hydrochloric acid and the approximate amount of thorium, as thorium nitrate solution, expected in the solutions to be tested. Add half the volume of *N* potassium hydroxide required for the blank solution and dilute to 25 ml. with water. To 10 ml. of the blank and 10 ml. of each of the uranium solutions add 2 ml. of 10 per cent. w/v solution of stannous chloride dihydrate in diluted hydrochloric acid (1 in 10), and 7 ml. of 50 per cent. w/v ammonium thiocyanate solution, and dilute to 25 ml. with water.

Centrifuge the solutions if a suspension is present, transfer to 20 mm. rectangular cells, and measure the transmittance in a colorimeter so adjusted as to give 100 per cent. transmittance with the blank solution. Plot the percentage transmittance against the weight of uranium present in the 25-ml. samples, preferably on semi-logarithmic graph paper. To determine the uranium content of unknown solutions, adjust the reaction of an aliquot of up to 10 ml. to *p*H 1·0, add 2 ml. of stannous chloride solution and 7 ml. of 50 per cent. w/v ammonium thiocyanate solution, dilute to 25 ml. with water, centrifuge, if necessary, to remove any suspension, measure the transmittance of the solution in a 2-mm. cell, and calculate the amount of uranium present by reference to the standard curve.

Quantities up to 2 mg. of iron do not interfere, as the stannous chloride reduces the iron to the bivalent state; the presence of 5 mg. of iron decreases the transmittance by about 2 per cent. and larger quantities further reduce it. Addition of a larger amount of stannous chloride will eliminate interference of amounts of tervalent iron in excess of 2 mg. Ten mg. of copper do not interfere, but 20 mg. cause a slight turbidity, and 50 mg. yield a heavy white precipitate. Centrifuging the solutions removes these precipitates and the transmittance is then the same as for similar solutions containing no copper.

A. H. A. ABBOTT

Technique for Obtaining Uncontaminated Small Samples of Ceramic Glazes and Other Hard Siliceous Materials. E. R. Caley (*Anal. Chem.*, 1947, **19**, 360)—Working with the aid of a magnifying glass and over-glazed paper, draw with a tungsten carbide pencil a series of very close parallel scratches in the glaze, and collect the powder and chips formed. Control the depth of the scratches to avoid cutting through the glaze. Samples of specific small areas of glaze may be obtained. The method may be extended to the sampling of discrete grains of rocks.

B. ATKINSON

Tetra-ethylenepentamine as a Colorimetric Reagent for Copper. T. B. Crumpler (*Anal. Chem.*, 1947, **19**, 325–326)—The specific extinctions of the blue complexes formed by ammonia, ethylenediamine, diethylenetriamine, triethylenetetramine, and tetra-ethylenepentamine with copper have been measured. The values for extinction per cm. per p.p.m. of cupric ion at the wavelength of maximum extinction rise in the above order from 0·00065 for ammonia to 0·0022 for tetra-ethylenepentamine. With the latter compound, the adsorption is independent of the amount of amine present in excess. For cupric ion concentrations from 0 to 160 p.p.m., with an excess of amine present, the colour reaction obeys the Beer - Lambert law. The colour of this complex is stable for at least 48 hr.

Estimation of copper with tetra-ethylenepentamine—The purification of the amine has been described by Jonassen *et al.* (*J. Amer. Chem. Soc.*, 1945, **67**, 1709). To prepare for analysis on the colorimeter 100 ml. of solution containing from 0 to 200 p.p.m. of copper ion use 10 ml. of a 2 per cent. aqueous solution of tetra-ethylenepentamine: adjust the *p*H of the test solution to 3·5 to 4·0 by means of sodium hydroxide. The ions that interfere are those that do so in the colorimetric determination of copper with ammonia (Mehlig, *Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 533). B. ATKINSON

[Spectrographic] Analysis of Silica - Alumina Cracking Catalysts. R. A. Burdett and L. C. Jones, jun. (*Anal. Chem.*, 1947, **19**, 238–241)—Recent general use of silica-alumina catalysts in the petroleum industry has necessitated a rapid method for determining the contaminants usually present in these materials. A procedure is described for the simultaneous determination of iron, sodium, vanadium, chromium, nickel, and copper by the internal standard method of analysis. An ARL - Dietert Multisource Unit supplies the electrical conditions for the high streaming velocity electrode system of Hasler and Harvey (*Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 540). These are used with a grating spectrograph and a rotating step sector mounted at the secondary focus of the grating.

Both aluminium and silicon are used as internal standards, although aluminium can only be used where catalysts of similar alumina content are to be compared. The line pairs used in the analysis, and the concentration range determined by this method are given in the following table.

Working curves were prepared from the spectrograms of catalysts previously analysed chemically for sodium and iron, and polarographically for vanadium. Determination of the nickel, chromium, and copper contents of these standards was accomplished by comparison with a set of synthetic standards, and working curves for these elements prepared.

All samples were prepared for arcing by mixing with an equal volume of ammonium chloride and packing the mixture into a graphite centre post electrode.

As an indication of the accuracy of the method the following figures for the average percentage deviation of spectrographic from chemical or polarographic analysis are quoted: iron ±2·9, sodium (high range) ±2·9, sodium (low range) ±3·5, and vanadium ±1·4. D. A. POYNTER

Contaminant	Wavelength in A.	Internal standard	Wavelength in A.	Range of concentration Percentage weight
Iron	2723·6	Aluminium	2568·0	0·04 to 0·7
Iron	2723·6	Silicon	2532·4	0·04 to 0·7
Sodium	3302·3	Silicon	2532·4	0·12 to 1·0
Sodium	3302·3	Aluminium	3066·2	0·04 to 0·12
Vanadium	3184·0	Aluminium	2568·0	0·01 to 0·07
Vanadium	3184·0	Silicon	2532·4	0·01 to 0·07
Nickel	3414·8	Silicon	2532·4	0·001 to 0·01
Chromium	4254·3	Silicon	2532·4	0·001 to 0·01
Copper	3274·0	Silicon	2532·4	0·0002 to 0·01

Physical Methods, Apparatus, etc.

Quantitative Analysis with the X-Ray Spectrometer. J. C. Redmond (*Anal. Chem.*, 1947, **19**, 773–777)—A method in which semi-quantitative and quantitative analysis of mixtures can be carried out by means of an X-ray spectrometer equipped with a Geiger counter is described.

It is claimed that results can be obtained in 10 min. by an almost untrained operator, once the necessary working curves have been established for the particular mixture being analysed.

Results obtained by applying the method to the quantitative analysis of mixtures of heavy metal carbides are given. An average deviation of ± 1·4 per cent. was obtained for a particular series of seven mixtures, the maximum deviation being +5·7 per cent.

Inability to eliminate orientation effects in the specimen may make it impossible to obtain as good results as in the examples given. E. G. STEWARD

Determination of Small Amounts of Water in Gases and Liquids by Infra-red Spectrometry. A. F. Benning, A. A. Ebert, and C. F. Irwin (*Anal. Chem.*, 1947, **19**, 867–868)—To estimate a small amount of water in a refrigerating agent the compound is liquefied in a pressure cell formed by a brass tube with quartz windows, and the absorption by the liquid of infra-red radiation at the 2·67-microns water-band is measured. Concentrations of water up to 10 p.p.m. may be

estimated with an accuracy of 1 p.p.m., but for higher concentrations the accuracy is slightly less. The method has been applied successfully to the estimation of water in Freons 12 and 11 and in carbon tetrachloride and tetrachloroethylene. Compounds containing hydrogen usually adsorb at 2·67 μ. as do Freons 113 and 114.

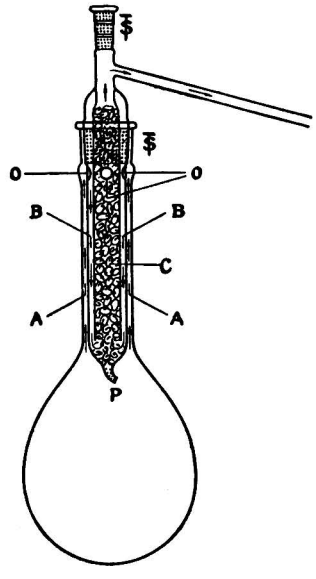
B. ATKINSON

Saturated Potassium Hydrogen Tartrate Solution as a pH Standard. J. J. Lingane (*Anal. Chem.*, 1947, **19**, 810–811)—Pure potassium hydrogen tartrate as obtained commercially is easily crystallised from water, and *p*H values of the solutions of the original material and of the first and second recrystallisates, obtained by shaking the solid for 2 to 3 min. with distilled water, agreed to within ±0·005 unit. The *p*H was not significantly affected by atmospheric carbon dioxide, and increased by only 0·03 unit after 1 year's standing in a stoppered Pyrex bottle; but its ease of preparation renders storage of the solution not worth while.

A saturated solution (0·034 *M* at 25° C.) has a *p*H of 3·57 ± 0·02, and a solution saturated at any temperature above 10° C. and brought to 25° C. for measurement has a *p*H within 0·02 unit of that of a solution saturated at 25° C.; thus, no special care is necessary in preparing the solution. The temperature coefficient of the *p*H has not been determined, but it is probably negligible. The solution has a greater buffering capacity than that of potassium hydrogen phthalate.

M. E. DALZIEL

Improved Trap for Analytical Distillations. F. L. Hahn (*Anal. Chem.*, 1947, **19**, 811–812)—Usual methods of reducing splashing of non-volatile materials prolong distillation time, increase the surface, and consequently reduce the efficiency of the distillation. In the droplet catcher shown, the



total air-space of the flask and trap is less than that of the empty flask, and also the active inner surface is increased while the outer surface remains unchanged. The apparatus is made of Pyrex glass and the neck of the flask and the two tubes of the trap should be as close as possible, to speed the passing of vapour and leave the maximum space in C, which is filled with helices. The outer trap-tube has two 4-mm. or three 3-mm. openings the bottom. In use, steam rises in A, passes through the openings at O, and down B, and then rises through C, the condensate being returned through P.

Distillation of 500 ml. of *N* sodium hydroxide in presence of zinc, with an external trap, gave a distillate of *p*H 8 to 9, but with the internal droplet catcher under the same conditions the *p*H of the distillate was 7 to 7·2 and the distillation rate 50 per cent. faster. With the droplet catcher the rate could be further increased by heating more strongly, whereas with the external bulb trap more rapid boiling led to increased contamination of the distillate.

M. E. DALZIEL

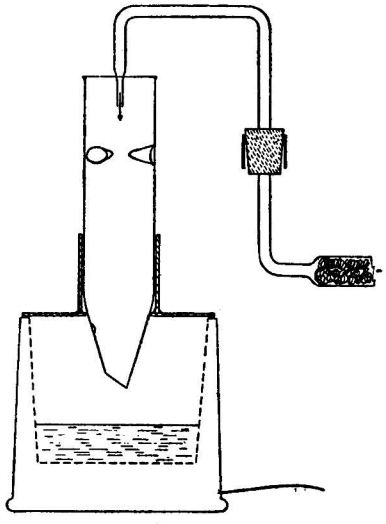
Melting-Point Bath Liquids Useful up to 440° C. L. M. White (*Anal. Chem.*, 1947, **19**, 432–433)—Three liquid silicones, type 550–84 centistoke grade, type 550–142 centistoke grade, and type 703–64 centistoke grade have been tested as high-temperature melting-point bath liquids in the apparatus of Conte (*Ind. Eng. Chem., Anal. Ed.*, 1930, **2**, 200) and in a modified form of that of Hershberg (*Ibid.*, 1936, **8**, 312). The liquids were heated repeatedly to 300° to 450° C. both in presence of air and under an atmosphere of nitrogen. All three silicones darken if the treatment is prolonged, but under an atmosphere of nitrogen the darkening is slower than in presence of air, and the silicones can be heated many times before they become too dark for use. The rise in the viscosity of the liquids caused by repeated heating may render them useless before they have become very dark. Compared with vegetable and mineral oils the silicones have relatively low viscosity coefficients and low freezing-points and may therefore be useful as melting-point bath fluids for use throughout the range −30° to 440° C.

B. ATKINSON

Quartz Microgram Balance. P. L. Kirk, R. Craig, J. E. Gullberg, and R. Q. Boyer (*Anal. Chem.*, 1947, **19**, 427–429)—This quartz torsion balance combines the torsion principle of Neher (see "*Procedures in Experimental Physics*," J. Strong, New York, 1942), the pan suspension of Steele and Grant (*Proc. Roy. Soc.*, 1909, *A*, **82**, 580), and the pan well of Pettersson ("*New Microbalance and its Use*," Dissertation, Stockholm, 1914), and incorporates a comparison microscope for determining the position of the beam. The balance is capable of weighing samples of up to 300 μg. and carrying a load of 0·1 to 0·2 g., the sensitivity of the instrument being at least 1 minute of arc per 0·005 μg. Over periods of months these balances have shown a high degree of reproducibility.

Constructional details of the balance are given in the paper. B. ATKINSON

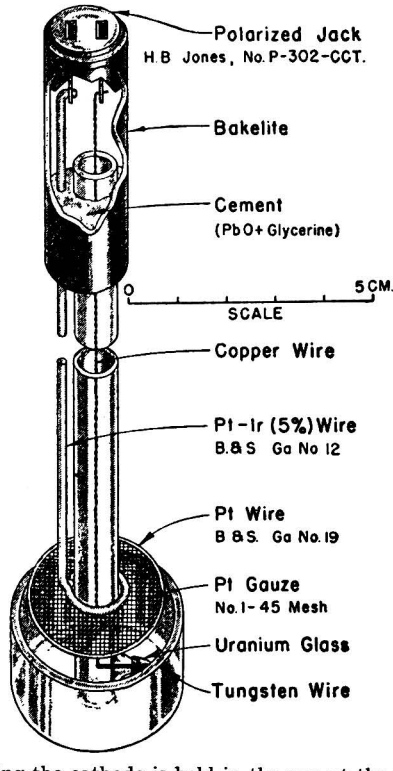
Simple Steam Micro-bath. C. L. Rulfs (*Anal. Chem.*, 1947, **19**, 1046)—The apparatus, while resembling the electrolyte solution steam micro-bath of Marion (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 82), [in which the heat generated by passing the current from a public supply through a 1 per cent. copper sulphate solution between two insulated electrodes of No. 12 copper wire raises the electrolyte to its boiling-point, the heat being regulated by



adjusting the submerged length of one of the electrodes, and the current cut off automatically when the level of the liquid is lowered by evaporation], is made of readily available parts, heating being effected through the electrical resistance of tap water itself; it is rapidly controlled and cuts off automatically when the water is depleted. The egg-cooker used boils a small volume of water in 1 min., and is refilled with distilled water to avoid accumulation of salts. A 3-in. water-bath ring supported on the bath will hold either a micro-beaker support or one for heating centrifuge cones. The filtered air blast technique speeds evaporation. M. E. DALZIEL

[Reporting Centrifuging Data]. D. P. Costello (*Science*, 1947, **105**, 474)—Centrifuging data presented in scientific papers are of little use unless the duration of centrifuging and the magnitude of the applied centrifugal force are given. The centrifugal force in terms of gravity can be calculated by means of the formula $c' = 4\pi^2 n^2 r/g$, where n = the number of revolutions per sec., r = the distance in cm. of the material from the centre of rotation, g = the force of gravity, and c' = the centrifugal force in terms of gravity. It is important that in determining r, the thickness of the base of the centrifuge tube and of any layer of supporting medium shall be allowed for. In scientific papers, c' or n and r should always be stated. B. ATKINSON

Unitised Mercury Cathode Apparatus for Electrolytic Removal of Metals. H. O. Johnson, J. R. Weaver, and L. Lykken (*Anal. Chem.*, 1947, **19**, 481–483)—Advantages of the mercury cathode described are that it may be washed quickly and easily, that the mercury can easily be changed, and that a high current density can be used. The combined anode and cathode unit is illustrated in the figure. The mercury



forming the cathode is held in the cup at the lower end of the glass tube and the anode is supported by a heavy platinum wire.

Procedure—Place in a 250-ml., tall-form beaker, 25 to 30 ml. of the solution to be electrolysed, fill the cathode with mercury to within 1 to 2 mm. of the top of the cup, and raise the beaker until the electrode almost touches the bottom. Connect the electrodes to a 6- to 12-volt supply and add concentrated sulphuric acid to the beaker until a current of 5 amp. is passing. Electrolyse for 30 min., adding distilled water to maintain the volume of solution constant. With the current on, lower the beaker, rinse the electrode with distilled water, replace the amalgam in the cup with clean mercury, and electrolyse for 20 min. Replace the mercury again and electrolyse for 10 min.

By the procedure given, zinc, iron, copper, chromium, and nickel are removed from solution almost completely, the amount of each metal left in solution being less than 100 μg. The procedure may be used for the removal of chromium, copper, cadmium, lead, and iron from solutions prior to the polarographic determination of sodium. When

separated by electrolysis, tervalent chromium amalgamates in the normal manner, but sexavalent chromium usually yields a fine black precipitate. Before the electrolysis, sexavalent chromium should preferably be reduced by boiling with hydrogen peroxide. Agitation of the mercury surface by the magnetically controlled motion of a glass bead with an iron core increases the rate of amalgamation of iron, copper, chromium, and nickel. B. ATKINSON

Review

REPORT ON COLOUR TERMINOLOGY. By A COMMITTEE OF THE PHYSICAL SOCIETY COLOUR GROUP. Pp. 56. London: The Physical Society. 1948. Price 7s.

In 1941 the Colour Group of the Physical Society set up a Committee with the following terms of reference:

(*a*) To place on record definitions of terms which have an accepted significance amongst any important group of colour workers; and

(*b*) To report on the possibility of co-ordinating the terms commonly used in the field of colour.

In order to allow the Committee to record some of the conclusions reached during its discussions, the terms of reference were extended to include (*c*).

(*c*) To make recommendations for a consistent terminology.

The Committee has now issued its report, with the hope that those who read it "will wish to use the existing terms to best advantage; the recommended terms being presented as a contribution towards a less ambiguous and more widely understood terminology."

This brief statement covering the beginning and end of the project hardly does justice to the magnitude of the task attempted. The fields covered include colour physics, colour vision, the Munsell and Ostwald systems, ordinary speech, industry (dyeing, paint and pigment, printing, colour photography, glass, the decorating trade) and contemporary artists. It is hardly believable that any common ground could exist amongst such diverse interests, but the Report shows that a surprising degree of agreement was reached during the course of the discussions. No recommendations have been attempted for terms used in ordinary speech or those used by artist painters, although such information as could be obtained is recorded for its general interest. A short but witty section explains why contemporary artists could hardly be expected to contribute to or make use of the Report.

The Committee has shown its wisdom by attempting to preserve the usages of ordinary speech wherever possible and by suggesting that where a change in terminology has to be made it should be imposed on the scientific rather than on the industrial user.

The Committee's recommendations are presented in the last section of the Report; these are tentative and are put forward "for further consideration by other interested bodies." In view of the agreement already reached among the diverse interests represented on the Committee it seems reasonable to hope that the recommended terminology will be accepted by chemists, technologists and physicists generally.

B. S. COOPER

MICROCHEMISTRY GROUP

A JOINT meeting of the Microchemistry Group with the Leeds Area Section of the Royal Institute of Chemistry and the Leeds University Chemical Society will be held at Leeds University on Tuesday, October 26th.

In the afternoon visits will be paid to various departments of the University.

At 6.30 p.m. there will be a symposium on Microchemical Methods in Forensic Analysis, in the Large Lecture Theatre of the Department of Chemistry. The following papers will be read—

"A General Account of Microchemical Methods in Forensic Investigations," by J. B. Firth, D.Sc., F.R.I.C., M.I.Chem.E., Director of the North-Western Forensic Science Laboratory, Preston.

"Microchemical Methods in Forensic Toxicology, with particular reference to Barbiturates," by G. E. Turfitt, B.Sc., Ph.D., F.R.I.C., Deputy Director of the Metropolitan Police Laboratory.

"Rapid Colorimetric Methods for the Detection and Estimation of Alkaloids and Related Compounds in Biological Material," by E. Pedley, M.Sc., Ph.C., A.R.I.C., Senior Scientific Officer, Home Office Laboratory, Preston.

Members of the North of England Section of the Society are cordially invited to attend. Further particulars may be obtained from the Acting Hon. Secretary of the Microchemistry Group, Mr. Donald F. Phillips, Central Research Laboratory, High Duty Alloys, Ltd., Slough, Bucks.

PHYSICAL METHODS GROUP

THE following meetings have been arranged for the session 1948–49.

November 30th, 1948—At the Imperial College of Science and Technology, London. Annual General Meeting of the Group, followed by a lecture on "The Measurement of Colour," by Mr. R. Donaldson.

January 25th, 1949—At the Imperial College of Science and Technology, London, S.W.7. A symposium on rheological methods.

April 1st, 1949—At University College, Nottingham. A symposium on electrophoretic analysis.

SYMPOSIUM ON METHODS OF PENICILLIN ASSAY: THEIR PURPOSE, SCOPE AND VALIDITY

CLOTH-BOUND reprints of this symposium, held at the joint meeting of the Physical Methods Group and the Biological Methods Group on January 29th, 1948, and since published in THE ANALYST, 1948, **73**, 197–216, 244–257, are now on sale by W. Heffer & Sons, Ltd., Cambridge. Price 3/6, plus 3d. postage.

NEW REPORTS OF THE ANALYTICAL METHODS COMMITTEE

THE Reports published in the June issue of THE ANALYST, viz.,

Metallic Impurities in Foodstuffs Sub-Committee:

Report No. 4. The Determination of Zinc in Foodstuffs.

Poisons Sub-Committee:

Report No. 4. The Assay of Yohimba.
Report No. 5. The Assay of Jaborandi.
Report No. 6. The Assay of Ephedra and of Ephedrine in Nasal Sprays.

are now available as separate reprints. Price 1s. 6d. to members; 2s. 0d. to non-members. Application for them, accompanied by remittance, should be made to the Editor, THE ANALYST, 7–8, Idol Lane, London, E.C.3.

MINISTRY OF SUPPLY invite applications for unestablished posts at the DEPARTMENT OF ATOMIC ENERGY, Springfields, Lancs., in the grades of Experimental Officer and Assistant Experimental Officer. Selected candidates will be required to carry out experimental work arising from the metallurgical development involved in the production of Atomic Energy.

Candidates must be British subjects and should have obtained a University degree in a science or engineering subject or in Mathematics, or be in possession of a Higher School Certificate with Mathematics or a science subject as principal subject, or an equivalent qualification. They should have knowledge and experience of metallurgical methods of investigation.

Inclusive salary ranges are:—

		£
Experimental Officer	Men	470–620
	Women	380–495
Asst. Experimental Officer ..	Men	200–410
	Women	200–330

Write, quoting F.391/48A, to Ministry of Labour and National Service, Technical and Scientific Register, K Section, York House, Kingsway, W.C.2 for application form which must be returned by 25th September, 1948.

THE Civil Service Commissioners invite applications for permanent appointments in the Chemical Inspectorate (Atomic Energy), Ministry of Supply, for analytical work involving modern physico-chemical methods.

SENIOR SCIENTIFIC OFFICERS. Candidates to be aged 31 or over and to have a good Honours degree in chemistry or physics. A sound knowledge of emission spectroscopy, or electronics, or radiation chemistry is needed.

SENIOR EXPERIMENTAL OFFICERS. Candidates to be aged 35 or over, to possess at least Higher School Certificate (or equivalent qualification) with a science subject as principal subject and to be well experienced in spectro-chemical or other modern methods of analysis.

Inclusive salary scales (London).

Senior Scientific Officer	Men:	£650—£850
	Women:	£525—£750
Senior Experimental Officer	Men:	£700—£900
	Women:	£575—£800

Provincial scales are slightly lower.

Further particulars and application forms from the Secretary, Civil Service Commission, Scientific Branch, 27, Grosvenor Square, London, W.1, quoting No. 2281. Completed applications must be returned by 7th October, 1948.

STAFFORD ALLEN & SONS LTD. require Ph.C. or B.Sc. for Nairobi laboratory. Applicant must have considerable experience in drug analysis. Long-term contract will include free passage to Kenya and back for self, also wife and child if applicable. Free medical attendance; annual local leave and, after tour of duty, 4 months home leave. Apply by letter to Department D/RPM, 20, Wharf Road, London, N.1.

PHARMACIST (B. Pharm., or Ph.C., preferred) required for research work in pharmaceutical investigation and development laboratory. Applicants should be under 30. Some hospital and/or manufacturing experience desirable, but not essential. The post offers excellent prospects with commencing salary according to qualifications and experience. Apply in writing (without testimonials at first) to Personnel Manager, Glaxo Laboratories Ltd., Greenford. Middlesex.

ASSISTANT to Chief Chemist required. Applicants should possess a Science degree in General Chemistry and/or Agriculture, and have a sound knowledge of bacteriology as applied to Milk, Milk Products and Water analyses. Applicants should set out details of qualifications, age, experience and salary required together with names and addresses of selected references. Box No. 3688, THE ANALYST, 47, Gresham Street, London, E.C.2.

CHEMIST required for analytical and control work in Laboratories of a Cable Manufacturers in London area, preferably graduate or equivalent. Age 20–30 with some industrial experience. Salary £400–450 per annum (gross). Write Box 3689, THE ANALYST, 47, Gresham Street, London, E.C.2.

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Printed and Published for the Society of Public Analysts and other Analytical Chemists by W. HEFFER & SONS Ltd., Cambridge, England. **Communications to be addressed to the Editor, J. H. Lane, 7–8, Idol Lane, London, E.C.3.** Enquiries about advertisements should be addressed to WALTER JUDD LTD., 47, Gresham Street, London, E.C.2

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