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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

THE CHEMICAL SOCIETY

The following Address was presented to The Chemical Society by the President, Mr. Lewis Eynon, at the opening ceremony of the Centenary Celebrations, at the Central Hall, Westminster, on Tuesday, July the fifteenth:—

FROM

THE SOCIETY OF PUBLIC ANALYSTS

AND

OTHER ANALYTICAL CHEMISTS

TO

THE CHEMICAL SOCIETY

On the occasion of the Celebration, on July 15th, 1947, of the Centenary of the Foundation of The Chemical Society, The President, Officers, Council and Members of the Society of Public Analysts and Other Analytical Chemists send Fraternal Greetings to the President, Officers, Council and Fellows of The Chemical Society.

We are glad to have this opportunity to express our friendship and goodwill to the oldest Chemical Society in the World which has so signally served the science of chemistry and to offer our best wishes for its continued prosperity.

(*Signed*) LEWIS EYNON (*President*).
G. TAYLOR (*Honorary Treasurer*).
K. A. WILLIAMS (*Honorary Secretary*).

Dated this Fifteenth Day of July
Nineteen Hundred and Forty Seven.

Seal of the Society of Public Analysts and Other Analytical Chemists

The Chemical Estimation of Nicotinic Acid in Cereals and other Foods

BY EIRENE M. JAMES, F. W. NORRIS, AND FRANK WOKES

CHEMICAL methods of estimating nicotinic acid in foods are generally based on the cyanogen bromide reaction, but vary considerably in the procedure adopted to extract the vitamin and to obviate difficulties caused by interfering substances. The rate of development and the stability of the characteristic yellow colour have been found to be affected by the particular aromatic amine employed as well as by certain other factors. In clinical tests British workers[1,2] have tried to eliminate interfering colours by treatment with permanganate, but did not apply this treatment to cereal samples, with certain of which the chemical results were considerably higher than would be expected from biological assays. American workers[3,4,5,6,7] have used adsorption techniques to separate the nicotinic acid from interfering

substances and have used different aromatic amines. Their results on wheat and other cereals have been in good agreement with the results of microbiological assays. But difficulty has arisen with a nicotinic acid "precursor" which occurs in different cereals and can be converted into microbiologically active nicotinic acid by acid or alkaline hydrolysis or even by incubation of the aqueous suspension at 37° C. at *p*H 1 or *p*H 8.[8,9,10] Various workers have shown the presence, in different cereals, of a precursor, which is converted into microbiologically active nicotinic acid when the cereals are germinated.[11,12,13]

The object of the present investigation was to study the effects of the various procedures used by different workers and devise a method which when applied to cereals would give satisfactory reproducibility and agreement with microbiological results.

In the last three years, working on a variety of unmalted and malted cereals and cereal products, we have adapted for use with the Spekker photo-electric absorptiometer a modified chemical method, based on the above British and American techniques, which has been submitted to the Chemical Panel of the Society's Vitamin Sub-Committee for trial by the members. The time seems opportune to publish details of this method as at present used, without of course attempting to anticipate the final decision of the Panel. A summary of some of the results was given in a communication read at a meeting of the Biochemical Society in September, 1945.[14]

Chemical method

Reagents—

Cyanogen bromide reagent—Prepare a saturated solution of bromine (AnalaR) in distilled water. Cool it below 10° C. and add, drop by drop, an ice-cold 10 per cent. solution of potassium cyanide (AnalaR) until it is just decolorised. Prepare this solution fresh each day and keep it in a cool dark place.

Stock nicotinic acid solution—Dissolve 100 mg. of nicotinic acid in 50 per cent. alcohol to make 100 ml.

Standard nicotinic acid solutions (20 and 10 μg./ml.)—Dilute the stock nicotinic acid solution to 1 in 50 and 1 in 100 with 25 per cent. alcohol. These standards should be prepared every month or so. The lower proportion of alcohol as compared with the stock solution is to avoid concentration by evaporation of the alcohol in the more frequently opened standard solutions.

Lloyd's reagent—Hydrated aluminium silicate obtained from Eli Lilly & Co. Each batch should be checked for adsorption and elution of nicotinic acid.

p-*Aminoacetophenone* ("*PAAP*")—Commercial samples may exhibit a strong yellow colour in acid solution and in that event should be heated in aqueous solution with "charcoal for adsorption" and recrystallised from water and tested to see that they give low reagent blanks.

Preparation of extract—

Mix 1 g. of sample (in No. 30 or 40 powder for barley or malt, and containing up to 150 μg. of nicotinic acid) with 5 ml. of concentrated hydrochloric acid and water to make 15 ml. and place in a boiling water bath for 40 minutes. Cool to room temperature and centrifuge (A). Decant the supernatant liquid, add to it 2·5 g. of Lloyd's reagent, shake for 1 minute and centrifuge (B). Reject the supernatant liquid B. Wash the residue from A with 10 ml. of 0·2 *N* sulphuric acid and centrifuge. Add the supernatant liquid from this to the residue from B. Shake this mixture for 1 minute, centrifuge and reject the supernatant liquid. Add to the residue 10 ml. of 0·1 *N* sodium hydroxide (more if necessary) to make alkaline to phenolphthalein. Shake vigorously for 1 minute and make up to 16·5 ml. (≡ 15 ml. of original acid extract). Foam can be dispersed by a drop of alcohol before adjusting to volume. Centrifuge, decant and reject the residue. To the supernatant liquid add very finely powdered lead nitrate (AnalaR) to bring the *p*H to between 8·0 and 9·0, using thymol blue as external indicator and keeping the liquid well stirred to avoid adding an excess. Centrifuge and add to the supernatant liquid one drop of phenolphthalein indicator. If a pink colour is not obtained, add sufficient solid tribasic sodium phosphate to produce a pink colour and centrifuge again. Take 5 ml. of the supernatant liquid and add 1 drop of 20 per cent. phosphoric acid and then tribasic sodium phosphate to bring the *p*H to 6·5 ± 0·3, using bromothymol blue as external indicator. Make up to 25 ml. (= "final extract").

DEVELOPMENT OF COLOUR—

Prepare the following mixtures:—

	Total reagent blank	"Unknown"		"Unknown" with nicotinic acid	
	TRB	U	U	UNA	U2NA
Final extract, ml.	—	5	5	5	5
Water, ml.	6	1	1	—	—
Nicotinic acid standard solution, 20 μg./ml. in 25% EtOH, ml...	—	—	—	—	1
Nicotinic acid standard solution, 10 μg./ml. in 25% EtOH, ml.	—	—	—	1	—
CNBr reagent, ml.	2	2	2	2	2

Heat in a water-bath at 56° to 58° C. for 4 minutes, cool to 20° C. for 5 minutes and then add to each:

PAAP, 10% solution in 96% EtOH, ml.	1	1	1	1	1
HCl, 6% w/v, ml.	1	1	1	1	1

Keep the mixtures in the dark, and 5 minutes after adding the PAAP and hydrochloric acid measure the optical density of U, UNA and U2NA against TRB in the Spekker absorptiometer as described below.

Prepare an *acid alcohol blank* (AAB) by mixing 20 ml. of alcohol, 20 ml. of 6 per cent. hydrochloric acid, 14·9 ml. of 0·1 *N* sodium hydroxide and water to make up 100 ml.

Prepare a *solution blank* (SB) by mixing 4 ml. of final extract with 4 ml. of the acid alcohol blank. Measure its colour against water mixed with an equal volume of the acid alcohol blank.

OPTICAL MEASUREMENTS—

Measure the characteristic yellow colour by means of a Spekker absorptiometer, using Chance's No. 7 and H503 filters before each photo-cell. The latter filter should be nearest to the photo-cell, which it protects against the disturbing effect of heat rays. Take four readings rapidly at the required time on each solution and use their mean for calculating results. To avoid errors arising from gradual variations in the response of the photo-cell, check the latter two or three times a day against a Chance's No. 6 filter wedged against a water-cell in focus on the side nearest to the photo-cell. Set the zero (with No. 7 and H503 filters immediately in front of each photo-cell as usual). Bring into focus a water-cell with no filter adjoining it and take four readings. Their mean may vary from 0·25 to 0·28 with different photo-cells and filters, but should not vary more than 0·002 from the average with a given photo-cell and filter. Undue exposure to light and heat (*e.g.*, by omitting to use the H503 filter) may cause variations of 0·01 to 0·02 or 0·025, leading to serious errors in results.

CALCULATION—

Let the mean colour of U against TRB be U/TRB, and similarly those of UNA and U2NA be UNA/TRB and U2NA/TRB. Let the colour of the solution blank against the diluted acid alcohol blank be SB/AAB.

Then the nicotinic acid content of the food is

$$\frac{\text{U/TRB} - \text{SB/AAB}}{\text{UNA/TRB} - \text{U/TRB}} \times \frac{15}{1} \times \frac{25}{5} \times \frac{10}{5}\ \mu\text{g./g.}$$

or

$$\frac{\text{U/TRB} - \text{SB/AAB}}{\text{U2NA/TRB} - \text{U/TRB}} \times \frac{15}{1} \times \frac{25}{5} \times \frac{20}{5}\ \mu\text{g./g.}$$

MICROBIOLOGICAL METHOD

The original method of assay with *Lactobacillus arabinosus* 17/5 as test organism[15] was later modified.[16,17,18] The method of the Microbiological Panel of the Vitamin Sub-Committee of the Society[18] is essentially that used in the present communication.

The method of computation of the results has given rise to some discussion (*cf.* Finney,[19] Wood[20]). The present results were calculated in the earlier stages by reference to the equation for the standard curve, which was calculated by the method of least squares. Later, results were calculated by comparison of the slopes of the calculated sample and standard curves. This method is to be preferred; but it should be pointed out that if the assay is valid, and sample and standard "blanks" are coincident, or very nearly so, then results calculated by either method will agree closely.

The accuracy of the method depends not only on the operator, but to some extent on inevitable slight variations of the conditions under which the living organisms grow. An arbitrary allowance of ± 10 per cent. of the mean has been considered reasonable; but experience shows that this margin may often be considerably reduced. The limits of accuracy were calculated in some instances by the method of Finney,[19] but this elegant method is somewhat lengthy when large numbers of assays are being performed.

Whatever the method of computation adopted in any particular case, no results are included which were derived from assays of doubtful validity. Thus, the microbiological results given in Table III are in every instance means of at least four valid assays, each calculated at three or four levels of sample dosage, the standard error being well within ± 10 per cent., and often within ± 5 per cent.

Results

Rate of formation and fading of colour—This depends on the aromatic amine used and also on the material and the medium in which it is being examined. Fig. 1 summarises results we have obtained in a large number of assays on cereals and cereal products. For convenience in comparison the optical density at different intervals after mixing is plotted as the percentage of the maximum value obtained. It will be seen that the colour develops slowly with metol, more rapidly with aniline or alcoholic *p*-aminoacetophenone (at least 80 per cent. of alcohol in the reaction mixture), or procaine in 10 per cent. hydrochloric acid, and still more rapidly with aqueous *p*-aminoacetophenone (not more than 10 per cent. of alcohol in the reaction mixture). In general, the more rapidly the colour develops the more rapidly it fades. The most rapid fading is with aqueous *p*-aminoacetophenone. Unfortunately, high concentrations of alcohol cause precipitation with malt extracts. We have had to use aqueous *p*-aminoacetophenone and, as can be seen from Fig. 1, this means

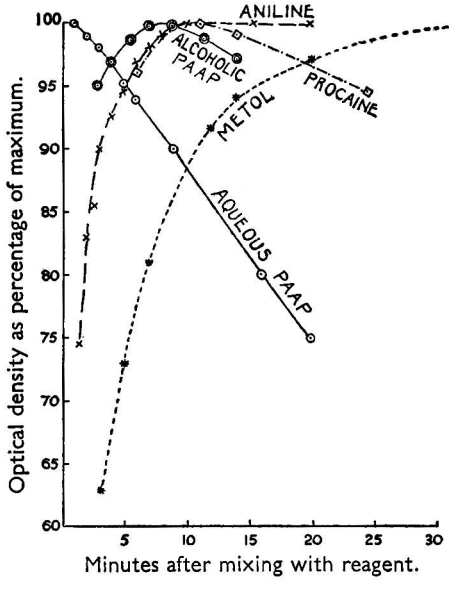

Fig. 1. Rates of development and fading of colour given by nicotinic acid with different aromatic amines used in chemical assays on cereal foods.

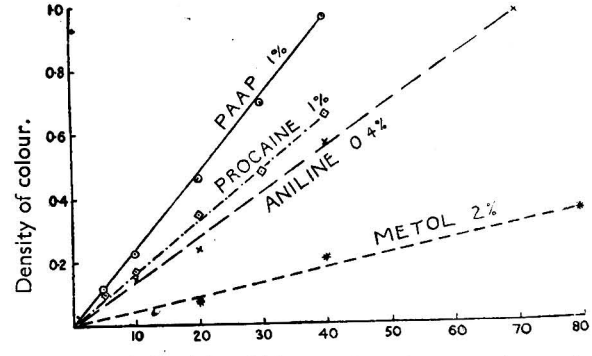

Fig. 2. Calibration curves for nicotinic acid, using *p*-aminoacetophenone, procaine, aniline or metol as aromatic amine, the percentage of each in the reaction mixture being stated.

that the 5-minutes' interval between mixing with reagent and taking readings must be controlled to within half a minute to reduce errors to 1 per cent. Even with alcoholic *p*-aminoacetophenone appreciable errors may be introduced if the readings are taken at different times during the first 15 minutes after addition of the reagent, as has been recommended,[1] and in our experience timing of the readings is also an advantage here. Since the colours develop much more rapidly than they fade (*cf.*[21,22,23]) readings should be taken *after* the maximum has been reached. This is ensured by an interval of 5 minutes when aqueous

p-aminoacetophenone is used, 10 minutes with alcoholic *p*-aminoacetophenone or procaine, 10 to 15 minutes with aniline and 40 to 60 minutes with metol. American workers[21,22] obtained a maximum colour with aniline in 6 minutes, but this might be due to their using higher temperatures. Our results were obtained at 20° to 25° C. Over this range we found no significant effect of temperature on the rate of development or of fading of colour. None of the extracts contained any nicotinamide, with which the colour develops and fades more rapidly than with nicotinic acid. Although the aniline curve in Fig. 1 reaches its maximum in about 10 minutes, statistically the results were not significantly below 100 % after 8 min.

CALIBRATION CURVES—Fig. 2 summarises our results in constructing calibration curves for nicotinic acid, using different aromatic amines. The percentage of each amine in the reaction mixture is stated in the figure. For aniline and metol these percentages are similar to those used by American workers. Higher readings on a given amount of nicotinic acid can be obtained by increasing these percentages, but, with our materials, this raised difficulties in regard to solubilities. For *p*-aminoacetophenone and procaine the percentages used were almost optimal, and small variations made no appreciable differences to the results. For instance, doubling the percentage of procaine increased the colour density by only 10 per cent. Because of these differences in solubilities we employed a reaction mixture of 19 ml. for metol and of 10 ml. for the other amines. Under these conditions, the optical density produced by metol was only a fifth of that produced by *p*-aminoacetophenone, less than a third of that produced by procaine and less than a half of that produced by aniline. Moreover, the results given by metol were much more variable, the coefficients of variation of a single observation being 11·9 as compared with 5·6 for aniline, 3·8 for procaine and 2·4 for *p*-aminoacetophenone. These results were obtained from readings taken at the time of maximum intensity, which varies widely in different experiments. In practice, therefore, it is more convenient when working with aqueous *p*-aminoacetophenone or with procaine to take readings 5 or 10 minutes, respectively, after mixing. Table I shows that this does not diminish the accuracy when *p*-aminoacetophenone is used, the standard deviation of 0·50 on 22·3 being equivalent to a coefficient of variation (for each point on the curve) of 2·2. If conditions are arranged so that the optical density readings lie between 0·2 and 0·7 the error can be reduced still further.

TABLE I

CALIBRATION OF ABSORPTIOMETER WITH PURE NICOTINIC ACID (N.A.) USING *p*-AMINOACETOPHENONE

Concn. of N.A., μg. in 10 ml.	Optical density (D) at max.	Optical density (D) at 5 mins.	D max. / concn. × 1000	D in 5 min. / concn. × 1000
5	0·118	0·113	23·7	22·6
10	0·226	0·213	22·6	21·4
20	0·462	0·439	23·2	22·0
30	0·697	0·663	23·2	22·1
40	0·962	0·919	24·1	23·2
		means	23·4	22·3
		standard deviations	0·57	0·50

Each curve in Fig. 2 was obtained at the optimal *p*H for the respective aromatic amine. For aniline this was that of the final extract (about 6·8). For the other amines it was more acid (about 1 for aminoacetophenone or procaine). The aniline colour is more stable but less intense at a more acid *p*H.

CHOICE OF AROMATIC AMINE—Satisfactory results were given with procaine (10 per cent. w/v in 10 per cent. w/v hydrochloric acid) and with *p*-aminoacetophenone (using not more than 10 per cent. alcohol in the reaction mixture, to avoid precipitation). Aniline is unsuitable for assaying animal extracts.[1] For cereals aniline appears satisfactory, although, like metol, it gives less intense and more variable colours under the given experimental conditions.

EFFECT OF PERMANGANATE—Wang and Kodicek,[2] using a visual method, recommended for clinical tests the use of potassium permanganate for removing interfering colours prior to development of the yellow colour given by nicotinic acid with cyanogen bromide and the aromatic amine. They did not apply the permanganate treatment to foods. We have found it to give fairly satisfactory results with unmalted barley, but obtained very variable results on applying it to malted preparations. This may be due to gradual development of

haziness in the solution, to which the photoelectric method is very sensitive, so that the absorptiometer readings, when plotted against time, do not show the characteristic fall and may eventually rise considerably above the initial value. Fig. 3 illustrates such results obtained with a typical malt extract and shows that the effect may be swamped by adding excess of nicotinic acid, indicating that it is due to interfering substances. It will be seen from the figure that this action of permanganate should lead to higher results, even if these are calculated on readings taken 5 minutes after mixing. This in fact occurred. In a large number of assays on the same malt extract we found that the result could vary from 80 to 170 μg./g. according to the amount of permanganate used (see Fig. 4). The microbiological result was 102 μg./g. In practice the amount of permanganate used would not of course vary so widely as this, but it might vary sufficiently to cause considerable errors in the results. Permanganate can also cause similar increases in the absorptiometer readings obtained in the assay of nicotinic acid in milk. Fig. 5 summarises readings obtained using aminoacetophenone and procaine with permanganate. When the same samples were assayed by our method using Lloyd's reagent, no such increase in readings occurred.

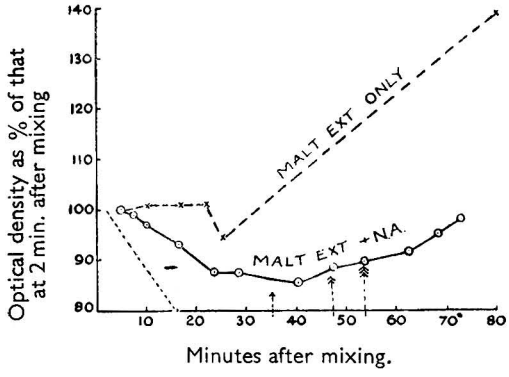

Fig. 3. Increase in density of colour given by nicotinic acid from malt extract on standing with PAAP after $KMnO_4$ had been used to decolorise interfering substances. Lower continuous curve obtained with same quantity of the malt extract but with added nicotinic acid (25 to 30 per cent. of total). Normal rate of fading in absence of $KMnO_4$ is shown by dotted curve on left, taken from Fig. 1. Arrows indicate times at which haziness was first detected visually by different observers.

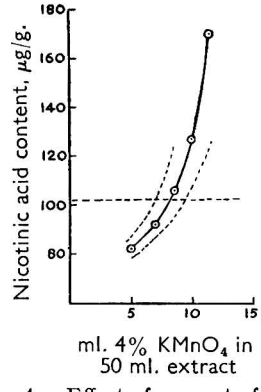

Fig. 4. Effect of amount of $KMnO_4$ used to decolorise malt extract on nicotinic acid content as found by chemical method. The broken curved lines represent twice the standard deviation. The broken horizontal line indicates the nicotinic acid content as found by microbiological assay.

IMPROVEMENT OF ACCURACY—When we first applied Wang and Kodicek's method to cereals and cereal products our chemical results averaged only 75 per cent. of the microbiological results and varied widely, as shown by the coefficient of variation of a single result sometimes exceeding 20. This was due to the use of too little permanganate and imperfect control of the amounts (see Fig. 4). We were able to improve the reproducibility of our results and bring them much closer to the microbiological results by standardising the amount of potassium permanganate used at 9 ml. of 4 per cent. solution for 50 ml. of reaction mixture, by fixing the temperature of the cooling water at 20° C. and by using Melnick's solution blank. The chemical results then averaged 109 per cent. of the microbiological results and the coefficient of variation of a single assay was reduced to 11·6. This improvement in accuracy was due mainly to control of the permanganate additions. Further improvement was sought by trying the American adsorption techniques.

REMOVAL OF INTERFERING SUBSTANCES BY ADSORPTION—Various adsorbents have been employed for this purpose by American workers. Charcoal was at one time recommended. Our experience with B.D.H. "charcoal for adsorption" gave unsatisfactory results owing to incomplete adsorption of nicotinic acid. For example, at *p*H 6·4 only 55 per cent. was adsorbed in a malt extract assay. The solution blank was 19 per cent. of the total colour, no better than was obtained with permanganate. Further experiments under different conditions still showed incomplete adsorption. We then tried Lloyd's reagent, which has been widely used in America and is recommended by a Niacin Assay Committee of representative American workers. In our first experiments with this we used 1 g. for each g.

of material containing up to 150 μg. of nicotinic acid. After heating 1 g. of the sample for 30 to 40 minutes in a boiling water bath with 3 *N* hydrochloric acid and cooling to room temperature, the supernatant liquid (which had a *p*H below 1) was shaken for 1 minute with 1 g. of Lloyd's reagent. The adsorbed nicotinic acid was eluted with 0·1 *N* sodium hydroxide and the resulting solution clarified with solid lead nitrate and trisodium phosphate as recommended by the American workers. Our results showed improved reproducibility (the coefficient of variation of a single assay having dropped to 6·5) but averaged only 91 per cent. of the microbiological results. By increasing the amount of Lloyd's reagent from 1 g. to 2·5 g. and introducing other slight modifications in technique we brought the average

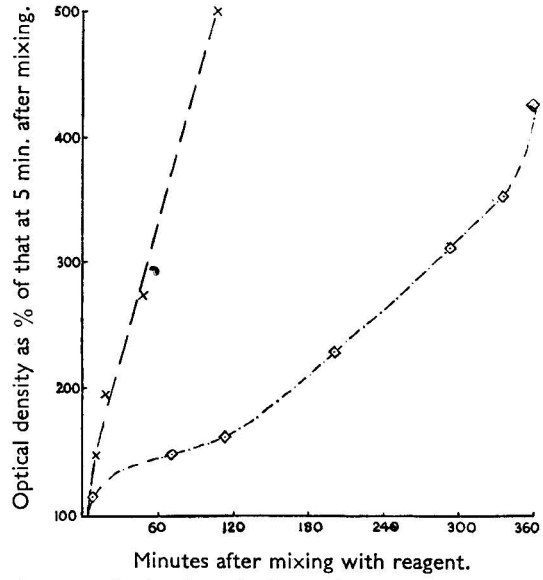

Fig. 5. Increase in density of colour given by nicotinic acid from milk on standing with reagent, when $KMnO_4$ had been used to decolorise interfering substances. Results obtained with PAAP indicated thus: × — — × ; results with procain thus: ⊡ — . — ⊡.

chemical results up to 101 per cent. of the average microbiological results. The coefficient of variation was reduced to below 6 and the recovery of nicotinic acid added to the original material was 98 per cent. No further increase in the chemical results was produced by raising the amount of Lloyd's reagent to 5 g. per gram. Decolorisation with zinc hydroxide in addition to lead hydroxide has been recommended by Swaminathan.[24] Our experiments with his technique have not given any better results than those obtained by our usual method employing Lloyd's reagent and lead hydroxide.

CORRECTION FOR INTERFERING SUBSTANCES—Interference with the colour given by the reaction between nicotinic acid and the reagent may be caused by:

(*a*) the colour of the reagent,
(*b*) the colour of the test solution,
(*c*) changes in colour produced by reaction between the reagent and non-specific substances in the test solution.

Correction for (*a*) is usually made by means of a reagent blank containing the amine and cyanogen bromide at the final *p*H. When *p*-aminoacetophenone is used a high reagent blank may be obtained, due mainly to the amine. This has led some workers[25] to substitute an amine blank for the total reagent blank, but in our experience this is not entirely satisfactory. The optical density of the total reagent blank may be 20 to 40 per cent. of the total optical density obtained in the assay.

Correction for (*b*) is usually made by means of the test solution at the final *p*H but containing no reagent. This test blank has also been termed "colour blank" or "solution blank" by different workers. The solution blank may vary widely with different foods and decolorising procedures and can have a colour greater than that produced by the nicotinic acid. Much work has therefore been done with the object of reducing its colour. Its

magnitude in relation to the total colour obtained in the assay has in fact been suggested for measuring the efficiency of decolorising procedures. When, however, we applied this criterion to compare the value of permanganate with Lloyd's reagent the differences were not very significant, as can be seen from Table II, which is based on over 200 assays on numerous samples. On the whole, Lloyd's reagent gave lower blanks. With wheat flour and meat extract the average blanks were considerably lower, but only two samples of each were examined. With cereals and other foods, of which more samples were examined, the differences were less evident.

TABLE II

EFFECT OF DECOLORISING PROCEDURE ON INTERFERING SUBSTANCES

	Optical density of solution blank as percentage of total optical density in assay after decolorisation with:	
	$KMnO_4$	Lloyd's reagent and $Pb(OH)_2$
Wheat flour	38	27
Barley	13	13
Malted barley	15	16
Malt extract	18	15
Yeast	22	21
Milk, dried	64	61
Meat extract	40	16
Maize (yellow corn meal)	31	30

Correction for (*c*) is a more complicated procedure involving the use of further blanks to determine the effect of amine and of cyanogen bromide separately on the test solution. This procedure has been carefully studied by Friedemann and his colleagues in America.[26] The application of Friedemann's methods to our materials has raised the question of the effect of germination, which will be considered in detail elsewhere. Here it will suffice to say that by using the reagent and solution blanks as described on p. 329 we have obtained good agreement with microbiological results on a wide variety of foods. Certain discrepancies (especially in low extraction flours) cannot be satisfactorily explained as due to different blanks, and other sources of error are being considered.

RECOVERY EXPERIMENTS WITH ADDED NICOTINIC ACID—These have been used by various workers to test the validity of Beer's law, the nicotinic acid being added after the decolorising treatment. Under these conditions we have obtained fairly complete recovery of added nicotinic acid but did not find the results of much value in correcting for interfering substances. On the other hand, we think it essential to test the behaviour of Lloyd's reagent with nicotinic acid under the experimental conditions, preferably by adding a known amount of nicotinic acid to the foodstuff being examined. Under these conditions also we have been able to obtain complete recovery.

COMPARISON OF CHEMICAL AND MICROBIOLOGICAL RESULTS—These are summarised in Table III (p. 335).

Taking into consideration the respective errors of the two methods, there was a highly significant difference with the two low extraction wheat flours, yellow corn meal and yeast extract. The chemical results were considerably higher, except on the yeast extract, which caused difficulties often encountered with yeast preparations. In the collaborative study by the American Niacin Assay Committee under the chairmanship of Dr. D. Melnick it was also found that chemical methods gave considerably higher results than microbiological methods on low extraction wheat flour, whilst satisfactory agreement was obtained between the two methods on whole wheat flour. It was suggested that this might be due to the presence of a nicotinic acid precursor becoming available to the micro-organism only after hydrolysis. In our work the question of this precursor is being studied in relation to the precursor in cereals which yields nicotinic acid on germination, and will be reported upon later. Results so far obtained indicate that the two types of precursors are probably different. The first two samples in Table III are samples No. 1 and No. 8 in the above American collaborative study. Our chemical and microbiological results on these are in excellent agreement with the average chemical and microbiological results obtained by leading American workers. The sample of maize (yellow corn meal) in Table III was supplied by Dr. Elvehjem, whose microbiological result of 19·5 μg./g. was in good agreement with our microbiological result of 22 μg./g. Our higher result, using the final chemical method, was not related to a

specially high solution blank, and some other explanation must be sought. Several of the other samples in Table III have also been examined by other workers, whose results confirmed ours, and we think that, apart from the low-extraction flours, maize and yeast, we have established satisfactory agreement between chemical and microbiological results in the range of samples examined.

TABLE III

NICOTINIC ACID CONTENT OF CEREALS AND OTHER FOODS

	Nicotinic acid μg./g. by	
	chemical assay	microbiological assay
Whole wheat flour	62	61
Whole wheat bread	68	62
Wheat germ	134	129
Flour, National	27	14
" white	47	20
Barley	109	111
Malted barley	126	127
Malt extract, commercial sample ..	102	95
" " " " ..	99	96
" " " " ..	90	96
Malt extract, dried	111	110
Yeast, dried	276	290
" "	346	320
" "	290	290
" "	332	331
Yeast extract	478	537
Dried meat	162	135
Meat extract	1027	1082
Yellow corn (maize) meal	43	22
Malt and yeast food	99	79
" " " "	113	116

Notes:

The nicotinic acid content is expressed as μg. per g. of the food as received, and each result is the mean of several assays. The following standard deviations of these means showed a highly significant difference between chemical and microbiological results on the National flour (chem. 2·2, microbiol. 0·2), white flour (chem. 3·6, microbiol. 0·4), yellow corn meal (chem. 3·0, microbiol. 0·4) and yeast extract (chem. 15·0, microbiol. 5·3) and a significant difference on the first sample of malt and yeast food (chem. 6·0, microbiol. 1·7). With the remaining sixteen samples there was no significant difference.

The accuracy of the chemical method with maize, yeast, and wheat flours of less than 90 per cent. extraction was found to be considerably lower than with the other foodstuffs examined. Thus, in 19 assays on 5 samples of the above the average coefficient of variation of a single assay was 12·7, as compared with an average of 5·8 in 55 assays on the remaining 16 samples. In calculating these coefficients the only results excluded were a few obtained mainly by Kodicek's method where development of cloudiness interfered with the Spekker readings. The lower standard deviations of the means for the microbiological method were due partly to its greater accuracy and partly to more assays being made on each sample.

These findings are in general agreement with the findings of most other workers both in this country and in America. Certain American workers[27,28] have reported that microbiological methods give considerably higher results than chemical methods (using Lloyd's reagent and *p*-aminoacetophenone or metol) on wheat, barley or malted barley, but that this discrepancy disappears when the material is oxidised with hydrogen peroxide before carrying out the microbiological assays. Their results have not been confirmed by other American workers[16] and need further investigation.

SUMMARY

Difficulties have been encountered in the chemical assay of nicotinic acid in cereals. A study, using the Spekker photo-electric absorptiometer, has been made of the following factors which might affect the results.

(*a*) Choice of and method of using aromatic amine, including *p*-aminoacetophenone in alcoholic or aqueous solution, procaine, aniline and metol.

(*b*) Procedures for removing or correcting for the presence of interfering substances by decolorisation or adsorption techniques or by the use of different blanks.

(*c*) Instrumental errors.

A chemical method has been devised which has given satisfactory reproducibility and agreement with microbiological results on a wide range of cereal products. This method gave considerably higher results than the microbiological method on low-extraction flours, maize, and some yeast preparations, but the discrepancy (which has also been encountered by other workers) cannot be explained by any of the above factors. The possible effects of nicotinic acid precursors have been left for further study.

We are indebted to Dr. D. Melnick and his colleagues on the Niacin Assay Committee, to Dr. E. R. Dawson for providing collaborative samples, and to Miss Janet Horsford for technical assistance.

The chemical work undertaken by one of us (E.M.J.) in this investigation forms part of the work for a Ph.D. thesis in the University of London.

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OVALTINE RESEARCH LABORATORIES
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A Note on the Chapman and McFarlane Method for the Estimation of Reducing Groups in Milk Powder

BY C. H. LEA

(*Read at the Meeting of the Society on April 2nd*, 1947)

WHILE peroxide, Kreis and aldehyde values of the fat, in addition to measurement of absorption of oxygen and production of carbon dioxide by the powder, have been used with some success in confirmation of organoleptic tests for fat deterioration in full cream milk powder[1,2,3] specific chemical tests for the protein type of spoilage which leads to the production of stale and "gluey" flavours and loss of solubility in separated milk powder or in full cream powder of high moisture content have been lacking. For this reason the colorimetric method for the estimation of reducing groups in milk powder recently proposed by Chapman and McFarlane[4] is of particular interest.

The method of Chapman and McFarlane depends on the reduction of potassium ferricyanide solution by heating with the powder for 20 minutes at 70° C. and *p*H 5·0, after which the protein is precipitated with trichloroacetic acid and the ferrocyanide produced estimated colorimetrically after development with ferric chloride. The duration and temperature of

heating were chosen to give a large difference between fresh and deteriorated powders, and the reaction does not proceed to completion.

Following Mirsky and Anson,[5] the authors conclude that the reducing groups responsible for the reaction of stale or heated milk powder, casein or lactalbumin are present in the protein molecule and become accessible on denaturation, and that these groups are probably mainly sulphydryl, assisted perhaps by tryptophan residues. Chapman and McFarlane therefore express their results as moles $\times 10^{-5}$ reducing groups per gram of powder, and calibrate against the SH group, using glutathione or cysteine as standard.* The values obtained did not obey Beer's law, apparently because the ferric ferrocyanide produced was not in true solution.

We have found that Beer's law is obeyed, and a straight line calibration curve obtained, provided that the coloured solutions are matched one minute after mixing instead of after 10 minutes as recommended in the original method. The occurrence of obvious flocculation in the stronger solutions on prolonged standing indicates the necessity for this modification. Using a Spekker photo-electric absorptiometer with a red filter, we obtained the same linear calibration curve passing through the origin and extending to an extinction coefficient of approximately 2·8 whether the colour was produced by known small quantities of potassium ferrocyanide or of ferrous ammonium sulphate, development of the colour in each case being with ferric chloride in the presence of potassium ferricyanide, phthalate buffer and trichloroacetic acid as in the test. Since evidence presented here and elsewhere[6] concerning the part played by sugar in causing deterioration of the protein leaves in doubt the precise nature of the groups causing reduction of the ferricyanide, it has been preferred to express the results as moles $\times 10^{-5}$ of ferricyanide reduced per gram of dry milk solids. Such values are not directly convertible to SH groups or to cysteine units since, under the conditions of the test, cysteine does not reduce exactly one equivalent of ferricyanide.

Results

Preliminary tests with the method showed that reasonable variation in the quantity of powder taken for the determination did not affect the result provided that the colour developed was not too deep. Fresh spray-dried powders showed a small reducing power which increased on storage either rapidly or very slowly according to the moisture content of the powder and the storage temperature.[6,7]

Nature of the reducing substances—

Mirsky and Anson[5] found SH groups and tryptophan and tyrosine residues to be mainly involved in the changes in reducing power that result from the denaturation of proteins. Chapman and McFarlane found that ferricyanide under the conditions of their test was reduced strongly by cysteine, feebly by tryptophan and not at all by tyrosine. The amount of cystine present in milk powder, however, even if all available as free cysteine, is not sufficient to account for as much as 10 per cent. of the reducing power of a badly deteriorated milk powder. Similarly, tests carried out with tryptophan and tyrosine in the proportions reputed to be present in separated milk powder show that these acids, even if entirely exposed in the protein, could not account for more than about 5 per cent. and 0·3 per cent. of the reducing power of fresh spray-dried powder, or about 0·2 per cent. and 0·0 per cent. of the reducing power of a badly deteriorated powder. Lactose showed no reducing power. Glucose also showed no reducing power, but after treatment with *N* sodium hydroxide at 40° C. for 5 minutes developed a pronounced brown colour and a reducing power of 6·3 moles $\times 10^{-5}$ of ferricyanide per gram.

Dialysis of a fresh milk powder of high (7·6 per cent.) moisture content showed that of its total reducing power of 0·9 moles $\times 10^{-5}$ ferricyanide per gram, about 0·6 was dialysable, while 0·3 remained associated with the protein. After storage at 37° C. for 6½ months the powder, which was now brown in colour and of very poor solubility, showed a total reducing value of 22·1 per gram of dry solids, of which about 9 units were dialysable and 13·1 undialysable. Approximately one-fifth of the undialysable fraction was still soluble, and there

* In a private communication received since completion of the present work, Professor R. A. Chapman states his belief that the substances responsible for the increase in reducing capacity in milk powders are either a complex formed between an amino group and a carbohydrate or decomposition products of such a complex.

had been no increase in the proportion of dialysable nitrogen during storage. These observations suggested that a non-dialysable protein - carbohydrate complex or its decomposition products was mainly responsible for the increased reducing power of the deteriorated milk powder, assisted in some degree by dialysable, non-nitrogenous reducing material, presumably degradation products of lactose.

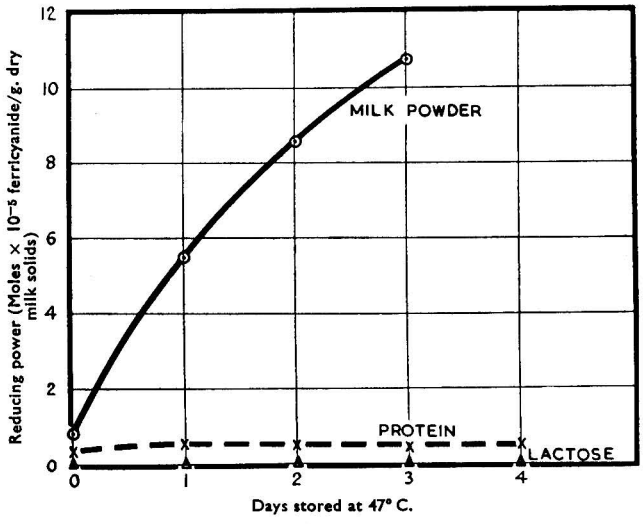

Fig. 1. Development of reducing power by (*a*) separated milk powder, (*b*) milk protein (dialysed separated milk powder) and (*c*) lactose, during storage at 47° C. and 55 % relative humidity.

STORAGE OF THE PROTEIN AND CARBOHYDRATE FRACTIONS OF MILK POWDER—

A quantity of fresh, spray-dried separated-milk powder was reconstituted, dialysed in cellophane against distilled water for 3 days at 0° C., and freeze-dried in high vacuum at a low temperature. Separate samples of this material, of pure α-lactose hydrate and of the control (undialysed) milk powder were stored in thin layers at 47° C. over sulphuric acid giving a relative humidity of 55 per cent. and examined daily for reducing power and moisture content. The results, calculated in all cases to moles × 10^{-5} ferricyanide per gram of dry, separated-milk solids are given in Fig. 1. The residual "protein" fraction and the lactose, when stored separately, showed practically no increase in reducing power under conditions which caused rapid deterioration of the separated-milk powder.

In a second experiment fresh, separated milk was dialysed at 0° C. for 7 days and the residual "protein" admixed with various sugars, frozen rapidly, freeze-dried, ground and stored at 37° C. and 55 per cent. relative humidity for 4 months. The compositions and reducing powers of the products are given in Table I. The *p*H of all samples lay between 7·00 and 7·05.

TABLE I

EFFECT OF VARIOUS SUGARS ON THE DEVELOPMENT OF REDUCING POWER

Sample	Sugar added (g./100 g. dry protein)	Reducing power (moles × 10^{-5}/g. protein)		Loss of free amino N during storage (mg./g. total N)*
		Initial	Increase during storage	
Control ..	—	0·6	0·2	0
G	1·9 glucose	0·5	16·1	7
L	144 lactose	0·4	31·0	33
S	144 sucrose	0·4	0·3	0
GL	1·9 glucose 144 lactose	0·5	34·4	35
GS	1·9 glucose 144 sucrose	0·4	13·4	7

* Initial value = 60.

It appears from these results that the ferricyanide reducing power of milk powder is an index of the formation and degradation of a protein - sugar complex, rather than of simple denaturation of the protein, that glucose is much more active than lactose in undergoing such reaction with protein, and that sucrose is inert. Since the protein - sugar reaction is now known to be a major factor in the causation of non-fatty deterioration in milk powder of high moisture content,[7] and probably in other foods such as dried egg,[8] the Chapman and McFarlane test, which is easily carried out as a routine procedure, appears to have definite possibilities for the control of quality. No single chemical test, however, can be expected to correlate accurately with flavour over a wide range of moisture contents, storage temperatures and storage atmospheres because at least two or three separate mechanisms appear to be involved in the development of "off" flavour under widely different storage conditions.

Summary

The increase in the reducing power towards potassium ferricyanide which develops during the storage of milk powder, particularly at high moisture contents and high storage temperatures, has been shown to be due to the formation and degradation of a protein-reducing sugar complex.

Technical assistance in this work was given by Mr. D. N. Rhodes. The work was carried out as part of the programme of the Food Investigation Board and is published by permission of the Department of Scientific and Industrial Research, Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge and Department of Scientific and Industrial Research.

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Discussion

Mr. E. B. Anderson said that work carried out in their laboratory confirmed Dr. Lea as to the formation of reducing substances in the heat treatment of milk, and the interaction of reducing sugars with amino groups. The new modification of the Chapman and McFarlane method would be of interest, as they had not had much success with the method. He would be interested to know the level of reducing substances as shown by this test in spray-dried powder from the high preheat treatment and also the level for roller powder.

Mr. A. L. Bacharach asked if there was any indication that fat in a full-cream milk powder was able to reduce protein deterioration, apart from its effect as a neutral diluent. In his view, available data pointed to the superiority of spray powder over roller powder in resistance to protein deterioration, though the reverse was true of fat spoilage. The problem of carbohydrate and protein combination, presumably primarily a reaction between the aldehyde group of a sugar and the amino group of an amino acid, and of the subsequent breakdown of intermediate products, deserved, in his view, fundamental study in which presumably modern physical methods could play a valuable part in helping to elucidate changes of configuration in the protein molecule. He trusted that Dr. Lea and his colleagues would themselves be undertaking such studies or would at any rate encourage others to do so.

Dr. Magnus Pyke said he was very interested in the subject and anxious to learn what was the chemistry of the reactions involved and to what extent they are related to the biological value of the protein. Any differences between the behaviour of vegetable and animal proteins on storage are of great interest and practical importance.

Mr. E. Barton Mann said he welcomed the work of Dr. Lea in so far as his method might assess the degradation of dried skimmed milk. Fraps, Meinke, Reiser and Sherwood (*Bull. Texas Agric. Exper. Station*, **1943, 637,** 23) had indicated that some animal proteins, including those of dried skimmed milk and dried buttermilk, may have "a high consuming power for carotene." In feeding tests on poultry it is no uncommon thing to find this depressant action of animal feeding stuffs, in respect to both carotene and vitamin A. If there is a linkage between the lactose and the protein of milk, it is obvious that the

denatured protein, as it cannot be utilised by the organism, would form food for attack by micro-organisms, most probably of the Gram-positive anaerobic type. Preponderance of these gives rise to intestinal putrefaction and mild toxicity and might perhaps interfere with vitamin and provitamin assimilation. As lactose enjoys the peculiar property of not being entirely absorbed until it reaches the end of the gut, if it is rendered non-effective by combination with protein, then there is always the possibility that nutriment may be withheld from lactic acid bacteria—the natural enemies of Gram-positive putrefactive anaerobes. Dr. Lea has recorded the lowered biological value of dried skimmed milk which by reason of age or other conditions has shown a higher lactose-protein complex than originally. Had he any reason to believe that this lowered value may be coupled with excessive bacterial action in the intestines on the lines suggested?

Dr. H. LIEBMANN asked if the amino acid - sugar reactions are catalysed by heavy metals.

Mr. H. W. HARTLEY said it had been found that in "sugar - dried egg" the sucrose exerts a protective influence on the protein, whereas in Dr. Lea's experiments, although sucrose had no adverse effect, no advantageous effects were apparent.

Dr. LEA, in reply to Mr. Anderson, said that he had not as yet specifically compared the level of reducing substances in spray-dried powders from low and high pre-heated milk. The level in roller powder was very much higher than in fresh spray powder, presumably as a result of the more severe heat treatment during drying.

In reply to Mr. Bacharach, he was not aware of any stabilising effect of the fat present in full-cream powders on protein deterioration, although there might very well be one. Separated milk powder, as a less valuable commodity, was often of higher initial moisture content (even on the fat-free basis) and was liable to be less carefully treated before, during and after drying than the full-cream product. A more fundamental attack on the reaction between pure proteins and sugars was being undertaken.

In reply to Dr. Magnus Pyke, he said that since lysine was the amino acid most concerned in the loss of biological value, the protein - sugar reaction was of particular interest with regard to the lysine-rich animal proteins that normally supplemented the cereal proteins in a mixed diet. The points raised by Mr. Barton Mann formed interesting subject-matter for speculation, but he was, at present, unable to carry them any further.

The simple amino acid - sugar reaction in the test tube is known to be catalysed by traces of heavy metals, and evidence is available that this is true also for deterioration of this type in certain dried vegetables.

In reply to Mr. Hartley, Dr. Lea stated that, although sucrose did not delay the primary reaction between reducing sugar and protein amino groups in milk powder, it did greatly delay the secondary change which resulted in loss of solubility of the protein. In this respect it would probably be found that dried milk and dried egg were similar.

The Photometric Determination of Dissolved Oxygen in Condensates and Feed Waters by means of the Starch-Iodide Complex

BY S. BAIRSTOW, J. FRANCIS, AND G. H. WYATT

THE increasing use of boiler plant having working pressures over 250 lb. per sq. in. has made the control of the dissolved oxygen content of the feed water of great importance. The concentration of dissolved oxygen that can be allowed with safety decreases rapidly with rise in pressure above this value, and it is generally accepted that the amounts should not exceed 0·03 ml. per litre at 400 lb. per sq. in. and 0·01 ml. per litre at 600 lb. per sq. in.[1]; at pressures of 1000 lb. per sq. in. and over, dissolved oxygen cannot be tolerated and must be removed, for example, by sodium sulphite. Since it is necessary to be able not only to show that the oxygen concentration is kept below the appropriate maximum value, but also to detect small differences due to leakages of air into the system before and after adjustment of the plant, it is desirable that the method of measurement used should be capable of determining amounts of dissolved oxygen considerably less than the maximum values specified. The classical method for this determination was devised by Winkler[2] and involves oxidation by dissolved oxygen of manganous hydroxide precipitated in the solution; the manganomanganic hydroxide (Mn_3O_4) so formed[3] reacts with potassium iodide to give free iodine which is determined volumetrically, with starch as indicator.

The Winkler method is subject to error because: (*a*) the end-point of the titration is indefinite; 0·01 ml. of dissolved oxygen per litre in a 250-ml. sample liberates iodine equivalent to only 0·045 ml. of 0·01 *N* thiosulphate solution, (*b*) the colour of the starch-iodide complex

disappears while some iodine is still present[4]; at ordinary temperatures this undetected iodine is equivalent to about 0·01 ml. of dissolved oxygen per litre, (*c*) no allowance is made for interference by substances other than dissolved oxygen. The method is suitable for the examination of natural waters, in which Winkler was interested, but for modern feed water supplies a more accurate process is necessary. Modifications have been described in the literature in which various ways of determining the liberated iodine have been proposed, interference by substances other than dissolved oxygen has been measured and the sampling apparatus has been improved. The present paper describes (1) the development of an accurate method for the determination of the free iodine by measurement of the intensity of the blue starch-iodide complex with the Spekker photo-electric absorptiometer, and (2) a convenient form of sampling apparatus in which may be carried out, without atmospheric contamination, the reactions liberating iodine, including those due to interfering substances. The complete process has been in use at this laboratory for about six months, during which period over four hundred routine determinations have been made.

Other methods—Consideration was given to the "dead-stop" method of titration first described by Foulk and Bawden[5] in which the end-point of the iodine - thiosulphate titration is detected accurately by electrical indication of the polarisation, by the thiosulphate, of platinum electrodes immersed in the solution. The procedure was applied to the determination of dissolved oxygen by Hewson and Rees[6] who did not, however, estimate interfering substances. This method involves a delicate titration and it was felt that elimination of this operation is highly desirable if routine determinations are to be made by hitherto untrained persons. This could be achieved by the application of colorimetry and an examination was first made of the proposal of W. Francis,[7] whereby the oxidation product of the precipitated manganous hydroxide is treated with hydrochloric acid to form free chlorine, which is determined by the well known colour reaction with *o*-tolidine.

This reaction appears *a priori* to be capable of measuring quantities of oxygen that would remain undetected by the Winkler titration, and it was found that a solution containing free chlorine equivalent to 0·002 ml. of dissolved oxygen per litre gave with *o*-tolidine a colour readily distinguishable from a blank. Pairs of samples of water were taken simultaneously from the same source and the dissolved oxygen was determined by the *o*-tolidine and Winkler methods; the results agreed within the limits of accuracy of the latter process. After the method had been in routine use for a few months, a decrease in the values found for the dissolved oxygen concentrations was observed. The measured values continued to diminish until finally no oxygen could be detected. During this period alterations had been made to the feed water system with a view to decreasing the oxygen content of the water, but its complete elimination appeared to be highly improbable. Comparison of the *o*-tolidine and Winkler methods was, therefore, repeated, and it was found that, although no free chlorine was detected by the former, the Winkler titration indicated the presence of 0·03 to 0·05 ml. of dissolved oxygen per litre. The oxygen contents of samples of feed water were artificially increased by introducing known amounts of water saturated with air, and it was found that *o*-tolidine first indicated the presence of free chlorine when the dissolved oxygen content of the sample, as determined by the Winkler method, was 0·07 to 0·08 ml. per litre. Further investigations were not made, but it is concluded that free chlorine was removed from solution immediately on liberation, probably by minute traces of organic matter in the feed water. A slight contamination of the condensates by the cooling water occurs continuously and it is thought to be significant that concordant results were obtained by the two methods during winter months, while the discrepancy was found in the Spring, when algal growths increase.

Starch-iodide methods—The *o*-tolidine reaction was abandoned and attention was directed to the measurement of the intensity of the blue colour of the starch-iodide complex formed in the same manner as in the Winkler method. Verbestel[8] refers to the use of the colour of the iodine solution for the determination of the dissolved oxygen content of water, and standard colour discs* may be obtained for this estimation. The change in depth of colour corresponding to 0·01 ml. of oxygen per litre is, however, small and the disc for feed waters contains only nine tinted glasses for the range of concentrations from 0·05 to 1·0 ml. per litre. Katayama[9] stated in 1907 that the concentration of starch-iodide is proportional to the iodine concentration if the starch concentration is kept constant, but he did not point out the possible analytical application of his discovery. In 1926 von Fellenberg[10] reported

* For use with the B.D.H. Lovibond Nessleriser.

that the intensity of the colour of the starch-iodide complex is not always proportional to the iodine concentration and that the reaction is of little value for the determination of iodine if salts are present; the latter contention was confirmed by Woodard.[11] On the other hand, Turner[12] found that the method is satisfactory for dilute solutions of iodine in absence of salts, but his paper has been adversely criticised.[13] A careful study of the reaction was made by Müller and McKenna,[14] from whose paper the extinction curve shown in Fig. 1 has been taken. At first no colour is produced; a range of iodine concentrations then follows over which the extinction curve is not straight, and finally the colour intensity is linearly related to the iodine concentration. Müller and McKenna point out that for the linear part of the curve the extinction E is related to the iodine concentration c by the relation $E = k(c - c_o)$ and state that knowledge of c_o allows calculation of the quantity of iodine that must be added to test solutions to ensure that only the linear part of the curve is used: it is clear from Fig. 1 that an iodine concentration c_1 is required. As early as 1887 Mylius[15] had shown that the blue colour is not obtained unless an iodide is present: it has since been stated[9,16] that the depth of colour is related to the iodide concentration in a complex manner, the iodide having a markedly greater effect at lower concentrations. Recently, however, it has been considered that the effect is due to a change in the electrical charge on the particles and it has been shown that salts other than iodides behave in a similar manner (for a full account of this and other work on the starch-iodide complex, with references prior to 1930, see Barger[17]; a later review is given by Meyer[18]). Results obtained during the present investigations, however, show that former emphasis on the effect of salts was too great. Biochemists have claimed successful applications of photo-electric adaptations of the reaction to the determination of iodine in blood[12,19] and protein-bound serum[20] and of chloride, iodate and iodide in protein-free solutions.[21] Most of these applications isolate iodine by distillation, oxidise it to iodate and then liberate iodine by the iodate - iodide reaction; by a similar reaction bromate in treated flour has been determined.[22] Simerl and Browning[23] have examined the colours produced by various kinds of starch and a number of starch derivatives with a view to the determination of starch in paper.

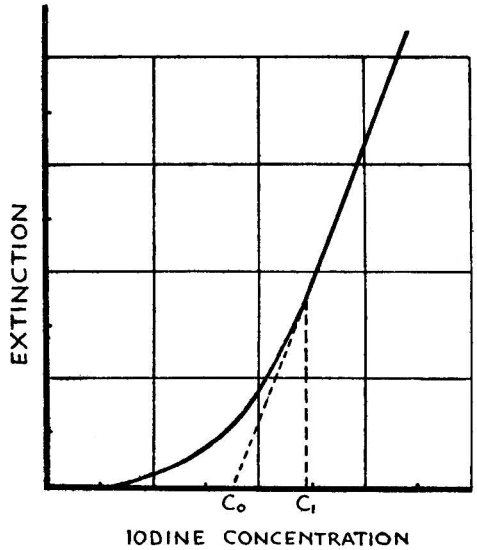

Fig. 1. Extinction curve.[14]

It is now recognised[24] that water often contains substances other than dissolved oxygen which may either liberate iodine from potassium iodide or absorb iodine, and it is therefore essential in the accurate determination of dissolved oxygen to include an "interference sample," in which is measured the effect of the interfering substances; the result obtained with the ordinary sample may then be corrected.

Experimental

Starch concentration—Preliminary experiments showed that it would probably be possible to determine up to 0·10 ml. of dissolved oxygen per litre of water by measuring the intensity of the blue colour of the starch-iodide complex by means of the Spekker photoelectric absorptiometer, using a 4-cm. cell in conjunction with a tungsten filament lamp, heat filters H503 and spectrum red filters H608. All the investigations described below were made with this equipment, but final calibration curves were drawn both for the tungsten filament and mercury vapour lamps. A 3-litre sample of feed water was collected under air-free conditions and 17·5 ml. of each of the three Winkler reagents alkaline iodide, sulphuric acid and manganous sulphate were added in that order. In these circumstances the small quantity of oxygen dissolved in the feed water does not react, since the formation of manganous hydroxide is prevented. (This solution is called the *blank solution* in the following account). Additions of standard iodine solution (approximately 0·001 *N*) were made to 250-ml. portions of the blank solution to give iodine contents equivalent to 0·01, 0·02 . . . 0·10 ml. of oxygen

per litre. The solutions were cooled to 15° C. and 5 ml. of 0·1 per cent. starch solution were added to each; after mixing and leaving to stand for 5 minutes, the absorptions were measured. It was found that insufficient starch had been added for the detection of more than about half the desired concentrations of oxygen. Accordingly, a family of curves, shown in Fig. 2, was constructed in a similar manner using starch solutions of various concentrations.* Points corresponding to 1·0, 1·5 and 2·0 per cent. starch solutions lie too close together to show clearly, so that curve *F* has been drawn to fit all the points concerned. In all subsequent determinations and investigations 1 per cent. starch solution was used. It is interesting to note that the ratios of the concentrations of starch and iodine at which curves *A*, *B* and *C* cease to be linear correspond to approximately 3·3 per cent. of iodine in the blue complex. Most of the late-nineteenth century researches on the constitution of the "compound" of starch and iodine attempted to prepare products with a maximum iodine content, but the value now found approximates to the minimum of 3 per cent. cited by Radley.[25]

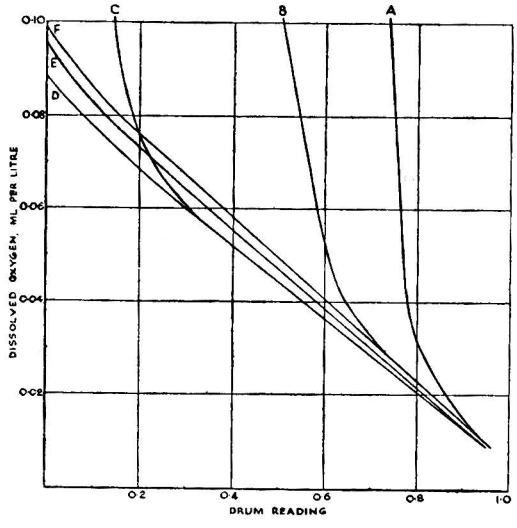

Fig. 2. Effect of starch concentration.

Curve A	..	0·05 % starch solution
,, B	..	0·1 % ,, ,,
,, C	..	0·2 % ,, ,,
,, D	..	0·4 % ,, ,,
,, E	..	0·7 % ,, ,,
,, F	..	1·0 % ,, ,,
		1·5 % ,, ,,
		2·0 % ,, ,,

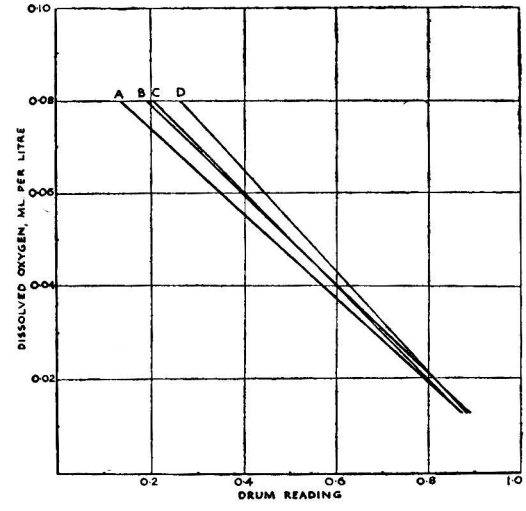

Fig. 3. Effect of different starch samples.

		Manufacturer	*Batch*
Curve A	..	a	1
		a	2
,, B	..	a	3
,, C	..	b	—
		c	—
,, D	..	d	—

Addition of starch—Throughout these investigations and in routine determinations care has been taken to use starch solutions prepared under standardised conditions, since the blue colour is usually considered to be due to a colloidal complex. However, wide differences in treatment with the same starch solution failed to produce any significant difference in the absorption of a series of blank solutions containing the same quantity of added iodine (see Table I).

The effect of using starch solution stored for various periods was also examined. The dissolved oxygen in pairs of water samples collected simultaneously was measured, using freshly prepared and stored starch solutions containing no preservative. The storage periods ranged from 1 to 8 days, but in no instance did the results with the stored starch solution differ from those with the fresh by more than 0·01 in drum reading, corresponding to 0·001 ml. of oxygen per litre—an insignificant difference. It was decided that for routine determinations the starch solution should be freshly prepared every week; since samples

* In this and all subsequent experiments the sample size was reduced to 50 ml., the volumes of reagents being reduced to correspond. The smaller sample is sufficient for the 4-cm. Spekker cell which, it was now apparent, would be used in the determination, and tubes suitable for collecting this small volume of water were constructed.

of feed waters are analysed on five days per week this allows a safety margin of at least three days.

TABLE I

EFFECT OF DIFFERENT METHODS OF ADDING STARCH SOLUTION

Method of addition of starch	Drum reading	Equivalent dissolved oxygen ml. per litre
Starch sample 1.		
A. Blown in rapidly from a pipette, then mixed by swirling	0·92, 0·90	0·009, 0·011
B. Run in from pipette with jet immersed, then mixed by swirling	0·91, 0·89	0·010, 0·012
C. Mixed by swirling during dropwise addition	0·89	0·012
Starch sample 2.		
A. Blown in rapidly from a pipette, then mixed by swirling	0·80	0·020
C. Mixed by swirling during dropwise addition	0·80	0·020

Quality of starch—In order to determine whether the position and slope of the calibration curve depends upon the batch of starch being used, six groups of three blank solutions, containing added iodine corresponding to 0·08, 0·05 and 0·03 ml. of oxygen per litre, were treated with starch solutions prepared from six different samples of soluble starch. The absorptions were measured and the linear parts of the calibration curves drawn (Fig. 3). Two batches of soluble starch over two years old, supplied by one manufacturer (*a*), gave a common curve *A* and a third batch from the same manufacturer, about one year old, gave the nearly parallel curve *B*. Using the difference method described on p. 348, the maximum error involved in reading from a single calibration curve for all three of these samples would be 0·002 ml. of oxygen per litre. Greater error will be probable if another manufacturer's soluble starch is used in conjunction with an existing calibration—see curves *C* and *D*, Fig. 3. It is strongly advised, therefore, that one bottle of soluble starch should be reserved for use in the dissolved oxygen determination and that when a fresh bottle is opened a new curve should be drawn.

Time of reaction—Bates, French and Rundle[16] studied the starch-iodide reaction by means of potentiometric titrations and observed that the activity of an iodine solution added to starch solution continues to decrease for several minutes. Talbot *et alia*[20] found that the colour was stable for at least 10 minutes but that it may fade after that time; on the other hand, Turner[12] claimed that there was no change during one hour. To a blank solution at 15° C. sufficient iodine, together with the appropriate amount of starch, was added to give a drum reading near the middle of the scale and the absorption was measured at known time intervals. The results obtained in duplicate experiments are shown in Fig. 4, from which it is seen that a constant reading is obtained between 2 and 5 minutes after mixing; for routine analyses a period of colour development of 5 minutes was selected.

Temperature effects—It has long been known that the colour of the starch-iodide complex is destroyed on warming the solution; Turner[12] reports that the fading is only slight at 60° C. and the colour disappears at about 70° C.; Talbot *et alia*[20] state that fading is perceptible at about 17° C. The effect of temperature under the conditions obtaining in the present method of determination was examined by adding to a number of blank solutions equal amounts of iodine, to give, on the addition of starch at 15° C., a drum reading near the centre of the scale. The temperatures of these solutions were adjusted to cover the range 5° to 50° C. before the starch was added; the absorptions were measured at the appropriate temperatures 5 minutes after the starch additions. (Sendroy[21] has stated that both the temperatures of mixing and of colour measurement should be controlled.) The results are shown in Fig. 5: since the blank solution without added iodine gave a drum reading 1·00 (at 15° C.) it will be appreciated that at 50° C. the starch-iodide complex had become almost colourless. It is possible that the above-mentioned differences between the various observers is due to an effect noticed by Pickering,[26] *viz.*, the temperature at which the colour disappears is dependent upon its intensity at ordinary temperatures. This feature was not examined experimentally because in the present method the range of iodine concentrations is small and the single set of observations near its centre should suffice. For routine determinations a temperature of 15° C. was selected as being both convenient and satisfactory.

Quantity of reagents—That the results obtained by the present method are not affected appreciably by possible inaccuracies in the amounts of alkaline iodide reagent, manganous

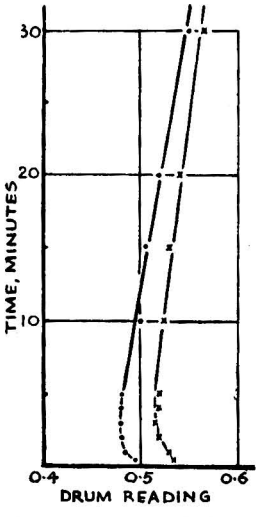

Fig. 4. Effect of time of colour development.

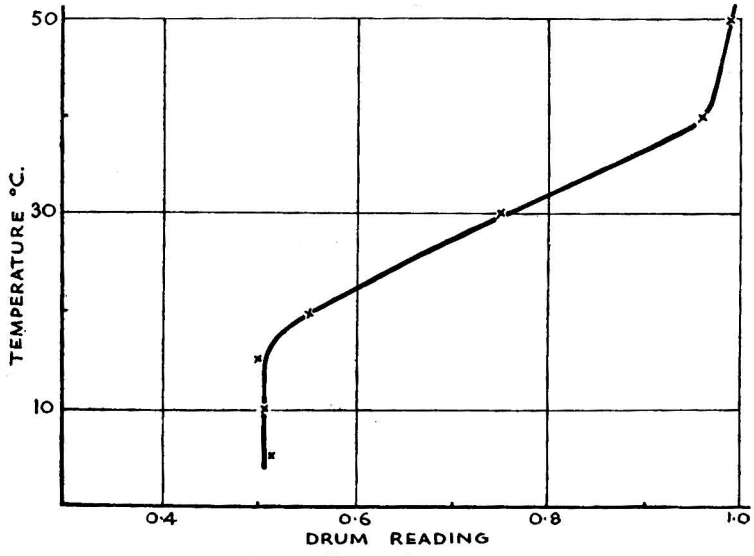

Fig. 5. Effect of temperature.

sulphate or sulphuric acid added was proved by halving and doubling the normal quantities. This test was made with blank solutions containing added iodine at four concentrations and the results are given in Table II.

TABLE II

EFFECT OF REAGENT CONCENTRATIONS

Iodine added equiv. to dissolved oxygen, ml. per litre	Drum readings for reagent concentration: × 0·5	Normal	× 2
0·01	0·93	0·93	0·91
0·03	0·70	0·71	0·69
0·06	0·35	0·36	0·35
0·08	0·12	0·13	0·12

The effect of variations likely to occur during routine determinations is obviously negligible. The reproducibility of the method was examined by taking several groups of three samples from one source at the same time and measuring the absorptions after development of the starch-iodide complex as described on p. 348. Typical results are given in Table III.

TABLE III

REPRODUCIBILITY OF RESULTS

Sample No.	Drum reading	Equivalent dissolved oxygen, ml. per litre
11 A, B, C	0·75, 0·73, 0·74	0·024, 0·026, 0·025
12 A, B, C	0·84, 0·82, 0·82	0·016, 0·018, 0·018
13 A, B, C	0·79, 0·77, 0·78	0·021, 0·023, 0·022

Calibration—A calibration curve for routine use was constructed by measuring the absorptions of a series of blank solutions (50 ml. each) to which were added 1 ml. portions of iodine solutions of concentrations to correspond to approximately 0·01, 0·02, 0·03, 0·06, 0·08 and 0·09 ml. of oxygen per litre when calculated on 50 ml., and 1 ml. of 1 per cent. starch solution. In this way solutions were obtained each having a total volume of 52 ml., *i.e.*, the same as in the routine method (see p. 348). The various iodine solutions were prepared by diluting 0·1 *N* iodine and their strengths were determined accurately by titration

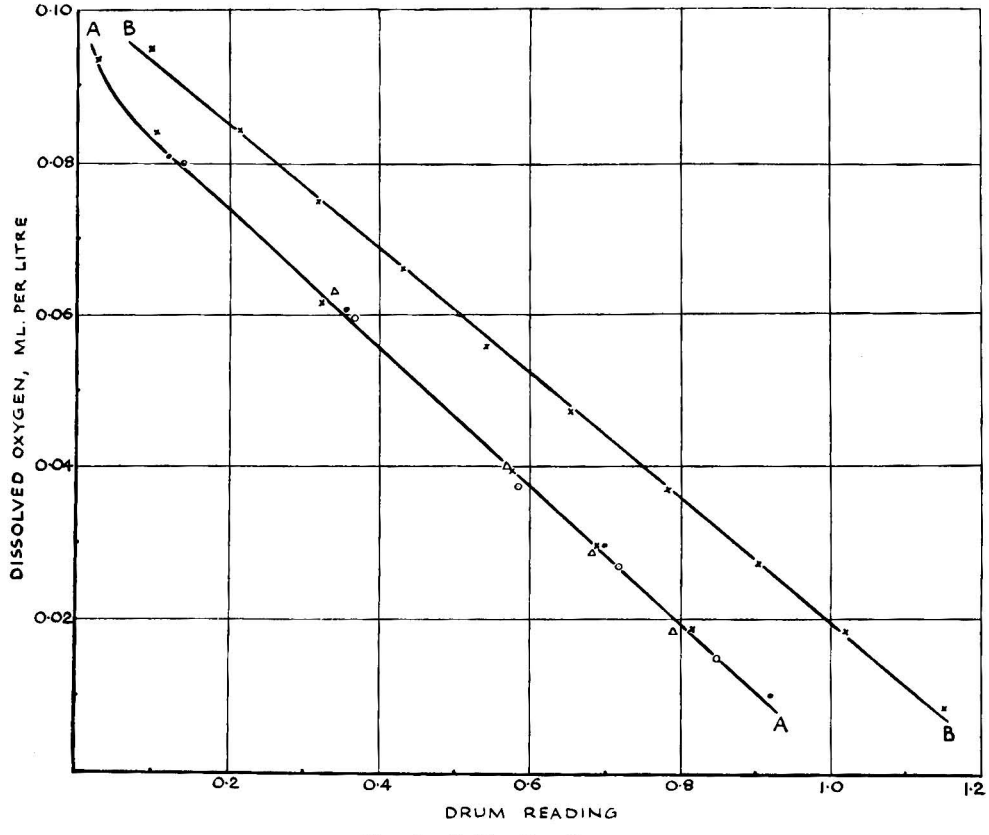

Fig. 6. Calibration Curves.

AA — Tungsten filament lamp. BB — Mercury vapour lamp.

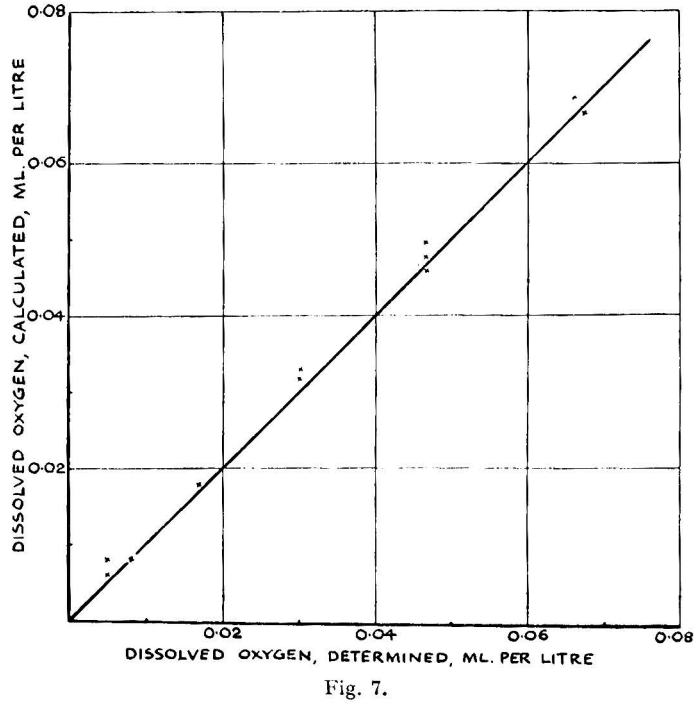

Fig. 7.

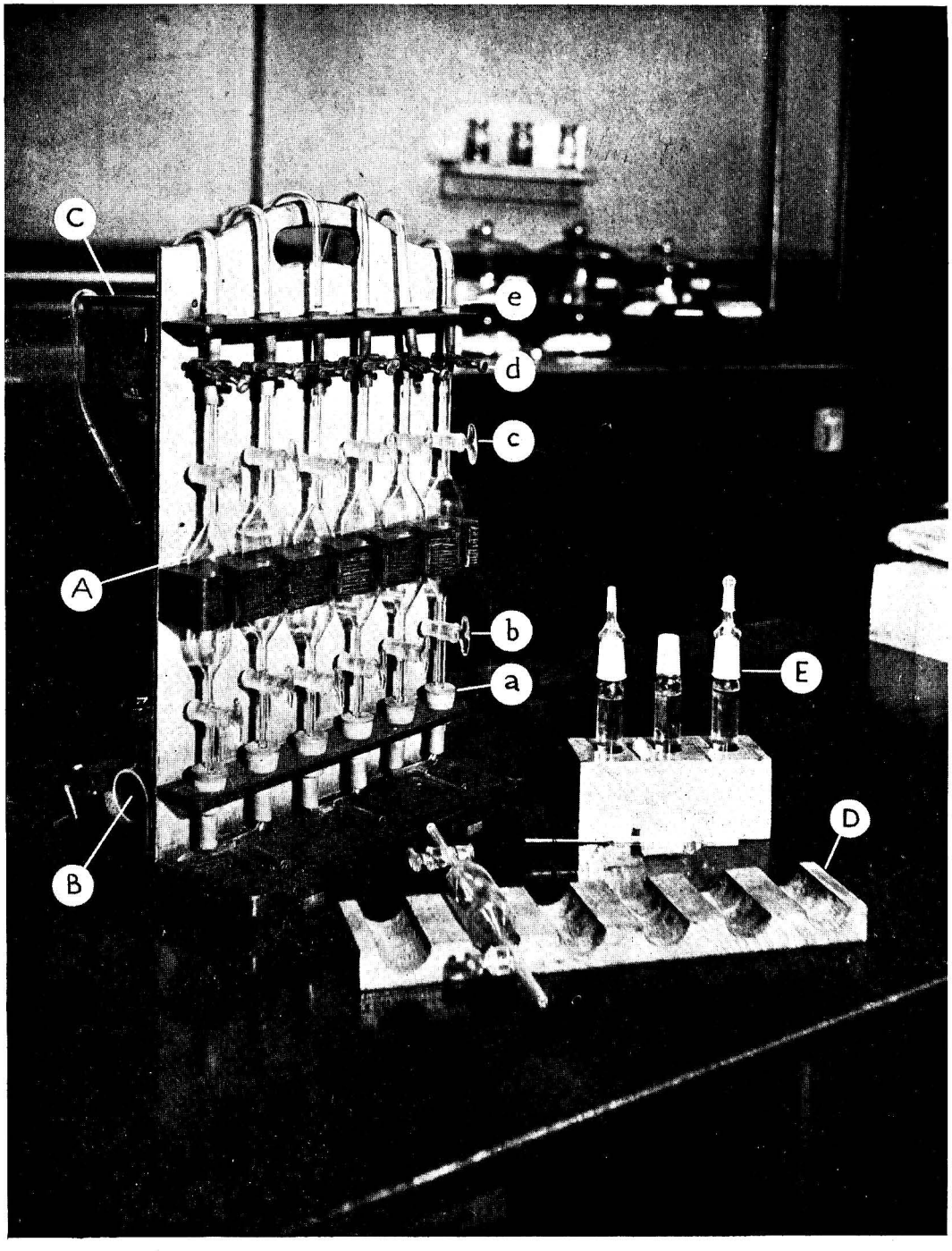

Fig. 8. Sampling Apparatus.

with 0·01 *N* sodium thiosulphate. The colours were developed for 5 minutes at 15° C. and the absorptions measured at the same temperature. The curve AA of Fig. 6 is drawn through four series of values obtained in this manner; it is linear over most of the range, but becomes curved when the oxygen concentration exceeds about 0·08 ml. per litre. For each of the above series of iodine concentrations six sample tubes were connected in series and filled with feed water, thus ensuring that each contained the same concentration of interfering substances. If a considerable period of time elapses between the collection of these groups of samples, changes in the interfering substances may occur and different amounts of iodine may be absorbed or liberated. For example, in one of the sets of determinations plotted on curve AA the absorptiometer drum readings corresponded originally to dissolved oxygen contents uniformly 0·003 ml. per litre greater than those shown. The adjustment made to this series is justified because the procedure for routine determinations, described on p. 348, measures the effect due to interfering substances and corrects the apparent oxygen content of the water accordingly; it is, therefore, a difference method and the slope of the calibration curve, and not its true position, is important. It is obvious that because of this fact only the linear portion of the curve should be used; it is also apparent that the determination of the effect of interfering substances is essential if accurate results are to be obtained. The linear part of the calibration curve may be extended by using a mercury vapour lamp instead of the tungsten filament type: the straight line BB of Fig. 6 was obtained in this manner, using heat filters H503 and spectrum yellow filters H606 in conjunction with a 4-cm. cell.

The following experiment shows that the blank solution used for constructing a calibration curve must be freshly prepared. A blank solution was prepared in the usual manner by collecting feed water under oxygen-free conditions and adding the Winkler reagents in the order: alkaline iodide, sulphuric acid and manganous sulphate. To 50 ml. aliquots of this solution were added 1-ml. portions of starch solution after intervals of 0, 1 and 2 hours; the absorptiometer drum readings obtained were 0·96, 0·94 and 0·90 respectively, showing that there is a slow, but progressive, liberation of iodine.

Interfering substances—When determining dissolved oxygen it is not practicable to eliminate the effects of interfering substances as was done by Turner,[12] who used water freshly distilled from caustic potash. Also the procedure of Sendroy[21] is inapplicable: he found that there were slight changes in the position of the calibration curve when different supplies of distilled water were used, but he did not ascertain the cause. Each time an analysis was made Sendroy fixed the position of the calibration curve by the determination of two points on it. Both of these authors measured the depth of the blue colour formed by iodine that had been isolated by distillation. This difficulty is avoided by taking two samples of the feed water simultaneously and treating them as follows. To the first sample (called the *oxygen sample*) add the Winkler reagents in the usual manner, followed by a known volume of iodine solution.* Under these conditions both the dissolved oxygen and the interfering substances react. To the second sample (called the *interference sample*) add the reagents in the order alkaline iodide, sulphuric acid and manganous sulphate, and then add the same volume of iodine solution as was added to the oxygen sample. Under these conditions manganous hydroxide is not formed and so dissolved oxygen cannot react, but the interfering substances take effect. The true dissolved oxygen content of the water may, therefore, be found from the difference between the iodine concentrations determined in the two samples. This statement should be qualified in that no allowance is made for the oxygen which is dissolved in the reagents and which is active only in the oxygen sample; it can be calculated that with the volumes of reagents and sample used in the present method this interference corresponds to approximately 0·005 ml. per litre, a figure which should be deducted from the experimental results. Sillars and Silver[27] determine the extent of this interference by the use of an extra sample in every analysis. One sample is treated as usual and to the other the Winkler reagents are added in double the normal amounts; the oxygen derived from the reagents is then found by the difference between the results. Sebald[4] uses equal amounts of the same reagents in both samples, but one of the latter has double the volume of the other.

* Schwartz and Gurney[29] appear to have been the first to add iodine to enable a correction to be made for the presence of interfering substances; these authors used potassium bi-iodate, which reacts with the iodide present in the sample. However, they made the addition only when no blue colour was developed in the normal procedure.

Finally, the method was tested by addition of known quantities of fully aerated water to samples of feed water and comparison of the calculated increases in dissolved oxygen content with the experimental values. The oxygen content of the aerated water was determined by the Winkler titration method and the desired volume of it was added to one of a pair of samples of feed water that had been collected simultaneously. The results are shown graphically in Fig. 7, which also shows the theoretical line. The maximum difference between the pairs of values is 0·003 ml. per litre, which is regarded as satisfactory since it includes all errors of sampling, manipulation and calibration.

The method which follows covers the range 0 to approximately 0·08 ml. of oxygen per litre when allowance is made for the iodine added to the interference sample. This is sufficient for the majority of determinations required in the control of feed waters and condensates for high pressure boilers, but occasionally it is necessary to determine higher concentrations. Experiments have shown that the sample may be treated with the Winkler reagents and diluted to twice the original volume either immediately before or after the addition of the starch solution and that the approximate results so obtained are of sufficient accuracy for usual requirements. The calibration curve for a mercury vapour lamp is, however, linear (BB, Fig. 6) and may be used in conjunction with 1-cm. or 0·5-cm. cells to cover the ranges 0 to 0·4 and 0 to 0·8 ml. of oxygen per litre respectively, but it is very improbable that the higher range would be necessary.

Method

Sampling apparatus—

The apparatus officially recommended[28] for the Winkler method has a capacity of 500 ml. The present authors previously used 250-ml. samples for this titration. The 4-cm. Spekker cell, however, has a capacity of about 30 ml. and it is therefore unnecessary to collect such large samples for the present method. This is an important consideration when an interference sample has also to be taken from each source and all waters for routine testing have to be carried to the laboratory. The equipment shown in Fig. 8 was therefore designed; it can accommodate six tubes *A* of capacity 55 ml. for three dissolved oxygen and interference samples, three Spekker flow-through cells *B* for *p*H measurements, and three 100-ml. bottles for conductivity samples (these are obscured in the photograph). The sample tubes for the oxygen determination resemble those described by Sebald[4] but are much smaller. Two graduations are etched on each of the narrow stems beyond the taps, the capacity between these marks being 0·15 ml., *i.e.*, the volume of reagents to be added to the contents. The lower graduation is situated a few mm. above the tap to leave a safety margin so that air is not admitted with the reagents. The lower ends of each pair of sample tubes are joined within rubber bungs *a*, held in the wooden frame, to a T-piece through which they are connected with the source to be sampled: a cooling coil should be used. The upper ends of the sample tubes are connected through rubber tubing carrying screw-clips *d* to outlet tubes, which are secured by rubber bungs *e* fixed in the wooden frame and which discharge into a metal tank *C*. The dimensions of the tank are 12 × 3 × 3 in., and it will accommodate water equivalent to the ten volume changes which it is necessary to run through the sample tubes in order to ensure thorough washing.

Collecting the sample—With clips *d* and taps *b* and *c* open, run the water through the sample tubes until tank *C* is almost filled. Reduce the rate of flow, close tap *b* and then screw-clip *d* on the tube that is to be used for the oxygen sample; all the water now passes through the tube to be used for the interference sample, on which tap *b* and screw-clip *d* are closed immediately after cutting off the water supply. Taps *c* are left open until the determination is made; contraction in volume due to fall in temperature will not then cause ingress of air because volume changes are accommodated by the flexibility of the rubber tubing. If cooling of the water below the prevailing temperature can be arranged, both taps *b* and *c* may be closed and the extra complication of the rubber tubing and screw-clips *d* may be avoided. The rubber tubing has not caused contamination of the samples and consequent erroneous results.

Reagents—

Alkaline iodide solution—Measure 250 ml. of distilled water: in part dissolve 35 g. of potassium iodide (AnalaR); in the remainder dissolve 150 g. of sodium hydroxide (AnalaR, stick). Mix the two solutions.

Manganous sulphate solution—Dissolve 113 g. of $MnSO_4.4H_2O$ (AnalaR) in 250 ml. of distilled water.

Diluted sulphuric acid (1 + 1).

Iodine solution—Approximately 0·0002 *N*: prepared by dilution of 0·1 *N* solution.

Starch solution—To 90 ml. of boiling distilled water add from a test tube a suspension of 1 g. of soluble starch (AnalaR) in 5 ml. of water; rinse the test tube with 5 ml. of water. Boil the solution for precisely one minute, cool and make up to 100 ml. with water. This solution must not be used when more than a week old.

PROCEDURE—

Close taps *c* and remove the rubber tubing and screw-clips *d* from the sample tubes; withdraw the latter from the bungs *a* and place on the wooden block *D*, which forms a safe and convenient stand for the tubes between the steps of the following procedure. The sample tubes do not carry distinguishing marks, but no confusion arises if they are kept in the same order on the sampling stand and on block *D*, *viz.*:

Sample 1		Sample 2		Sample 3	
Oxygen tube	Interference tube	Oxygen tube	Interference tube	Oxygen tube	Interference tube

Treat each sample tube in rotation as follows. Remove water remaining in the stem *d* by means of a glass jet connected to a water pump and fill stem *d* with alkaline iodide reagent from the appropriate dropper *E*, the container for which is kept filled with solution so that the dropper fills without application of suction. Withdraw excess reagent by means of the glass jet until the meniscus is at the outer of the two graduations. Hold the tube vertically with end *d* uppermost, open tap *c* and then cautiously open tap *b* until the reagent meniscus falls to the lower graduation; close taps *b* and *c* in that order (0·15 ml. of reagent has then been added to the sample). By means of a second jet rinse out reagent remaining in stem *d*. Mix the contents of the sample tube by rocking movements and replace it on block *D* in the reverse position from that which it formerly occupied. Add subsequent reagents in a similar manner, but use stem *a* to avoid mixing with alkaline iodide solution remaining in the bore of tap *c*. The oxygen samples are treated with manganous sulphate solution* followed by sulphuric acid: the same reagents are added in the inverse order to the interference samples. Both oxygen and interference samples may be exposed to atmospheric oxygen without affecting the results after complete solution of the manganese hydroxide precipitate has taken place; both are treated as follows. Hold the sample tube vertically with stem *a* uppermost; open the upper tap and rinse out the lower stem with a few ml. of the contents by opening the lower tap. Measure 50 ml. of the solution in a graduated flask and transfer to a conical flask, cool to 15° C. and add 1 ml. of 0·0002 *N* iodine and 1 ml. of starch solution. After leaving for 5 minutes for the blue colour to develop, measure the absorptions, using 4-cm. cells with heat filters H503 and spectrum yellow filters H606 in conjunction with a mercury vapour lamp; drum setting, water - water, 1·20. From the calibration curve read the oxygen equivalents of the absorptions of the oxygen and interference samples and from the difference between these values deduct 0·005 ml. per litre to correct for the oxygen dissolved in the reagents; the result is the true dissolved oxygen content of the water. When the samples have been collected, the determination of the dissolved oxygen in three sources of water occupies about 30 minutes.

SUMMARY

Existing methods for the determination of oxygen dissolved in feed waters and condensates are critically reviewed and a more convenient, yet accurate, procedure is proposed. The new method depends upon the measurement of the intensity of the blue starch-iodide colour by means of the Spekker photo-electric absorptiometer. A 55-ml. sample suffices for the determination and apparatus has been devised for its collection.

The authors thank the London, Midland and Scottish Railway Company for permission to publish these investigations.

* The oxidation of manganous hydroxide is comparatively slow, but the reaction is completed if the addition of the sulphuric acid is made about 3 minutes after that of the manganous sulphate.

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L. M. & S. RAILWAY CO.
RESEARCH DEPARTMENT
STONEBRIDGE PARK

March, 1947

The Determination of Nitrates in Water

BY F. W. ALLERTON

THE various methods available for the determination of nitrates in water fall into three groups: (*a*) nitrometer methods; (*b*) direct colorimetric estimations; (*c*) reduction methods with subsequent colorimetric estimation.

Of the first group, Crum's nitrometer method is the only one of importance and it has long been superseded by safer and more rapid methods. The second group includes the phenol-sulphonic acid and indigo-carmine methods. The former, depending upon the production of picric acid and subsequent colorimetric estimation, is by no means rapid and is influenced by any considerable amount of chlorides present in the water; furthermore, the evaporation of the sample to dryness may involve losses of nitrogen. The indigo-carmine method based upon the oxidation of the dye to a colourless compound is largely used, but is stated by Sutton[1] to give approximate results only. Both of these colorimetric methods are criticised by Thresh[2], who states that reduction methods yield the most satisfactory results, especially when the reduced nitrogen is separated by distillation prior to nesslerisation.

Thresh reduces the nitrates in faintly acid solution for one hour with zinc-copper couple and subsequently distils. Free ammonia is first removed from the sodium hydroxide or carbonate employed by boiling, and the zinc-copper couple is carefully excluded from the distillation flask, presumably to prevent reduction of any nitrates contained in the alkali.

I have found this method to give satisfactory results, but the preparation of the zinc-copper couple and the reduction period needed detract from the method as a routine laboratory process.

In an attempt to overcome these disadvantages the possibility of using Devarda alloy was investigated. This alloy has been used for a number of years for the routine estimation

of nitrates in manures, and Donald[3] has made a thorough investigation of the best conditions for dealing with quantities of sodium nitrate of the order of 1 g. There appears, however, to have been no adaptation of this method to quantities of nitrogen of the order of 0·0001 g., such as are found in convenient quantities of most natural waters.

Apparatus—For this purpose, the following simple apparatus was used. A 250-ml. flask with ground-glass delivery was connected to a vertical water condenser, the whole being assembled so that a 100-ml. Nessler glass could be adjusted as required beneath the delivery of the condenser.

Experimental—As a preliminary trial, half the quantities of ammonia-free water, A.R. sodium hydroxide and Devarda alloy recommended by Donald were used and nitrate equivalent to 0·00005 g. of nitrogen was taken for estimation. The standard nitrate solution was prepared from A.R. potassium nitrate and contained 0·00001 g. of nitric nitrogen per ml. Despite careful heating, frothing was so marked that a satisfactory distillation was impossible. Consequently, the effect of smaller amounts of alloy and alkali was investigated.

With 0·5 g. of alloy and an amount of sodium hydroxide necessary to give a concentration of 0·05 *N* there was still considerable frothing, but careful heating enabled a normal distillation to be carried out. By nesslerising an aliquot portion it was found that 0·00007 g. of nitrogen was present in the distillate. There was, therefore, despite the use of A.R. sodium hydroxide, a very large error due to ammonia or nitrates in the reagents.

In order to locate the source of the ammonia, a 5 per cent. sodium hydroxide solution was prepared from carefully cleaned sodium metal and ammonia-free water. When this was used the distillate contained 0·000062 g. of nitrogen, which, since the caustic soda could be assumed nitrogen-free, pointed to the presence of nitrogen in the Devarda alloy. A blank test confirmed this conclusion.

A quantity of the alloy was therefore boiled with three successive portions of 100 ml. of distilled water and then carefully washed on a filter with ammonia-free water. An estimation with 0·00005 g. nitrogen and the washed and dried alloy (0·5 g.) gave 0·0000529 g. of nitrogen.

It seemed probable that the error could be still further decreased and the frothing diminished by using smaller quantities of alloy and alkali.

At this point, the following technique was adopted—

The required number of ml. of standard nitrate solution were pipetted into the 250-ml. flask, and a known volume of the 5 per cent. sodium hydroxide solution was added. Amounts of Devarda alloy ranging from 0·20 g. to 0·5 g. were then added and the volume was made up to 120 ml. with ammonia-free water. The distillation was regulated so that about 15 minutes elapsed before the flask boiled, in order to prolong the reduction period. Fifty ml. of distillate were now rapidly collected. Since at the beginning of heating an appreciable volume of hydrogen was evolved, which, it was thought, might carry over ammonia gas, the end of the condenser was made to dip into 2 ml. of ammonia-free water in the Nessler cylinder. After a few minutes the cylinder was lowered into the normal position.

Tables I and II illustrate the results of using various amounts of nitrogen as nitrates with different concentrations of alkali and weights of Devarda alloy.

TABLE I

Devarda alloy, 0·25 g.; water 120 ml.

Nitric nitrogen taken g.	Nitrogen found, with 0·1 *N* NaOH g.	Nitrogen found, with 0·05 *N* NaOH g.	Volume distilled ml.
0·00005	0·0000501	0·0000491	50
	0·0000502	0·0000494	
0·00010	0·0000992	0·0000982	50
	0·0000996	0·0000980	
0·00020	0·0002036	—	50
	0·0002020		
0·00050	0·000498	0·000489	100
0·00100	0·000963	0·000976	100

It will be seen from Tables I and II that—

(1) 0·05 *N* sodium hydroxide is inclined to give incomplete reduction, especially of the larger amounts of nitrates; and

(2) there is no advantage in using 0·5 g. of Devarda alloy for less than 0·00020 g. of nitrogen.

With smaller quantities of nitrogen, where a larger proportion of the distillate is nesslerised, 0·5 g. of alloy appears to give slightly high results owing to remaining traces of ammonia or nitrates as impurity.

TABLE II

Devarda alloy, 0·50 g.; water, 120 ml.

Nitric nitrogen taken g.	Nitrogen found, with 0·1 *N* NaOH g.	Nitrogen found, with 0·05 *N* NaOH g.	Volume distilled ml.
0·00005	0·0000502	0·0000496	50
0·00010	0·000101	0·0000988	50
0·00020	0·000206	0·000196	50
0·00050	0·000500	0·000494	100
0·00100	0·00105	0·000975	100

With quantities of nitrogen from 0·00020 g. to 0·0010 g. the ammonia was not completely distilled into 50 ml. and a further 50 ml. was necessary. It is apparent that a balance must be struck between excess ammonia due to impurities in the reagents and incomplete reduction of the nitrates to ammonia.

With quantities of nitrogen above 0·00020 g. a small proportion only of the distillate is nesslerised and hence the influence of reagent impurities is negligible with reagents prepared as stated. Rather higher concentrations of alkali might be used to ensure complete reduction in a shorter time, but I have found that the advantages so gained are more than offset by increased frothing.

In order to test the method without the bias possible from the use of known quantities of nitrogen, an assistant was instructed to add an amount of the standard solution between 5 ml. and 20 ml. to a flask containing 0·25 g. of Devarda alloy and 120 ml. of 0·1 *N* sodium hydroxide. The amount found was 9·3 ml. instead of 9·2 ml. added—an error of little more than 1 per cent.

SUMMARY

The method described is claimed to be rapid and accurate providing that the reagents are especially freed from nitrates and ammonia. The most suitable quantities appear to be: nitrate nitrogen 0·000050 to 0·00020 g., Devarda's alloy 0·25 g. and sodium hydroxide concentration 0·1 *N*.

Thus, in 10 ml. of waters containing from 0·5 to 2·0 parts of nitrate nitrogen per 100,000, the amount can be estimated at least within the accepted accuracy of the Nessler method.

Note—It is of course assumed that an allowance is made for free ammonia and nitrites in waters abnormally high in these substances.

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The Analysis of Nickel - Cobalt - Iron Alloys Used in Glass-to-Metal Seals*

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INTRODUCTION—

THE methods for the construction of many modern thermionic devices involve the use of a glass-to-metal seal. Nickel - iron alloys have been used for this purpose for many years where the use of "soft" glasses was permissible. In many of the modern applications it is necessary to use low-expansion glasses and, for sealing to these glasses, the metals molybdenum and tungsten have been used. Apart from the cost, which is high, there are other features of the use of these two metals which do not make them wholly desirable and new low-expansion alloys have been developed for the purpose. These new alloys contain nickel, iron and

* Communication from the Staff of the Research Laboratories of The General Electric Company Limited, England.

cobalt as their main constituents and have become well known in the U.S.A. under the commercial names "Kovar" and "Fernico."

The need for these materials greatly increased during the war and their manufacture and their use in this country have already reached considerable proportions. It is essential for the manufacture of good seals that the physical properties of the alloy, more especially the expansion characteristics, shall be closely controlled and, although the only completely satisfactory check is by making a test seal, the alloy manufacturer and the user have both been concerned to set up some specification of chemical composition. Specifications were drawn up in the U.S.A. and in this country, but there was some suggestion, certainly in America and possibly here also, that the tolerances allowed were not as great as the available analytical methods required. Indeed, in these laboratories for some time it was considered inadvisable to make use of the analytical figures obtained, as the extent of their error was unknown.

This paper discusses the analytical problem, makes some reference to the experimental difficulties and goes on to describe the methods that have been found satisfactory for the accurate analysis of this type of alloy.

The analytical problem—

The specifications for the American and the British alloy compositions are given in Table I, and it will be seen that in the British specification the "nickel plus cobalt" content is specified with a tolerance of ± 0·5 per cent. and the nickel alone with the same tolerance. In the American specification the nickel, iron and cobalt are all given as ranges, the extremes of which add up to from 98·9 to 100·4.

The form in which these figures are presented suggests that the analytical methods available do not give results of high precision.

Table I

	American	British
Nickel	28·7–29·2 per cent.	29 ± 0·5 per cent.
Cobalt	17·3–17·8 »	17 »
Nickel + cobalt	—	46 ± 0·5 »
Iron	52·9–53·4 »	—
Manganese	0·50 max. »	0·3 »
Silicon	0·20 max. »	0·15 »
Carbon	0·06 max. »	0·05 max. »
Sulphur	—	0·01 max. »
Phosphorus	—	0·01 max. »

With iron, nickel and cobalt all present in substantial proportions, the analytical problem may be stated thus:—

(1) Nickel and cobalt are not quantitatively separated by dimethylglyoxime or α-nitroso-β-naphthol, without the use of special methods.

(2) A clean separation of iron from nickel and cobalt is not achieved by the usual precipitants such as ammonia or zinc oxide, and a new problem is introduced in that the presence of iron further complicates the separation of nickel from cobalt.

Attention was therefore initially directed to recently published work on analogous problems.

Experimental

(i) Using synthetic solutions containing iron, nickel and cobalt to simulate the composition of the alloys, the scheme outlined by B. S. Evans[1] was investigated. This depends on the precipitation of nickel and cobalt as cobalticyanides to separate these metals from iron, and the subsequent determination by cyanide titration of nickel, and of nickel plus cobalt. The cobalt is thus obtained as the difference between two determinations.

Various difficulties were encountered, but the principal objection to the method, and the one that led to its ultimate rejection, was the nature of the cobalticyanide precipitate, which was always difficult and often impossible to filter. It was clear that the method was not directly applicable to alloys of the particular composition with which we were concerned.

(ii) The next experiments were concerned with adapting the methods of W. R. Schoeller[2] to our particular problem. Schoeller's scheme involves the separation of iron as ferric phosphate in acetic acid solution; subsequent precipitation of the cobalt, together with

some of the nickel, as cobalt ammonium phosphate; and determination by cyanide titration of the nickel in the main portion and also of the nickel co-precipitated with the cobalt. Here again the cobalt is obtained as the difference between two determinations.

Applying these methods to synthetic solutions whose composition simulated the alloys it was not found possible to obtain clean separations, even with reprecipitations, and as was also the case with Evans's method, it was impossible to achieve any sort of precision, let alone accuracy, for the determinations. Thus results obtained for nickel in a number of experiments varied from 29 to 31 per cent. and for cobalt from 14 to 19 per cent.

(iii) DETERMINATION OF NICKEL—

In view of these difficulties, attention was redirected to the determination of nickel with dimethylglyoxime in presence of iron and cobalt.

Although bivalent cobalt forms complexes with dimethylglyoxime, these are soluble and nickel may be satisfactorily determined in presence of cobalt alone provided that sufficient dimethylglyoxime is added to combine with both metals. An alternative method is to oxidise the cobalt with hydrogen peroxide in ammoniacal solution, destroy the excess hydrogen peroxide by boiling and precipitate the nickel with dimethylglyoxime, with which the oxidised cobalt does not combine. Nickel can also be determined in presence of iron, using tartrate or citrate to prevent precipitation of iron in ammoniacal solution. The ease with which separation of either of these binary mixtures may be made will depend, for any particular mixture, on the relative proportions of the constituents. When nickel is present with both cobalt and iron a new problem arises and the relative proportions of the three metals as they occur in the Kovar type of alloy appear to raise the problem in a very acute form. Thus, when an attempt is made to precipitate nickel with dimethylglyoxime from such a mixture in the usual manner, brown slimy precipitates difficult to filter are produced, the nickel compound being grossly contaminated with both cobalt and iron. The difficulty cannot be overcome by oxidation of the cobalt as mentioned above, because this method is not effective in presence of tartrate.

Examination of the contaminated precipitates by X-ray diffraction methods showed that a distinct second phase was present in addition to the nickel dimethylglyoxime complex. A search of the literature indicated that the contaminant might be the compound reported by J. G. Weeldenburg,[3] and said to contain one atom of iron, one of cobalt and three molecules of dimethylglyoxime. The formation of this compound will be discussed later in the paper.

Methods for preventing the co-precipitation of iron and cobalt were suggested as long ago as 1913 by O. Brunck.[4,5] These methods were rediscovered 24 years later by G. Balz,[6] as pointed out by H. Diehl.[7] It was claimed that the iron - cobalt - dimethylglyoxime compound was formed only by ferric iron, and that the formation of this compound could be prevented by reduction of iron to the ferrous state. The next experiments were therefore directed along these lines.

We were able to confirm Balz's findings that the separation is improved by (1) preliminary reduction of the iron with sodium sulphite, (2) neutralisation with sodium acetate instead of ammonia after addition of dimethylglyoxime to the acid solution, (3) precipitation at 70° C. instead of at the boiling point and (4) shorter times of standing.

The following method, based on these observations, affords a clean separation in a single precipitation. Examination by X-ray diffraction technique of precipitates obtained by this method indicated that the nickel dimethylglyoxime complex was the only phase present; chemical tests by colorimetric methods confirmed the absence of cobalt and iron.

METHOD FOR THE DETERMINATION OF NICKEL

Weigh 0·2 g. of the sample into a 400-ml. beaker, add 10 ml. of diluted nitric acid (1 + 1) and warm until it is dissolved.

Add 20 ml. of diluted sulphuric acid (1 + 1), evaporate to fuming to remove the nitric acid and fume for 5 minutes. Cool, add 75 ml. of water and heat to boiling to dissolve the salts. Filter, if necessary, through a No. 40 Whatman filter paper and wash with hot water. Cool somewhat, add diluted ammonia solution (2 + 1) until a slight permanent precipitate is formed and then clear with diluted sulphuric acid (1 + 6) followed by 20 drops in excess. Add 2·0 g. of sodium sulphite and boil for a few minutes to reduce $Fe^{'''}$ to $Fe^{''}$. Cool, add 2·0 g. of tartaric acid, neutralise with ammonia and re-acidify with diluted sulphuric acid (1 + 6) adding 20 drops in excess.

Dilute to 250 ml., adjust the temperature to 70° C. and precipitate the nickel with 75 ml. of 1 per cent. alcoholic dimethylglyoxime, followed by 4 g. of sodium acetate crystals dissolved in a small volume of water, to neutralise the mineral acid and ensure complete precipitation of the nickel complex. Stir vigorously, and then allow to stand for 15 minutes on the bench.

Filter the precipitate through a weighed No. 3 grade sintered glass crucible and wash thoroughly with warm water. Suck as dry as possible, dry at 110° C. for 1 hour and re-weigh.

Table II shows results for nickel obtained on synthetic solutions made to correspond in composition to the alloys.

TABLE II

DETERMINATION OF NICKEL IN PRESENCE OF COBALT AND IRON

Iron present mg.	Cobalt present mg.	Nickel taken mg.	Nickel found mg.
106	34	59·88	59·80
"	"	59·88	60·04
"	"	59·88	59·94
"	"	59·88	59·72

(iv) DETERMINATION OF COBALT—

For the determination of cobalt in presence of iron and nickel the method of Dickens and Maassen[8] appeared to be promising. This consists in an electrometric titration of the cobalt in ammoniacal solution with potassium ferricyanide, which oxidises cobalt to the tervalent state; citrate is added to prevent precipitation of iron. The only common element that causes interference is manganese, which is present in these alloys as a minor constituent. In these circumstances the authors recommend addition of excess ferricyanide and back-titration with standard cobalt solution; by this method the sum of manganese plus cobalt is determined and the manganese is subsequently determined by another method. Tomicek and Frieberger[9] recommend the removal of manganese prior to titration of the cobalt, and Tomicek and Kalny[10] indicate that manganese can be oxidised to the tervalent or to the quadrivalent state, according to the conditions.

In absence of manganese the method of Dickens and Maassen worked admirably, but in presence of even small amounts of manganese the end-points were poor and considerable loss in accuracy resulted. It was therefore decided to remove manganese prior to titration and for this purpose the precipitation of manganese dioxide with nitric acid and potassium chlorate proved effective. With this method precipitation may fail for very small amounts of manganese (of the order of 1 mg.) but the method has always been found to work satisfactorily for quantities likely to cause significant errors in the cobalt determination. For the final titration of the cobalt a back-titration technique has been adopted, as better end-points are obtained in this way.

METHOD FOR THE DETERMINATION OF COBALT

Apparatus—For the electrometric titration the electrodes are (1) platinum electrode and (2) normal calomel electrode, which are connected to a galvanometer through the sliding contact of a potentiometer. The potentiometer is connected to a 1·5-volt cell in an external circuit and this arrangement permits rapid adjustment of the galvanometer by means of the potentiometer. A Tinsley portable galvanometer, full scale deflection ≡ 4 micro amps., was found to be of adequate sensitivity. A mechanical stirrer is used during the titration.

The circuit diagram is as shown in the figure (p. 355).

Reagents—(1) Cobalt nitrate solution: 10 g. of $Co(NO_3)_2.6H_2O$ per litre (1 ml. ≡ approx. 0·002 g. Co). Standardise the cobalt content by precipitating with α-nitroso-β-naphthol, ignite to oxide and reduce to metal in a stream of dry oxygen-free hydrogen.

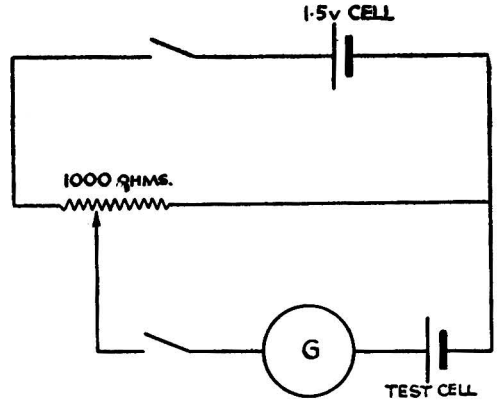
(2) Potassium ferricyanide solution: 11·17 g. of $K_3Fe(CN)_6$ per litre. Standardise against the cobalt solution and adjust to equivalency. Store in a dark bottle and re-standardise periodically.

(3) Ammonium citrate solution: 300 g. per litre.

Standardisation of ferricyanide solution—Introduce 100 ml. of ammonium citrate solution and 50 ml. of "880" ammonia into a 600-ml. beaker and dilute to 300 ml. with water. The solution should be cool (about 15° C.). Add 25 ml. of the ferricyanide solution, immerse the electrodes and commence stirring. Adjust the galvanometer to zero and titrate the solution slowly with the cobalt nitrate solution. As the titration proceeds, the galvanometer spot

light moves slowly across the scale and it will be necessary to re-adjust to zero to continue observation of the spot light. Continue the titration until one drop produces an observable deflection of the galvanometer. Continue the titration dropwise, observing visually the deflection produced for each drop added. The deflection per drop increases to a sharp maximum at the end-point, beyond which a sudden fall in the deflection per drop is observed. During the dropwise part of the titration it may again be necessary to adjust the galvanometer to zero.

Procedure—Dissolve 0·2 g. of the sample in 10 ml. of diluted nitric acid (1 + 1) in a 250-ml. beaker, and evaporate on the hot plate to a syrupy consistency. Add 100 ml. of nitric acid and boil to remove any oxides of nitrogen. Then remove the beaker from the source of heat and add, in small quantities, 5 g. of potassium chlorate with the aid of a glass spoon. During the addition the cover should be kept on as much as possible to minimise



losses from the solution. When the addition is complete boil the solution vigorously until the volume is reduced to about 25 ml. Wash down the cover and sides of the beaker, add 40 ml. of water and cool quickly.

Filter the precipitate of manganese dioxide on filter paper pulp in a Gooch crucible, and wash with cold 2 per cent. nitric acid. Evaporate to remove the bulk of the nitric acid, add 100 ml. of water, warm to dissolve salts and cool.

Into a 600-ml. beaker introduce 100 ml. of ammonium citrate solution, 50 ml. of "880" ammonia, and 50 ml. of water, and cool the solution in running water. To this ammoniacal citrate solution add 25 ml. of the ferricyanide solution, and then slowly, and with stirring, add the sample solution. Immerse the electrodes, commence stirring, adjust the galvanometer to zero and titrate the excess of ferricyanide with cobalt nitrate solution, observing the end-point as for the standardisation.

If manganese is absent the separation with nitric acid and potassium chlorate may be omitted.

Results obtained on synthetic solutions by the method described above are shown in Table III.

TABLE III

DETERMINATION OF COBALT IN PRESENCE OF NICKEL AND IRON (MANGANESE PREVIOUSLY REMOVED AS MnO_2)

Iron present mg.	Nickel present mg.	Manganese present mg.	Cobalt taken mg.	Cobalt found mg.
62	34	0	20·0	20·0; 20·1
"	"	0·5	20·0	19·9; 20·2
"	"	1·0	20·0	20·0; 19·9
"	"	2·0	20·0	19·9; 20·0
"	"	5·0	20·0	20·2; 20·2

(v) DETERMINATION OF IRON—

It was considered desirable to determine all three major constituents of the alloys rather than employ the difference method, particularly in view of the presence of manganese and

other minor constituents. The possibility of separating iron from nickel and cobalt to permit subsequent reduction and titration with dichromate was therefore investigated.

Precipitation with ammonia or zinc oxide, or with phosphate as in Schoeller's method, was not satisfactory even with reprecipitations. However, the classical "basic acetate" separation afforded a precipitate of iron almost completely free from nickel and cobalt, the amounts of these metals present being small enough to have no effect on the subsequent titration of the iron. A further reprecipitation with ammonia proved to be of little advantage, and a single "basic acetate" precipitation, carried out with care, gave satisfactory results.

Method for the determination of iron

Reagents—Ammonium carbonate solution: 20 per cent. w/v. Ammonium carbonate solution: 1 per cent. w/v. Acetic acid: 2 *N*. Ammonium acetate: 2 *N*. Stannous chloride solution: 5 g. dissolved in 7 ml. of hydrochloric acid and diluted to 100 ml. with water. Mercuric chloride solution: 5·6 per cent. w/v. Barium diphenylamine sulphonate solution: 0·15 per cent. w/v in water.

Procedure—Dissolve 0·5 g. of the sample in 20 ml. of diluted hydrochloric acid (1 + 1) and 5 ml. of diluted nitric acid (1 + 1) in a 600-ml. beaker. Evaporate nearly to dryness, add 10 ml. of concentrated hydrochloric acid and again evaporate nearly to dryness to remove the nitric acid. Add 10 ml. of diluted hydrochloric acid (1 + 4), warm to redissolve salts, dilute to 100 ml. and cool.

Add ammonium carbonate solution, drop by drop, using first the 20 per cent. solution and finally the 1 per cent. solution, until a faint precipitate not redissolving in 2 minutes is obtained. Add 3 ml. of 2 *N* acetic acid, dilute to 400 ml. with water and boil for 1 minute. (If the ammonium carbonate addition has been correctly carried out, the addition of acetic acid gives a clear solution, but, on heating, the bulk of the iron is precipitated before the boiling temperature is reached.) Add 8 ml. of 2 *N* ammonium acetate and again boil for 1 minute.

As soon as the precipitate has settled, filter through an 11-cm. No. 41 Whatman filter paper, washing at first with hot water containing 2 ml. of 2 *N* ammonium acetate and 1 ml. of 2 *N* acetic acid per 100 ml. and finally with hot water alone.

Return the precipitate and paper to the original beaker, add 30 ml. of diluted hydrochloric acid (1 + 1) and leave on the steam bath until the paper has disintegrated. Heat to boiling and add the stannous chloride solution dropwise until the yellow colour of the ferric chloride disappears and then add 5 drops in excess. Cool quickly, add 5 ml. of mercuric chloride solution and allow to stand for 1 minute. Dilute to 150 ml., add 10 ml. of phosphoric acid and 10 drops of barium diphenylamine sulphonate indicator and titrate with 0·1 *N* potassium dichromate until a purple colour is obtained.

Results obtained on synthetic solutions by the method described above are shown in Table IV.

Table IV

Determination of iron after separation by the "basic acetate" process

Nickel present	Cobalt present	Iron taken	Iron found
g.	g.	g.	g.
0·138	0·081	0·2529	0·2545
"	"	0·2529	0·2534
"	"	0·2529	0·2529
"	"	0·2529	0·2542

(vi) Determination of manganese—

Preliminary experiments were directed towards a titration of the cobalt plus the manganese with ferricyanide followed by a separate determination of the manganese, the cobalt being determined by difference.

As mentioned under section (iv) on the determination of cobalt, it was found that a preliminary separation of the manganese gave more satisfactory results in the cobalt determination.

An absorptiometric method for the determination of manganese was therefore adopted. This made it possible to use a larger sample than would have been convenient if cobalt

had also to be determined, and had the outstanding advantage of absorptiometric methods in that no preliminary separation of the manganese was necessary.

Method for the determination of manganese

Dissolve 0·5 g. of the alloy in 25 ml. of diluted nitric acid (1 + 1) in a 100-ml. beaker and heat to boiling to eliminate nitrous fumes. Add 5 ml. of phosphoric acid (to prevent subsequent precipitation of sparingly soluble ferric periodate), 10 ml. of water and 0·3 g. of potassium periodate. Heat to boiling and boil gently for 10 minutes to ensure complete oxidation of the manganese, cool, and make up to 100 ml. with water in a standard flask. Complete the determination with the Spekker absorptiometer as follows.

Fill a 1-cm. glass cell with the coloured solution, insert it in the instrument and place heat-absorbing and Ilford spectrum green filters in position. Set the drum to zero, switch on the tungsten lamp light source and adjust the left-hand iris until the galvanometer deflection is nil. To a second 1-cm. cell add 1 drop of 2 per cent. sodium nitrite solution followed by the test solution, allow time for the permanganate colour to be discharged and then insert in the "Spekker." Switch on the lamp and rotate the drum until a zero galvanometer deflection is obtained and take the drum reading.

Preparation of standards—

Pipette 10 ml. aliquots of each of the following three solutions into 100-ml. beakers to simulate Kovar type alloys. *Iron*—6·625 g. of pure iron dissolved in a minimum amount of diluted nitric acid (1 + 1) and diluted to 250 ml. *Cobalt*—10·6 g. of cobalt nitrate (AnalaR) dissolved in water and made up to 250 ml. *Nickel*—18·6 g. of nickel nitrate (AnalaR) dissolved in water and made up to 250 ml.

Prepare standard manganese solution by dissolving 0·25 g. of pure manganese metal in 5 ml. of diluted nitric acid (1 + 1) and diluting to 1 litre, so that 1 ml. ≡ 0·25 mg. Mn.

Prepare a series of standards by adding 0 to 10 ml. of the manganese solution to solutions of the mixed nitrates contained in 100-ml. beakers, to cover the range 0 to 0·5 per cent. Mn. Add 10 ml. of nitric acid and 5 ml. of phosphoric acid to each beaker and oxidise the manganese to permanganate, following the procedure already described. Before plotting a graph relating manganese content with drum readings, correct the readings for any manganese found in the blank solution, so that the curve shall pass through the origin.

Results on commercial alloys

The methods outlined for the determination of nickel, cobalt, iron and manganese have been successfully applied to the analysis of commercial alloys. Table V gives duplicate results for the analysis of a number of these alloys and indicates the precision that the methods afford.

Table V

Analysis of commercial alloys

Sample No.	Nickel %	Cobalt %	Iron %	Manganese %	Mean total %
1.	28·81	17·37	53·44	0·18	99·81
	28·87	17·32	53·44	0·18	
2.	28·88	17·32	53·55	0·29	100·05
	28·89	17·25	53·62	0·29	
3.	29·36	16·79	53·44	0·46	99·89
	29·34	16·81	53·11	0·46	
4.	29·19	17·22	53·31	0·30	100·03
	29·22	17·19	53·33	0·30	
5.	29·27	17·09	53·34	0·39	100·19
	29·36	17·02	53·51	0·39	
6.	29·43	16·97	53·34	0·07	99·84
	29·29	16·95	53·56	0·07	
7.	29·43	17·01	53·16	0·33	100·00
	29·68	17·03	53·03	0·32	

THE IRON - COBALT - DIMETHYLGLYOXIME COMPLEX

As mentioned in an earlier part of the paper, when attempts were made to precipitate nickel with dimethylglyoxime from synthetic solutions containing nickel, iron and cobalt in proportions similar to those of the alloys with which we were concerned, brown slimy precipitates were obtained, difficult to filter and differing greatly in appearance from the characteristic bright red nickel compound. These precipitates were examined by X-ray diffraction methods. For reference, X-ray patterns were also obtained for the pure nickel dimethylglyoxime compound and for dimethylglyoxime reagent; both of these substances gave well-defined patterns and were found to be essentially crystalline.

The brown precipitates obtained initially gave X-ray patterns showing a mixture of phases of which two were predominant, one being the pure dimethylglyoxime compound, the other what was presumed to be the iron - cobalt - dimethylglyoxime complex mentioned by Weeldenburg.[3] No alteration of the lattice parameters of the nickel complex was observed.

It was found that similar brown precipitates with dimethylglyoxime could be obtained from solutions containing iron and cobalt only; the amount of the precipitate and the ease with which it formed varied according to the ratios of iron to cobalt in the solution and to the length of time of standing.

The investigation covered a range of compositions from 100 per cent. cobalt to 100 per cent. iron and the results are summarised in Table VI.

TABLE VI

Ratio of iron to cobalt in solution Fe : Co	Composition of precipitate*	Iron and cobalt in precipitate % Fe	% Co	Ratio of iron to cobalt in precipitate by weight
0 : 100	No precipitate			
10 : 90	Mainly β phase Some α			
25 : 75	Mainly β phase Some γ	5·8	9·3	0·62:1
48·7 : 51·3 (Equi-atomic)	Mainly β phase	8·2	10·2	0·8:1
75 : 25	Mixture of β and γ phases	16·2	9·5	1·7:1
90 : 10	Mainly "free" dimethylglyoxime			
100 : 0	No precipitate			

* Most of the precipitates contained, in addition, some "free" dimethylglyoxime in varying proportions.

The exact composition of the precipitates obtained from the various mixtures was affected to some extent by the time of standing but, in general, it was found that cobalt-rich mixtures tend to give precipitates consisting mainly of one phase, referred to in Table VI as the β phase, the material which constituted the major contaminant of the nickel precipitates we obtained in our initial experiments. Iron-rich mixtures give precipitates consisting of a mixture of two phases, referred to in the Table as β and γ; the latter is probably richer in iron.

Most of the precipitates obtained consisted of more than one phase and, after long standing, most contained significant quantities of free dimethylglyoxime. It was not possible, therefore, to determine the exact composition of the various phases in terms of iron - cobalt - dimethylglyoxime ratios, although it was established that the iron-to-cobalt ratio varied considerably according to the composition of the reacting solution.

X-ray examination of the precipitate obtained from the equi-atomic iron - cobalt mixture, however, gave a single β phase, contaminated only with free dimethylglyoxime.

According to Weeldenburg, the compound containing 1 atom of iron, 1 atom of cobalt and 3 molecules of dimethylglyoxime, to which he ascribed the formula $FeCoC_{12}H_{19}N_6O_6$, is obtained by precipitation from solutions containing iron and cobalt in equi-atomic proportions and precipitation is said to be quantitative. Because of the presence of free dimethylglyoxime in the precipitate it was not possible to determine the ratio of metal to oxime, but the ratio of iron to cobalt was found to be 0·8 : 1.

While our results, in the main, confirm the observations of Weeldenburg, Brunck, and Balz, they amplify them in the sense that the nature and degree of contamination of the

nickel complex when precipitated from mixtures containing iron and cobalt is determined by the relative proportions of iron and cobalt in the mixture. Our results differ from theirs in that no constant ratio of iron to cobalt has been found in any of the iron - cobalt complexes, even from solutions containing the metals in equi-atomic ratio.

The substance that we have called the β phase, obtained either alone or in admixture over the greater part of the range of iron - cobalt mixtures, probably corresponds to the compound reported by Weeldenburg. Our results suggest that while it is possible that the ratio of metal atoms to molecules of dimethylglyoxime in the β phase obtained from solutions over a range of compositions remains constant at 2 : 3, iron and cobalt may replace each other to a considerable extent without giving rise to any very obvious changes in the X-ray pattern.

It was observed that when the ratio of iron to cobalt in a solution exceeds certain limits, in either direction, the colloidal β phase gives place to a crystalline α phase in the case of high cobalt, or a colloidal γ phase in the case of high iron ratios, but the compositions of these phases are not known.

The small crystal size as shown by the X-ray patterns probably explains and accounts for the slimy nature of these iron - cobalt complexes and for the difficulty experienced in filtering them. Even slight contamination of the nickel precipitate is evidenced by a distinct change in the characteristic bright red colour of the dimethylglyoxime complex, but the iron - cobalt complex is not isomorphous with that of nickel and, provided the conditions set out in the paper are established, it would seem probable that a clean separation of nickel can be made from mixtures with iron and cobalt over a wide range of compositions.

Summary

Methods are given for the accurate determination of nickel, cobalt, iron and manganese in low-expansion alloys used for glass-to-metal seals.

In the determination of nickel, interference is caused by the formation of an insoluble complex containing iron and cobalt associated with dimethylglyoxime. This can be prevented by a preliminary reduction of the iron to the ferrous state and nickel can then be precipitated in the normal way, free from iron and cobalt.

Cobalt is determined by electrometric titration with potassium ferricyanide after removal of manganese by precipitation as dioxide.

Iron is separated by a basic acetate process, reduced and titrated.

Manganese is determined absorptiometrically after oxidation to permanganate.

Duplicate analyses of a number of commercial alloys are given.

The nature and formation of the iron - cobalt - dimethylglyoxime complex are discussed in some detail.

Thanks are due to Mr. R. H. Jones of the Mond Nickel Co. for drawing our attention to the electrometric method for the determination of cobalt, and to Mr. J. T. Minster, of the M-O. Valve Co., for supplying details of the circuit.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Colorimetric Determination of DDT in Milk and Fatty Materials. M. S. Schechter, M. A. Pogorelskin, and H. L. Haller (*Anal. Chem.*, 1947, **19**, 51–53)—Attention has recently been directed to the possible danger to public health from contamination of such materials as milk, butter, eggs, meat, and fats when farm animals consume feeding stuffs treated with DDT. Pharmacological investigations (Woodard *et al.*, *Science*, 1945, **102**, 177) have shown that ingested DDT accumulates as such in the fatty tissues of experimental animals and can be excreted in the milk. Some of the DDT is metabolised to bis(*p*-chlorophenyl)acetic acid (Grummit *et al.*, *J. Amer. Chem. Soc.*, 1945, **67**, 156; White and Sweeney, *U.S. Pub. Health Repts.*, 1945, **60**, 66) which is excreted in the urine (Neal *et al.*, *Ibid.*, 1946, **61**, 403; Ofner and Calvery, *J. Pharmacol.*, 1945, **85**, 363; White and Sweeney, *loc. cit.*). When high dosages of DDT are administered to goats and rats, the milk may become toxic enough to kill other animals drinking it (Telford, *Soap Sanit. Chem.*, 1945, **21**, 161; Telford and Guthrie, *Science*, 1945, **102**, 647).

Determination of DDT in food material is complicated by the large amount of fat accompanying it when it is extracted by organic solvents. Application of chromatography was not successful, and low-temperature precipitation of the fat by cooling the solution with solid carbon dioxide led to difficulties in filtration. Saponification methods have the disadvantage that DDT is converted into its dehydrochlorinated derivative, but are applicable when it is not necessary to ascertain whether the DDT is present as such or has decomposed into its dehydrochlorinated derivative. Observation of the solubility of fat and the insolubility of DDT in sulphuric acid led to the present method.

Procedure—To 100 g. of milk add an equal volume of 95 per cent. ethanol and divide the mixture equally between two 200-ml. centrifuge bottles (unless larger bottles are available). When the concentration of DDT is more than 5 p.p.m., take correspondingly smaller samples. Add 50 ml. of light petroleum (Skellysolve B, b.p. 60° to 70° C.) to each portion, cover the bottles with rubber caps, shake vigorously, and centrifuge at 2000 r.p.m. for 15 min. Pour the contents of both bottles into a 500-ml. separating funnel, and drain the lower layer in equal portions into the same bottles. Pass the upper petroleum layer through a 5-cm., tightly-packed, cottonwool plug, held in a glass Gooch crucible holder, into a 500-ml. Erlenmeyer flask with a standard ground-glass joint. Extract the contents of the bottles as before with two successive 25-ml. portions of light petroleum and a final 50-ml. portion, centrifuging for about 10 min. each time. Drain the aqueous layer into the centrifuge bottles, and pass the petroleum layer through the plug into the flask. Place a glass bead in the flask and remove the light petroleum by distillation in an all-glass apparatus, inserting a tube connected with a vacuum line to remove the last traces of solvent. Wash the residue quantitatively into a 500-ml. separating funnel with 150 ml. of chloroform.

In the analysis of butter or fat, a 5-g. sample, or an extract thereof from which the solvent has been removed, takes the place of this residue.

Place 100 ml. of chloroform in a second separating funnel and extract the chloroform solution in the first funnel successively with (i) 50 ml. of sodium sulphate and sulphuric acid mixture (100 g. of anhydrous sodium sulphate in 1 litre of conc. sulphuric acid), (ii) 50 ml. of sodium sulphate and sulphuric acid mixture, (iii) 50 ml. of fuming sulphuric acid and sulphuric acid mixture (equal volumes of fuming sulphuric acid containing 20 to 30 per cent. of sulphur trioxide and conc. sulphuric acid), and (iv) 50 ml. of sodium sulphate and sulphuric acid mixture. Repeat the last extraction if it does not yield a light-coloured extract. Drain each acid washing from the first funnel into the second funnel and finally into a 250-ml. cylinder. The extraction in the second funnel minimises loss of DDT by slight emulsification of chloroform in the acid liquids. The funnels should be shaken vigorously each time and allowed to stand for 10 to 15 min. before the acid layer is drained off. On the rare occasions when the emulsion does not break, the mixture may be centrifuged.

Filter the chloroform from the first funnel and then that from the second funnel through a 5-cm., tightly-packed, cottonwool plug into a third separating funnel. Pipette off any chloroform that has risen to the surface from the combined acid washings in the cylinder and run it through the plug of cottonwool. Rinse the two funnels and the plug with 50 to 100 ml. of chloroform. Add enough 5 per cent. sodium bicarbonate solution (40 ml.) to the combined chloroform filtrates so that the mixture will remain alkaline after vigorous shaking. After about 10 min., filter only the chloroform layer through a similar plug into a 500-ml. Erlenmeyer flask with a standard joint. Wash the sodium bicarbonate solution left in the funnel with two successive 30-ml. portions of chloroform, and run these also through the plug into the flask. If the filtrate is not clear, repeat the filtration.

Introduce a glass bead into the flask and reduce

the volume of the solution by removal of chloroform to about 10 ml. Wash this quantitatively into a large test tube (25 × 200 mm. or larger) with acetone, add a glass bead, and cautiously evaporate the solvent on the steam-bath, removing the last traces by means of a tube connected with a vacuum line. Nitrate the residue with 5 ml. of nitrating mixture and complete the analysis as described by Schechter *et al.* (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 704). Since there will still be some interference from the raffinate from the butter fat, it is advisable to make spectrophotometric measurements at 600, 620, and 640 mμ. and to average the results. Below 600 mμ., there may be enough interference to cause high results, so that it may be impossible to calculate the amount of *p,p'*-DDT and *o,p'*-DDT and add them to obtain the amount of DDT. It is therefore desirable to use a standard sample of the same DDT as was used in the feeding experiments, or, failing this, DDT of the same type and grade.

Biological tissues may be treated with sodium sulphate as described by Smith and Stohlman (*U.S. Pub. Health Repts.*, 1944, **59**, 984) or Ofner and Calvery (*loc. cit.*). After evaporation of the solvent used for extraction, the residue may be dissolved in chloroform and submitted to the sulphuric acid extraction described. *p,p'*-DDA [bis(*p*-chlorophenyl)acetic acid] is removed by the sulphuric acid treatment and does not interfere. If its determination is required, it should first be separated from the ether solution of the sample (or an extract thereof) with sodium bicarbonate solution, which is then separated, acidified, and extracted with fresh ether. After removal of the ether, the residue containing the *p,p'*-DDA can usually be nitrated directly and determined by comparison with *p,p'*-DDA standards by the Schechter - Haller colorimetric procedure or, with small amounts, the residue may be submitted to a modified sulphuric acid treatment in which the fuming sulphuric acid and the sodium bicarbonate washes are omitted, four sulphuric acid and sodium sulphate washes and one wash with 40 ml. of water being used.

Dehydrochlorinated DDT [1:1-dichloro-2,2-bis-(*p*-chlorophenyl)ethylene] is not removed by the sulphuric acid treatment and its presence may be detected by its effect on the absorption spectrum of the developed colour in the Schechter - Haller colorimetric test. It gives a red colour, with an absorption spectrum different from that of the blue colour given by DDT. *o,p'*-DDT and its dehydrochlorinated derivative would likewise be unaffected by the sulphuric acid treatment.

Recovery experiments with milk containing known amounts of DDT were satisfactory. The blank value given by the milk used was 0·38 p.p.m. (calculated as DDT), but the characteristic blue colour did not appear. The DDT content of milk from cows fed with DDT-treated material ranged from 3 to 26 p.p.m., that of butter ranged from 456 to 534 p.p.m., that of lean steak was 4 p.p.m., and that of the fat from the steak, 178 p.p.m. Milk containing 1 p.p.m. of DDT gives an easily discernible blue colour. A. O. J.

Determination of DDT in Milk by Determination of Organic Chlorine. R. H. Carter (*Anal. Chem.*, 1947, **19**, 54)—Mix 200 ml. (or 200 g.) of well homogenised milk with 200 ml. of ethyl alcohol in a separating funnel, and shake the liquid gently with 250 ml. of a mixture of 75 per cent. of ethyl ether and 25 per cent. of light petroleum (Skellysolve B, b.p. 60° to 70° C.). Draw off the aqueous layer and repeat the extraction three times with 100-ml. portions of the solvent mixture, shaking vigorously for 5 min. Discard the aqueous phase. Transfer the combined extracts to a suitable flask, remove the solvent on the steam-bath, and remove the last traces of water by addition and distillation of two 50-ml. portions of benzene.

To the residue in the flask add 150 ml. of *iso*-propanol and about 6 g. of metallic sodium cut into small pieces. Heat under refluxing conditions for 2 hr. with moderate boiling. Add 25 ml. of ethyl alcohol, allow time for complete reaction with the remaining sodium, and dilute with 100 ml. of water poured through the condenser. Evaporate the liquid to low bulk in a beaker on the steam-bath, dilute to about 400 ml. with water, and warm if necessary to dissolve the soap. Acidify with sulphuric acid, cool with ice or water, and remove the precipitated fatty acids by filtration, washing them twice with water. Extract the combined filtrate and washings twice with 100-ml. portions of the mixed solvent (*supra*). Make the aqueous solution alkaline to phenolphthalein with 2 *N* potassium hydroxide and, if necessary, reduce the volume to about 300 ml. by evaporation. Acidify the solution with a slight excess of nitric acid and determine the chloride ion by any of the standard methods. Calculate the amount of DDT by multiplying the amount of chlorine by 2.

The recovery of known amounts of DDT added to milk was not less than 95 per cent. Determination of organic chlorine in milk from cows fed on a diet free from DDT showed less than 0·2 p.p.m. of chlorine. In milk from cows receiving DDT in the diet, the corresponding figure ranged from 1 to 12 p.p.m., and these results were confirmed by determination of DDT by the colorimetric method of Schechter *et al.* (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 704; *cf.* preceding abstract).

The method presented is simple, rapid, and reasonably sensitive, but is not specific for DDT. It is possible that other halogen-containing organic insecticides, fungicides, and herbicides, *e.g.*, Spergon

(tetrachlorobenzoquinone), 2:4-dichlorophenoxyacetic acid (2 : 4-D), *p*-dichlorobenzene, and benzene hexachloride may also be absorbed and stored in milk and meat products. A. O. J.

Colorimetric Assay of Digitoxin by the Modified Raymond Method. R. C. Anderson and K. K. Chen (*J. Amer. Pharm. Assoc., Sci. Ed.*, **1946, 35**, 353–355)—A chemical estimation, based upon the method of Raymond (ANALYST, 1938, **63**, 478; 1939, **64**, 113), depending upon the reaction of *m*-dinitrobenzene with digitoxin is described and compared with the method of Bell and Krantz (*J. Pharmacol.*, 1946, **87**, 198) and with the U.S.P. biological assay on cats. The latter method is the more accurate, but a chemical method is desirable for checking intermediate manufacturing stages and the final products.

Procedure—Take 10 ml. of dilute digitoxin solution in 47·5 per cent. ethyl alcohol, add 1 ml. of a 1 per cent. solution of *m*-dinitrobenzene in absolute alcohol and place in an ice-bath. After 5 to 10 min., add 2 ml. of a 20 per cent. solution of sodium hydroxide. Mix and return the solution to the ice-bath. An indigo-blue colour is produced and is measured in a colorimeter with an orange filter, exactly 5 min. after the addition of the sodium hydroxide. The colour fades fairly rapidly. Construct a standard curve using volumes of 0·75 ml., 1 ml., and 1·25 ml. of a 0·1 per cent. solution of digitoxin, diluted to 50 ml. with 47·5 per cent. ethyl alcohol. Standards so produced correspond to concentrations of 0·015, 0·020, and 0·025 mg. of digitoxin per ml. Treat samples of each solution in the manner described above, using the colorimeter readings to construct a calibration curve.

When a series of dilutions of digitoxin were assayed by this method the results obtained were within ± 5 per cent. of the values obtained by the Bell and Krantz method, and agreed with the theoretical value to the same extent. When repeated tests were made upon the same sample of digitoxin, assayed by the U.S.P. cat method, the largest experimental errors were − 3·2 per cent. and + 2·6 per cent. with the modified Raymond method and − 4·5 per cent. to + 4·2 per cent. with the Bell and Krantz method. The relative potencies of 21 different batches of digitoxin were determined by all three methods. The maximum variations from the biological assay were, by the method described, + 12 per cent. and − 14 per cent., and by the Bell and Krantz method, + 14 per cent. and − 11 per cent. All assay results were within the limits of tolerance of ± 20 per cent. required by U.S.P., and the extreme difference between the two colorimetric methods did not exceed 18 per cent.

Assay of tablets—Take tablets equivalent to 6 mg. of digitoxin, triturate, and transfer to a 60-ml. centrifuge tube, add 60 ml. of 80 per cent. ethyl alcohol, securely stopper the tube, and agitate mechanically for about 12 hr. Centrifuge, and then take 5 ml. of the supernatant liquid and dilute to 25 ml. with 47·5 per cent. ethyl alcohol. Develop the colour as described. This solution theoretically contains 0·02 mg. per ml. Results obtained using eleven batches of tablets in this way gave results closely in agreement by either colorimetric method. In contrast with the Bell and Krantz method, it was found that lactose used in the preparation of the tablets did not interfere with the reaction in spite of the prolonged extraction with the alcohol. Neither colorimetric method is specific for digitoxin since similar colour reactions take place with other cardiac glycosides, and therefore their principal value is in manufacturing control. The method was also applied to ampoules with satisfactory results. R. H. T.

Chemical Estimation of Nicotinic Acid. A. Mueller and S. H. Fox (*J. Biol. Chem.*, 1947, **167**, 291–292)—In the cyanogen bromide - aniline method for the estimation of nicotinic acid, organic amine is not necessary for colour formation if an ammoniacal buffer solution is used. The canary-yellow colour obtained by reaction with cyanogen bromide and ammonia has a high extinction coefficient ($E^{1\%}_{1\,cm.}$ is 4500 to 4600) with a wavelength maximum at 410 mμ. The colour reaches maximum intensity within 2 to 2·5 min., and then fades rapidly. Nicotinamide reacts more slowly and gives a less intense coloration. The wavelength maximum is at 398 mμ., reaching maximum intensity in 6 min. with a slightly lower $E^{1\%}_{1\,cm.}$ value than that of the acid.

Procedure—To 1 ml. of nicotinic acid solution, containing 1 to 15 μg., add 2 ml. of ammonia buffer (0·1 *M* ammonia, 0·5 *M* dipotassium phosphate, and 2·0 *M* ammonium chloride), followed by 5 ml. of 10 per cent. cyanogen bromide solution. Prepare a reagent blank with 1 ml. of water in place of the nicotinic acid solution, and evaluate the colour at 410 mμ. at intervals between 2 and 3 min. after addition of the reagents. Calculate the nicotinic acid content from the maximum value thus obtained. F. A. R.

Biochemical

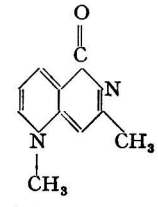
Microbiological Method for the Estimation of Nicotinic Acid based on the use of Proteus HX19. N. Grossowicz and E. Sherstinsky (*J. Biol. Chem.*, 1947, **167**, 101–105)—*Method*—Maintain cultures of *Proteus* HX19 on standard agar slants and make transfers at monthly intervals, incubating at 30° C. for 24 hr. and then storing in the refrigerator. Prepare a basal medium of the following composition: ammonium sulphate, 0·75 g.; potassium dihydrogen phosphate, 4·50 g.; potassium chloride, 0·50 g.; sodium nitrate, 1·00 g.; water,

750 ml. Adjust to *p*H 7·4 by addition of 2 to 2·5 ml. of 10 *N* sodium hydroxide, transfer 7·5-ml. quantities to test tubes, plug with cotton-wool, and autoclave at 15 lb. for 30 min. To each tube, add the following sterile solutions under aseptic conditions: 30 per cent. glucose solution, 0·2 ml.; 0·1 per cent. ferric citrate solution, 0·1 ml.; 0·5 per cent. magnesium sulphate solution, 0·1 ml.; 10 per cent. acid-hydrolyzed casein, 0·1 ml. (This is prepared as follows: To 50 g. of casein, add 250 ml. of 25 per cent. sulphuric acid, autoclave for 10 hr. at 15 lb. pressure, and remove the sulphuric acid with baryta. Carefully remove the excess of baryta by addition of sulphuric acid to *p*H 4 to 5 and then stir the filtrate with 2 per cent. of Norit for 30 min., to adsorb traces of nicotinic acid, and filter. Adsorption is complete if the organism shows only scanty growth in absence of nicotinic acid and full growth after addition of 0·15 μg. of nicotinic acid per 10 ml. of medium. Finally, adjust the hydrolysate to *p*H 7·0 with concentrated sodium hydroxide solution and dilute or concentrate to give a final concentration of 10 per cent.) Add graded amounts of a standard nicotinic acid solution containing 0·0, 0·005, 0·01, 0·02, 0·03, 0·04, 0·06, 0·08, and 0·1 μg. of nicotinic acid per 10 ml., or the solution to be tested, and make up the volume in each tube to 10 ml. If sulphonamides are present, add 0·1 ml. of a 0·1 per cent. solution of *p*-aminobenzoic acid. Suspend a loopful of *Proteus* organisms in sterile 0·9 per cent. saline to give a suspension just barely turbid, and add 1 ml. to 100 ml. of saline. Add 2 drops of the resulting suspension to each tube and incubate in a sloping position (angle, 10° to 15°) for 40 hr. at 30° C. Measure the growth of the organism in each tube turbidimetrically or by means of a photo-electric colorimeter, and calculate the nicotinic acid content of the test solution from the curve obtained with the standard solutions. Nicotinamide and nicotinic acid are equally effective, and recovery experiments gave results with an error not exceeding ± 10 per cent. Some other pyridine derivatives stimulate the growth of the organism, but other members of the vitamin B complex do not. F. A. R.

Rapid Fluorimetric Method for the Estimation of the Total Pyridine Nucleotides in the Red Blood Cells. N. Levitas, J. Robinson, F. Rosen, J. W. Huff, and W. A. Perlzweig (*J. Biol. Chem.*, 1947, **167**, 169–175)—The alkali-acetone reaction for N^1-methylnicotinamide (*cf.* preceding abstract) yields with both pyridine nucleotides a highly fluorescent product that can be used for the estimation of the co-enzymes in amounts as small as 1 μg.

Procedure—Collect 5 ml. of blood in a tube containing dry ammonium oxalate (6 mg.) and potassium oxalate (4 mg.). Transfer 2·0 ml. of the blood to a centrifuge tube containing 2·0 ml. of 25 per cent. trichloroacetic acid and 6·0 ml. of water, leave for several minutes, and then centrifuge for 5 to 10 min. at 3000 r.p.m. and filter through a No. 30 Whatman paper. Put 0·50 ml. of the filtrate into each of three fluorimeter cuvettes, A, B, and C. To A add 0·5 ml. of water; to B, 1·0 ml. of water; and to C, 0·50 μg. of N^1-methylnicotinamide in 0·50 ml. of standard solution. To tubes A and C, add 0·5 ml. of acetone, and then to each of the three tubes add 0·20 ml. of 6 *N* sodium hydroxide and mix immediately. Leave at room temperature for 5 min. and then add 0·3 ml. of 6 *N* hydrochloric acid and mix at once. Immerse the tubes in boiling water for 2 min., add 1 ml. of 20 per cent. potassium dihydrogen phosphate solution, and dilute to 10 ml. with water. Measure the fluorescence of each solution as described for N^1-methylnicotinamide, and calculate the result in a similar manner. F. A. R.

Determination of N^1-Methylnicotinamide in Urine. J. W. Huff and W. A. Perlzweig (*J. Biol. Chem.*, 1947, **167**, 157–167)—When N^1-methylnicotinamide is treated with acetone and alkali in the cold, and then heated in acid solution, a highly fluorescent and stable compound is formed. This has been identified (Huff, *J. Biol. Chem.*, 1947, **167**, 151) as 1: 7-dimethyl-5-oxo-(1: 5-dihydro-1: 6-naphthyridine) hydrochloride:



On the formation of this compound has been based a much more sensitive and simple method of estimating N^1-methylnicotinamide in urine than any previously described.

Procedure—Put 2·00 ml. of the urine and 0·4 ml. of glacial acetic acid into each of two 25-ml. graduated cylinders, and to one of them add 1 ml. of a standard solution containing 10 μg. of N^1-methylnicotinamide. Dilute the contents of both cylinders to 20 ml. (or a volume such that the test solution contains 0·5 to 1·2 μg. of N^1-methylnicotinamide per ml. and 2 per cent. acetic acid) with water. To each cylinder add 0·1 g. of charcoal, shake, and filter immediately through a No. 30 Whatman paper. Put 1·00 ml. of the diluted urine filtrate into each of two cuvettes, A and B, and 1·00 ml. of the diluted urine filtrate containing the standard into another cuvette, C. Add to A and C, 0·5 ml. of acetone (freed from fluorescent substances by distillation over potassium permanganate), and to B, 0·5 ml. of water, mix each, and then add to each tube 0·20 ml. of 6 *N*

sodium hydroxide, mix immediately, and leave at room temperature for 5 min. To each tube add 0·3 ml. of 6 *N* hydrochloric acid, and mix at once. Immerse in boiling water for 2 min., cool, and add 1 ml. of 20 per cent. w/v potassium dihydrogen phosphate solution and dilute with water to a volume of 10 ml. Adjust the galvanometer reading of the fluorimeter to 100 with a solution containing 0·3 μg. of quinine sulphate per ml., and then measure the fluorescence of tubes A, B, and C. Then, if *A*, *B*, and *C* are the readings obtained with these tubes, and if the acidified urine was diluted to 20 ml. (see above), the amount of N^1-methylnicotinamide in milligrams in the total volume of urine

$$= \frac{A - B}{2(C - A)} \times \frac{\text{vol. of urine, ml.}}{0{\cdot}1}$$

The normal excretion by an adult on an adequate diet ranged from 3 to 17 mg., with an average value of 7 mg. per day. F. A. R.

Application of Sendroy's Iodimetric Chloride Titration to Protein-containing Fluids. D. D. Van Slyke and A. Hiller (*J. Biol. Chem.*, 1947, **167**, 107–128)—*Method*—Put 1 ml. of plasma or serum into a 50-ml. centrifuge tube and add 25 ml. of phosphoric and tungstic acid reagent (dissolve 6 g. of $Na_2WO.2H_2O$, reagent grade, containing less than 0·01 per cent. of chloride, in 1 litre of 0·15 *M* phosphoric acid; if chloride-free tungstate is not available, substitute 2 g. of picric acid). Add about 0·3 g. of silver iodate and shake *vigorously* for 40 sec. During this period, proteins are precipitated and the reaction between silver iodate and chloride ion is completed: $AgIO_3 + Cl' = IO_3' + AgCl$. Centrifuge for 1 min. at 2500 r.p.m., and transfer a 10-ml. portion of the supernatant liquid to a 50-ml. conical flask. Add 1 g. of sodium iodide and titrate the liberated iodine with 0·02303 *N* sodium thiosulphate solution, using 2 drops of 1 per cent. starch solution as indicator. Calculate the amount of sodium chloride in grams per litre by multiplying the titre by the factor 0·5845.

The method can also be applied to Folin - Wu or Somogyi filtrates. To 1 volume of 1 : 10 filtrate, add 1·5 volume of 0·15 *M* phosphoric acid, shake with 0·3 g. of silver iodate per 10 ml. of filtrate, centrifuge, and titrate as described above. In the analysis of whole blood, the phosphoric and picric acid reagent should be used (dissolve 5 g. of picric acid in 1 litre of water and 10 ml. of phosphoric acid). The analysis of urine or gastric juice is carried out exactly as described for plasma, except when less than 50 mg.-mols. of chloride per litre are present. A sample 5 times that normally employed is then used, the volumes of reagent being kept the same. Milk is analysed in the same way as low-chloride urine. F. A. R.

Organic

Isolation and Identification of Fatty Acids as bis-(*p*-dimethylaminophenyl)-ureides. F. L. Breusch and E. Ulusoy (*Arch. Biochem.*, 1946, **11**, 489–498)—Free fatty acids are isolated and characterised as the crystalline ureides of bis-(*p*-dimethylaminophenyl)-urea; the derivatives are formed directly by boiling a solution of the fatty acid with a solution of the urea derivative in ether. Most of the ureides are only slightly soluble in ether, and crystallise directly from the ether solution even in the presence of large quantities of neutral fat.

Preparation of the reagent—The bis-(*p*-dimethylaminophenyl)-urea was prepared via dimethylaniline → *p*-nitrosodimethylaniline → *p*-aminodimethylaniline (+ carbon disulphide) → bis-(*p*-dimethylaminophenyl)-thiourea → free base (Zetsche and Röttger, *Ber.*, 1939, **72**, 1599, 2095). The free base forms white crystals, m.p. 86° to 89° C., and is soluble at 21° C. in ether (6·5 per cent.), acetone (14·2 per cent.), alcohol (0·6 per cent.), and acid-free ethyl acetate (12·1 per cent.); it is insoluble in water, but readily soluble in acids.

Preparation of ureides—Boil under refluxing conditions for 2 to 3 hr. a 1 to 4 per cent. solution of the fatty acid in ether with approximately the necessary molar amount of a saturated, clear solution of the base in ether. The ureides separate chiefly during boiling, but sometimes after cooling in an ice-box or after partial evaporation of the ether. The derivatives are always the mono-ureides, containing one mol. of acid and one mol. of base. The yield is about 80 per cent.; the presence of free water decreases the yield. The ureides are readily soluble in 5 per cent. hydrochloric acid, but not in the 0·1 per cent. acid; they can be extracted quantitatively from ether solutions by means of the 5 per cent. acid, and by precipitation with cold alkali the crystalline ureide is regained unchanged. Attempts to titrate them in alcohol-benzene solution with hydrochloric acid failed.

Direct separation of fatty acids from neutral lipids—Application to propionic acid mixed with triolein—Three hundred mg. of propionic acid, 5·0 g. of pure triolein, and 1 g. of the free base were heated in 40 ml. of ether for 2 hr. under refluxing conditions. After cooling for 2 hours in an ice-box, 1·11 g. of crystalline propionic acid ureide (m.p. 155° to 158° C.) could be isolated by filtration. After one recrystalisation from acetone, the m.p. was 161° C. Excess of free base could readily be eliminated from the ether solution by shaking with dilute hydrochloric acid, leaving a practically pure ether solution of unaltered triglyceride.

Separation of ureides from lipids—As traces of a formed ureide, unprecipitated because of its solubility in ether may in some cases remain in a

lipoid solution, extraction with dilute hydrochloric acid was used to remove practically all ureide. For example, a solution of 250 mg. of pure stearic acid ureide (m.p. 122° C.) in 100 ml. of pure ether was extracted 4 times with 50-ml. portions of 5 per cent. hydrochloric acid. After separation, the evaporated ether layer was found to contain only 5 mg. of crystals, showing that 98 per cent. of the ureide had been removed. The stearic acid ureide could be precipitated unaltered (m.p. 121° C.) from the clear solution in hydrochloric acid by addition of alkali.

The properties of the bis-(*p*-dimethylaminophenyl)-ureides of the normal fatty acids from C_1 to C_{20} (except C_{13}, C_{15}, C_{17}, and C_{19}); of the α,β-unsaturated fatty acids crotonic, β-methylcrotonic, 1-pentenoic, 1-nonenoic, 1-decenoic, 1-hendecenoic, 1-dodecenoic, 1-tridecenoic, and 1-tetradecenoic; of the unsaturated acids 10-hendecylenic, oleic, stearolic, ricinoleic, 9 : 11-linoleic, 9 : 12-linoleic, and 9 : 12 : 15-linolenic; and of racemic mixtures of the *d*- and *l*-acids of β-hydroxypelargonic, β-hydroxycapric, β-hydroxyhendecylic, β-hydroxylauric, and β-hydroxytridecanoic acids, are tabulated. E. M. P.

New Specific Colour Reaction of Hexuronic Acids. Z. Dische (*J. Biol. Chem.*, **1947, 167,** 189–198)—*Method*—Mix, with cooling, 1 ml. of the solution; containing 5 to 100 μg. of uronic acid, with 6 ml. of concentrated sulphuric acid and heat for 20 min. in boiling water. Cool to room temperature and add 0·2 ml. of a 0·1 per cent. alcoholic solution of pure carbazole with shaking. After a few minutes, a pink colour appears; the intensity increases for 2 hr. and remains practically unchanged for at least an hour. Later, the colour slowly decreases and loses about one-third of its intensity after 24 hr. The pink colour is sensitive to water and, if 3·8 ml. of water are added, with cooling, to the reaction mixture, the colour fades rapidly and disappears completely in about 3 min. If the mixture is left at room temperature for many hours, a weak violet colour appears; this reaction is common to all sugars. The formation of the pink colour, however, is characteristic of uronic acids, true sugars giving no visible reaction at dilutions of the order of 0·01 per cent. The reaction is also negative with ascorbic, mucic, hydroxypyruvic, 2-keto hexonic, pyruvic, acetoacetic, lactic, fumaric, succinic, malic, acetic or formic acid, acetone, urea, or guanidine. Positive reactions are given by conjugated acids and polyuronides, and the intensity corresponds approximately to their hexuronic acid contents. F. A. R.

Determination of Dipentaerythritol in Mixtures of Pentaerythritol and Dipentaerythritol. J. A. Wyler (*Ind. Eng. Chem., Anal. Ed.*, **1946, 18,** 777–778)—The method is based on the crystallisation of dipentaerythritol from aqueous solutions containing an approximately 1 : 1 mixture of di- and mono-pentaerythritols. This ratio is obtained, if necessary, by adding pure dipentaerythritol to the solution of the sample before analysis. The precipitated dipentaerythritol is weighed directly, and corrections are applied for the quantity of dipentaerythritol added and that remaining in solution.

Procedure for the analysis of a mixture of 80 *parts of pentaerythritol and* 20 *parts of dipentaerythritol*—Weigh exactly 2·500 g. of the sample and 2·500 g. of pure dipentaerythritol in a tared aluminium dish, and transfer to a 125-ml. Erlenmeyer flask weighed to the nearest 0·1 g. Add exactly 60 g. of distilled water, shake gently, and heat on an electric hot-plate to complete solution; prevent undue loss of water by placing a clean rubber stopper in the neck of the flask and loosening a few times to relieve pressure. Continue heating and shaking for 3 to 5 min. Weigh the flask and, if necessary, add water drop by drop to ensure the presence of 60 g. of water. Allow the flask to stand at room temperature for half an hour, with occasional gentle shaking, and then leave it overnight in a water-bath at 18° C. (not below 15° C.). Check the weight and, if necessary, add a few drops of water to ensure that the weight of water present is 60 $\pm$ 0·1 g. Place the flask in a water-bath at 25° C. and shake gently. Remove the stopper and stir the separated crystals of dipentaerythritol, keeping the flask at 25° C. for at least 10 min. Transfer the crystals to a weighed, 15-ml. Gooch-type fritted-glass crucible of medium porosity, effecting the transfer by means of exactly 40 ml. of denatured alcohol applied from a small wash-bottle. Wash the crystals with 10 ml. of ethyl ether and dry at 110° C. for 1 to 2 hr., or to constant weight.

Percentage of dipentaerythritol

$$= 100\,(A - 2{\cdot}500 + B)/2{\cdot}500,$$

where A = weight of dry crystals obtained and B is the solubility correction for the dipentaerythritol remaining in the filtrate and the washings; for the conditions specified this correction is 0·145 g.

The added dipentaerythritol must be of high purity, and the conditions followed accurately. It may sometimes be advisable to run a blank determination, a known mixture of about the same composition as the unknown being used, to test the solubility correction. The precipitated crystals should not be allowed to cake to a hard mass, but should be present as relatively fine, individual plates readily removable by means of a stirring rod and a stream of alcohol from a wash-bottle. The sample taken for analysis should be representative of the mixture under examination; dipentaerythritol has a very low packing density and a

pronounced tendency to segregate when mixed with the denser and more compact pentaerythritol crystals. E. M. P.

Identification of Acidic Oxycellulose by means of Aromatic Amines and Diamines. Matya Krajčinović and Mladen Krajčinović (*J. Text. Inst.*, 1947, **38**, T11–T12)—The test depends on the formation of additive compounds between oxycellulose and aromatic amines or diamines, the additive compounds being identified by introduction of an azo group as chromophore in the benzene radicle and development of a dye. With the diamines, the amino group not combined with the carboxyl group of the oxycellulose was diazotised and the product was coupled to the oxycellulose with β-naphthol or other developer. The monoamines used were aniline, dimethylaniline, and α-naphthylamine, and the dyes were developed with diazotised *p*-nitraniline, *m*-nitraniline, and α-naphthylamine. As diamines there were used benzidine, *p*-phenylenediamine, *m*-toluylenediamine, and chloro-*m*-phenylenediamine, the coupling agents being β-naphthol, the α-naphthalide of 2 : 3-oxynaphthoic acid, Naphthol AS-BO, resorcinol, ethyl-β-naphthylamine, and phenylmethylpyrazolone.

Diamine giving the diazo-component	Developer used as coupling component: β-Naphthol	Naphthol AS-BO	Phenylmethyl-pyrazolone	Resorcinol	Ethyl-β-naphthylamine
Benzidine	reddish-violet	pale violet	orange-yellowish	yellowish-brown	pale pink
p-Phenylenediamine ..	pale reddish-violet	pale violet	orange	brownish-yellow	pale pink
m-Toluylenediamine ..	pale reddish-violet	pale violet	orange-yellowish	brownish-yellow	pale brown
Chloro-*m*-phenylene-diamine	pink	violet-pink	orange-brown	pale brown	faintly pink

Method, using benzidine and β-naphthol. Moisten about 0·5 g. of fibre with 2 ml. of 0·1 *M* alcoholic benzidine solution and rinse well in water after 1 or 2 min. Diazotise by applying 15 ml. of 3 per cent. hydrochloric acid and 5 ml. of 3 per cent. sodium nitrite solution, and rinse well after 2 or 3 min. Develop the colour by adding a few drops of 0·1 *M* β-naphthol in alkaline solution, and wash the sample. The alcoholic benzidine solution must be neutral before testing, and the fibre must be treated with 0·5 per cent. hydrochloric acid for 30 min. and washed until neutral.

The above table shows the results given with diamines after diazotisation and coupling with the developers indicated. E. M. P.

Cellulose in Wood. T.A.P.P.I.Tentative Standard T17 m–46. Anon. (*Paper Trade J.*, 1946, **123**, Dec. 26th, *T.A.P.P.I. Sect.*, 217–218)—Weigh accurately approximately 2 g. of air-dry, disintegrated wood of known moisture content, in a tared, 40-ml. alundum crucible (porosity, R.A.98) contained in a weighing bottle. Extract for at least 6 hr. in a Soxhlet apparatus with a mixture of 33 volumes of 95 per cent. ethyl alcohol and 67 volumes of C.P. benzene, evaporate off the solvent, and wash the residue well with hot water, using suction. Transfer the residue to a 40-ml., glass crucible with a fritted base (porosity, Pyrex M), from the base and top of which glass tubes lead, through 3-way taps (connecting with the air), to a Hempel gas pipette and to a Hempel precision gas burette, respectively. The crucible and gas burette are water-jacketed at 23·5° to 32° C., and the burette and pipette should contain saturated calcium chloride solution, saturated with chlorine at room temperature. Remove surplus moisture from and distribute residual moisture throughout the sample by suction, first at the base and then at the top of the crucible. Pass approximately 230 ml. of chlorine, from the burette, up through the sample and into the pipette; this first chlorination should be completed in 3 to 4 min. Remove the crucible, wash the contents successively with 50 ml. each of water, approximately 3 per cent. sulphur dioxide solution, water, and freshly prepared 2 per cent. sodium sulphite solution, and transfer them to a 250-ml. Pyrex beaker by means of a pointed glass rod. Rinse out the last traces from the crucible, using, firstly four 15-ml. portions of the sulphite solution; then place 10 ml. of the solution in a watch glass and draw it into the crucible by suction from the top, repeating this operation three times. Place the beaker in boiling water for 30 min., replace the fibres in the glass crucible and wash them with 250 ml. of water. Repeat the above chlorination and extraction operations until only a faint pink colour results on adding the sulphite solution, taking only 2 to 3 min. for the chlorination stages, to avoid hydrolysis of the cellulose. Finally, wash the lignin-free wood, in the alundum crucible, successively with 500 ml. of hot water and 50 ml. each of 95 per cent. alcohol and ether, dry it at 100° to 105° C., and weigh it

with the crucible, in the stoppered weighing bottle, to 1 decimal place; report the result as a percentage of the moisture-free, unextracted wood. J. G.

Qualitative Analysis of Mixtures of Cyanamide Derivatives. A. A. Korinfski (*Zavod. Lab.*, 1945, **11**, 816–821)—Cyanamide, dicyandiamide, melamine, urea, thiourea, guanidine, biguanide (diguanidine) $C_2H_7N_5$, dicyanodiamidine (guanylurea), amidodicyanic acid (cyanourea) $C_2H_3ON_3$, ammeline $C_3H_5ON_5$, ammelide $C_3H_4O_2N_4$, sulphides, and thiocyanates may be detected in complex mixtures by the following methods.

Cyanamide—To 5 to 10 ml. of a solution neutralised to litmus with dilute sulphuric acid add cadmium hydroxide in excess, shake, and leave for 3 hr.; filter 2 or 3 ml. of the solution and add 5 or 6 drops of ammoniacal silver nitrate solution to give a yellow precipitate if cyanamide is present. In absence of sulphides or thiourea the cadmium hydroxide precipitation is unnecessary. If very small amounts of cyanamide are to be detected in presence of large amounts of thiourea add, instead of ammoniacal silver nitrate, a few drops of aqueous ammonia and some lead hydroxide. In presence of cyanamide the lead hydroxide assumes a yellow colour. Cadmium hydroxide is prepared by precipitating cadmium acetate solution with sodium hydroxide and washing the precipitate with hot water until the washings give no colour with phenolphthalein. Lead hydroxide is prepared by precipitation with aqueous ammonia.

Urea—The urease reaction should be used; other methods are not sufficiently selective.

Thiourea—Acidify 3 to 4 ml. of the solution with acetic acid, add 0·5 ml. of 5 per cent. cadmium acetate solution, shake, and filter. To the filtrate add sodium hydroxide to precipitate cadmium hydroxide, and boil. A yellow coloration of the hydroxide indicates thiourea (minimum amount detectable 0·02 mg., limiting concentration 1 in 150,000).

Dicyandiamide, $H_2N.C(:NH).NHCN$—The test is based on the formation of crystalline silver monocyanoguanidine picrate (*cf.* Harger, *Ind. Eng. Chem.*, 1920, **12**, 1107; Gelhaar, *Tekn. Tidsk.*, 1933, 23). Acidify 1 or 2 ml. of the solution with acetic acid, add an equal volume of a saturated aqueous solution of picric acid and a few drops (an excess must be used) of 5 per cent. silver nitrate solution, shake, leave undisturbed for 10 to 20 min., and then transfer a drop of the solution, together with the precipitate, to a microscope slide. In presence of dicyandiamide the crystals are in the form of oblong tablets (for sensitivity, see Table).

Melamine—The picrate crystallises from neutral or acetic acid solution as a mass of entangled fibres (rapid crystallisation) or as very fine and long yellow needles (slow crystallisation from warm solution), which may be distinguished under the microscope from the picrates of ammeline and dicyanodiamidine. Melamine picrate alone may be obtained by treating 2 ml. of the solution under test with 2 ml. of glacial acetic acid and 4 ml. of saturated picric acid solution, and then shaking and leaving for 30 min. (see Table).

Ammeline—From slightly acid solution the picrate forms fat, yellow needles. Since ammeline is only slightly soluble in cold water the mixture under test should be extracted with hot water or dilute sodium hydroxide solution. As potassium picrate would precipitate later on, potassium hydroxide should not be used.

Ammelide—In absence of ammeline, treat with 2 per cent. sodium hydroxide solution, filter, and neutralise the filtrate with acetic acid, to give a white amorphous precipitate of ammelide (minimum amount detectable 0·1 mg., limiting concentration 1 in 2500). If congo red indicator is added before neutralisation the ammelide on precipitation adsorbs the indicator and becomes dark red while the solution is decolorised. In presence of ammeline, add to the solution (2 to 3 ml.) of the sample in sodium hydroxide 3 ml. of saturated picric acid solution, heat to boiling, add acetic acid until it gives a faint odour, again heat to boiling, shake, and leave for 30 min. Ammeline picrate and amorphous ammelide are easily distinguished under the microscope.

Guanidine—Add a few drops of aqueous ammonia (avoid an excess since ammonium picrate may precipitate later) to 2 to 3 ml. of the neutral solution of the sample and then an equal volume of saturated picric acid solution, shake, and leave for 1 hr. Guanidine picrate gives fine oblique plates (rapid crystallisation), or long columns (slow cooling).

Biguanide—Garby's reaction with nickel salts (*Ind. Eng. Chem.*, 1926, 819) is used.

Dicyanodiamidine, $H_2N.C(:NH).NH.CO.NH_2$—The picrate from dilute acetic acid or neutral solution consists of small rounded polyhedra. In presence of large amounts of melamine, it is best to use Grossmann's reaction (*Ber.*, 1906, **39**, 3357).

Amidodicyanic acid, $H_2N.CO.NHCN$—In dilute acetic acid solution (*p*H 4) a colourless, amorphous precipitate of $AgC_2H_2ON_3$ is obtained with silver nitrate.

Thiocyanates, by ferric chloride; *and sulphides*, by cadmium acetate.

Mixtures—With a mixture containing all these substances take 2 to 3 g. of the powdered material, shake for 30 min. with 100 to 150 ml. of water, filter, and wash 2 or 3 times with small amounts of water; then treat the filter and insoluble matter with 5 to 6 ml. of 2 per cent. sodium hydroxide solution, filter, and test the filtrate for ammeline and ammelide. On separate portions of the

aqueous extract carry out tests for urea, thiocyanate, ammonium, and cyanamide (cadmium hydroxide - lead hydroxide method) and then apply the following scheme to 25 to 30 ml. of the solution—Neutralise and add 1 ml. of acetic acid and 2 ml. of 5 per cent. cadmium acetate solution (a precipitate of cadmium sulphide indicates sulphide); to the filtrate add sodium hydroxide dropwise to give a white precipitate, heat to 75° to 80° C. and leave for 30 min. (yellow colour of precipitate indicates thiourea); filter, and to the filtrate add an equal volume of saturated picric acid solution and aqueous ammonia (to faint odour), examine a drop containing precipitate under the microscope (guanidine and biguanide indicated); filter, and add to the filtrate 25 ml. of 5 per cent. nitron in 5 per cent. acetic acid (the precipitate is nitron picrate and nitron thiocyanate); filter, and add 10 per cent. ammonium nitrate solution to precipitate nitron completely, filter, and add to the filtrate 5 ml. of 5 per cent. silver nitrate solution and exactly neutralise to methyl orange (a precipitate indicates amidodicyanic acid); filter, and add to the filtrate 1 ml. of nitric acid [concentration not stated], 5 ml. of 5 per cent. silver nitrate solution, and an equal volume of saturated picric acid solution (a precipitate indicates dicyandiamide).

SOLUBILITIES OF PICRATES IN G. PER LITRE AT 20° C. AND (IN BRACKETS) LIMITING CONCENTRATIONS

Substance	Solution A	Solution B	Solution C	Solution D
Melamine	< 0·0005 (1 : 250,000)	> 0·4000 (no ppt.)	0·0006 (1 : 200,000)	0·0024 (1 : 50,000)
Ammeline	0·0039 (1 : 40,000)	0·2032 (~ 1 : 600)	0·0034 (1 : 45,000)	0·0160 (1 : 10,000)
Dicyanodiamidine	0·0048 (1 : 30,000)	0·3148 (no ppt.)	0·0060 (1 : 25,000)	> 0·2000 (no ppt.)
Guanidine	— (1 : 4000)	— (1 : 7000)	— (no ppt.)	— (no ppt.)
Dicyandiamide*	0·0012 (1 : 80,000)	0·2264 (1 : 1000)	0·0012 (1 : 150,000)	not determined do.

* The solubilities refer to silver monocyanoguanidine picrate, the limiting concentrations to dicyandiamide.

The Table shows the solubilities at 20° C. of the picrates of melamine, ammeline, dicyanodiamidine, and silver monocyanoguanidine, and the limiting concentrations detectable through the picrates, in half-saturated aqueous picric acid (A), in half-saturated aqueous picric acid containing 1 per cent. of NH_4 (B), in half-saturated aqueous picric acid containing 5 per cent. acetic acid (C), and in half-saturated aqueous picric acid containing 25 per cent. acetic acid (D). G. S. S.

Determination of Certain Cyanamide Derivatives in Complex Mixtures. A. A. Korinfski (*Zavod. Lab.*, 1946, **12**, 418–421)—

Melamine—Precipitation of the picrate in 5 per cent. acetic acid (Zavarov, *J. Chem. Ind. Russ.*, 1945, part 2 (?)) is unsatisfactory for determining melamine in presence of large amounts of ammeline and dicyanodiamidine. In 25 per cent. acetic acid, however, dicyanodiamidine picrate does not precipitate at all, whilst ammeline picrate may be washed from the precipitate (its solubility is 0·016 g. per 100 ml.), but a small correction has to be made for the solubility of melamine picrate. The method may be used in the presence of ammelide, guanidine, dicyandiamide, and urea. *Procedure*—Mix 0·2 to 0·3 g., or 0·5 g. if the melamine content is less than 30 per cent., of the finely ground powder with 100 ml. of water by stirring with a glass rod for 20 min., filter into a 250-ml. graduated flask through a small filter, wash the insoluble matter on to the filter by means of a small quantity of water, and then wash the filter twice with cold water (this minimises the amount of ammeline in solution). Make up to the mark, mix, take 50 ml., add 50 ml. of glacial acetic acid, heat to 80° C., add 100 ml. of a saturated aqueous solution of picric acid, and leave for 2 hr. in the cold with occasional shaking. Filter by suction through a glass filtering crucible (No. 2), use the filtrate to wash the precipitate on to the filter, then wash four times with a saturated aqueous solution of melamine picrate and three times with ether. Dry for 2 hr. at 100° to 105° C., cool, and weigh. Calculate the melamine percentage from the formula, $(a + 0{\cdot}0048) \times 0{\cdot}355 \times 500/w$, where a is the weight of precipitate and w the weight of sample taken.

Ammeline—The determination of ammeline as picrate in presence of melamine and dicyanodiamidine is based on the low solubility of ammeline in water. *Procedure*—Add 100 ml. of water to a sample containing from 0·1 to 0·15 g. of ammeline in a 250-ml. conical flask, stopper, and shake for 30 min. Filter and wash two or three times with

small portions of cold water, place the filter paper and insoluble matter in a 250-ml. graduated flask, add 15 ml. of 2 per cent. sodium hydroxide solution, shake for 10 to 15 min., make up to the mark, mix, and filter. Take 50 ml. of the filtrate, add 55 ml. of saturated aqueous picric acid solution, heat to boiling, add 5 ml. of glacial acetic acid, and leave at room temperature for not less than 8 hr. Filter by suction through a weighed, glass filtering crucible (No. 2), use the filtrate to transfer the precipitate, wash with saturated ammeline picrate solution, and dry the residue at 90° C. for 2 hr. Ammeline, per cent., $= (a + 0{\cdot}0037) \times 0{\cdot}3395 \times 500/w$, where a is the weight of picrate, 0·0037 a correction for solubility, and w the sample weight. The method is unsuitable in presence of ammelide.

Thiourea—in presence of cyanamide and its derivatives, of sulphides, and of thiocyanates (*cf. Idem., ibid.*, 1945, **11**, 816). *Procedure*—Place a sample containing 0·1 to 0·2 g. of thiourea in a 250-ml. graduated flask, dissolve it by shaking with 150 ml. of water, acidify with acetic acid, add 10 ml. of 10 per cent. cadmium acetate solution to remove sulphide ions, make up to the mark, mix, filter off 50 ml., add to it 50 ml. of water, and then gradually 20 per cent. sodium hydroxide solution to precipitate cadmium hydroxide and to give an excess of 1 to 2 ml. Heat to 80° C., stirring with a glass rod, set aside to cool for 1 hr., acidify with acetic acid, filter through an ashless paper, and wash five or six times with water. Place the filter and precipitate in a beaker, break up the paper by means of a glass rod, add 50 ml. of 0·1 *N* iodine solution and 5 ml. of concentrated hydrochloric acid, and titrate with 0·1 *N* sodium thiosulphate, using starch as indicator. One ml. of 0·1 *N* iodine is equivalent to 0·0038 g. of thiourea.

Dicyandiamide, $H_2N.C(:NH).NHCN$—(i) If thiocyanates are present, dissolve a sample containing 0·1 to 0·2 g. of dicyandiamide in 100 ml. of water, acidify with acetic acid, add 10 ml. of a 5 per cent. solution of nitron in acetic acid, filter into a 500-ml. graduated flask, wash the filter four times with water, treat the filtrate with 15 ml. of 10 per cent. ammonium nitrate solution, shake, make up to the mark with water, mix, and filter. Take 100 ml. of the filtrate and determine dicyandiamide by the volumetric picric acid - silver nitrate method (the precipitated complex silver picrate is dissolved in nitric acid and the silver is determined by titration as in the U.S.S.R. Specification 10175—39). (ii) If sulphides and thiourea are present, dissolve a sample containing 0·1 to 0·2 g. of dicyandiamide in 100 ml. of water in a 250-ml. graduated flask, exactly neutralise the solution to methyl red by means of acetic acid and sodium hydroxide, add 20 to 25 ml. of cadmium hydroxide emulsion (Korinfski, *Ibid.*, 1945, **11**, 816), heat to 80° C., allow to stand for 1 hr., make up to the mark, mix, and filter. Carry out the volumetric determination on 50 ml. of the filtrate. G. S. S.

Determination of Hydroperoxides in Rubber and Synthetic Polymers. H. A. Laitinen and J. S. Nelson (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 422–425)—The method is intended for the determination of relative quantities of hydroperoxides in natural rubber and various synthetic polymers and co-polymers, and is based on the reaction between the hydroperoxide and ferrous iron in benzene - methanol solution, the excess ferrous iron being determined colorimetrically by means of *o*-phenanthroline as reagent. Interference by antioxidants is prevented by the presence of phosphoric acid.

Reagents—Ferrous iron solution—Remove the dissolved oxygen from 1 litre of methanol by bubbling nitrogen through it for 30 min. Dissolve 19·605 g. of ferrous ammonium sulphate hexahydrate in the methanol containing 13·9 ml. of concentrated sulphuric acid to make the solution about 0·25 *M* with respect to sulphuric acid. Stored under nitrogen, this solution is stable for a long time. For use over a period of a few days, 10-ml. portions may be allowed to stand in air. Each day, prepare a 0·002 *N* solution by diluting 2 ml. of the 0·05 *N* solution to 50 ml. with methanol, but without adding sulphuric acid. *Nitric acid - methanol solution*—Add 1 ml. of concentrated nitric acid to 20 ml. of methanol slowly and with cooling. *Phosphoric acid - methanol solution*—Prepare a molar stock solution by adding 6·75 ml. of 85 per cent. phosphoric acid (14·8 *M*) to methanol, to make 100 ml. of solution. Dilute the molar solution to 0·04 *M* for use as a working solution; 0·5 ml. of 0·04 *M* solution provides 0·02 mg.-mol. of phosphoric acid. *o-Phenanthroline solution*—Dissolve 1 g. of *o*-phenanthroline monohydrate in 1 litre of thiophene-free benzene.

Analysis of unknown samples—Pipette 1 ml. of rubber solution, containing about 1 g. of polymer in 100 ml. of benzene, into a 50-ml. volumetric flask. Add about 25 ml. of thiophene-free benzene, washing any polymer from the sides of the flask. Add 1 ml. of nitric acid - methanol solution and 0·5 ml. of the 0·04 *M* phosphoric acid solution, shaking well after each addition. Add 1 ml. of 0·002 *N* ferrous iron solution, shake well, and allow to stand for at least 15 min. Add 3 to 5 ml. of *o*-phenanthroline solution and make up to 50 ml. with thiophene-free benzene. Read the transmission value at 508 mμ. with a spectrophotometer or use a photo-electric colorimeter with a filter showing maximum transmission at 500 mμ. or 510 mμ. (preferably not 520 mμ.), comparing the transmission with that of benzene. If the transmission value is between 50 and 70 per cent., calculate the

active oxygen content as shown below. Otherwise, estimate the necessary adjustment in sample size and repeat the determination. With samples of entirely unknown peroxide content, vary the sample size, using 0·1, 1, and 10 ml. of polymer solution in the preliminary estimation. If a 10-ml. sample is used, or if the sample is highly coloured, use a diluted benzene solution of the polymer instead of benzene as the comparison liquid in the optical measurement.

Construction of calibration curve—Using a graduated 1-ml. pipette, add 0·2, 0·4, 0·6, 0·8, and 1·0 ml. of 0·002 *N* ferrous iron solution to a series of 50-ml. volumetric flasks. Add about 25 ml. of benzene to each flask, washing the walls carefully. Form the ferrous iron complex by adding 3 to 5 ml. of the *o*-phenanthroline solution and make up to 50 ml. with benzene. Take colorimeter or spectrophotometer readings relative to benzene. Compute the optical density of each solution from the equation $D = \log I_0/I$, and construct a graph of optical density against concentration of iron, expressed as equivalents of ferrous iron per 50 ml. A straight line, corresponding to Beer's law, should be obtained.

Calculations—From the calibration curve read the amount of iron remaining unoxidised and determine by difference the amount of iron oxidised. Each g.-equivalent of iron corresponds to one g.-equivalent or 8 g. of active oxygen. Calculate the active oxygen as p.p.m. of solid polymer. If the polymer is only partly soluble, determine the concentration of the soluble polymer by evaporating an aliquot of the solution, and base the calculation on the soluble part only. $(A - B) \times 8 \times 10^6/C \times V$ = micrograms of active oxygen per g. of polymer = p.p.m. of active oxygen, where A = g.-equivalents of ferrous iron taken; B = g.-equivalents of ferrous iron remaining; C = concentration of polymer in g. per ml. of benzene; V = ml. of polymer solution used.

The results are reproducible to about 20 per cent: on a given rubber solution, but sampling difficulties may cause larger variations on solid samples. The method is sensitive to 10 or 20 p.p.m. of active oxygen, calculated on the solid polymer, and can be made sensitive to 1 p.p.m. by increasing the sample size and compensating for its colour by using a polymer solution instead of benzene as the comparison liquid. For results of absolute significance, standardisation by means of *tert.*-butyl hydroperoxide is recommended. The method is applicable only to hydroperoxides and can be used generally for compounds of low molecular weight as well as for high polymers. E. M. P.

Polarographic Investigations of Oxalate, Citrate, and Tartrate Complexes of Ferric and Ferrous Iron. J. J. Lingane (*J. Amer. Chem. Soc.*, 1946, **86**, 2448–2453)—The ferric ion gives rise to a polarographic reduction wave in acid tartrate, citrate, or oxalate solutions, of which the citrate medium is best for analytical purposes. Between *p*H 5 and *p*H 7 the reaction is reversible and the ferrous ion can be determined by its anodic wave.

In 0·5 *M* sodium citrate solution containing 0·005 per cent. of gelatin, at *p*H 6, the diffusion current constant, $i_d/(Cm^{2/3}t^{1/6})$, where i_d is the diffusion current, C the concentration of ferric, or ferrous, ion, m the mass of mercury flowing per sec., and t the drop time, is constant to within ± 1·5 per cent., and has a value of 0·93 ± 0·01 micro-amp./millimole/litre/mg.$^{2/3}$sec.$^{-1/2}$ at 25° C. for the cathodic wave of the ferric ion. The corresponding value for the anodic wave of the ferrous ion is 0·90 ± 0·01. At *p*H 6, the half-wave potential is − 0·22 v. *versus* the saturated calomel electrode.

Similar waves are obtained in 0·5 *M* sodium tartrate and 0·5 *M* sodium oxalate, under the same conditions, the values of the diffusion current constants of the cathodic ferric waves being 1·11 ± 0·03 and 1·50 ± 0·03 micro-amp./millimole/litre/mg.$^{2/3}$ sec.$^{-1/2}$, respectively.

In carrying out determinations on solutions containing the ferrous ion, it is necessary to add a measured volume of ferrous solution to a known volume of supporting electrolyte that has been freed from dissolved air by hydrogen or nitrogen, owing to the ease of oxidation of the ferrous complexes. J. G. W.

Inorganic

Micro-determination of Chlorides by means of Mercurous Iodate. K. Avaliani (*Zavod. Lab.*, 1946, **12**, 179–182)—Soluble chlorides react with solid mercurous iodate to give mercurous chloride and soluble iodate equivalent to the amount of chloride initially present in solution. After filtration, the soluble iodate may be determined by addition of potassium iodide and titration of the liberated iodine with thiosulphate.

Procedure—To 10 ml. of the solution under examination (preferably a sulphuric acid solution of *p*H 2·2) in a test tube, add from the end of a spatula about five times the theoretical quantity of powdered mercurous iodate, stopper the tube, shake for 2 min., filter through a dry paper, rejecting the first portion of the filtrate, and titrate 1 ml. with 0·01 *N* sodium thiosulphate after adding potassium iodide. Calculate the chloride content according to the equation

$$Hg_2(IO_3)_2 + 2NaCl = Hg_2Cl_2 + 2NaIO_3,$$

making a small correction, if necessary, as indicated below, to allow for the fact that the amount of iodate formed is a few tenths of 1 per cent. less than the theoretical.

The reagent is obtained from potassium iodate

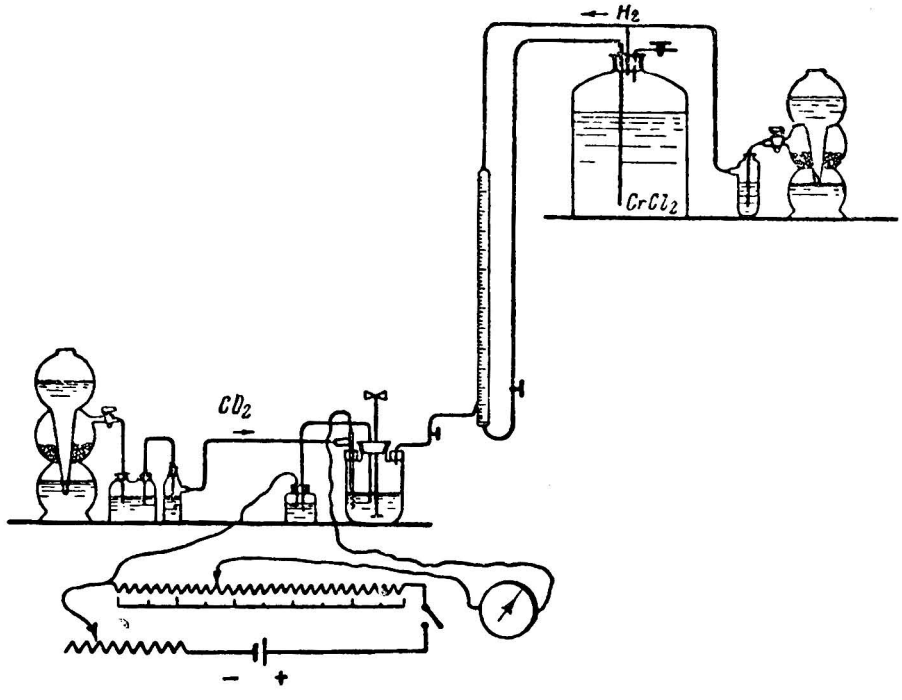
and mercurous nitrate by double decomposition. The salt is then washed with water until successive washings give the same colours with sulphuric acid and potassium iodide; it is dried over sulphuric acid and phosphorus pentoxide, and kept in a finely powdered form in the dark. The solubilities of mercurous iodate at 25° in water, and in 0·01 *N* sulphuric acid (*p*H 2·2), are too small to have any effect upon the results.

Within the range of chloride content 1 to 12 mg.-mols. per litre (or 0·058 to 0·701 g. of sodium chloride per litre), results are about 99·4 per cent. of the theoretical. G. S. S.

Bivalent Chromium Solution for the Volumetric Determination of Tungsten. Determination of Tungsten in Tungsten Concentrates and Ferro-tungsten. J. A. Tschernichov and V. G. Gorjuschina (*Zavod. Lab.*, **1946**, **12**, 397–411)—The authors have shown (*Ibid.*, 1945, **11**, 137) that chromous chloride reduces saxavalent tungsten quantitatively to quinquevalent tungsten with great speed in concentrated hydrochloric acid in the cold. Usually chromous chloride titrations require the use of hot solutions, and Flatt and Sommer (*Helv. Chim. Acta*, 1944, **27**, 1518) have recently described a method for tungsten which suffers from this disadvantage. From a study of various factors influencing the reaction, details are now given of the conditions necessary for determining tungsten by means of a standard solution of chromous chloride.

Apparatus—The reservoir for the titrating solution is a 3-litre bottle of dark glass. It is fitted with a three-hole rubber bung carrying a siphon connected to the bottom of a burette, a tube connecting the space in the bottle with both a hydrogen generator and the top of the burette, and a tube with a tap. The titration vessel is a bottle of 400-ml. capacity with four necks; the central neck carries a rubber bung with a hole which is used both for the insertion of a stirrer and for outlet of the carbon dioxide which is passed, during the titration, through the solution. Through the other three necks pass (i) a carbon dioxide inlet tube and a platinum indicator electrode, (ii) a saturated potassium chloride bridge connected with a saturated calomel half-cell, and (iii) the jet of the burette. Electrical connections are made in the normal manner for potentiometric titrations (see Fig.).

Effect of complex formation—Heteropoly compounds—In presence of phosphoric acid, the potential jump at the end-point of the titration is less definite; thus with 5 ml. of 0·1 *M* sodium tungstate in 100 ml. of concentrated hydrochloric acid, not more than 10 ml. of phosphoric acid (sp.gr. 1·7) may be present. Silicic acid, even in large amounts, has no effect. *Organic acids*—Titration curves obtained with concentrated hydrochloric acid solutions of tungsten are not affected in any way by the presence of citric, tartaric, oxalic, or formic acid. In more dilute hydrochloric acid, correct results may be obtained with oxalic acid present, although the



potential at the equivalence point is lower and the technique of titration is more difficult, but not with the other organic acids, owing to formation tungsten blue.

Replacement of hydrochloric acid by sulphuric acid—Without a reduction in the total acidity, clear solutions may still be obtained by using a mixture of 50 ml. of concentrated hydrochloric acid and 30 ml. of concentrated sulphuric acid in place of 100 ml. of the former acid when 5 ml. of 0·5 *M* sodium tungstate are taken. A somewhat smaller potential jump occurs at the end-point. Quantitative reduction does not occur when hydrofluoric or phosphoric acid is used instead of sulphuric acid.

Effect of various elements—Since only a single stage is involved in the reduction of tungsten and the factor for calculation is large, the method can be recommended only for the determination of large amounts of tungsten, *e.g.*, in tungsten concentrates and in ferro-tungsten. The possible effects of iron, molybdenum, chromium, and copper are important. *Iron*—The redox potentials of Fe^{II}/Fe^{III} (+ 0·75 v.) and W^{V}/W^{VI} (+ 0·26 v.) are so different that two clearly defined potential jumps are observed; thus, even with 10 ml. of 0·1 *N* iron alum solution the titration of 5 ml. of 0·1 *M* sodium tungstate in 100 ml. of concentrated hydrochloric acid is unaffected. If oxalic acid is also present, the reduction of iron and tungsten occurs simultaneously and slowly, and titration gives the combined iron and tungsten content. *Molybdenum*—In concentrated hydrochloric acid solution, molybdenum alone gives indications of the transitions $Mo^{VI} \rightarrow Mo^{V}$ and $Mo^{V} \rightarrow Mo^{III}$, but with tungsten also present no steps are found on the titration curves before the tungsten end-point, which then corresponds to reduction of tungsten to the quinquevalent stage and molybdenum to the tervalent stage. *Chromium*—The redox potential of CrO_4''/Cr''' is + 1·3 v.; chromates are therefore reduced well before tungsten and have no effect on the determination. *Copper* is first reduced to cuprous chloride and the conditions are then unfavourable for reduction to metal; no interference with the tungsten determination is observed.

Titration of reduced tungsten with an oxidising agent—For the determination of molybdenum and titanium in steels, etc., Klinger, Stengel, and Koch (*Arch. Eisenhüttenw.*, 1934–35, **10**, 433) reduced first with zinc and later with chromous chloride in excess; on addition of an oxidising agent two points were obtained on the titration curve, the first indicating oxidation of the excess of chromous chloride and the second, oxidation of the sought-for element. In a similar case, Sirokomski and Shukov (*Zavod. Lab.*, 1945, **11**, 373) used air to oxidise the excess of reducing agent. These methods are, however, unsuitable for tungsten, since in the former case the transition $Cr^{II} \rightarrow Cr^{III}$ is not detectable and in the latter, air oxidation may be used only when the redox potential is not less than + 0·4 v.; hence quinquevalent tungsten (+ 0·26 v.) is oxidised before the titrant is introduced. It is recommended, therefore, to reduce with chromous chloride under potentiometric control so that no excess is added, and to titrate the tungsten in the same apparatus by means of an oxidising agent (dichromate). For this, a standard solution of chromous chloride is not required; chromous chloride serves merely in place of solid reducing agents (zinc or amalgams), but its use, with a potentiometric end-point, ensures complete reduction of tungsten and avoids the need for separating the liquid. The oxidimetric titration is preferable to the reductometric since interference by other elements is less and fewer separations are required.

Procedure—Decomposition of the material (i) *Scheelite concentrate.* Heat 0·25 g. of the finely ground material in a small porcelain dish with 4 to 5 ml. of concentrated hydrochloric acid (addition of nitric acid is unnecessary), evaporate nearly to dryness, treat with 3 ml. of 20 per cent. sodium hydroxide solution, and, after solution of the tungstic acid, add 10 ml. of saturated oxalic acid solution to ensure a clear solution later, particularly in the presence of much iron. Transfer the solution to the titration vessel which already contains 100 ml. of concentrated hydrochloric acid. (ii) *Wolframite concentrate.* Dissolve 0·25 g. of the material, finely-ground in an agate mortar, in 25 ml. of concentrated hydrochloric acid in a 75- to 100-ml. beaker under cover. Heat gently and, after decomposition, add 3 ml. of concentrated nitric acid to attack pyrites. Evaporate nearly to dryness, and add formalin dropwise until the evolution of brown fumes ceases. Add to the nearly dry residue 3 ml. of 20 per cent. sodium hydroxide solution, mix to dissolve the tungstic acid, add 10 ml. of saturated oxalic acid solution, and pour into the titration vessel containing 100 ml. of concentrated hydrochloric acid. (iii) *Ferro-tungsten.* Treat 0·2 g. of the finely ground material with 2 or 3 ml. of hydrofluoric acid and a few drops of concentrated nitric acid in a platinum dish, and, after dissolution, add a few drops of formalin, and then 2 ml. of concentrated hydrochloric acid when the nitric acid has been fully decomposed. Evaporate nearly to dryness, add sodium hydroxide, and continue as described above.

Titration—(i) Direct titration. Connect up the titration cell, etc., as indicated above and titrate with 0·1 *N* chromous chloride solution (0·1 ml. is equivalent to 0·0184 g. of W or 0·0232 g. of WO_3) taking as initial point the potential jump due to iron. Standardise the chromous chloride solution each day on 0·1 *N* copper sulphate solution, using

10 to 15 ml. in 100 ml. of water containing 5 ml. of concentrated hydrochloric acid, heated to boiling during the titration. (ii) Dichromate titration of the reduced solution. Titrate potentiometrically with chromous chloride solution, which need not be standardised, until the potential jump due to tungsten is obtained. Replace the burette by one containing 0·1 *N* potassium dichromate and titrate to re-oxidise tungsten. G. S. S.

Determination of Gold by a Back-titration Method. L. I. Tzobar (*Zavod. Lab.*, 1946, **12**, 506–507)—A rapid, accurate, and economical method of determining gold in gold alloys is based on reduction with an excess of standard ferrous iron solution and titration of the residual ferrous iron with permanganate.

Procedure—Dissolve 0·1 g. of the sample in 4 to 5 ml. of *aqua regia* by heating on a sand-bath, evaporate to a small bulk, and, if the alloy contains no copper, add 2 to 3 ml. of 0·1 *N* cupric chloride, and then run in from a burette saturated sodium (or ammonium) bicarbonate solution until a precipitate forms and the yellowish liquid turns greenish. Clear the solution with 8 to 10 drops of 4 *N* sulphuric acid, boil and then cool, add 25 ml. of 0·1 *N* ferrous ammonium sulphate in dilute sulphuric acid, and shake for 30 sec. The precipitated gold quickly settles (the presence of silver chloride from any silver present in the alloy assists). Introduce 2 to 2·5 ml. of the Zimmermann-Reinhardt reagent, and titrate the excess of ferrous salt by means of 0·025 *N* potassium permanganate. G. S. S.

Silver Sulphide method for Determining Cadmium. N. N. Lapin (*Zavod. Lab.*, 1946, **12**, 158–160)—Although cadmium can be precipitated quantitatively by means of hydrogen sulphide, volumetric or other methods of determining cadmium based upon a determination of sulphide in the precipitate fail because the latter always contains compounds other than CdS, *e.g.*, Cd_2Cl_2S. Determination of cadmium in the precipitate by gravimetric means takes, however, considerable time. It is now shown that a rapid volumetric determination, accurate to 1 part in 100, can be carried out by treating the washed precipitate with a known volume of standard silver nitrate solution, filtering, and titrating the residual silver nitrate. The cadmium sulphide is transformed completely into insoluble silver sulphide and any chloride in the precipitate forms silver chloride, so that the amount of precipitated silver salts corresponds to that of the cadmium compounds in the precipitate decomposed.

Procedure—In 100 ml. of the solution (*e.g.*, of 0·3 g. of cadmium nitrate) mixed with 5 ml. of concentrated hydrochloric acid, precipitate cadmium sulphide by means of hydrogen sulphide, filter, and wash the precipitate until soluble chlorides and sulphides are removed. Transfer the precipitate and paper to a conical flask, add 30 ml. of 0·1 *N* silver nitrate, close the flask, shake vigorously for 3 to 5 min. to break up the paper and precipitate, remove and wash the stopper, heat the solution for 5 min. on an electric hot-plate, filter into a titration flask, washing all the soluble silver salts into the flask, and titrate with potassium thiocyanate solution, after adding 2 ml. of 10 per cent. iron alum solution and 2 ml. of concentrated nitric acid. Calculate the cadmium content from the amount of silver nitrate consumed. G. S. S.

Determination of Nickel and Cobalt in Iron Ores with Preliminary Removal of Iron by means of Sodium Fluoride. I. V. Tananaev and V. G. Silnitschenko (*Zavod. Lab.*, 1946, **12**, 140–141; *Metallurgia*, 1946, **35**, 122)—When iron has to be separated before a determination of nickel or cobalt, sodium fluoride may be used to give a dense, crystalline white precipitate of $5NaF.2FeF_3$ which is easily filtered off; it contains all the iron and negligible amounts of nickel and cobalt.

Procedure—With iron ores, boil 1 g. of the sample with 15 ml. of concentrated hydrochloric acid to complete decomposition, dilute to 100 ml. with water, and add aqueous ammonia until a precipitate appears. Clear the solution by adding a small amount of hydrochloric acid, and treat the hot solution with 100 to 120 ml. of a hot 4 per cent. solution of sodium fluoride. Filter after 8 to 10 min. In the filtrate determine (i) nickel by means of dimethylglyoxime, after adding aqueous ammonia or sodium acetate, or (ii) cobalt by making ammoniacal and electrolysing. G. S. S.

Separation of Niobium and Tantalum from Titanium. V. A. Oschman (*Zavod. Lab.*, 1946, **12**, 154–157)—Hydrolysis of niobium and tantalum salts in sulphuric acid solutions of low *p*H always gives incomplete precipitation of the earth acids, whilst at high *p*H, contamination by other components of the solution is apt to occur. In hydrochloric acid solutions of high concentration, however, these difficulties are largely removed, but large amounts of titanium and zirconium still interfere. It is now shown that, if the titanium is reduced to the tervalent state before hydrolysis, considerable quantities of titanium may be present without contaminating the earth acid precipitate obtained in hydrochloric acid solution, since tervalent titanium salts are more stable towards hydrolysis than are quadrivalent titanium salts (*cf.* ferrous and ferric salts). In the presence of large amounts of both titanium and zirconium, niobium is incompletely precipitated, but with zirconium alone, or in small amount accompanied by titanium, the interference is not serious.

Procedure—Treat 0·2 to 2 g. of sample (*e.g.*, ilmenite concentrate containing 10 per cent. of

earth acids, $Nb_2O_5 + Ta_2O_5$, and up to 80 per cent. of titania, together with iron, etc.) with hydrofluoric acid and sulphuric acid to remove silica, fuse the dry residue with pyrosulphate, and extract with a mixture of 10 ml. of concentrated hydrochloric acid, 4 ml. of 30 per cent. hydrogen peroxide, and 36 ml. of water, warming gently but avoiding, as much as possible, decomposition of the peroxide. Cool, filter, and repeat the fusion on the insoluble matter if it is considered desirable. Add 5 ml. of hydrochloric acid (if a second fusion has been carried out, extract the melt with a mixture containing only 5 ml. of acid, and after mixing with the first extract, add no additional acid), transfer to a Someya reduction apparatus (or to a separating funnel, capacity 300 to 500 ml., joined below the tap to a test tube by means of a short rubber tube), containing 200 g. of pure zinc amalgam, remove air by means of carbon dioxide, close the reductor, and shake for 8 to 10 min. after the brown colour of ferric chloride has disappeared. During the shaking, the colour changes through violet (tervalent titanium) to green if niobium is present, the latter colour serving as an indication that reduction of titanium is complete; with tantalum minerals no green colour may appear, but 10 min. may be considered sufficient for the reduction of 1 g. of titanium dioxide if freshly prepared amalgam has been used (normally the amalgam may be used 5 to 6 times before replacement). Remove the amalgam from the reductor. If the separating funnel is used, the amalgam is allowed to flow into the test tube and is washed by the upward flow of water contained in the test tube, and the volume of the tube and rubber connection should be just sufficient to hold all the amalgam. Pour the solution into a beaker, if necessary through a filter, dilute with boiling water to 500 ml., heat quickly to the boiling point, and maintain the solution at this point for 20 min., then add some paper pulp, and filter through an 11-cm. paper containing paper pulp, washing with hot, diluted hydrochloric acid (4 : 100) until sulphates are removed. Ignite the paper and precipitate to constant weight, fuse the residue with 3 g. of pyrosulphate, extract the melt with 20 ml. of diluted sulphuric acid (1 : 3) containing 3 ml. of perhydrol, make up to 50 ml., and determine the titanium colorimetrically. To determine zirconium in the extract, add 10 ml. of 20 per cent. ammonium phosphate solution, maintain at 60° C. for 2 hr. (or, better, overnight), filter, wash the precipitate with 5 per cent. sulphuric acid containing perhydrol, and then with 4 per cent. ammonium nitrate solution, and ignite and weigh as ZrP_2O_7. Deduct from the weight of impure earth acids the weights of TiO_2 and ZrO_2 calculated from the results of the titanium and zirconium determinations.

Some typical results are—(i) Zirconium absent. $(NbTa)_2O_5$ taken 1·0 mg., found 1·0, 1·2 (TiO_2 500 mg.); taken 14·9 mg., found 15·3 (TiO_2 500 mg., Fe_2O_3 100 mg.); taken 197·9 mg., found 200·0 (TiO_2 1000 mg.). (ii) Moderate amounts of zirconium present. $(NbTa)_2O_5$ taken 1·0 mg., found 1·5 (TiO_2 200 mg., ZrO_2 10 mg.); taken 2·0 mg., found 2·1 (TiO_2 nil, ZrO_2 6 mg.); taken 4·9 mg., found 5·0 (TiO_2 0·1 mg., ZrO_2 15 mg.); taken 9·9 mg., found 9·4 (TiO_2 0·1 mg., ZrO_2 30 mg.). (iii) Titanium and zirconium present in large amounts. $(NbTa)_2O_5$ taken 7·9 mg., found 3·0 (TiO_2 400 mg., ZrO_2 50 mg.); taken 29·7 mg., found 13·6 (TiO_2 500 mg., ZrO_2 80 mg.); taken 59·4 mg., found 33·5 (TiO_2 500 mg., ZrO_2 150 mg.). G. S. S.

Agricultural

Rapid Semi-micro Volumetric Determination of Potash in Leaf Ash. G. W. Chapman (*J. Agric. Sci.*, 1947, **37**, 29–31)—Results with a maximum error of 1 per cent. may be obtained rapidly by the decomposition of potassium cobaltinitrite, precipitated by the usual methods, in the presence of disodium monohydrogen phosphate to prevent the formation of insoluble cobalt compounds during the formation of sodium nitrite. The cobalt phosphate formed is readily soluble in the dilute sulphuric acid used in the permanganate titration.

Procedure—Treat a sample containing 2 mg. of potassium oxide with 6 drops of a mixture of 1 part of sulphuric acid, 1 part of water, and 2 parts of hydrochloric acid, and digest for 1 hr. on the water-bath, and then for 1 hr. on an air-bath at 160° to 200° C. There should be no appreciable excess of sulphuric acid. Add 5 ml. of water, shake occasionally during 15 min., and pipette 2 ml. of the clear liquid into a 15-ml. centrifuge tube. Add 2 ml. of cobaltinitrite reagent (Lewis and Marmoy, *J. Soc. Chem. Ind.*, 1933, **52**, 177) quickly, and leave to stand for about 4 hr. to complete precipitation; decomposition occurs on standing for some days. Add a few drops of a 70 per cent. solution of camphor in ethanol to sink any precipitate that floats, and centrifuge for 5 min. in a centrifuge of 9-inch radius at 3000 r.p.m. Decant most of the liquid, add 5 ml. of 70 per cent. ethanol and centrifuge again. Drain off the liquid and wash twice more with ethanol, then drain thoroughly and dry in a water-oven.

Add 5 ml. of 30 per cent. disodium monohydrogen phosphate solution, stir, and digest on the water-bath for 15 min. to 1 hr. Transfer to a test tube, add an equal volume of diluted sulphuric acid (1 in 8), and titrate with standard permanganate. Use a 10-ml. burette graduated to 0·02 ml., and keep a layer of permanganate on the liquid surface until the approach of the end-point, which may be regarded as the formation of a colour lasting one minute.

The titration factor for the permanganate should be determined at the working temperature, a variation of 1·9 per cent. being obtained between results at 2° and 30° C. The cobaltinitrite reagent is stable at ordinary temperatures after an initial vigorous aeration for 3 hr. Loss of nitric oxide causes results obtained by titration in an open beaker to be 1·2 per cent. low. Calcium, magnesium, iron, and phosphorus, in normal amounts, do not interfere, but calcium gives a considerable positive correction if present in a several-hundred-fold excess. Ethanol, weaker than 70 per cent., gives less efficient centrifuging. M. E. D.

Water Analysis

Rapid Electrometric Determination of the Alkalinity of Sea Water. D. H. Anderson and R. J. Robinson (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 767–769)—The alkalinity of sea water (defined as the number of mg.-equivalents of hydrogen ion neutralised by one litre of sea water at 20° C.) is primarily due to bicarbonates, with lesser amounts of borates, phosphates, etc., forming a buffer system of *p*H about 8. Sea water is about as alkaline as 0·002 *N* sodium bicarbonate solution.

As measured with the glass electrode, $pH = -\log C_{H^+} f_{H^+}$, where f_{H^+} is an empirical constant determined as follows. Titrate 100-ml. samples of sea water of various ionic strengths with 0·01 *N* hydrochloric acid, using the glass electrode as indicator. Take the equivalence point (about *p*H 4·5) as occurring with the greatest change in e.m.f. per unit volume of added acid. Continue the titration to a measured *p*H of about 3 and calculate the normality C_{H^+} of the excess acid from C_{H^+} = (ml. of excess HCl) × (normality of HCl)/(ml. of sample + ml. of HCl). Then calculate f_{H^+} from the final *p*H.

The value of f_{H^+} depends upon the ionic strength of the sample and upon its *p*H at measurement. For a given ionic strength, however, f_{H^+} is sensibly constant in the approximate range *p*H 3 to 3·6. Examination of natural and artificial sea waters ranging in ionic strength from about 0·07 to 1·0 (corresponding to about 2 to 29 g. of chloride per litre) showed that f_{H^+} fell rapidly from 0·844 to about 0·76 (at a chloride concentration of approximately 7 g. per litre) and then remained sensibly constant. A graph or table relating its value to the chloride concentration may thus be prepared.

Procedure—Deliver 100 ml. of sea water into a glass-stoppered, 135-ml. bottle (previously aged for several months with hydrochloric acid of *p*H about 3·5) containing exactly 25 ml. of 0·01 *N* hydrochloric acid. The sample may then be retained for examination ashore. Bring to the desired temperature, measure the *p*H by means of the glass electrode and hence obtain $C_{H^+} f_{H^+}$. Then

$$\text{Alkalinity} = 2{\cdot}500 - 1250\,(C_{H^+} f_{H^+})/f_{H^+},$$

where f_{H^+} has the appropriate value for the sample. Alternatively, tables or graphs relating *p*H and alkalinity may be prepared, or the *p*H meter may be calibrated to read the alkalinity directly. The probable error is about 1 per cent.

The procedure should be applicable to effluents, biological fluids, etc., which are fairly uniform as to ionic strength and concentration of unknown material. J. T. S.

Method for Determining the Total Hardness of Water by means of Soap Solution. E. N. Teterkin (*Zavod. Lab.*, 1946, **12**, 177–178)—Total hardness is determined by comparing the amount of soap solution required to give a stable foam with the sample under test with that required with standards of known hardness. The method has certain advantages over the normal processes: any kind of commercial soap may be used; the standards are prepared from ordinary laboratory chemicals; the end-point is definite; and the difficulties due to the presence of magnesium in the sample and its absence in the standard are overcome by introducing magnesium ions into the standard.

Reagents and standards—Make up a soap solution about 1 to 2 per cent. (calculated after drying the soap for 24 hr. at 30° to 40° C.) in 40 per cent. alcohol, allow to stand for 1 to 2 days, and then filter through a thick filter paper. Prepare an artificial standard of hardness 100 degrees (German) containing magnesium corresponding to the quantity present (say, 25 per cent.) in the waters to be examined in the following way. Obtain a saturated solution of calcium oxide in water, filter, titrate 100 ml. of the filtrate with 0·1 *N* hydrochloric acid, calculate the result to German degrees of alkalinity (say A°), and place 75,000/A ml. in a graduated litre flask. Neutralise with the calculated quantity of 0·1 *N* hydrochloric acid, add 620 mg. of magnesium sulphate heptahydrate (dried at 140° C. to constant weight), and make up to the mark with distilled water. This solution has a hardness of 100 degrees. Construct calibration curves by taking different volumes of this solution corresponding to hardnesses from 1 to 15 degrees with 1 degree intervals, diluting each to 100 ml., and titrating at 18° to 20° C. with the soap solution to a stable foam which does not disappear from the surface during 5 min.; deduct the volume of soap solution required to give a foam with the distilled water used in the preparation of the standards. If the soap solution deposits flakes on keeping, clarify the solution at 30° to 50° C. before use.

Procedure—Titrate with the soap solution at 18° to 20° C., as for the standards and read off the hardness value from the calibration curve.

The method can be applied directly to water of hardness 15 German degrees and less; the error does not exceed 0·10 to 0·25 degree. It cannot be used for water containing much organic matter. G. S. S.

Review

TEXTBOOK OF PHARMACOGNOSY. By T. E. WALLIS. Pp. vi + 504. London: J. and A. Churchill. 1946. Price 28s.

"Pharmacognosy is the objective study of crude drugs of animal and vegetable origin treated scientifically." This sentence from the opening chapter of Dr. T. E. Wallis's new volume is the keynote of the book. His aim has been to produce a book on pharmacognosy giving a new presentation of the subject. The late Professor H. G. Greenish's "*Materia Medica*" was for many years one of the standard works and the publishers suggested that Dr. Wallis should use some of the figures and material from it. The result is that though this book may be regarded as the successor of Professor Greenish's volume, so well known to students, it is now brought up to date and in line with modern views on the subject.

In this work drugs exhibiting cellular structure are classified morphologically, while dried juices, extracts, etc., are grouped according to the method of their preparation. The simpler structures are dealt with in earlier chapters, and the author proceeds successively to more complex structures in the later portions of the book. Final chapters deal with Commerce and Cultivation, Transport and Marketing, the Acquisition of Diagnostic Characters, Deterioration and Storage, Insect and Other Pests, Adulteration and its Detection. An appendix deals with Calcium Oxalate Crystals. The book is well indexed and a glance through it shows the wide range of drugs covered.

Descriptions are systematised and the particulars arranged approximately according to their use in the identification and evaluation of the material in the group. Individual drugs are described under a number of headings in the following order: (1) Origin, (2) Cultivation and Preparation, (3) Characters, macroscopic and microscopic, (4) Constituents and Tests, (5) Evaluation, (6) Adulterants and Substitutes, and (7) Uses. The information given is comprehensive in its scope and fully up to date. The 213 illustrations and histological drawings are noteworthy, the latter (many the work of the author) being exceptionally clear.

Quantitative microscopy, for which the author is so well known and much of which he originated, is stressed where it is of diagnostic importance.

The work is well produced and of convenient size, and the use of different kinds of type makes it easy to pick out the information required under the appropriate heading. Both the author and the publishers are to be complimented on this volume, which will be of use not only to advanced students and workers in pharmacognosy, but also to analysts, pharmacists and others whose work is concerned with crude drugs. S. K. CREWS

SUPPLIES OF LABORATORY CHEMICALS

A FURTHER letter has been received from the Board of Trade to the effect that discussions have taken place with Chemical Suppliers and that a number of difficulties have been disposed of. It is believed that about half of the chemicals reported by members as being unobtainable are now available again. Difficulties presented by the other half are being tackled and it is hoped that the majority will be available in reasonable quantities in the not too distant future.

In order to be satisfied how effectively the position has been improved the Board has asked me to bring to their notice any information that comes before me showing if and where difficulty is still being found.

K. A. WILLIAMS,
Hon. Secretary