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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

NORTH OF ENGLAND SECTION

THE Twenty-fifth Annual General Meeting of the Section was held at Manchester on Saturday, January 28th, 1950. The Chairman, Mr. J. G. Sherratt, presided over an attendance of 35. The Hon. Secretary presented the Report and Financial Statement, which were adopted. Appointments for the forthcoming year were made as follows:—*Chairman*—Mr. J. G. Sherratt. *Vice-Chairman*—Mr. A. A. D. Comrie. *Hon. Secretary and Treasurer*—Mr. Arnold Lees, 87, Marshside Road, Southport, Lancs. *Elected Committee Members*—Messrs. J. R. Edisbury, A. O. Jones, C. H. Manley, R. K. Matthews, F. Morris and J. E. Sands. *Hon. Auditors*—Messrs. C. J. House and J. R. Walmsley.

A number of matters of professional interest were subsequently discussed.

The Estimation of Tomato Solids in Tomato Products by a Method Involving the Determination of Lycopene by Absorption Spectroscopy

BY F. G. STOCK

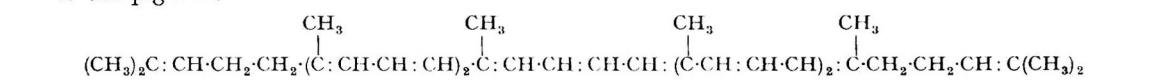
SYNOPSIS—A method is proposed for the estimation of tomato solids in tomato products by the determination of lycopene by absorption spectroscopy. The history of work done on the tomato pigment is traced. The theoretical aspects of the determination of lycopene in the presence of carotenes are discussed in the light of experiments carried out by the author. A method giving consistent and reproducible results has been evolved for the extraction of lycopene from tomato products. A suggestion is advanced that the origin of the extracted pigment, whether from a ripe or green tomato, is indicated by the shape of the absorption curve obtained. A series of five graphs is used as an illustration. The lycopene content of (*a*) English tomatoes in various stages of ripeness, and (*b*) commercially available concentrated purées are given. The method is applied to sauces and ketchups at present available.

THE Food Standards (Tomato Ketchup) Order, S.R. and O. No. 1817, 1949,* prescribes a standard for tomato ketchup, catsup, sauce and relish. A tomato solids content of not less than 6 per cent. by weight is specified, and the Order comes into operation for sales by retail on October 1st, 1950.

The characteristic red pigment of the tomato was first investigated in 1876 by Millardet,[1] who obtained it in a crystalline state, and also observed the crystals in the flesh of the ripe fruit. He established that absorption in the visible portion of the spectrum of a carbon disulphide solution of the pigment was characterised by two bands in the green region and one in the blue. Later investigators[2,3,4,5] found that carotene was present in tomatoes,

*See *Analyst*, 1950, **75**, 112.

and considered it to be identical with the pigment obtained by Millardet. The tomato pigment was again studied in 1903 by Schunck,[6] who found that the red colouring matter was clearly distinguishable from carotene in appearance, crystal form, solubility and absorption spectrum. The first chemical investigation of lycopene was undertaken by Montanari[7] in 1904. His results led him to consider that the tomato pigment was a condensation product of two molecules of carotene, having the formula $C_{52}H_{74}$. In 1910, Willstätter and Escher[8,9] isolated from Italian tomato conserve a relatively large quantity of the pure crystalline red pigment, together with a smaller quantity of the yellow pigment, carotene. They found lycopene to be an unsaturated hydrocarbon and to possess the same composition and molecular weight as carotene, namely $C_{40}H_{56}$. It was less soluble than carotene in ether, carbon disulphide, light petroleum and alcohol, and oxidised and bleached more readily. Xanthophylls and xanthophyll esters were also reported to be present. As a result of the work of Karrer and others,[10,11,12,13,14,15] the following structural formula has now been assigned[13] to the pigment—



Willstätter and Escher, as well as Montanari, carried out their investigations on the pigment isolated from Italian-grown varieties. Matlack and Sando[16] proved the pigments contained in Italian-grown tomatoes and in American red and purple tomatoes to be identical.

Lubimenko[17] considered lycopene to be formed by the oxidation of chlorophyll, but Euler *et al.*[18] found that green tomatoes kept at 20° to 21° C. ripened normally in a few days, developing the usual red lycopene colouring. At 30° C. they developed only a yellow colour, and tests showed the presence of carotene, xanthophyll and an unidentified flavone dye, but no lycopene. At 37° C. they turned yellow and spoiled rapidly. Neither light nor its absence had any effect on the rate of colouring.

It is believed that lycopene is formed by an enzyme action which is inhibited by the higher temperature. Kuhn and Grundmann[15] stated that the chlorophyll content of the green fruit is far too small to account for the formation of lycopene from it. Smith[19] showed that protection from intense light favours lycopene formation, whereas light is conducive to the maximum production of carotene. Since lycopene is present in mature fruits which have been grown in the complete absence of light and which have therefore never contained chlorophyll, lycopene cannot be an oxidation product of chlorophyll as has been assumed by some investigators.

The oil-soluble colour of the tomato has been suggested as a basis for an approximate estimation of tomato solids in tomato products.[20] The natural colour of the tomato was extracted with light petroleum, and its visual density determined by means of a Lovibond tintometer fitted with the Rothamsted device.

Three analytical techniques are of use in the study of a problem of this nature, namely—

(*a*) The separation of carotenoids by the partition technique between immiscible solvents as described in detail in Thorpe's Dictionary, volume II.[21]

(*b*) Chromatographic adsorption.

(*c*) Spectroscopic criteria using absorption maxima in different solvents. These afford much assistance in the task of characterising carotenoids.

Experimental

A chromatographic study of the pigment extracted from ripe tomatoes by light petroleum of b.p. 80° to 100° C., was made in this laboratory, using an alumina column. Three zones separated—

(i) At the bottom of the column, a rather indistinct, yellowish, diffuse zone, *viz.*, α- and β-carotenes.

(ii) A well-defined reddish zone, *viz.*, lycopene, in the middle.

(iii) At the head of the column, a pinkish ill-defined zone, *viz.*, xanthophylls.

A rough idea of the respective proportions present could be obtained from the depths of the various zones. Very approximately, the pigment appeared to be composed of about 90 per cent. of lycopene, the remainder being carotenes with only a very small amount of xanthophylls. This assumption is borne out by the work of Kuhn and Grundmann on the constitution of lycopene.

The spectral absorption curve of the extracted pigment was next examined. For work

in the visible region, a light petroleum (b.p. 80° to 100° C.) solution was employed, and a chloroform solution for the investigation of absorption in the ultra-violet. Much general absorption was observed in the ultra-violet, and it was found necessary to extract the chloroform solution with 90 per cent. methanol, after which satisfactory absorption curves were

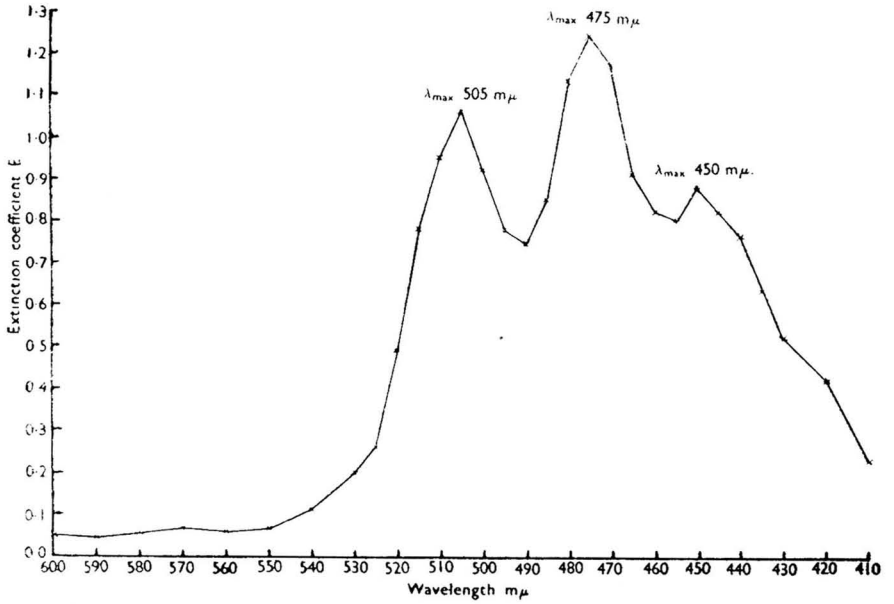


Fig. 1. Absorption curve in visible spectrum for light petroleum (b.p. 80° to 100° C.) solution

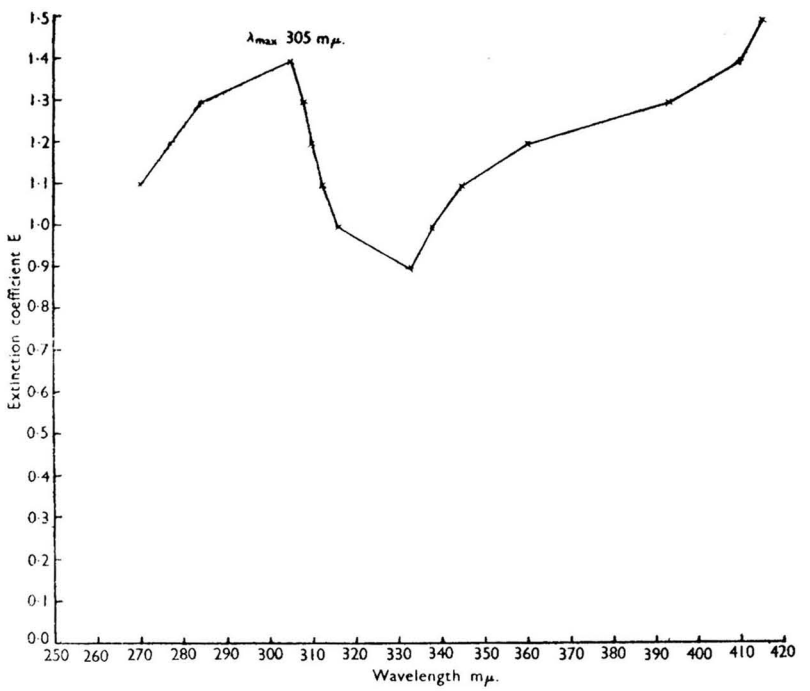


Fig. 2. Absorption curve in ultra-violet spectrum for methanol-washed chloroform solution

obtained with very little irrelevant absorption. The spectral absorption curve of the pigment dissolved in light petroleum (b.p. 80° to 100° C.) was next studied (Fig. 1). Solutions in chloroform and in carbon disulphide were also examined. The following maxima were recorded—

Solution in	Observed maxima, mμ.				Recorded maxima, mμ.*		
Light petroleum	505	475	450		506	474	444
Chloroform	510	480	455	*cf.*	513	480	451
Carbon disulphide	545	505	—		548	507	—

* Morton,[22] chart V, p. 60.

This identified the pigment as being chiefly lycopene. As further evidence, the ultra-violet curve of a chloroform solution, extracted with 90 per cent. methanol to remove substances giving irrelevant absorption, was determined (Fig. 2). This showed a well-defined maximum at 305 mμ., which is in agreement with recorded data. Furthermore, the extinction coefficient at 305 mμ. was one-third of that at 475 mμ., which again is in accordance with published results.[23]

The shape of the lycopene curve is extremely characteristic. The absorption curves obtained in different solvents have the same shape, but there is a "shift" in the wavelengths of the maximum absorption peaks of the curves. If we compare the carbon disulphide and light petroleum curves, we find that the 505 mμ. maximum in light petroleum is displaced to 545 mμ. in carbon disulphide, *i.e.*, towards the red end of the spectrum. The solution does, in fact, appear quite red in carbon disulphide, whereas in light petroleum it is yellowish-red.

The effect of carotene on the shape of the lycopene curve was studied using a light petroleum extract of carrots as a source of carotenes. The absorption curve of this solution is shown in Fig. 3. The point of interest is that the absorption at 505 mμ. is practically

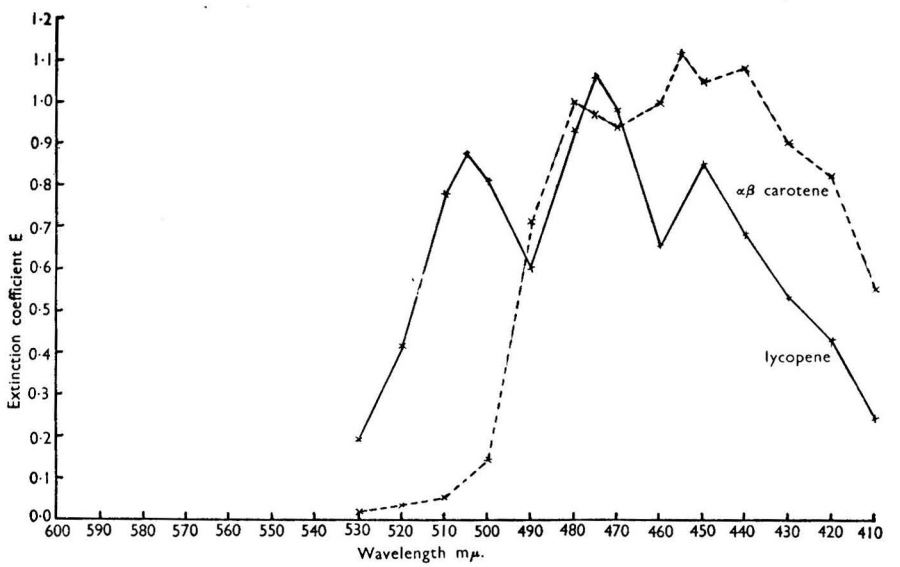


Fig. 3. Comparison of a mixed α- and β-carotene curve and a lycopene curve (light petroleum solutions)

negligible, and the first absorption maximum is reached at 485 mμ., thus enabling lycopene to be estimated spectroscopically in the presence of carotenes. As confirmation, equal volumes of two solutions of approximately equal concentration, as indicated by the extinction coefficients, were mixed, and the mixed solution evaporated to the same volume. The shape of the absorption curve for this solution is shown in Fig. 4. The actual maximum at 505 mμ. was practically unaltered, but the shape of the curve is vastly different. If we assume that the lycopene/carotene ratio in the pigment from ripe tomatoes approximates to 9/1, then Fig. 4 is the curve of a mixture of lycopene and carotenes in the ratio of approximately 9/11.

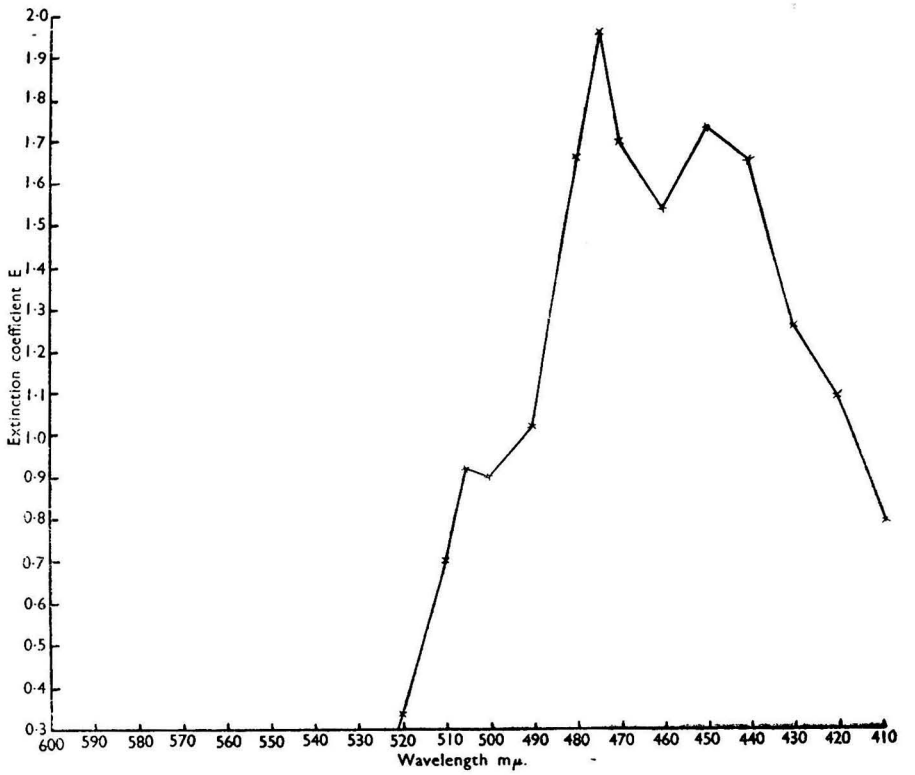


Fig. 4. Effect on the lycopene curve of the addition of α- and β-carotenes (light petroleum solution)

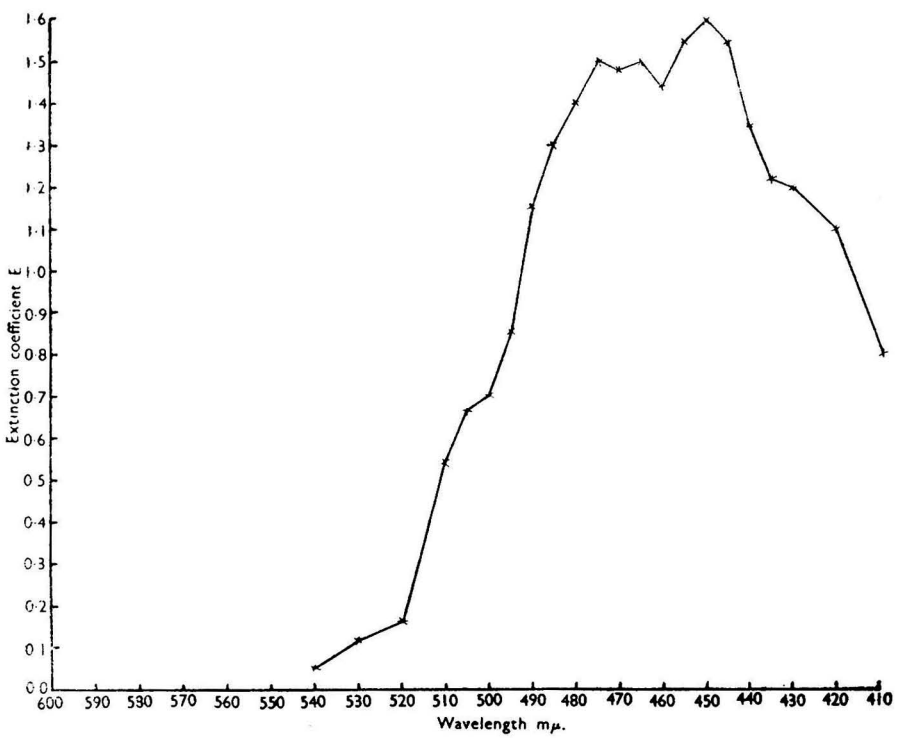


Fig. 5. The absorption curve of the pigment extracted from a green tomato (light petroleum solution)

This confirms the findings of Mills,[24] that, by selecting suitable wavelengths, accurate quantitative spectral analysis of carotenoid mixtures can be made with an error of less than 2·5 per cent.

Another question arising here is the possibility of determining by the shape of the curve whether a given pigment originates from a green or a ripe tomato. This would be possible if the relative proportions of lycopene and carotene altered upon ripening. Kuhn and Grundmann in their work on the constitution of lycopene gave the following figures for the lycopene, carotene and xanthophyll content of the tomato pigment.

	Green, mg. per 100 g.	Half ripe, mg. per 100 g.	Fully ripe, mg. per 100 g.
Lycopene	0·11	0·84	7·85
Carotene	0·16	0·43	0·73
Xanthophyll	0·02	0·03	0·06
Xanthophyll esters	0·00	0·02	0·10

This indicates that by inspection of the shape of the curve an inference regarding the origin of the pigment may be drawn. The ratio E_{max} 505 mμ./E_{max} 475 mμ. is approximately 8/10 in a normal ripe tomato pigment, and the use of this ratio is now suggested as a criterion. The curve obtained from the pigment of a green tomato is shown in Fig. 5, and its shape is obviously very different from that obtained for the ripe fruit.

In order to obtain results on a statistical basis for the lycopene content of tomatoes and tomato products, a method was evolved which gave consistent and reproducible results. Extraction with light petroleum of an aqueous solution gave rise to troublesome emulsions, and it is also doubtful whether a complete extraction of the lycopene is obtained in this way. The use of anhydrous sodium sulphate, followed by hot extraction in a Bolton extractor, was also unsatisfactory.

Proposed method

The method finally decided upon was to dry the material, well mixed with sand, in a vacuum desiccator, and then, after powdering, to transfer to a Bolton extractor. Acetone was used as solvent, light petroleum not being very successful, owing to a form of chromatographic separation taking place on the sand column. Further, the penetration of the column did not seem as effective with light petroleum as with acetone.

Procedure

Weigh accurately about 5 g. of the purée, sauce or ketchup into a stainless steel or porcelain dish. Incorporate carefully, and thoroughly, sufficient sand to make a powdery mass. Allow to dry in an efficient vacuum desiccator over calcium chloride for at least 16 hours. Transfer to a mortar and powder thoroughly. Pack tightly into the liner of the Bolton extractor. Extract with acetone for 3 hours, using a 100-ml. Quickfit pyrex flask, keeping it away from bright sunlight. Evaporate the acetone until nearly dry, then blow the last drops off in a current of air, with the flask removed from the water-bath. Add light petroleum (b.p. 80° to 100° C.) and heat to boiling on the water-bath, cool, and make up to a convenient volume, usually 150 ml.

Determine the absorption at 505 mμ. in a 1-cm. cell, using a constant-deviation spectrometer in conjunction with a Hilger photometer, a ribbon filament lamp being employed as light source. The full absorption curve may be plotted if required, special note being made of the $E_{max.}$ 475 mμ. Normally, however, only one determination at 505 mμ. is required, and this is made in duplicate.

If the solution is left in the dark overnight, the E alters very little, but it is advisable to make the determination without delay. A light petroleum (b.p. 80° to 100° C.) solution of the pigment was refluxed for 3 hours and gave the same reading before and after the refluxing. So for all practical purposes, there is very little destruction of the lycopene under the conditions of the experiment.

It was found in a series of experiments that approximately 70 to 80 per cent. of the lycopene was extracted in a few minutes, and 85 to 90 per cent. in about half an hour. To obtain a 95 to 98 per cent. extraction, however, 3 hours were required. It was possible to obtain still more complete extraction by prolonging the time beyond 3 hours, but the further amount extracted, even after prolonged boiling, was proportionately very small. The method outlined above will extract at least 95 per cent. of the lycopene present, and since all the

determinations are made under the same conditions, the figures obtained are comparable. The method will give consistent results on the same sample. The question of complete extraction is closely connected with the distribution of lycopene in the tomato. The bulk is easily extracted, but a proportion appears to be held in the fruit in some way. Information on this question is difficult to find, but it seems probable that, with a more complete extraction, the figures obtained by Darbishire on commercial purées, using a Lovibond tintometer, would have shown a smaller variation than the 40 per cent. he reported.[20] Extraction for 3 hours gives the acetone solutions a cloudy appearance, but if the experimental details regarding the evaporation of the acetone are closely followed, the resulting light petroleum solution is quite clear. Light petroleum (b.p. 80° to 100° C.) was used as final solvent because of its comparatively high boiling-point, so that there is less risk of concentration due to evaporation when transferring the solution to the 1-cm. cell of the photometer. Furthermore, the 505 mμ. maximum is a very easy one to determine.

In calculation it is assumed that the $E^{1\%}_{1cm.}$ 505 mμ. for lycopene in light petroleum is approximately 2000, and the $E^{100\%}_{1cm.}$ is calculated for the product under examination. In the case of tomato purées, the total solids, and salt, if any, were also determined, and hence the true tomato solids content. The lycopene content was expressed as (*a*) micrograms of lycopene per gram of product, or (*b*) micrograms of lycopene per gram of tomato solids (on the purées only).

Results

A number of determinations were made on tomatoes in various stages of ripeness, after preparing purées by mashing and passing through a sieve to obtain a purée free from skins and seeds. The results obtained are given in Table I. It will be observed that the lycopene

Table I

The lycopene content of purées prepared from English-grown tomatoes in various stages of ripeness

Description of tomatoes	Total solids	$E^{100\%}_{1cm.}$ 505 mμ.	Lycopene per g., assuming $E^{1\%}_{1cm.}$ 505 mμ. = 2000, μg.	Lycopene per g. of tomato solids, μg.
Green	5·4	3·2	16·0	296
Red-green	4·8	3·3	16·5	344
Ripe, red-green	5·4	5·6	28·0	519
Red, firm (very small)	10·8	12·8	64·0	593
Red, firm	7·0	15·8	79·0	1128
Red, firm	5·2	12·5	62·5	1201
Ripe, red, firm	4·4	12·9	64·5	1466
Very ripe, some rotten	5·2	22·0	110	2115

content gradually increases, with a very considerable variation between green and over-ripe tomatoes. It is worth noting that, for an average ripe, red, firm tomato, the concentration of lycopene approximates to 1500 μg. per g. of tomato solids. With respect to the actual commercial products available to the sauce manufacturers, it should be remembered that nearly all, if not all, concentrated tomato purées used in this country are imported, and consist of a product manufactured from ripe tomatoes. Unripe tomatoes are used for tomato chutney rather than for tomato sauce or ketchup. In a bulletin issued by the National Canners' Association Research Laboratory of the U.S.A.,[25] reference is made to the exclusion of greenish tomatoes in the preparation of purée, because of the development of an objectionable colour. Good pulp can be made only from thoroughly ripe, sound tomatoes. Not only do green tomatoes, or tomatoes with green portions, not have the desired amount of red colouring matter, but the yellow and greenish particles mask and dull the red colour present. Also the amount of pectin naturally occurring in tomatoes is greatest at the time of complete ripeness; this is important, because the pectin present in the finished product contributes to its viscosity. The avoidance of over-ripe tomatoes also is in the interests of the manufacturer, because the absence of mould is of great importance. The colour of tomato products is an important index of their quality, and greatly influences their commercial value. The manufacturers of purées have a financial interest in avoiding the use of green, partly green and over-ripe tomatoes, and it is highly improbable that they will do anything likely to decrease the commercial value of their products. Thus we may be reasonably assured of a

fairly uniform concentrated purée being commercially available to sauce manufacturers, and this is borne out by Table II.

TABLE II

THE LYCOPENE CONTENT OF MANUFACTURERS' CONCENTRATED PURÉES AND ALSO OF TWO SAMPLES OF UNCONCENTRATED PURÉE

Origin	Total solids, %	NaCl, %	Tomato solids, %	$E^{100\,\%}_{1\,cm.}$ 505 mμ.	Lycopene per g., μg.	Lycopene per g. of tomato solids, μg.
(*a*) MANUFACTURERS' PURÉES						
Italian "Rago"	33·9	2·3	31·6	108	540	1709
Italian "Valnure"	26·4	1·9	24·5	87	435	1775
Italian "Helvia"	26·7	1·6	25·1	106	530	1677
Italian "Suprema"	33·1	3·1	30·0	91	455	1517
Italian "Soriso"	32·5	0·7	31·8	107	535	1683
Italian "Catalano"	28·3	1·5	26·8	87	435	1624
Portugese "Toiro"	37·9	2·4	35·5	110	550	1550
Hungarian "Gschwindt"	28·0	0·2	27·8	85	425	1529
Hungarian "Helios"	25·8	0·2	25·6	95	475	1856
Hungarian "Globus"	27·2	0·2	27·0	82	410	1518
Hungarian "Golden Pheasant"	27·6	0·2	27·4	87	435	1588
French "U.D.C."	30·7	2·3	28·4	70	350	1552
French "Gourmet"	27·5	0·3	27·2	92	460	1691
French "Rolli"	28·1	4·5	23·6	69	345	1462
Canadian "Smith"	25·9	0·3	25·6	97	485	1505
South African "Barclay Vale"	27·4	0·4	24·0	77	385	1604
Australian "O.T. Ltd."	23·8	1·9	21·9	75	375	1712
					Average	1620
(*b*) CANNED UNCONCENTRATED PURÉES						
Australian	11·0	0·7	10·3	31	155	1505
New Zealand	11·2	0·9	10·3	33	165	1601

The variation in lycopene content is surprisingly small, and this is all the more remarkable when we consider that these purées represent random samples from the produce of seven different countries, covering practically the whole world. It would seem a reasonable assumption that 1 g. of tomato solids contains a minimum of 1500 μg. of lycopene, and it is proposed that this figure should be used as a means of estimating the tomato-solids content of an unknown sauce or ketchup. In the manufacture of such products, colouring matter may occasionally be used to maintain a standard colour. In this case, however, a water-soluble dye is used, which is not extracted by the proposed method. In any event, the shape of the absorption curve of lycopene is so characteristic that an analyst, if ever confronted with an oil-soluble dye, would be able to detect its presence from the shape of the curve. It is very unlikely, however, that this would be encountered. A number of sauces and ketchups were analysed, with the results given in Table III.

The samples giving low figures were further investigated, and in every case the shape of the absorption curve was the normal curve for lycopene. The E values at 475 mμ. bore normal relationships to the E values at 505 mμ., so that the use of green tomatoes was excluded.

To test the accuracy of the method, a number of samples of ketchup of known tomato-solids content were obtained. A series of five samples of ketchup, obtained at different stages in the manufacture of a single batch of tomato ketchup, was analysed, and the lycopene content was found to be practically constant, showing that there is very little loss during the manufacture. Furthermore, about a dozen different purées were included in this batch of over a ton of ketchup, and the tomato-solids content of each one was known, so that the theoretical composition of the batch could be calculated. The calculated tomato solids approximated to 12·5 per cent., and the actual percentage found by lycopene determination averaged 11·5 per cent. over the five samples. The manufacturers themselves from time to time had analysed their product by Morpeth's method,[26] and this gave figures ranging between 11 and 12 per cent. A ketchup containing exactly 33 per cent. of tomato purée was obtained from one manufacturer, together with a sample of the purée from which it was made. The $E^{100\,\%}_{1\,cm.}$ 505 mμ. of the purée was 94 and that of the ketchup 29. The solids content of the

purée was 27·0 per cent. after allowance for a small amount of salt. The calculated tomato-solids content of the ketchup was therefore 9·0 per cent., and the amount found by lycopene determination was 9·7 per cent.

TABLE III

APPLICATION OF THE METHOD TO THE ESTIMATION OF THE TOMATO-SOLIDS CONTENT OF A NUMBER OF TOMATO SAUCES AND KETCHUPS AVAILABLE FOR RETAIL SALE

(*a*) TOMATO SAUCES

Sample	1	2	3	4	5	6	7	8	9
$E_{1\,cm.}^{100\,\%}$ 505 mμ.	14	27	21	17	2·1	24	0·5	15	18
Lycopene, μg. per g.	70	135	105	85	10·5	120	2·5	75	90
Tomato solids, assuming 1500 μg. lycopene = 1 g. of tomato solids	4·7	9·0	7·0	5·7	0·7	8·0	0·2	5·0	6·0

Sample	10	11	12	13	14	15	16	17
$E_{1\,cm.}^{100\,\%}$ 505 mμ.	14	20	34	5·8	21	24	17	2·1
Lycopene, μg. per g.	70	100	170	29	105	120	87	11
Tomato solids, assuming 1500 μg. lycopene = 1 g. of tomato solids	4·7	6·6	11·0	1·9	7·0	8·0	6·2	0·7

(*b*) TOMATO KETCHUPS

Sample	18	19	20	21	22	23	24	25	26
$E_{1\,cm.}^{100\,\%}$ 505 mμ.	26	26	43	30	24	36	12	31	15
Lycopene, μg. per g.	130	130	215	150	120	180	60	155	75
Tomato solids, assuming 1500 μg. lycopene = 1 g. of tomato solids	8·7	8·7	14·3	10·0	8·0	12·0	4·0	10·3	5·0

Sample	27	28	29	30	31	32	33	34
$E_{1\,cm.}^{100\,\%}$ 505 mμ.	18	26	30	31	32	55	22	25
Lycopene, μg. per g.	90	130	100	155	160	275	110	125
Tomato solids, assuming 1500 μg. lycopene = 1 g. of tomato solids	6·0	8·7	6·7	10·3	10·7	18·3	7·3	8·3

From a series of six samples of tomato ketchup supplied by another manufacturer the following results were obtained. Two samples made from Hungarian purée to contain 15·3 per cent. of tomato solids were found to give $E_{1\,cm.}^{100\,\%}$ 505 mμ. of 40 and 39, corresponding to a tomato-solids content of 13·3 and 13·0 per cent. respectively. Two samples made from French purée to contain 15·0 per cent. of tomato solids were found to give $E_{1\,cm.}^{100\,\%}$ 505 mμ. of 48 and 47, corresponding to a tomato-solids content of 16·0 and 15·7 per cent. respectively. A further two samples made from French purée to contain 13·7 per cent. of tomato solids both gave $E_{1\,cm.}^{100\,\%}$ 505 mμ. of 42, corresponding to 14·0 per cent. of tomato solids.

One important point remains to be stressed, namely the desirability of defining "tomato solids." In the experiments on the lycopene content of tomatoes and concentrated purées, approximately 5 g. were taken for the solids determination, a little water was added, and the mixture was dried to an even film on the water-bath for approximately 1 hour, followed by drying at 100° C. in the oven for 1 hour. Various methods are given in the literature for tomato-solids determination,* *e.g.*, vacuum desiccation, refractometer methods, etc. It would be in the interests of all concerned if the Food Standards (Tomato Ketchup) Order, S.R. and O. No. 1817, 1949, defined "tomato solids," and prescribed a standard method for determination.

SUMMARY AND CONCLUSIONS

It has been demonstrated that the lycopene content of the tomato and tomato products can be ascertained by a spectroscopic method using the spectral absorption at 505 mμ. as a criterion. Furthermore, the effect of carotene on the absorption maximum has been shown to be negligible at 505 mμ., even though the shape of the remainder of the absorption curve

*See report of A.M.C. Tomato Products Sub-Committee, *Analyst*, 1941, **66**, 319.

alters as the percentage of carotene increases. It is suggested that the relationship between $E_{max.}$ 505 mμ. and $E_{max.}$ 475 mμ. can be used as a criterion of the degree of ripeness of the tomatoes used. A method of extracting and determining the lycopene which gives consistent results has been developed. A suggested factor is given, based on a number of determinations of the lycopene content of English-grown tomatoes in various stages of ripening, and also of commercially available tomato purées from which sauces and ketchups are normally manufactured. The factor converts micrograms of lycopene to grams of tomato solids. A table is given of the results obtained in the application of the method to a number of ketchups and sauces at present available. The method does not claim extreme accuracy, but it gives a definite indication of tomato-solids content, and is undoubtably a good "sorting out" test, which may be used in conjunction with other methods of analysis. The analytical procedure involved is very simple, and the determination rapid.

The author is indebted to the Birmingham Public Health Committee for facilities for carrying out this work, and also wishes to record his thanks to Mr. H. H. Bagnall, the Birmingham City Analyst, for his very helpful encouragement. The writer is very grateful to Mr. D. J. Munns, the Chief Chemist at H.P. Sauce Ltd., Birmingham, for supplying the samples of commercial purée.

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November, 1949

A Photo-Electric Method of Determining the Colour of Flour as Affected by Grade, by Measurements of Reflecting Power

By D. W. KENT-JONES and W. MARTIN

(*Read at the meeting of the Society on Wednesday, October 5th, 1949*)

Synopsis—A piece of apparatus that has been designed for assessing flour colour as an indication of flour grade is described. Its readings are independent of the effect of natural or artificial bleaching. It consists in a source of light rays that are directed by a lens system on to standard white surfaces of magnesium oxide, the reflected light from which is received by two screened photo-electric cells with filters having their main transmission in the 530 mμ. band.

After balancing the optical system by means of mechanically operated shutters, one of the standard surfaces is replaced by a similar surface of flour paste and the decrease in the amount of reflected light determined by reducing the light that reaches the other standard surface by means of a cam-operated shutter until the system is again balanced. The duller the flour, the greater the "cut-off" required.

The apparatus is easy to manipulate and a numerical evaluation of the flour colour can be obtained in five minutes.

The problem of evaluating flour colour, which is influenced by the length of extraction and is therefore a measure of the grade of the flour, is of commercial importance, but the difficulties involved in making useful and sufficiently exact measurements are considerable. In the main, the factors controlling the colour of flour are—

(1) The grade of the flour, which depends upon the extent of the contamination of the ground-up endosperm by fragments of the outer coverings of the grain, *i.e.*, bran and associated substances.
(2) The degrees of yellowness due to the amount of the natural yellow colouring matter present and the extent to which this has been removed by natural and artificial bleaching, or by both.
(3) The granularity; the more granular the flour the darker and duller it appears. Dullness from this cause will not persist in the crumb of the resulting loaf.
(4) The presence of dirt, smut (*Tilletia tritici*) and other extraneous matter.

In pre-war days, when white flour was the normal product commercially made, minor differences in colour often affected the price by several shillings per sack. Even to-day, when 85 per cent. extraction has to be made, there is still considerable interest and competition in obtaining the best possible colour in flour. Despite considerable work on the subject, there is as yet no simple and satisfactory method available for measuring and recording numerically the colour of flour as an index of grade. What is known as the Pekar test, which involves the visual comparison of compressed slabs of flour, has remained the stand-by of millers and bakers for the assessment of the comparative brightness of flour samples. This test is open to much criticism, as is indicated by Kent-Jones, Amos and Martin.[1]

It is true that the literature abounds with methods which have been suggested for the measurement of flour colour but, as is pointed out by Kent-Jones, Amos and Martin,[1] these have not been found sufficiently rapid and reliable to gain general acceptance. A useful distinction between the yellowness due to natural yellow pigments and dullness principally due to bran powder contamination of the endosperm was made by Kent-Jones and Herd.[2] This convenient division of flour colour has been generally accepted and has been adopted in this investigation.

The degree of yellowness of flour can be easily determined by extracting the unoxidised carotene, under standardised conditions, and measuring the intensity of the yellowness acquired by the solvent. Many such methods have been described.[2,3,4,5,6,7,8,9,10] Some criticism of the early method of Kent-Jones and Herd[2] was made by Visser't Hooft and

de Leeuw,[11] but this is not applicable to the modified procedure of Kent-Jones and Amos.[12] The dullness factor, which Kent-Jones and Herd[2] called the "grade colour" as opposed to the creaminess factor, is much more difficult to measure and has received much less attention. Kent-Jones and Herd[2] devised a method of measuring this factor by extracting the bran pigments, but certain weaknesses in this method were pointed out by Markley and Bailey.[13] It has been our experience that this grade colour—or "brightness" as millers and bakers call it, and which is so noticeable in the crumb of the loaf—is correlated with the extent to which a smooth surface of flour in paste form reflects light. The method of measuring this aspect of flour colour which is described in this paper relies upon this correlation. Preliminary work revealed that the influence of differences in granularity could be overcome by the use of a flour paste and that the effect of differences in degree of bleach could be largely eliminated by the employment of light of a prescribed wavelength. In this paper we shall describe the apparatus which was eventually evolved and the technique advised for the accurate comparison and numerical recording of grade colour by the measurement of the reflecting power of the surfaces of flour pastes. We, and those associated with us, have studied this problem for many years, and, although not unaware of the difficulties involved and the almost impossible task of devising a method to which no objection could be raised, we have found that the procedure described in this paper is of considerable practical use.

PRINCIPLE OF THE METHOD—

The principle of the method is to utilise a balanced circuit containing two photo-electric cells to measure the amount of light of a particular wavelength which, under the conditions of the test, is reflected from the surface of a paste prepared from the flour. The amount of light reflected is not measured in absolute units but is recorded as a proportion of the amount of light of the same wavelength which is reflected from a standard white surface. As explained previously, the determination is performed upon a paste of the flour in order to obviate the differences which would otherwise arise between flours which were similar in grade and colour but which differed in granularity. Whereas previous methods have suffered from the defect of insensitivity, the apparatus and technique described in this paper enables the operator, uninfluenced by personal judgment, to assess flour colour quite as accurately as can the experienced and skilled miller and baker and moreover to express the result numerically. The data recorded in this paper reveal that different operators obtain results in close agreement and, as a simple method of standardisation is available, all instruments should give, within the normal experimental error, the same results.

APPARATUS—

The apparatus (Fig. 1) consists of a source of light, L, which is a short filament 36-watt lamp (12 volts) fed from the normal A.C. mains supply via a transformer. This lamp projects light via the lens systems, X and Y, which are designed to give parallel light, on to two standard surfaces, S_1 and S_2, which consist of glass cells, approximately 5 cm. square and 1 cm. thick, containing heavy magnesium oxide. The reflected light from the standard surfaces is picked up by the photo-cells, C and D. Immediately in front of each photo-cell is a filter, W (Wratten No. 58), having its main transmission in the 530 mμ. waveband. It has been established that the reflected light from the surface of a flour paste transmitted by this filter is not influenced to any appreciable extent by the degree of bleach, whether natural or artificial. Thus the amount of light transmitted by the filter is substantially dependent purely upon the grade of flour, *i.e.*, upon the amount and nature of the bran powder present. This green filter was selected after considerable experimental work with an earlier form of colorimeter, namely, that of Bolton and Williams,[14] from which the principle of comparing the light reflected by the surface of a sample under test against that reflected by a standard white was adopted. The photo-cells are connected in the usual way to a galvanometer with a tapping key in circuit so that the null point can be easily obtained.

In front of lens X is a shutter B (Figs. 1 and 2) operated by the "set zero" control, K, by means of which a fixed portion of the beam of light passing to cell S_1 can be cut off when K is raised. It is found necessary to have this arrangement because of the marked difference in reflecting power of the standard surface and the surfaces presented by the flour pastes. If this cut off were not used, the dial G would have to be rotated considerably before the null point was reached, even with a flour of high grade, and hence an appreciable portion of the scale on dial G would be wasted. By suitable adjustment of the amount of light cut off

by the "set zero" control, K, which can be adjusted by means of screw F (Fig. 2), the range of the instrument can be altered to suit the materials being tested.

There is a second shutter, E, in front of the lens X, which is operated by a screw terminating in a knob, J, on the panel and this serves to effect the initial balance of the current after the "set zero" knob has been raised.

In front of the lens Y is a cut-off screen, A, operated by the calibrated dial G, through a linear camshaft. The total movement of the cam is very small, being approximately 0·16 inch for a complete revolution of the dial.

The setting of the instrument adopted by the authors at the present time will cover all grades of flour from the brightest patent flour in the mill (C flour) to flours as dull as those

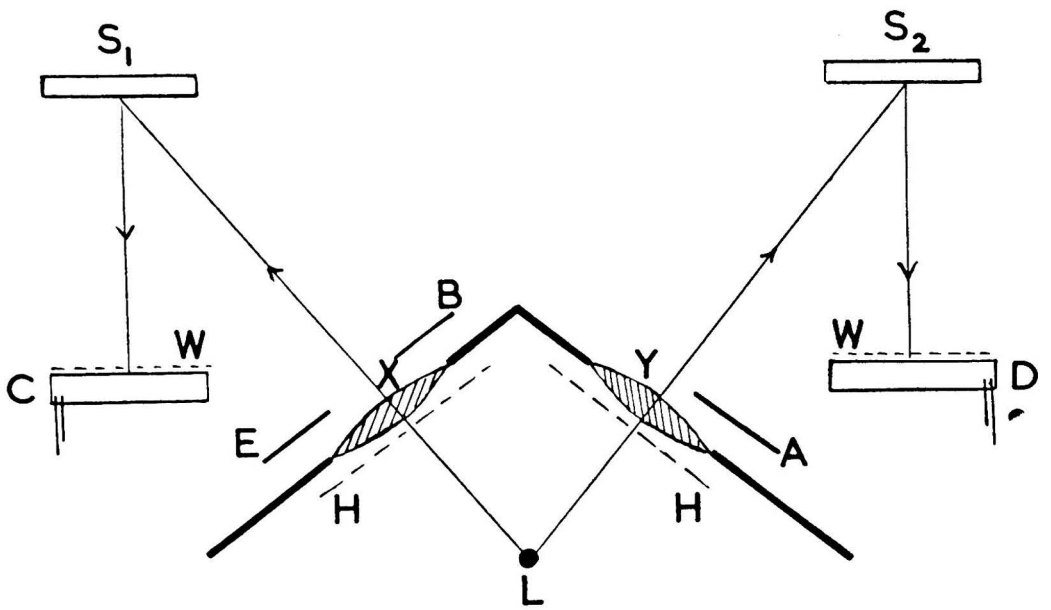

Fig. 1

given by flour of 90 per cent. extraction. With this setting, there should be a fixed reading on the dial when a Kodak gelatin filter of 80 per cent. transmission is placed in front of the standard surface S_1 (heavy magnesium oxide), and the instrument operated as if testing flour paste. In practice, with flour, we have selected the dial reading of 80. If a reading of 80 is not obtained, the screw adjustment F must be rotated (this alters the extent of the movement of the shutter B operated by the "set zero" control K), until the instrument, upon being rebalanced as usual for zero, does give a reading of 80 when the 80 per cent. transmission filter is in position in front of surface S_1. The differences obtained in dial readings when different samples of Kodak filters of 80 per cent. transmission are used are very small, so that all instruments can be standardised to yield substantially the same results provided that the magnesium oxide is of the same quality and does not change.

The range covered by this instrument is naturally intimately bound up with the cam movement and the original cam fitted to the instrument had only half the movement of the one at present in use. With this very fine cam movement the instrument was, of course, even more sensitive than it is at the moment, but the range of brightness which could be measured was naturally much narrower; in our opinion the present cam gives a more useful form of instrument.

Bolton and Williams[14] pointed out that it was necessary to remove infra-red light in order to obtain a good result with this type of instrument. They accomplished this by using a cell of dilute copper sulphate solution. We have not used a copper sulphate cell in our instrument on account of practical disadvantages, such as the need for fairly frequent replacement of the solution and the difficulty of maintaining the inner surfaces of the cell in a clean condition. Instead, we have incorporated in the light path of our instrument

a special glass (Chance's No. ON19), labelled in Fig. 1, H, which has the power of absorbing a high proportion of the infra-red rays.

The extreme sensitivity of the instrument is shown by the fact that mere reversal of the faces of the cell may give a reading difference as great as 10° on the dial—the dial being calibrated 0° to 360°. It is, therefore, most important to make sure in any series of tests that the same cell face is always used.

We have found that normal mains fluctuations do not call for the use of a stabilising transformer but, if the supply were rather abnormal, a transformer of this type might be necessary.

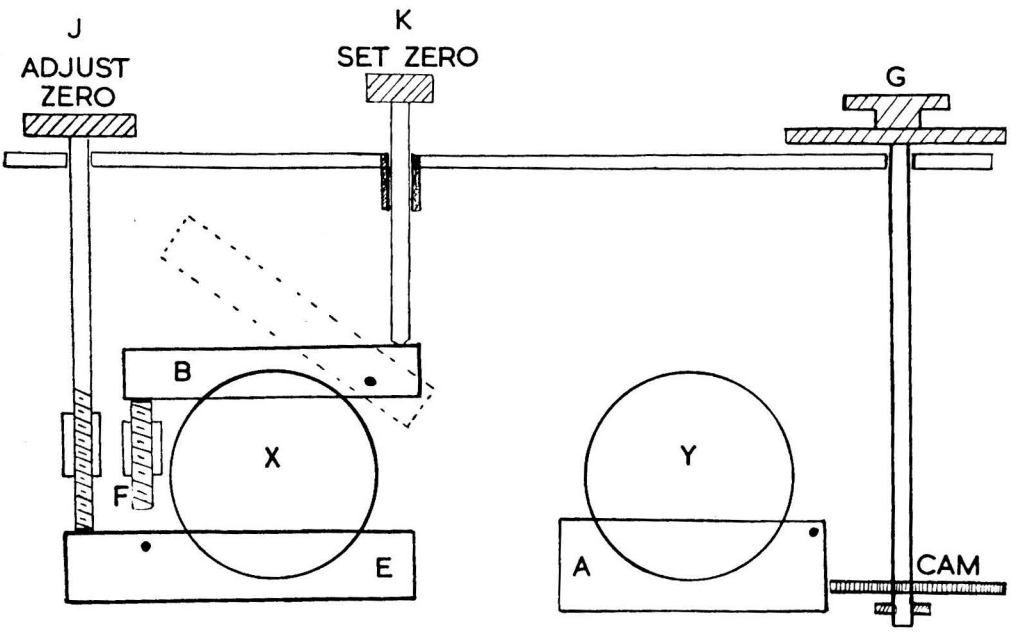

Fig. 2

Method

Switch on the lamp L and raise the "set zero" control K, thereby cutting down by a pre-determined amount the light falling on the standard surface S_1 and thus on the photo-cell C. Set the dial G at zero and balance the photo-cells by adjusting the zero control J (Fig. 2) until, on using the tapping key (not shown in diagram as it is part of the electrical circuit), there is no movement on the galvanometer, which indicates that both cells are receiving the same amount of light. Remove the standard cell S_1 and replace by a similar cell into which has been poured a flour paste prepared by mixing together, until smooth and homogeneous, 30 g. of flour and 50 ml. of distilled water. Depress the "set zero" control K, which then allows the full amount of light to fall on the sample cell, and rotate the calibrated dial G from its zero setting, cutting down the light from S_2 until a null point is obtained on the galvanometer, indicating that both cells are again receiving the same amount of light. Record the degrees through which the scale G has been rotated.

General remarks on the method—

The figures obtained from the calibrated dial are purely empirical, but they do serve to give a numerical representation of the grade of flour; the higher the dial reading, the lower the grade of the flour, *i.e.*, the duller the colour. The results can be very easily duplicated and, as bleaching has practically no effect at the wavelength of light employed, flours can be re-checked, if necessary, after a lapse of time, such as several weeks, although in the interim they will have undergone natural bleaching.

The reproducibility of results with a given instrument is quite satisfactory, as can be seen from the data of the tables which follow. The concordance of the results provided by different models of this instrument will, of course, be influenced by the accuracy with which

the cam and its associated shutter is manufactured. Naturally we have no experience of the "spread" of the data which would be provided by different models of this instrument, but we do not contemplate any serious difficulty in obtaining reasonably close duplication with different models. This is essentially a matter for the manufacturers and depends upon the uniformity of material and construction.

With accuracy in making the cam and with standard magnesium oxide to the specification indicated, there is no reason why all instruments checked by the Kodak transmission filter should not give reasonably similar results. It is, however, important to bear in mind two matters. Firstly, the variation which might arise with different photo-electric cells. We have, in fact, worked with other matched cells and these have given results almost identical with the cells originally used. Secondly, there may be errors introduced from the nature of the filaments in various lamps as the light thrown on the surface being examined is not completely uniform. This is an important matter and lamps must be selected so as to have the filament in the same relative position, which can be conveniently judged by the operator. An arrangement for pre-focussing the lamp assists in overcoming the trouble when lamp replacement becomes necessary.

We have not, so far, encountered any serious difficulties due to differences in power of reflection of the heavy magnesium oxide, provided the material has approximately the same granularity. We used B.D.H. heavy magnesium oxide, all of which passes a No. 25 flour silk (approximately 197 meshes per linear inch or 38,809 per square inch).

It has been our practice to test a flour paste within a few minutes of its being mixed and, when a series of flours is to be tested, we find it convenient to weigh out all the samples at one sitting and then to mix each of them with water when its turn for testing arrives. It is necessary to maintain absolute cleanliness in the sample cell surfaces because very small differences in reflecting power are being recorded.

DISCUSSION OF RESULTS AND SUMMARY—

The degree of reproducibility attainable is revealed in Table I, which gives the dial readings furnished by a number of flours at different times. The dial can be read to half a degree. It should be noted that the readings in this and subsequent tables are those read directly from the dial and not the corresponding figures of the empirical scale recommended in the paper by Kent-Jones, Amos and Martin.[1]

TABLE I

REPRODUCIBILITY OF RESULTS

Dial readings obtained on separate flour pastes

Sample	First day	Second day	One week later
Long patent flour	48, 48, 48·5	49, 50, 50·5	50·5, 50, 51
Lower grade flour	211·5, 210·5, 212	212·5, 210, 210·5	208, 207, 206

Little effect is noticed if the amount of water used in making the paste is varied. Thus, if instead of 50 ml. of distilled water, 60 ml. is used for the 30 g. of flour, there is no appreciable change in the dial reading, as is shown in Table II.

TABLE II

EFFECT OF VARYING QUANTITY OF WATER USED IN MAKING FLOUR PASTE

Sample	Quantity of water added to 30 g. flour: 50 ml.	60 ml.
Flour 1	203	202
2	48	45
3	121	120

TABLE III

REPRODUCIBILITY BY DIFFERENT OPERATORS

Sample	Operator I	Operator II	Operator III
4	37	38	39
5	255·5	254	257
6	70	69	71

The differences in readings obtained on a flour by different operators is remarkably small, as is shown by the figures in Table III.

As indicated earlier, we use the paste as soon as it is made, but there is no marked change in the result if the test is carried out within half an hour of the initial mixing. Table IV gives the results obtained on the same paste tested after intervals of 30 minutes.

TABLE IV

EFFECT OF TIME ON THE READING

Sample	Readings		
	As made	30 minutes later	1 hour later
A	50·5	51·5	57
B	71	74	81
C	155	158	166

Table V gives results indicating in a broad way that the readings of grade colours furnished by this apparatus are substantially independent of the degree of bleach. This matter is, however, discussed in more detail by Kent-Jones, Amos and Martin.[1]

TABLE V

EFFECT OF BLEACH

Sample	Reading
A. Unbleached	50·5
A. Bleached	48
B. Unbleached	70
B. Bleached	72
C. Unbleached	155
C. Bleached	153

The nature of the skins of the grain or of the branny particles present in the flour does have some influence on the colour of the resulting flour. Thus, branny particles from white wheats have rather less deleterious effect with respect to colour than those of red wheats. The difference in reading obtained, when finely ground white and red bran is added at the rate of approximately 6 per cent. to a sample of flour, is of the order of 15°.

Also bran powder, as opposed to the same weight of bran in larger form, gives a poorer colour to the flour as assessed by commercial judgment. It is, therefore, of interest to check the results obtained under these conditions, although it should be pointed out that normally the differences in commercial flours in these respects are not great. Our broad experience is that when one flour is brighter than another, solely because of commercial differences in the bran size, there is a difference in dial reading of less than 10°.

It is claimed that, by means of this instrument, it is possible to express, on a reliable numerical scale, the commercial evaluation of flour colour and grade, uninfluenced by the effect of bleach. The method is rapid (5 minutes per sample) and gives easily reproducible results.

We wish to record our thanks to Mr. F. Widdis of Messrs. H. Tinsley & Co., Ltd., for his interest in this work and his great assistance in the final design of the apparatus, to Dr. A. J. Amos for his advice and assistance in preparing this paper, and to Mr. R. Donaldson of the National Physical Laboratory for helpful criticism of the instrument.

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Experiments in the Photo-Electric Recording of Flour Grade by Measurements of Reflecting Power

BY D. W. KENT-JONES, A. J. AMOS AND W. MARTIN

(*Read at the meeting of the Socicty on Wednesday, October 5th*, 1949)

SYNOPSIS—The reliability and usefulness of the instrument described at the same meeting by Kent-Jones and Martin for the determination of the grade colour of flour has been investigated. It is recommended that the grade colour of a flour should be expressed as the dial reading in degrees divided by 10 and corrected to the nearest 0·5 unit.

Statistical analysis of the data of reproducibility shows that if the grade colour figure for a given flour is assessed by different operators on different days the results can be expected to agree within 0·5 unit.

With flours of ash content below 1 per cent., a range that embraces flours from high-grade patent to 90 per cent. extraction flour, the effect of bleach is within the experimental error of the method.

The dial readings of the instrument are linearly related to the logarithms of the corresponding ash contents and the correlation coefficient between dial reading and ash content is 0·959.

Some of the aerating ingredients used for self-raising flour influence the dial reading, but it is shown that this influence can be annulled by using a buffer solution in place of water for making the paste. With this modification the instrument gives reliable results with self-raising flour.

THE quality of flour is determined by two factors, namely, the suitability of the flour for its intended purpose, *e.g.*, bread-making or cake-making, and the colour of the flour. This latter aspect of quality is of such importance that in pre-war days it regulated to a great extent the price of flour. The "colour" of flour is, however, a complex characteristic that is largely governed by two independent factors; these are, first, the brightness or dullness, which is related to the extent to which the endosperm has become contaminated with powdered wheat skins and associated substances during the milling process and, secondly, the degree of creaminess which is dependent upon the amount of carotene in the original wheat and the extent to which this has undergone natural or artificial bleaching. The former aspect of flour colour, *i.e.*, the dullness or brightness, is referred to as the "grade" colour because it is correlated with the degree of extraction, that is, the grade of the flour.

Since the two factors which together mainly determine the colour of flour are quite independent, any degree of brightness, or dullness, can be associated with any tint between the full creaminess of an unbleached flour and the extreme whiteness of a highly bleached one. The possible variations in the over-all colour of flour, therefore, cover a very extensive range from a very bright and very white flour on the one hand to a very dull and fully creamy flour on the other.

The great commercial importance which attaches to flour colour has led to an evaluation of this characteristic becoming a very frequently applied test in mill and bakery. The earliest method of assessing flour colour, and one which is still the standby of millers and bakers, was a visual test—the Pekar test—in which a comparison is made between the surface of compressed slabs of flour before and after they have been dipped in water. The immersion of the slabs in water tends to magnify the colour differences through the action of enzymes,

probably oxidative in nature, which exist in the flour. The visual appearance of such a compressed slab of flour depends, however, not only upon the over-all colour of that flour, but also upon other factors such as the degree of compression to which the slab has been subjected, the moisture content of the flour, the conditions of the immersion of the slab in water and the length of time which has elapsed since the immersion. Although this test does undoubtedly serve a useful purpose in the flour industry, it is only a comparative test, which does not lend itself to numerical expression and which relies upon personal judgment.

Attempts have been made to devise methods of evaluating the over-all colour of flour which permit the results to be recorded on a numerical basis. Jago[1] described a method in which the colour exhibited by the surface of a compressed slab of flour was matched visually against a combination of red and yellow glasses in a Lovibond tintometer. Baker, Parker and Freese[2] decided that the over-all colour of flour embraced four colour factors, namely, yellow, red, black and white and that a satisfactory match could be obtained by visual comparison with Maxwell discs rotated at high speed. These methods, however, have never gained popularity in the cereal field and no method of measuring the over-all colour of flour on a numerical basis has become generally accepted.

In the milling industry, of course, interest is taken not only in the over-all colour of flour, but in each of the two main independent colour factors, since one, the grade colour, reflects the efficiency with which the milling operation has been performed, and the other, the creaminess, is a measure of the degree of bleach. It is only natural, therefore, that attention should have been given to the independent determination of each of these factors. The determination of the degree of creaminess is a relatively simple matter involving only the extraction of the carotene with a suitable solvent and the measurement of the intensity of colour in the resulting solution. It is not surprising, therefore, that numerous methods of performing this test have been recorded. Investigations into this determination have been reported by Kent-Jones and Herd[3]; Markley and Bailey[4]; Binnington, Hutchinson and Ferrari[5]; Binnington, Sibbett and Geddes[6]; Ferrari and Bailey[7,8,9]; Simpson[10]; Visser't Hooft and De Leeuw[11]; Kent-Jones and Herd[12]; Kent-Jones and Amos.[13]

The determination of the "grade" colour of flour is a more complex problem, but it also has received attention in the past. Kent-Jones and Herd[3] reported a method involving the extraction of the pigments of the admixed bran powder, *i.e.*, the powdered wheat skins and the measurement of the colour of the resulting solution against a standard solution. This method furnished reliable and reproducible results with flours of 75 per cent. extraction or less, but did not prove to be so satisfactory when applied to long extraction flour such as the present 85 per cent. National flour. The grade colour or brightness of flour is, however, related to the extent to which a smooth surface of the flour reflects light—as is evident from the long-used Pekar test—and Kent-Jones and Martin[14] have recently utilised this relationship as the basis of a new procedure for measuring grade colour. They have devised and described an instrument which is capable of measuring fine differences in the proportion of incident light reflected by a flour surface and, by employing a flour paste and light of a prescribed wavelength, have made the measurement independent of variations in the granularity of flour and in the degree of bleach.

The technique of Kent-Jones and Martin[14] is an advance upon previous procedures for assessing grade colour in two directions. First, the method is identical in principle with the procedure employed throughout the flour industry for judging flour colour in that it depends upon the reflecting power of a flour surface and, secondly, relying as it does upon the reactions of photo-electric cells, it is independent of personal judgment. A numerical evaluation of the grade of flour as provided by the instrument of Kent-Jones and Martin,[14] supplemented by a numerical measure of the degree of bleach obtained, for example, by the method of Kent-Jones and Amos,[13] should provide a complete picture of the over-all colour of the flour and, moreover, reveal the extent to which each of these two main colour factors contributes to the whole.

The purpose of this paper is to present the data which we have accumulated during our experience with the method of Kent-Jones and Martin, and to illustrate the value which the method can have for those in the various sections of the cereal industry who are called upon to make frequent assessments of flour colour. Although we have restricted our present review to wheat flours, the instrument can be used to advantage for measuring the colour of many other cereal products and undoubtedly has possibilities in other fields.

METHOD OF EXPRESSING RESULTS—

The circular scale carried by the Kent-Jones and Martin instrument and from which the final readings are taken is calibrated in degrees. With the instrument adjusted so as to give a reading of 80° when balance is effected after an 80 per cent. transmission screen has been inserted in front of the standard cell, as advocated by Kent-Jones and Martin, pre-war patent flours give readings of about 15° to 40°, while flours of 85 per cent. extraction give readings of about 100° to 140°. There is much to be said for the use in the cereal industry of a less widely spread scale, particularly as many millers in this country are accustomed to the grade colour scale of Kent-Jones and Herd,[3] which ranged from 4·5 to 6·0 for patent flour to 8·5 to 10·0 for basic grades. A scale in this region for the grade colour, which is easily understood by the miller, has the further advantage that it falls in line with the widely used bleach figure scale of Kent-Jones and Herd[3] which runs from about 2 to 10. With these considerations in mind we decided to narrow the scale and we have done this by accepting as the "grade colour figure" the figure obtained by dividing the dial reading in degrees by 10 and correcting to the nearest 0·5.

REPRODUCIBILITY OF DIAL READINGS—

In view of the possibility of this method being adopted by chemists in both the milling and the baking industries, that is by representatives of buyers and sellers of flour, it is most important that the results it gives should show good reproducibility. It is not sufficient that duplicate readings on a given flour paste should show good agreement; the spread of results obtained upon a flour by different operators on different days must be small. The reliability of the method from this angle was therefore determined by statistical analysis of appropriate experimental data. The design of this experiment involved the determination of the dial readings of five flours by two operators on each of 4 days. The operators were unqualified assistants accustomed to performing routine tests. The experimental results appear in Table I.

TABLE I

DIAL READINGS OF FLOURS DETERMINED BY TWO OPERATORS ON DIFFERENT DAYS

	Dial readings Flour No.				
	1	2	3	4	5
Operator A					
1st day	104	122	146	159	34
2nd »	108	120	150	161	33
3rd »	101	121	147	158	35
4th »	96	118	143	157	32
Operator B					
1st day	100	123·5	153	160	—
2nd »	98	119·5	150	158	31·5
3rd »	111	132	148	161	32
4th »	102	126	146	158	32·5

Statistical analysis of these data shows that the ratio of "between-operators" variance to the error variance is 2·01, and the corresponding figure for the "between-days" variance is 2·13; for these ratios to attain significance at a probability level of 0·05 they would need to exceed 4·84 and 3·59 respectively. The standard deviation is 3·2°.

These data mean that if the grade colour figure for a given flour is assessed by different operators on different days, the results (expressed as dial readings divided by 10) may be confidently expected to agree within ±0·5 unit.

INFLUENCE OF DEGREE OF BLEACH ON DIAL READINGS—

If the instrument of Kent-Jones and Martin does in fact do what it purports to do, that is measure the grade colour or brightness of a flour, then it is essential that the dial reading given by a flour should be uninfluenced by any bleach, natural or artificial that has been conferred upon the flour. Kent-Jones and Martin state that they have rendered the dial reading substantially independent of degree of bleach by the insertion of a filter in the light path. In their paper they quote three tests in support of their contention but state that further tests may be desirable. Since the maximum difference between the dial readings before and after treatment obtained in the tests quoted by Kent-Jones and Martin was

less than the standard deviation established by our experiments, we extended the study to a wider range of flours. A wider discrepancy than that recorded by Kent-Jones and Martin between the readings given by a flour in the unbleached and in the bleached state would not be perturbing, provided it was still within the experimental error of the method.

In these experiments flours of various ash contents, obtained from several mills, were tested on the instrument in the unbleached state and also after the flours had been bleached by the addition of one of the bleaching agents in use in the milling industry. The bleaching agents were applied in the proportions in which they are commonly used in the industry. The data obtained in these experiments are given in Table II.

TABLE II

EFFECT OF BLEACHING REAGENTS ON THE DIAL READING

Sample	Ash, %	Dial readings		Colour figures (dial reading divided by 10)	
		Bleached	Unbleached	Bleached	Unbleached
		Benzoyl peroxide, 1/15 oz. per 280 lb.of flour			
K1	0·49	54	53·5	5·5	5·5
K2	0·61	77	72·5	7·5	7·0
K3	0·67	86	84	8 5	8 5
K4	0 75	93	93	9 5	9·5
K5	0·88	116	114·5	11·5	11·5
B1	0·43	42	31·5	4 0	3·0
B2	0·99	126·5	124·5	12·5	12·5
S1	0·42	34	28	3·5	3·0
S2	0·46	48	40	5·0	4·0
S3	1·15	124	119·5	12·5	12·0
		Nitrogen trichloride, 7 g. per 280 lb. of flour			
S1	0·42	34	29·5	3·5	3·0
S2	0·46	48	43	5·0	4·5
S3	1·15	124	119	12·5	12·0
		Chlorine, 1 oz. per 280 lb. of flour			
S1	0·42	34	31	3·5	3·0
S2	0·46	48	41·5	5·0	4·0
S3	1·15	124	119·5	12·5	12·0

As we had anticipated, differences appreciably greater than the 2·5° quoted by Kent-Jones and Martin were obtained in this extended series of tests of unbleached and bleached samples. The encouraging feature of the tests is, however, that the effect of bleach upon flours with ash contents below 1 per cent.—a range which embraces flours from high grade patent flours to 90 per cent. extraction flours—is within the experimental error of the method, *i.e.*, ±0·5 units.

CORRELATION BETWEEN DIAL READING AND ASH CONTENT—

We have explained in the introduction to this paper that the "grade" colour of flour, which Kent-Jones and Martin claim to be measurable by their instrument, is related to the extent to which the endosperm of the grain has become contaminated with powdered wheat skins and associated substances during the milling process. Provided a flour contains no extraneous mineral matter, the proportion of powdered wheat skins it contains is correlated with its ash content, because the ash content of pure endosperm is 0·3 per cent. or less, whereas the ash content of pure wheat skin is in the region of 8 to 10 per cent. It follows, therefore, that if this instrument is to provide a reliable measure of the "grade" colour of flours, there must be a correlation between dial reading and natural ash content. Our next step in this investigation, therefore, was to determine the dial readings and the ash contents of various mill stocks from several mills. As the instrument was designed for use with commercial flours the stocks used in this experiment were restricted to those with ash contents not substantially greater than 1 per cent. The data obtained in this series of tests are given in Table III.

TABLE III

DIAL READINGS AND ASH CONTENTS OF A SERIES OF MILL STOCKS

Sample	Ash, %	Dial reading
Mill 1		
A flour	0·52	61
B "	0·54	59·5
C "	0·46	49
D "	0·80	99
B_2 "	1·11	121
Mill 2		
A flour	0·43	40·5
B "	0·42	36
C "	0·43	47
D "	0·87	108
Mill 3		
A flour	0·53	54
B "	0·42	38·5
C "	0·47	50·5
D "	1·08	122
B_2 "	1·06	110
Mill 4		
A flour	0·44	55
B "	0·44	56
C "	0·51	62·5
D "	0·68	98
B_2 "	1·08	130
Mill 5		
A flour	0·37	39
B "	0·43	39·5
C "	0·43	39·5
D "	0·66	96
E "	1·10	149·5
B_2 "	0·79	99
Mill 6		
A flour	0·47	48
B "	0·44	43·5
C "	0·43	40·5
D "	0·74	93·5
1st Bk. flour	0·60	82
Mill 7		
A flour	0·44	36·5
B "	0·42	37·5
C "	0·47	51
Mill 8		
A flour	0·48	56
C "	0·44	39·5
D "	0·66	80·5
B_1 "	0·50	57
B.M.R.	0·77	96
Mill 9		
A flour	0·46	47·5
B "	0·52	51·5
C "	0·47	48
D "	0·58	68
A, B, C, D flours	0·51	54
Mill 10		
A flour	0·45	39
B "	0·43	39
C "	0·49	47
D "	0·61	66
1st Bk. flour	0·59	80
2nd Bk. flour	0·59	75·5
B_2 flour	0·59	70·5

Statistical analysis of these data reveals that for these experiments the correlation coefficient between dial reading and ash content is 0·959. This very high degree of correlation leaves no doubt that the colour figure of a normal uncontaminated flour furnished by this instrument is a reliable index of the grade of that flour.

When the dial readings of Table III were plotted against the corresponding ash contents, the scatter diagram thus produced showed that the relationship between the two parameters was not linear. Re-examination of the data established that a linear relationship did exist, however, between dial reading and the logarithm of the ash content. The regression of log ash on dial reading was therefore calculated and was found to be—

$$\text{Log ash} = 0{\cdot}0044 \times \text{dial reading} - 0{\cdot}5454.$$

It must be emphasised that this equation connecting dial reading with log ash applies only to the instrument we used and at its present setting. Each setting and each instrument, therefore, will have its own regression equation.

In Fig. 1 the regression line corresponding to this equation has been drawn and on the same diagram the dial readings of Table III have been plotted against the logarithms of the corresponding ash contents. Although the degree of correlation between dial reading and ash content is so high, as can be seen from the scatter diagram of Fig. 1, it is possible that two flours of significantly different ash contents may furnish the same dial reading and, conversely, two flours of identical ash contents may yield significantly different dial readings. Examples from Table III are A flour from Mill 5 and A flour from Mill 10, flours which give the same dial reading of 39 but have ash contents of 0·39 and 0·45 per cent. respectively; D flour from Mill 5 and B.M.R. flour from Mill 8, each of which gives a dial reading of 96 but which have respectively ash contents of 0·66 and 0·77 per cent.; B flour from Mill 4 and A flour from Mill 7, each of which has an ash content of 0·44 per cent. and yet which give

dial readings of 56 and 36·5 respectively; and D flour from Mill 5 and D flour from Mill 8 which are identical in ash content of 0·66 per cent., but which give respectively dial readings of 96 and 80·5.

Occasional apparent discrepancies of this nature are not surprising and are, in fact, to be expected. The ash contents of different types of wheat show an appreciable spread and the ash content of the pure endosperm of one wheat may be different from that of another. Furthermore, the various skins on a wheat kernel vary among themselves in both ash content and colour, and hence the relationship between the dial reading and the ash content of an individual mill stock can be influenced by the origin of the stock in question.

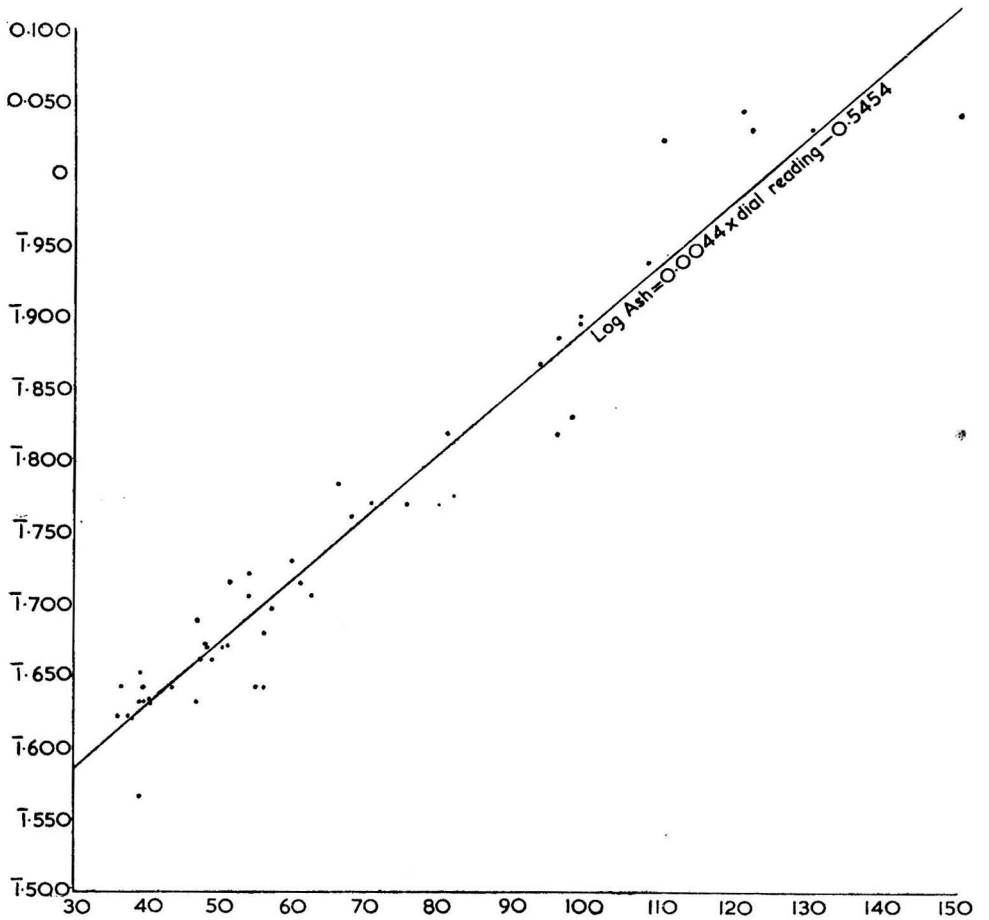

Fig. 1

Although these facts have not been unrealised in the past, there has been a tendency to ignore them and to accept ash content as a definite measure of flour colour. Our experience, however, has proved that ash content is not always a fully reliable index of the brightness of the flour as determined by its ability to reflect light, which is what is meant by "grade" colour.

The position is, in our opinion, as follows. If an instrument is to be a reliable means of measuring the "grade" colour of flour, the readings it gives will bear a high degree of correlation with the corresponding ash contents, but since the ash content is not always a sure index of the colour, occasional deviations from the relationship should occur. This instrument conforms to these expectations and it is our opinion that providing, as it does, a direct measure of reflecting power, it is a more reliable means of evaluating "grade" colour

than is the indirect estimate from the ash content, and hence, where a discrepancy between the two methods occurs, it is the instrument that provides the truer index of the colour.

As has been mentioned, the miller in pre-war days paid considerable attention to the ash content of his flour because this figure was accepted as an index of the grade colour. To-day, however, the miller is without this guidance because by Government decree all bread flour contains a proportion of added calcium carbonate (Creta praeparata) and the reported ash content of a flour is not, therefore, a measure of the natural ash content of the flour. Furthermore, it is not possible to apply a standard correction for the effect of the addition of the statutory proportion of calcium carbonate because it is impossible to effect completely uniform distribution of this addendum. This new method for the measurement of grade colour should, therefore, prove exceptionally valuable at the present time in that it will enable the miller to obtain a numerical assessment of the success of his milling operations despite the influence of added mineral matter upon the ash determination.

In Table IV are given the grade colour figures, *i.e.*, dial readings divided by 10, of a series of commercial samples of 85 per cent. National flour received recently at our laboratories for analysis and it will be seen that between the worst and the best of these, for samples covering the extreme range likely to be encountered under normal conditions, there is a spread of 4 units of grade colour. Included also in this table are the ranges of colour figures to be expected from pre-war white flours and from 90 per cent. extraction flours of the type made for a short period during the war.

TABLE IV

TYPICAL GRADE FIGURES FOR COMMERCIAL FLOURS

Sample	Grade colour figure
Pre-war patent flours (ash contents 0·32 to 0·40 per cent.)	1–4·0
Pre-war straight-run flours (72 per cent. extraction) (ash contents 0·44 to 0·50 per cent.)	4·5–6·5
National flour (85 per cent. extraction)	9·5
" " " "	10·5
" " " "	11·0
" " " "	9·0
" " " "	10·5
" " " "	13·0
" " " "	10·0
" " " "	9·5
" " " "	11·0
" " " "	13·5
" " " "	10·0
" " " "	10·0
" " " "	12·0
" " " "	9·5
90 per cent. extraction flour	13·0–15·0

DETERMINATION OF GRADE COLOUR OF SELF-RAISING FLOURS—

The evaluation of the grade colour of self-raising flour has always been a problem. The inclusion of over 7 lb. of mineral aerating ingredients in 280 lb. of flour and the possibility of minor variations in the distribution of the ingredients renders the ash content of a self-raising flour valueless as an index of colour. Separation of the added mineral ingredients by high-speed centrifuging prior to the determination of ash content has been suggested,[15] but the method has not been widely adopted. The present colour method, therefore, seemed to offer a simple solution to the problem, provided any interference caused by the added chemicals could be overcome.

A preliminary experiment (Table V) revealed that the addition of acid calcium phosphate and sodium bicarbonate to a flour in the proportions used for self-raising purposes significantly diminished the dial reading given by a flour by the normal technique. The addition of normal proportions of acid sodium pyrophosphate and sodium bicarbonate did not, however, seriously alter the dial reading.

In view of the interference caused by the presence of acid calcium phosphate, which is the commonly used acid ingredient of self-raising flours, it was decided to investigate the effect of using a buffer solution in place of water in the preparation of the flour paste. Experiments showed that if the paste were made with a citrate buffer solution, the reading of a plain

TABLE V

DIAL READINGS OF FLOURS CONTAINING MINERAL ADDITIONS
PASTE MADE WITH WATER

	Flour			
	A	B	C	D
Natural ash content, %	0·41	0·56	0·78	1·20
Dial reading with water (normal procedure)	39	68	105	169
Dial reading with water after addition of 3¼ lb. $NaCO_3$ and 4½ lb. A.C.P. per 280 lb. (normal self-raising flour)	25	48	87	151
Dial reading with water after addition of 3¼ lb. $NaCO_3$ and 6½ lb. proprietary pyrophosphate mixture per 280 lb. (normal self-raising flour)	39	62	105	168

flour was not altered and, moreover, the conversion of the flour to a self-raising flour by the addition thereto of sodium bicarbonate and acid calcium phosphate or acid sodium pyrophosphate did not markedly affect the dial reading. Furthermore, when the buffer solution was used to make the paste, a flour containing a marked excess of acid calcium phosphate, acid sodium pyrophosphate or sodium bicarbonate, gave a dial reading not vastly different from that of the untreated flour. A series of results from these experiments is given in Table VI.

TABLE VI

DIAL READINGS OF FLOURS CONTAINING MINERAL ADDITIONS
PASTE MADE WITH BUFFER SOLUTION

	Flour			
	A	B	C	D
Natural ash content, %	0·41	0·56	0·78	1·20
Dial reading with water (normal procedure)	39	68	105	169
Dial reading with buffer	41	65	103	172
Dial reading with buffer after addition of 3¼ lb. $NaHCO_3$ and 4½ lb. A.C.P. per 280 lb. (normal self-raising flour)	40	67	106	164
Dial reading with buffer after addition of 3¼ lb. $NaHCO_3$ and 6½ lb. proprietary pyrophosphate mixture per 280 lb. (normal self-raising flour)	39	62	105	168
Dial reading with buffer after addition of 4½ lb. A.C.P. per 280 lb. (excess of acid)	41	70	—	173
Dial reading with buffer after addition of 6½ lb. proprietary pyrophosphate mixture per 280 lb. (excess of acid)	39	—	—	174
Dial reading with buffer after addition of 3½ lb. $NaHCO_3$ per 280 lb. (excess of alkali)	35	—	—	—

A stock buffer solution is prepared by dissolving 110 g. of disodium hydrogen phosphate and 77 g. of citric acid in water and making up to 1 litre. When required for testing self-raising flour this solution is diluted fivefold.

These tests show, therefore, that the method can be applied to self-raising flours equally as well as to plain flours provided the flour is made into a paste not with water but with a citrate buffer solution of specified strength. By this technique it is possible to obtain a reliable numerical assessment of the colour of a self-raising flour. This instrument, therefore, provides a long-felt want in that it enables the grade colour of flour to be expressed on a numerical basis, even when that flour contains mineral additions which invalidate the use of the ash test as a measure of colour.

SUMMARY

The instrument of Kent-Jones and Martin enables the grade colour to be expressed numerically and the results it gives have good reproducibility in the hands of different operators (irrespective of the day upon which the test is made). The "grade colour figure" obtained by dividing the dial reading by 10 can be determined within ±0·5 unit irrespective of the degree of bleach on the flour.

The dial readings obtained upon this instrument are linearly related to the logarithms of the corresponding ash contents and there is a very high degree of correlation between dial reading and ash content.

The grade colour of self-raising flours can be determined by this instrument by employing a buffer solution in the preparation of the paste.

It is with pleasure that the authors place on record their appreciation of the ready willingness of Dr. E. C. Wood to guide them in the theoretical and practical issues involved in the statistical aspects of this paper.

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Discussion

Mr. A. L. Bacharach called attention to the difference between "dial readings" for bleached and unbleached flour. Although the maximum difference between pairs of readings was 10, and although this represented only twice the expected difference from the mean (5 units), the effect of bleaching might well be significant because all the differences produced by it were in the same direction.

Dr. J. H. Hamence asked whether there was any means of checking the instrument against a standard.

Mr. D. M. Freeland pointed out that the correlation of colour units with ash content demonstrated by the authors could apply only these days to "pure" mill streams. Most users of flour found ash figures were increased because of the presence of 14 oz. of prepared chalk per 280 lb. of flour and the ash - colour relation was thereby disjointed. Would not some approximate ranges of colour units to the usual observations of the Pekar test be more useful to the flour buyer?

Mr. E. Seal said that, as the grade colour was really that of the flour-water paste and not the flour itself, it was not exactly a measure of flour colour. A soft flour would appear much brighter in the dry state than a strong granular flour of equal grade. Had Dr. Amos observed these actual differences between weak and strong flours of equal grade-figure as measured on this instrument? He also asked whether the use of the buffer with plain flours would extend the period of time during which a measurement could be accurately made on the paste.

Dr. C. R. Jones endorsed the claim that there was need for a method of measuring brightness of flours, and considered the results given most encouraging, subject to the important question already raised as to reproducibility of results with different instruments. He suggested the term "flour-water suspensions" be used in place of "flour pastes" to avoid confusion with gelatinised preparations.

Dr. A. Green said that he considered that the reference by the authors to the presence of carotene in wheat flour was not justified. Both Zechmeister and von Euler had failed to find it (see also Bacharach, *Analyst*, 1941, **66**, 36).

The degree of bleaching of the test flours was not in general that which is used in commercial practice. Would the application to the more branny stocks of heavier treatments than those described by the authors still yield practical identity in light reflection for the same mill product when unbleached and when bleached?

In view of the fact that nitrogen trichloride and chlorine dioxide have different effects on the pigments of bran, had the authors tested the performance of their instrument on mill products containing finely divided bran, before and after these products had been treated with chlorine dioxide? It is possible that light of a different wavelength might be needed in this case.

Dr. K. A. Williams mentioned that he used a very similar method in determining the colour of oils and fats. In that case he had preferred to determine the ratio of the transmissions at two wavelengths rather than a figure at only one wavelength. Attention had recently been paid to this method in America.

Dr. J. Straub called attention to the fact that greyness of flour is measured by the apparatus much more accurately than it could be assessed visually. He felt that such precision would prove its usefulness in research in preventing greyness of flour. When trying out new techniques of milling for the removal of

greyness, the slightest invisible change in greyness would show whether progress was being made. The laboratory was to be congratulated on its results.

DR. N. L. KENT asked whether a high correlation between dial reading and ash content was obtained if a series of flours taken right down the reduction of a mill, from A to K, L or M, *i.e.*, including both high grade and low grade flours, was used.

MR. F. T. HOLDEN said that he was a miller from British East Africa. He felt that the method which had been described for measuring the colour of flour in a positive way would be welcomed by millers, and more particularly by the miller abroad. For the first time it would be possible for him to compare the colour he obtains in a simple and reliable way with that achieved by millers in this country. Therefore the speaker asked for an assurance that no local factors would upset the figures he might obtain, thus preventing a true comparison with the figures obtained in this country. He asked whether the character of the water would affect the result, and whether the temperature of the water would alter the readings.

DR. AMOS said in reply that the fact that the slight effect of bleach upon the dial readings had always been in one direction might have some significance, but the purpose of the quoted figures was to show that the magnitude of this effect upon the readings was within the experimental error of the test.

The instrument could be standardised by two methods. One of these, which was mentioned in the paper, was to insert an 80 per cent. transmission screen in one light path and to adjust the instrument so that a given reading was obtained upon the dial. The other method was to adjust the instrument so that the readings of two or three flours of known ash contents were those required by the regression equation for the instrument in question. The ideal method would probably be calibration against a standard white surface, but this would necessitate a standard which was absolutely permanent, and it had not been possible to meet this essential criterion.

Mr. Freeland had perhaps misunderstood the position. The authors had demonstrated that their dial readings were closely correlated with the *true* ash contents of flours as proof that their readings were a measure of *grade* colour. The fact that this correlation would not be apparent in National flours because of the presence of chalk was beside the point; the instrument was designed to measure grade colour, and this it did whether chalk was present or absent.

Although the reading was made on a flour paste, it was nevertheless a true measure of the grade colour of the flour, as had been shown by the high degree of correlation between the readings and ash contents. Certainly a soft flour appeared brighter than a strong flour of equal grade because of the influence of granularity, and it was for this very reason that the authors had eliminated the influence of granularity by the use of a paste. The use of the buffer with plain flours had not been tried, but there seemed no point in investigating the possibility of extending, by this means, the time that a paste could be kept before the reading was taken, since with a water paste no change in the reading occurred in 30 minutes.

The rates of bleaching treatment that were employed were average values for the commercial bleach-treatment of straight-run flours, and they had not significantly affected the readings when applied to flours ranging from patent grade to a grade equivalent to 90 per cent. extraction. It was true that in commercial practice certain very low-grade stocks would receive a much higher dosage, but this point had not arisen because in this paper the authors had restricted their investigations to stocks with ash contents not significantly greater than 1 per cent. The use of chlorine dioxide was not included in the bleaching experiments because it was not in commercial use in this country, but in view of the fact that it was likely to be so used at some time in the future, tests with this product were in hand.

The correlation coefficient between dial reading and ash content quoted in the paper applied to a series of flours ranging from high patent grade to those with ash contents slightly higher than 1 per cent. Tests with low-grade stocks having ash contents of 3 per cent. and over had shown a very good degree of correlation.

The nature of the water used to make the paste would not be likely to influence the results seriously; the authors had tried distilled water and tap water and had found no difference in their results. Tests made during the summer had not been affected by the temperature, but the high temperature experienced in some countries overseas might lead to a slight difference in the readings; this could be tested and due allowance made.

THE CALCULATION OF THE BOTANICAL COMPOSITION OF WHEAT FLOURS AND OFFALS FROM THE CHEMICAL ANALYSIS

AT the meeting at which the above two papers were read, a lecture with this title was delivered by J. Straub, Chem. Ing., of the Central Institute for Nutrition Research, Utrecht.

The following is a summary of Mr. Straub's lecture, which has been published in full in *Rec. Trav. Chim. Pays-Bas*, 1950, **69**, 141.

A method was described for calculating the botanical composition, in terms of the percentage of pericarp, aleurone and endosperm in wheat flours and offals, from the amounts of crude fibre, phosphorus and starch in the milled product; these three constituents

being characteristic respectively of the pericarp, the aleurone and the endosperm. Hence, from a knowledge of the amounts of fibre, phosphorus and starch that characterise the different botanical tissues it is possible to calculate the relative proportions of each that might be present in a sample, without having to separate them in a pure state.

As a further consequence of the work it was possible to calculate the protein and fat content of the botanical fractions. It was also shown that the endosperm consists of two distinct parts, an inner and an outer, of widely differing starch and protein contents, and of which the proportions could be determined in milled products.

Lactose and Chloride Contents of Egyptian Cows' and Buffaloes' Milks

By A. M. EL-SOKKARY and H. A. HASSAN

Synopsis—Previous studies of Egyptian milks have not included the direct chemical estimation of lactose and chloride contents. An investigation has therefore been carried out, as an extension of previous work, into the lactose and chloride contents of the milks of Egyptian cows and buffaloes, 100 of each animal being taken at random.

Buffaloes' milk was found to have a significantly higher lactose content and lower chloride content than cows' milk. Mean values, ranges of variation and frequency distributions of values have been calculated for both milks.

The chloride - lactose relation, calculated by the Mathieu and Ferré formula, was found to be approximately the same for both milks, although the value was lower than had originally been supposed.

Owing to the lack of adequate data on the subject, there is still considerable scope for an investigation of the milks of Egyptian cows and buffaloes. In a previous paper,[1] the authors have given some results for both milks, but lactose and chloride contents were not given, since they were not determined. Lactose is generally obtained by difference methods in routine milk analysis, and thus there are very few available data based on its actual chemical determination, as compared with the data concerning the other constituents of cows' milk. This inadequacy is even more noticeable in the case of buffaloes' milk, owing to the small amount of work that has been carried out on it. It was decided, therefore, to carry out an investigation on both milks to complete the previous study.

The complementary relationship between lactose and chloride in milk has been recognised for over 50 years, and Mathieu and Ferré's formula[2] is still looked upon as a valuable means of assessing the part played by lactose in the variation of non-fatty solids in milk. The chloride content of milk is taken as a criterion of the performance of the animals' glands in milk secretion, since a low chloride content denotes a high level of lactose secretion, and thus indicates a good condition of the secreting tissue.

Recent investigations,[3,4] however, although still indicating a close negative correlation between the lactose and chloride contents of milk, show wide variation in Mathieu and Ferré's value or the "simplified molecular constant."

The problem, therefore, needed further investigation, particularly with respect to the Egyptian milk animals, in which field this paper is the first to deal with the subject.

Experimental

This study was carried out on individual samples taken from the well-mixed milk from the morning milking of 100 cows and the same number of buffaloes kept in Cairo and on neighbouring farms. Samples were taken at random, but animals with clinical cases of disease were avoided.

Lactose and chloride contents were determined, and their relationship calculated according to the Mathieu and Ferré formula[2]:

$$\text{Lactose, per cent.} + 19{\cdot}6 \times \text{chloride, per cent.} = \text{constant (7)}.$$

Methods of analysis—Lactose was determined by the gravimetric method of A.O.A.C. for milk,[5] and chloride by the wet digestion method developed by Davies.[6]

Results

Table I shows the means, S.D., S.E.M. and significance of differences in lactose content, chloride content and chloride - lactose number, for both milks. The significance of differences is calculated by the *t*-test.[7]

Table I

The significance of differences between cows' and buffaloes' milks

	Lactose, %		Chloride, %		Chloride - lactose number	
	Cows'	Buffaloes'	Cows'	Buffaloes'	Cows'	Buffaloes'
Mean	4·71	4·87	0·0765	0·0649	6·20	6·15
Range of variation	3·75–5·39	3·91–5·46	0·0403–0·1492	0·0338–0·1190	5·73–6·89	5·83–6·58
S.D.	0·33	0·27	0·0209	0·0149	0·20	0·18
S.E.M.	0·03	0·03	0·0021	0·0015	0·02	0·02
Difference between means, C-B ..	− 0·16		+ 0·0116		+ 0·05	
t	3·7324		4·5356		1·8746	
Probability, P ..	< 0·01		< 0·01		0·1–0·05	
Significance* ..	+		+		−	

* Plus sign (+) = significant; minus sign (−) = insignificant.

The frequencies with which the three values occur were calculated, and are given in Tables II, III and IV.

Table II

Frequency distribution of percentage of lactose

Intervals ..	3·40 to 3·80	3·80 to 4·20	4·20 to 4·60	4·60 to 5·00	5·00 to 5·40	5·40 to 5·80	Total
Cows' milk	2	5	32	39	22	—	100
Buffaloes' milk ..	—	1	13	52	33	1	100

Table III

Frequency distribution of percentage of chloride

Intervals ..	0·0300 to 0·0500	0·0500 to 0·0700	0·0700 to 0·0900	0·0900 to 0·1100	0·1100 to 0·1300	0·1300 to 0·1500	Total
Cows' milk	7	35	38	14	3	3	100
Buffaloes' milk ..	12	51	33	3	1	—	100

Table IV

Frequency distribution of chloride - lactose number

Intervals ..	5·6 to 5·8	5·8 to 6·0	6·0 to 6·2	6·2 to 6·4	6·4 to 6·6	6·6 to 6·8	6·8 to 7·0	Total
Cows' milk ..	2	8	41	36	9	1	3	100
Buffaloes' milk ..	—	16	54	20	8	2	—	100

Discussion of results

Lactose content—

It can be seen from Table I that the lactose content of cows' and buffaloes' milks ranged from 3·75 to 5·39 per cent. and 3·91 to 5·46 per cent. respectively; the mean values being 4·71 per cent., S.E.M. 0·03, and 4·87 per cent., S.E.M. 0·03. It is thus observed that buffaloes' milk has a higher lactose content than that of cows; the difference averaging 0·16 per cent. This difference, though little, is consistent, and, as calculated by the *t*-test, has a P of less than 0·01, which indicates its high significance. This was also proved by Anantakrishnan *et al.*,[3] who found that Indian buffaloes' milk contained more lactose than did cows' milk.

Table II shows that the highest frequency distribution of lactose lies in the class 4·60 to 5·00 per cent. in both milks, but for that of cows this highest frequency represents 39 per cent. of the samples, while it represents 52 per cent. of the samples of buffaloes' milk. It is also noticeable that for cows' milk, 39 per cent. of the samples are below this class, while

for buffaloes' milk, the corresponding percentage is only 14, and 34 per cent. of the samples are above the 4·60 to 5·00 per cent. class. This distribution clearly shows that the value tends to be higher for buffaloes' than for cows' milk. Results given by Ghosb and Datta-Roy[8] show that lactose in buffaloes' milk ranged from 4·0 to 5·3 per cent., and that of cows' milk ranged from 3·8 to 5·3 per cent. These results are in close agreement with those of the present investigation, and with most of the available data for European and other breeds of cows.

CHLORIDE CONTENT—

It is well known that the chloride contents of individual samples of cows' milk show wide variation. This is demonstrated by the magnitude of the variations in Table I. The mean values are 0·0765 per cent., S.E.M. 0·0021, and 0·0649 per cent., S.E.M. 0·0015, for cows' and buffaloes' milks respectively. In the former, the value varied from 0·0403 to 0·1492 per cent., while in the latter it varied from 0·0338 to 0·1190 per cent., thus indicating that, on the average, cows' milk is 0·0116 per cent. higher in its chloride content than that of buffaloes. The difference is statistically significant, as shown in Table I.

This observation, together with the higher content of lactose in buffaloes' milk and its higher value of casein nitrogen as a percentage of the total nitrogen - casein number (El-Sokkary and Hassan[1]), indicates that buffaloes have a more efficient secreting tissue than cows have.

It can also be seen, by comparing the present results with those given by Davies,[9] that Egyptian cows' milk tends to contain less chloride than does the milk of other breeds of cows.

Table III shows that the highest percentage distributions of the chloride value for both cows' and buffaloes' milks lie in the classes 0·07 to 0·09 per cent. and 0·05 to 0·07 per cent. respectively, representing 35 and 51 per cent. of the samples. For cows' milk, 58 per cent. of the samples had chloride values above the highest frequency distribution, while for buffaloes' milk, only 37 per cent. were above it; hence the significance of the difference between the two milks is explained.

CHLORIDE AND LACTOSE RELATIONSHIP—

Owing to the fact that chloride and lactose concentrations in milk account for approximately 75 per cent. of its osmotic pressure, many attempts have been made to establish the correlation between them on a complementary basis. Among these attempts are those of Kopatschek[10] and Koestler,[11] but their ratios, however, are not so commonly used as that given by the formula suggested by Mathieu and Ferré,[2] known as the "simplified molecular constant." It is stated that if the value is below 7, the milk sample may be suspected of containing added water.

Since these workers published their results many investigations have been carried out on the subject, and these showed the value to vary over a remarkably wide range. Richmond *et al.*[12] state that the value is said to lie, for most milks, between 7·4 and 7·9, but they quoted a report of a range of 6·92 to 8·28.

Recently, some investigators have given results which show wider variations. Mathieu,[13] working on the milk of cows in Alpine regions, found that the ratio, in most samples, was higher than 8·0. Anantakrishnan *et al.*[3] found that, with Indian cows' and buffaloes' milks, the lactose - chloride relationship did not hold. Moreover, working out the ratio from results given by Roadhouse and Henderson[4] on milk from American breeds of cows, the authors found that, for one set of results, the ratio varied from 6·19 to 7·34, corresponding to lactose and chloride contents of 4·74 and 0·074 per cent. respectively for the lower ratio, and 4·16 and 0·162 per cent. respectively for the higher ratio. In another set, a ratio as low as 5·99 is given, corresponding to 5·05 per cent. lactose and 0·048 per cent. chloride.

These latter results agree to a large extent with those of the present study, where the mean value for the ratio in cows' milk is 6·20, S.E.M. 0·02, ranging from 5·73 to 6·89, and that of buffaloes' milk is 6·15, S.E.M. 0·02, ranging from 5·83 to 6·58. Difference between both means is shown to be insignificant (see Table I).

Table IV gives the frequencies with which values are distributed among the various classes. The highest percentage distributions for both milks lie in the same class, namely 6·0 to 6·2, and represent 41 per cent. of the cows' milk samples and 54 per cent. of those of buffaloes' milk.

SUMMARY—

Buffaloes' milk was found to have significantly higher lactose and lower chloride contents than cows' milk. The following are the respective means for cows' and buffaloes' milks:

Lactose: 4·71 per cent., S.E.M. 0·03, and 4·87 per cent., S.E.M. 0·03.

Chloride: 0·0765 per cent., S.E.M. 0·0021, and 0·0649 per cent., S.E.M. 0·0015.

Using the formula suggested by Mathieu and Ferré the lactose - chloride relation was calculated, and was found to be approximately the same for both milks, although the value was lower than had originally been supposed.

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July, 1949.

The Infra-Red Spectroscopic Estimation of 4-Methyl-2:6-Ditertiary Butyl Phenol in Mixtures Containing 2-Methyl and 3-Methyl-4:6-Ditertiary Butyl Phenols

BY J. L. HALES

SYNOPSIS—Industrial requirements set the problem of finding a rapid, accurate method for the estimation of 4-methyl-2 : 6-ditertiary butyl phenol in crude and refined products. It was also desirable to obtain a check on the type and amount of impurity in the products.

A description is given of an infra-red spectroscopic technique whereby this may be obtained. The limitations inherent in the method because of the presence of certain impurities are indicated.

Experimental values are given for estimations on several synthetic mixtures and on a crude product, and the magnitude of the errors introduced by impurities is indicated.

INTRODUCTION—Weinrich[1] has shown that although *m*- and *p*-cresol form mixtures which are difficult to separate, the two corresponding ditertiary butyl derivatives have a marked difference in volatility. Should, however, a cresol fraction containing *o*-cresol in addition to the *m*- and *p*- derivatives be ditertiary butylated, the product will contain, in addition to the 4-methyl-2 : 6-ditertiary butyl phenol (b.p. 191° C./100 mm.) and 3-methyl-4 : 6-ditertiary butyl phenol (b.p. 211° C./100 mm.), a proportion of 2-methyl-4 : 6-ditertiary butyl phenol (b.p. 194° C./100 mm.) which will render more difficult the separation by distillation of the first-mentioned component in a reasonably pure state.

The 4-methyl-2 : 6-ditertiary butyl phenol has technological applications as a softener and anti-oxidant, and the present purpose was to work out a rapid infra-red spectroscopic method of analysing mixtures in which it is the preponderant component, but which contain the other two isomerides, and in addition small quantities of monobutyl derivatives of the three cresols.

A method is described which gives an accurate value for the quantity of the desired component, and also a check on the amounts of the other two components present. A correction may be applied to allow for errors introduced due to the presence of monobutyl cresols in the crude mixture.

EXPERIMENTAL

Samples of the following were available—

(*a*) The three individual dibutyl cresols.
(*b*) A crude mixture of all three dibutyl cresols.
(*c*) A fraction representative of the monobutyl cresol content of (*b*).

The spectra of these samples and of their solutions in *cyclo*hexane were obtained on a Hilger D209 double-beam infra-red spectrometer,[2] using a rocksalt prism (see Fig. 1).*

The region from 700 to 800 cm.$^{-1}$ was found to be the most useful for analytical purposes, since it includes key bands suitable for the estimation of each of the three components. Furthermore, rocksalt has a high dispersion in this region. Since all the aromatic ring frequencies lie in this range, the analysis is liable to interference from aromatic impurities, such as unchanged or monobutylated cresols. This is referred to later in the paper.

*cyclo*Hexane, which is fairly transparent in this region, was chosen as the solvent for this analysis. Standard solutions of various concentrations of the individual components were made up and their spectra were measured. Sample cell thickness was 1·00 mm. for all analytical measurements.

Wide slits (1·20 mm., corresponding to spectral slits of 6 cm.$^{-1}$) were used with the spectrometer, so as to give a high signal-noise ratio for the thermopile, and to avoid resolving the key band at 775 cm.$^{-1}$ (Fig. 1) into two bands at 771 and 776 cm.$^{-1}$, which would complicate the analysis.

Optical density at the three key frequencies for each of the three components was plotted against concentration (Fig. 2). Scattered radiation was allowed for, using a mica shutter and a 1-mm. thick cell filled with *cyclo*hexane, so as to approximate closely to the experimental conditions.

A number of synthetic mixtures were prepared, their optical density at the key frequencies measured, and from the curves illustrated in Fig. 2 the analytical figures were worked out by the method of successive approximations.[3] The results are given in Table I.

TABLE I

SYNTHETIC MIXTURES

	Ditertiary butyl phenols		
	4-methyl-2 : 6-, g./100 ml.	2-methyl-4 : 6-, g./100 ml.	3-methyl-4 : 6-, g./100 ml.
Found	1·81	2·48	2·42
Found (corrected)	1·67	1·78	1·81
Taken	1·72	1·68	1·69
Found	1·75	1·30	1·08
Found (corrected)	1·70	0·75	0·73
Taken	1·72	0·71	0·64
Found	1·55	0·99	0·87
Found (corrected)	1·51	0·51	0·60
Taken	1·52	0·44	0·55
Found	2·10	0·83	0·53
Found (corrected)	2·07	0·26	0·27
Taken	2·05	0·23	0·22

Corrected figures take into account the overlapping of key bands.

It should be noted (Fig. 2) that the key band at 775 cm.$^{-1}$, for the 4-methyl-2 : 6-ditertiary butyl phenol has an extinction coefficient considerably higher than those of the bands at the key frequencies for the other two components. This enhances the accuracy and reliability of the estimation of this component, particularly as it is the major component

* The spectrometer that was used incorporated a D.C. amplifying system, and slight drifts in the latter were the main factor limiting the consistency of the quantitative results.

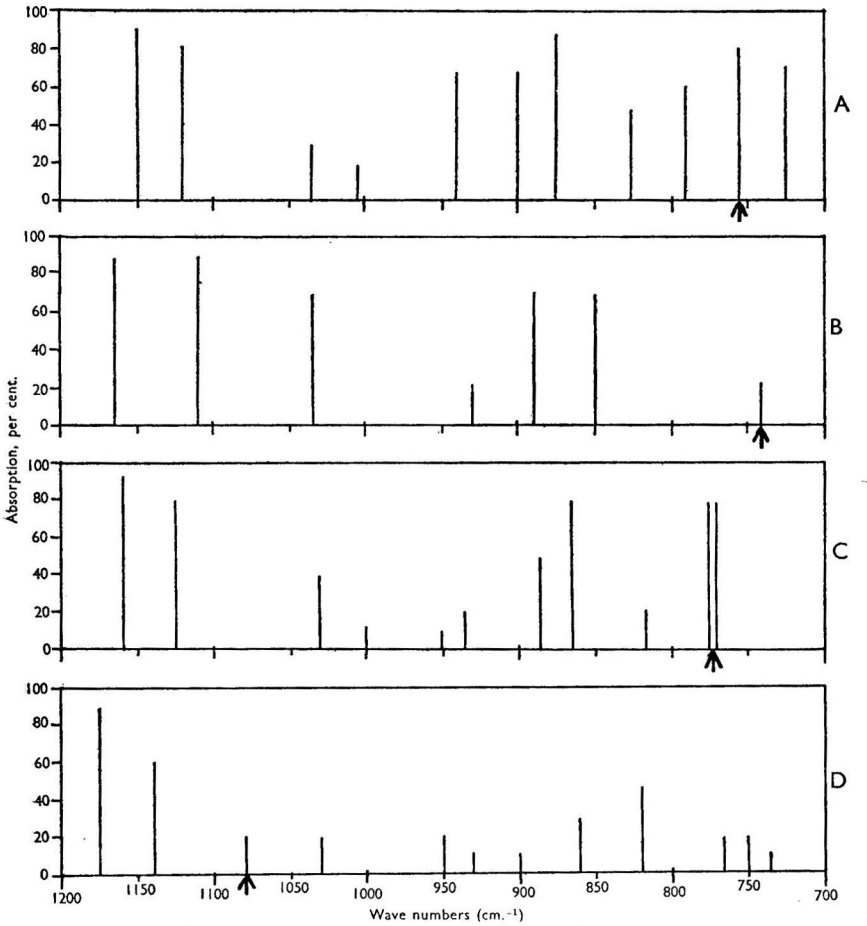

Fig. 1. Absorption Spectra. The arrows indicate analytical bands.
A: 2-Methyl-4:6-ditertiary butyl phenol. C: 4-Methyl-2:6-ditertiary butyl phenol.
B: 3-Methyl-4:6-ditertiary butyl phenol. D. Monobutyl cresol fraction.

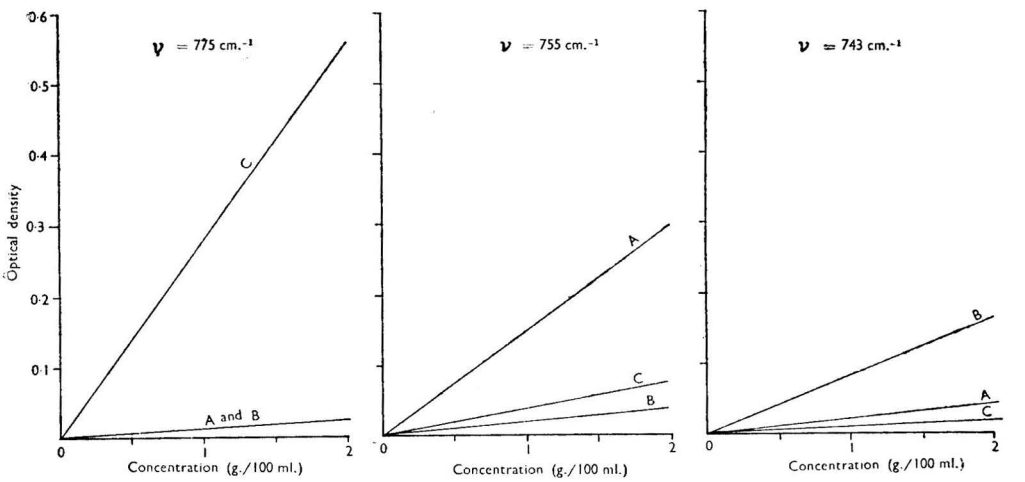

Fig. 2. Analytical curves for ditertiary butyl phenols.
A: 2-Methyl-4:6 ditertiary butyl phenol. B: 3-Methyl-4:6-ditertiary butyl phenol.
C: 4-Methyl-2:6-ditertiary butyl phenol.

in the present case. In Table I the uncorrected figures illustrate the error introduced when no allowance is made for the overlapping of the key bands, and it is seen that the effect is smallest in the case of the band at 775 cm.$^{-1}$. These considerations are substantiated by the relative magnitude of the errors in the figures given in the table.

MONOBUTYL CRESOL FRACTION—This fraction had several absorption bands near 775 cm.$^{-1}$ (Fig. 1) and the presence of monobutyl cresols in the crude product to be analysed will thus reduce the accuracy of estimation of the dibutyl cresols.

A correction for errors due to the presence of monobutyl cresols may be applied using an "internal reference" method. The monobutyl cresol fraction had a fairly intense band at 1081 cm.$^{-1}$ which does not coincide with any bands in the dibutyl cresols, and this can be used to detect and estimate the effect of small quantities of monobutyl cresols in the crude mixture. However, a 1-mm. layer of *cyclo*hexane absorbs strongly at 1081 cm.$^{-1}$, so that any comparison of optical densities must be made on the undiluted components. Although this may introduce deviations from Beer's law, the correction under consideration need not be known to a very high accuracy. Hence the optical density of the band at 1081 cm.$^{-1}$ was compared with the densities at the three key frequencies for the monobutyl cresol fraction, and from the density of the 1081 cm.$^{-1}$ band in the crude dibutyl cresol mixture, the respective corrections for density at the three key frequencies could be estimated. Their magnitude is shown by the figures in Table II, which gives the results of two analyses on the crude mixture.

TABLE II

ANALYSIS OF CRUDE DIBUTYL CRESOL MIXTURE

	Ditertiary butyl phenols		
	4-methyl-2 : 6-, g./100 ml.	2-methyl-4 : 6-, g./100 ml.	3-methyl-4 : 6-, g./100 ml.
Found	1·28	0·40	0·18
Corrected for presence of monobutyl cresols	1·24	0·26	0·01
Found	1·26	0·41	0·19
Corrected for presence of monobutyl cresols	1·22	0·27	0·02

Total crude material was 2 g. per 100 ml. in each case.

Thanks are due to the Esso European Laboratories for providing the specimens of pure and crude materials required for this investigation. The work has been carried out as part of the research programme of the Chemical Research Laboratory, and this paper is published with the approval of the Director of the Laboratory.

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The Estimation of Iron by Dichromate

By D. STOCKDALE

Synopsis—The relative merits of diphenylamine, diphenylbenzidine, barium diphenylamine sulphonate, ferrous phenanthroline and potassium ferricyanide as indicators in the estimation of iron by dichromate have been examined. The source of the iron was either ferrous ammonium sulphate or an ore, and the titrations were made in both the presence and in the absence of orthophosphoric acid.

All these indicators proved efficient when the conditions were suitable. Barium diphenylamine sulphonate was found to be the best, because its colour change is marked and because the end-point that it gives is the least affected by the conditions of the titration.

Numerous indicators, both internal and external, and a wide range of conditions have been recommended for this estimation. Because of certain discrepancies in the literature and occasional failures in the laboratory, it seemed desirable to make a systematic examination of the position.

The indicators used were as follows—

Diphenylamine (DA)	0·04 ml. of a 1 per cent. solution in concentrated sulphuric acid.
Diphenylbenzidine (DB)	0·04 ml. of a 1 per cent. solution in concentrated sulphuric acid.
Barium diphenylamine sulphonate (BaDS)	1·0 ml. of a 0·2 per cent. solution in water.
Ferrous phenanthroline (FeP) ..	0·05 ml. of an aqueous solution 0·025 *M* with respect to both ferrous sulphate and 1 : 10 phenanthroline monohydrate.
Potassium ferricyanide	Dilute solutions, used externally.

Electrochemistry

Since the pioneering work of J. H. Hildebrand[1] the electrochemistry of the oxidation of iron by dichromate and of the indicators has been much explored, notably by I. M. Kolthoff[2,3,4] and G. F. Smith[5] and their collaborators. Only the principal points need be recapitulated here.

When a bright platinum wire is placed in a solution of ferrous and ferric ions, the half-cell potential at 25° C. is given by

$$E = E_o - 0{\cdot}059 \log_{10}\frac{[Fe^{\cdot\cdot}]}{[Fe^{\cdot\cdot\cdot}]}.$$

E_o, the standard electrode potential at infinite dilution, is −0·771 volt.[6] However, as the ferric ion is very liable to form complex ions, the formal electrode potential in a solution of significant concentration, such as would be obtained by dissolving ferrous and ferric sulphates in the ratio of their equivalent weights in *N* sulphuric acid, is substantially less than E_o. It is about −0·69 volt when the ferrous and ferric salts are each about 0·05 *N* in either *N* sulphuric or *N* hydrochloric acid. In general, the lower the pH of the solution, the lower this potential, and it can be depressed further by the addition of some compound, such as orthophosphoric acid, which forms complexes with the ferric ion even more readily than do the common strong acids.

The electrode potential for the reduction of the dichromate ion,

$$Cr_2O_7'' + 14H^{\cdot} + 6e \rightarrow 2Cr^{\cdot\cdot\cdot} + 7H_2O,$$

is similarly given by

$$E = E_o - \frac{0{\cdot}059}{6} \log_{10}\frac{[Cr^{\cdot\cdot\cdot}]^2}{[Cr_2O_7''][H^{\cdot}]^{14}}.$$

The standard electrode potential of this reaction is −1·36 volts, but more usually the activity coefficients of the ions are far from unity, and the potential obtained by adding 50 ml. of

0·1 *N* dichromate to 25 ml. of 0·1 *N* ferrous solution is about −1·10 volts when the final concentration of strong acid is formally normal. This potential is much influenced by the pH, as the equation suggests, and will be increased by increasing the hydrogen ion concentration of the solution in which the reduction is taking place.

These points are illustrated by curves which have been obtained during the present investigation (Fig. 1). These curves show the results obtained when 25 ml. of 0·1 *N* ferrous salt in a solution of acid of the nature and concentration indicated were titrated with 50 ml. of 0·1 *N* potassium dichromate containing such an excess of the acid that the formal concentration of acid remained constant at that shown for each curve until the equivalence-point was reached. The volume at the equivalence-point was 50 ml., except with phosphoric acid present, when it was 60 ml., since 10 ml. of 50 per cent. by volume of orthophosphoric acid had been added initially. The horizontal lines near the curves mark the potentials of first colour change for the diphenylamine group of indicators and the formal electrode potential of ferrous phenanthroline in *N* sulphuric acid.

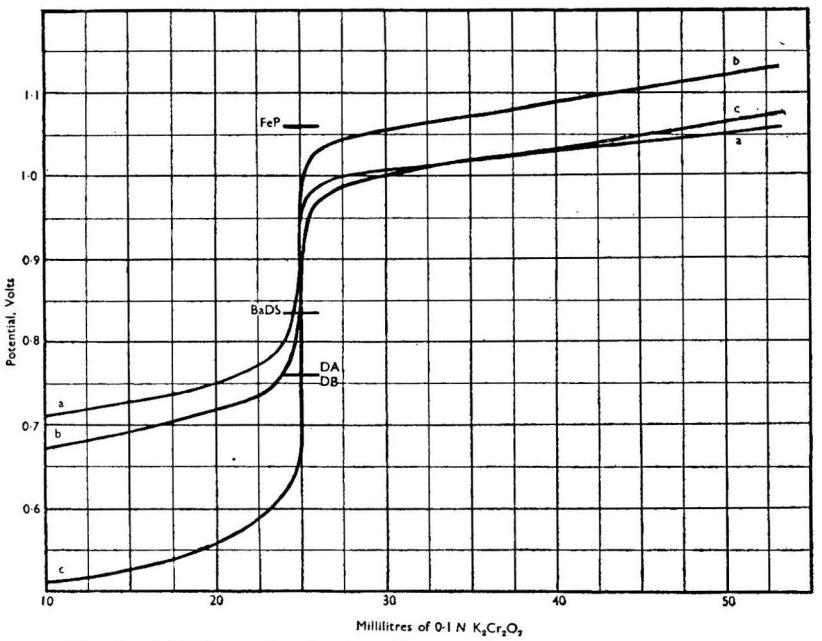

Fig. 1. Millilitres of potassium dichromate plotted against 25 ml. of 0.1 *N* solution of Fe^{++}. Curve (*a*) 0.1 *N* hydrochloric acid; curve (*b*) *N* sulphuric acid; curve (*c*) *N* sulphuric + orthophosphoric acids

The rate of change of potential per unit volume of dichromate was found to be greatest at −0·92 volt in *N* acid in the absence of phosphoric acid, and this was accepted as the equivalence-point potential. Fig. 1 shows that the first colour change of DA and DB should occur substantially before, and that of BaDS slightly before this point. It is to be expected that the maximum intensity of colour would be reached at potentials about 0·1 volt more than those of the first colour change. If this were so, the end-points of these indicators assessed on maximum intensity, not on first colour change, would coincide substantially with the equivalence-point. These potentials were found to be −0·86 volt for DA, −0·91 volt for BaDS and −0·94 volt for DB, the first two results being in accordance with expectation. The result for DB, which should be the same as that for DA because DB is the first oxidation product of diphenylamine, is high, presumably because DB is so insoluble (0·06 mg. per litre in water at 25° C.[2]). Most of this indicator is precipitated when a drop of it is added to the ferrous solution. When that remaining in the solution has been oxidised, more will slowly enter into solution and be oxidised in turn. The maximum colour intensity, therefore, can be obtained only slowly, and an estimation of the oxidation potential made during a titration carried out at a practicable rate will necessarily be high.

Fig. 1 shows that FeP is an unsuitable indicator when in a normal solution of a strong acid. However, as Smith and Richter[5] have shown, its oxidation potential is decreased by increasing the concentration of acid. The oxidation potential of the dichromate system is increased by the same means. It should therefore be possible to lower the line representing the potential of FeP relatively until it cuts the vertical part of the titration curve (Fig. 1), and under this condition the indicator would prove efficient. In an experiment using 2 *N* sulphuric acid, FeP was fully red at −0·91 volt. The addition of 0·05 ml. of 0·1 *N* dichromate at 25 ml. raised the potential to −1·05 volts and caused an immediate and noticeable change in the intensity of the colour. The red tinge faded out completely in about 30 seconds. In 10 *N* sulphuric acid the indicator was fully red at −0·87 volt, and a further addition of 1 drop of dichromate raised the potential to −1·00 volt, causing the indicator to change colour rapidly and completely. It would seem that 2 *N* is approximately the minimum concentration of sulphuric acid in which it is possible to make this titration. A final concentration of 2·5 *N* was later successfully adopted as standard. Results at the lower concentrations of hydrochloric acid were similar. With 1·5 *N* acid a titration was just possible, provided it was made slowly. With 2·5 *N* acid, 1 drop changed the potential from −0·84 volt (red) to −1·06 volts (colourless), and the oxidation of the indicator took place in about 10 seconds. In 6 *N* solution, on the other hand, it was almost impossible to obtain a result, because the greater part of the indicator was precipitated on the electrode tubes and on the sides of the beaker, and because the yellow colour of the ferric chloride ion made the final colour change difficult to see.

In these experiments, drops of the solutions were withdrawn from time to time and tested with potassium ferricyanide. It was found that in a normal solution of a strong acid the critical potential for this indicator was near −0·85 volt, a potential very slightly lower than that required for DA and definitely lower than those needed for the other indicators. It is also lower than the value of −0·92 volt taken as the equivalence-point potential. These results suggest that potassium ferricyanide ceased to give a blue colour when about 1 part per thousand of the bivalent ion remained unoxidised. This corresponds to a concentration of approximately 3 mg. per litre. This conclusion is confirmed by later work (see Tables I and II). There is, therefore, an appreciable indicator error with potassium ferricyanide, and it can be used in exact estimations only when a solution of dichromate is used to connect a known with an unknown quantity of iron, the two ferrous solutions being of approximately the same concentration.

Experiments with ferrous ammonium sulphate

Portions from 1·2 to 1·4 g. of the salt were weighed into conical flasks, dissolved in 100 ml. of approximately *N* sulphuric acid, and titrated with 0·1 *N* potassium dichromate (exact, assuming the salt to be pure). The final volume was 150 ml. and the final concentration of sulphuric acid was approximately 0·65 *N*. In a parallel set of experiments, 10 ml. of 1 : 1 by volume orthophosphoric acid was added in each titration, the final volume again being 150 ml., and the weight of sulphuric acid being the same as before. The above conditions apply for all the indicators except FeP, when 100 ml. of 4 *N* sulphuric acid was used to give a final concentration of 2·5 *N* with respect to this acid in a final volume of 150 ml.

The results, each the arithmetical mean of four titrations, are given in Table I.

Table I

	Without H_3PO_4, ml.	With H_3PO_4, ml.
Diphenylamine	25·49 ± 0·015	25·50 ± 0·01
Diphenylbenzidine	25·48 ± 0·02	No result possible
Barium diphenylamine sulphonate	25·49 ± 0·01	25·50 ± 0·01
Ferrous phenanthroline	25·48 ± 0·01	—
Potassium ferricyanide	25·44 ± 0·01	—

Table I gives the volume in ml. of 0·1 *N* potassium dichromate found to be equivalent to 1 g. of ferrous ammonium sulphate. The ferrous ammonium sulphate used was referred by potassium permanganate to pure sodium oxalate. Its equivalent weight was found to be 392·9 (for pure salt, 392·1). One gram of the salt should be oxidised by 25·45 ml. of 0·1 *N* potassium dichromate. It seems reasonable to assume that the sample of potassium dichromate was reasonably pure and that the end-points as shown by the various indicators

(Table I) are all close to the true equivalence-point. The standard deviations are given in the table; they mean only that if the groups of experiments were repeated under the same conditions the probability that the arithmetical means would fall within the range indicated is about 60 per cent. It was hoped that a deviation would serve as a measure of the value of the indicator, but, surprisingly, this was far from being the case. All the deviations are small, and the spread of results in any group can be entirely accounted for by the errors inherent in volumetric analysis with the type of apparatus used. The numerical results show that all the indicators worked perfectly; personal impressions of their behaviour were quite different. Some gave clear colours, with sharp changes; other gave muddy colours with the intensity of the colour changing noticeably with time after the addition of the oxidant, in such a way that considerable personal judgment seemed to be required in making an assessment. It seemed probable that such judgment could not be free from considerable error. It would appear, however, that satisfactory results can be obtained in volumetric analysis, even when using what seems to be an indifferent indicator and with the observer in doubt about his gauging of the end-point.

Notes on indicators

Diphenylamine is first oxidised irreversibly to DB, which is then oxidised reversibly to diphenylbenzidine violet. The behaviour of BaDS is similar. The end-points given in Table I are those of maximum intensity. It is probably advisable to assess them without reference to a blank, partly because of the difficulty of obtaining equal concentrations of indicator when such small volumes of indicator solution are used, and partly because the diphenylbenzidine violet is itself further oxidised by a small excess of dichromate to compounds of less intense colour. This further oxidation under the experimental conditions was slow, usually taking not less than an hour for the removal of the violet colour, and it is unlikely that it interfered with the assessment of the point of maximum intensity, but the colour of a spent and oxidised solution is often not permanent enough to be reliable as a standard in a subsequent titration.

The results given in Table I show that when an end-point is obtainable reasonably quickly with one of these three indicators it makes no difference to the numerical result which indicator is used, or whether orthophosphoric acid is present or not.

Diphenylamine—This indicator behaved somewhat erratically, particularly in the earlier stages of its oxidation in the absence of phosphoric acid. In two of the titrations the violet colour appeared early and deepened over a range of about 0·6 ml. in a titration of some 30 ml. In two others, the range was only about 0·25 ml. Usually a dirty muddiness appeared in the solution, often as much as 1 ml. before the end-point. Despite these variations the end-point was consistent. In the presence of phosphoric acid the solution remained clear, the colours were bright, and the range was approximately 0·04 ml. In some cases, however, the colour at the end-point was blue, not violet. Titrations without phosphoric acid are perhaps to be preferred, because of the warning given of the approach to the end-point.

Diphenylbenzidine—The colours, in the absence of phosphoric acid, were much brighter than with diphenylamine. Blue, not violet, predominated, and the blue colour changed to violet when the solution was allowed to stand after the titration. The range was approximately 0·7 ml. The titration must be carried out slowly with this indicator, because the colour deepens only slowly after the addition of the dichromate. This is perhaps due to its marked insolubility. In all cases a precipitate was produced when the indicator was added initially. Conversely, it also reacted slowly when ferrous iron was added to a solution of potassium dichromate.

When phosphoric acid was present there was a further slowing of the oxidation of the indicator to such an extent that to obtain consistent end-points was impracticable.

Barium diphenylamine sulphonate—Without phosphoric acid the approach to the end-point was marked by the appearance of a slight muddiness in the solution. The full rose-violet colour was developed after its first appearance by about 0·05 ml. of dichromate, and the range from first appearance of muddiness to maximum colour was about 0·1 ml. With phosphoric acid the colours were clear and the full colour was developed by about 0·03 ml. of dichromate. The indicator proved pleasant to use by either method, the element of doubt in judging the end-point being almost entirely absent.

Ferrous phenanthroline—The oxidation of this indicator, and indeed of the other oxidation - reduction indicators discussed,[3] appears to be complex, with ferrous iron playing

some part in the mechanism. It is possible to prepare a solution of FeP containing an appreciable excess of dichromate, the indicator being obviously in the reduced form, and to discharge the red colour by the addition of a small quantity of a solution containing ferrous iron. It follows that FeP is a more satisfactory indicator when the ferrous solution is run into the dichromate. This will usually entail the use of a standard iron solution in a titration, the stages being the oxidation of the iron for estimation with excess dichromate, followed by titration of this excess with standard iron. With DA and BaDS this process would seem to be unnecessary, because so little trouble arises in the direct titration, and, indeed, the direct titration with FeP is quite practicable provided a generous concentration of sulphuric acid is present.

Experiments with iron ore

These experiments were made to find out whether the elements likely to be present in a mineral or the compounds introduced by its reduction with stannous chloride interfered in any way with the indicators. The material used was Iron Ore A supplied by British Chemical Standards, and contained 58·20 per cent. of iron, together with very small quantities of sulphur, phosphorus, arsenic, copper and titanium, in addition to lime, magnesia, alumina and silica.

About 10 g. of the mixed and dried ore were digested with concentrated hydrochloric acid, potassium chlorate being added as a saturated solution from time to time in the later stages of the attack, a total of about 1 g. of the solid being used. The excess of hydrochloric acid was removed by evaporation, and the contents of the dish were baked at 110° C. for 1 hour. The ferric chloride was extracted with 1 : 1 hydrochloric acid, and the residue was fused in a platinum crucible with 3·5 g. of sodium carbonate. After treatment with hydrochloric acid, the residue was evaporated to dryness and baked again at 110° C. for 1 hour. This second extraction in hydrochloric acid was filtered into the main solution, which was diluted to 1 litre. For each titration 25 ml. of this solution was used. The iron was reduced with approximately 0·4 *M* stannous chloride in hydrochloric acid, and the small excess of this reagent was oxidised by 0·25 g. of mercuric chloride added as a solution. The final volume was about 120 ml., and was approximately normal with respect to hydrochloric acid, except that twice this quantity of acid was used for FeP. When phosphoric acid was present the volume added was 10 ml. of 1 : 1 acid in a final volume of 120 ml. Titrations were made with the sample of potassium dichromate used previously in 0·1 *N* solution. The results are given in Table II.

Table II

Dichromate required for 25 ml. of solution of iron ore

	Without H_3PO_4, ml.	With H_3PO_4, ml.
Diphenylamine	26·67 ± 0·005	26·61 ± 0·005
Diphenylbenzidine	26·66 ± 0·005	No result possible
Barium diphenylamine sulphonate ..	26·66 ± 0·01	26·65 ± 0·015
Ferrous phenanthroline	—	26·69 ± 0·015
Potassium ferricyanide	26·57 ± 0·03	—

Comments on indicators

The various substances present in the more complex solution made little difference to the behaviour of the indicators. The diphenylamine group always gave a violet colour at the end-point, whereas a blue was sometimes obtained with pure ferrous ammonium sulphate. The colours faded more rapidly in the presence of a small excess of the oxidising agent.

Diphenylamine—Phosphoric acid reduced the indicator range from about 0·2 to about 0·03 ml. It also improved the quality of the colour change, which was, however, moderately good in its absence. The reduction by the phosphoric acid of the volume of dichromate equivalent to the ore remains unexplained. The effect was checked by titrating a volume of the solution to just short of the end-point. It was then divided, and phosphoric acid was added to one half. One drop of dichromate caused this portion to change colour, but 2 drops were required to develop the full colour in the other.

Diphenylbenzidine—As before, the full violet colour was slow to develop, and therefore the final additions of the dichromate had to be made slowly. Phosphoric acid again delayed

the oxidation to such an extent that the use of this indicator in conjunction with this acid was not possible.

BARIUM DIPHENYLAMINE SULPHONATE—The end-point in the absence of phosphoric acid appeared to be somewhat indefinite and the colours obtained were again muddy. The indicator range was about 0·25 ml. In all titrations made in the presence of phosphoric acid the colours were clear and the change at the end-point was marked. The indicator range was only about 0·03 ml. in this case.

FERROUS PHENANTHROLINE—The colour of the $FeCl^{\cdot\cdot}$ ion masked the red colour of the indicator to such an extent that it was almost impossible to obtain an end-point. Even when phosphoric acid was present the colour change was not particularly obvious, and it was found advisable to compare the colour of the solution under titration with the colour of a similar solution containing a small quantity of ferrous iron. The end-point was taken at the first sign of lightening.

POTASSIUM FERRICYANIDE—The end-point with this particular sample of ore proved somewhat troublesome. It will be noticed that the standard deviation for this indicator, though still small, is larger than those for the others.

PERCENTAGE OF IRON IN THE ORE

With 25·49 ml. of potassium dichromate equivalent to 1 g. of ferrous ammonium sulphate of equivalent weight 392·9 (Table I), and 26·66 ml. of the dichromate equivalent to 25 ml. of the solution of the ore at 10·244 g. per litre (Table II), the percentage of iron in the ore is 58·05. The average value returned by the fourteen analysts co-operating with British Chemical Standards is 58·20 ± 0·02. The organiser's own value is 58·09 per cent. Better agreement is not to be expected, in view of the length of the standardisation chain—sodium oxalate, potassium permanganate, ferrous ammonium sulphate, potassium dichromate, ore. Interference by the foreign substances present during the titration of the solution of the haematite can, therefore, only have been small.

CONCLUSIONS

Of the indicators examined, barium diphenylamine sulphonate is the best for use in the estimation of iron with dichromate. Kolthoff and Sandell[7] have previously arrived at the same conclusion. The indicator gives a satisfactory result when the solution is at least 0·5 *N* with respect to hydrochloric or sulphuric acid. If orthophosphoric acid is present the colours are clear and the range of colour change is small. In the absence of this acid, titrations must be continued until the violet colour of the indicator is fully developed. Without phosphoric acid the colours may be somewhat muddy, but the colour change takes place over a rather larger range of dichromate, so giving some warning of the approach of the end-point. Both methods give satisfactory results, and which is selected seems largely to be a matter of personal choice.

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THE UNIVERSITY CHEMICAL LABORATORY
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The Accurate Determination of Cobalt

By J. T. YARDLEY

Synopsis—Methods for the accurate determination of cobalt have been surveyed, special attention being paid to the potentiometric titration of cobaltous salts with standard potassium ferricyanide. Working conditions and technique were adjusted to give the highest degree of repeatability and search was made for a reliable "reference" salt for use as a check on the accuracy of the method. This was found in potassium cobalticyanide. A number of small batches of the salt were prepared and found on analysis to be identical within the known limits of repeatability of the methods applied. The conditions for the attainment of maximum accuracy in the potentiometric titration are given. Nickel in amounts similar to that of cobalt does not interfere, but the presence of similar quantities of iron under these conditions decreases accuracy, although no difficulties appear if the iron does not exceed one-twentieth of the cobalt. The results given by the potentiometric method are in excellent agreement with those of the gravimetric determination as anthranilate.

In the course of routine work in these laboratories it became necessary to survey the methods available for the accurate determination of cobalt. The primary aim of the examination was to decide upon the method most suited to the assay of highly pure cobaltous salts, as a semi-routine undertaking. Earlier experiments had indicated that electro-deposition in ammoniacal solution gave low results, owing to incomplete deposition and the occasional formation of a slight anodic deposit in the absence of reducing agents. When reagents were added to inhibit anodic oxidation, results tended to become too high owing to contamination of the cathode plate. The gravimetric determination as cobalt anthranilate was found to be quite satisfactory and precipitation of the α-nitroso-β-naphthol complex, followed by ignition in hydrogen, was found to give moderately successful results when the greatest possible care was exercised, but neither method was ideally suited to the work. The precipitate obtained with phenylthiohydantoic acid, which like the nitroso-naphthol complex is extremely useful for separation purposes, was excessively bulky and defied accurate evaluation, except very indirectly via the anthranilic acid method.

At an earlier date the potentiometric titration of cobaltous salts with standard potassium ferricyanide according to Tomicek and Freiberger[1] and Dickens and Maassen[2] had received passing attention by the author, but without yielding wholly satisfactory results. Nevertheless, subsequent papers[3,4,5] dealing more particularly with the application of the method to the analysis of complex substances provided the incentive for a reconsideration of the method, and because of previous experiences the re-examination was conducted along slightly different lines. These fresh experiments at once gave very encouraging results, which were followed up despite doubts cast upon the completeness of the reaction by the recent work of Bagshawe and Hobson.[5]

The working conditions and technique were first adjusted until the highest degree of repeatability was achieved, and concurrent experiments to establish a reliable "reference salt" for use as a check on the accuracy of the method were carried out. The common hydrated cobaltous salts are not suitable as standards and electrolytic cobalt, in our experience, is invariably slightly impure. Ignition of the sulphate at temperatures between 400° and 550° C. always failed to produce a pure anhydrous salt, the product being either deficient in sulphate or containing traces of water. An attempt was made to prepare a pure metal by ignition of the oxalate in either hydrogen or carbon dioxide, but thisw as unsuccessful. The product obtained after the hydrogen treatment was so highly pyrophoric as to be unusable.

Potassium ferricyanide was already being used as a standard in the cobalt titration (see below) and this raised the possibility of purifying the somewhat analogous cobalticyanide and sulphating weighed quantities of this anhydrous complex salt to provide standard cobaltous sulphate solutions. A number of small batches of this salt were prepared from AnalaR materials, as described below, and after repeated recrystallisations the separate batches gave analyses which were identical within the known limits of repeatability of the

methods applied. The potentiometric titration of standard potassium ferricyanide with approximately 0·05 *M* cobaltous solutions, derived from this purified cobalticyanide under the conditions to be described, was found to give results which were, with one exception, within ±0·06 per cent. of the calculated values.

The conditions which were found desirable for the attainment of maximum accuracy with the potentiometric titration were—(1) The cobaltous solution should be added *to* the ferricyanide solution in the presence of approximately those quantities of ammonia and ammonium citrate given in the experimental section (see below). (2) The cobaltous solution should be about 0·05 *M* in strength. (3) The reactants should be covered with a layer of inert immiscible liquid in order to exclude air. This covering also serves to shield the operator and the equipment from the strongly ammoniacal vapours and to minimise the formation of encrustation on the burette tips. (4) It was essential to employ efficient mechanical stirring throughout the titration.

Experimental

Preparation of potassium cobalticyanide as reference salt—A solution of 48 g. of $CoCl_2.6H_2O$ (AnalaR) in 100 ml. of hot water was added slowly to a nearly boiling solution of 80 g. of potassium cyanide (AnalaR) in 100 ml. of hot water. When effervescence had ceased, 5 *N* hydrochloric acid was added until the mixture was acid to litmus and the whole was boiled for some minutes. Further small amounts of solid potassium cyanide were added until the slight precipitate redissolved. The solution was again made acid and the alternate addition of potassium cyanide and acid was continued until the faintly acid solution was almost clear and yellow-green in colour. A further 5 ml. of 5 *N* hydrochloric acid was added and the solution boiled until solid commenced to separate. The crude product was filtered, washed with alcohol and, after air-drying, recrystallised from the minimum amount of water, the hot solution being filtered through asbestos. This recrystallisation was repeated two, three and four times respectively with different batches and the purified material washed with alcohol and ether and dried *in vacuo*.

Reference-standard solutions of cobalt sulphate—These were prepared by careful decomposition of the cobalticyanide with concentrated sulphuric acid, in a Kjeldahl flask, using about 20 ml. of acid for each 5 g. of the salt. After evaporation to dryness the residues were dissolved in water and diluted to suitable standard volumes at the temperature of standardisation.

Preparation of standard 0·10 *N potassium ferricyanide solution*—32·925 g. of potassium ferricyanide (AnalaR), previously recrystallised twice from water and dried at 80° C., was dissolved in water and diluted to 1 litre in a standard flask at the temperature of standardisation. Check titrations were carried out iodometrically and the normality factors, referred to standard potassium iodate solutions (via $Na_2S_2O_3$), were invariably identical with those calculated from the weights of ferricyanide taken.

Apparatus—Titration assembly—Baird and Tatlock potentiometric titration apparatus. (Titration assembly only.)

Electrodes—Saturated calomel and bright platinum, of the type supplied with the above apparatus.

Potentiometer—Cambridge portable Cole potentiometer.

Procedure

Dilute 25 ml. of 0·10 *N* $K_3Fe(CN)_6$ with 100 ml. of 5 *N* ammonia solution containing 5 g. of citric acid (AnalaR). Cover the solution with a layer of about 25 ml. of light petroleum (b.p. 100° to 120° C.) and titrate, rather slowly, with the cobalt sulphate solution. (The potential falls slowly from an initial value of about 300 mV. (calomel −ve).) When a reading of about 100 mV. has been reached, further additions should be made in half drops and sufficient time should be allowed for the potential to become steady before proceeding to the next addition. Immediately after the end-point has been passed the solution becomes negative with respect to the calomel and if a complete titration curve is required it is necessary to reverse the galvanometer connections (calomel +ve). Under the conditions given, however, the end-point invariably occurs before this reversal of polarity and it is always possible to locate it by plotting dE/dV against V_x, without proceeding beyond the point of equipotential. The inclusion of a reversing key in the circuit is therefore not essential, and in practice it is convenient to terminate titrations at this point.

Results

Table I

Expt.	Co solution	Co present, g. per 50 ml.	Co found, g. per 50 ml.	Remarks
1	A	0·1804	0·1805	Solution A prepared from first batch of $K_3Co(CN)_6$ after three recrystallisations
2	"	"	0·1805	
3	"	"	0·1805	
4	B	0·1777	0·1774	Solution B prepared from second batch of $K_3Co(CN)_6$ after four recrystallisations
5	"	"	0·1778	
6	"	"	0·1777	
7	"	"	0·1776	
8	"	"	0·1776	
9	C	0·1762	0·1763	Solution C prepared from third batch of $K_3Co(CN)_6$ after five recrystallisations
10	"	"	0·1762	
11	"	"	0·1763	
12	D	0·1762 +0·18 g. Ni	0·1763	Solution D was derived from solution C by evaporating an aliquot, adding $Ni(NO_3)_2.6H_2O$ and then restoring original volume of aliquot

The experiments in the above table were conducted at temperatures varying from 18·5° to 29·5° C.

Table II

Cobalt (g. per 50 ml.) determined by		
Electro-deposition in ammoniacal solution	Gravimetrically as Co anthranilate	Potentiometric titration of $K_3Fe(CN)_6$
0·2646	0·2630	0·2630
0·2645	0·2632	0·2630

A typical complete titration curve is reproduced below.

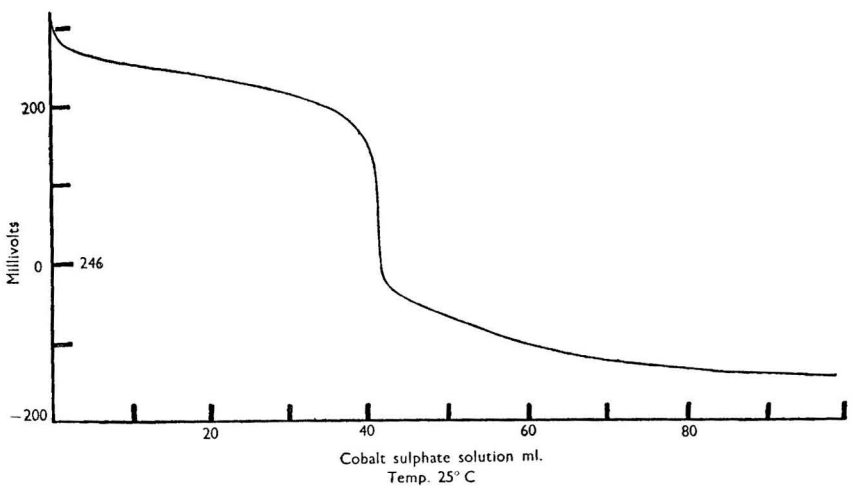

Fig. 1. A typical titration curve

The lower portion of the curve ($Co^{\cdot\cdot\cdot}/Co^{\cdot\cdot}$) invariably had the rather singular shape shown and during titrations it was always observed that there was a tendency for the recorded potential to drift slightly to more negative values in this region. In the absence of the covering layer this phenomenon was more pronounced and its occurrence is presumably connected with the higher concentration of oxidisable ions (ferrocyanide and $Co^{\cdot\cdot}$) present in the solution after the end-point. A certain amount of potential drift was also encountered on the upper portion of the curve when no covering layer was added.

This curve and the relevant experimental results appear to be incompatible with the view of Bagshawe and Hobson[5] that the cobalt - ferricyanide reaction must always be a balanced one and cannot proceed to completion. Their low value of $4{\cdot}2 \times 10^3$, for the equilibrium constant, was derived from potentials shown by the two systems, separately, oxygen being used to oxidise the cobalt. The predicted "overlap" of the two systems was not encountered under the changed conditions by the present author, possibly because of the considerable influence of ionic strength of the environment upon the redox systems involved.

In order to satisfy the curve reproduced above, a value for the equilibrium constant of about 2×10^6 would be required.

Further experiments—The addition of an amount of nickel equal to that of the cobalt in solution was not found to influence the titration (see Experiment 12), but the presence of similar amounts of iron reduced the accuracy of the determinations under these conditions. Smaller amounts of iron, up to 1/20 of the amount of cobalt present, produced no difficulties.

In order to test the accuracy of the method when applied to cobalt nitrate, a solution of $Co(NO_3)_2.6H_2O$ (AnalaR) was prepared and an aliquot was converted into the sulphate by repeated treatment with sulphuric acid. After restoring the original volume of the aliquot, precise agreement was obtained between titrations employing the two solutions. The potentiometric titration of a solution of cobalt bromide also gave a result in excellent agreement with that obtained by the gravimetric determination as anthranilate.

SUMMARY—

The cobalt - ferricyanide reaction has been re-examined and found to give accurate results under the conditions described. Potassium cobalticyanide has been used as a reference salt and concentrations of cobaltous solutions derived from this complex salt have been indirectly related to the concentrations of potassium iodate solutions. The conclusions of Bagshawe and Hobson[5] concerning the incompleteness of the reaction are discussed and contrasted with the findings of this investigation.

NOTE—

After the completion of the above work an account of the potentiometric titration of cobalt in bright nickel-plating solutions was published by Carter,[6] who, under somewhat different conditions, obtained results which were in good agreement with determinations by the α-nitroso-β-naphthol method.

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The Determination of Iron, Copper, Lead and Mercury in Fabricated Polyvinyl Chloride

By W. T. REES

Synopsis—Sensitive methods for the determination of microgram quantities of iron, copper, lead and mercury have been adapted to the determination of these metals in polyvinyl chloride plastics intended to be used in contact with pure hydrogen peroxide.

The methods described are absorptiometric, requiring the use of a filter absorptiometer or a spectrophotometer. The development of an efficient wet oxidation procedure is described. In particular, attention is given to the preparation of the sample for the determination of mercury. A special distillation apparatus has been designed to avoid loss of this volatile metal during the digestion.

The linear calibration graphs obtained for the four methods are characterised by the parts per million of the metal in the sample producing an extinction of 0·010. Typical results are also included for the recovery of the metals added to portions of the plastic.

Polyvinyl chloride plastics frequently contain small traces of metals which can catalyse the decomposition of pure hydrogen peroxide in contact with the plastic. To investigate the desirable limits for the concentration of such metallic impurities, it has been necessary to develop suitable methods for the accurate determination of microgram quantities. The methods are absorptiometric, and require the use of a filter absorptiometer of the Spekker type or, preferably, a spectrophotometer of the Beckman type.

The stability of polyvinyl chloride to hydrogen peroxide suggests that it is not particularly amenable to wet oxidation. Nevertheless, a satisfactory procedure has been devised, using sulphuric and nitric acids. The destruction of the organic matter by ashing was not considered, as it is well known that losses of metal may occur during the process. Even with a wet digestion procedure, mercury may be lost if an open Kjeldahl flask is used. The special distillation apparatus which is described has been found to prevent loss of mercury during the wet destruction of the plastic.

Wet oxidation of the sample

Samples were prepared for wet oxidation by cutting into small cubes of about $\frac{1}{8}$-inch sides. Boiling concentrated nitric acid alone merely bleached samples of the plastic, but concentrated sulphuric acid charred the material when the acid began to fume. Much frothing occurred, and careful control of heating was thus necessary to prevent loss of sample through the neck of the Kjeldahl flask. The carbonaceous matter was then oxidised by the addition of small portions of nitric acid to the fuming sulphuric acid digest. This method was adopted for the determination of mercury, since the volume of nitric acid used was kept to the minimum, an advantage in the subsequent procedure.

Further investigation showed that preliminary digestion with a mixture of equal volumes of concentrated nitric and sulphuric acids accelerated the decomposition somewhat. When the sulphuric acid began to fume, the oxidation was completed by the addition of further nitric acid, as previously described. This quicker method was used for the determination of iron, copper and lead.

In both methods the nitric acid oxidation was continued until a clear, pale yellow solution was obtained, which remained clear and became colourless on cooling. Occasionally, although oxidation was apparently complete, white crystals formed or darkening occurred as the mixture cooled. (The crystals were probably a nitroderivative of a constituent of the plastic.) In such cases further digestion and treatment with nitric acid were necessary.

The acids used for the wet oxidation were mainly responsible for the reagent blank, even though AnalaR quality acids were employed. Experience showed that 2 g. of polyvinyl chloride plastic, taken in a 50-ml. Kjeldahl flask, required at least 10 ml. of sulphuric acid (or 20 ml. of mixed acids) for the digestion. The volume of nitric acid necessary to complete the oxidation was from 12 to 15 ml. A reagent-blank experiment may therefore be run

simultaneously with the test, by including 15 ml. of nitric acid while adding at least this volume to the test. Further nitric acid may be subsequently added to the blank if more than 15 ml. is used for the test. The blank is thus kept to a minimum.

The reagent blanks and test results obtained by analysis of a typical sample of the plastic are shown in Table I. The high value of the reagent blank for the iron determination was traced to the strong acids used in the wet digestion. This blank could not be conveniently reduced, but was found to be strictly reproducible.

TABLE I

COMPARISON OF "BLANK" AND "TEST" RESULTS

		Iron	Copper	Lead	Mercury
Reagent blank	(*a*) Final extinction reading ..	0·20	0·02	0·02	<0·01
	(*b*) Corresponding p.p.m. of metal	20	1·5	4	1
Metal content of the sample, p.p.m.		30	1	40	4

The volatility of mercury compounds introduces difficulties in the preparation of organic samples for the determination of this metal. The presence of chloride in the plastic accelerates the loss of metal during the digestion process. Thus, mercury added to a portion of the plastic in an open Kjeldahl flask was completely lost. Kozelka[1] overcame such loss by using a closed flask with a side-arm leading to a condensing receiver. He distilled mercury quantitatively by passing chlorine through the digest. The use of chlorine is inconvenient, but recovery of mercury, using apparatus similar to that described by Kozelka, was unsatisfactory, even though the Kjeldahl solution was treated with the distillate. A Friedrich condenser fitted to the Kjeldahl flask was inefficient in the present work, presumably because of the rapid evolution of vapour which occurred when portions of nitric acid were added to the fuming sulphuric acid mixture. It was also necessary to remove water from the Kjeldahl flask to keep the sulphuric acid fuming.

A modified apparatus was therefore designed which consisted of a 50-ml. Kjeldahl flask, closed with a standard-joint stopper carrying a tap funnel and fitted with a side-arm. The latter was connected to a receiving flask to collect the distillate, and this flask was also fitted with a reflux condenser (see Fig. 1). The flask served to buffer the effect of the sudden evolution of vapour from the Kjeldahl flask following the addition of nitric acid, and also to collect the water which distilled. Mercury added to a portion of the sample was not completely distilled from the Kjeldahl flask. Satisfactory recovery was obtained, however, by combining the solution from the Kjeldahl flask with the distillate before proceeding with the determination.

THE DETERMINATION OF IRON AND COPPER

A composite method was evolved for the determination of iron and copper, involving the wet oxidation of 2 g. of the sample. The Kjeldahl solution was transferred to a beaker, evaporated to a volume of approximately 1 ml., cooled, transferred to a 15-ml. calibrated flask, and diluted to the mark at 20° C. The acidity of this solution was determined on a convenient aliquot in order that appropriate adjustment could be made in the subsequent procedures.

Iron was determined by taking a 5-ml. aliquot of this solution in a 10-ml. calibrated flask and applying the well-known thiocyanate reaction, following the recommendations of Ovenston and Parker.[2] Thus, further sulphuric acid was added to render the final 10-ml. volume 1·5 *N*, and then 1 ml. of 0·5 per cent. w/v ammonium persulphate was added. After standing for a minute, 1 ml. of 20 per cent. w/v ammonium thiocyanate was added, the solution diluted to the mark and kept at 20° C. for 15 minutes. The extinction of this solution was measured in a 1-cm. cell at 475 mμ. If a filter absorptiometer is employed, Ilford spectrum filters No. 603 or 604 are suitable; No. 604 allows greater precision, but with a sensitivity reduction of about 50 per cent.

Copper was determined in a second 5-ml. aliquot of the same solution as that used for the iron determination. Dithizone was used to isolate the copper, the final determination being made with sodium diethyldithiocarbamate.

Copper can be extracted from a 0·1 *N* mineral acid solution by a carbon tetrachloride solution of dithizone. Palladium, gold, silver, mercury and bismuth are co-extracted. The oxidation of some of the dithizone by any ferric iron present is of no consequence in an extraction process. Of these metals, therefore, only mercury and bismuth need be considered as sources of interference in the present work. Mercury will be absent owing to its volatility during the Kjeldahl digestion; and bismuth could be separated by extraction of a mixture of the dithizonates with 2 per cent. w/v potassium iodide in 0·01 *N* hydrochloric acid.[3] Bismuth was not detected in the compositions examined; thus this extra step would not normally be necessary.

The diethyldithiocarbamate extraction was made from an aqueous phase similar in composition to that used by Haddock and Evers,[4] but carbon tetrachloride was chosen as the organic phase as recommended by Sandell.[5]

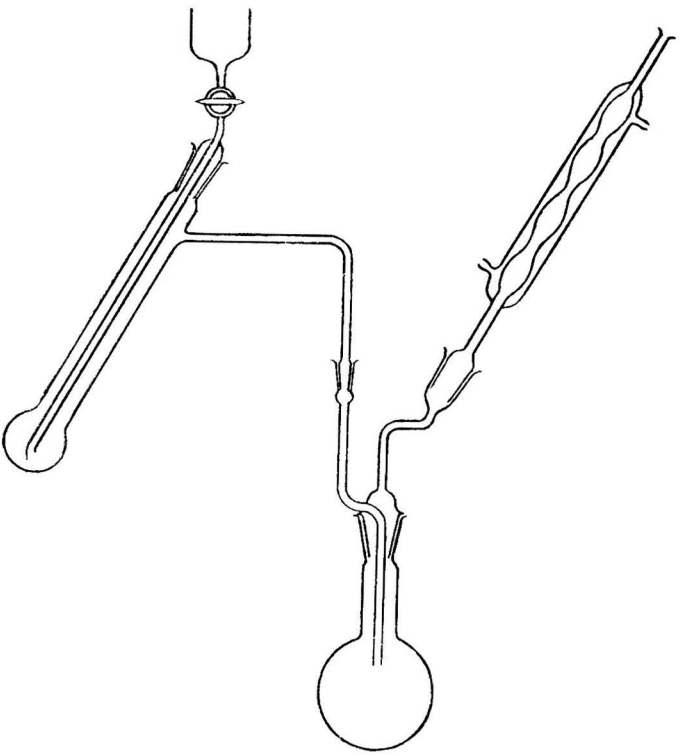

Fig. 1. Modified Kjeldahl apparatus

The 5-ml. aliquot was adjusted to 0·1 *N* acidity by appropriate dilution with water, and extracted with three 5-ml. portions of 0·01 per cent. w/v dithizone in carbon tetrachloride, the extracts being collected in a micro-Kjeldahl flask. The solvent was removed and the residue digested with 0·25 ml. of concentrated sulphuric acid and a few drops of concentrated nitric acid, to obtain a clear colourless solution. This solution was transferred to a separating funnel, and 2 ml. of copper-free citric acid solution (77 per cent. w/v of the monohydrate) were added, followed by 3·3 ml. of 14 *N* ammonium hydroxide and 1 ml. of sodium diethyldithiocarbamate solution (1 per cent. w/v in water). The mixture was diluted to about 15 ml., and extracted with a 2-ml. portion of carbon tetrachloride, which was then run off, through a small filter of glass wool previously wetted with the solvent, into a 10-ml. calibrated flask. The extraction was repeated with three further 2-ml. portions of carbon tetrachloride, running each into the 10-ml. flask. A further 1 ml. of solvent was then used to wash the filter and the stem of the funnel. The solution was adjusted to 20° C., and diluted to the mark. The extinction of this solution was then measured in a 1-cm. cell at 436 mμ. If a filter absorptiometer is employed, Ilford spectrum filters No. 601 are suitable.

The determination of lead

The method adopted for the determination of lead involves the wet oxidation of 2 g. of the sample, followed by a dithizone extraction, and finally absorptiometric application of the sodium sulphide method.

The extraction of lead by dithizone has been studied by many workers, including Clifford and Wichmann[6] and Biefeld and Patrick.[7] Such extraction is possible from a slightly basic solution, using a chloroform or carbon tetrachloride solution of the reagent. Citrate is included to prevent precipitation of other metallic hydroxides, and the presence of cyanide precludes the co-extraction of all other metals except bismuth, stannous tin and thallium. Bismuth may be separated by the potassium iodide procedure previously described, and a special volatilisation procedure is available for the removal of tin.[6,8] Thallium is unlikely to occur in compositions of the type under consideration. The minimum pH necessary for extraction of lead from an ammonia - citrate - cyanide medium is 9·5.

In view of the difficulties associated with dithizone colorimetry, the sulphide method was chosen as being sufficiently sensitive and reliable for the present purpose. A combination of the lead and mercury methods was undesirable, on account of the special apparatus used in the determination of mercury.

The wet digestion product, of volume about 5 ml., was diluted with 10 ml. of water and cooled. A 1-ml. portion of 50 per cent. w/v citric acid (monohydrate) was added, and the mixture adjusted to the required pH by addition of ammonium hydroxide (sp.gr. 0·880) until the blue end-point of thymol blue indicator was reached. The solution was transferred to a separating funnel, 1 ml. of 10 per cent. w/v potassium cyanide was added, and the mixture was extracted successively with 7·5, 5 and 5 ml. of 0·1 per cent. w/v dithizone in chloroform. After washing the combined extracts with 10 ml. of water to which 1 drop of ammonium hydroxide had been added, the organic solution was run into a 50-ml. Kjeldahl flask. The aqueous phase was extracted with 5 ml. of dithizone solution and the extract added to the Kjeldahl flask. The solvent was removed and the residue digested with 1 ml. of concentrated sulphuric acid, with the dropwise addition of 20 per cent. w/v hydrogen peroxide, until a clear colourless solution was obtained. One gram of solid ammonium persulphate was then added, and the mixture was heated strongly for 30 minutes. The cooled solution was transferred to a 50-ml. calibrated flask, 4 ml. of 50 per cent. w/v ammonium acetate were added, followed by 2 ml. of 50 per cent. w/v citric acid (monohydrate), 5 ml. of ammonium hydroxide (sp.gr. 0·880) and 0·5 ml. of 10 per cent. w/v potassium cyanide. This mixture was diluted to the mark at 20° C., and the extinction measured with a filter absorptiometer in a 4-cm. cell, using Ilford spectrum filters No. 601 in conjunction with Calorex heat filters. The extinction measurement was repeated after the addition of 2 drops of 10 per cent. w/v sodium sulphide solution. Thus correction was possible for any absorption shown by the solution before addition of the reagent.

The determination of mercury

Laug and Nelson[9] have described a dithizone method for the determination of mercury. The metal is separated from lead, cadmium, zinc and nickel by extraction from *N* acid solution with dithizone in chloroform. Copper accompanies the mercury, but may be separated by shaking the mixture of the dithizonates with an acid bromide solution. Mercury enters the aqueous phase as the feebly dissociated $HgBr_4''$ ion. If the pH of the separated aqueous phase is then adjusted to 6, mercury may be re-extracted by the dithizone reagent. This method was adopted for the present purpose.

The final photometric measurements were made by the reversion procedure of Irving and co-workers,[10] acid bromide being used as the reversion reagent. The whole method is given in detail below.

Special solutions required (Sandell,[5] p. 325)—

(*a*) *Hydroxylamine hydrochloride, 20 per cent. w/v*—Extract a 20 per cent. w/v aqueous solution with portions of 0·01 per cent. w/v dithizone solution in chloroform until the chloroform layer remains pure green in colour. Then extract with three portions of pure chloroform to remove the last traces of dithizone.

(*b*) *Potassium bromide, 40 per cent. w/v*—To 500 ml. of a 40 per cent. w/v aqueous solution add 1 drop of 6 *N* sodium hydroxide solution, and extract with 0·01 per cent. w/v dithizone solution in chloroform until the chloroform layer remains pure green in colour.

Make the aqueous phase just acid, extract the last traces of dithizone by means of pure chloroform, and then make just alkaline again.

(*c*) *Buffer solution*—Dissolve 75 g. of disodium hydrogen phosphate (Na_2HPO_4) and 19 g. of anhydrous potassium carbonate in water and dilute to 500 ml. Extract with 0·01 per cent. w/v dithizone solution in chloroform until the chloroform layer remains pure green in colour. Then extract with generous portions of pure chloroform to remove the last traces of dithizone.

(*d*) *Dithizone, 0·1 per cent. w/v*—Dissolve the weighed amount of dithizone (which should be good reagent quality, but need not be specially purified) in the appropriate volume of pure chloroform (chloroform of AnalaR or equivalent grade is recommended).

(*e*) *Dithizone, 0·01 per cent. w/v and 0·001 per cent. w/v*—Prepare by diluting the 0·1 per cent. w/v solution with chloroform as required.

(*f*) *Standard mercury solution*—Dissolve mercuric chloride (AnalaR grade or equivalent) in *N* hydrochloric acid to give a solution containing 0·1 per cent. w/v of mercury metal. Dilute this stock solution as required with *N* hydrochloric acid to give a standard solution containing 0·0002 per cent. w/v of mercury metal. (One millilitre of this standard solution contains 2 μg. of Hg^{++}.)

WET OXIDATION OF THE SAMPLE—

Transfer 2 g. of the comminuted sample to the Kjeldahl flask of the special apparatus previously described. Add 10 ml. of concentrated sulphuric acid (AnalaR) through the tap funnel, and heat the mixture to fuming over a small flame. Run in approximately 2-ml. portions of nitric acid (sp.gr. 1·42) (AnalaR), during the digestion, and heat more strongly when frothing subsides. Continue the nitric acid oxidation until a clear and almost colourless solution is obtained; allow to cool. (When cool, the solution should remain clear and appear colourless if oxidation is complete.) Run in 10 ml. of water and simmer until fuming begins; allow to cool.

DITHIZONE EXTRACTION—

Dismantle the apparatus and wash the solution from the Kjeldahl flask into a 500-ml. calibrated flask. Add the solution from the round-bottomed flask and rinse out the apparatus thoroughly, combining the rinsings with the solution in the calibrated flask. Dilute in this to the mark at 20° C.

Pipette a 50-ml. aliquot of the solution into a 100-ml. conical flask and boil gently for 10 minutes on a hot-plate. Cool and pass a rapid stream of sulphur dioxide gas through the solution for 1 minute (as recommended by Kozelka[1]). Cool again if necessary, then add 0·25 ml. of 0·025 per cent. w/v methyl orange indicator solution and neutralise with strong ammonia solution, cooling as necessary during the neutralisation. Transfer the solution to a 100-ml. separating funnel and dilute to about 65 ml. with water. Add 5 ml. of 13 *N* sulphuric acid and 5 ml. of hydroxylamine hydrochloride reagent. (Occasionally the volume of the solution exceeds 65 ml. after transference to the separating funnel. In such a case the volume of 13 *N* acid added should be proportionately increased.)

Extract the aqueous mixture with four 10-ml. portions of 0·001 per cent. w/v dithizone reagent, running the extracts into a second 100-ml. separating funnel. Add 50 ml. of 0·25 *N* hydrochloric acid to the combined extracts, shake vigorously, and separate the chloroform solution into another 100-ml. separating funnel. Wash the hydrochloric acid solution with 5 ml. of 0·001 per cent. w/v dithizone reagent, and add the separated chloroform phase to the previous extracts. Discard the aqueous phases.

Add 50 ml. of 0·25 *N* hydrochloric acid and 5 ml. of potassium bromide reagent to the chloroform solution. Shake vigorously and discard the separated chloroform phase. Wash the aqueous phase with 10 ml. of chloroform and separate the chloroform as completely as possible. Reject this chloroform washing. Add 10 ml. of buffer solution to the aqueous solution, and mix. Pipette into the separating funnel 10·0 ml. of 0·001 per cent. w/v dithizone reagent. Shake carefully, with frequent release of pressure. Run the chloroform phase through a small filter of glass wool into a suitable receiver.

APPLICATION OF THE REVERSION PROCEDURE—

Using a photo-electric spectrophotometer, measure the extinction of the chloroform solution in a 1-cm. cell at 605 mμ. (corresponding to a maximum in the absorption spectrum of dithizone in chloroform solution). If a filter absorptiometer is employed, Ilford spectrum

filters No. 607 are appropriate with a tungsten-filament lamp. Conserve the solution as far as possible by using clean dry cells.

Pour the solution from the cell into the receiver containing the remainder of the chloroform extract, and transfer to a 100-ml. separating funnel containing 50 ml. of 0·25 *N* hydrochloric acid and 5 ml. of potassium bromide reagent. Shake vigorously, and run off the chloroform phase through a small filter of glass wool into a suitable receiver. (Avoid wastage of chloroform solution at each stage to permit complete filling of cell and guard against evaporation.) Measure the extinction of this solution under exactly the same conditions as before. Subtract the first extinction reading from the second.

Obtain a "reagent blank" by repeating the whole procedure described above, beginning with 10 ml. of concentrated sulphuric acid in the Kjeldahl flask, and adding a volume of nitric acid equal to that required for the wet oxidation of the sample.

Deduct the final difference reading so obtained from the corresponding reading for the test, and deduce the mercury content of the sample by reference to the calibration graph.

CALIBRATION GRAPH—

Place quantities of the standard mercuric chloride solution covering the range 0 to 15 μg. of Hg^{++} in 100-ml. separating funnels. Make up the volume in each case to 12·5 ml. by addition of *N* hydrochloric acid, and then dilute to 50 ml. with water. To each solution add 10 ml. of buffer reagent, and mix. Pipette 10·0 ml. of 0·001 per cent. w/v dithizone reagent into each funnel, and proceed from this stage as already described for the test solution.

Subtract from the difference readings so obtained that corresponding to no addition of mercury. Plot the corrected difference readings against concentration of mercury.

APPLICATION OF THE METHODS

The absorptiometric procedures were applied directly to solutions of suitable salts of the metals, and linear calibration graphs were obtained. These graphs may be characterised by the parts per million of metal in the sample corresponding to an extinction reading of 0·010 in the final measurement when the methods described are applied. The figures thus relate to 1-cm. cells, except in the case of lead, when a 4-cm. cell is employed. This value approaches the experimental error of the Spekker absorptiometer, and is well above that observed with the Beckman spectrophotometer. The data for the iron, copper, lead and mercury methods are shown in Table II.

TABLE II

P.P.M. OF METAL IN SAMPLE GIVING E = 0·01

	Iron	Copper	Lead	Mercury
Beckman spectrophotometer	1·0	0·7	—	1·1
Spekker absorptiometer (*a*) Mercury lamp ..	1·0	0·8	2·0	—
(*b*) Tungsten lamp ..	1·0	0·9	1·8	1·6

Standard metallic salt solutions were prepared, each 0·1 per cent. w/v with respect to the metal, as follows—

Iron—ferrous ammonium sulphate (AnalaR) in *N* sulphuric acid.

Copper—copper sulphate (AnalaR) (avoiding crystals showing any efflorescence) in 0·1 *N* sulphuric acid.

Lead—lead nitrate (AnalaR), dried for 2 hours at 105° C., in 1 per cent. v/v nitric acid.

Mercury—mercuric chloride (AnalaR) in *N* hydrochloric acid.

Immediately before use these solutions were diluted to 0·002 per cent. w/v with respect to the metals. Known quantities of the metals, as aliquots of the dilute solutions, were added to 2-g. portions of a polyvinyl chloride plastic, and analysed by the prescribed procedures. The calibration graphs so obtained, after correction for the appropriate reagent blanks, were found to be parallel to those obtained directly on solutions of the metallic salts. The intercepts on the extinction axes corresponded to the metal content of the plastic used in these experiments. Examples of the recovery of metals added to the plastic and treated in this way, after due allowance for the metals present in the plastic itself, are given in Table III.

The procedures described should be of general application to similar compositions. This

may be confirmed in any particular instance by checking recovery of the metals added as described above.

The composition used in developing these methods was reasonably homogeneous, but when this is not the case suitable control of sampling is obviously necessary. A sheet of polyvinyl chloride plastic which was "mottled" gave more than the normal variation in results

TABLE III

TYPICAL ANALYTICAL RESULTS

Iron	Added p.p.m.	29	58	87
	Recovered p.p.m.	29	59	87
Copper	Added p.p.m.	15	30	45
	Recovered p.p.m.	15	31	45
Lead	Added p.p.m.	23	39	62
	Recovered p.p.m.	23	39	58
Mercury	Added p.p.m.	8	38	69
	Recovered p.p.m.	7	38	64

for the determination of iron. Determination of a calibration graph in the presence of the sample is helpful in such cases, the extinction intercept giving an average figure. The fact that such a graph should be parallel to that obtained directly on solutions of the metallic salts is of assistance in this respect.

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The Determination of Antimony and Tin in Cable Sheathing Alloys

BY R. M. BLACK

SYNOPSIS—The paper describes in detail the procedure employed for the determination of antimony and tin in cable-sheathing alloys, employing a mixture of hydrogen peroxide and acetic acid as reagent for the dissolution of the alloy. The antimony is determined volumetrically with potassium bromate, and the tin by the method of B. S. Evans, both methods being slightly modified to ensure the complete decomposition of peroxide.

THE analysis of alloys of lead with antimony and tin is of considerable importance in the cable industry, and has been the subject of much study, mainly directed to the development of a satisfactory and rapid method for bringing the metals into solution. Such analyses are often required quickly, as a verification of results obtained by spectrographic methods, for example. Unfortunately, the usual methods for dissolution of the alloys are laborious and time-consuming.

The treatment of the alloy with a mixture of concentrated hydrochloric acid and bromine requires subdivision of the sample, and the process of solution may take an hour or more. There is also the danger of loss of tin in the form of the halide, owing to the temperature being fairly high, in the region of the boiling-point of the acid mixture. The use of concentrated sulphuric acid increases the rate of solution, but suffers from the disadvantage that the antimony is difficult to dissolve, and therefore heating at boiling-point for 15 minutes

is required. Sulphur separates, and lead sulphate is precipitated on dilution. Evans[1] has recommended the use of a mixture of phosphoric and perchloric acids; this procedure takes about 15 minutes and special precautions must be taken against loss of tin. Different solvents are recommended for the estimation of antimony, namely a mixture of hydrochloric and perchloric acids.

The ideal solvent for the determination of antimony and tin would be hydrochloric acid, but this alone is not only slow in action but also liable to errors because of the volatility of the tin on boiling the concentrated acid. Lead appears to dissolve rapidly in only those materials which have readily available oxygen, and it was shown by Hamilton,[2] after a number of experiments which included fusion with sodium peroxide, that 30 per cent. hydrogen peroxide attacked lead and sheathing alloys with great vigour.

Accordingly, a mixture of hydrogen peroxide and acetic acid has been adopted in these laboratories as solvent for bringing the antimony and tin into solution. The reagent itself is based on one of the etching solutions commonly used to disclose lead structure.

DISSOLUTION OF THE ALLOY—

The following method is used for the dissolution of the alloy. Dissolve about 2·5 g. of the sample, which can be in the form of lumps, in a mixture of 10 ml. of 30 per cent. hydrogen peroxide, 10 ml. of distilled water and 5 ml. of glacial acetic acid. The best results are obtained when the reagent is mixed prior to addition to the weighed alloy. The addition of acetic acid, while not strictly necessary, hastens the decomposition of the alloy. The reaction commences immediately on the addition of the reagent, and in some cases the dissolution is so rapid that appreciable quantities of heat are evolved and cooling becomes necessary. The final disintegration of the alloy is easily observed after 2 to 15 minutes, depending on the composition and to some extent on the crystal size or grain structure of the lead.

When antimony is absent, the resulting solution contains a white suspension which is thought to be lead hydroxide and basic lead acetate. The presence of antimony gives this suspension a grey coloration, due to metallic antimony which is insoluble in the reagent (or gives a grey deposit in the absence of a suspension), and this forms a useful "spot" test for antimony.

Complete solution cannot therefore be attained with acetic acid alone, and for the purposes of the determination the addition of 25 ml. of concentrated hydrochloric acid is made. This addition decomposes the greater part of the hydrogen peroxide, oxides of chlorine being evolved, dissolves the antimony and converts the other metals into chlorides. The antimony and tin may then be determined by the standard methods, providing that one or two modifications are made in the procedure to ensure the complete decomposition of the hydrogen peroxide.

THE DETERMINATION OF ANTIMONY—

The antimony in the hydrochloric acid solution is reduced with sulphur dioxide and determined by titration with standard potassium bromate solution. The method is the usual one, originally described by Györy[3] and, with modifications by Siedler, by Nissensen and Rowell.[4] The procedure used is further modified in the following manner.

Warm the yellow solution produced on the addition of hydrochloric acid, as already described, on a hot-plate for a few minutes to encourage the decomposition of the peroxide, then remove from the hot-plate and add 25 ml. of 20 per cent. sodium sulphite solution. Stand the flask (500-ml. conical, Quickfit B.24 neck) on a steam or boiling-water bath for 15 minutes. Boil off the excess sulphur dioxide, 15 minutes boiling on a low hot-plate usually being sufficient to remove the last trace, and then add 50 ml. of distilled water. Allow the flask to cool, and titrate the solution with 0·1 *N* (or weaker) potassium bromate from a micro-burette. Two-thirds of the way through the titration (this volume can be estimated if the approximate antimony content of the alloy is known), add a few drops of methyl red solution and continue the titration, adding the bromate a drop at a time, and shaking the flask after each addition. The end-point is indicated by the decoloration of the methyl red.

1 ml. of 0·1 *N* potassium bromate ≡ 0·006088 g. of antimony.

THE DETERMINATION OF TIN—

The method for the determination of tin is that due to Evans,[1,5] with slight modification.

After the antimony determination as just described, insert a B.24 male joint into the top of the flask, and add 25 ml. of hydrochloric acid, 1 ml. of saturated mercuric chloride

solution and 2 g. of sodium hypophosphite. Boil the solution for 15 minutes. During this period, boil 250 ml. of water containing 10 ml. of 4 per cent. potassium iodide in a separate flask, to remove dissolved air. After the lapse of 15 minutes, add the iodide solution to the tin solution, drop a small piece of solid carbon dioxide (Drikold) into the flask, and stand it in water to cool, adding further pieces of carbon dioxide from time to time to ensure the exclusion of oxygen from the flask. When the solution has reached room temperature, add a few drops of freshly prepared starch solution, and titrate the tin with 0·01 *N* iodine solution; the end-point being indicated by the usual grey or bluish tint of the solution. The addition of small pieces of carbon dioxide not only ensures the exclusion of oxygen, but also stirs the solution, and increases the rate of cooling; this reduces the time for a determination.

1 ml. of 0·01 *N* iodine solution ≡ 0·0005935 g. of tin.

NOTES ON THE DETERMINATION—

It is important that, after dissolution of the alloy with the hydrogen peroxide reagent, all traces of hydrogen peroxide and other peroxides should be removed. This is particularly important for the determination of tin, and in the case of other alloying components when a polarographic method is to be employed. This is accomplished by the sulphite, but it was found that the addition of sulphite solution followed by the boiling off of the sulphur dioxide was insufficient to destroy all peroxide, and in consequence low results for antimony and a bad end-point for the tin determination were obtained. Heating for 15 minutes on a steam-bath will, however, obviate these difficulties.

A series of standardised alloys has been available, and the following analyses made by this method are given in Table I.

TABLE I

	Percentage composition			
	Standard methods		Above methods	
Alloy	Sb	Sn	Sb	Sn
A	—	1·98	—	1·98
B	0·88	—	0·86	—
	1·00	—	1·03	—
C	—	0·4	—	0·41
D	0·47	—	0·46	—
E	0·2	0·4	0·21	0·39
	0·15	—	0·153	—

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BRITISH INSULATED CALLENDER'S CABLES, LTD.
RESEARCH DEPARTMENT
38, WOOD LANE, LONDON, W.12

July, 1949

Notes

USE OF CHLOROPHYLL FOR BREAKING EMULSIONS IN ALKALOIDAL ASSAYS OF SOLANACEOUS DRUGS

SINCE it was noticed, in the course of extracting alkaloids by the pharmacopoeial methods from alkaline solution with chloroform, that emulsification became worse with each successive extraction, it was thought that some constituent, which prevented emulsification, was being extracted along with the alkaloids.

The preparations being assayed all contained chlorophyll. In order to test its preventive action, quantities ranging from a few drops to a few ml. of a 5 per cent. solution of chlorophyll in chloroform were added to the emulsions. Breaking began immediately on shaking, and was complete within a short time. This treatment was used with success on tinctures and extracts which contain chlorophyll. Commercial spirit-soluble chlorophyll (Wm. Ransom and Son Ltd.) was used, a blank determination showing it to have no effect on the final titre.

Normally there is a slight tendency to emulsification in the subsequent acid extractions, and the added chlorophyll increases this somewhat; but no trouble is experienced if the acid layers are bulked together with any emulsion and shaken. One extra chloroform wash may be needed to remove all the chlorophyll.

PHARMACEUTICAL LABORATORIES
KEMPTHORNE PROSSER AND CO.'S NEW ZEALAND DRUG CO., LTD.
DUNEDIN, NEW ZEALAND

I. C. EDMUNDSON
B. J. WILKINS
July, 1949

A COLOUR TEST FOR COCAINE

JAMES and Roberts[1] consider nitration of the benzene ring to be the fundamental reaction of the Vitali test for the solanaceous alkaloids, atropine, hyoscine and hyoscyamine. In the case of these alkaloids, nitric acid alone is sufficient for the nitration of the benzene ring; nitric acid alone has no action on cocaine, but a mixture of nitric and sulphuric acids easily brings about the nitration of the benzene ring in cocaine. The colour reaction of this nitro-compound with alkalis is the basis of the colour test for cocaine.

To about 0·5 mg. of the substance in a test tube add about 100 mg. of potassium nitrate and 10 drops of concentrated sulphuric acid, and heat in a boiling water-bath for 10 minutes. Cool, dilute with water to about 30 ml., extract once with chloroform, and discard the chloroform. Make alkaline with ammonia and extract again with chloroform. Evaporate off the chloroform, dissolve the residue in about 2 ml. of acetone and add 1 to 2 drops of a 10 per cent. solution of sodium hydroxide. Cocaine gives an intense purple colour. Cocaine hydrochloride (0·25 mg.) gave a strong purple colour; a light purple colour was obtained with 0·05 mg.

The colour appears to be specific for cocaine. Atropine, homatropine and a few cocaine substitutes were examined by this method. The colour reactions obtained with these using 0·25-mg. quantities are given below.

Amylocaine hydrochloride	No colour
Procaine hydrochloride	Light reddish-violet colour, changing quickly through brown to greenish-yellow
Benzocaine	No colour
Homatropine hydrobromide	Light reddish colour
Atropine sulphate	Strong violet colour

In the case of amylocaine, the acid - chloroform extract gave a purple colour similar to, but very much weaker than, that with cocaine. The purple colour with cocaine and the violet colour with atropine appear to remain unchanged for a long time.

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GOVERNMENT ANALYST'S LABORATORY
COLOMBO, CEYLON

E. RATHENASINKAM
July, 1949

WATER-SOLUBLE MATTER IN VEGETABLE TANNED LEATHER

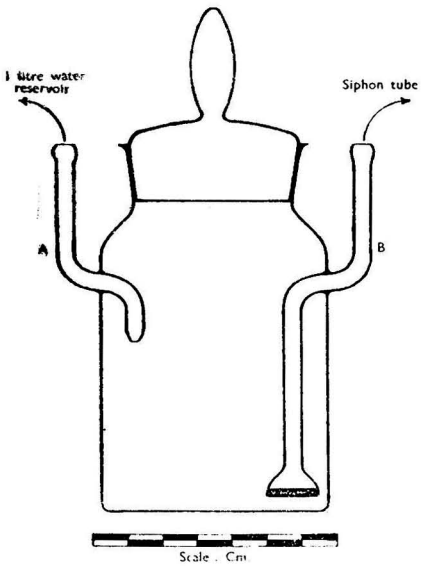

Fig. 1

THE International Society of Leather Trades' Chemists describes an official method[1] for the determination of the water-soluble matter in vegetable-tanned leather, and this has been reproduced by the British Standards Institution.[2] In both of these publications it is stated that a Proctor extractor shall be used: a diagram is given in the first reference, but the second does not describe the extractor or the manner in which it should be used.

In this laboratory it has been found to be more convenient to extract the leather in the apparatus shown in Fig. 1. This was made from Pyrex glass, and consists of a stoppered bottle with inlet and outlet tubes sealed into the side. The inlet tube A is drawn into a jet, and the outlet tube B has at its lower end a sintered glass disc of porosity No. 1; the sintered disc is about 5 mm. above the bottom of the bottle. To use the extractor, connect A with a reservoir of distilled water by a rubber sleeve fitted with a screw clip, and B with a siphon tube ending in a jet—the rubber connection carries another screw clip. Place a layer of purified sand[1] in the bottle, using sufficient to cover the filter disc; add the fat-free leather, and water, and continue as described in the references cited. The outlet siphon B is easily filled by closing the bottle and blowing through the inlet tube A. This apparatus was designed to reproduce as closely as possible the action of the Proctor extractor, but with the number of separate components reduced to the minimum practicable. It is thought, however, that equally satisfactory results would be obtained if a finer porosity sintered disc were used and the sand eliminated.

The author thanks the Railway Executive for permission to publish this note.

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RAILWAY EXECUTIVE
LONDON MIDLAND REGION
SCIENTIFIC RESEARCH DEPARTMENT
STONEBRIDGE PARK

G. H. WYATT
July, 1949

GENERATOR FOR AIR-FREE CARBON DIOXIDE (OR HYDROGEN)

THE generator already described[1,2] has proved its usefulness, particularly as a source of air-free carbon dioxide in the micro-determination of nitrogen by the Dumas method, of methoxyl by the Zeisel method, and so on. Its use as an air-free hydrogen generator was handicapped, however, by the fact that since pure zinc (at least arsenic-free) had to be used, the liberation of hydrogen (on treatment with diluted hydrochloric acid) was inevitably slow, and once started continued for some time after the use of the apparatus had been discontinued. This drawback has now been completely eliminated by the use of the zinc - copper couple. In practice this is easily prepared by covering the zinc *in situ* with a 3 to 5 per cent. solution of copper sulphate; the zinc - copper couple is immediately formed, and by running in acid from the reservoir, spent liquor can be expelled as described formerly. In such a condition hydrochloric acid (1 vol. of concentrated hydrochloric acid/1 vol. of water) acts immediately on the zinc; in fact, the evolution of hydrogen is nearly as rapid as that of carbon dioxide from the action of acid on marble and, furthermore, evolution of hydrogen ceases when the acid stream is turned off. This adaptation has rendered the generator invaluable as part of a permanent set-up in the apparatus used for the estimation of unsaturation by catalytic micro-hydrogenation and also to replace the usual hydrogen cylinder,

with its numerous disadvantages, in catalytic hydrogenation when dealing with quantities of 50 g. or less of material. Since the 5-litre container of the generator can hold 5 kg. of zinc, one charging lasts a long time.

It has been the practice to use with the generator a purification train[2] consisting of solid potash, aqueous silver sulphate and alkaline potassium permanganate; but latterly the silver sulphate has been replaced by mercuric chloride (to remove acetylenes *inter alia*). The hydrogen delivered is of a high degree of purity.

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CHEMISTRY DEPARTMENT
THE UNIVERSITY, GLASGOW

S. HORWOOD TUCKER
June, 1949

Reviews

OUTLINES OF BIOCHEMISTRY. By ROSS AIKEN GORTNER. Third Edition. Edited by R. A. GORTNER jun. and W. A. GORTNER. Pp. xvi + 1078. London: Chapman & Hall, Ltd. 1949. Price 60s.

The first edition of this well-known book was written about twenty-five years ago. The original preface says: "In most of the Universities of America the development of the field of biochemistry has been left very largely to the group interested in the medical aspects. Accordingly, in a very large measure the biochemistry of the American Universities is not biochemistry in its strictest sense. . . . The purpose of the present volume is that those students who are interested in biological phenomena may have an insight into the roles which organic chemistry and physical chemistry play in living processes." A second edition came out in 1938 and this third edition is a co-operative effort; out of ten contributors, only one seems to be connected with a medical school. The book retains the original inspiration in that it is described as "a complete discussion of the fundamental organic and physico-chemical reactions of plant and animal organisms and the colloidal systems in which they take place."

The new edition is a large book of well over half a million words. The allocation of space to the more important sections is instructive: colloids 25 per cent., proteins 22 per cent., carbohydrates, etc. 22 per cent., lipids and essential oils 7 per cent., plant pigments 3·5 per cent., biochemical regulators 12 per cent.

Whatever "biochemistry in its strictest sense" may be taken to mean in 1950, the subject needs to preserve strong links with general chemistry. There is a large overlap between this book and many treatises on both physical and organic chemistry; that is not necessarily a bad thing, although there is here a decided tendency to lay more stress on results than on the methods of reaching them.

The subject index contains no reference to respiration, respiratory quotient, calories and nutrition, and the discussion of specific dynamic action is "in the air" when more elementary matters are omitted. The "hormones" of the thymus gland and the pineal body are described perhaps uncritically since the latest references quoted are in the years 1935–38. There is little or nothing about biological assays, statistical methods or physiological mechanisms and "detoxication" processes are given only cursory treatment.

As a conspectus of contemporary biochemistry the book must be judged defective. The gesture of independence twenty-five years ago may well have been a salutary protest against too great a pre-occupation with medical biochemistry, but the new edition perhaps errs in the opposite sense.

Nevertheless, this third edition has great merit and even at 60s. it is good value. Its emphasis is on the fundamental chemistry, organic and physical, and on the corpus of biochemical knowledge viewed primarily through chemical spectacles. The student who has mastered a large part of his book will be well equipped to proceed to the study of other treatises which reflect more clearly the present position of biochemistry; that is as a discipline firmly resting on chemical foundations but reaching out in other directions and having debts to acknowledge as well as achievements to its credit. Perhaps, however, the only thing to cavil at in this excellent volume is its title, which now scarcely corresponds with its content.

R. A. MORTON

TECHNIQUE OF ORGANIC CHEMISTRY. Volume I. PHYSICAL METHODS OF ORGANIC CHEMISTRY. Part II. Edited by A. WEISSBERGER. Second Edition. Pp. xi + 1023. New York and London: Interscience Publishers, Ltd. 1949. Price 100s.

This work in seven volumes is planned on a large scale. Volumes I and VII (organic solvents) are now in second editions. The first edition of Volume I came out in 1946 (see *Analyst*, 1946, **71**, 451). This edition of Part II contains a new chapter on electrophoresis by D. H. Moore; there are new sections on nephelometry and turbidometry and the determination of light scattering; the chapters on the determination of dipole moments, radio-activity and mass spectrometry have been entirely rewritten and other chapters have been revised.

X-ray diffraction is covered by L. Fankuchen, electron diffraction by L. O. Brockway; refractometry by N. Bauer and K. Fajans; spectroscopy, spectrophotometry, colorimetry and fluorimetry by W. West; polarimetry by W. Heller; dipole moments by C. P. Smyth; potentiometry by L. Michaelis; polarography by O. H. Müller; magnetic susceptibility by L. Michaelis; radio-activity by W. F. Balfe and J. F. Bonner jun.; and mass spectrometry by D. W. Stewart.

There can be few persons competent to make a critical assessment of the whole book; the subjects with which the present reviewer is familiar are very well done. There is an interesting note on errors in spectrophotometry due to fluorescence (p. 1329) and the sections on light sources, infra-red spectroscopy and Raman spectroscopy are informative, but the treatment of ultra-violet absorption spectra is much too brief in relation to the space devoted to techniques. Several other sections are very sketchy in respect of examples of uses in organic chemistry. The chapter on refractometry is a very well finished piece of work, possibly because there is no great pressure of new work waiting to be assimilated; indeed, few of the references in this section are less than ten years old. Polarimetry is in much the same case. The chapter on dipole moments, although now much expanded, is still reasonably brief but does not take too much for granted. T. Shedlovsky's account of conductometry is very clearly written. The treatment of magnetic susceptibility illustrates the difficulty in a work of this kind of striking the right balance between experimental methods, pure theory and application to organic chemical problems. The accounts of radio-activity and mass spectrometry are practical and serviceable for chemists.

Printing, paper, binding and illustrations are excellent, and even at £5 this Part is not unreasonably priced.

The absence of a section on chromatography will be noticed. This is probably because Volume V (by H. G. Cassidy) is entitled "Adsorption."

Many will still feel that (as was pointed out in the earlier review) a 1000-page volume on varied methods could with general advantage be subdivided into smaller volumes.

R. A. MORTON

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Mr. Ingram would therefore like to have names of all who are interested by May 1st, 1950. Messrs. Thomas Cook will then write to each individual separately.

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