THE ANALYST

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THE ANALYST

THE ANALYTICAL SECTION OF THE INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

AT the close of the September, 1951, meeting of the International Union of Pure and Applied Chemistry in New York, the Analytical Section officially came into being. After a period of some four years of effort, originating at the London meeting in 1947, punctuated by meetings in Amsterdam in 1949 and at Graz in 1950, a provisional Section Committee formed at Amsterdam worked with the Paris headquarters of the Union, and formulated Rules for the Section. These were submitted to the Section membership and adopted at New York. At the same time the existence of Sections within the Union became official. It is interesting to analytical chemists to know that their Section meeting in New York was the first official Section meeting under Union auspices.

The Analytical Section is composed of a Section Committee, a group of Commissions working on problems of general interest to analytical chemists, and Sub-commissions working under Commission auspices. The officers of the Section, elected at the meeting in New York, are: *President*—C. J. van Nieuwenburg (Delft), 1951–53; *Vice-President*—I. M. Kolthoff (Minneapolis), 1951–53; *Vice-President*—P. E. Wenger (Geneva), 1951–55; and *Secretary*—S. E. Q. Ashley (Pittsfield), 1951–55. They constitute the Executive Committee. The Section Committee, which is responsible for the general conduct of Section activities, is made up of the following membership—

The Officers of the Section.

The President, Secretary and Treasurer of the Union.

F. E. Beamish	(Toronto)	1951–1953
G. Charlot	(Paris)	1951–1953
R. C. Chirnside	(London)	1951–1955
F. Feigl	(Rio de Janeiro)	1951–1955
J. Gillis	(Ghent)	1951–1953
J. I. Hoffman	(Washington)	1951–1955

The Commissions that at present are carrying on the work of the Section are as follows—

COMMISSION ON ANALYTICAL REACTIONS (*Commission des Réactions Analytiques*)—

R. Belcher (Birmingham); F. Burriel-Martí (Madrid); N. D. Cheronis (Brooklyn); C. Duval, *Secretary* (Paris); J. Gillis, *Chairman* (Ghent); A. Okác (Brno); P. Rây (Calcutta); M. Servigne (Paris); P. W. West (Baton Rouge); J. H. Yoe (Charlottesville).

COMMISSION ON MICROTECHNIQUES (*Commission des Microméthodes*)—

E. Abrahamczik (Ludwigshafen am Rhein); A. A. Benedetti-Pichler (Flushing); P. L. Kirk, *Vice-chairman* (Berkeley); H. Lieb (Graz); H. Malissa, *Secretary* (Graz); P. E. Wenger (Geneva); C. L. Wilson (Belfast); M. K. Zacherl, *Chairman* (Vienna).

COMMISSION ON PHYSICO-CHEMICAL DATA OF ANALYTICAL INTEREST (*Commission des Données Physico-chimiques d'intérêt analytique*)—

R. G. Bates (Washington); W. R. Brode (Washington); G. Charlot (Paris); G. Duyckaerts (Liège); I. M. Kolthoff, *Chairman* (Minneapolis); H. A. Laitinen, *Secretary* (Urbana); G. Schwarzenbach (Zürich); L. G. Sillén (Stockholm).

COMMISSION ON TERMINOLOGY AND EXPRESSION OF ANALYTICAL RESULTS (*Commission de Terminologie et de Symbolistique Analytiques*)—

D. H. Dodd (Thorncliffe, near Sheffield); R. J. Forbes, *Chairman and Secretary* (Amsterdam); R. Gauguin (Paris); F. D. Tuemmler (Emeryville); G. T. Wernimont (New York).

The Commission on Analytical Reactions has been in existence since 1934 and has issued the following publications—

Tables of Reagents for Inorganic Analysis (Table des Réactifs pour l'Analyse minérale).

First Reports of the "International Committee on new Analytical Reactions and Reagents" of the "Union Internationale de Chimie."

Leipzig, Akademische Verlagsgesellschaft M.B.H., 1938.

Réactifs pour l'Analyse Qualitative Minérale recommandés par la Commission Internationale des Réactions et Réactifs analytiques nouveaux de l'Union Internationale de Chimie. Deuxième Rapport. Rédacteur: P. E. Wenger et R. Duckert.

Bâle, Wepf et Cie, 1945.

Reagents for Qualitative Inorganic Analysis, Second Report of the International Committee on New Analytical Reactions and Reagents of the International Union of Chemistry.

P. E. Wenger and R. Duckert, editors.

New York, London, Amsterdam, Brussels: Elsevier Publishing Co., Inc., 1948.

Tables of Reagents for Inorganic Analysis. Third Report of the "International Committee on new Analytical Reactions and Reagents" of the "Union Internationale de Chimie."

C. Duval, editor.

Paris, Libraire Istra, 1948.

Réactifs pour l'Analyse Qualitative Minérale. Recommandés par la Commission Internationale des Réactions et Réactifs analytiques nouveaux de l'Union Internationale de Chimie pure et appliquée (1937–1947).

P. E. Wenger and Y. Rusconi, editors.

Paris, Société d'Edition d'Enseignement Supérieur, 1950.

The Commission on Microtechniques was formally established at the meeting in Graz. It has begun to study the better organisation of analytical literature, certain questions of standardisation of microchemical apparatus, and the organisation of international meetings on microchemistry. The Commission has established the following Subcommission—

Subcommission on Standardisation of Microchemical Apparatus—H. K. Alber (Philadelphia); Ronald Belcher (Birmingham); Georg Gorbach (Graz); D. Monnier (Geneva); M. K. Zacherl, *Chairman* (Vienna).

The Commission on Physico-Chemical Data of Analytical Interest has divided its work among several Subcommissions concerned with the following—

Subcommission on Polarographic Data—J. Heyrovský (Prague); H. A. Laitinen, *Chairman* (Urbana); G. Semerano (Padova); J. K. Taylor (Washington); S. Wawzonek (Iowa City).

Subcommission on Emission Spectrography—W. R. Brode, *Chairman* (Washington); J. R. Churchill (New Kensington); A. Gatterer (Castel Gondolfo); J. Gillis (Ghent); B. F. Scribner (Washington).

Subcommission on Absorption Spectrography—W. R. Brode (Washington); G. Duyckaerts, *Chairman* (Liège); J. Lecomte (Paris); M. G. Mellon (Lafayette); A. C. Menzies (London).

Subcommission on Potentiometric Data—R. G. Bates, *Chairman* (Washington); H. T. S. Britton (Exeter); N. H. Furman (Princeton); L. G. Sillén (Stockholm).

Subcommission on Oxidation - Reduction Potentials—R. G. Bates (Washington); G. Charlot, *Chairman* (Paris); H. A. Laitinen (Urbana); M. Pourbaix (Brussels); L. G. Sillén (Stockholm); P. Souchay (Paris); E. R. Swift (Pasadena).

Subcommission on Stability Constants of Complexes—J. Bjerrum (Copenhagen); D. N. Hume (Cambridge); G. Schwarzenbach, *Chairman* (Zürich).

Subcommission on Solubility Data—W. Feitknecht (Bern); I. Leden (Göteborg); A. Ringbom (Åbo); L. G. Sillén, *Chairman* (Stockholm).

It is the intention of this Commission to sponsor the publication of critically selected values of constants and numerical data of importance to the work of analytical chemists, and to provide analytical chemists with authoritative data and consistent conventions for reporting results.

The Commission on Terminology and Expression of Analytical Results has begun the study of the format of analytical methods, symbols and units for expressing analytical results, the definition of terms relating to sampling, precision and accuracy, quality control, design of experiments and so on. A glossary of terms with equivalents in various languages is also under consideration.

Co-operative efforts will also be undertaken, for instance, in connection with the development of test methods for evaluating chemical reagents, for the proper use of symbols and other terminology, on spectrographic data and so on.

An important function of the Section's activity is the co-ordination of international meetings and Congresses on subjects of interest to analytical chemists. Without such co-ordination, there is danger that Congresses may overlap in interest, become too frequent, or may not obtain general support. Effective co-ordination can greatly reduce travel for those interested in attending, and can assist in procuring the necessary financial support for an effort of this kind.

The next meeting of the Section, its Section Committee and the Commissions will take place in Oxford, England, at the time of the Congress on Analytical Chemistry.

No programme has yet been formulated for the publication of Commission findings, although the five books mentioned above have appeared as a result of the work of the members of the Commission on Analytical Reactions.

Suggestions for other fields of activity may be submitted to the Analytical Section by writing to the Secretary, S. E. Q. Ashley, at 100, Woodlawn Avenue, Pittsfield, Mass., U.S.A. These suggestions will receive the careful attention of the Section Committee, which has broad geographical representation and determines policy for the Section.

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS
AND OTHER ANALYTICAL CHEMISTS

The Use of Cylinder Oxygen in the Organic Micro-Determination of Nitrogen

BY H. SWIFT AND E. S. MORTON

(*Presented at the meeting of the Microchemistry Group on Wednesday, April 23rd, 1952*)

A practical method is described for the determination of nitrogen in substances that burn with difficulty. It consists in burning the substance in carbon dioxide and then in a controlled flow of oxygen to remove final traces of unburnt material.

DURING the course of nitrogen determinations at this University various types of compounds have been encountered that have burnt only with difficulty. The figures obtained from such determinations have been reproducible within experimental error, but have given nitrogen values of 3 to 5 per cent. less than those calculated. Several methods have been tried, notably the use of potassium dichromate,[1] potassium chlorate[2] and copper acetate,[3] but with only moderate success. The method described below has, therefore, been adopted and has been in constant use for over a year. This consists in burning the substance in a stream of carbon dioxide and in removing final traces of unburnt material by a controlled flow of pure oxygen. Unterzaucher[4] used this principle and derived oxygen from the catalytic decomposition of hydrogen peroxide. In the following method cylinder oxygen of medicinal purity was used. The rate of combustion is considerably increased by comparison with the standard Pregl method.

Colson[5] also has recently described a rapid micro-Dumas method of determining nitrogen.

METHOD

APPARATUS—

The combustion tube, Fig. 1, is of the usual form, except that it is 80 cm long. To prepare the tube for use, it is cleaned and dried, and the permanent filling is added in the following order—

A small wad of silver wool, to fill a 5-mm length of the tube. This is compressed against the neck of the tube with a long glass rod. The wool prevents the asbestos plug from choking the neck of the tube.

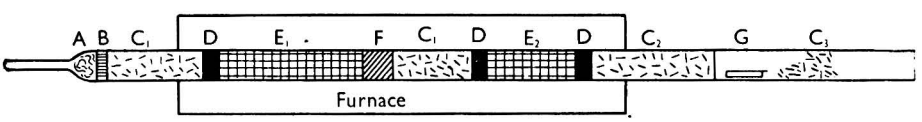

Fig. 1. Combustion tube

A—Silver wool, 5 mm
B—Asbestos plug, 2 to 3 mm
C_1—Coarse copper oxide, 10 cm
C_2—Coarse copper oxide, 13 cm
C_3—Coarse copper oxide to within 3 cm from end of tube
D—Oxidised copper spiral plug, 5 mm
E_1—Reduced copper spiral, 15 cm
E_2—Reduced copper spiral, 10 cm
F—Platinum gauze spiral, 1 cm
G—Nickel boat containing sample

A plug of ignited asbestos, to fill 2 to 3 mm of the tube.

Ignited copper oxide to extend 10 cm along the tube, held in position by an oxidised copper gauze roll 5 mm long.

A 15-cm reduced copper spiral held in place by a 5-mm oxidised copper spiral plug.

A piece of platinum gauze 1 cm long.

Coarse copper oxide, to fill 10 cm of the tube, held in position by a 5-mm oxidised spiral plug.

A reduced copper spiral 10 cm long held by another 5-mm oxidised plug.

Gas supply, Fig. 2—The supply is regulated by a three-way tap, the single limb of which is attached by thick-walled pressure tubing to a jet that delivers the gas to the combustion tube. The two branch tubes are each bent in a horizontal plane at an angle of 30° to the tap. One of these tubes is attached by thick-walled pressure tubing to the carbon dioxide supply, A, that can be from "dry ice"; the other carries a graduated gasometer, B, and a mercury pressure regulator, C, the two being separated by a glass tap, D. Stout

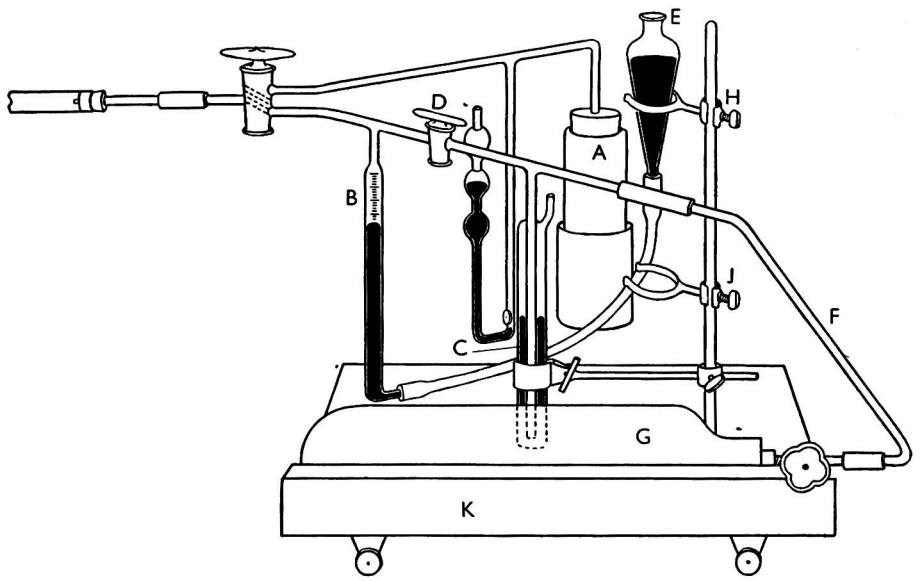

Fig. 2. Gas supply

rubber connections join the pressure regulator, by a glass delivery tube, F, to the cylinder of pure oxygen, G. The gasometer is graduated to hold a total of 10 ml of oxygen, which is compressed by placing the connecting mercury reservoir, E, in the split ring clamp, H. When drawing oxygen in, the reservoir is placed in a similar clamp, J. The difference in mercury levels of the gasometer and reservoir when in the raised position is 20 cm. The gas regulator is a tube 15 cm long with an internal seal where the stem of the T-piece enters; an exit tube 6 cm long (for the excess of gas) is provided as shown in Fig. 2, and the regulator is filled to one-third of its height with mercury. The entire oxygen apparatus is mounted on a small wooden trolley, K.

PROCEDURE—

Place ignited coarse copper oxide, to a length of 13 cm, in the combustion tube and introduce immediately behind the oxide a tared nickel boat containing 2 to 3 mg of the sample. By means of a glass piston insert coarse copper oxide into the tube so that the oxide occupies the space immediately behind the boat to within 3 cm of the end of the tube. This operation is carried out with the combustion tube lying across the split furnace (45 cm long). Before attaching the three-way tap to the combustion tube, turn on the cylinder of pure oxygen, shut the tap connecting the mercury valve to the reservoir, and open the three-way tap to the oxygen circuit. Raise the mercury level in the gasometer to its full height by means of the reservoir. With the excess of oxygen blowing through the valve, open the connecting stopcock to the valve and gasometer, and allow the oxygen to sweep the limb free of air. Close the three-way tap and place the reservoir in the lower ring clamp in order to draw oxygen into the gasometer. Turn off the connecting tap and close the oxygen valve. Raise the

reservoir to maintain a pressure on the gas in the gasometer and place it in position in the upper ring clamp.

Attach the combustion tube to the three-way tap. Connect the neck of the tube to the azotometer in the usual way, allowing 6 to 8 cm of the neck end of the combustion tube to protrude from the furnace. Sweep out the tube by turning the three-way tap to the carbon dioxide supply until micro-bubbles are observed. This takes about 5 minutes. Fill the azotometer by raising the reservoir and regulate the flow of carbon dioxide to 3 to 5 bubbles per second by means of the micro-tap leading from the combustion tube to the azotometer. Switch on the furnace adjusted to 730° C, and allow it to attain a red heat.

Partly decompose the sample in a stream of carbon dioxide by moving the combustion burner, fully opened to the air, in the usual manner but at a rate of 2 cm per minute. Some nitrogen is given off and passes into the azotometer. Follow the first combustion with a second using the usual iron gauze tunnel and burn in a similar fashion until the sample is reached. At this point allow the tube to attain a red heat, then open the three-way tap to the oxygen supply and allow 2 ml of oxygen to pass slowly. Close the oxygen supply tap and open the tap to the carbon dioxide circuit. After 30 seconds restart the oxygen supply and allow a further 2 ml of oxygen to pass. Repeat a third time. Continue heating the combustion tube at 2 cm per minute until the furnace is reached. Turn off the gas burner and switch off the furnace. Sweep out the tube till micro-bubbles are observed in the azotometer, which is read in the usual way.

Notes—

A new tube is conditioned in the same way as a short Dumas tube.

A blank determination is carried out on a standard sample. This gives a steady blank of approximately 0·01 to 0·03 ml.

It will be observed that no fine copper oxide is used for oxidation purposes.

Results

The results shown in Table I are of typical analyses by the oxygen method.

Table I

Results of analyses by the proposed method

	Nitrogen	
	theory, %	found, %
4-Amino-uracil	33·3	33·6
4-Amino-uracil (N in ring ^{15}N)	33·55	33·6
4:4′-Diamino-6:6′-azocinnoline dimethochloride	25·1	25·05
4-Acetamido-7-nitrocinnoline	24·15	23·9
7-Amino-4-phenoxycinnoline	17·75	17·5
Barbituric acid	21·9	22·1
N-(*p*-Acetamido-xy-dinitrophenyl)-phthalimide	15·15	15·35
4:4′-Diamino-3-3′-dinitroazobenzene	27·8	27·6
3:3′:4:4′-Tetraminoazobenzene	34·7	34·7
2-Formamido-4-nitroacetophenone	13·4	13·3
1-Acetyl-2:3-dimethyl-4-nitro-indole	12·1	12·25
3-Acetyl-4′-nitroazobenzene	15·6	15·95
Methyl ethyl ketone 2:4-dinitrophenylhydrazone	22·2	22·15
2-Methylsulphonyl-4-tetra-acetylglucosylamino-6-acetamido-pyrimidine	10·0	9·9

A few comparison figures for the proposed method against those given by the methods previously used are shown in Table II.

Several sizes of reduced spiral were tried in the initial experiments, but the two sizes given in the method were found to be the most convenient. Initially it was considered necessary to reduce the 10-cm spiral after five determinations. This, however, has proved to be unnecessary and twenty or more determinations may be carried out before both spirals are renewed. To facilitate the removal of the plugs and spirals, the copper gauzes are wound round stout copper wire bent at the ends to form hooks. The platinum gauze was found to reduce the blank value, so it was positioned in the most satisfactory place, *i.e.*, behind the

first spiral. The volume of oxygen used may be varied with each set of determinations, after obtaining a satisfactory blank value, but it is advisable not to exceed 8 ml per determination. Platinum boats were found to give erratic results in some determinations, hence

TABLE II

COMPARISON OF RESULTS OF NITROGEN DETERMINATIONS

Compound	Nitrogen			
	Calculated, %	By proposed method, %	By standard techniques	
			%	Procedure
4-Amino-uracil	33·3	33·6	30·6	Mean of 5 results by micro-Dumas method
4-Acetamido-7-nitrocinnoline ..	24·15	23·9	22·45	By micro-Dumas method
6-Nitro-4-amino-quinazoline ..	29·5	29·5	27·15	Mean of 3 results obtained in other laboratories
6-Nitro-4-*iso*propylidene-hydrazinocinnoline	28·7	28·95	25·65	
7-Nitro-4-acetamido-quinazoline	24·1	24·1	21·9, 24·5	Results obtained in other laboratories

the use of nickel ones. In addition to samples that burn with difficulty, normal samples can be treated in a similar fashion to give satisfactory results. At the moment an apparatus that incorporates an air-free flowmeter and uses a mixture of carbon dioxide and oxygen is being designed and this should reduce the blank value.

The authors wish to express their gratitude to Professor E. R. H. Jones for facilities to carry out the work. In addition they wish to thank Mr. G. Wood for assistance with the analyses.

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DEPARTMENT OF CHEMISTRY
THE UNIVERSITY OF MANCHESTER
MANCHESTER, 13

DISCUSSION

DR. W. T. CHAMBERS said that he had observed over the past five years that Drikold contained variable amounts of occluded air. The degree of purity of the gas evolved was frequently such as to warrant the application of a blank correction. Apart from obvious inconveniences the blank was not constant during the life (4 to 5 days) of the Drikold charge in the quart-size Dewar vessel used.

He had now reverted to the Hein-Kipp apparatus, which readily gave carbon dioxide of the requisite purity. He asked what brand of solid carbon dioxide was used by Mr. Swift and if it was consistently satisfactory, *i.e.*, required no blank correction.

DR. C. L. WILSON said, with reference to Dr. Chambers' comment, that they in Belfast had abandoned the use of Drikold, owing to the very variable blank. One sample might give a very low blank indeed, whereas the next one might be completely unsatisfactory. They had found the Tucker gas generator to be by far the most satisfactory source of carbon dioxide.

MR. SWIFT said, with reference to the Tucker gas generator, that there was no doubt that the purest form of carbon dioxide was produced from marble and hydrochloric acid. Nevertheless, he had found that Drikold, as supplied by the Carbon Dioxide Co. Ltd., was entirely satisfactory and the blank negligible when the rapid combustion method was used. Enquiries had shown that Drikold was prepared by the combustion of coke, with subsequent removal of flue gases, and not from fermentation gas.

MR. G. INGRAM asked what the flow-rate of gases was during the combustion.

MR. SWIFT said that each 2 ml of oxygen should pass at about the same rate as the carbon dioxide, namely, 3 to 5 bubbles per second, although that rate can be slightly exceeded with no untoward effect.

MR. C. M. LAVENDER asked if it was necessary to renew the second copper gauze before each determination to prevent oxygen reaching the nitrometer.

MR. SWIFT said that it was unnecessary to renew the second copper gauze spiral very frequently as 50 or so determinations could be made with the same one.

DR. A. LACOURT said that she was very satisfied with the quality of carbon dioxide produced from marble and hydrochloric acid in a Kipp apparatus that had been fitted with a valve to prevent air from diffusing back into the apparatus. She admitted that the Unterzaucher method was quite successful for dealing with substances in which nitrogen estimation was difficult; the carbon dioxide being bubbled through diluted hydrogen peroxide solution.

MR. SWIFT said in conclusion that he had recently adapted the Unterzaucher reverse-flow principle to his method and, in addition, had included an air-free flowmeter for use with a liquid paraffin excess pressure valve. The valve gave a very steady pressure and, when used with the flowmeter, eliminated the necessity for a micro-tap at the azotometer end. Oxygen was admitted from the burette through a micro-tap and the determination was carried out (as in Unterzaucher's method) with the tube permanently in the furnace, which was left on.

Cylinder oxygen might be preferable to hydrogen peroxide as no dilution of the oxygen concentration would then take place.

Analytical Uses of the Isosbestic Point Exhibited by Vitamin A on Oxidation

BY H. H. BAGNALL AND F. G. STOCK

Vitamin A on oxidation exhibits a so-called isosbestic point, or point of constant absorption, at 290 mμ. The phenomenon is exhibited by fish-liver oils as well as by solutions of the pure vitamin, and use is made of this to estimate approximately the original vitamin-A content of oxidised fish-liver oils, so as to decide whether a failure to comply with a declaration of vitamin-A content was the result of oxidation or of insufficient original potency. The method is best used for groups of oils rather than for individual samples.

Examples are given of the application of the method to oxidised fish-liver oils.

IT is well known that the vitamin-A content of a fish-liver oil decreases on storage, this fact being reflected in a corresponding decrease in the gross value of $E^{1\%}_{1\,cm}$ at 328 mμ and an increase in the absorption on the short-wave side of $\lambda_{max.}$. It is believed that the formation of vitamin-A epoxide, with an absorption maximum in the 270 to 280 mμ region, causes this phenomenon. An interesting feature of the oxidative process, which can be clearly demonstrated by its artificial acceleration, is the appearance of a so-called isosbestic point, or point of constant absorption, at about 290 mμ, to which Bolomey[1] has already called attention. It has suggested itself to us that this phenomenon might possibly be of value in deriving an approximate estimate of the original vitamin A in an oxidised high-potency oil. In some instances recently, it would have been of interest to know whether or not the amount of vitamin declared had ever been present in an oil before oxidation.

The fact that fish-liver oils, as well as solutions of the pure vitamin, exhibit this phenomenon implies that for any particular oil the irrelevant absorption is a static quantity, the only change occurring on oxidation being the conversion of vitamin A to its epoxide. In examples of extreme oxidation, the epoxide itself decomposes and at the same time the isosbestic point becomes indefinite.

EXPERIMENTAL RESULTS—

The occurrence of the isosbestic point on the halibut-liver oil absorption curve at 290 mμ was demonstrated by the use of accelerated oxidation tests. A weighed amount of halibut-liver oil was heated in a wide-necked flask on a water-bath in a current of air, and after $\frac{1}{2}$ hour the absorption curve of a *cyclo*hexane solution of the oxidised oil was determined. This process was repeated with another weighed quantity of oil for an exposure time of 1 hour. The data for the original absorption curve and the curves obtained after oxidation are given in Table I and illustrated in Fig. 1. By the use of geometrical correction procedures the true

vitamin-A contents were estimated, and the subtraction curves for the irrelevant absorption were deduced; these results are shown in Table I and illustrated in Fig. 2. The isosbestic point is clearly indicated in Fig. 1, and Fig. 2 shows the characteristic absorption features of vitamin-A epoxide.

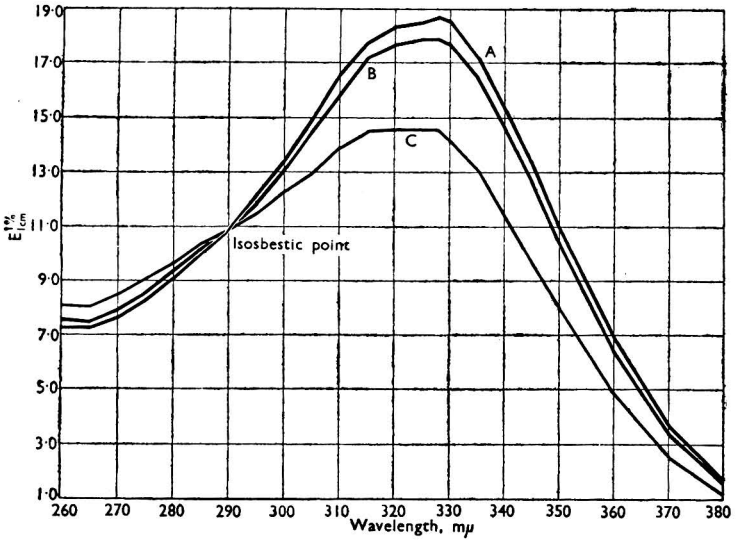

Fig. 1. Demonstration of isosbestic point by accelerated oxidation tests with halibut-liver oil. A, original oil; B, after oxidation for 30 minutes; C, after oxidation for 1 hour

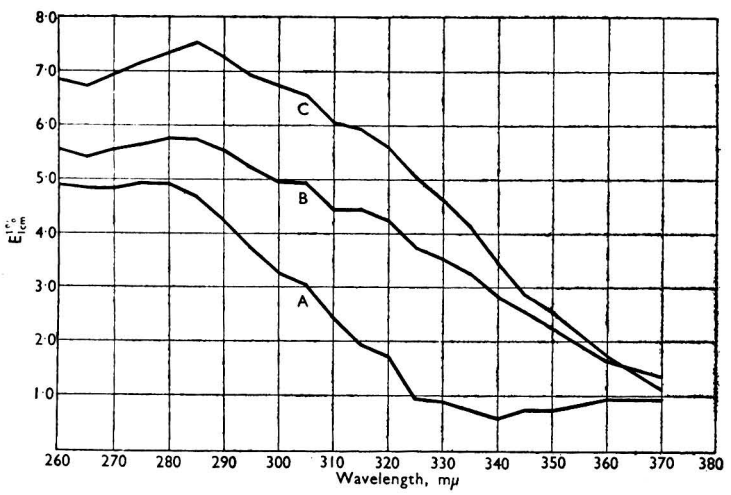

Fig. 2. Accelerated oxidation tests with halibut-liver oil. Calculated irrelevant absorption showing presence of vitamin-A epoxide. A, original oil; B, after oxidation for 30 minutes; C, after oxidation for 1 hour

DISCUSSION OF RESULTS—

It has been found possible by the use of this isosbestic point to obtain an approximate indication of the original vitamin-A content of an oxidised high-potency oil. The fact that the gross value of $E^{1\%}_{1cm}$ at 328 mμ × 1600 gives an approximate estimation of the vitamin-A content of an unoxidised oil has long been accepted, but the use of the $E^{1\%}_{1cm}$ at

290 mμ presents a rather more difficult problem. To arrive at an appropriate factor by which to multiply the value of $E^{1\%}_{1\,cm}$ at 290 mμ would require the determination of the ratio of E at 328 mμ to E at 290 mμ for a large number of unoxidised high-potency fish-liver oils; subsequent multiplication of this ratio by 1600 would give the necessary factor for conversion of the value of $E^{1\%}_{1\,cm}$ at 290 mμ to International Units of vitamin A. Unfortunately it has not been possible to determine a factor directly in this way, owing to the fact that so many of the samples examined by us under the Food and Drugs Act had oxidised to various extents. Nevertheless an approximate factor has been arrived at by other means, and examples of its usefulness are given later.

TABLE I

HALIBUT-LIVER OIL ACCELERATED OXIDATION DATA

	$E^{1\%}_{1\,cm}$ value					
	*cyclo*Hexane solution of oil			Calculated irrelevant absorption		
Wavelength, mμ	Original oil	Oxidised oil, ½ hour	Oxidised oil, 1 hour	Original oil	Oxidised oil, ½ hour	Oxidised oil, 1 hour
260	7·30	7·51	8·13	4·90	5·56	6·83
265	7·30	7·40	8·05	4·86	5·43	6·73
270	7·65	7·83	8·49	4·86	5·57	6·97
275	8·35	8·43	9·04	4·94	5·67	7·19
280	9·12	9·21	9·64	4·91	5·79	7·36
285	10·00	10·07	10·40	4·70	5·78	7·52
290	10·87	10·88	10·86	4·28	5·55	7·29
295	12·03	11·91	11·44	3·74	5·21	6·95
300	13·37	13·15	12·24	3·28	4·98	6·77
305	14·85	14·53	12·99	3·04	4·97	6·59
310	16·50	15·85	13·86	2·44	4·47	6·06
315	17·68	17·20	14·45	1·97	4·48	5·93
320	18·25	17·66	14·55	1·74	4·29	5·60
325	18·43	17·88	14·55	0·96	3·74	5·08
328	18·63	17·88	14·51			
330	18·49	17·77	14·17	0·90	3·54	4·63
335	17·12	16·52	13·02	0·77	3·29	4·16
340	15·23	14·67	11·41	0·60	2·82	3·48
345	13·23	12·70	9·64	0·74	2·58	2·86
350	10·87	10·45	8·02	0·76	2·26	2·54
360	6·81	6·43	4·90	0·96	1·69	1·73
370	3·61	3·54	2·60	0·94	1·38	1·15
380	1·72	1·73	1·20			

DEDUCTION OF AN APPROXIMATE FACTOR—

Table II gives results obtained for the vitamin-A content of twelve samples of a product that was stated to contain 27,000 International Units per gram. From a consideration of these it is obvious that extensive oxidation has occurred and is very marked in samples number 1, 7 and 11. The interesting question now arises as to whether these samples originally contained 27,000 International Units of vitamin A per gram. Although some of the samples are evidently still genuine, or nearly so, others show large differences in gross values of $E^{1\%}_{1\,cm}$ at 328 mμ, but the remarkable consistency of the gross values of $E^{1\%}_{1\,cm}$ at 290 mμ suggests that originally all the oils were of approximately the same potency. These twelve samples originated from ten different oils, and numbers 5, 6 and 10, 11 were duplicates from the same original batches, but when examined by us the members of each pair exhibited different degrees of oxidation. For numbers 5 and 6 the gross values of $E^{1\%}_{1\,cm}$ at 290 mμ for the two samples are identical, while numbers 10 and 11, in spite of the fact that 11 is very badly oxidised, show the remarkably close values of 9·41 and 9·30, respectively. This table gives a striking example of the use of the isosbestic point in analytical practice for helping to form an opinion as to whether a failure of a group of samples to comply with a declaration is due to oxidation or to insufficient original potency. Clearly in this example it is due to oxidation, and there is every reason to be satisfied that the original potency was in compliance with the declaration. It was ascertained that the average gross value of $E^{1\%}_{1\,cm}$ at 328 mμ of these ten oils at the date of manufacture was 17·41, which corresponds to 28,000 International Units of vitamin A per gram. The average gross value of $E^{1\%}_{1\,cm}$ at 290 mμ

from Table II is 9·14 and the appropriate 290 mμ factor will be 28,000/9·14 or about 3000. For a fresh unoxidised oil, gross $E^{1\%}_{1\,cm}$ at 290 mμ × 3000 is approximately equal to gross $E^{1\%}_{1\,cm}$ at 328 mμ × 1600.

TABLE II

HALIBUT-LIVER OILS DECLARED TO CONTAIN 27,000 I.U. OF VITAMIN A PER GRAM

Sample number	Gross $E^{1\%}_{1\,cm}$ at 328 mμ	"Corrected" $E^{1\%}_{1\,cm}$ at 328 mμ	Vitamin A, I.U. per gram	Gross $E^{1\%}_{1\,cm}$ at 290 mμ
1	2·94	0	0	9·32
2	14·97	11·88	22,480	8·55
3	18·63	17·68	33,600	10·81
4	16·13	13·14	24·980	8·75
5	15·92	12·96	24,620	8·67
6	14·35	10·92	20,750	8·67
7	5·20	1·03	500	8·97
8	10·64	7·47	14,200	8·87
9	15·81	14·27	27,110	8·50
10	15·38	11·71	22,270	9·41
11	8·12	4·71	7000	9·30
12	15·92	12·68	24,090	10·00

It is as well to consider briefly the accuracy of such a procedure. Gross $E^{1\%}_{1\,cm}$ at 328 mμ × 1600 approximately equals "corrected" $E^{1\%}_{1\,cm}$ at 328 mμ × 1920; hence the average ratio of the absorption by vitamin A to the irrelevant absorption is 5:1, *i.e.*, there is an average of 16·7 per cent. of irrelevant absorption at 328 mμ. At 290 mμ this ratio is much less; for pure all-*trans* vitamin-A acetate in *cyclo*hexane, E at 290 mμ/E at 328 mμ is 0·350; hence it follows that $E^{1\%}_{1\,cm}$ at 290 mμ × 5490 = International Units of vitamin A for the pure vitamin. If, as we have suggested from the figures in Table II, multiplication of the gross value of $E^{1\%}_{1\,cm}$ at 290 mμ of a high-potency fish-liver oil by 3000 gives an approximate estimate of the original vitamin-A content, then the average ratio of the absorption by vitamin A to the irrelevant absorption at 290 mμ approaches unity. It is obvious that the estimation of five parts of vitamin A in the presence of one part of irrelevant absorption is much more accurate than the estimation of one part of vitamin A in the presence of one part of irrelevant absorption, and hence, for individual oils, the expression, gross $E^{1\%}_{1\,cm}$ at 290 mμ × factor, can never give more than an approximate estimation of the vitamin-A content. We wish to stress this fact, and to suggest that the isosbestic point is of more value in the study of a group of oils, as illustrated by the examples given, than in helping to form an opinion on an isolated oil.

CONFIRMATION OF THE FACTOR—

Table III records the results on thirty-three high-potency fish-liver oils, chiefly halibut, and it is obvious from the ratio, "corrected" $E^{1\%}_{1\,cm}$ at 328 mμ/gross $E^{1\%}_{1\,cm}$ at 328 mμ, which on unoxidised oils approximates to 0·83, that many of these show evidence of oxidation; this was confirmed by the absorption curves obtained. The mean values of the ratios "corrected" $E^{1\%}_{1\,cm}$ at 328 mμ/gross $E^{1\%}_{1\,cm}$ at 328 mμ, and $E^{1\%}_{1\,cm}$ at 290 mμ/gross $E^{1\%}_{1\,cm}$ at 328 mμ, are 0·79 and 0·58, respectively. For unoxidised oils the expected ratios would be 0·83 and 0·53, and oxidation would result in a decrease in the former and an increase in the latter, which has indeed happened. If, however, for the purpose of calculating the average values of these two ratios, we use only the 16 oils showing little oxidation, with a "corrected" $E^{1\%}_{1\,cm}$ at 328 mμ/gross $E^{1\%}_{1\,cm}$ at 328 mμ ratio of 0·80 or more, the results are 0·84 and 0·53, respectively, which are in almost perfect agreement with the expected values.

APPLICATIONS OF THE METHOD—

The vitamin-A contents of samples 17 to 21 (Table III) were declared as 36,000 International Units per gram. The average potency was only 26,400 International Units per gram, but on multiplying the $E^{1\%}_{1\,cm}$ at 290 mμ value by 3000 the value 37,600 International Units per gram was obtained, and this result, together with the evidence of oxidation shown by the absorption curves, led to the reasonable conclusion that the oils at the time of manufacture conformed with the declaration.

Samples 24 and 25 were vitamin-A and D capsules containing 3 minims of oil with a declared potency of 4500 International Units of vitamin A per capsule. This required the

oil to contain 27,000 International Units of vitamin A per gram, but the amounts present were, respectively, 20,290 International Units per gram and nil. The calculations from the values of $E^{1\%}_{1cm}$ at 290 mμ, however, resulted in figures of 26,550 and 25,140 International Units per gram, and again we were able to assume that at the time of manufacture

TABLE III

COMPARISON OF GROSS $E^{1\%}_{1cm}$ AT 328 mμ, "CORRECTED" $E^{1\%}_{1cm}$ AT 328 mμ AND GROSS $E^{1\%}_{1cm}$ AT 290 mμ VALUES AND DATA CALCULATED THEREFROM

Sample number	$E^{1\%}_{1cm}$ at 328 mμ	$E^{1\%}_{1cm}$ at 328 mμ × 1600	Corrected $E^{1\%}_{1cm}$ at 328 mμ	Corrected $E^{1\%}_{1cm}$ at 328 mμ × 1920	"Corr." $E^{1\%}_{1cm}$ at 328 mμ / Gross $E^{1\%}_{1cm}$ at 328 mμ	$E^{1\%}_{1cm}$ at 290 mμ	$E^{1\%}_{1cm}$ at 290 mμ × 3000	$E^{1\%}_{1cm}$ at 290 mμ / Gross $E^{1\%}_{1cm}$ at 328 mμ
1	19·95	31,930	16·06	30,830	0·80	12·55	37,650	0·63
2	18·44	29,490	15·14	29,070	0·82	9·01	27,000	0·49
3	18·45	29,520	15·01	28,820	0·81	9·20	27,600	0·50
4	18·32	29,320	14·29	27,450	0·78	10·23	30,690	0·56
5	19·40	31,030	15·30	29,380	0·79	10·11	30,330	0·52
6	17·11	27,380	12·45	23,910	0·73	11·10	33,300	0·65
7	31·20	54,240	24·08	46,230	0·77	17·67	53,010	0·57
8	25·44	40,700	22·38	42,930	0·88	12·07	36,210	0·47
9	24·97	39,950	20·46	39,280	0·82	12·54	37,620	0·50
10	25·93	41,490	22·35	42,910	0·86	12·12	36,360	0·47
11	16·91	27,060	14·70	28,220	0·87	8·02	24,060	0·47
12	29·04	46,480	25·35	48,670	0·87	13·39	40,170	0·46
13	27·93	44,680	23·28	44,700	0·83	13·91	41,730	0·50
14	15·10	24,160	11·99	23,020	0·79	8·39	25,170	0·56
15	9·72	15,550	7·70	14,790	0·79	6·57	19,710	0·68
16	8·86	14,180	7·12	13,680	0·80	5·87	17,510	0·66
17	20·05	32,070	15·07	28,940	0·75	12·37	37,110	0·62
18	17·77	28,430	11·46	22,010	0·65	13·19	39,570	0·74
19	17·82	28,510	12·94	24,850	0·73	11·89	35,670	0·67
20	19·35	30,960	13·89	26,680	0·72	13·11	39,330	0·68
21	20·72	33,150	15·33	29,440	0·74	12·09	36,540	0·58
22	18·14	29,020	16·03	30,770	0·88	8·35	25,050	0·46
23	15·84	25,340	14·41	27,670	0·91	7·59	22,770	0·60
24	13·36	21,380	10·57	20,290	0·79	8·85	26,550	0·66
25	3·07	4910	0	0		8·38	25,140	2·73
26	18·05	28,880	14·47	27,770	0·80	10·95	32,850	0·61
27	11·32	18,110	8·00	15,360	0·71	8·64	25,920	0·76
28	15·05	24,070	11·16	21,420	0·74	9·20	27,600	0·61
29	18·24	29,180	14·71	28,240	0·81	11·43	34,290	0·63
30	17·46	30,550	14·61	28,050	0·84	10·23	30,690	0·59
31	20·58	32,940	16·24	31,380	0·79	11·90	35,700	0·60
32	3·05	4880	1·71	3280	0·56	3·24	9720	1·04
33	18·56	29,700	16·16	31,020	0·87	8·45	25,350	0·46

the vitamin-A content was as declared and that oxidation as confirmed by the absorption curves was the cause of the discrepancies. It should be emphasised that caution is necessary when assessing samples similar to number 25, where the vitamin content is nil, as the epoxide itself may have partly decomposed, and false deductions may be drawn from the value of $E^{1\%}_{1cm}$ at 290 mμ.

With sample number 6 of halibut-liver oil, a declaration of conformity with the B.P. minimum standard of 30,000 International Units per gram was given. The value found was 23,910 units, but an estimated original content of 33,000 units was obtained by the use of the formula $E^{1\%}_{1cm}$ at 290 mμ × 3000.

Sample 27 consisted of 3 minim capsules stated to contain 4500 International Units per capsule; this implied that the oil contained 27,000 units per gram. Although the vitamin content was only 15,360 International Units per gram and oxidation was obvious, the use of the 290 mμ value indicated an original content of 25,900.

Sample 32 was an oil declared to contain 6000 International Units per ml. The amount found was 3300, with evidence of gross oxidation, the 290 mμ value was 9700 and so originally the vitamin potency was in all probability well in excess of the stated 6000 International Units per ml.

CONCLUSIONS—

It is suggested that the gross $E^{1\%}_{1cm}$ at 290 mμ × 3000 for high-potency fish-liver oils in *cyclo*hexane solution gives an approximate estimate of the original vitamin-A content of an oxidised oil. The use of this statement is best confined to groups of oils rather than individual samples, for which the inaccuracies entailed in its use may be too great to make possible a useful opinion.

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Paper Chromatography of Some Starch Conversion Products

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White malt syrup—a product obtained from starch by enzymatic conversion—is indistinguishable from liquid glucose, the acid conversion product, by the ordinary methods of copper reduction and polarimetry. Three special methods have been used for comparing the compositions of these articles. The discrepancies between the results are discussed and reasons are given for preferring those obtained by paper chromatography. It is demonstrated that by this procedure the two articles can readily be distinguished, either alone or when present as ingredients in confectionery, etc. Figures hitherto reported for the maltose content of starch conversion products and similar goods are shown to have been inflated by the inclusion of substances other than maltose.

UNDER the name of "White Malt Syrup" a product has recently been imported in considerable quantities from Holland, where it is said to be produced from starch by the action of micro-organisms or enzymes. It is a clear, almost colourless, viscous syrup closely resembling liquid glucose, for which it is offered as a substitute. According to the Dutch Pure Food Law, white malt syrup must contain more than 80 per cent. by weight of total solids; more than 50 per cent. of the solids as maltose, and less than 5 per cent. of the solids as dextrose. Considerable difficulty has been experienced in drawing a distinction between this product and liquid glucose, as their optical rotations and copper reducing powers are substantially identical (Table I).

TABLE I

OPTICAL ROTATION AND REDUCING POWER OF MALT SYRUP AND LIQUID GLUCOSE

Malt syrup		Liquid glucose	
Rotation of a 10% solution, °S	Reducing power as percentage of invert sugar	Rotation of a 10% solution, °S	Reducing power as percentage of invert sugar
70·6	36·6	69·0	39·7
71·2	41·3	65·2	37·9
70·4	36·7	68·3	39·8
69·2	39·4	67·2	40·5

With the object of facilitating this distinction, three methods have been explored: selective oxidation by copper acetate (a modified Barfoed method); selective fermentation; and paper chromatography.

SELECTIVE OXIDATION—

Barfoed's copper acetate solution is said to be reduced by monosaccharides, but to be unaffected by disaccharides. It was found, as expected, that malt syrup reacted with Barfoed's solution to a very much smaller extent than did liquid glucose, and consequently

the two products were distinguishable. But three difficulties remained. The procedure was lengthy and not more than three samples could be handled simultaneously by one worker. The presence of invert sugar vitiated the method, which could not therefore be extended to confectionery. And some uncertainty was felt as to the reliability of the figures obtained for dextrose content when determined in presence of a ten-fold preponderance of maltose. Some trial determinations of dextrose carried out on synthetic mixtures of dextrose and maltose and of dextrose and lactose indicated that the reducing effect of the disaccharides on Barfoed's solution, although slight, was appreciable, and greater with maltose than with lactose. However, the validity of these results was open to question, since we had at this time no means of proving that the apparent reducing effect of the maltose was not due to traces of dextrose in the sample of laboratory reagent maltose.

SELECTIVE FERMENTATION—

The Dutch Government specification for malt syrup prescribes a method of analysis involving the hydrolysis of the sample by prolonged boiling with hydrochloric acid (*a*) the malt syrup as received, (*b*) after fermentation for 48 hours with *Candida pseudotropicalis* and (*c*) after fermentation for 48 hours with *Saccharomyces cerevisiae*, followed in each instance by determination of reducing sugars. The difference between (*a*) and (*b*) is a measure of dextrose, the difference between (*b*) and (*c*) is a measure of maltose, and (*c*) is a measure of "dextrin."

We have attempted to apply this method, using yeasts obtained specially from the Dutch National Collection at Delft. Our experiments have been too few to justify criticism of the method, although in our inexperienced hands it has shown poor agreement between duplicates. It is, however, beyond doubt that it is time-consuming and would be quite unsuitable for the examination of a number of routine samples. Like the Barfoed method it would be inapplicable to confectionery.

We are grateful to Mrs. B. Kirsop of the Brewing Industry Research Foundation for having obtained the yeasts for us from Holland and for advice and assistance with the fermentation experiments.

PAPER CHROMATOGRAPHY—

Details of the apparatus and procedure used are given in the Appendix on p. 404.

The chromatograms obtained with malt syrup and with liquid glucose are shown in Fig. 1, with chromatograms obtained from milk toffees prepared in the laboratory from the same recipe, but with the two different ingredients. Diagnostic features are: (1) in the malt syrup chromatogram, the intensity of the maltose spot is ten to twenty times that of the dextrose spot, whereas in the liquid glucose chromatogram the dextrose spot is the stronger, and (2) in the liquid glucose chromatogram the spot marked (*d*) is not very different in size and intensity from the maltose spot, whereas with the malt syrup chromatogram it is only one-tenth as strong at most and is commonly indiscernible as in the chromatogram illustrated. Criterion (2) is the more dependable when invert sugar is present, since the intensity of the dextrose spot is thereby increased, but as the illustration of the toffee chromatograms shows, the distinction can still be readily drawn in the presence of the other common sugars.

DISCUSSION OF RESULTS—

A sample of white malt syrup furnished the following analytical results—

	By Barfoed's method	By fermentation method	By chromatography
Dextrose, %	4·3, 4·3	4·6, 7·4	2½, 3

The lack of agreement between the two fermentation results, which were simultaneous duplicates, rules them out from consideration. It seems reasonable to attribute the discrepancy between the Barfoed and the chromatographic results—a discrepancy which was found to be of the same sign and of very similar magnitude with several other samples of malt syrup—to the reducing effect of maltose on the Barfoed reagent. This is confirmed, as paper chromatographic analysis of the laboratory reagent maltose revealed the presence of only 1 per cent. of dextrose—much less than would have been required to account for its observed reducing action on Barfoed's solution. For these reasons we have preferred the results obtained by chromatography.

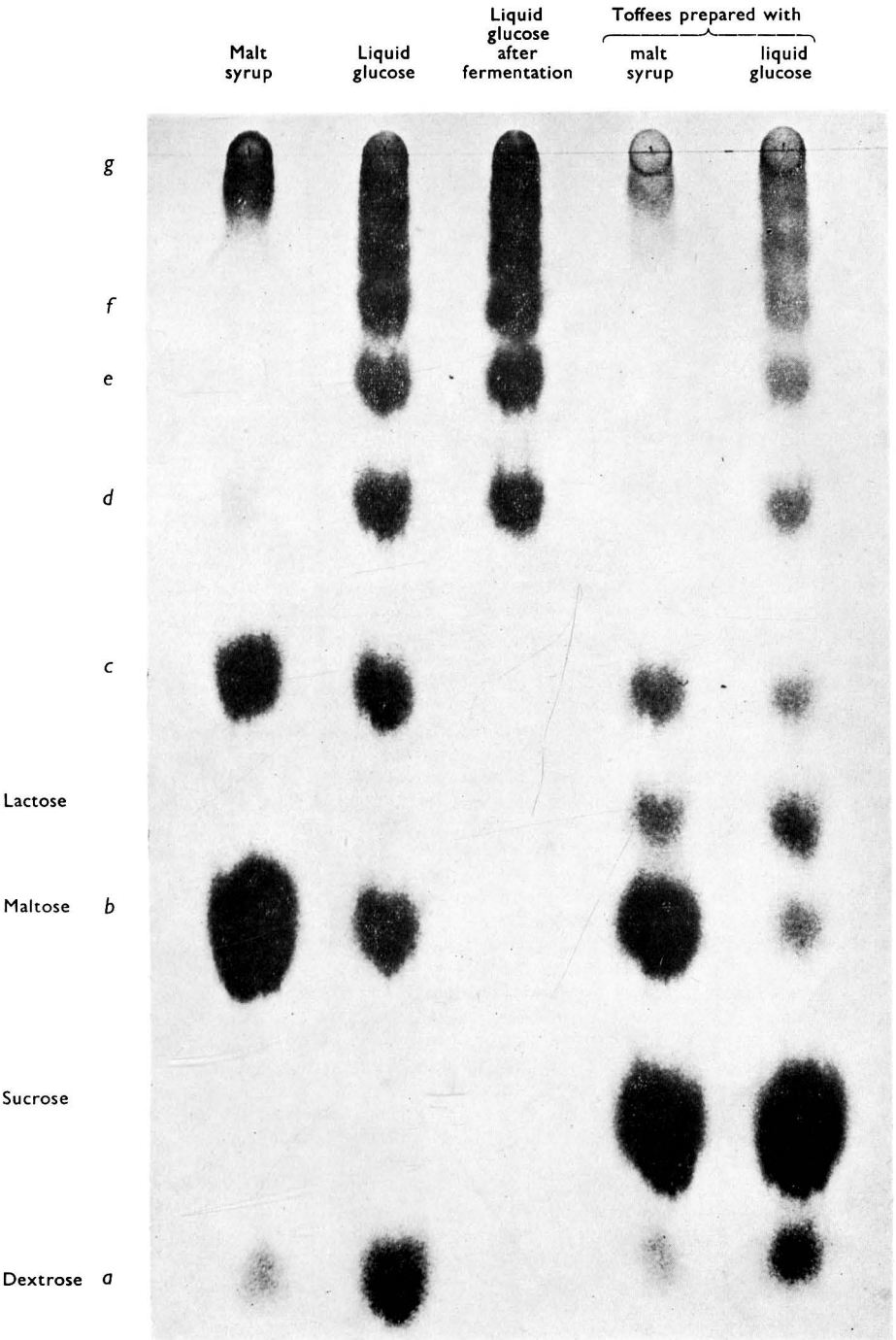

Fig. 1. Chromatograms of starch conversion products

COMPOSITION OF STARCH CONVERSION PRODUCTS—

Some interesting observations resulted when attempts were made to verify by chromatography the usually accepted figures for the maltose content of liquid glucose. All of the half dozen samples of British-made liquid glucose that have been examined have been found to have maltose contents of some 10 to 15 per cent., whereas by the traditional methods the same samples gave results of 25 to 30 per cent. (Table II). Figures for the maltose content of American corn syrup have recently been reported by McDonald and Perry[1] that are in close agreement with our chromatographic findings on liquid glucose of similar reducing power.

TABLE II

MALTOSE CONTENT OF LIQUID GLUCOSE

Anhydrous maltose, %		
By fermentation	By hypo-iodite	By chromatography
22·7	29·6	13 ± 2
20·3	25·6	9·5 ± 2
24·0	30·6	14 ± 2

The figures for maltose by fermentation in Table II were arrived at by determining the polarisation and the reducing sugars on each sample before and after complete fermentation with bakers' yeast. The differences between these two pairs of results were regarded as due to dextrose plus maltose, and the proportion of each was worked out from their known polarisations and reducing powers. In the hypo-iodite experiments, the total aldoses were estimated by the iodine absorbed in Kline and Acree's procedure.[2] The difference between this and the Barfoed result was calculated as maltose.

It has always been recognised that the methods of estimating maltose in starch conversion products, such as those outlined above, involve arbitrary assumptions, but it is nevertheless surprising to find how widely the results diverge from the truth. To shed some further light on these divergences the following experiments were made.

A 10 per cent. solution of liquid glucose was prepared and a portion of it, after being sterilised by boiling, was inoculated with bakers' yeast and incubated at 35° C for 14 days. At the end of this time it was clarified by the addition of dialysed iron, and chromatographed. A portion of the original solution was chromatographed simultaneously for comparison. The two chromatograms, which are reproduced in Fig. 1, show that not only have dextrose and maltose been removed from the solution by the process of fermentation, but so also has the sugar that causes the spot next above the maltose spot. This sugar is probably maltotriose (*cf.* Sugihara and Wolfrom[3]). The existence of this third fermentable sugar accounts for the inaccuracy of the figures for maltose content obtained by fermentation.

A further portion of the original solution was submitted to the Kline and Acree[2] hypo-iodite oxidation treatment, and was afterwards concentrated to its original bulk and chromatographed. No sugar spots at all were obtained. It appears, therefore, that all of the substances that give coloured spots with the spraying reagent are oxidised by iodine under these conditions. This accounts for the still greater inaccuracy of the figures for maltose content obtained by hypo-iodite, since they are based on the assumption that dextrose and maltose are the only iodine-absorbing substances present in the sample.

From a third portion of the liquid glucose solution, "dextrin" was precipitated by the addition of a large excess of alcohol (industrial methylated spirits). The precipitate was washed with alcohol, taken up in water and chromatographed. One spot only appeared, that being at the point of application of the sample. Hence, only substance (*g*) of those distinguished in Fig. 1 is precipitated by alcohol, and methods for the determination of maltose based on the weighing of the precipitated "dextrin" or on the determination of the polarisation or reducing power of the solution remaining after precipitation of "dextrin" will be liable to much the same errors as is the hypo-iodite method. The fact, therefore, that these two independent methods furnish similar results is not surprising, and casts no doubt upon the validity of the chromatographic findings.

Appendix

Apparatus and method for paper chromatography of starch conversion products—

We have thought it desirable to describe in some detail the apparatus and procedure that we have used, especially as we do not know whether full working details are anywhere readily available in a collected form. For the most part we have followed the recommendations of Partridge[4] as modified by de Whalley, Albon and Gross,[5] and no claim to originality is made for our procedure. We acknowledge our indebtedness to Mr. Albon and Dr. Gross of Tate and Lyle Ltd., who very kindly demonstrated their technique to us, and suggested the use of mixtures of *n*-propanol, ethyl acetate and water as eluting liquid.

Table III

Conditions for chromatography of starch conversion products

Paper used	Whatman No. 4
Solvent	*n*-Propanol, 60 parts by volume Ethyl acetate, 10 parts by volume Water, 30 parts by volume
Time in cabinet	16 to 18 hours
Spraying reagent	A 4 per cent. solution of aniline in alcohol, 5 volumes A 4 per cent. solution of diphenylamine in alcohol (prepared fresh each day), 5 volumes Syrupy phosphoric acid, 1 volume
Heating	10 minutes at 80° C

Our papers are cut to a total length of 42 cm, with a starting line ruled 11 cm from the top edge. The sample solutions are applied to the paper by means of a capillary pipette (Fig. 2) made by drawing out tubing of 2-mm bore and 1-mm wall thickness. The bulb is about 7 mm long and contains approximately 5 μl. The volume delivered by the pipette is constant, provided that grease is totally excluded. For this reason the pipette is kept in chromic acid when not in use. It is filled by capillarity or by closing the rubber tube with the finger and simultaneously squeezing it. After the exterior has been wiped dry, the meniscus is adjusted to the mark by touching the jet against the edge of a piece of filter-paper. The chromatogram is supported on either side of the point where the sample is to be applied, the jet is applied to the surface and the measured volume allowed to run out. Ten per cent. solutions of liquid glucose or of malt syrup are convenient for chromatography. If quantitative estimates of the individual sugars are required, suitable standard solutions are prepared and spotted on the chromatogram at the same time as the solutions of the samples. The spots should be spaced about $2\frac{1}{2}$ cm apart along the starting line.

As chromatographic cabinet, we use a glass accumulator vessel 9 × 7 inches × 16 inches high with a glass plate ground on to it to form an airtight lid. For our particular purpose, grafting such vessels together in pairs as was done by de Whalley, Albon and Gross[5] is not necessary. The troughs are made by sealing both ends of a suitable length of $1\frac{1}{4}$-inch Pyrex tubing and then either grinding away the whole of one side on a carborundum wheel or cutting a slit parallel to the axis of the tube with a small carborundum cutter. Either process is tedious and many fractures occur. Each trough is borne on a pair of glass slats, supported in turn on a scaffold constructed of glass tubing. In the open-type troughs the papers are held in position by wrapping the top end round a glass slat 3 cm wide. With the slit type no such support is necessary. In either instance the papers are folded so that the starting line is outside the trough and about 1 inch below the top of it. The trough is as close as possible to the top of the cabinet, and the length of the paper is such that it hangs just clear of the pool of solvent in the bottom of the cabinet. The paper is cut to a slight point at the bottom to encourage the descending solvent to drip off.

When the eluting process has continued for the proper length of time, the paper is removed and hung to dry in a special chimney, 8 × 5 inches (internal dimensions) × 28 inches high, constructed of hardboard on a wood framework. The walls are double, some $\frac{1}{2}$-inch apart, the inner terminating half an inch below the outer at the top. The walls on one of the 8-inch sides are glazed with old photographic plates. A hot-air blast is injected at the bottom of the chimney and passes up its centre and down the cavity between the walls, escaping at the bottom through holes in the outer walls into the atmosphere or the fume chamber.

This arrangement produces an enclosure in which the temperature is uniform to within two or three degrees. The hot-air blast is produced by a fan-type electric blower of similar type and size to that usually incorporated in a domestic hair dryer, blowing into an asbestos box, $6\frac{1}{2}$ inches cube, containing a 600-watt heating element. The blast passes out of the box into the chimney through twenty-five $\frac{3}{8}$-inch holes bored in one side of the box. The temperature can be varied by altering the setting of a rheostat in the blower circuit.

When the paper is thoroughly dry it is sprayed quickly and evenly, first on one side and then on the other. The sprayer, Fig. 3, works well with compressed air at a pressure of about 10 cm of mercury, producing a fine mist. An air reservoir is placed between the sprayer and the compressor to absorb pulsations from the latter. A foot bellows has been used with

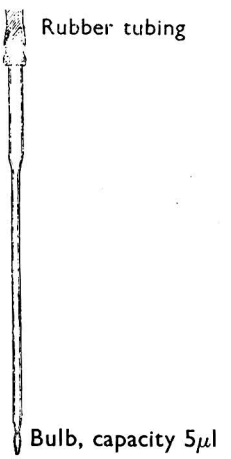

Fig. 2. Capillary pipette

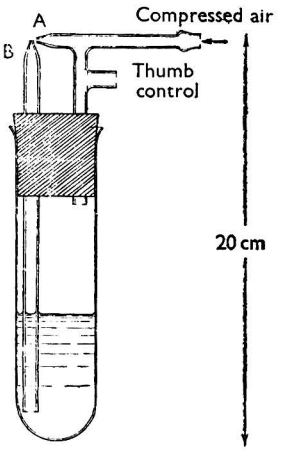

Fig. 3. Sprayer. A, air jet about 1 mm in diameter; B, liquid jet of diameter 0.1 mm or less

success, but a mechanical compressor is preferable. The paper is sprayed until it is thoroughly damp all over, but it is not allowed to become wet. The chromatogram is then returned to the chimney for the final heating to develop the coloured spots. After the prescribed time the intensities of the spots are compared by transmitted light. The comparison must be made within a reasonable time, as the background darkens on exposure.

The mixed aniline - diphenylamine phosphate reagent is superior to any other that we have tried for the location and estimation of aldoses and ketoses on the same chromatogram. It produces blue, violet-blue or green-blue spots with aldoses and brown spots with ketoses. It is about equally sensitive to dextrose and to laevulose, 5 μl of a 0·2 per cent. solution being detectable.

On an ideal chromatogram a number of spots of the same sugar should appear in a straight horizontal line. We have found, however, that these lines are sometimes inclined to the horizontal, and may also be markedly curved. Consden, Gordon and Martin[6] refer to "the irregularity of the band across the width of a wide paper . . . due to the inhomogeneity of the paper" and report a variation of the distance travelled by a particular species of ± 2 per cent. on Whatman No. 1 paper. On Whatman No. 4 paper, which we have mostly used, we have sometimes found much greater variation than this; for example, a row of dextrose spots $2\frac{1}{4}$ cm apart travelled 11·3, 11·9, 12·3, 11·8 and 11·8 cm. The maximum variation here is over 8 per cent. This irregularity is often greater on one side of a sheet of paper than on the other, and it varies from sheet to sheet and still more from packet to packet of paper. For many purposes the effect is unimportant, but it renders uncertain the identification of unknown sugars by means of the chromatogram. It is worth while, therefore, to make several preliminary tests on each batch of paper and to reserve for the most important work those batches whose behaviour is uniform. As a result of limited experience we believe that Whatman No. 2 paper is virtually free from this irregularity and can with advantage

replace the No. 4 paper. The disadvantage is that the spots travel much more slowly on the Whatman No. 2 paper, and the running-time has to be increased to 48 hours.

We are grateful to Mr. A. H. Rheinlander, M.Sc., for his interest and advice, and to the Government Chemist, Dr. G. M. Bennett, C.B., F.R.S., for permission to publish this paper.

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April, 1952

A Uniform-Temperature Drying Oven for Quantitative Chromatography

BY N. ALBON AND D. GROSS

A drying oven that will maintain a highly uniform temperature for paper chromatograms is described. The temperature variations within the working space are not more than $\pm 1°$ C and only $\pm 0{\cdot}5°$ C across the width. This ensures uniform heating of standards and samples in quantitative chromatographic work.

IN our recommended procedure for the chromatographic determination of raffinose,[1,2] it is essential that standards and sample be treated under strictly identical conditions until the time comes to compare the spots visually and to estimate them. This can be ensured by making the separation of standards and sample on the same sheet of paper and under the same conditions. Spraying must be done evenly, but this can be effected without difficulty. Nevertheless, in drying the sprayed chromatograms, variations of temperature may cause some spots to be heated to a higher temperature than is intended and consequently may cause a colour of greater intensity to develop. This in turn will necessarily lead to errors in the estimation and will impair the accuracy of the method. Measurements of the temperature in various places inside a conventional type of chromatographic oven show that quite considerable variations in temperature do occur. The exact effect of these variations on the development of the spots can be determined only by ensuring stable heating conditions and by comparing the results. If conditions can be created with nearly all differences in temperature between standards and sample obviated, effects of temperature and heating time can be studied and the optimum conditions for development laid down. With this in mind, an oven was designed and constructed to give not so much a constancy, as a high uniformity, of temperature; as long as all the spots on the same sheet are subjected to the same temperature, the same rate of development of colour can be expected and constancy of temperature is not essential. If the temperature can be kept steady within a few degrees during the drying process and the uniformity inside the drying space can be maintained to 1 or 2 degrees, then no appreciable differences in the development of colour will arise and the correct comparison of sample with standards is ensured.

To design a drying oven for chromatograms is not easy, because the evaporated organic solvent has to be exhausted into a fume cupboard. Air has continually to be drawn in, heated to the desired temperature, made to flow round the suspended moist chromatogram and exhausted at a fairly rapid rate. Temperature control, even to a reasonably fine degree, is difficult with air as heating medium. Even with uniform air velocity and temperature across the air-stream and with a thermometric device located at a point representative of the temperature to be measured, there is still the relatively slow response of each known

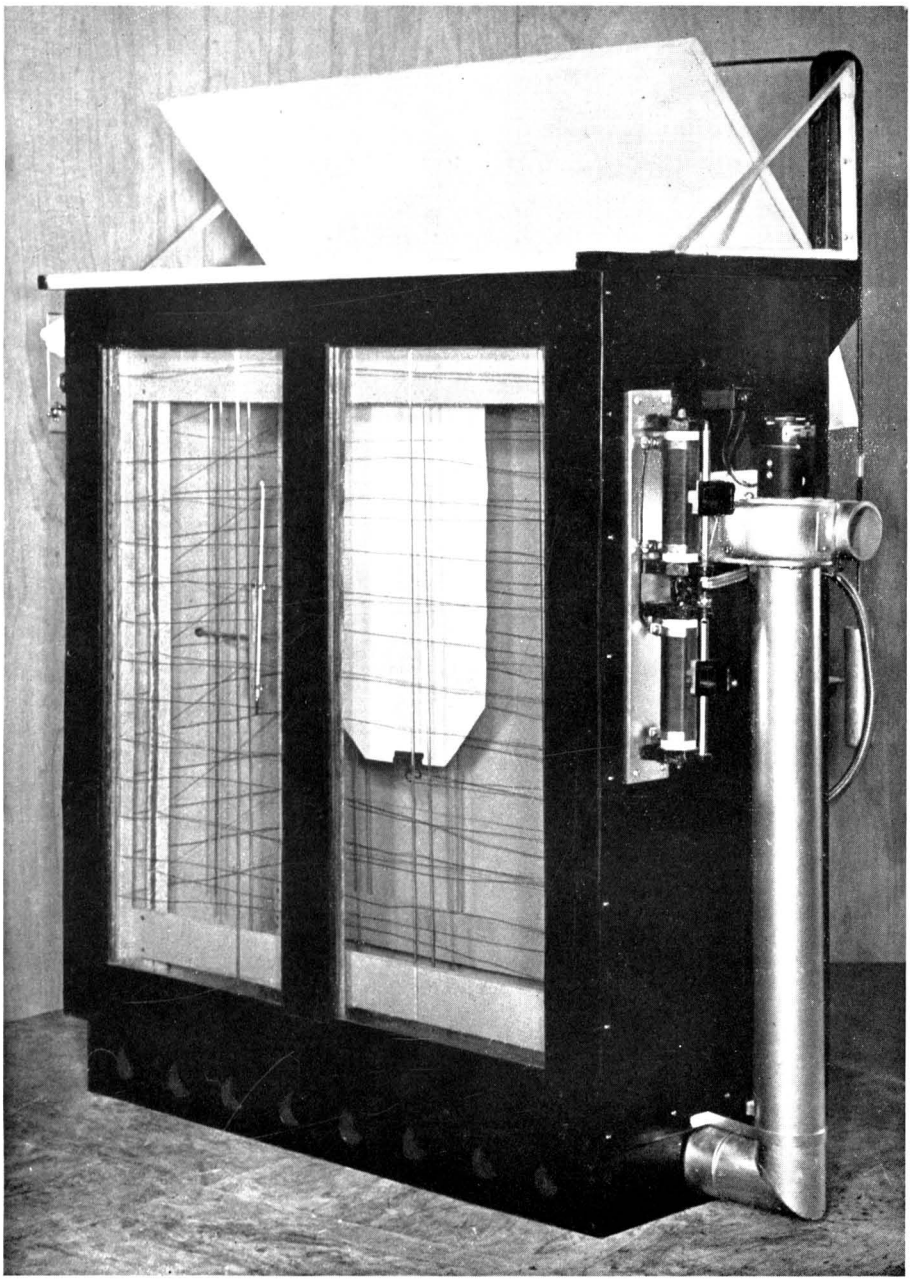

Fig. 1. Drying oven for full-size chromatograms

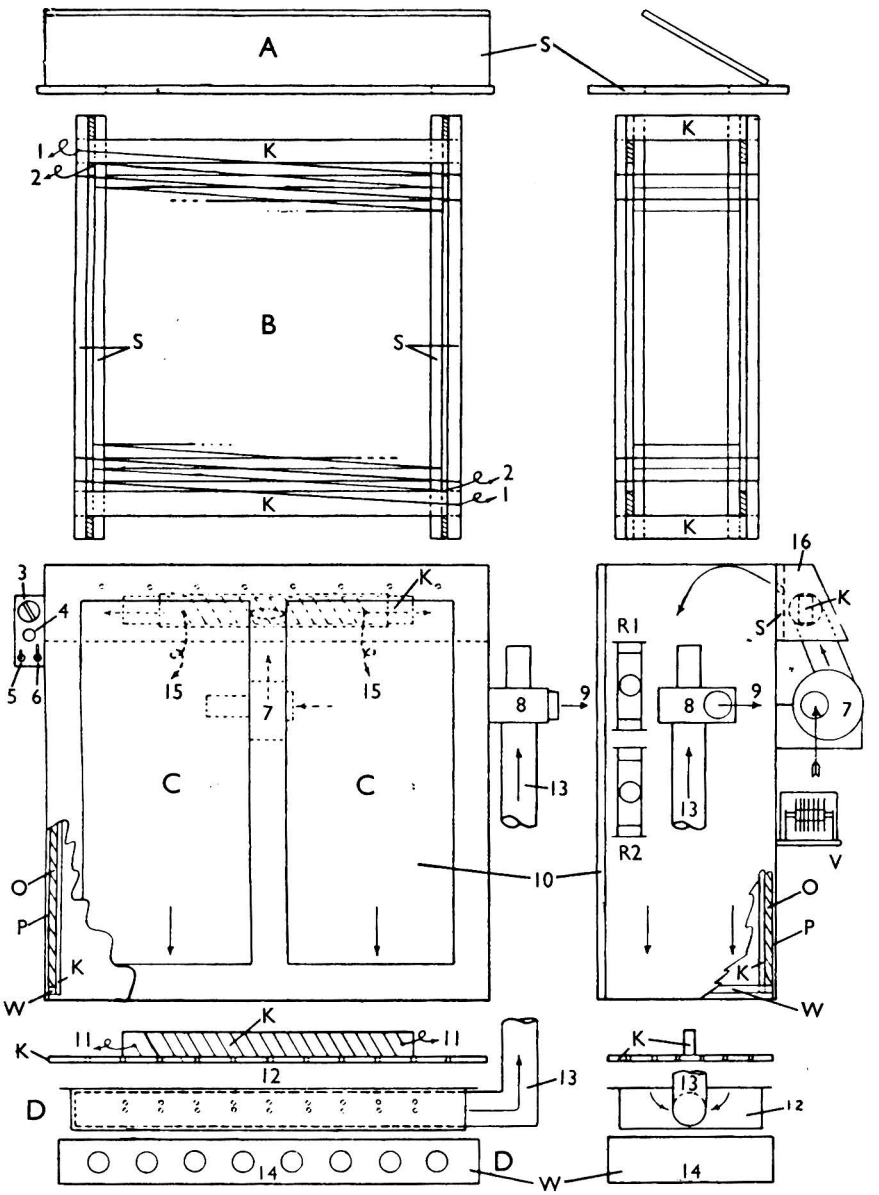

Fig. 2. Detail of chromatogram oven. A, top cover and lid; B, frame heaters; C, main structural assembly; D, base fittings

1	Outer Heater Wire	5	Switch for No. 1	9	To Fume Cupboard	13	Exhaust Pipe
2	Inner Heater Wire	6	Switch for No. 11	10	Double Glass Window	14	Oven Support
3	Simmerstat Control	7	Air Input Fan	11	Booster Heater Wire	15	Preheater Wire
4	Neon Lamp	8	Exhaust Fan	12	Metal Tray	16	Metal Cover

K	"Kimoloboard"	R2	Input Fan Rheostat
O	"Onozote" Insulating Board	S	Grey "Sindanyo" Board
P	Plywood	V	24v. D.C. Supply for Fans
R1	Exhaust Fan Rheostat	W	Wood

thermo-sensitive element in air to contend with. This thermometric time lag is more marked at temperatures between 70° and 100° C, *i.e.*, in the range for which the oven is designed. The response and time lag depend greatly upon the heat conductivity of the medium surrounding the thermometric device and upon heat transfer from the medium to the sensitive element. Naturally, the response depends very much upon the permissible temperature fluctuations, and the aim was to keep fluctuations within 2° C. Amongst the several temperature-sensitive elements tried and tested with regard to rate of response were mercury - toluene regulators of suitable bulb size, electrical-contact thermometers, bi-metallic thermometers and thermocouples. Even under the best conditions the response was too slow and fluctuations were too great.*

Since, with this type of chromatogram oven, elaborate means of measuring and controlling temperatures are hard to justify, it was finally decided to control the input of heat by a Sunvic Simmerstat device; although this is not automatic and needs occasional adjustment, it proved capable of keeping the temperature steady within a few degrees for several hours and certainly ensured constancy of temperature for shorter periods. The Simmerstat control switches the current on and off at certain times, which can be pre-selected on a dial. Once the oven has attained the requisite temperature the operator, after a little practice, can balance the heat input against the heat loss by adjusting the Simmerstat control. The temperature drops by 1° to 2° C when the lid is opened, but it can be quickly restored to its previous level.

As automatic control was found to be impracticable, three points that appeared important as a means of improving temperature uniformity were closely watched in designing the oven: efficient mixing of the air, uniform heating inside the oven and good thermal insulation to reduce heat leakage. Fig. 1 shows one of the ovens that has been in use satisfactorily in our laboratory for nearly a year without any deterioration in its performance. The rather large size was originally chosen as being suitable for dealing with several sheets of paper 24 inches square. These sheets have to be so placed that they are dried in that space of the oven which shows good uniformity of temperature, that is, at least 1·5 inches from heating wires or from the top or bottom of the oven. These dimensions can be scaled down if the oven is designed for smaller or narrower sheets.

Fig. 2 shows a detailed drawing of the oven and Fig. 3 the electrical circuit used.

As can be seen in Fig. 2, vertical down-flow air circulation is used, whereby the cold air is drawn in by a fan (7), flows over a pre-heater (15), enters the oven near the top and is forced to descend to the bottom. A second fan (8) assists this movement and finally exhausts the air. The temperature of the pre-heated air is kept at about 5° to 10° C below the oven temperature and this apparently promotes efficient circulation and eliminates high temperature gradients. The heating arrangement inside the oven consists of two heating elements wound on a support to a height and width approximating to the dimensions of the inner oven space. The heating wire wound on the outside of the frame (1) is switched on permanently at working temperatures of 70° to 100° C, but is not of a sufficient wattage to maintain these temperatures. The heating wire wound on the inside of the frame (2) is connected to the Simmerstat control (3), which switches the current on and off automatically as required. This additional input of heat maintains the working temperature at the desired level. The air passing through is uniformly heated until it leaves the oven through holes in the bottom. There is a 1-kilowatt booster heater (11) to help bring the oven quickly to working temperature. This appreciably cuts down the time required for preparing the oven for drying operations. This booster heater is switched off as soon as the temperature has reached the desired level, after which the temperature is sustained by the outer and inner heaters. It takes less than 30 minutes to raise the temperature of a cold oven to 90° C.

The two fans used for regulating the air flow can be adjusted by two rheostats, R1 and R2, to give the optimum flow-rate of air for any particular temperature. Once the suitable flow-rate has been found the fans need scarcely any further adjustment for quite considerable periods of time, given the same heating conditions for the same temperature level. The fans

* Since this was submitted, experiments with a more sensitive bi-metallic thermostatic device, the Sunvic Thermostat TS.3, 22 inches stem (without pocket), together with a Hotwire Vacuum Switch, Type F 102/3, both supplied by Sunvic Controls Ltd., have led to more satisfactory results. After some minor adjustments of the heating elements, thermostatic control was made possible, although the uniformity of temperature was not quite as good as that achieved by Simmerstat control.

are Government surplus material and run off 24-volt D.C., which necessitates a transformer and rectifier assembly. These fans are rated at 15 watts.

The insulation of the oven was given special attention, as elimination of heat leakages increases the uniformity of the temperature in the oven. For three walls, a sandwich-type of insulation was chosen. The inner layer is Onozote, faced by Kimoloboard on one side and plywood on the other side. Onozote is an expanded rubber-type insulating material

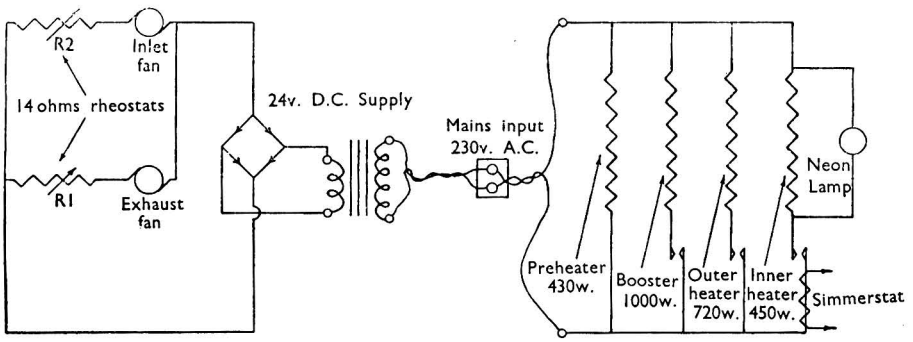

Fig. 3. Circuit diagram of chromatogram oven

and is protected from the heat of the oven by the Kimoloboard, which is an asbestos-type insulating material. The bottom is of Kimoloboard and wood; the top cover and lid, which had to be of a heavier material for structural reasons, are of grey Sindanyo, which is also an asbestos-type board. All these materials are commercially available in this country. A double glass window at the front permits easy inspection and ensures fairly good insulation. Temperature gradients can be smoothed out and a more favourable air circulation can be attained by plugging or opening holes provided at the top and bottom when a new oven is taken into use.

By measuring the temperatures within the proper working space at many points simultaneously, we found that the temperature variations could be kept easily within 2° C whilst the variations across the width are usually within 1° C.

We should like to express our appreciation of the valuable assistance rendered by W. Underwood in designing and constructing the oven and we wish to thank the Directors of Tate and Lyle Limited for permission to publish this paper.

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March, 1952

The Chromatographic Determination of Raffinose in Raw Sugars

Improvements in Technique

By N. ALBON and D. GROSS

An improved chromatographic procedure for determining raffinose in raw sugars is described. A solvent mixture that gives better separation of raffinose and sucrose and also of mixtures of other sugars is proposed. Experimental R_F values for various sugars are given. Preparation of standards is simplified, uniform heat treatment of paper chromatograms in a drying cabinet capable of maintaining a highly uniform temperature is ensured and the stability of the intensity of the spots is improved.

Since the publication of our procedure for determining raffinose in raw sugars[1,2] efforts have been made to improve the method in four respects: to find a suitable solvent mixture that would not contain pyridine, which is undesirable; to find an alternative for raw cane sugar - raffinose standards; to ensure more uniform heat treatment, which would result in more uniform development of colour spots; and to find a means of counteracting, or at least of retarding the fading of the spots produced on spraying the chromatograms with α-naphthol. Success in all these endeavours has made the method more suitable for quantitative routine work and has increased its accuracy.

Solvent—

Apart from its price and its unpleasant smell, pyridine has the objectionable feature of being difficult to remove from the paper, and residual traces of it inhibit the reaction with α-naphthol and necessitate heating the chromatogram at 90° C for 1 hour before spraying. A mixture of solvents without these disadvantages, but with similar separating power will reduce the time of heating considerably. Many solvents and solvent mixtures were tested on a small scale, with strips measuring 14 cm × 5 cm, to determine their capacity for separating sucrose and raffinose mixtures. As a result, the following solvent mixture was found to be the most suitable from several points of view.

Recommended solvent—*n*-propanol, ethyl acetate and water in the ratio of 7:1:2 by volume.

Compared with the mixture previously used, this solvent is cheaper, has no unpleasant smell and can be removed from the chromatogram by heating for only a few minutes; it also has a somewhat higher separating power and produces sharper spots. Furthermore, it is possible to separate larger quantities of sugar mixtures than before. The single-phase composition of the mixture makes it less susceptible to temperature fluctuations. The same solvent is used for both the trough and the bottom of the chromatographic tank. The use of *n*-propanol, mainly in mixtures with water, has been described by several authors for separating amino-acids and Hirst and Jones[3] have mentioned *iso*propanol - water mixtures for separating some carbohydrates. Tests with *n*-propanol - water mixtures did not give satisfactory results, but the admixture of ethyl acetate or *n*-butanol modified the R_F values of the tested sugars sufficiently to produce good separations. The final composition of the solvent mixture has been adjusted for maximum resolution of sucrose and raffinose, and results so far prove it to be satisfactory also for mixtures of other sugars. For instance, excellent separations of the glucose and maltose in starch hydrolysis products have been effected with it. The solvent travels fairly quickly down the paper and by varying the water content of the mixture the R_F values of the individual sugars can be considerably altered and so made suitable for any given separation whilst still retaining the sharpness of the spots. Table I shows the R_F values of several sugars with this solvent mixture as measured on Whatman No. 1 (chromatographic grade) paper at 13° C. The R_F values at 20° C are higher. The distance of travel of each sugar spot was taken as the distance from the starting line to the centre of the spot.

STANDARDS—

The choice of standards consisting of raw cane sugar solutions admixed with raffinose pentahydrate was necessitated by the comparatively high ash content of raw beet sugar samples. Owing to the ash present in raw beet sugar, raffinose is compressed into a small spot and, to make the comparison of spots as precise as possible, standards have to be used that produce a similar effect. The large proportion of sucrose present affects the spots in a similar way, but to a much smaller extent. If the raw sugar samples are de-ionised by passing them through mixed beds of ion exchange resins, standards of pure sucrose and raffinose

TABLE I

R_F VALUES AT 13° C WITH THE RECOMMENDED SOLVENT

Solvent front moved 38·2 cm in a 16-hour run

Ketose sugars (α-naphthol reagent)			Aldose sugars (amine reagent)		
Sugar	Distance moved, cm	R_F value	Sugar	Distance moved, cm	R_F value
d-Allulose	11·4	0·30	Arabinose	10·6	0·28
Diheterolevulosan I*	4·4	0·12	Cellobiose	3·1	0·081
Diheterolevulosan II*	7·3	0·19	Dextrose	8·5	0·22
Gentianose	5·6	0·15	Galactose	7·5	0·20
Kestose†	2·2	0·058	Gentiobiose	2·3	0·060
Levulose	10·5	0·27	Lactose	2·3	0·060
Melezitose	2·2	0·058	Maltose	3·1	0·081
Raffinose	1·3	0·034	Mannose	11·1	0·29
Sorbose	10·8	0·29	Melibiose	2·1	0·055
Stachyose‡	0·4	0·015	Rhamnose	17·5	0·46
Sucrose	5·2	0·14	Ribose	14·5	0·37
			Xylose	12·2	0·32

* Sample received from National Bureau of Standards, Washington, D.C.

† Kestose is a trisaccharide synthesised during invertase inversion of sucrose, and consists of two fructose and of one glucose units. See *International Sugar Journal*, 1951, **53**, 247; 1952, **54**, 127.

‡ Sample received from East Malling Research Station; 1952, **54**, 127.

can be used. Much improvement was given by alternative standards composed of sucrose, raffinose and potassium chloride at suitable concentrations. This eliminates the need for raw cane sugar, which may not be available in some laboratories; the procedure is also more flexible. The standards are prepared with a salt concentration of the same order as that of the raw sugars under test, where the ash content is known; otherwise, 0·5 per cent. of potassium chloride on sucrose can generally be used satisfactorily. Standards of this composition have been used in the analysis of a large number of raw beet sugar samples, with good results. Vavruch uses potassium lactate in a similar procedure.[4]

HEAT TREATMENT—

The method of visually comparing raffinose spots from the test solution with those from the standards demands that samples and standards should, for accurate quantitative work, be treated identically during the whole procedure. As the actual separations on the same chromatogram are made under identical conditions, the only operations that need careful attention are spraying and drying. It is very important to spray the sheet evenly by making use of a suitable spraying bottle, preferably one that is operated by compressed air; this can then easily be accomplished. For identical development of the colour of a series of spots it is essential that the temperature in the drying cabinet should be fairly constant to ensure that all spots are heated to the same temperature for the same length of time. An investigation was also made to determine the effect of temperature fluctuation on the accuracy of quantitative work. A drying cabinet was designed and constructed to give maximum uniformity of temperature in the heating space and, especially, across its width. As samples and standards are compared along one horizontal line across the sheet, it follows that differences of temperature across the width of the cabinet will have a much greater effect than any that might exist between the vertical extremes. The design proved successful and a high degree of temperature uniformity was achieved.[5] The temperature anywhere in the space provided

for heating the chromatograms never varied by more than 2° C, and was usually steadier. The difference across the width was about 1° C, often less. Hence it was possible to check the effect of temperature variations on the development of the coloured spots and to find the optimum temperature and optimum heating time.

The results indicated that a variation of 5° C did not cause significant errors and that a temperature of 65° to 75° C and a heating time of 10 to 15 minutes were advisable. The chromatogram needs to be watched through the window and removed before the background shows coloration. Higher temperatures make the colour appear too rapidly and may cause the background to become unduly coloured, although the heating time will then be shorter. The new drying cabinet ensures uniform heat treatment during the development of the coloured spots, so every possible discrepancy between the colour intensities of samples and standards is eliminated.

COLOUR STABILITY—

Amongst the many reagents tested for raffinose, α-naphthol with phosphoric acid was the most sensitive for paper chromatograms. The only disadvantage with it was in the rapid fading of the developed colour after removing the chromatogram from the drying cabinet. On exposure to the atmosphere the paper absorbed moisture and fading occurred. The different rates of fading observed are probably due to variations in humidity. If the chromatogram is placed in a desiccator (silica gel) immediately after its removal from the drying cabinet, the colour remains unaltered for at least 2 weeks. Further, if the chromatogram is placed between two glass sheets, the spots can be preserved for several hours. A simple viewing box affording even illumination based on this principle has been constructed and found useful.

We should like to thank Mr. H. C. S. de Whalley, Director of Research, for valuable advice and interest in this work and the Directors of Tate and Lyle Limited, for permission to publish this paper.

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March, 1952

The Determination of Water in Nylon

By J. HASLAM and M. CLASPER

A method is described for the determination of water in nylon. It is based on heating the nylon *in vacuo* at 260° C for a definite period of time, collecting in a cold trap the water evolved and subsequently determining it with the Karl Fischer reagent.

The amount of residual water in nylon is an important factor in the plant control of the finished product and, although there are very few references in the literature to the determination of water in nylon, various methods have been used in practice. These methods

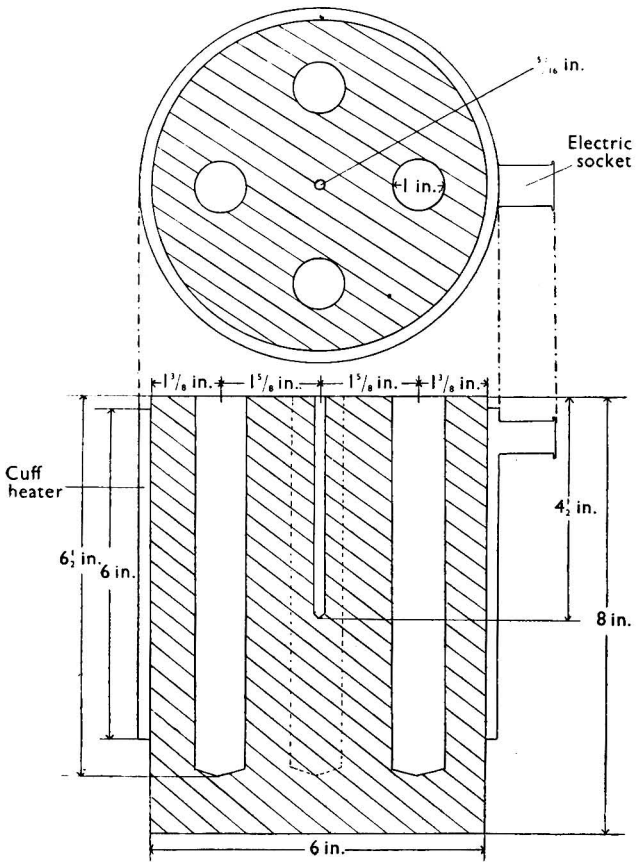

Fig. 1. Aluminium block fitted with cuff heater and insulated on sides and bottom. Heater-band diameter 6½ inches, width 6 inches, 1000 watts

have not been found wholly satisfactory. In general they depend on estimating the loss sustained by nylon under various heat treatments by weighing the nylon before and after heating. This loss need not necessarily be due to the removal of water.

A satisfactory method was required for determining the true water content of nylon; indeed, the possibility was pointed out to us of working out such a method based on heating the sample *in vacuo* at 260° C for a specified period of time, collecting the water given off and subsequently determining this by means of the Karl Fischer reagent. The purpose of this paper is to describe in full detail a working method for this determination. The method

described is carried out quite readily, a test result being available within 1 hour. The blank on the apparatus and reagents is only of the order of 0·02 per cent., when calculated as water content of the original nylon. It is probable that the range covered by the test is adequate for all samples, for it has been shown that recoveries of water have been satisfactory between 0 to 1 per cent. Full details of the test with all the manipulative operations and apparatus are given below.

METHOD

APPARATUS—

The heating unit consists of an aluminium block (8 inches high and 6 inches in diameter) with a cuff heater (6 inches wide and of 6½ inches diameter) of 1000 watts capacity (see Fig. 1). There is a central thermometer hole in the block and four equally spaced holes, the dimensions and positions of which are shown in Fig. 1. For insulation the block is placed in a cylindrical asbestos box, and the temperature of the block is maintained at 260° C by means of a Variac transformer. The cooling-bath consists of a Dewar flask of 1 litre capacity filled with methanol

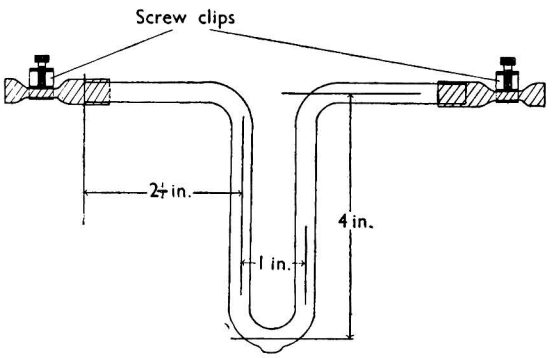

Fig. 2. Collecting tube

that is cooled to −80° C by solid carbon dioxide. The traps are made from 8-mm tubing to the dimensions indicated in Fig. 2. The vacuum is maintained by a rotary vacuum pump and the pressure is measured by means of a mercury manometer. The samples of nylon are heated in 1 × 8-inch test tubes.

The apparatus is fitted up as shown in Fig. 3 and the assembly of the apparatus, with four determinations running concurrently, is shown in Fig. 4.

The Fischer apparatus that has been used is based on that of Bonner,[1] except in that the burettes for the Fischer reagent and the alcohol - water solution have solenoid-controlled stopcocks. Stirring is accomplished by standing the titration vessel on a magnetic stirrer, and the end-point in the Fischer titration is obtained electrometrically by use of an applied potential of about 20 millivolts and a "magic eye" indicator. This form of Fischer apparatus, although not essential to the test, will form the subject of a subsequent independent communication by Messrs. Payne and Soppet of our Research Department.

REAGENTS—

Karl Fischer reagent—One millilitre of the reagent is equivalent to 0·0035 to 0·0040 g of water.

Alcohol - water solution—One millilitre of the solution is approximately equivalent by titration to 1 ml of the Karl Fischer reagent.

PROCEDURE—

Connect the empty test tube and the trap together (see Fig. 3) and, in order to ensure that they are completely dry, evacuate to a pressure of less than 4 mm of mercury for at least 5 minutes by connecting to the rotary pump, which, in order to maintain the vacuum, is allowed to run throughout the determination. Then place the trap in the Dewar cooling-flask to a depth of 3 inches and allow 5 minutes for it to attain a temperature of −80° C. Close the screw-clip on the test-tube side of the cooling-trap, remove the stopper from the test tube and place 20 g of nylon, previously weighed, in the tube. Re-insert the stopper in the test tube and re-open the screw-clip to connect with the vacuum in the rest of the apparatus, which is again

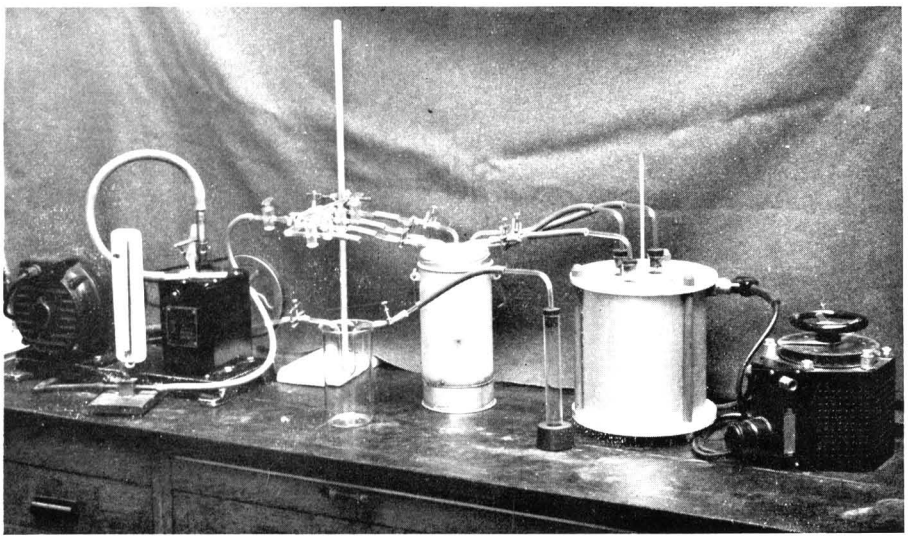

Fig. 4. Assembly of apparatus

evacuated to a pressure of less than 4 mm of mercury by the rotary vacuum pump. After 5 minutes place the test tube in the heating-block at a temperature of 260° C and continue to heat for 30 minutes. At the end of this time close the screw-clips on each side of the trap and disconnect the trap from the rest of the apparatus. On removing the trap from the cooling-bath, release the vacuum in the trap by opening one of the screw-clips; then close the screw-clip and allow the trap to attain room temperature. Remove the rubber tubing carrying the screw-clips from each end of the trap, which is carefully washed out three times with approximately 2 ml of dry alcohol. Collect the washings in a previously dried flask

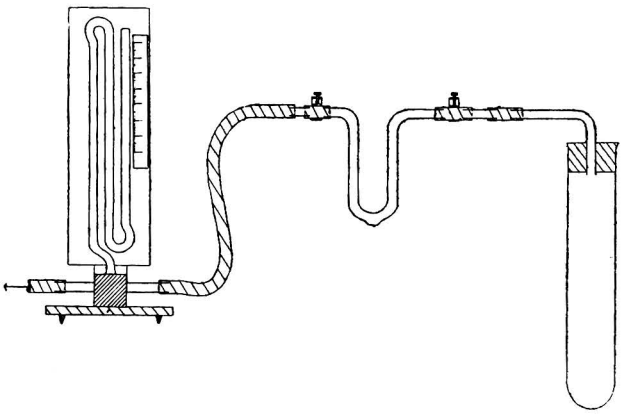

Fig. 3. Flow diagram

that fits into the closed system of the Fischer apparatus, add an excess of Fischer reagent and titrate the excess with the standard alcohol - water solution, using the dead-stop iodine end-point method.

The alcohol - water solution is previously standardised against the Fischer reagent so that 1 ml is approximately equal to 1 ml of the Fischer reagent. The water equivalent of the Fischer reagent is previously determined by titrating a known weight of hydrated sodium acetate ($CH_3.COONa.3H_2O$).

Make a blank determination alongside that of the sample and calculate the water content of the sample from the amount of Fischer reagent used in the test after allowing for the blank.

Results

Various amounts of water were placed in the test tube, and the recoveries on 20-g samples by applying the above procedure were as follows—

Water added, %	0·10,	0·16,	0·53,	0·81,	1·15
Water found, %	0·08,	0·18,	0·49,	0·74,	1·06

On applying the procedure to commercial samples of Nylon 66 the following results were obtained—

Water in sample 1, %	0·10,	0·11
Water in sample 2, %	0·14,	0·14

It should be realised that if amounts of water of the order of 1 per cent. are present, the Fischer titration obtained on application of the test to 20 g of sample will be of the order of 55 ml. In all such instances it is obviously desirable to carry out the test on smaller quantities of sample.

The proposed method has been applied chiefly to the determination of water in Nylon 66, but it has been found to be equally applicable to Nylon 610 and Nylon 6 (caprolactam). With both these nylons, however, the temperature of the aluminium block is kept at around 220° C.

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Horizontal Paper Chromatography

By P. MEREDITH and H. G. SAMMONS

A method of paper chromatography for one- or two-dimensional work is described. The paper is held horizontally on a glass frame 1 to 2 inches above a large liquid surface, so that the vapour phase is of approximately the same composition as the liquid phase. By means of this apparatus all the water-soluble constituents of nerve lipid hydrolysates have been separated. It is also possible to separate and identify the amino-acids in protein hydrolysates or artificial amino-acid mixtures on paper $10\frac{1}{2}$ inches square. The apparatus is compact, and control of temperature is easy. Two-dimensional chromatograms can be developed in a day.

The apparatus described in this paper was designed to separate the water-soluble constituents of nerve lipids after hydrolysis with methanolic hydrochloric acid.

In the first attempts to separate these substances by paper chromatography neither the ascending nor the descending technique was satisfactory on account of variable R_F values and the considerable "tailing" that took place. It was possible that these phenomena were due to lack of uniformity in composition between the vapour and liquid phases and in the vapour phase itself, a subject that has been stressed by Bentley and Whitehead.[1] To overcome these defects a large liquid surface is necessary, with but a small air space between the liquid and the paper throughout the whole of its length. With these conditions in mind, a method of horizontal chromatography has been devised, and the water-soluble fraction of a nerve lipid hydrolysate used to illustrate its application to biological problems.

Experimental

Apparatus—

The apparatus consists of a glass trough with a loose glass frame inside it to carry the paper. The top edges of the trough are ground flat and covered with a loose glass plate. The dimensions are arbitrary, $12 \times 12 \times 4$ inches being convenient external dimensions. The apparatus is shown assembled but without the glass cover in Fig. 1. The frames are shown diagrammatically in Figs. 2 and 3.

Fig. 2 shows the frame suitable for one-dimensional chromatography. This frame can carry up to four papers. It is constructed from Pyrex glass rod with protruding points to keep the paper strips separated. Fig. 3 shows the frame suitable for two-dimensional studies. The frames are designed so that each paper is held taut. The supporting rods do not interfere with the development of the chromatogram.

Solvent systems—

(1) For one-dimensional separations—
n-*Butanol*—4 parts by volume.
Ethylene glycol monoethyl ether—4 parts by volume.
Water—4 parts by volume.
Ethanol—1·5 parts by volume.
Ammonium hydroxide, sp.gr. 0·880—1·25 parts by volume.

(2) For two-dimensional separations (based on the method of Consden, Gordon and Martin[2])—

First solvent—Phenol saturated with water and stabilised with 5 per cent. v/v of ethanol. The alcohol makes the system less sensitive to temperature.

Second solvent—Lutidine - collidine (1 + 1), saturated with water and stabilised with 5 per cent. v/v of ethanol.

Spraying reagents—All the reagents except the 2 per cent. aqueous ammoniacal silver nitrate solution were prepared by the method of Chargaff, Levine and Green.[3]

Standards—Monoethanolamine, DL-serine, L(+)-glutamic acid, choline chloride, inositol, galactose and sphingosine.

Paper—Whatman No. 54, either 24×2 inches, $24 \times 4\frac{1}{2}$ inches or $10\frac{1}{2}$ inches square.

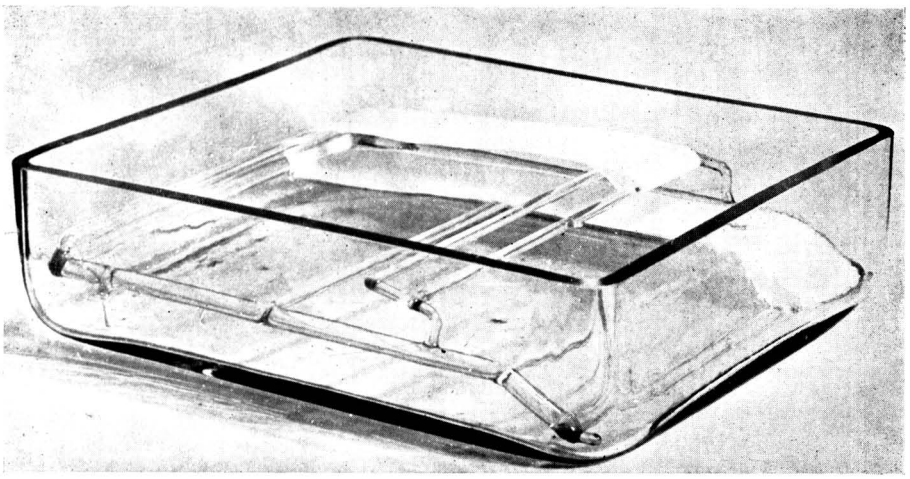

Fig. 1. Apparatus assembled for horizontal chromatography

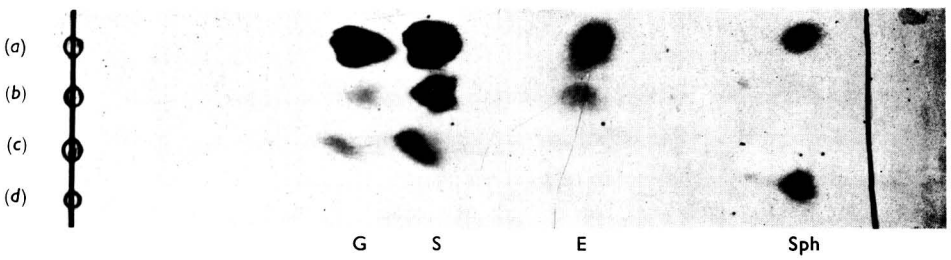

Fig. 4. Horizontal paper chromatogram. (*a*) Standard mixture; (*b*) nerve lipid hydrolysate; (*c*) sphingolipid hydrolysate after chloroform extraction; (*d*) chloroform extract of sphingolipid hydrolysate. G, glutamic acid; S, serine; E, ethanolamine; Sph, sphingosine

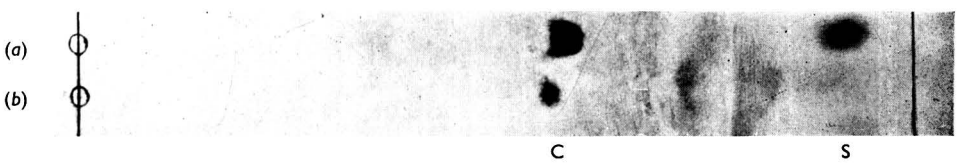

Fig. 5. Horizontal paper chromatogram. (*a*) Standard mixture; (*b*) nerve lipid hydrolysate. C, choline; S, sphingosine

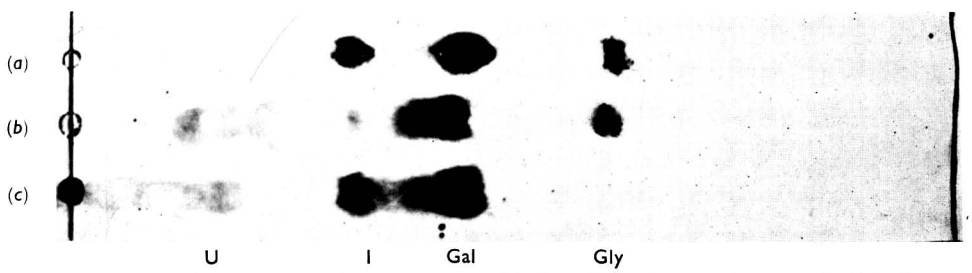

Fig. 6. Horizontal paper chromatogram. (*a*) Standard mixture; (*b*) nerve lipid hydrosate; (*c*) sphingolipid hydrolysate. U, unidentified; I, inositol; Gal, galactose; Gly, glycerol

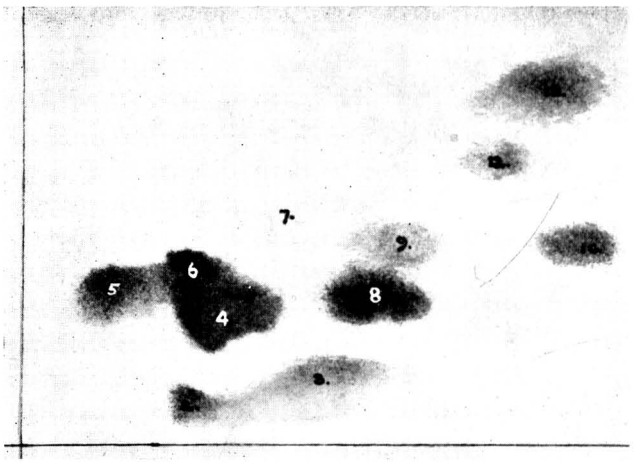

Fig. 7. Chromatogram of an artificial mixture of amino acids

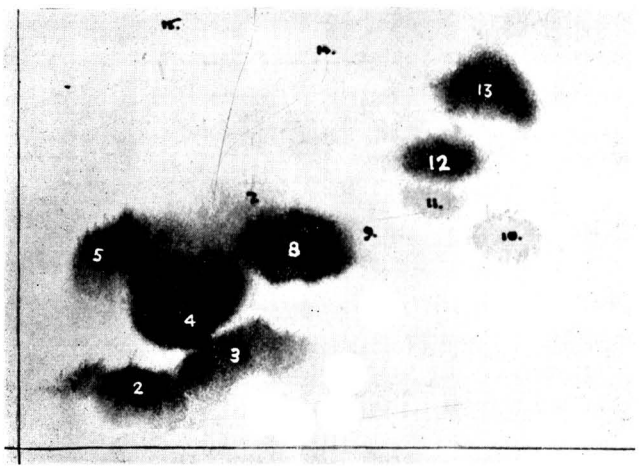

Fig. 8. Chromatogram of a gelatin hydrolysate

The probable interpretation of Figs. 7 and 8 is—1, sodium aspartate (?); 2, lysine; 3, arginine; 4, glycine; 5, aspartic; 6, serine and glutamic acid; 7, threonine; 8, alanine; 9, hydroxyproline; 10, proline; 11, histidine; 12, valine; 13, leucine and *iso*leucine; 14, tyrosine; 15, salt (?)

THE PREPARATION OF SUBSTANCES USED TO ILLUSTRATE THE METHOD—

The lipid was extracted under nitrogen with chloroform from dried bovine optic nerve tissue. It was hydrolysed for 3 hours with 6 *N* methanolic hydrochloric acid. After removal of the methanol and hydrochloric acid under reduced pressure, the residue was partitioned between ether and water. The aqueous phase was separated, dried by evaporation and re-dissolved in a small volume of *N* hydrochloric acid.

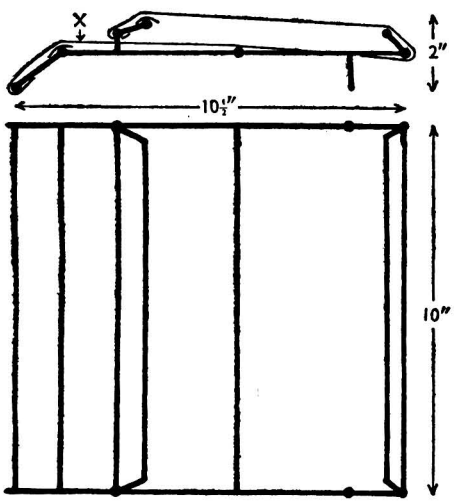

Fig. 2. Frame for one-dimensional chromatography

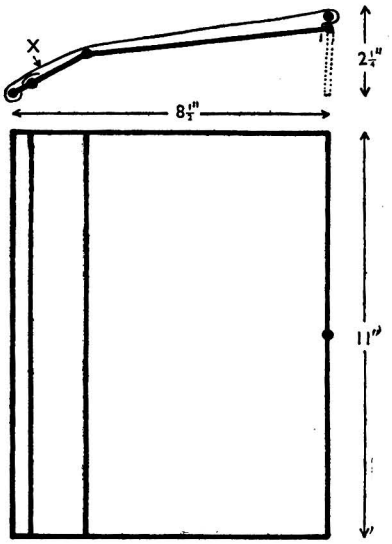

Fig. 3. Frame for two-dimensional chromatography

The sphingolipid was prepared by the method of Carter, Haines, Ledyard and Norris.[4] It was hydrolysed as described above. After the ether - water partition had been effected the aqueous phase was extracted with chloroform, which removed the sphingosine (McKibbin and Taylor[5]).

The amino-acid mixture was based on an analysis of collagen given by Bowes and Kenten.[6] The gelatin sample was hydrolysed with 3·6 *N* sulphuric acid for 18 hours, neutralised with barium hydroxide, filtered and diluted to a definite volume.

METHOD

By means of a micro-syringe, place the spot, containing 30 to 60 μg of the main components, on the paper. Dry the solution as it is placed on the paper by means of a stream of warm air from a hair-dryer.

Pour the appropriate solvent into the trough to a depth of about a quarter of an inch.

Place the frame in position and cover the trough with the glass plate. Use silicone stopcock grease to seal the joint. Leave strips for 16 hours, and squares for 3 hours, each way. If necessary for two-dimensional separations the time for the second run can be extended.

After development, remove the papers still attached to the frame, dry them and spray with the appropriate reagent. Typical results are shown in Figs. 4 to 8.

The compounds that react with ninhydrin are shown in Fig. 4, those precipitable by phosphomolybdic acid in Fig. 5 and those reacting with ammoniacal silver nitrate in Fig. 6. After extraction with chloroform the sphingolipid hydrolysate shows no sphingosine, Fig. 4 (*c*). Its extraction by chloroform is confirmed in Fig. 4 (*d*). The solvent used for one-dimensional work gave suitable R_F ratios for serine, ethanolamine and glutamic acid; for sphingosine and choline; and for inositol, glycerol and galactose. Ammonia in the solvent was used to intensify the spots. Methods for the quantitative estimation of all the substances separated are under investigation.

The authors thank Professor A. C. Frazer and Professor J. Elkes for their interest in this work; also Dr. E. Hecht of the Physiology Department, Utrecht, for a specimen of pure sphingosine, and Mr. R. G. Baker of this department for samples of amino-acid mixtures and help in interpreting the two-dimensional chromatograms.

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DEPARTMENT OF PHARMACOLOGY
MEDICAL SCHOOL
UNIVERSITY OF BIRMINGHAM

March, 1952

The Colorimetric Determination of Iron in Raw and Treated Municipal Water Supplies by Use of 4:7-Diphenyl-1:10-Phenanthroline

BY G. FREDERICK SMITH, W. H. MCCURDY, JUN.,* AND HARVEY DIEHL

An organic analytical reagent, 4:7-diphenyl-1:10-phenanthroline (bathophenanthroline), which is specific for iron, is described for the ultra-micro spectrophotometric determination of iron. The reagent has been applied to the determination of amounts of iron ranging from 1 to 10 μg in 100 ml of water. It forms a *tris* type molecular complex ferrous ion whose wavelength of maximum absorption is 533 mμ and molecular extinction coefficient is 22,400. An extraction procedure for the isolation of the coloured complex from its aqueous solution increases the sensitivity and extends the specificity to the point where no known interference by either metal cations or common anions such as chloride, nitrate, acetate, sulphate or chlorate occurs. The method has been applied to the determination of raw and purified well water provided by municipal water supply systems. Copper does not interfere.

THE ferroin reaction, which involves the formation of a red complex of ferrous iron with certain heterocyclic nitrogen compounds, has been extensively used for the colorimetric determination of small quantities of iron. The iron is first reduced to the ferrous state and then reacted with 1:10-phenanthroline, dipyridine, terpyridine or certain substituted derivatives of these compounds. References to the various procedures can be found in the work of Smith and Richter,[1] Brandt and Smith[2] and Welcher.[3] The method is in routine use for the determination of iron in wine, beer, foods, chemicals and many other products. It has been adopted as the official method for determining iron in water.[4]

In the determination of iron in treated water 1:10-phenanthroline, although a great improvement upon thiocyanate, is still not sufficiently sensitive as the lower limit is about 0·5 parts of iron per million, which is greater than the figure for iron in treated water. A new, substituted 1:10-phenanthroline, prepared by Case[5] and studied by Smith and McCurdy,[6] has now been found to be sufficiently sensitive to solve this problem. The new reagent is 4:7-diphenyl-1:10-phenanthroline and has been given the short name "bathophenanthroline." This name has been assigned because the absorption maximum of the ferrous derivative lies at a greater wavelength than that of 1:10-phenanthroline, *viz.*, 533 mμ compared with 510 mμ (a bathochromic shift).

Not only is the molar extinction coefficient of the ferrous bathophenanthroline ion (22,400) greater than that of ferrous 1:10-phenanthroline (11,100) but the new reagent can also be extracted from aqueous solutions with certain immiscible solvents, such as *iso*amyl alcohol and *n*-hexyl alcohol. Two important advantages are gained from this:

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the iron in large samples can be easily concentrated into a small volume for measurement and it is easy to free the necessary reagents from iron, which eliminates the blank correction.

METHOD

REAGENTS—

Bathophenanthroline, 0·001 *M*—Prepare a 50 per cent. ethyl alcohol solution of bathophenanthroline by dissolving 0·0334 g of the reagent ($C_{24}H_{16}N_2$, mol. wt. 334) in 50 ml of ethyl alcohol and by diluting with 50 ml of iron-free water. Store this solution in a glass-stoppered 125-ml Pyrex bottle. Alternatively, the reagent may be dissolved in the alcohol extracting solvent (without addition of water) and the complexing agent, together with a part of the extracting solvent, can be added to the test liquid. The first procedure is preferred. The reagent is also easily soluble in glacial acetic acid.

Hydroxylamine hydrochloride—A 10 per cent. solution. Reagent grade hydroxylamine hydrochloride contains appreciable amounts of iron. Prepare 100 ml of a 10 per cent. aqueous solution of hydroxylamine hydrochloride and add 3 to 4 ml of the 0·001 *M* bathophenanthroline solution. Place the solution in a 125-ml conical separating funnel and add 10 to 20 ml of *iso*amyl alcohol. Shake the contents of the flask and allow 5 minutes for the supernatant alcohol solution of the extracted red iron impurity to collect. Draw off the colourless aqueous bottom layer into a second separating funnel and repeat the separation to insure complete removal of iron. Store the iron-free hydroxylamine solution in a 125-ml glass-stoppered Pyrex reagent bottle. The solution has a pH of 1·5 to 1·75. It has been shown that all excess of bathophenanthroline is extracted from the reagent purified in this manner. The small amount of extracting solvent left in the reagent is not detrimental.

Sodium acetate solution—Transfer to a 125-ml conical separating funnel 100 ml of a 10 per cent. solution of reagent grade sodium acetate solution and 3 to 4 ml of bathophenanthroline reagent. Add 2 ml of the 10 per cent. hydroxylamine solution to reduce the iron present. Extract the red iron complex by adding 10 to 20 ml of *iso*amyl alcohol and shaking the contents of the separating funnel thoroughly. When the two immiscible liquids have separated completely (after 5 minutes) draw off the lower aqueous layer into a second separating funnel and repeat the separation to ensure complete removal of iron. Store the acetate reagent in a 125-ml glass-stoppered Pyrex bottle.

iso*Amyl alcohol*—Reagent grade *iso*amyl alcohol can be used with no preliminary preparation. Technical grade *iso*amyl alcohol must be distilled before use. *n*-Amyl, *iso*amyl or *n*-hexyl alcohol may be used. The distribution coefficient of the 4:7-diphenyl ferroin complex between these alcohols and water could not be readily determined because of the great solubility of the complex in alcohol. However, the ferrous complex is more completely removed by one extraction with *n*-hexyl alcohol than by similar treatment with *iso*amyl or *n*-amyl alcohol. This is probably the result of *n*-hexyl alcohol being the least water-soluble alcohol of the group. Under ordinary conditions two extractions with *iso*amyl alcohol are sufficient to completely recover the iron. *n*-Hexyl alcohol may be preferable where the volume of sample is large and minute amounts of iron are present. In these circumstances one extraction is generally adequate. The distribution coefficient of the ferrous complex ion formed from 2:9-dimethyl-1:10-phenanthroline between various immiscible alcohols and water are as follows: *n*-amyl alcohol, 490 to 1; *iso*amyl alcohol, 1570 to 1; and *n*-hexyl alcohol, 3420 to 1. For bathophenanthroline the same order of magnitude of results should be attained.

Ferrous iron solutions—Samples of crystalline ferrous ammonium sulphate hexahydrate (Mohr's salt) or ferrous ethylenediamine sulphate tetrahydrate (Oesper's salt) can be used for preparing standard solutions. To prepare a standard solution containing 0·01 mg of iron per ml, accurately weigh 0·0702 g of Mohr's salt or 0·0684 g of Oesper's salt and dissolve it in conductivity water with 2·5 ml of concentrated sulphuric acid. Transfer this solution to a 1000-ml graduated flask, dilute to volume with conductivity water and mix thoroughly.

APPARATUS—

Separating funnels—For many applications 60 and 125-ml separating funnels are adequate. In some respects it is preferable to use a separating funnel with a cock of smaller bore than is usual and with the section above the cock longer and more tapered. This refinement is not, however, a prerequisite. Separating funnels with standard-taper ground stoppers and stopcocks are a material advantage.

Nessler tubes and rack—For 0·01 to 0·1 parts of iron per million, Nessler tubes are adequate and indeed preferable to ordinary spectrophotometric instruments such as the Cenco-Nelson spectrophotometer or similar instruments, because even a glass cell with a 5-cm light path is inadequate. For alcoholic solutions of the colour complex made from 0·01 to 0·06 parts of iron per million in increments of 0·01 p.p.m., Nessler tubes are required. An observation rack with 10 tube positions provides for 8 standard tubes, a blank and for a tube for the unknown.

Volumetric flasks and pipettes—A good stock of 10 and 50-ml calibrated volumetric flasks and pipettes of 1, 2, 5 and 10-ml capacity is required. The flasks should be of the standard-taper glass-stopper type.

PROCEDURE FOR THE DETERMINATION OF IRON IN TREATED WATERS—

Preparation of standard solutions—This type of determination involves 0·001 to 0·01 mg of iron in 100-ml water samples (1 to 10 parts of iron in 100 million parts of water).

Place 99 ml of iron-free water in a 125-ml separating funnel and add, by means of a pipette, 1 ml of standard iron solution containing 0·001 mg of iron. Add 2 ml of 10 per cent. hydroxylamine solution and 4 ml of 10 per cent. sodium acetate solution. This gives a solution whose pH is approximately 4. Add 4 ml of 0·002 *M* bathophenanthroline. The red ferrous complex appears at once and the excess of reactant forms a white turbidity because of its reduced solubility in the water solution. Add 10 ml of *iso*amyl alcohol, stopper the separating funnel and shake the contents thoroughly. Set the reactants aside for at least 5 minutes, transfer the lower aqueous layer to a second 125-ml separating funnel, repeat the addition of 10 ml of *iso*amyl alcohol, shake and set aside again for 5 minutes and finally draw off the aqueous lower layer and discard it. Transfer the coloured alcohol extracts to a 50-ml graduated flask, rinsing each separating funnel and stopper with generous portions of ethyl alcohol from a 5-ml pipette. Dilute the contents of the graduated flask to volume with ethyl alcohol, stopper the flask and mix its contents thoroughly. Transfer the coloured *iso*amyl alcohol solution of bathophenanthroline ferrous complex to a 50-ml Nessler tube.

Repeat this procedure in turn with 2·0, 3·0, 4·0, 5·0, 6·0, 8·0 and 10 ml of standard iron solution and prepare a blank containing all the reagents except the iron solution, giving a total of nine standards of reference. The composition of the solutions and their colour intensities remain unaltered for long periods if the tubes are protected against evaporation.

Preparation of test samples of purified water supplies—Municipal water plants generally supply city water mains with a product that is sufficiently free of iron to fall within the range covered by the standard Nessler tubes, whether the water samples are taken at the pumping station source or from the tap at the point of consumption. Treat 100-ml samples of water exactly as described above for the preparation of the standard Nessler tubes. The iron is readily estimated to the nearest hundredth part per million by comparing the unknown *iso*amyl extractions of the test samples with the standard Nessler tubes. By test, starting with additional known concentrations of iron added to 100 ml of iron-free water, duplicate colour intensities of the unknown solutions with standards was found.

Sampling of raw well water—This type of product may contain from one-tenth of a part to five parts per million of iron, although it is generally at or near the higher value. For this range a moderately efficient spectrophotometer is required for determining colour intensities. Hence, take 1 to 10-ml samples of raw water for analysis. It is important to sample the water at the source and to take samples immediately before analysis. If this is not possible, use a completely full sample bottle in order to help retard oxidation of iron in contact with atmospheric oxygen. If an appreciable length of time is likely to elapse between sampling and analysis, add 2 ml of iron-free hydrochloric acid solution to the sampling bottle before sampling to ensure no precipitation of iron and other impurities.

Preparation of standard reference data for the estimation of total iron in raw well water—By use of the standard iron solution containing 0·01 mg of iron per ml, the procedure just described can be applied to iron solutions at higher concentrations. At these concentrations a 60-ml separating funnel is used, as follows.

Add to five 60-ml separating funnels 5·0, 6·0, 7·0, 8·0 and 9·0-ml portions of iron-free water. With calibrated pipettes, add to these tubes 5·00, 4·00, 3·00, 2·00 and 1·00 ml, respectively, of standard iron solution containing 0·01 mg of iron per ml. Then add 2 ml of 10 per cent. iron-free hydroxylamine solution, 4 ml of 10 per cent. iron-free sodium acetate

No. 7 September, 1952

THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

BULLETIN

FORTHCOMING MEETINGS

Ordinary Meeting of the Society, October 1st, 1952

AN Ordinary Meeting of the Society will be held at 7 p.m. on Wednesday, October 1st, 1952, in the Meeting Room of the Chemical Society, Burlington House, London, W.1.

The following papers will be presented and discussed—

"Spectroscopic Properties of Vitamin A_2. Application to the Assay of Cod Liver Oil," by H. R. Cama, B.A., M.Sc., Ph.D., and Professor R. A. Morton, Ph.D., D.Sc., F.R.S., F.R.I.C.

"The Estimation of Carbonyl Compounds by Semicarbazide and Hydroxylamine with Special Reference to Fatty Acid Oxidation Products," by A. J. Feuell, B.Sc., A.R.I.C., and J. H. Skellon, M.Sc., Ph.D., F.R.I.C.

"Simultaneous Determination of Pentose and Hexose," by W. R. Fernell, B.Sc., and H. K. King, M.A., Ph.D., F.R.I.C.

Ordinary Meeting of the Society, November 5th, 1952

AN Ordinary Meeting of the Society, organised by the Biological Methods Group, will be held at 7 p.m. on Wednesday, November 5th, 1952, in the Meeting Room of the Chemical Society, Burlington House, London, W.1.

The subject of the meeting will be "Vitamin Assay."

Meeting of the North of England Section, October 25th, 1952

AN Ordinary Meeting of the North of England Section will be held on Saturday, October 25th, 1952, at the City Laboratories, Mount Pleasant, Liverpool, 3.

Meeting of the Scottish Section, November 5th, 1952

AN Ordinary Meeting of the Scottish Section will be held at 7.15 p.m. on Wednesday, November 5th, 1952, in Room 246, Royal Technical College, Glasgow.

The following paper will be presented and discussed—

"Quantitative Microscopy in Relation to Plant Tissue," by Francis Fish, B.Pharm., Ph.C.

PAPERS ACCEPTED FOR PUBLICATION IN *THE ANALYST*

THE following papers have been accepted for publication in *The Analyst*, and are expected to appear in the near future. It is not possible to enter into any correspondence about any of them.

"The Chemical Composition of Milk Between 1900 and 1950," by J. G. Davis.

It has been suggested, during the past few years, that the chemical quality of milk in this country has deteriorated over the last twenty or thirty years. The difficulties encountered in an attempt to answer this question are discussed in some detail. One of the most important requirements is the systematic testing of samples selected at random all over the country by the same analytical methods, over the period under consideration. There is insufficient information to allow any dogmatic assertion about the trends in milk quality. In the present paper, the results of over half-a-million samples tested in various parts of England during the period 1900 to 1950 have been tabulated and examined. It appears that there has been a slight rise in fat content, with a peak at 1926 to 1930, and subsequently a slight fall. The solids-not-fat values appear to have risen steadily from 1900 to 1921-1925 and since then to have fallen appreciably. Further evidence is produced to suggest that there has been a similar fall in the quality of milk in Wales, whereas the fat content of milk in Scotland has increased.

Methods for maintaining or improving the quality of milk are discussed, and it is suggested that the logical solution is a universal method of quality payment. The total solids content is suggested as being the most suitable from chemical aspects and also from the points of view of justness, simplicity, and methods of utilisation. The evidence available from other countries suggests that where milk is bought on a quality basis, the fat or solids-not-fat contents, or both, are at least maintained, if not improved, and where payment is independent of quality, the chemical composition tends to deteriorate.

"The Polarographic Determination of Titanium in Aluminium Alloys," by R. P. Graham and A. Hitchen.

A precise and accurate method for the polarographic determination of titanium in a variety of aluminium-base alloys is described. The supporting electrolyte is *M* tartaric acid, 0·5 *M* sulphuric acid and 1·2 *M* ammonium sulphate.

A sample of the alloy is first leached with sodium hydroxide, and then the titanium is extracted from the residue with sulphuric acid. The solution, after neutralisation and the addition of appropriate amounts of tartaric and sulphuric acids, gives a well-formed polarographic wave, the height of which is directly proportional to the concentration of titanium.

When applied to a wide range of standard samples from several laboratories, the method gave results in excellent agreement with the certified values.

"A Plate-Assay Technique for Biotin, Nicotinic Acid and Pantothenic Acid," by S. Morris and A. Jones.

Methods have been devised for the assay of biotin, nicotinic acid and pantothenic acid by the plate assay technique.

During the course of the investigatory work, parallel assays by the tube technique were made at the same time.

The methods proposed show that the plate assay can be used within certain limits.

The results are significantly lower than those of the tube assay, although for such practical purposes as plant control and laboratory control of pharmaceutical products, the loss of accuracy is unimportant compared with the gain in time.

The plate assay has been used for nicotinic and pantothenic acids in vitamin tablets with results in good agreement with the tube assay.

"The Detection of Preservatives in Beverages by a Fermentation Test, with Special Reference to Brominated Compounds," by D. A. A. Mossel and A. S. de Bruin.

A fermentation test has been developed for detecting preservatives other than benzoic and sulphurous acids in soft drinks. The substrate is brought to a pH value of 5·4 ± 0·1, and, if necessary, is enriched with 0·25 per cent. of Difco yeast extract. Thereupon it is inoculated with approximately 10^4 cells per ml of bakers' yeast (*Saccharomyces cerevisiae*). The gas formed is measured after 36 and 48 hours at a temperature of 24° ± 1° C.

Brominated acetic acid derivatives with bromine concentrations as low as 2 mg per kilogram can be detected. Sulphurous and benzoic acids at concentrations of 75 mg per kilogram do not interfere with the test, owing to the rather high pH value. Eosin, a food-colouring material that is used in the Netherlands, only inhibits the fermentation when more than 50 mg of it is present per kilogram of sample.

Essential oils, tannins or other compounds present in natural fruit juices do not interfere at the concentrations that occur in fruit drinks.

Citrus terpenes, which are present in some beverages, can be inactivated by adding liver particles to the fermentation medium. Under these circumstances threshold levels of detection of the various brominated preservatives vary from 1 to 10 mg of bromine per kilogram.

"Some New Applications and Techniques of Ion-Exchange Resins in Chemical Analysis," by G. H. Osborn.

Details are given of experimental techniques for the analytical use of ion-exchange resins and examples are also given of some analytical problems solved with the help of these resins.

"Bibliography on the Use of Ion-Exchange Resins," by G. H. Osborn.

"The Determination of Small Amounts of Calcium in Plant Material," by A. C. Mason.

Small quantities of calcium (10 to 500 μg) can be estimated quickly and accurately by titration with an aqueous solution of sodium ethylenediamine tetra-acetate, with Murexide as indicator. Phosphates, which interfere, are removed by ion exchange on a resin column. The design of such a column and its reliability in effecting the complete and rapid removal of phosphate is discussed. The method, which is free from interference by magnesium in amounts usually encountered, and iron or manganese, gives results that are in good agreement with the oxalate method. The standard error for 16 determinations on a plant ash solution is 2·84 μg in 212 μg, which corresponds to a coefficient of variation of 1·35 per cent.

"The Determination of Magnesium and Aluminium in Titanium Metal," by J. A. Corbett.

This paper deals with the separation of titanium from aluminium and magnesium by precipitation with cupferron and extraction with chloroform. The aluminium is determined colorimetrically with "aluminol" and the magnesium gravimetrically with ammonium phosphate after separation from the titanium.

"A Nomogram for the Calculation of Urinary 17-Ketosteroids," by E. R. Cook and Margaret E. Rooks.

If 24-hour urine specimens are diluted to multiples of a specified volume, the total urinary 17-ketosteroids can be calculated without loss of accuracy by the use of a nomogram, the construction of which is described. The graph can be used for several different methods by taking suitable volumes of urine and solvent.

The accuracy of the correction by equation and by nomogram has been tested, and the close agreement of corrected values obtained by the procedures of Callow, Callow and Emmens (1938), Hamburger (1948) and Cook (1952) is shown.

NOTICES

Second International Congress on Rheology

THE British Society of Rheology, supported by the Joint Commission on Rheology of the International Council of Scientific Unions, is arranging the Second International Congress on Rheology to be held at St. Hilda's College, Oxford, England, from the evening of Sunday, July 26th, until the afternoon of Friday, July 31st, 1953. The President is Sir Geoffrey Taylor, F.R.S.

The programme will include a Presidential Address, a number of Invited Lectures, a Discussion on the International Organisation of Rheology, and papers offered for presentation by Rheologists attending the Congress.

The Congress will cover the whole field of the study of the deformation and flow of matter, except such specialised subjects as have come to be regarded as branches of applied mechanics, *e.g.*, the classical theory of elasticity, aerodynamics.

Fuller details of the arrangements, and forms for provisional application for accommodation and for the offer of papers for presentation, can be obtained from the Hon. Organising Secretary, Dr. G. W. Scott Blair, The University, Reading, England. Provisional application forms should be completed and returned to the Hon. Organising Secretary not later than October 1st, 1952.

German Symposium on Fat Chemistry and Allied Subjects, 1952

THE fourth post-war Annual General Meeting and Symposium of the Deutsche Gesellschaft für Fettwissenschaft, Münster, Germany, will be held in Düsseldorf from October 6-10th, 1952. Enquiries about this Symposium and membership may be addressed to Dr. L. Ivanovszky, F.R.I.C., M.I.Chem.E., "Glenbrook," 68 Park Street, Bridgend, Glam.

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solution and 4 ml of the bathophenanthroline colour reagent. After mixing these components well, add 8 ml of *iso*amyl alcohol and shake the stoppered flask thoroughly. After 5 to 10 minutes (preferably 10 minutes) draw off the lower aqueous layer. Transfer the remaining coloured *iso*amyl alcohol to a 10-ml graduated flask. Using a 1-ml pipette, rinse the inner walls of the separating funnel, collect the rinsings in a graduated flask and dilute to the mark with ethyl alcohol. Determine the transmittance and optical density and plot the transmittance as ordinate and parts per million as abscissae to give a smooth graph. When the ordinates are optical density values, a straight line indicates conformity with Beer's law.

The analysis of raw well water—Treat samples of from 1·00 to 5·00 ml of raw water in a manner similar to that described for the preparation of the reference standard amyl alcohol extractants. Measure the optical density and determine the number of parts of iron per million present from the calibration graph. Typical experimental calibration data are shown in Table I.

TABLE I

THE TRANSMITTANCE AND OPTICAL DENSITY OF EXTRACTS OF STANDARD IRON SOLUTIONS IN THE *iso*AMYL ALCOHOL SOLUTION OF THE BATHOPHENANTHROLINE - FERROUS COMPLEX

Volume of extracted *iso*amyl alcohol - ethyl alcohol solution = 10 ml

Iron concentration, μg	Transmittance, %	Optical density
1·0	88·2	0·056
2·0	80·1	0·097
4·0	67·0	0·174
6·0	57·8	0·239
8·0	50·3	0·298
10·0	41·5	0·384

RESULTS

The data shown in Table I were obtained with a Beckman model B spectrophotometer and 1-cm quartz cells, at 533 mμ. Water was used to standardise the instrument. This accounts for the failure of the graphical representation of the data of Table I to give a straight line intercepting the horizontal and vertical axis at the origin. Less elaborate spectrophotometers such as the Cenco-Nelson photolometer give results almost as accurate. To enhance the absorption measurement, the following filters, in addition to the Corning "Alka" infra-red filter, have been found suitable: sextant green (No. 401); heat resistant yellow-green (No. 450); dark theatre blue (No. 503); colorimeter blue-green (No. 978); dark shade blue (No. 430); and signal green (No. 440). The first two are preferable, although the remainder are satisfactory and render the method only slightly less sensitive.

FACTORS CONTRIBUTING TO THE SUPERIORITY OF THE BATHOPHENANTHROLINE - FERROIN REACTION—

Specificity—The familiar acid anions of hydrochloric, sulphuric, nitric, acetic and perchloric acids do not interfere. If copper is present, certain anions such as iodide, thiocyanate, cyanide, thiosulphate, sulphide and phosphate may cause precipitation in the aqueous solution upon addition of bathophenanthroline, but such precipitates do not interfere with the quantitative extraction of the ferrous iron complex.

The following metal cations: Li, Na, K, Be, Mg, Ca, Sr, Ba, $Ce^{\cdots\cdot}$, $Ce^{\cdots}$, $Pr^{\cdots}$ and the rare earth metals in general, including $Th^{\cdots\cdot}$, $Ti^{\cdots\cdot}$, $Zr^{\cdots\cdot}$, V as vanadate and vanadyl ion, $Cr^{\cdots}$, $W^{\cdots\cdots}$, $U^{\cdots\cdots}$, $Mn^{\cdot\cdot}$, $Fe^{\cdots}$, $Ru^{\cdots}$, $Os^{\cdots\cdots\cdot}$, $Ni^{\cdot\cdot}$, $Pd^{\cdots}$, $Pt^{\cdots\cdot}$, $Ag^{\cdot}$, Zn, Cd, $Hg^{\cdot\cdot}$, $Hg^{\cdot}$, $B^{\cdots}$, $Al^{\cdots}$, $Ga^{\cdots}$, $Tl^{\cdot}$, $Sn^{\cdots\cdot}$, $Sn^{\cdot\cdot}$, $Pb^{\cdot\cdot}$, $P^{\cdots\cdot\cdot}$, $As^{\cdots\cdot\cdot}$, $As^{\cdots}$ and $Be^{\cdots}$ do not interfere. Tellurates and selenates are reduced to the metal by hydroxylamine. Cobalt forms a light yellow colour but this is not extracted from acid solution.

Copper also forms a yellow complex bathophenanthroline complex ion that is a *bis*- rather than a *tris*-complex. This *bis*bathophenanthroline - cuprous complex is formed in neutral or alkaline solution and is transformed on acidification to the colourless *mono*-bathophenanthroline - cuprous complex. Therefore, with the conditions of acidity used (pH 4·0) the *mono*-cuprous complex extracted by *iso*amyl alcohol is colourless and without

interference in the iron determination. The molecular extinction coefficient of the *bis*-bathophenanthroline - cuprous complex is 12,140 and represents the most delicate ferroin reaction complex at present described for use in the spectrophotometric determination of copper.

The present procedure for the determination of iron has been applied in the presence of copper with results as shown in Table II.

TABLE II

THE SPECTROPHOTOMETRIC DETERMINATION OF IRON IN PRESENCE OF COPPER

Measurements taken with a Carey recording spectrophotometer

Iron taken, mg	Copper present, mg	Optical density at 535 mμ	Molecular extinction coefficient found
0·09521	0·0000	0·777	22,700
0·02345	0·2578	0·212	22,200
0·04720	0·1281	0·385	22,750
0·09467	0·0623	0·801	22,850
0·19804	0·0289	1·575	22,200
		Mean =	22,540

The value found for the molecular extinction coefficient of the *tris*bathophenanthroline - ferrous complex in the presence of copper compares favourably with that found in the absence of copper (22,400). The reagent reacts with no known metal ion, other than Fe^{++}, to give a coloured complex that can be extracted by *iso*amyl alcohol at pH 4·0.

Purification of reagents—A necessary accompaniment to the development of an extremely delicate reagent for isolating and determining a given element is that all reagents be entirely free from the element being determined. The more sensitive the reagent, the greater the necessity for purity of the accessory chemicals.

By the use of bathophenanthroline the accessory reagents, hydroxylamine, and sodium acetate in the form of their 10 per cent. aqueous solutions may be easily freed from iron, and the customary laboratory stocks of water and alcohols may be readily obtained iron-free from stock supplies or may be easily purified by simple distillation.

Recovery of reagents—*iso*Amyl alcohol as well as extracting the bathophenanthroline - ferrous complex also extracts the excess of organic reagent quantitatively from the aqueous solution, so all of the reagent can be recovered from the alcohol extract and, at the same time, the alcohol, if subjected to distillation, can be simultaneously recovered. The reagent can be recovered by a similar procedure to that of Smith and Cagle.[7]

Colour stability—A series of colour standards such as that described above is stable for long periods of time.

Colour sensitivity—The most important applications of bathophenanthroline are in the determination of iron at extremely low concentrations. The most effective range of concentrations for which it should be used is 0·001 to 0·01 p.p.m. For these concentrations, tall-form 375-mm Nessler tubes, which are recognised standard water analysis equipment, should be used.[4] The next best range is 0·01 to 0·1 p.p.m. and 300-mm Nessler tubes should be used, as described in this paper. The third range is 0·1 to 1·0 p.p.m. and 1-cm cells and a photolometer with suitable filters or a spectrophotometer operating at 533 mμ should be used. For higher concentrations it is proper to substitute 1:10-phenanthroline for bathophenanthroline. The limit of sensitivity of the more sensitive bathophenanthroline - ferroin reaction in the lowest range given above is the colour intensity of the blank determination, *i.e.*, it depends on the degree to which the reagents can be freed from iron.

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A Chemical Method of Determining Penicillin in Culture Fluids

By ANNE BELOFF-CHAIN and F. DENTICE D'ACCADIA

A rapid method of determining penicillin in culture fluids or crude preparations is described. The method involves the extraction of penicillin from an aqueous phase with amyl acetate at a pH value of 2 and the re-extraction of an aliquot of the amyl acetate solution by a known volume of phosphate buffer at a pH of 7. The penicillin is then assayed by the iodimetric titration method. A correction factor is introduced for substances in the culture fluid that are extracted and that take up iodine under these conditions.

The accuracy of the method is illustrated by a series of determinations with known quantities of penicillin in phosphate buffer and culture fluids.

The only chemical method described in the literature for determining penicillin in culture fluids is that of Pénau, Hagemann and Saias.[1]

A rapid chemical method for the determination of penicillin, applicable to culture fluids and crude preparations, is of great practical importance in routine analysis during the different stages of the penicillin production process. The method described in this paper is simpler and more rapid than that of Pénau *et al.*; it was worked out two years ago and has been in routine use here and in other penicillin plants for about eighteen months. The method is based on well known principles, but it should be of practical interest to those concerned with penicillin production.

The iodimetric method first described by Alicino[2] was used. The time necessary for alkali inactivation and iodine uptake was considerably shorter, however, than that given in the original work so the procedure was modified.

Method

Reagents—

Amyl acetate.

Phosphate buffer—An 0·2 *M* solution of Sørensen's di-sodium hydrogen phosphate and potassium dihydrogen phosphate buffer of pH 7.

Hydrochloric acid—An approximately normal solution and a 1·2 *N* solution.

Sodium sulphate—Anhydrous.

Sodium hydroxide, N.

Sodium thiosulphate—An 0·01 *N* solution accurately standardised against potassium iodate.

Iodine—An 0·01 *N* solution.

Sodium starch glycollate indicator,[3] 0·2 *per cent.*

Procedure—

Take a 20-ml sample of culture fluid and carry out the following extraction in duplicate. Transfer exactly 5 ml of this fluid to a 25-ml separating funnel previously cooled in an ice-bath, and add 10 ml of amyl acetate. When the culture fluid has cooled to between 3° and 4° C, acidify it to about pH 2 with approximately *N* hydrochloric acid and extract the penicillin immediately with the amyl acetate by shaking vigorously. Replace the separating funnel in the ice-bath until the two liquid phases have separated. Remove the aqueous phase and transfer the amyl acetate to a dry test tube provided with a ground glass stopper. If any large drops of the aqueous phase are present in the amyl acetate they should be removed with a Pasteur pipette. Introduce about 1 g of anhydrous sodium sulphate into the amyl acetate and shake the tube until the amyl acetate is clear. Transfer an aliquot, usually 7 ml, of amyl acetate to another 25-ml separating funnel containing 5 ml of 0·2 *M* phosphate buffer of pH 7. Thoroughly mix the two phases again by shaking vigorously and then allow them to separate. Transfer most of the aqueous phase containing the penicillin to a 10-ml Erlenmeyer flask leaving behind about 0·5 ml in the separating funnel to prevent the risk of it mixing with any of the amyl acetate.

Transfer 1·0 ml of the penicillin solution (containing 150 to 1200 units of penicillin per ml) to a 25-ml Erlenmeyer flask with a ground glass stopper, add 2 ml of *N* sodium hydroxide and set the mixture aside for 5 minutes. After this time add 2 ml of 1·2 *N* hydrochloric acid solution and 5 ml of 0·01 *N* iodine solution. After 10 minutes titrate the iodine with 0·01 *N* thiosulphate solution and 0·2 per cent. sodium starch glycollate as indicator.

BLANK—

A blank run must be made to correct for substances other than penicillin present in the culture fluid that are extracted and that take up iodine under the specified conditions. The blank value will of course vary according to the composition of the culture fluid. The procedure for the blank run is as follows. Transfer 6 ml of culture fluid to a 10-ml Erlenmeyer flask and acidify to about pH 2 with approximately *N* hydrochloric acid solution. Immerse the flask in boiling water for 2 minutes and then cool to room temperature. All the penicillin will be completely inactivated by this treatment. Extract 5 ml of this culture fluid and assay it as described above.

CORRECTION FACTOR FOR INACTIVATED PENICILLIN—

It has been found that some of the inactivation products of penicillin are extracted by amyl acetate and take up iodine; therefore, a correction factor depending on the penicillin present in the original broth must be subtracted from the titration value of the blank. With the culture fluid used in this work the variation of the blank during a fermentation depended only on the correction factor, *i.e.*, on the concentration of penicillin present at any given time. This variation, however, should always be tested for any other culture fluid used.

CALCULATION—

The difference in the titration values of the blank (corrected) and of the penicillin extract gives the iodine uptake due to penicillin. As shown by Alicino,[3] 1 mg of sodium benzyl penicillin will consume 2·52 ml of 0·01 *N* iodine solution. The titration value, therefore, is divided by 2·52 to convert it into milligrams and multiplied by 1600 to convert it into units of penicillin per ml.

If the value obtained is y units and x ml is the aliquot of the amyl acetate extracted with buffer solution, the total units of penicillin per ml will be—

$$y + \frac{10 - x}{x} y.$$

If x is 7 ml, then the titration value is multiplied by 907 (10/7 × 1600/2·52) to give the total units per ml.

RESULTS AND DISCUSSION

In order to determine the accuracy of the method, a series was assayed and the results are shown in Table I.

A standard solution of penicillin was prepared from a crystalline preparation with an activity of 1500 units per mg. The solution contained 4 mg per ml, *i.e.*, 6000 units per ml; from this a series was made by diluting it with phosphate buffer of pH 7 to give 13 solutions (8 of which are presented in Table I) with concentrations varying from 150 to 1950 units per ml. From each solution, eight 1·0-ml samples were taken and assayed by the iodimetric technique as described above. Four of these samples were inactivated by alkali for 5 minutes with 10 minutes allowed for iodine uptake and four were inactivated for 15 minutes with a period for iodine uptake of 25 minutes. The means of the titration values are shown in Table I, columns (4) and (2) respectively. From these values it is evident that the inactivation is complete in 5 minutes and iodine uptake in 10 minutes.

The number of units of penicillin per ml has been calculated in each instance from the mean of the titration values, and the results are shown in columns (3) and (5).

A similar series was prepared from the original penicillin standard solution, but this time by diluting with culture fluid containing 6 per cent. of corn steep liquor, 3 per cent. of lactose and 0·17 per cent. of sodium hydroxide instead of phosphate buffer. Two 5-ml portions were taken from each solution and extracted in the manner described above. Four 1·0-ml samples of each extract were assayed for penicillin. The mean of the titration values

are shown in Table I, column (7). The penicillin content of both extracts was calculated from the mean of the titration values subtracted from the blank; the number of units of penicillin per ml is shown in column (8). The correction factor for the blank at each concentration of penicillin assayed is shown in column (6). These values were determined by

TABLE I

THE DETERMINATION OF PENICILLIN IN CULTURE FLUIDS

	Iodimetric assay					Assay from broth extract		
	Hydrolysis time, 15 min. Iodination time, 25 min.		Hydrolysis time, 5 min. Iodination time, 10 min.			Hydrolysis time, 5 min. Iodination time, 10 min.		
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Penicillin taken, units per ml	Titrations* with 0·01 *N* iodine, ml	Penicillin found, units per ml	Titrations* with 0·01 *N* iodine, ml	Penicillin found, units per ml	Correction factor, ml	Titrations* with 0·01 *N* iodine, ml	Penicillin found, units per ml (corrected for blank)	Error, %
150	0·25	147	0·25	147	0·08	0·26	136	−9
300	0·49	298	0·48	279	0·10	0·41	290	−3
600	0·92	603	0·91	597	0·15	0·64	590	−2
900	1·48	925	1·48	925	0·20	1·04	861	−4
1200	1·90	1210	1·88	1200	0·26	1·35	1152	−4
1500	2·36	1504	2·36	1504	0·29	1·63	1406	−7
1800	2·83	1803	2·80	1784	0·37	1·94	1690	−6
1950	3·07	1955	3·06	1942	0·41	2·12	1850	−5

* The titration values are given as millilitres of 0·01 *N* iodine consumed. They are means of four titrations.

the inactivation of standard solutions of pure crystalline penicillin in water or buffer according to the method described for the blank determination. Extracts of the inactivated solution were then titrated by the iodimetric method.

The percentage errors, as calculated from the theoretical values by weight are shown in column (9).

From these results one concludes that for all normal routine penicillin fermentation work this rapid method of chemical assay is sufficiently accurate between the limits of 150 and 1200 units per ml. At concentrations of less than 150 units per ml it is advisable to use the biological assay method. When the concentration exceeds 1200 units per ml it is necessary to dilute the culture fluid before assaying by this method. The application of this method has made it possible to know the penicillin content of the culture fluid at any given time during a fermentation 30 to 40 minutes after taking a sample.

We wish to thank Professor E. B. Chain, F.R.S., for having suggested the method developed in this work, and for his interest throughout the progress of the work. We are also indebted to Mr. C. Paolini and Mr. A. Romagnoli for valuable technical assistance.

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Observations on the Use of Girard's Reagents in the Preparation of Crude Urine Extracts for Analysis of 17-Ketosteroids

By BARBARA M. BRAY*

Three methods for isolating the ketonic fraction from a mixture of urinary steroids by means of Girard's reagents are compared with respect to the time required for the formation of the hydrazone, the degree of dryness necessary in the reaction mixture and their reproducibility within the range required in routine clinical practice.

Condensation with Girard's reagent T for 3 minutes in a bath of boiling water gave an average recovery of 95 per cent. for amounts of dehydro-*iso*-androsterone between 0·5 and 2·5 mg, and 94 per cent. for the same steroid added to a urinary extract. Girard's reagent P gave lower results. Drying the urinary extract for 12 to 36 hours *in vacuo* did not improve the recovery.

The whole of the estimation can be completed in a day; but the work may be allowed to stand overnight by keeping the extract of the ketonic fraction in a desiccator, or the hydrolysis product of the hydrazone under ether.

In the application of the Zimmermann reaction to the estimation of 17-ketosteroids, the colours given by crude extracts of urine are not always comparable with those given by standards of pure steroids. The discrepancies are due to coloured substances which absorb light in the green region of the spectrum and to chromogens other than 17-ketosteroids (Engstrom and Mason[1]). A blank on the extract and alkali can be used to compensate for the coloured substances, and Talbot, Berman and MacLachlan[2] have suggested the use of a factor to correct for interfering chromogens. The Medical Research Council's Committee on Clinical Endocrinology[3] has recently (1951) proposed the use of this factor in their standard method. Experience in this laboratory has shown that there is not always good agreement between the value obtained by correction of the results given by a crude extract and that found on estimation of the ketonic fraction of the same specimen of urine.

In routine practice the Holtorff - Koch method[4] of estimation that involves aqueous alkali has been found preferable to Callow, Callow and Emmens' method.[5] Nathanson and Wilson[6] compared four modifications of the colorimetric estimation of 17-ketosteroids with alkaline *m*-dinitrobenzene and recommended the Holtorff - Koch procedure because it involves less technical difficulty and avoids the inconvenience and loss of time required for frequent preparation of alcoholic potassium hydroxide. Engstrom and Mason[1] found that mathematical correction factors could not be applied to the Holtorff - Koch method of estimation, but that this and Callow's method gave identical results without further correction when applied to the ketonic faction.

The derivation of any correction factor is necessarily empirical and the results of its application can only be an approximation to the true 17-ketosteroid value, since the nature and relative concentrations of the interfering chromogens cannot be assumed to be constant. For these reasons, in this laboratory, for research and cases of special clinical interest, the isolated ketonic fraction is used.

The ketonic fraction can be easily and elegantly isolated from the crude urine extract by the method of Girard and Sandelescu[7] in which either Girard's reagent P (pyridinium-aceto-hydrazide-chloride) or T (trimethyl-amino-aceto-hydrazide chloride) is used to form water-soluble hydrazones of the steroid hormones which can then be separated from the insoluble non-ketonic steroids.

As there are many minor variations in the technique for the preparation of these steroid hydrazones (see Table I), it was necessary to establish which of these most efficiently combined economy of time and the degree of accuracy required in routine practice. The variations in question are briefly described in Table I.

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The variables in these methods appear to be the dryness of the reaction mixture and the time required for the formation of the hydrazone, the methods involving alcohol requiring a

TABLE I

METHODS FOR PREPARING WATER-SOLUBLE STEROID HYDRAZONES

Author	Technique
Girard and Sandelescu[7] (1936)	The residue is dissolved in absolute alcohol containing 10 per cent. of glacial acetic acid and 5 to 10 per cent. of Girard T or P. Heated to boiling on a water-bath under a reflux condenser for 30 to 60 minutes.
Talbot, Butler and MacLachlan[8] (1940)	The residue is dissolved in 4 ml of 95 per cent. alcohol, 0·5 g of Girard T and 0·5 ml of glacial acetic acid added and then heated to boiling on a water-bath under a reflux condenser for 60 minutes.
Talbot, Butler, MacLachlan and Jones[9] (1941)	0·2 g of Girard T and 0·5 ml of glacial acetic acid are added to the dry residue containing not more than 50 mg of the ketonic fraction. The flask is stoppered and rotated in boiling water for 10 minutes.
Butt, Morris, Morris and Williams[10] (1951)	The residue is left overnight *in vacuo* over phosphorus pentoxide. To the dry residue, containing about 0·1 mg of the ketonic fraction, is added 0·02 ml of a freshly prepared solution containing 20 mg of Girard T in 0·2 ml of glacial acetic acid. The tube is stoppered and rotated in boiling water for 2 minutes.
Zygmuntowicz, Wood, Christo and Talbot[11] (1951)	To the residue containing about 3 mg of the ketonic fraction is added 0·2 g of Girard P and 0·5 ml of glacial acetic acid. The flask is stoppered and heated in boiling water for 3 minutes.

longer time. It is the purpose of this paper to report the results of experiments comparing recoveries of dehydro-*iso*androsterone (DHA) after various modifications in the method of hydrazone formation.

EXPERIMENTAL

The method of Talbot, Butler and MacLachlan[8] was followed for the preparation of the urine extract, except that emulsions were avoided during the extraction by a preliminary treatment of 300 ml of urine with about 5 g of zinc sulphate, filtering and using 250 ml of the filtrate for the estimation.† The extract was washed, the hydrazone hydrolysed and the free steroid recovered as in the paper referred to above (Talbot *et al.*[8]). The ketosteroids were estimated by the method of Holtorff and Koch,[4] with a 60-minute period for colour

TABLE II

COMPARISON OF RECOVERIES BY THREE METHODS OF PREPARING THE HYDRAZONE FROM 5 mg OF DHA

	Method		Recovery	
	Reagents	Procedure	mg	%
(*a*)	Girard T, 0·5 g Glacial acetic acid, 0·5 ml Absolute alcohol,* 4 ml	Heated for 1 hour on a bath of boiling water under a reflux condenser with the upper end closed by a $CaCl_2$ tube	4·54	91
(*b*)	Girard T, 0·2 g Glacial acetic acid, 0·5 ml	Stoppered and heated for 3 min. in a bath of boiling water	4·91	98
(*c*)	Girard P, 0·2 g Glacial acetic acid, 0·5 ml	Stoppered and heated for 3 min. in a bath of boiling water	3·95	79

* Redistilled over sodium.

development, and in one experiment DHA was estimated by the method of Munson, Jones, McCall and Gallagher.[12] In practice it has been found possible to dispense with the "urine blank" of the extract and the alkali recommended by Holtorff and Koch,[4] as practically all the coloured substances are removed in the non-ketonic fraction. The absorptions of test samples and standards were read against the reagent blank by means of an "EEL" photo-electric colorimeter with a green filter. Standards were prepared with 0·1 and 0·2 ml of an alcoholic solution of DHA containing 0·5 mg per ml.

† Zinc acetate was originally used by Bisset, Brooksbank and Haslewood,[13] but zinc sulphate was found to be effective.

CONFIRMATION OF THE ADEQUACY OF PROCEDURE (*b*) FOR VARIOUS QUANTITIES OF PURE DHA—

Aliquots containing from 0·5 to 2·5 mg of DHA in alcoholic solution were evaporated to dryness in 100-ml flasks and the Girard T hydrazone prepared as in (*b*) above. The recovered steroid was estimated by the Zimmermann reaction and by the Pettenkofer reaction as modified by Munson, Jones, McCall and Gallagher,[12] see Table III.

TABLE III

RECOVERY OF DHA AFTER PREPARATION OF THE GIRARD-T HYDRAZONE BY THE METHOD OF ZYGMUNTOWICZ

Estimated by the methods of Zimmermann and of Pettenkofer

	Zimmermann		Pettenkofer	
DHA, mg	Found, mg	Recovery, %	Found, mg	Recovery, %
0·5	0·54	108	0·51	102
1·0	1·07	107	1·05	105
1·5	1·26	84	1·21	81
2·0	1·97	99	1·98	99
2·5	2·34	94	2·45	98

EFFECT OF DRYING THE EXTRACT BEFORE PREPARATION OF THE HYDRAZONE—

An extract from 1500 ml of urine was washed, dried by shaking with anhydrous sodium sulphate and evaporated to dryness. The residue was dissolved in 20 ml of benzene and six 3-ml aliquots were transferred, by means of a pipette, into 100-ml flasks. To three of these were added 1 ml of an alcoholic solution containing 2 mg of DHA per ml and the contents of the flasks evaporated to dryness under reduced pressure. The hydrazones of two aliquots, one with and one without added DHA, were prepared immediately, a second pair after one night *in vacuo* over calcium chloride and the third pair after two nights. The three-minute procedure (*b*) with Girard T was used throughout. The recoveries were estimated by the Zimmermann method with the results shown in Table IV.

TABLE IV

THE EFFECT ON THE RECOVERY OF DHA OF DRYING THE URINARY EXTRACT

Method of drying	Extract only, mg	Extract plus 2 mg of DHA, mg	Recovery mg	Recovery %
In water bath ..	1·05	2·84	1·79	89·5
In vacuo, 12 hours ..	1·22	3·05	1·83	91·5
In vacuo, 36 hours ..	1·25	3·00	1·75	87·5

REPRODUCIBILITY OF RECOVERY OF DHA ADDED TO URINE EXTRACTS—

The residue from a benzene extract of 2000 ml of urine was dissolved in 25 ml of benzene and eight 3-ml aliquots were transferred, by means of a pipette, into 100-ml flasks. To three of these were added 0·2-ml portions of an alcoholic solution of DHA containing 5 mg per ml. All the aliquots were evaporated to dryness, the Girard T hydrazones prepared immediately by the three-minute procedure (*b*) of Zygmuntowicz and the recovered steroid estimated by Zimmermann's method. The results are shown in Table V.

METHOD

The method recommended for the isolation of the ketonic fraction from crude extracts of urine is as follows—

Evaporate the extraction solvent in a 100-ml flask, if necessary in several successive small quantities, so that the residual solid is concentrated in a small area. Add 0·2 g of Girard's reagent T and 0·5 ml of glacial acetic acid to the residue. Stopper the flask securely (a strip of self-adhesive cellulose tape over the stopper ensures this) and rotate in a bath of boiling water for 3 minutes.

Cool the flask on ice and transfer the contents quantitatively to a separating funnel with the help of 40 ml of ice-cold water. Add 3 ml of 10 per cent. w/v sodium hydroxide solution and extract the non-ketonic fraction three times with 20 ml of ether. Wash the combined ether extracts with three successive 20-ml volumes of water.

Hydrolyse the hydrazone by the method of Talbot *et al.*,[8] as follows—

Add to the combined water-washings 1 ml of concentrated sulphuric acid and mix with the original aqueous phase. Add 20 ml of ether to the aqueous solution of the hydrazone and allow 2 hours for its complete hydrolysis. Separate the ether layer and extract the

TABLE V

RECOVERY OF DHA ADDED TO URINARY EXTRACTS

Urinary steroids, mg	Urinary steroids plus 1 mg of DHA, mg
2·50	3·35
2·57	3·60
2·20	2·95
2·20	
2·35	
Average 2·36	Average 3·30
Standard deviation 0·17	Average recovery { 0·94 mg / 94 per cent.

liberated sterone a further three times with 20 ml of ether. Combine the ethereal solutions and wash them three times with 20 ml of water. Dry the ethereal solution with anhydrous sodium sulphate and evaporate it to dryness. Dissolve the residue in a suitable volume of alcohol for estimation of the sterone by the method of Zimmermann.

It is a pleasure to thank Dr. W. H. H. Merivale for his helpful suggestions and criticisms, and Professor G. A. D. Haslewood for reading the manuscript.

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An Investigation of Anionic Interference in the Determination of Small Quantities of Potassium and Sodium with a New Flame Photometer

By G. C. COLLINS and H. POLKINHORNE

The paper describes the design, construction and performance of a new commercial flame photometer, with particular reference to the interference of the commoner inorganic anions in the determination of small quantities of potassium and sodium.

It is now generally accepted that estimations of sodium and potassium can be made much more rapidly by means of a flame photometer than by standard chemical methods and, further, that the results so obtained are accurate.

Hitherto most workers in this country have constructed their own apparatus and a number of flame photometers have been described in the technical literature.

As long ago as 1940, Berry, Chappell and Barnes[1] pointed out that the results obtained with flame photometry were to some extent dependent upon ions other than those being determined, and more recently Brealey[2] has emphasised that the order of "the interference effect" obtained with certain ions is a characteristic of the particular instrument used.

This paper describes and analyses the interference effects observed during tests with a commercial flame photometer recently produced by Evans Electroselenium Ltd.

Description of the instrument—

The "EEL" flame photometer is illustrated in Fig. 1 and a diagrammatic view of the components is shown in Fig. 2.

Compressed air is supplied to a small annular-type atomiser (1), through a control valve (2), at a pressure of 10 lb. per sq. inch as indicated on the gauge (3) mounted on the front of the instrument. The air supply can be derived from either a cylinder with a reducing valve or from a suitable compressor. The flow of air through the atomiser draws the sample from the beaker (4) up the stainless steel capillary tube (5) and sprays it as a fine mist through the ebonite plug (6) into the mixing chamber (7). Here the larger droplets fall out and flow to waste through the drain tube (8). Gas is introduced into the mixing chamber through the inlet tube (9) from the internal gas pressure stabiliser (10) and control valve (11). The gas - air mixture passes to a multi-jet burner mounted above the mixing chamber where it burns as a broad flat flame, and the hot gases pass up a well-ventilated chimney (12). Coal gas, butane, propane or any of the proprietary bottled gases, such as Calor gas or Botto-gas, can be used without any modification of the instrument.

The light emitted by the flame is collected by a reflector (13) and focused by a lens (14) through the interchangeable optical filters (15) on to an "EEL" barrier-layer photocell (16). The current generated by this cell is taken through a potentiometer (17) to a Tinsley taut-suspension galvanometer unit (18). A glass window (19) is interposed between the lens and filter for cooling purposes.

The "EEL" barrier-layer photo-cell is used for both the sodium and potassium determinations, the optical filter only being changed, and the sensitivity of the instrument is such that full-scale deflection is obtained with 5 p.p.m. of sodium or 10 p.p.m. of potassium.

Gelatin filters, cemented between glass, are used; the relative over-all spectral responses of the filter - photo-cell combinations are indicated in Fig. 3.

The coal gas - air flame was used partly for convenience, but primarily because it was expected to give results showing lower mutual interference effects between the alkali metals themselves than would be experienced with, for example, an oxy-acetylene flame.

Operation—

With the flame burning at constant air and gas pressures, and with the appropriate filter in position in the instrument, the 10-ml Pyrex glass beaker containing a standard solution

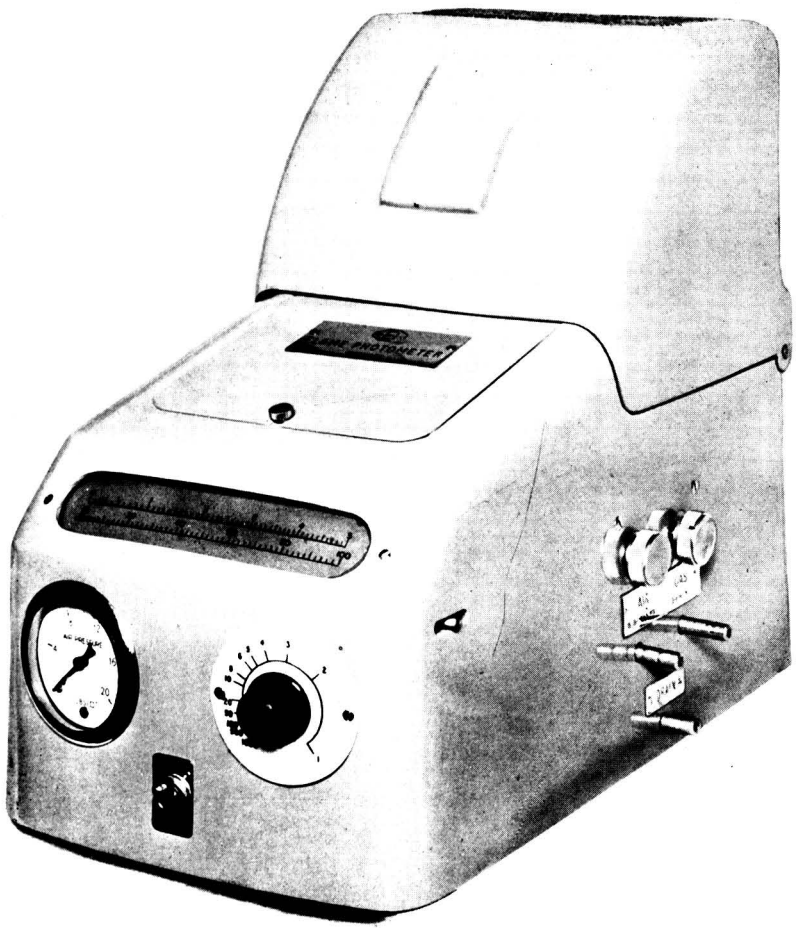

Fig. 1. Flame photometer made by Evans Electroselenium Limited

is moved up a recessed plate (20) in the side of the instrument. This automatically positions it relative to the capillary tube, up which the liquid is then drawn.

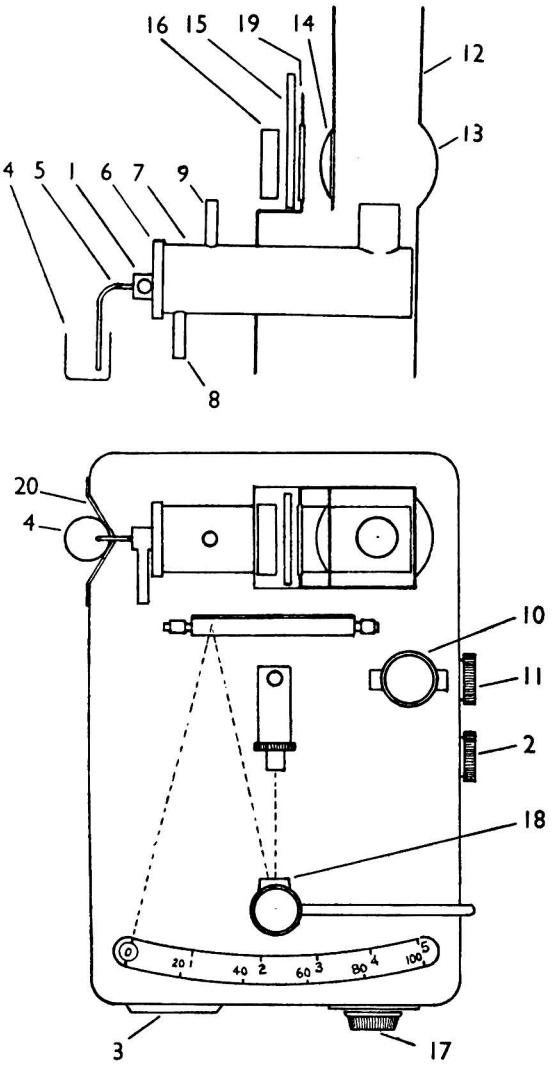

Fig. 2. Diagram of the components of the flame photometer

1, atomiser; 2, control valve; 3, pressure gauge; 4, beaker; 5, stainless steel capillary tube; 6, ebonite plug; 7, mixing chamber; 8, draining tube; 9, gas inlet tube; 10, pressure stabiliser; 11, control valve; 12, chimney; 13, reflector; 14, lens; 15, optical filters; 16, photocell; 17, potentiometer; 18, taut-suspension galvanometer unit; 19, window; 20, plate

The sensitivity control is adjusted to give a convenient reading on the galvanometer scale, and the standard solution is then replaced by the test sample. The new reading is noted and compared with that given by the standard.

Experimental

Preliminary survey—

Prepared solutions were tested, and the errors caused by the presence of other ions on the estimation of sodium and potassium were determined. The anionic interference effects

of the sulphate and phosphate ions were observed by adding the appropriate ammonium salt to standard solutions of sodium and potassium. All reagents were checked to ensure that they were free from sodium and potassium. The results obtained are shown in Table I.

TABLE I

INTERFERENCE EFFECT OF VARIOUS IONS

Interfering ion	Concentration of interfering ion, p.p.m.	Percentage interference in determination of 10 p.p.m. of sodium	Percentage interference in determination of 10 p.p.m. of potassium
$Na^{\cdot}$	100	—	0
	1000	—	0
$K^{\cdot}$	100	0	—
	1000	+3	—
SO_4''	100	0	0
	1000	0	0
PO_4'''	100	0	0
	1000	−2	−11
Interfering acids	**Concentration of acid**		
HCl	0·01 *N*	0	0
	0·1 *N*	−15	−40
	1·0 *N*	−24	−50
H_2SO_4	0·01 *N*	0	0
	0·1 *N*	0	0
	1·0 *N*	−6	−6
H_3PO_4	0·01 *N*	−2	−9
	0·1 *N*	−15	−48
	1·0 *N*	−34	−78
HNO_3	0·01 *N*	0	0
	0·1 *N*	0	0
	1·0 *N*	0	0

It is interesting to observe the large differences in interference with *N* solutions of the various acids, phosphoric causing a 78 per cent. depression in the reading for potassium and nitric having no effect. It would appear, therefore, that neither the hydrogen ion nor the nitrate ion cause interference at this concentration. Hence, the interference effect of the other acids is probably due to the anion, and one might then expect a *N* solution of a salt

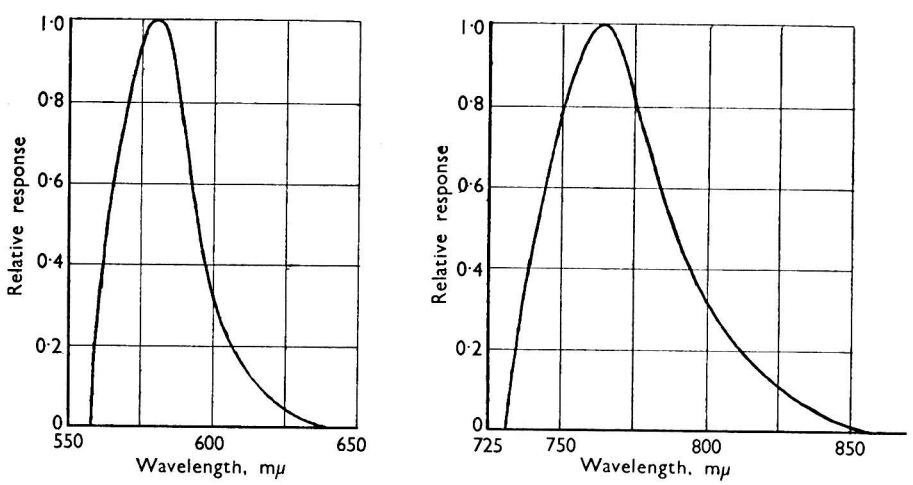

Fig. 3. Combined cell and filter response curves for (*a*) sodium and (*b*) potassium

to give the same degree of interference as a N solution of the corresponding acid, subject to the cation producing no interference. As far as was known this aspect of flame photometry had not been explored and seemed worthy of further investigation.

INVESTIGATION OF ANIONIC INTERFERENCE—

It was found that sodium and potassium-free ammonium hydroxide solution (sp.gr. 0·88) gave no interference; consequently pure ammonium salts were satisfactory for this investigation.

Solutions were prepared each containing 10 p.p.m. of potassium but containing different and known concentrations of a spectrographically-pure ammonium salt. A similar range

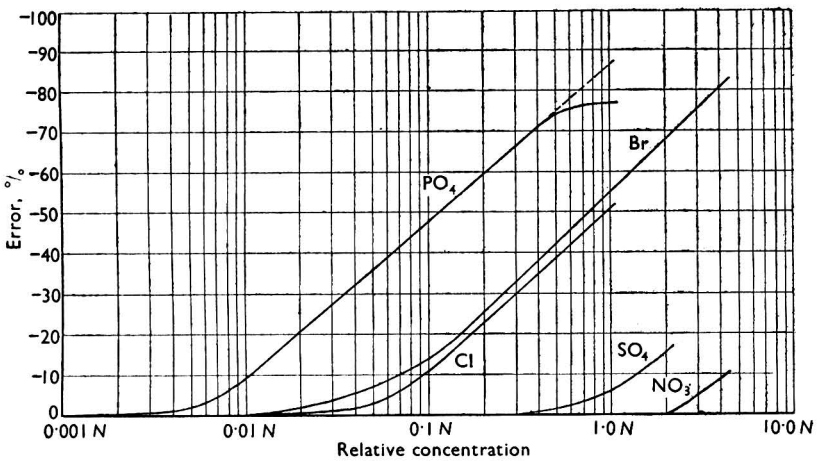

Fig. 4. Interference errors in the determination of 10 p.p.m. of potassium

of solutions was prepared with the corresponding acid, and the complete "double series" was repeated with solutions each containing 10 p.p.m. of sodium.

With such solutions the interference effect was determined for the following ions: NO_3', SO_4'', PO_4''', Cl′ and Br′, derived firstly from the ammonium salt and secondly from the acid. The concentrations covered a range greater than that met with normally.

Exactly the same interference resulted from the addition of an ammonium salt as resulted from the appropriate acid at the same anionic concentration.

This alone is of practical value since if a test sample is known to contain a high proportion of phosphate ion, it would be necessary to use standards having a similar phosphate concentration. Commercially available salts have generally an appreciable sodium or potassium content which sometimes renders them useless as flame photometer standards. The acids are, however, generally less contaminated and, as is shown, these can be used to balance standards to the level of the interfering anion.

Further, a broader view can be taken of anionic interference as a whole. For example, any salt containing phosphorus can be expected to exhibit anionic interference to a degree dependent upon the phosphorus concentration since, in the flame, which is oxidising in character, the phosphorus will give rise to phosphate ions.

The same argument applies to the other anions giving interference, namely, NO_3', SO_4'', Cl′ and Br′.

In a subsequent test it was shown that carbonate and hydroxide ions did not interfere at concentrations of 5 N and 11 N, respectively.

RESULTS AND DISCUSSION

ANALYSIS OF RESULTS—

When the percentage error in measurements of 10 p.p.m. of potassium or 10 p.p.m. of sodium were plotted against $\log_{10}$ (concentration) of interfering anion, an approximately linear relationship was obtained. The various curves had the same slope and differed only

in respect of their position on the relative concentration axis (Figs. 4 and 5). From these graphs equivalent strengths can be assigned to the various interfering anions, and also the ionic concentrations below which interference is negligible can be read. The latter are shown in Table II.

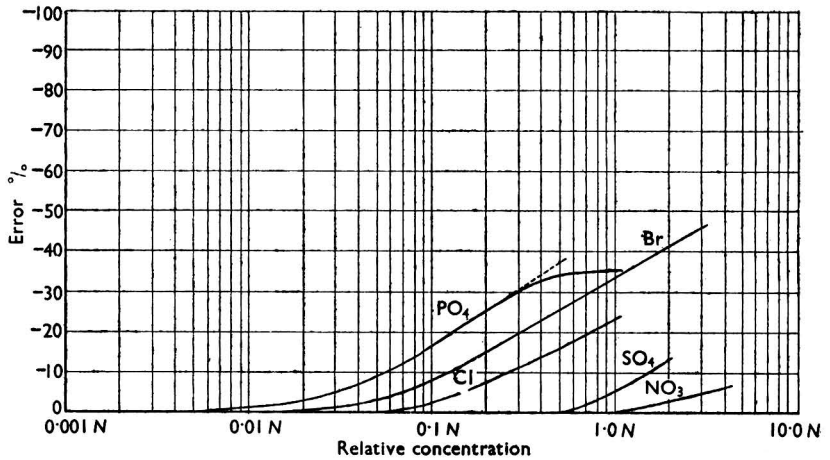

Fig. 5. Interference errors in the determination of 10 p.p.m. of sodium

TABLE II

LIMITING ANIONIC CONCENTRATIONS FOR ZERO INTERFERENCE

Interfering ion	Limiting concentrations for determination of 10 p.p.m. of sodium, p.p.m.	Limiting concentrations for determination of 10 p.p.m. of potassium, p.p.m.
NO_3'	40,000	100,000
SO_4''	18,000	56,000
Cl'	1400	1200
Br'	1000	2100
PO_4'''	600	400

POSSIBLE MECHANISM OF ANIONIC INTERFERENCE—

The studies of Belcher and Sugden[3] have indicated that when an alkali metal is added to a flame of a similar type to the present one, it gives rise to a number of reactions which can be regarded as being in equilibrium at the flame temperature. Although their measurements involved the absorption of short-wave radiation by the free electrons produced by ionisation of a small amount of the added metal, rather than the emission of light energy by the excited metal atoms, the considerations in both instances are similar.

We can suppose that when a solution of a salt of an alkali metal is added to a flame an equilibrium is set up between undissociated salt (say NaX) and its constituents Na and X. The anionic portion X may be the original one, *e.g.*, as with halides, or may be produced by modification of the original, *e.g.*, orthophosphate will become metaphosphate in the flame—

$$NaX \rightleftharpoons Na + X;\ K = \frac{[Na][X]}{[NaX]}.$$

With a considerable excess of, say, the ammonium salt, X can be considered to arise from this source only, the anionic contribution of the original sodium salt being negligible. If the total sodium added be expressed as a concentration $[Na]^0$ then—

$$[Na]^0 = [Na] + [NaX]$$
$$= [Na]\left(1 + \frac{[X]}{K}\right)$$

The light intensity, I emitted is proportional to the amount of free sodium [Na], whilst [X] is proportional to the concentration of the added anion, if, as is the case, this is much greater than that of the sodium solution. Hence, with a given total of sodium applied to the flame per unit time, a plot of $1/I$ against the concentration of the anion should give a straight line.

Fig. 6 shows such curves derived from Figs. 4 and 5; it will be observed that linearity obtains at the higher relative concentration values. Hence it can be considered that at

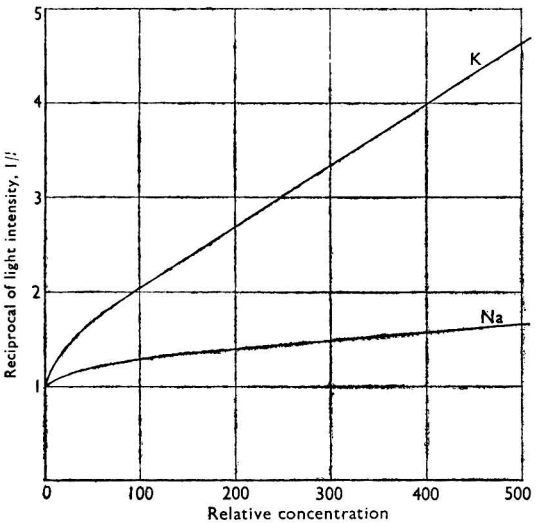

Fig. 6. Effect of anion concentration on light intensity

these values, for the substances tested, anionic interference results primarily from the combination of the alkali metal with the interfering anion to form the appropriate salt.

CONCLUSIONS—

(1) It has been shown that anionic interference results in a suppression of emission of the element being determined, and is the same whether the interfering anion be present in the form of the acid or as the corresponding ammonium salt.

(2) In general, the same concentration of an interfering anion suppresses the emission of potassium to a greater degree than that of sodium.

(3) For each anion there is a limiting concentration above which interference is found to occur, the relationship between those limiting concentrations being as follows—

For determinations of potassium—

N phosphate $\simeq 6{\cdot}2\ N$ bromide $\simeq 8\ N$ chloride $\simeq 134\ N$ sulphate $\simeq 400\ N$ nitrate.

For determinations of sodium—

N phosphate $\simeq 2\ N$ bromide $\simeq 6\ N$ chloride $\simeq 29\ N$ sulphate $\simeq 100\ N$ nitrate.

These different levels of limiting concentration can be considered to be functions of the stability of these particular salts of the alkali metals in the flame in question.

(4) When percentage interference is plotted against $\log_{10}$ (concentration), the resulting curves have similar slopes.

(5) It is inferred that anionic interference results from a reduction in the number of free metal ions, owing to their combination with the ionised anion. It has been shown that when the anion is present in excess the predominating equilibrium is then—

$$A^{\cdot} + Y' = AY;\ K = \frac{[A^{\cdot}][Y']}{[AY]},$$

where A = alkali metal and Y = interfering anion.

It is emphasised that any work on interference effects should be carried out with spectrographically pure reagents and that the results can only be applied with accuracy to the particular instrument on which they were determined.

Should at any time the interference effects of other cations be investigated the salt used should be specified, particularly with cations of the lighter elements. If the chloride, say, of lithium be used, the anionic interference of the chlorine ion might possibly exceed the cationic effect of the lithium ion.

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Notes

THE DETERMINATION OF TRACES OF MERCURY IN URINE BY THE REVERSION TECHNIQUE

In the course of another investigation it was found necessary to determine traces of mercury in urine, amounts of 20 μg per litre being considered significant, when only small samples of urine, approximately 25 ml, were available for analysis. Milton and Hoskins[1] have determined mercury by a single colour dithizone method in litre samples of urine. In view of the large amounts of urine required in that procedure it was considered unsuitable when only 25-ml samples, containing as little as 0·5 μg of mercury, were available for the determination.

The reversion technique for determining mercury with dithizone developed by Irving, Andrew and Risdon[2] is a refinement in the use of the reagent that has considerably increased its value. Arthington and Hulme[3] have used the reversion method for determining mercury in the peel of apples. This note describes the application of the reversion technique to the determination of mercury in urine.

The method adopted consists in digesting the urine with permanganate in sulphuric acid solution, removing the excess of permanganate by adding hydroxylamine hydrochloride and extracting the resulting solution, when cool, with a solution of dithizone in chloroform. The transmission of the dithizone extract is measured in a colorimeter, the extract is reverted and the transmission measured again.

The digestion procedure used for this determination is that of Hubbard.[4] Only one dithizone extraction of the mercury is necessary as the acidity of the digest is sufficiently high (approximately 1·5 *N* with respect to sulphuric acid) to prevent the formation of copper dithizonate.

Method

Reagents—

All reagents should be of recognised analytical purity.

Dithizone solution—Dissolve 5 mg of dithizone in a litre of chloroform. This solution should not be more than 10 days old as high results are given with old solutions. The chloroform used should be heated under a reflux condenser for 3 to 4 hours and then distilled; 1 per cent. of pure ethyl alcohol is added to the distillate. Chloroform can be recovered by the method of Biddle,[5] but heating must be done under a reflux condenser for 3 to 4 hours before distilling.

Hydroxylamine hydrochloride solution—A 50 per cent. solution. Dissolve 50 g of hydroxylamine hydrochloride in 50 ml of water.

Diluted sulphuric acid solution (1 + 1)—Mix 500 ml of sulphuric acid with 500 ml of distilled water.

Reversion solution—Mix 10·2 g of potassium hydrogen phthalate and 30 g of potassium iodide in distilled water and make up to 500 ml. Add a few drops of sodium thiosulphate solution to remove liberated iodine and shake the solution with dithizone solution to remove metals.

PROCEDURE—

Add an exactly known volume of urine (25 ml or less) to a 250-ml flask and dilute to 50 ml with water. Add 1 g of potassium permanganate and 10 ml of sulphuric acid, fit a condenser to

TABLE I

DETERMINATION OF MERCURY IN TWO SAMPLES OF URINE

Sample A		Sample B	
Mercury added, μg	Mercury found, μg	Mercury added, μg	Mercury found, μg
0·25	0·25	2·5	2·6
0·25	0·30	5·0	5·1
1·0	1·0	nil	0·25
2·0	2·25	nil*	nil
4·0	3·8	—	—
4·0*	3·6	—	—
nil	0·1	—	—

* The samples contained 1 mg of added copper.

TABLE II

INSTRUMENT READINGS FROM DETERMINATIONS OF MERCURY IN URINE

Transmission before reversion (T)	Transmission after reversion (T_0)	"Density" before reversion (D)	"Density" after reversion (D_0)	Reversion ($D_0 - D$)	Mercury in 25 ml of urine, μg
56·5	42·75	0·248	0·369	0·121	2·6
45·5	40·75	0·342	0·390	0·048	1·0
55·25	41·0	0·254	0·387	0·133	2·8
57·5	40·5	0·240	0·392	0·152	3·3
42·5	41·0	0·372	0·387	0·015	0·3
52·75	48·75	0·278	0·312	0·034	0·6

the flask and boil under a reflux condenser for 30 minutes. If the solution is decolorised whilst boiling, cool slightly, remove the condenser, add 0·5 g of potassium permanganate, replace the condenser and boil for 30 minutes. If the solution is again decolorised repeat the process until the permanganate discoloration persists after boiling for 30 minutes. Add 1 ml of hydroxylamine hydrochloride to the solution and boil under reflux for another minute. Cool and dilute the solution to 100 ml.

Shake 25 ml of this solution with 10 ml of dithizone solution in a separating funnel. If the colour of the chloroform layer indicates a low mercury content add the balance (75 ml) of the solution and shake again. If the mercury content is high, dilute a suitable aliquot of the solution to 100 ml and add sufficient sulphuric acid and hydroxylamine hydrochloride to restore their concentrations to their original values. Shake this solution with dithizone solution as before. Allow the chloroform layer to separate and run it through a wad of cotton wool inserted in the stem of the separating funnel into a colorimeter cell. Measure the transmission (T) of the filtrate in a colorimeter with a 620 mμ filter. Transfer the solution to a separating funnel containing 10 ml of reversion solution, shake, allow to settle, filter through a wad of cotton wool as before into a cell and measure the transmission (T_0).

Convert the transmission values to "density" values by applying the following formulae—$D = 2 - \log T$ and $D_0 = 2 - \log T_0$. Calculate the reversion value ($D_0 - D$) and read the equivalent amount of mercury from a calibration curve prepared with a series of solutions containing known amounts of mercury. The determination of the calibration curve is made under conditions similar to those of the analysis.

An Evelyn colorimeter has been used for this work. The cells have a minimum volume of 6 ml and a light path of 2 cm.

Results

Known amounts of mercury were added to 25-ml portions of two samples, A and B, of urine. The mercury content was determined by the procedure outlined to give the results recorded in Table I. Table II shows instrument readings obtained in the course of the analysis of six samples of urine containing different amounts of mercury.

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February, 1952

THE USE OF PAPER CHROMATOGRAPHY FOR DETECTING BENZOIC ACID AND ITS DERIVATIVES IN FOOD PRODUCTS

In Great Britain benzoic acid and sodium benzoate may be used as preservatives for certain classes of foodstuffs. Manufactured food products of foreign origin may contain *p*-chlorobenzoic acid, salicylic acid or *p*-hydroxybenzoic acid or its salts and esters as preservatives. It is possible to detect the presence of any of these four acids in an extract of the foodstuff by paper chromatography, with one of the buffered solvent systems recommended by Fewster and Hall.[1]

It was found that 0·25 mg of benzoic acid, or one of its derivatives, can be readily detected on a chromatogram. Hence, if 50 g of a foodstuff is extracted and the whole extract applied to the paper in the manner described below, it is possible to detect 5 parts of benzoic acid per million in the foodstuff. However, these preservatives are usually encountered at higher concentrations than this and the following method has been devised to deal with concentrations of between 20 and 200 p.p.m.

For liquids and for products that can be macerated to the consistency of a thin slurry, it is convenient to weigh out 50 g of material, to acidify it with 2 ml of 5 *N* sulphuric acid and to extract it with chloroform in a suitable liquid - liquid extraction apparatus. The particular apparatus used in this laboratory is a modification of that illustrated by Hughes.[2] *p*-Chlorobenzoic and *p*-hydroxybenzoic acids are only slightly soluble in chloroform, but the addition of 20 ml of ethanol to the sample enables them to be extracted. With solid products it is necessary to steam-distil and extract the distillate. The extract is evaporated to dryness in a water-bath at 100° C and the residue is dissolved in 1 ml of ether. This evaporation must be done with great caution as benzoic acid is easily lost at this stage.

At different points on a line and 3 cm from the edge of a sheet of Whatman No. 3 filter-paper, 0·1 and 0·2-ml aliquots are applied. A further 0·4 ml of the solution is diluted to 2 ml and aliquots (0·1 and 0·2-ml) of this diluted solution are also applied to the paper. These four quantities should ensure that at least one of them will result in satisfactory separation and identification of the acids if they are present at concentrations in the range 20 to 200 p.p.m.; for higher concentrations further dilution of the ether solution is desirable. The chromatogram is developed overnight with the solvent system: *n*-butanol (40 parts), ethanol (11 parts) and ammonium hydroxide - ammonium carbonate buffer (19 parts), as described by Fewster and Hall.[1] The developed chromatogram is dried at 150° C for at least 5 minutes and sprayed with a mixture of methyl red, bromophenol blue and phosphate buffer (Fewster and Hall[1]). Benzoic acid and its derivatives show up as distinct pink spots. An alternative spraying reagent is an 0·025 per cent. solution of 2:6-dichlorophenol-indophenol in phosphate buffer (pH 7·2) as used in the estimation of ascorbic acid, when the acids show up as pink spots on a blue background. In addition, salicylic acid can be detected by the intense light blue fluorescence of the unsprayed paper when the paper is examined in ultra-violet light. With the above solvent system the following R_F values have been obtained under the experimental conditions—*p*-chlorobenzoic acid, 0·59; salicylic acid, 0·59; benzoic acid, 0·50; *p*-hydroxybenzoic acid, 0·31.

Reliance should not be placed solely on R_F values for identification, and a control spot of pure benzoic acid or salicylic acid (0·1 ml of a solution in chloroform or ether containing 2 mg

of the acid per ml) should be placed on the same sheet of paper and developed alongside the unknown sample.

A sample of imported canned tomato sauce containing approximately 200 parts of sodium benzoate per million was treated by the above method. The chromatogram showed a distinct spot corresponding to benzoic acid. When salicylic acid was added to another portion of the same sample, both acids were detected.

Quantities of sodium benzoate, calculated to give concentrations of 20 to 200 p.p.m., were added to different portions of grapefruit juice. By varying the volume of ether (or chloroform) used for dissolving the residue and varying the volume applied to the paper, in accordance with the figures given above, benzoic acid was satisfactorily detected at the concentrations used. Mixtures of sodium benzoate with sodium *p*-hydroxybenzoate or methyl *p*-hydroxybenzoate and salicylic acid or *p*-chlorobenzoic acid were also added to grapefruit juice, the final concentration of each acid being in the range of 40 to 120 p.p.m. In each instance the three acids appeared as distinct and separate spots on the developed chromatogram; salicylic acid was distinguished from *p*-chlorobenzoic acid by its strong fluorescence.

The author wishes to thank the Directors of the Metal Box Company Limited for permission to publish this note, and Dr. H. Liebmann for his interest and advice.

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2. Hughes, E. B., *Chem. and Ind.*, 1942, 103.

THE METAL BOX COMPANY LIMITED
ACTON, LONDON, W.3

T. L. PARKINSON
February, 1952

THE DETECTION AND ESTIMATION OF SMALL AMOUNTS OF PHENOL IN CRESYLIC ACID

REFINED cresylic acid consists chiefly of mixtures of *o*-, *m*-, *p*-cresols and xylenols either with or without a percentage of phenol. The relative proportions of each constituent can vary over a wide range.

For the estimation of up to 10 per cent. of phenol the method described under Serial No. C.C. 15–50 "Standard Methods for Testing Tar and its Products" (1950 edition) is generally used. This colorimetric method, due to R. M. Chapin, is based on the bleaching action of formaldehyde on colours formed by treating phenol with Millon's reagent. The colours given by cresols and xylenols are unaffected. By its nature, this colorimetric reaction does not lend itself to the detection and estimation of small amounts, *i.e.*, about 2 per cent., of phenol.

The test described here, which is done on a semi-micro scale, is based on the chromatographic separation, on filter-paper, of the azo dyes formed by coupling the phenols, in the presence of alkali, with diazotised sulphanilic acid. As little as 0·5 per cent. of phenol can be detected with certainty and amounts up to 3 per cent. estimated. The test has proved useful when a limit test is required for 2 per cent. of phenol, and a number of samples can be examined with the minimum of manipulation and attention.

Hossfeld[1] has reported the qualitative application of this separation and in the procedure described here the amount of phenol is estimated by comparing the chromatogram with a series of standard chromatograms prepared from a synthetic cresylic acid to which has been added known amounts of phenol. A modification of the technique in paper chromatography described by Rutter[2] is used, a strip of paper, 20 mm wide, replacing the usual circular filter-paper. The conditions of the test and certain dimensions of the apparatus and paper have been standardised in such a way that comparable chromatograms are produced in which the colour intensity of the yellow dye due to phenol gives a measure of the amount of this constituent.

A known quantity of cresylic acid, in the form of its sodium salt, is deposited at one end of the paper strip. The strip is then pressed against a filter-paper wetted with an alkaline solution of diazotised sulphanilic acid, coupling with the phenol, cresols and other phenolic compounds taking place *in situ*. By this means a compact and uniformly coloured zone of azo dye is formed at one end of the strip, the remaining portion being left wet with alkaline diazo solution. The strip is dried and then irrigated with a solvent, as recommended by Rutter. With methyl ethyl ketone containing 9 per cent. of water as solvent the original orange coloured dye formed from a

mixture of phenol with *o*-, *m*- and *p*-cresols is separated into a series of coloured bands spaced, in relation to the position of the original colour, in the following order: (1) bright yellow from phenol, (2) orange from *o*,*m*-cresol and (3) dark red from *p*-cresol. If xylenols are added to this mixture the colour band (red-brown) due to these occupies a position intermediate between (2) and (3).

In general the method can be applied to commercial cresylic acid with no preliminary treatment of the sample. If, however, the sample is dark coloured a small portion, 5 to 10 ml, should be redistilled to pitching point and the test carried out on a portion of the well-mixed nearly colourless distillate.

Method

Apparatus—

A unit consists of a glass petri dish 90 mm in diameter and 14 mm deep covered by a glass plate 120 mm square and 1·5 mm thick in which a central hole 4 mm in diameter has been drilled. On this rests another glass plate of similar dimensions but without a hole.

If several units are to be used it is important that these dimensions should be uniform throughout.

Reagents—

Sulphanilic acid—Dissolve 1·91 g of colourless recrystallised sulphanilic acid in 250 ml of distilled water.

Sodium nitrite—Dissolve 0·85 g of pure sodium nitrite in 250 ml of distilled water.

Diluted sulphuric acid—Sp.gr. 1·270 at 20° C.

Sodium hydroxide solution, 2 N.

Methyl ethyl ketone—Use "purified" quality. The specific gravity at 20° C should be not less than 0·804 or greater than 0·806. In 100 ml, not more than 5 ml should distil at 79·3° C and not less than 95 ml at 80° C.

Aqueous methyl ethyl ketone—Place 9 ml of water in a 100-ml calibrated flask and make up to the mark with methyl ethyl ketone reagent.

Procedure—

Preparation of diazo reagent—Mix 10 ml of sulphanilic acid solution with 2 ml of diluted sulphuric acid solution in a glass-stoppered tube and cool the tube in ice. Place 10 ml of sodium nitrite solution in another stoppered tube and cool the tube in ice. Mix the contents of both tubes and keep the mixture cool in ice. Just before using the mixture make it alkaline by adding 11 ml of sodium hydroxide solution to it.

Preparation of paper—Draw a pencil line across the diameter of a 12·5-cm Whatman No. 2 filter-paper. Draw two other lines on either side of the first at a distance of 10 mm from it to form the outline of a strip of paper 20 mm wide and approximately 125 mm long. Make a pencil mark on the middle line 25 mm from one end.

Prepare a solution of the sample of such concentration that a 12-μl micro-pipette will contain 20 μg of sample. Dissolve the sample in a slight excess of sodium hydroxide solution before making up to a known volume with water. Fill the pipette with this solution and hold it lightly in contact with the filter-paper strip at the point shown by the pencil mark. When the contents have been completely transferred allow the paper to dry in the air for 5 to 10 minutes.

Formation of dye—Immerse a 12·5-cm Whatman No. 2 filter-paper in a shallow layer of the alkaline diazo reagent. Transfer the saturated paper to a slab of thick glass and remove the excess of liquid by applying moderate pressure with two forward strokes and one backward stroke of a rubber roller squeegee, of the type used in photographic work. Lay the filter-paper that contains the sample on top of the paper damped with diazo reagent, and place a third clean filter-paper on top of both of them. Press the sandwich of papers so formed by two forward strokes and one backward stroke of the squeegee, which is previously washed clean and wiped dry. Remove the middle paper and allow the dye, which is in the form of a compact circular zone around the pencil mark, to develop its full colour by setting the paper aside for 5 to 10 minutes. This is best done in a damp atmosphere, by placing the filter-paper circle over a petri dish containing a little water and covering the paper with another inverted petri dish. Dry the paper by placing it on a steam-heated plate for a few minutes and then cut out the 20-mm strip.

When the spot of azo dye is formed in the manner described, neither the upper nor the lower filter-paper becomes stained with dye.

Development of chromatogram—The chromatogram is developed in a room whose temperature is in the range of 17° to 19° C.

Trim the strip of paper as shown in Fig. 1. Cut a narrow tail, 15 mm long and not exceeding 2 mm in width, and cut the rest of the paper at this end of the strip away. Condition the strip for one hour in air at a relative humidity of 60 per cent. This can conveniently be done by suspending the paper from the stopper of a wide-mouthed glass bottle in which has been placed a shallow layer of an aqueous solution of glycerin containing 73 per cent. by weight of glycerin. Bend the tail back at right angles to the plane of the paper and then pass it through the central hole of the glass plate; place another glass plate of similar size but without a hole on top of the strip. Dip the tail into 1 ml of developing solvent (aqueous methyl ethyl ketone) contained in a shallow watch-glass, 40 mm in diameter, placed on the bottom of the petri dish. During development of the

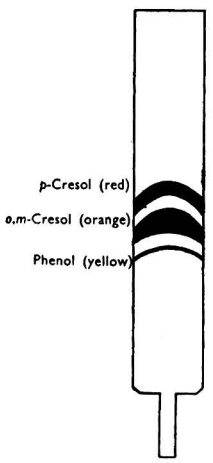

Fig. 1. Developed chromatogram

chromatogram, keep the two glass plates gently pressed together by a 100-g weight, or its equivalent, placed on the top plate. By capillary action a slow but uniform flow of developing solvent passes along the strip and moves the circular orange-coloured spot in such a way that it assumes an arc shape before it resolves into a number of similarly shaped but differently coloured bands. Allow the chromatogram to develop until the dye bands are well separated and compact; the time required for this varies from 2 to 2½ hours.

Match the colour intensity of the narrow yellow band due to phenol against the corresponding band in a series of standard chromatograms prepared from synthetic cresylic acid to which has been added known amounts of phenol. A synthetic acid containing 10 per cent. of *o*-cresol, 55 per cent. of *m*-cresol and 35 per cent. of *p*-cresol is used, although the relative proportions of these constituents may be varied. The yellow colours of the narrow band due to phenol are well gradated and whilst 0·5 per cent. is easily visible, no difficulty has been experienced in assessing quantities of phenol up to 3 or 4 per cent. Standard chromatograms, if stored between glass plates and in the dark, keep for several weeks without fading.

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

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1. Hossfeld, R. L., *J. Amer. Chem. Soc.*, 1951, **73**, 852.
2. Rutter, L., *Analyst*, 1950, **75**, 37.

S. A. ASHMORE
H. SAVAGE
GOVERNMENT LABORATORY
STRAND, LONDON, W.C.2

March, 1952

Ministry of Food

STATUTORY INSTRUMENT*

1952—No. 1124. The Meat Products (No. 2) Order, 1952. Price 9d.

This Order, which came into operation on June 15th, 1952, replaces the Meat Products Order, 1952 (S.I., 1952, No. 507; Analyst, 1952, 77, 275). The principal changes are—

The restriction on the use of soya in the manufacture of sausages is removed.

The provisions that skimmed milk powder and fat of vegetable origin may in certain circumstances count towards the meat content of sausages are removed.

The definition of "meat product" is amended to include "soup."

Mortadella sausages and beef brawn are added to the list of excepted products.

British Standards Institution

NEW SPECIFICATIONS†

B.S. 611:1952. Petri Dishes. Price 2s.
B.S. 770:1952. Methods for the Chemical Analysis of Cheese. Price 2s.
B.S.1079:1952. Haemoglobinometers Haldane Type. Price 2s.

DRAFT SPECIFICATIONS

A FEW copies of the following draft specifications, issued for comment only, are available to interested members of the Society, and can be obtained on application to the Secretary, Society of Public Analysts and Other Analytical Chemists, 7–8, Idol Lane, London, E.C.3.

Draft Specifications prepared by Technical Committee LBC/1—Volumetric, Mouldblown and Lamp-blown Glassware.

CO(LBC)2503—Draft B.S. for Lunge Nitrometers.
CO(LBC)2501—Draft B.S. for Weighing Pipettes.
CO(LBC)2543—Draft B.S. for Soxhlet Extractors.

Draft Specification prepared by Technical Committee OSC/24—British National Committee on Soaps, Fats, etc.

CO(OSC)2495—Draft B.S. Methods of Analysis of Oils and Fats. (Draft Addendum No. 1 to B.S. 684).

Draft Specification prepared by Sub-Committee DS/13/13—Definitions for Upholstery and Bedding Fillings.

CO(DS)2681—Draft B.S. for Glossary of Terms for Fillings and Stuffings for Bedding, Upholstery, Toys and Other Domestic Articles.

Draft Specification prepared by Sub-Committee PVC/2/7—Road Paints.
CO(PVC)2328—Draft B.S. for White Line Road Paint.

* Obtainable from H.M. Stationery Office. Italics indicate changed wording.
† Obtainable from the British Standards Institution, Sales Department, 24, Victoria Street, London S.W.1.

Book Reviews

DIE CHEMISCHE ANALYSE. Edited by WILHELM BÖTTGER. Band XXXIII. NEUERE MASSANALYTISCHE METHODEN. By E. BRENNECKE, K. FAJANS, N. F. FURMAN, R. LANG and H. STAMM. Third Edition. Pp. xx + 347. Stuttgart: Ferdinand Enke, Verlag. 1951. Price DM 46 (paper); DM 49 (cloth boards).

The first edition of this well-known book on volumetric analysis, published in 1935, consisted of six monographs dealing respectively with the elimination of errors in acidimetric titrations (Brennecke), ceric sulphate as an oxidising reagent (Furman), iodate and bromate methods (Lang), chromous salts as reducing reagents (Brennecke), oxidation - reduction indicators (Brennecke) and adsorption indicators (Fajans). A second edition in 1937 had an additional short chapter on the use of alkaline permanganate (Stamm). The American translation ("Newer Methods of Volumetric Analysis," London, Chapman and Hall, Ltd., 1938) of the second edition was reviewed in the *Analyst* (1939, **44**, 309). Professor Böttger, the editor of the series, died in 1949 when the third edition of this book was nearly ready for publication. The editorial work of completing the volume was undertaken by Dr. E. Brennecke, who has also written four new chapters.

The present volume is about double the size of the first edition. It includes the subject-matter of the earlier editions, arranged as previously, with subsequent developments added. The plan of the book, therefore, remains unchanged, the main emphasis being, in general, more practical than theoretical. The chapter in the earlier editions on acid - alkali titrations is expanded to three chapters on titration errors, fluorescence indicators and the titration of weak bases with perchloric acid in glacial acetic acid solution. These are followed by a chapter on the determination of small amounts of water, depending upon estimations of the acid liberated by the hydrolysis of acid chlorides or of anhydrides or of aluminium chloride or by an iodimetric process (Karl Fischer reagent), based upon the reaction of iodine with water in the presence of sulphur dioxide and pyridine in methyl alcoholic solution. A new chapter has also been added on reductions with liquid amalgams, which were introduced by Nakazono in 1921 and have subsequently been used by many other Japanese workers. In this chapter, the advantages are considered of using liquid amalgams of zinc, cadmium, lead, bismuth and tin, instead of a column of solid amalgamated zinc as in the Jones reductor, and apparatus is described for both macro- and micro-determinations.

Most of the procedures are based upon visual methods for the determination of end-points, but electrometric methods are described and the chapter on chromous titrations deals exclusively with potentiometric methods.

Comprehensive bibliographies are included at the end of each chapter. The expansion of the literature of the subject since the appearance of the first edition is striking. To quote a single example, there are forty-eight references to papers dealing with titrations using *tris-o*-phenanthroline-ferrous sulphate, first introduced in 1931, as an oxidation - reduction indicator, compared with eight references in the first edition.

The volume contains a well arranged subject index. Micro-methods, which have been much developed since the appearance of the first edition, are indexed under the headings of micro-determinations and micro-titrations. The title of a book of this kind, dealing with "newer" methods tends to become less apt when the subject-matter of earlier editions is included unchanged after a considerable span of years. The revision is, however, thoroughly effective in bringing the volume up to date. This new edition will no doubt be widely consulted by analysts, and may be expected to find its place on library shelves amongst the standard books on chemical analysis.

A. M. WARD

Publications Received

THE PHASE RULE AND ITS APPLICATIONS. By ALEXANDER FINDLAY. Revised by A. N. CAMPBELL and N. O. SMITH. Ninth Edition. Pp. xii + 494. New York: Dover Publications Inc. 1951. Price $1.90 (paper); $5.00 (cloth boards).

MASS SPECTROMETRY. Pp. vi + 205. London: The Institute of Petroleum. 1952. Price 30s. *Report of a Conference organised by the Mass Spectrometry Panel of the Institute of Petroleum and held in Manchester, April 20th to 21st, 1950.*

INDUSTRIAL AND MANUFACTURING CHEMISTRY. Part I. ORGANIC. By GEOFFREY MARTIN, D.Sc., Ph.D., F.R.I.C., F.C.S. Revised by EDWARD I. COOKE, M.A., B.Sc., A.R.I.C. Seventh Edition. Pp. xxi + 752. London: The Technical Press Ltd. 1952. Price 90s.

BRITISH STANDARDS INSTITUTION YEARBOOK, 1952. Pp. 460. London: British Standards Institution. 1952. Price 7s. 6d.

THE CORROSION RESISTANCE OF TIN AND TIN ALLOYS. By S. C. BRITTON, M.A. Pp. 82. Greenford, Middx.: Tin Research Institute. 1952. Price 3s. 6d.

DENTAL PRACTITIONERS' FORMULARY, 1952. Pp. 28. London: The Pharmaceutical Press; The British Medical Association. 1952. Price 1s. 6d. (ordinary edition); 3s. (interleaved).

IODINE PHARMACEUTICALS. A CHECKLIST OF IODINE COMPOUNDS AND PREPARATIONS USED IN HUMAN MEDICINE. Pp. vi + 78. London: Chilean Iodine Educational Bureau. 1952. Price 2s. 6d.

HETEROCYCLIC COMPOUNDS. Volume III. POLYCYCLIC DERIVATIVES OF PYRROLE; POLYCYCLIC SYSTEMS WITH ONE NITROGEN COMMON TO BOTH RINGS; PYRINDINE AND RELATED COMPOUNDS. Edited by ROBERT C. ELDERFIELD. Pp. vi + 442. New York: John Wiley & Sons Inc. London: Chapman & Hall Ltd. 1952. Price $12; 96s.

HETEROCYCLIC COMPOUNDS. Volume IV. QUINOLINE, *iso*QUINOLINE AND THEIR BENZO DERIVATIVES. Edited by ROBERT C. ELDERFIELD. Pp. vi + 674. New York: John Wiley & Sons Inc. London: Chapman & Hall Ltd. 1952. Price $17; 136s.

NATURAL RUBBER LATEX AND ITS APPLICATIONS. No. 1. AN INTRODUCTION TO ITS ORIGIN, PROPERTIES AND MANUFACTURE. By HENRY P. STEVENS, M.A., Ph.D. Pp. 72. London: The British Rubber Development Board. 1952. Free of charge on request.

PORTRAITS OF PAST PRESIDENTS

THE custom of supplying Portraits of Past Presidents to Members of the Society and subscribers to *The Analyst*, which was discontinued during and since the war years, is to be restored.

Unfortunately it is no longer possible to supply these photogravure reproductions with all copies of *The Analyst*, as was done in the past, but a sufficient number will be printed to supply gratis copies to all who make application in advance to the Editor, *The Analyst*, 7 & 8, Idol Lane, London, E.C.3.

The first to be prepared is that of Mr. F. W. F. Arnaud, and orders for this portrait, on which the printing number will depend, should be sent in before August 30th, 1952.

CROWN AGENTS FOR THE COLONIES

LABORATORY TECHNICIAN (Male) required by the Government of Uganda for the Department of Agriculture for one tour of 30 to 36 months on Provident Fund terms with prospect of permanency. Commencing salary (including temporary allowance) according to qualifications and experience in the scale £687 rising to £1,050 a year. Outfit allowance £30. Free passages and liberal leave on full salary. Candidates not over 35 years of age should have gained Higher National Certificate or pass degree in Chemistry and be capable of supervising routine analytical work on soils and crops. Apply at once by letter, stating age, full names in block letters and full particulars of qualifications and experience and mentioning this paper to the Crown Agents for the Colonies, 4, Millbank, London, S.W.1, quoting on letter M.32115.G. The Crown Agents cannot undertake to acknowledge all applications and will communicate only with applicants selected for further consideration.

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A VACANCY exists for a Chemist/Assayer (27106/57/52) in Northern Rhodesia. The appointment is on agreement for one tour of three years in the first instance with salary in the scale £690–£1320 according to qualifications and experience. In addition a cost of living allowance of 19% of salary is payable. Income tax at low local rates; Water, light and sanitary fees also payable; Rent for Government quarters with heavy furniture, when available, not more than 10% of salary; Free passages for officer, wife and up to the cost of one adult return passage for children; Free medical attention for officer and family within Northern Rhodesia; Five days vacation leave for each completed month of resident service. Candidates, preferably between 25 and 30 years of age, should possess an Honours degree in Chemistry and would be required to undergo two years' special post-graduate training before taking up appointment. Duties will be to carry out assays and analyses of ores, minerals and rocks in the laboratory of the Geological Survey Department. Intending candidates should apply in writing to the Director of Recruitment (Colonial Service), Colonial Office, Sanctuary Buildings, Great Smith Street, S.W.1, giving brief details of their age, qualifications and experience. They should mention this paper and quote the reference number (27106/57/52).

CHEMIST with bacteriological experience required at Cambridge Factory or Bacteriologist prepared to work on Industrial Effluent disposal. Salary according to qualifications and experience. Write (quoting Ad.714), stating age, experience and salary required to Personnel Manager, Pest Control Limited, Bourn, Cambridge.

GRADUATE (Male) in Chemistry, Physics or Engineering, aged 25–30, with industrial experience not necessarily in Packaging. Starting salary according to age and experience, but not less than £550 p.a. Participation in F.S.S.U. The position entails the speedy assessment and solution of current problems in industrial packaging and the ability to write reports for the layman is essential. Applications in writing quoting reference DPTO to Printing, Packaging & Allied Trades Research Association, Randalls Road, Leatherhead, Surrey.

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

Milk Products Sub-Committee:

Report No. 1. Analysis of Condensed Milks (1).
Report No. 2. Analysis of Condensed Milks (2).
Report No. 3. Analysis of Sweetened Condensed Milk in which the Sucrose has altered during Storage. *Out of print.*
Report No. 4. Determination of Water, of Total Solids and of Fat in Dried Milk.

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

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

Essential Oil Sub-Committee:

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

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

Report No. 4. Determination of Zinc.

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

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

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

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

Fluorine in Foods Sub-Committee:

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

Sub-Committee on Vitamin Estimations. Microbiological Panel:

Report on the Microbiological Assay of Riboflavine and Nicotinic Acid.

Sub-Committee on Vitamin Estimations. Carotene Panel:

The Determination of Carotene in Green-Leaf Material. Part 1. Fresh Grass.
The Determination of Carotene in Green-Leaf Material. Part 2. Green-Leaf Materials other than Grass. (Gratis).

Sub-Committee on Vitamin Estimations. Aneurine Panel:

The Chemical Assay of Aneurine in Foodstuffs.

Tragacanth Sub-Committee:

Report No. 1. Evaluation of Powdered Tragacanth.
Report No. 2. Evaluation of Flake Tragacanth.

Soapless Detergents Sub-Committee:

Examination of Detergent Preparations.

Meat Extract Sub-Committee:

Analysis of Meat Extract.

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