THE ANALYST

A Monthly Publication dealing with all branches of Analytical Chemistry: the Journal of the Society of Public Analysts and Other Analytical Chemists

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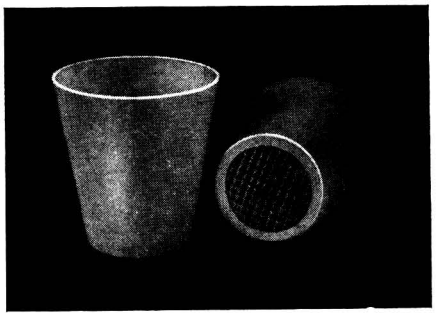

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No. 4 March, 1952

THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

BULLETIN

ANNUAL GENERAL MEETING, MARCH 7th, 1952

THE seventy-eighth Annual General Meeting of the Society was held at 5 p.m. on Friday, March 7th, 1952, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. J. R. Nicholls, C.B.E., D.Sc., F.R.I.C. The financial statement for 1951 was presented by the Honorary Treasurer and approved, and the Auditors for 1952 were appointed. The Report of the Council for the year ending March, 1952, was presented by the Honorary Secretary and adopted.

The Scrutineers reported that the following had been elected Officers for the coming year—

President—J. R. Nicholls, C.B.E., D.Sc., F.R.I.C.

Past Presidents serving on the Council—Lewis Eynon, E. B. Hughes, G. W. Monier-Williams and George Taylor.

Vice-Presidents—R. C. Chirnside, D. W. Kent-Jones and Eric Voelcker.

Honorary Treasurer—J. H. Hamence.

Honorary Secretary—K. A. Williams.

Other Members of Council—The President declared the following to have been elected Ordinary Members of Council for the ensuing two years—C. A. Adams, N. L. Allport, B. S. Cooper, N. Heron, H. E. Monk and H. C. S. de Whalley.

D. C. M. Adamson, A. J. Amos, T. McLachlan, G. H. Osborn and E. C. Wood, having been elected members of the Council in 1951, will, by the Society's Articles of Association, remain Ordinary Members of Council for 1952. Owing to the death of Dr. H. E. Cox it was necessary for only five members of the Council to retire this year and it has been decided, in accordance with Article 35, that A. A. Smales shall remain an Ordinary Member of Council until March, 1953.

A. A. D. Comrie (Chairman of the North of England Section), H. C. Moir (Chairman of the Scottish Section), C. L. Wilson (Chairman of the Microchemistry Group), J. Haslam (Chairman of the Physical Methods Group) and H. O. J. Collier (Chairman of the Biological Methods Group) will be *ex-officio* members of the Council for 1952.

FORTHCOMING MEETINGS

Ordinary Meeting of the Society, April 2nd, 1952

AN Ordinary Meeting of the Society will be held at 7 p.m. on Wednesday, April 2nd, 1952, in the Meeting Room of the Chemical Society, Burlington House, London, W.1.

The following papers will be presented and discussed—

"The Determination of Traces of Arsenic in Germanium Dioxide and Tetrachloride," by S. T. Payne.

"Inorganic Chromatography on Cellulose. Part IX. The Determination of Thorium by Chromatography on Alumina and Cellulose Adsorbents and the Simultaneous Determination of Thorium and Uranium in Minerals and Ores," by A. F. Williams, B.Sc., F.R.I.C.

"Inorganic Chromatography on Cellulose. Part X. The Spectrographic Determination of Micro Quantities of Thorium Separated by Chromatography from Minerals and Ores," by G. W. J. Kingsbury and R. B. F. Temple, D.Phil.

Joint Meeting of the Microchemistry Group with the Bristol and District Sections of the Chemical Society, the Royal Institute of Chemistry and the Society of Chemical Industry, April 23rd, 1952

A JOINT Meeting of the above bodies will be held on Wednesday, April 23rd, 1952, at Bristol.

The following paper will be presented and discussed—

"The Use of Cylinder Oxygen in the Organic Micro-Determination of Nitrogen," by H. Swift and E. S. Morton.

This will be followed by a discussion on "Standard Substances for Use in Organic Micro Analysis."

Afternoon visits to the Long Ashton Research Station and to Messrs. J. S. Fry & Sons, Ltd., have also been arranged.

Meeting of the Physical Methods Group, April 8th, 1952

THE Thirty-sixth Ordinary Meeting of the Physical Methods Group will be held at 6.30 p.m. on Tuesday, April 8th, 1952, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. This meeting has been organised by the Polarographic Discussion Panel.

The following papers will be presented and discussed—

"The Measurement of Diffusion Current with Special Reference to the Tinsley Pen-Recording Polarograph," by W. Furness, B.Sc., F.R.I.C.

"Hypochlorites and the Dropping Mercury Electrode," by E. N. Jenkins, M.Sc., A.R.I.C.

"The Polarographic Determination of Tellurium in Selenium," by G. H. Osborn, F.R.I.C., A.M.Inst.M.M., and J. G. C. Cobb.

The next meeting will be held on Friday, May 16th, in Swansea, on the subject of "Ion Exchange Resins."

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 Evaluation of Amoebicidal Substances *in vivo*," by L. G. Goodwin.

> The occurrence of *Entamoeba histolytica* in man and other hosts is described. The effect of drugs on this infection in man and animals, and particularly of emetine, diodoquin or carbarsone on rats, is detailed.
>
> The interference of *E. muris* in the treatment of amoebiasis is also mentioned.

"The Evaluation of Chemotherapeutic Agents Directed Against Trypanosome Infections," by E. M. Lourie.

> Refined statistical methods have been invaluable for the development of techniques of biological standardisation, but in the search for new compounds of clinical value they are useless or deceptive unless appropriate *experimental* procedures are used.
>
> In the control of sleeping sickness in Africa, "mass-treatment" is tending to be replaced by "mass-chemoprophylaxis." The compounds now used for this purpose are highly effective, but are not necessarily the best that might be devised. There is accordingly need for a simple and rapid method for screening compounds as to their prophylactic activity.
>
> There is good reason to believe that the composition of certain trypanocides of the melaminyl series, already fairly extensively used in the field, cannot be guaranteed by chemical or physical means. It is therefore necessary that standard preparations of these substances should be established and that batches intended for use in man should be biologically compared with those standards.

"Rapid X-Ray Quantitative Analysis of Crystalline Powders with Particular Reference to Titanium Dioxide Pigments," by W. Hughes and H. Smith.

A simple X-ray diffraction camera is described, the action of which is based on the flat powder layer method. The camera can be constructed easily and it gives a tenfold reduction in exposure times compared with a normal 9-cm powder camera. Specimen preparation is simplified, and where large numbers of specimens are involved there is considerable saving of time in processing the film. The instrument has proved useful for very rapid qualitative and quantitative examinations of crystalline powders. The accuracy with rutile - anatase mixtures is about $\pm 2{\cdot}5$ per cent.

A second method of quantitative analysis by means of a Geiger-counter X-ray spectrometer is described. No photographic processing is required and the accuracy with rutile - anatase mixtures is probably better than ± 1 per cent.

"An Extrapolation Procedure for the Assay of Aneurine," by S. J. Prokhovnik.

A procedure for the determination of aneurine in the presence of inhibiting factors is outlined. It involves only an acidified water extraction and the oxidation of the aneurine with mercuric chloride under carefully controlled conditions. The aneurine concentration is obtained by a simple extrapolation of the "apparent aneurine" readings given by the test substance at various dilutions.

The method is quick and simple, although special care must be taken with regard to reagents and technique. It gives results that are reproducible to within 5 per cent. of each other, and gives good agreement with independent assays including the official one.

"A Polarographic Method for the Estimation of Tetrachloronitrobenzene Residues on Potatoes," by J. G. Webster and J. A. Dawson.

A method is described for the polarographic estimation of small amounts of tetrachloronitrobenzene. Details are given of the application of this method to the determination of tetrachloronitrobenzene residue on potatoes. Recovery rates are shown to be of the order of 84 to 93 per cent. from tubers treated with 10 to 500 μg of tetrachloronitrobenzene.

"The Determination of Higher Alcohols in Whisky and other Potable Spirits," by G. H. Osborn and O. E. Mott.

A critical examination of the method for the determination of higher alcohols, recommended in the report of the Royal Commission on Whisky and Other Potable Spirits (1908 Appendix Q), has been carried out. The cause of the discrepancies in reported results has been found and a modified method that gives consistent results is described. The colour developed by higher alcohols in presence of furfural and sulphuric acid can be measured photometrically or visually and can be related to the amounts of higher alcohols present.

"The Micro-Determination of Magnesium in Plant Materials with 8-Hydroxyquinoline," by J. Davidson.

A micro-method is described for estimating magnesium in plant materials. Magnesium is precipitated as the oxinate, which is then determined in acid solution by measuring the absorption at 358 mμ with a spectrophotometer.

Amounts of magnesium from 20 to 200 μg in 1 to 4 ml of solution can be estimated with an accuracy of ± 1 μg. Recovery of magnesium added to various plant materials and ash solutions ranged from 98·8 to 100·4 per cent.

Limits of interference by phosphate, oxalate and manganese have been investigated and methods for eliminating interference have been devised.

"The Determination of Sub-Microgram Quantities of Arsenic by Radioactivation. Part II. The Determination of Arsenic in Sea-Water," by A. A. Smales and B. D. Pate.

The application of radioactivation to the determination of arsenic in small samples of sea-water, taken from points off the south-west coast of Cornwall, is described. An average value of 2·6 μg of arsenic per litre, with a range of 1·6 to 5·0 μg per litre, was found.

"The Determination of Sub-Microgram Quantities of Arsenic by Radioactivation. Part III. The Determination of Arsenic in Biological Material," by A. A. Smales and B. D. Pate.

The determination of minute amounts of arsenic in "normal" biological material such as human hair, nails, urine and blood and in the internal organs of a mouse by the radioactivation method is described and results are tabulated. Amounts of arsenic as small as 10^{-10} g can be determined so that very small samples suffice. For example, the "normal" level of arsenic in human blood or urine can be readily determined in only one drop of sample.

The general advantages of the activation method are summarised.

"The Separation of Silver from Copper by Electro-Deposition from Ammoniacal Solution," by Harvey Diehl and John P. Butler.

The method of controlled cathode potential electro-deposition can be used to separate silver from copper rapidly and quantitatively. Deposition is made from an ammoniacal solution. Passage of oxygen through the solution during the electrolysis of silver prevents the formation of metallic silver throughout the solution itself and renders unnecessary the addition of hydrogen peroxide suggested earlier. The method has been applied with success to the analysis of silver solder.

'The Analysis of Zinc Residues," by E. F. Pellowe and F. R. F. Hardy.

A rapid chemical method is described for the analysis of galvaniser's ash or other zinc residues containing metallic zinc, zinc oxide and zinc chloride.

The zinc residue is shaken with ammonium acetate solution to dissolve zinc oxide and chloride. Zinc chloride is determined by titration with silver nitrate; zinc oxide plus chloride is determined by titration with potassium ferrocyanide in presence of sulphuric acid and the amount of zinc oxide is given by difference between the two determinations. Metallic zinc is determined on the residue from the dissolution stage.

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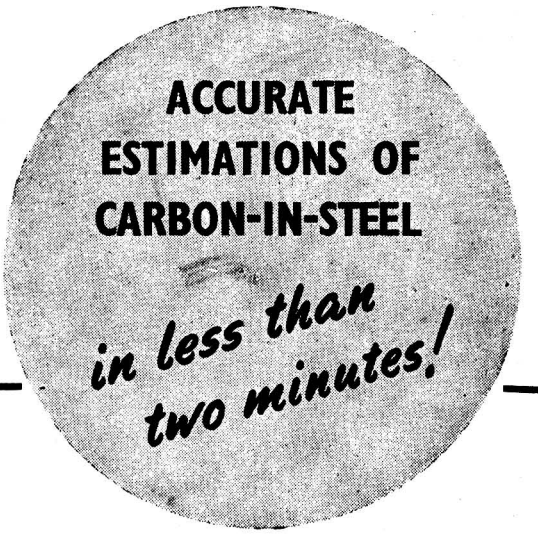

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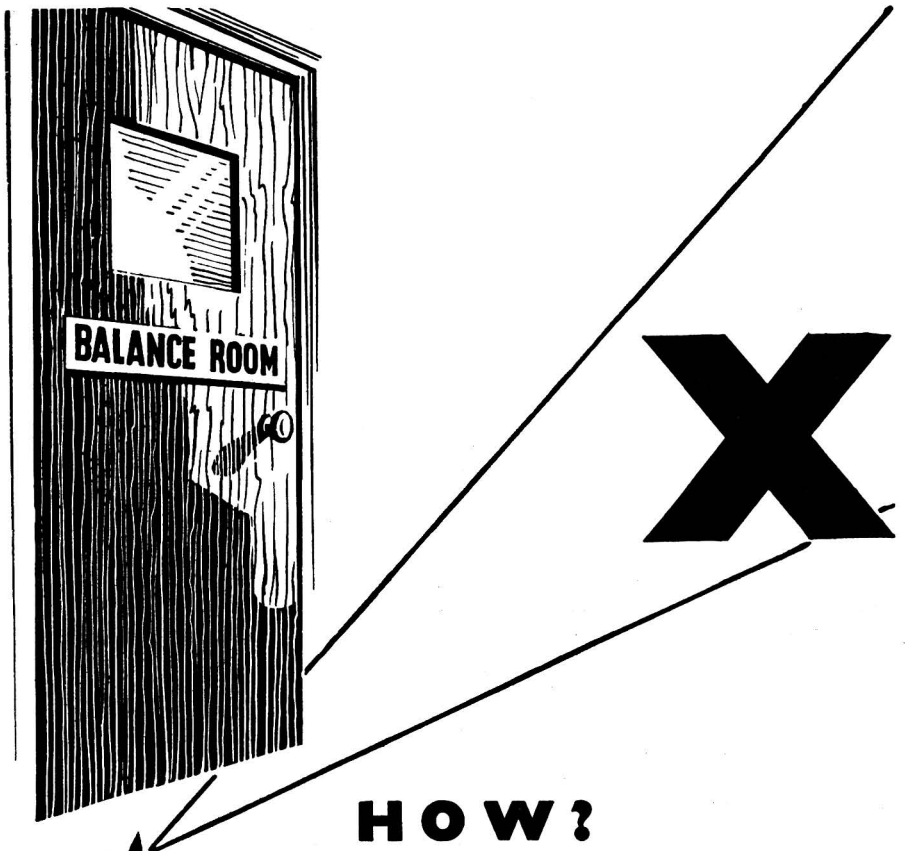

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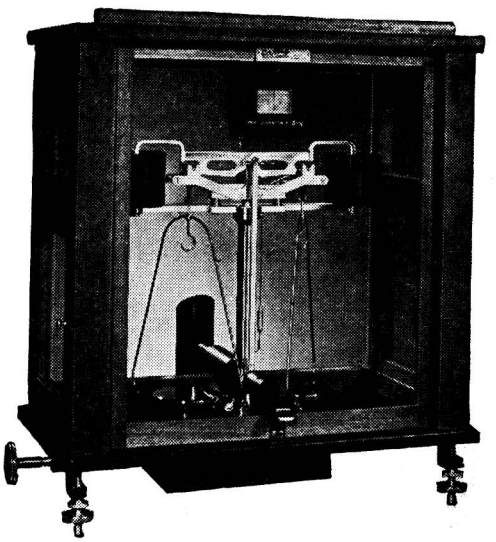

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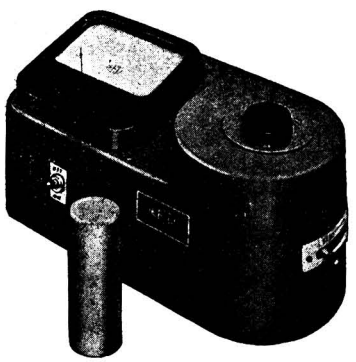

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

INFORMAL DINNER

A DELEGATION appointed by the Council of the Society, consisting of the President, Dr. J. R. Nicholls, C.B.E., Mr. R. C. Chirnside, Vice-President, and Dr. K. A. Williams, Honorary Secretary, attended the XIIth International Congress of Pure and Applied Chemistry and the 75th Anniversary Meeting of the American Chemical Society held in New York in September, 1951.

A report of these meetings was given by the members of the Delegation at an Informal Dinner held at the Park Lane Hotel, Piccadilly, London, W.1, on Tuesday, December 18th, 1951.

NEW MEMBERS

Sydney Abbey, B.Eng.Chem. (McGill); George Albert Barber, B.Sc. (Manc.); Jack Harold Defrates; Marcus Alan Ellis, M.P.S., Ph.C.; John Stanley Lea, B.Sc. (Lond.), A.R.I.C.; Bernard Milgrom; Brian Walter Mitchell, B.A. (Cantab.), B.Sc. (Lond.), A.R.I.C.; James Harry Shelton, A.R.I.C.

DEATH

WE regret to record the death of

Archibald Robert Jamieson

The Assay of Analgesic Drugs on Man

BY C. A. KEELE

(*Presented at the meeting of the Biological Methods Group on Monday, March 12th,* 1951)

The effects of morphine, amidone, pethidine and the drugs of the dithienylbutylamine series on man are described. The methods of measuring their intensity are reviewed and mechanisms for their action are suggested. The effects of the new dithienyl analgesics on ischaemic muscle pain have been studied in two ways; results and side effects are noted. A comparison of the amounts of pain relief given by different analgesics is shown.

THE effect of analgesic drugs on man is much more difficult to test, and the results are less satisfactory, than is that of antihistaminics, for which Professor Bain's methods[1] provide a high pitch of precision. The reason is simply that pain is a subjective affair, whereas the actions of antihistaminics can be demonstrated by procedures independent of a patient's opinions or feelings. It has been suggested that one could make measurements of the intensity of pain objective by recording associated reflex responses or by measuring changes in respiration, heart rate, blood pressure or sweating; such reactions are very variably related to the intensity of pain and are more closely connected with a person's reaction to pain than with the intensity itself. It is well known that increases in heart rate and sweating can occur in some people from mere anticipation of pain, for example, when sitting in a dentist's chair.

By such visceral reactions no basis for an unbiassed assessment of pain can be provided. Moreover, analgesic drugs might influence these responses by actions distinct from those by which they relieve pain.

It is thus evident that there is no satisfactory substitute for a patient's, or an experimental subject's, description of his pain. The problem is how to devise procedures that will test the reliability of a subject's statements about pain; many techniques have been used for this purpose in recent years. The tests may be divided into those suitable for studying the actions of analgesic drugs in normal volunteers and those for evaluating such drugs when they are given to patients suffering pain.

Tests in normal volunteers

Most of the methods involve the measurement of the intensity of a stimulus applied to an accessible part of the body, the smallest required to produce a sensation of pain being termed the "threshold" intensity. Among the stimuli used have been a faradic current applied to the skin (Macht, Herman and Levy[2]), graded pressure with needle pricks (Seevers and Pfeiffer[3]), radiant heat applied to the blackened skin of the forehead (Hardy, Wolff and Goodell[4]) and an alternating current applied to a dental filling (Goetzl, Burrill and Ivy[5]).

Other less precise methods include the recording of the number of muscular contractions in the forearm required to produce pain when the blood supply to this region is temporarily occluded (a method used by Harrison and Bigelow[6] in studying analgesics) and an adaptation of this ischaemic muscle pain method by Hewer and Keele,[7] which will be mentioned shortly.

The most widely used of all these tests has been the radiant heat method of Hardy, Wolff and Goodell,[4] which has also been applied to studies on animals. It must, however, now be admitted that this procedure is by no means satisfactory, since variable results have been reported by different workers studying the same drug.

A limitation applying to all the tests so far described is that analgesic drugs may relieve pain in patients by mechanisms that cannot be revealed by studies made on normal persons. Wolff, Hardy and Goodell[8] have, for example, emphasised that morphine relieves pain in three ways—

(1) By raising the pain threshold (as shown by the techniques already discussed).
(2) By detaching the reaction to pain from the perception of pain. Pain is still perceived and recognised as pain, but the accompanying apprehension is allayed. This effect is very similar to that following the operation of leucotomy, in which the nerve tracts connecting the frontal lobe with the rest of the brain are cut. Indeed, one might suggest that morphine acts by producing a "reversible pharmacological leucotomy." One cannot devise experimental conditions for assessing this type of drug action.
(3) By inducing lethargy and sleep.

It may be of value to describe the results of investigations made at the Middlesex Hospital by my colleagues and myself on some drugs recently given to me by the Wellcome Research Foundation for tests of their analgesic actions on man. They include some derivatives of amidone (Physeptone) and also a new series of dithienylbutylamine compounds, the analgesic effects of which on dogs have been reported by Adamson and Green.[9]

Actions on ischaemic muscle pain—

The effects of the new dithienyl analgesics on ischaemic muscle pain have been studied in two ways, (*a*) by observing the effects on maintained ischaemic muscle pain, the drug being injected intravenously, and (*b*) by observing the effects on the number of muscular contractions required to produce ischaemic muscle pain, the drug being injected intramuscularly.

Maintained ischaemic muscular pain—The method used here is to make the subject clench and unclench his fist once a second after the circulation to the arm has been occluded by raising, to about 220 mm of mercury, the pressure in a sphygmomanometer cuff applied to the upper arm. After about 30 to 50 contractions, pain is felt in the muscles of the forearm; when it is described as being of slight or moderate intensity the contractions are stopped, with the occlusion still maintained. The subsequent course of the intensity of the pain as experienced by a well-trained subject is shown in Fig. 1 in the curve labelled "control." The pain is assessed subjectively and in the control curve an intravenous injection of saline was

given when the pain was of moderate intensity. This injection had no influence on the steady increase in pain, which became very severe 6½ minutes after stopping the muscular contractions. At this point the cuff was released and the pain characteristically fell to zero within a few seconds.

The effect of an intravenous injection of 1 mg of morphine sulphate is also shown in Fig. 1. With this the pain of moderate intensity was almost abolished two minutes after starting the injection, and then the intensity of pain increased rapidly. By this procedure the analgesic potency of the dithienylbutylamines has been compared with that of morphine, amidone and pethidine in five persons having much experience of the technique. With each drug the aim was to find the threshold dose that would give significant relief of pain, each dose being given on a separate day. Table I shows the figures obtained with subject J. W. M. The threshold doses of the various drugs required to relieve pain can be seen from the table and the differences in potency are obvious. The occurrence of side effects will be discussed later.

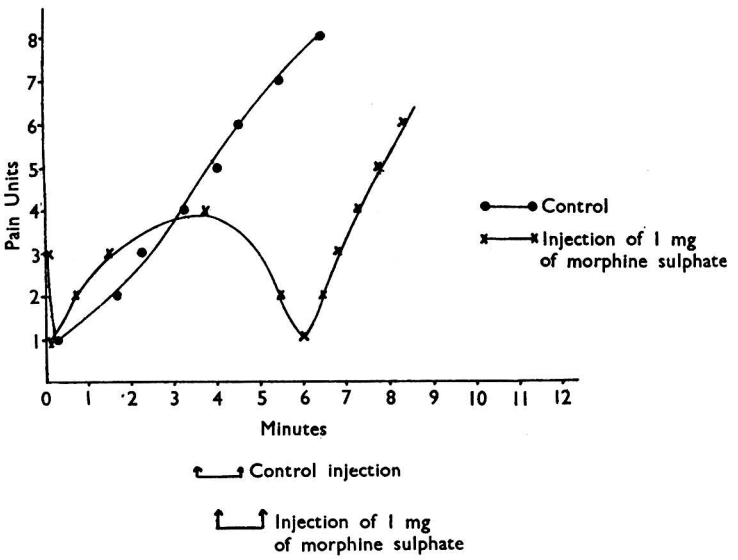

Fig. 1. Effect of morphine on ischaemic muscular pain (subject J. W. M.)

The blood supply to the forearm was occluded by means of a sphygmomanometer cuff applied to the upper arm. After 50 ischaemic muscular contractions the arm was allowed to rest with circulation still occluded. Injections given intravenously during one minute. Control: 0·9 per cent. solution of sodium chloride. Pain units: a subjective pain scale

In Table II the threshold doses are recorded for all five subjects studied. The data are incomplete, but the drugs have been set out in the table in their approximate order of potency with morphine, the most potent, on the left and pethidine, the weakest, on the right. This estimate of the analgesic potency of the dithienyl compounds places them somewhere between morphine and amidone, on the one hand, and pethidine on the other. This information is most helpful in choosing the dose for intramuscular injection, and subsequent work on patients has confirmed this estimate of the relative potency of these new compounds.

Muscular contractions producing ischaemic muscle pain—The actions of one of the dithienylbutylamines (191 C 49) were compared with those of morphine by the technique described by Harrison and Bigelow.[6] The blood supply to the forearm was occluded and the number of ischaemic muscular contractions, at a rate of 1 per second, required to produce slight and moderate pain were counted. After a preliminary run the drug was injected intramuscularly and the test as described above was repeated every half hour for 3 to 4 hours. This method was used on twenty student volunteers and an example of an effective analgesic action is shown in Table III. It will be noted that after injection of 10 mg of morphine sulphate and 25 mg of compound 191 C 49 the number of ischaemic muscular contractions

required to produce each grade of pain was greater than before the drugs were given; injection of 0·9 per cent. sodium chloride solution showed that the number of contractions required to produce pain in the absence of drugs tended to diminish in the course of 3 hours.

TABLE I

THE EFFECT OF ANALGESIC DRUGS ON ISCHAEMIC MUSCULAR PAIN IN SUBJECT J. W. M.

Drug	Amount, mg	Pain relief	Side effects
Morphine	1·0	marked	marked
	0·5	none	none
Amidone	3·0	marked	marked
	1·0	marked	none
	0·5	marked	none
	0·5	doubtful	doubtful
Pethidine	5·0	marked	marked
	2·5	none	doubtful
Dithienylbutylamine series—			
191 C 49	7·5	marked	marked
	5·0	slight	doubtful
	2·5	none	none
268 C 49	5·0	marked	none
	2·5	doubtful	doubtful
489 C 49	7·5	slight	marked
	5·0	none	marked
1 C 50	5·0	marked	marked
	2·5	marked	none

However, it must not be assumed that the results recorded with subject H. J. C. are typical. Indeed, less than half the normal subjects in these tests gave consistent results and a few even showed less response to an analgesic drug than to saline. Mr. P. A. Young, statistician to the Wellcome Research Foundation, analysed the results of this part of the work and suggested that the figures gave more information about the psychological make-up of the subjects than about the analgesic potency of the drugs used. This conclusion seems correct and it should be emphasised that it applies perhaps even more to patients when they are used for tests on analgesic drugs.

TABLE II

EFFECTS OF INTRAVENOUSLY INJECTED ANALGESICS ON MAINTAINED ISCHAEMIC PAIN IN FIVE SUBJECTS

Subject	Threshold doses of drugs						
	Morphine, mg	Amidone, mg	I C 50, mg	268 C 49, mg	191 C 49, mg	489 C 49, mg	Pethidine, mg
J. W. M.	1·0	0·5	<2·5	5·0	5·0	7·5	5·0
R. L. M. D. ..	1·0	2·5	7·5	>2·5	5·0	7·5	10·0
H. J. C.	>2·0	2·0	1·0	2·5	—	5·0	10·0
F. D.	>0·5	3·0	5·0	5·0	—	>5·0	7·5
L. P.	>2·0	2·0	>2·5	2·5	—	2·5	5·0

NOTES—The drugs are arranged in order of decreasing potency from left to right. The signs < and > are used to indicate that the threshold value has not been accurately found, but is below or above the value given.

The side effects of these drugs are summarised in Table IV. The figures require a few comments. The occurrence of drowsiness or sleep after injection of saline in seven out of sixteen persons was undoubtedly the result of the experiments being done during the Christmas vacation, when late nights were frequent and possibly encouraged by the prospect of an opportunity for quiet repose during the tests on the following afternoon. However, we concluded that the hypnotic effect of compound 191 C 49 was greater than that of morphine. The occurrence of nausea in two subjects and vomiting in the third of three subjects who were given the dithienyl compound 268 C 49 raises a difficult problem of assessment. At

first we decided that this drug should not be tested further, because the production of nausea and vomiting in such trials is bad for the morale of the team, but later we decided to give 268 C 49 another chance, and it has since been given to about twenty-five students and patients, with no further instances of vomiting that could be attributed to the drug. From observations on only a few human subjects it is particularly difficult to draw valid conclusions.

In connection with gastric complications, it may be legitimate to digress for a few moments to describe a test on sensitivity to oral administration of piperidyl*iso*amidone. It is well known that administration of amidone by mouth frequently causes nausea and vomiting, so experiments were done to determine whether or not piperidyl*iso*amidone would

TABLE III

EFFECTS OF ANALGESICS ON THE NUMBER OF ISCHAEMIC MUSCULAR CONTRACTIONS REQUIRED TO PRODUCE PAIN IN SUBJECT H. J. C.

	Number of contractions required to produce pain with 10 mg of morphine sulphate		Number of contractions required to produce pain with 25 mg of 191 C 49		Number of contractions required to produce pain with saline (0·9% NaCl)	
Time, hours	Slight pain	Moderate pain	Slight pain	Moderate pain	Slight pain	Moderate pain
0	47	57	55	62	55	62
0·5	53	64	59	67	45	56
1·0	57	66	61	82	51	58
1·5	60	67	61	73	54	60
2·0	49	60	72	83	47	55
2·5	65	78	66	79	49	65
3·0	59	67	62	79	40	55
3·5	50	58	57	82	—	—
4·0	—	—	43	61	—.	—

NOTE—Injections were given intramuscularly at time 0.

be better tolerated. It was known that the latter was about one-fifth as potent an analgesic as amidone, so we compared the effects of 5 and 10 mg of amidone with 25 and 50 mg of piperidyl*iso*amidone, lactose being used as a control. The tablets containing the three different materials were identical in appearance and were labelled A, B and C. The tests were carried out on eight student volunteers and two patients, the students taking their tablets during the evening after they had reached home, so that when nausea and vomiting did occur the inconvenience was minimal. Oral administration of 50 mg of piperidyl*iso*amidone produced nausea in all normal subjects and caused vomiting in six, but an equianalgesic dose (10 mg) of amidone caused nausea in three normal subjects and vomiting

TABLE IV

SIDE EFFECTS AFTER INTRAMUSCULAR INJECTION

Drug	Number of subjects	Effect				
		Drowsiness or sleep	Euphoria	Dizziness	Nausea	Vomiting
191 C 49	22	17	8	9	1	0
Morphine	13	7	1	4	3	0
268 C 49	3	3	2	2	2	1
Saline	16	7	0	0	0	0

in only one. A 5-mg dose of amidone administered to the eight normal subjects produced no nausea or vomiting. The two patients were given doses of 25 and 50 mg of piperidyl*iso*amidone; nausea and vomiting were produced in both. Thus in this instance we were able to affirm that piperidyl*iso*amidone was worse, not better, tolerated than amidone.

OBSERVATIONS ON PATIENTS WITH PAIN

All methods used for studying the actions of analgesic drugs in normal persons have finally to be checked by studies on patients with pain severe enough to require relief by drugs. Indeed, the methods so far described are to be regarded as simply introductory to the clinical investigation, although they serve a most useful purpose in indicating the dosage

that can be safely and effectively given to patients. However, clinical studies have difficulties of their own and it is worth while considering how they may be tackled.

First, one has to realise that the pain of disease is often highly variable in its course and that many factors influence it, so that the background against which the drug action is to be evaluated is a changing one. Secondly, pain being subjective, one must devise methods to check the reliability of a patient's observations. Finally, there is the question of the

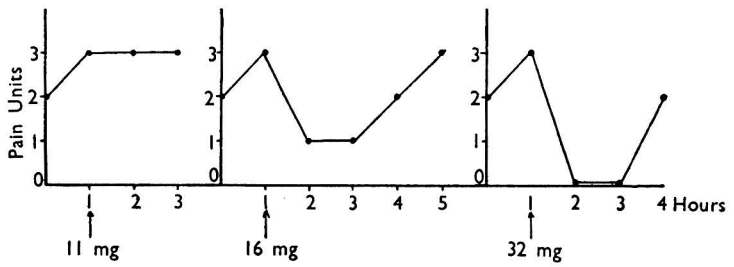

Fig. 2. Effects of morphine on pain associated with carcinoma of the lip (patient M. J.)

Injections of morphine sulphate at times indicated by arrows. Pain units: 1 = slight pain; 2 = moderate pain; 3 = severe pain

number of patients necessary for a satisfactory answer. My own view is that it is best at first to restrict the observations to a few carefully selected patients with chronic pain who are likely to require repeated administrations of analgesic drugs for a few weeks. They must, of course, be co-operative, sufficiently intelligent and not too ill to keep accurate records or pain charts. There are few patients who can fulfil these requirements, but it is worth waiting for them, as the information they can give about a new drug, which is at first likely to be available only in small quantities, may be most helpful in deciding whether or not further investigations should be made.

These points can be illustrated by reference to some observations recorded on one patient during the studies on the dithienyl compounds. The effects of some of these drugs were compared with those of morphine on a man of 48 who had a carcinoma of the lip that,

TABLE V

EFFECT OF INTRAMUSCULAR INJECTION OF MORPHINE SULPHATE ON PATIENT M. J.

Dose		Pain		Number of occasions
mg	grains	Before injection	After injection	
11	$\frac{1}{6}$	severe	severe	2
16	$\frac{1}{4}$	severe	none	6
16	$\frac{1}{4}$	severe	slight	7
16	$\frac{1}{4}$	severe	moderate	4
16	$\frac{1}{4}$	severe	severe	0
16	$\frac{1}{4}$	moderate	none	2
32	$\frac{1}{2}$	severe	none	6
32	$\frac{1}{2}$	severe	slight	2
32	$\frac{1}{2}$	severe	moderate	0

in spite of excision, had extended to destroy part of the right lower jaw and had also invaded the floor of the mouth and the neck. The growth was still active and caused severe persistent pain in the right jaw and right side of the mouth, and spread thence to the neck. Analgesic therapy was urgently required as no further excision of the growth was possible. The patient was most willing and able to co-operate in keeping pain charts and Fig. 2 shows typical records obtained after the intramuscular injection of 11, 16 and 32 mg of morphine sulphate. The "Pain Units" refer to the intensity of pain as described by the patient; 1 = slight pain, 2 = moderate pain and 3 = severe pain. The patient did not know that the doses of different injections varied, so his variations in response provide good testimony of his discriminatory powers. In order to show that these charts are representative, his responses to many doses of morphine have been summarised in Table V. The figures in the table refer to the number of occasions on which a given dose of morphine sulphate caused different degrees of relief.

The intensity of pain before and after injection of the drug is recorded and the right-hand column shows how often the various responses were observed.

The effects of three of the dithienyl compounds were similarly recorded and Fig. 3 shows the effect of compound 489 C 49 of this series. The most noteworthy feature of its action was the production of sleep as the pain was relieved, an effect that was confirmed by observations on a few other patients and agrees with our findings on the student volunteers.

Thus the work on normal persons and on selected patients had shown that these dithienylbutylamines had an analgesic action usually associated with a definite hypnotic effect. Such properties would be most useful in treating post-operative pain or in helping to procure sleep in patients kept awake by pain during the night. As Keats, Beecher and Mosteller[10] have emphasised, the only really satisfactory way of studying the effectiveness

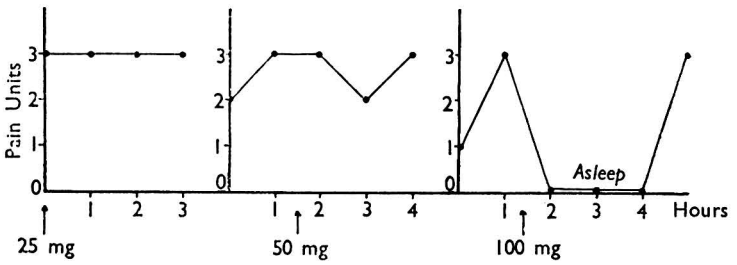

Fig. 3. Effects of the dithienylbutylamine compound 489 C 49 on pain associated with carcinoma of the lip (patient M. J.)

Injections given at times indicated by arrows. Pain units: 1 = slight pain; 2 = moderate pain; 3 = severe pain

of analgesic drugs is to observe their actions on patients under conditions likely to evoke the drugs' therapeutic value. An investigation on these lines is now being carried out at the Middlesex Hospital.

There is an aspect of this type of clinical trial worthy of further comment. In many such investigations stress has been rightly laid on the need for controlling not only the patient's but also the observer's records, this being done by concealing the identity of the drug being given. When the actions of two well-tried drugs are being compared this is doubtless a justifiable procedure; but when a new drug, whose properties are not fully known, is being compared with a standard drug, it is essential that the nature of the drugs given should be known to the observer in the early stages of the clinical trial so that he can watch for any undesirable side actions and detect them as soon as possible. In any event the most important factor is the accurate recording of the findings, and in such pilot experiments one may come across actions of new drugs that at once show them to have no future in therapeutics. It is then unnecessary to go to the trouble of making a more elaborate controlled therapeutic trial.

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The *Staphylococcus aureus* Plate Assay of Penicillin: Effect of Sugars on Zone Edges and Accuracy of Results

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The presence of sugars in penicillin test solutions effects markedly the definition at the edges of the zones of inhibition in the cylinder plate assay method. The incorporation of 0·1 per cent. of sucrose in a typical *Staphylococcus aureus* nutrient agar medium greatly improves the definition of the zone edges. The presence of sugars in penicillin test solutions increases the zone diameters and may cause fictitiously high results to be recorded. For accurate work the standards must be compensated by incorporating appropriate sugars in similar concentrations to those present in the test solutions. The regression line obtained by plotting the logarithms of the concentrations against the zone diameters in a medium containing 0·1 per cent. of sucrose has a slight positive curvature, approximating to a maximum error of 3 per cent. if the curvature is ignored in calculating results over the range 2 to 8 units per ml.

In assaying penicillin by the plate method it is of the greatest importance that the edges of the zones of inhibition shall be sharply defined so that their diameters can be accurately measured, for very small differences in zone diameters correspond to large differences in potency of the test solution. When *Bacillus subtilis*[1] is used as the test organism, the edges of the zones are extremely well defined and eminently suitable for accurate measurement. According to the British Pharmacopoeia and the Food and Drug Administration of the U.S.A.,[2] *Staphylococcus aureus* has to be used as the test organism in penicillin assays, although the strain to be used is not stipulated in the British Pharmacopoeia. The most commonly recognised are the Oxford strain NCTC 6571 and the American F.D.A. strain NRRL 313. The former usually gives poorly defined zones and the latter somewhat better zones when used as a surface inoculum. Surface inoculation, however, needs considerable care if accurate results are to be obtained and is uneconomical in time and effort, especially when used on a large scale in industrial laboratories. The strains of *S. aureus* mainly used are therefore those that best lend themselves to bulk inoculation and at the same time give the required sensitivity. Under routine working conditions, however, even the best strains of *S. aureus* do not give zones of inhibition equal to those obtained with *B. subtilis*; the most commonly occurring type of zone possesses a halo and some of them resemble Liesegang rings. These phenomena are no more than a nuisance to the analyst, but have led to much interesting work, notably by Pulvertaft, Greening and Haynes[3] and more recently by Ingram[4]; so far, however, no specific effect appears to have been traced to any particular substance in the test solutions or in the nutrient agar.

In the course of routine assay work we observed that the edges of the zones of inhibition varied in definition and general appearance with different samples. The possibility that these effects were produced by differences in working conditions such as variation in inoculum culture, degree of refrigeration, rate of warming in an incubator and so on, were eliminated by producing various types of zones on the same plate with penicillin solutions that contained different substances. Further work was therefore undertaken to trace the agents causing variation and to find suitable agents for the production of zones of inhibition with sharply defined edges.

It is well known that when certain dissolved substances are present in penicillin test solutions larger zones are obtained than when they are absent; these larger zones give fictitiously high assay results if normal simple solutions of penicillin are used as standards. In practice, the effect of dissolved substances on results has been largely ignored with such products as penicillin lozenges,[2] perhaps because it was assumed that the degree of dilution was sufficiently large to reduce errors from this source to negligible amounts. The work described in this paper shows that this is not always so, and that sometimes the assay results can be significantly too high if the effect of sugars is ignored. In the course of work on

lozenges it was observed that the test solutions frequently produced zones of inhibition with well defined edges that often consisted of a single ring of increased bacterial growth permitting measurement of the zone diameters to be accurately made. Preliminary investigation of this phenomenon showed that the causal agent was sucrose. Work was carried out to determine more precisely the effects of the commonly used sugars, sucrose, lactose and dextrose, on the size of the zones of inhibition and on the definition of the zone edges.

Experimental

The cylinder plate assay method was used. The medium was as follows—

Agar	2·0 per cent.
Lemco	0·3 "
Peptone (Oxoid)	1·0 "
Marmite (yeast extract)	0·15 "
Sodium chloride	0·3 "
Tap water to	100 "

The pH was adjusted to 7·5 before sterilising.

The effect on zone diameters and zone edges of sugars in test solutions—

Solutions containing 0·5 unit of benzyl penicillin (sodium salt) per ml and 0·05, 0·1, 0·2 or 0·4 per cent. of sucrose, lactose or dextrose in 0·01 *M* phosphate buffer were compared with a standard solution (0·5 unit per ml) of penicillin in 0·01 *M* phosphate buffer on each of twenty plates. Typical results in terms of mean zone diameters together with observations on the zone edges are shown in Table I.

Table I

Mean diameters of zones of inhibition of solutions of different sugars each containing 0·5 unit of penicillin per millilitre

	Concentration of sugar, %	Zone diameter		Mean increase in zone diameter, mm	Appearance of zone edges
		Expt. 1, mm	Expt. 2, mm		
Sucrose—	0·0	12·81	13·22	—	Standards had haloes with diffuse inner edges. Sucrose solutions gave zones with very strong inner edges, decreasing in intensity with decreasing concentrations of sucrose
	0·05	13·06	13·31	0·17	
	0·10	13·17	13·75	0·45	
	0·20	13·46	14·10	0·77	
	0·40	14·27	14·91	1·58	
Lactose—	0·0	13·41	15·62	—	Standards and lactose solutions occasionally gave zones alike in appearance, but on other occasions well defined zones resulted, especially with higher concentrations of lactose
	0·05	13·43	15·58	−0·04	
	0·10	13·45	15·65	0·04	
	0·20	13·35	15·80	0·06	
	0·40	13·38	15·84	0·10	
Dextrose—	0·0	13·50	14·34	—	The zones of the dextrose solutions had more sharply defined edges than the standards but were less pronounced than those of the sucrose solutions. In some experiments three Liesegang rings were observed
	0·05	13·46	14·69	0·16	
	0·10	13·53	14·76	0·23	
	0·20	13·98	15·16	0·65	
	0·40	14·55	15·56	1·14	

It can be seen from these figures that a marked increase in zone diameter occurred with sucrose and glucose in solution. The zone diameter increased with increase in concentration of the sugar. The effect of sucrose was a little greater than that of glucose. It is interesting to note that lactose gave no significant increase in zone diameter at any of the concentrations used.

The effect of the sugars on the zone edges was remarkable. The effect varied according to the nature of the sugar used and also according to its concentration. It was found to be important to make use of efficient refrigeration between the operations of pouring and potting the plates, or ill-defined zone edges mostly resulted; many of these zone edges had a miscellaneous variety of Liesegang ring effects. Under well controlled conditions it was found that

sucrose generally gave clearly defined circles, often with a well defined edge of heavy bacterial growth. Glucose produced a similar effect, but the circles were usually less well defined than those obtained with sucrose, and occasionally Liesegang rings were observed. Lactose usually had less effect, but sometimes zone edges were sharper and occasionally Liesegang rings were formed.

Similar effects were produced with concentrations of penicillin of 1·0, 2·0 or 8·0 units per ml, but with 8·0 units per ml the zone edges as a rule were less well defined. Generally an increase of sucrose or glucose concentration had the effect of reducing the width of the halo.

EFFECT OF SUCROSE IN THE NUTRIENT MEDIUM ON THE DEFINITION OF ZONE EDGES—

In view of the results obtained with sugars present in the test solutions, experiments were made to find out whether sucrose in the medium produces the same effects on zone edges as when present in the test solutions. Various concentrations of sucrose were added to the medium. Media were prepared containing 0·025, 0·05, 0·1 or 0·2 per cent. of sucrose. Plates were prepared from each of these media in the usual way. The cylinders were filled with standard solutions of penicillin, ranging in potency from 0·25 to 4·0 units per ml, in 0·01 *M* phosphate buffer and the plates were placed in an incubator overnight at 37° C.

The plates containing 0·025 or 0·05 per cent. of sucrose in the medium had zones of inhibition with haloes and indistinct edges; those with media containing 0·1 per cent. of sucrose gave zones with haloes, but the inner edges of the zones possessed a very dark narrow ridge of heavy bacterial growth, which was ideal for measuring zone diameters; those with 0·2 per cent. of sucrose in the medium had zones similar to those given by 0·1 per cent. of sucrose, but the inner dark ridge of growth was slightly blurred, although thicker.

When a medium containing no sugars is used, the outer edge of the halo is usually easier to read than the inner edge and is regarded as the zone perimeter, but when sucrose is added to the medium the inner edge becomes the easier one to read and in some instances there is no definable outer edge at all. This has the apparent effect of reducing the zone diameter, but the zone diameters of the corresponding edges are greater when sucrose is incorporated in the medium.

Further experiments incorporating glucose or lactose into the medium showed that lactose usually improved the zone edges, but glucose, although it gives considerable improvement, was not so efficient as sucrose.

EFFECT OF SUGARS IN TEST SOLUTIONS ON ZONE DIAMETER WITH NUTRIENT MEDIA CONTAINING 0·1 PER CENT. OF SUCROSE—

This experiment was made to determine whether or not the increase in zone diameters caused by the presence of sugars in the test solutions, with a normal medium, still occurred when a modified medium containing 0·1 per cent. of sucrose was used.

Four solutions were prepared each containing 1·0 unit of penicillin per ml in 0·01 *M* phosphate buffer. One of these solutions also contained 0·2 per cent. of glucose, another 0·2 per cent. of sucrose and a third 0·2 per cent. of lactose; the fourth solution was kept for a blank determination. A fifth solution containing 0·5 unit of penicillin per ml in 0·01 *M* phosphate buffer was prepared to enable the slope of the regression line to be found. The concentration of sugar (0·2 per cent.) in each solution was chosen because it can occur when testing lozenges, but is unlikely to be exceeded in any other normal assay.

The results of seventeen replicate tests show that under these conditions the apparent potency of penicillin is 19 per cent. too high in a solution containing 0·2 per cent. of sucrose, and 10 per cent. too high in a solution containing 0·2 per cent. of glucose. No apparent increase was obtained with 0·2 per cent. of lactose. The effect of the sucrose and glucose on the zone edges was marked, the zones being extremely sharply defined in most tests.

In order to determine the effects of greater dilutions of sucrose, a further experiment was made in which sucrose solutions each containing 1·0 unit of penicillin per ml were assayed against the 0·01 *M* buffer containing 1·0 unit of penicillin per ml. The concentrations of sucrose used were 0·4, 0·2, 0·1, 0·05 and 0·025 per cent. and the six solutions were compared on each of twenty plates. The results are shown in Table II. By plotting the values of "zone diameter" against "concentration of sucrose" a nearly linear regression line is given. It follows, therefore, that the effects of sucrose cannot be eliminated by dilution and consequently accurate results can only be obtained by adequately compensating the standards.

The effect of sucrose in solutions containing higher concentrations of penicillin, *viz.*, 4·0 and 8·0 units per ml, was also determined and the results, together with those at the 1 unit per ml level, are shown in Fig. 1. In these solutions up to 1 per cent. of sucrose was used; the zones produced were exceedingly well defined at these high concentrations, but the effect of sucrose on apparent potency was still present, although relative to the actual potency of the solution the effect becomes smaller with increase in penicillin potency.

TABLE II

EFFECT OF SUCROSE IN TEST SOLUTIONS CONTAINING 1 UNIT OF PENICILLIN PER MILLILITRE ON ZONE DIAMETER

Concentration of sucrose, %	Zone diameter, mm	Mean zone diameter, mm	Mean apparent potency, units per ml
0·4	17·11, 17·31, 16·53	16·98	1·47
0·2	16·62, 16·53, 15·84	16·33	1·22
0·1	16·30, 16·30, 15·47	16·02	1·12
0·05	16·10, 16·11, 15·34	15·85	1·06
0·025	16·08, 15·92, 15·28	15·76	1·03
nil	15·95, 15·89, 15·18	15·67	1·00

The effect of sucrose on results obtained with normal and compensated standards in assays of lozenges was verified on a number of samples of lozenges in the following manner.

Samples of penicillin lozenges were assayed after dissolving approximately 1·2 g of sample in 0·01 *M* buffer and diluting to 500 ml. As the lozenges were composed chiefly of sucrose, the resulting concentration of sucrose was about 0·2 per cent. The penicillin standard solutions used for the tests contained (*a*) 0·01 *M* buffer and (*b*) 0·01 *M* buffer and 0·2 per cent. of sucrose. The results obtained for both conditions are given in Table III and show the marked difference between normal and compensated standards. It is apparent from this and the previous work that, in the assay of these lozenges, the standards must contain the same amount of sucrose as the sample. In view of the difference in effect on zone diameter produced by different sugars, it is necessary to know the composition of the lozenges before accurate compensation can be made. For normal production samples this is already known and no difficulty arises, but for other samples, an analysis of the sugars in the lozenge base must be made before the assay of penicillin is attempted.

TABLE III

ASSAYS OF PENICILLIN LOZENGES WITH AND WITHOUT COMPENSATED STANDARDS

	Potency, units per lozenge	
Sample	With normal standards	With compensated standards containing sucrose
A	600	500
B	640	550
C	620	500
D	600	490
E	650	540
F	700	600
	635 (Mean)	530 (Mean)

LINEARITY OF DOSE - RESPONSE CURVE FOR MEDIUM CONTAINING 0·1 PER CENT. OF SUCROSE—

Before accepting the increased accuracy available from the improved definition of the zone edges arising from the use of a nutrient medium containing 0·1 per cent. of sucrose, it was necessary to check the effect of the modified medium on the linearity of the dose - response curve. For this purpose the mean zone diameters obtained over a range of concentration of penicillin from 0·5 to 16 units per ml were measured. Six concentrations of penicillin, 16, 8, 4, 2, 1 or 0·5 units per ml, were tested on each of twenty plates on several days. The mean diameters measured on each twenty replicate plates were as follows—

Concentration of penicillin, units per ml	16	8	4	2	1	0·5
Mean zone diameter, mm	24·99	23·18	21·31	19·26	16·96	14·26
Difference between intervals, mm		1·81	1·87	2·05	2·30	2·70

The regression line exhibits a considerable degree of curvature, but if a narrow range only is used in the assay the error from this source is small. With standards of 2 and 8 units per ml a working range for the assay of 1·5 to 16 units per ml is possible with a maximum error due to curvature of less than 4 per cent.; if three standards, *viz.*, 2, 4 and 8 units per ml are taken, and the mean of the three is used in calculating the potency of the unknown, the error due to curvature becomes less than 3 per cent. Practical tests with the second of these

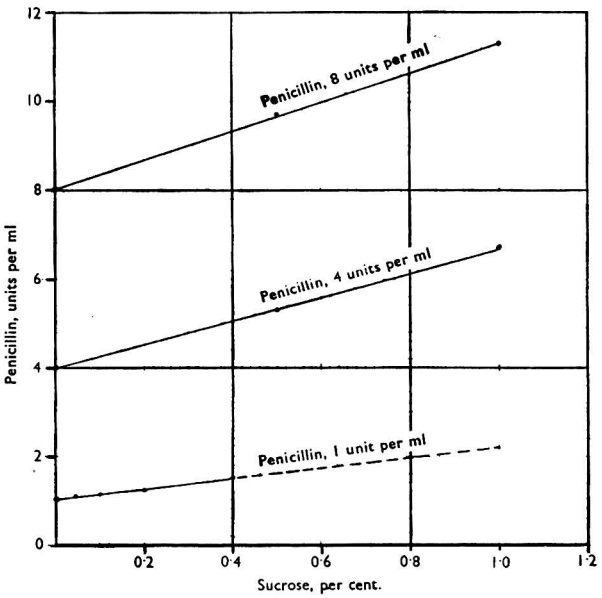

Fig. 1. Effect of sucrose in solution on apparent potency of penicillin as determined by the *S. aureus* plate assay. Full lines determined by experiment; broken line indicates extrapolation

two procedures, made by repeated assays on a large number of samples, show that the standard error of the method with six plates each carrying three zones of unknown and one of each of the three standards is approximately 4 per cent.

When compensated standards are used for tests on solutions containing considerable amounts of sugars (up to 1 per cent.), a much smaller "slope" is obtained over the range 2 to 8 units per ml, so that the method becomes less accurate. It follows, therefore, that solutions should if possible be assayed at such a concentration of penicillin that excessively high concentrations of sugars are avoided.

Conclusions

Sugars in penicillin solutions assayed by the cylinder plate method have in some instances the effect of increasing the zone diameters produced by penicillin. This leads to fictitiously high results if ordinary penicillin standards are used. Of the three sugars examined, sucrose produces the largest effect and dextrose a somewhat smaller effect; up to 0·4 per cent. of lactose is almost without effect on the size of the zones. Consequently in the assay of penicillin lozenges or other samples containing sugars the standards used must be compensated by adding the appropriate sugars.

The presence of sucrose in assay solutions gives an improved zone edge in an ordinary nutrient agar medium. A similar effect can be obtained by incorporating 0·1 per cent. of sucrose into the nutrient agar. This greatly increases the accuracy with which the zone diameters can be measured and consequently also the accuracy of the method as a whole. The curvature of the regression line for zone diameter on the logarithm of the concentration

in the sucrose medium is sufficiently small over the range 2 to 8 units per ml to permit calculation of results on a theoretically linear relationship without introducing serious error. The maximum error from this source is about 3 per cent.

Compensation of the standards for the presence of sugars in lozenges or other samples must be made by adding amounts of the appropriate sugars to make the concentrations the same as in the test solutions.

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The Determination of *p*-Nitrophenol and *p*-Nitrophenyl-O-S-diethyl Thiophosphate in Parathion

By J. C. GAGE

A method is described for the analysis of a mixture of parathion, the S-ethyl isomer of parathion and *p*-nitrophenol. The mixture is reduced, the *p*-aminophenol and reduced S-ethyl isomer are successively extracted, coupled with *o*-cresol and estimated colorimetrically as an indophenol dye. The reduced parathion is extracted, diazotised, coupled with N-sulphatoethyl-*m*-toluidine and determined colorimetrically.

In a previous communication[1] a method was described for the determination of the insecticide O-*p*-nitrophenyl-O-O-diethyl thiophosphate, usually known by the names parathion or E605. In that method parathion was reduced to the corresponding amino compound, which was then diazotised and coupled to give an azo dye, the intensity of which was measured in a suitable colorimeter. The method is not specific for parathion; a similar colour is given by many nitro compounds that react in the same manner, some of which occur in the commercial product as impurities or may be derived from parathion by chemical or physical treatment or by metabolic processes. An investigation to discover a method that would be more specific for parathion and would enable the compounds associated with it or derived from it to be separately determined was undertaken in connection with studies of residual parathion on treated crops and with metabolism experiments on laboratory animals. This paper describes a method for determining *p*-nitrophenol and O-*p*-nitrophenyl-O-S-diethyl thiophosphate, herein termed the S-ethyl isomer of parathion. *p*-Nitrophenol is derived by hydrolysis from parathion slowly in aqueous solution, but more rapidly in the presence of alkali[2]; O-*p*-nitrophenyl-O-S-diethyl thiophosphate is obtained from parathion by isomerisation above 140° C.[3]

Method

Reagents—

Toluene—"Sulphur-free" toluene purified as described below.
Hydrochloric acid—Concentrated and 0·5 *N* solutions.
o-*Cresol*—A 2 per cent. w/v solution in 0·2 *N* sodium hydroxide.
Sodium acetate—Analytical reagent quality.
iso-*Amyl alcohol*—Analytical reagent quality.

Procedure—

Reduction of nitro group—Boil "sulphur-free" toluene under reflux for 20 minutes with 2·5 per cent. of benzoic acid, 2·5 per cent. of zinc dust and a trace of aniline. Cool, filter

and wash well with 0·5 *N* hydrochloric acid. This procedure is designed to remove impurities that not only produce a high blank value but also inhibit colour development; the toluene retains sufficient benzoic acid to be suitable for the reduction without further addition of acid. Dissolve the sample to be analysed in the prepared toluene. Add about 0·5 g of zinc dust to 20 ml of the toluene solution and boil gently under an air condenser for 20 minutes. Cool and filter through a plug of cotton wool into a separating funnel; wash the residual zinc with a few millilitres of toluene and pass the washings through the filter. Shake the filtrate for 3 minutes with 10 ml of 0·5 *N* hydrochloric acid and transfer the aqueous layer into a 6 × 1-inch tube; wash the toluene layer with a few millilitres of water and add the washings to the tube.

Determination of p-*nitrophenol*—The reduced *p*-nitrophenol is coupled with *o*-cresol in presence of alkali to give an indophenol dye, which is determined colorimetrically.[4]

Add 1 ml of 2 per cent. *o*-cresol solution and 2 ml of ammonium hydroxide, sp.gr. 0·880, to the acid extract obtained after the reduction procedure. After 10 minutes pour back the whole of the solution into the separating funnel with the toluene used in the reduction; shake for 3 minutes, add 2 ml of 20 per cent. sodium hydroxide solution and finally shake for 1 minute. By this treatment the blue indophenol colour that develops if *p*-aminophenol is present remains in the aqueous phase, and other compounds that are to be subsequently determined pass into the toluene layer. Run the aqueous layer into a 25-ml flask, and wash the toluene layer with a few millilitres of water. Make the contents up to volume and, 30 minutes after the addition of sodium hydroxide, read the optical density at 615 mμ against a reagent blank. Determine the amount of nitrophenol present by comparing the reading of the optical density with a calibration curve made from readings with standard solutions of nitrophenol subjected to the same procedure.

Determination of S-ethyl isomer—Extract the original toluene layer with 10 ml of 0·5 *N* hydrochloric acid by shaking for 3 minutes in a separating funnel, run the lower layer into a tube and wash the toluene layer with a few millilitres of water. Add 1 ml of *o*-cresol solution and 2 ml of 20 per cent. sodium hydroxide solution to the acid solution. If the S-ethyl isomer is present a blue indophenol colour develops owing to hydrolysis of the reduced compound to *p*-aminophenol by the alkali. The colour reaches its maximum in about 45 minutes. After 1 hour transfer the solution back to the separating funnel containing the toluene, shake for 3 minutes, run the lower layer into a 25-ml volumetric flask and make up to volume as before. Read the optical density at 615 mμ against a reagent blank and determine the amount of S-ethylisomer present by comparing the reading with a calibration curve made for this component.

Determination of parathion—Reduced parathion is not hydrolysed by the alkaline conditions in the previous stages and is determined by extracting the resulting toluene layer with 0·5 *N* hydrochloric acid and subjecting the acid layer to the diazotisation and coupling procedure previously described.[1] Reduced parathion is not completely extracted from toluene by the procedure used in the previous stages and it is necessary to extract several times with dilute acid. Successively extract the toluene layer with 20, 20 and 10 ml of 0·5 *N* hydrochloric acid; collect the aqueous layers in a 50-ml volumetric flask and make up to 50 ml. If the total amount of parathion present is known to be greater than 100 μg, add 1 ml of 0·25 per cent. sodium nitrite to 10 ml of this acid solution and, after 15 minutes, 2 ml of 1 per cent. N-sulphato-ethyl-*m*-toluidine and 4 ml of 30 per cent. w/v sodium acetate solution. After 15 minutes acidify the solution with 0·5 ml of concentrated hydrochloric acid, add 5 ml of ethanol and make up to 25 ml with distilled water. If the amount of parathion present is less than 100 μg, diazotise and couple the whole of the acid solution with five times the amount of reagents stated above, and after acidification extract the azo dye into analytical reagent *iso*amyl alcohol, with two successive portions of 10 and 5 ml; pour these extracts into a 15-ml volumetric flask and make up with ethanol.

With either method read the optical densities of the solutions at 510 mμ against a reagent blank and determine the amount of parathion present by comparing the reading with a calibration curve made for solutions of parathion subjected to the appropriate procedure.

AMOUNT TAKEN FOR ANALYSIS—

For the investigation a Unicam DG spectrophotometer was used with 1-inch cylindrical cells. A suitable concentration range in the acid solution after reduction was found to be

between 1 and 5 μg per ml for parathion and *p*-nitrophenol, and between 2 and 10 μg per ml for the S-ethyl isomer. When mixtures are being analysed, it may be that the concentration necessary for an adequate optical density from a minor component will involve an excessive concentration of a major component. It is possible to obtain an approximation to the amount of the major component by diluting its solution after colour development, but a more accurate procedure is to dilute the acid solution at the appropriate stage with 0·5 *N* hydrochloric acid before colour development and add proportionally larger amounts of reagents. After the completion of the reaction the whole solution is again extracted with toluene if a further stage is required, and the aqueous phase is diluted to the appropriate volume.

RESULTS

The S-ethyl isomer undergoes a slow hydrolysis in the ammonia solution used during the determination of *p*-nitrophenol and if much is present a high value for *p*-nitrophenol will be obtained. The extent of this has been shown to be of the order of 2 per cent. by repeating this stage of the estimation instead of passing on to the determination of S-ethyl isomer. A correction for this has been applied in the results given below.

Samples of parathion and S-ethyl isomer have been kindly supplied by Albright and Wilson Ltd.; parathion had been purified by a chromatographic procedure and the S-ethyl isomer synthesised from *p*-nitrophenyl ethyl chlorophosphate and sodium ethyl mercaptide. The sample of parathion when received was found to contain 0·1 per cent. of *p*-nitrophenol and 0·4 per cent. of S-ethyl isomer; four months later the *p*-nitrophenol content had risen to 1·7 per cent. I am indebted to Mr. B. Topley for the method used by Albright and Wilson Ltd. to establish the purity of the sample of S-ethyl isomer. The elementary analyses for phosphorus and sulphur were correct, and the absence of a P = S group was demonstrated by the nitric acid oxidation method.[3] The theoretical amount of *p*-nitrophenol could be obtained by alkaline hydrolysis, and when the extent of hydrolysis at a fixed pH value was measured at progressive time intervals, the calculated hydrolysis constant showed no variation. By the procedure described above the sample was found to contain 3·7 per cent. of *p*-nitrophenol and 2·5 per cent. of parathion, or of substances with similar properties calculated as parathion. Mixed solutions of these two samples together with *p*-nitrophenol in toluene were prepared and analysed; Table I shows that the calculated results for three such mixtures agree well with those expected.

TABLE I

ANALYSES OF MIXTURES OF *p*-NITROPHENOL, S-ETHYL ISOMER AND PARATHION

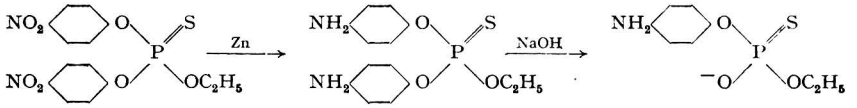
p-Nitrophenol		S-Ethyl isomer		Parathion	
expected, mg per 100 ml	found, mg per 100 ml	expected, mg per 100 ml	found, mg per 100 ml	expected, mg per 100 ml	found, mg per 100 ml
0·86	0·98	1·12	1·08	9·80	9·76
0·207	0·235	1·52	1·37	5·2	5·08
11·5	11·7	4·2	5·3	50·5	50·1

DISCUSSION

The method described in this paper was that used by Diggle and Gage[5] in their study of the inhibition *in vitro* of cholinesterase by a variety of samples of parathion; inhibition was found to be proportional to the content of S-ethyl isomer in the samples.

As the S-phenyl isomer of parathion, paroxon and *bis*-*p*-nitrophenyl ethyl thiophosphate behave in a manner similar to parathion in the procedure described above, they will, if present, be reported as parathion. *bis*-*p*-Nitrophenyl ethyl thiophosphate is stated to be present in the commercial parathion manufactured in this country, and some experimental work has been directed toward its determination. After reduction, *bis*-*p*-nitrophenyl ethyl thiophosphate gives an azo colour, as does parathion in the above procedure; if the acid solution of the reduced compound is made alkaline and heated it still produces an azo colour of about the same intensity, although it can be shown that the alkali treatment splits off *p*-aminophenol if zinc dust is added to prevent oxidation. It is probable that the course

of the reaction is as follows, and that the final ionised thiophosphoric acid derivative is stable to further alkaline hydrolysis—



When reduced parathion solution is heated with alkali, the greater part is hydrolysed to *p*-aminophenol, but in all samples examined the solution still gives an azo colour equivalent to about 10 per cent. of the parathion. If a solution of reduced S-ethyl isomer is heated with alkali the proportion apparently stable is about 25 per cent., although if the same sample is subjected to the complete analytical procedure described above, only a negligible trace of azo colour can be developed if the acid solution for the determination of parathion is heated with alkali. It seems likely, therefore, that with these two compounds the alkali not only attacks the nitrophenyl group but also the ethoxy or the ethylmercaptide group; this reaction would give an anion similar to that suggested for *bis*-*p*-nitrophenyl ethyl thiophosphate.

When a solution of S-ethyl isomer is submitted to the analytical procedure described above, the optical density reading, if applied to a *p*-nitrophenol standard curve, is equivalent to about two-thirds of the calculated figure for combined *p*-nitrophenol. It is probable that a dual hydrolytic attack on the molecule occurs in the presence of alkali in the determination of S-ethyl isomer stage and forms, in part, an alkali-stable component, which does not give an indophenol colour and which, by virtue of the presence of an ionisable hydrogen atom, is not extracted into toluene from alkaline solution. It has been found that if at this stage *o*-cresol is not added to a sample of reduced S-ethyl isomer, an appreciable azo colour can be developed in the solution after it has been extracted with toluene. It has been shown that this is not due to the presence of a component with an appreciable partition between the two phases, as the intensity of the azo colour is not significantly changed if the volume of the toluene is increased five-fold. As the analytical results with the S-ethyl isomer are reproducible, it can be assumed that the ratio of the end-products is not influenced by normal variations in experimental conditions.

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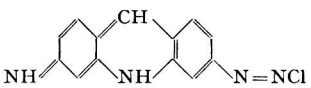
The Colorimetric Estimation of Small Quantities of Proflavine Hemisulphate

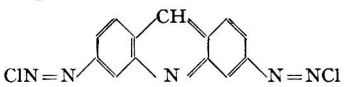
By W. H. C. SHAW and G. WILKINSON

A method is described for the colorimetric estimation of proflavine hemisulphate (2:8-diaminoacridine hemisulphate) by conversion to the quinone-imine form of 2-aminoacridyl diazonium chloride. A proflavine solution is treated with a limited excess of nitrous acid, under controlled conditions of pH and temperature, and the excess of nitrous acid is subsequently removed by sulphamic acid. Coupling in acid solution with N-(1-naphthyl)-ethylenediamine dihydrochloride then gives a stable purple colour. This procedure is an improvement on the use of the weak and unstable purple colour of the quinone-imine itself, which has been previously proposed for the estimation of 2:8-diaminoacridines. The method detailed gives a rectilinear relation of optical densities and concentrations for quantities of proflavine hemisulphate up to 300 μg and it can be applied without modification to euflavine and acriflavine in similar amounts. Details are also given for the application of the method to pharmaceutical preparations.

The development of a purple colour by interaction of nitrous acid and 2:8-diaminoacridine (proflavine) was reported by Grandmougin and Smirous,[1] who attributed this effect to the formation of a quinone-imine—



In alkaline solution this coupled with R-salt to give a red dye, whereas excess of nitrous acid was considered to lead at least to partial formation of the *bis*-diazo compound—



Schulte[2] has described a colorimetric method for the determination of acriflavine based on the development of a purple colour produced by addition of nitrous acid, but the method has the disadvantage that the colour fades gradually, even in the dark. The object of the work described here was to apply the reaction with nitrous acid to the estimation of small quantities of proflavine hemisulphate with the elimination, if possible, of the disadvantages of Schulte's method.

Experimental

Formation of quinone-imine—

A standard solution containing the equivalent of 0·02 per cent. w/v of anhydrous proflavine hemisulphate was prepared from a sample of proflavine hemisulphate B.P. Suitable quantities of this solution were treated under different conditions with sodium nitrite solution, and the optical densities of the resultant solutions were measured on a Spekker absorptiometer with a 1-cm cell and No. 3 orange - yellow glass filters. These filters were selected because comparable amounts of both proflavine hemisulphate solution and of the completely decomposed quinone-imine gave readings close to zero, while the purple quinone-imine exexhibited appreciable absorption.

Effect of concentration of nitrous acid—A series of solutions was prepared in 50-ml graduated flasks. Each solution contained 5 ml of 0·02 per cent. w/v proflavine hemisulphate solution diluted to about 43 ml. Five millilitres of 0·1 *N* hydrochloric acid were added to each flask to ensure a final (the optimum) acid concentration of 0·01 *N* and the solutions were cooled to 0° C. Various quantities of 0·1 *N* or 0·01 *N* sodium nitrite solution were

added and the solutions diluted to 50 ml. The values of the optical densities of the resulting solutions, measured at intervals from the time of mixing, are given in Fig. 1 and show the formation and decomposition of the quinone-imine colour. The optimum amount of nitrite was found to be contained in 1 ml of 0·01 *N* sodium nitrite solution.

Effect of variation of pH—The development of colour with 0·01 *N* nitrite solution in the presence of various concentrations of hydrochloric acid was examined in a similar experiment. A series of curves like those shown in Fig. 1 led to the conclusion that 0·01 *N* was the most effective concentration of acid.

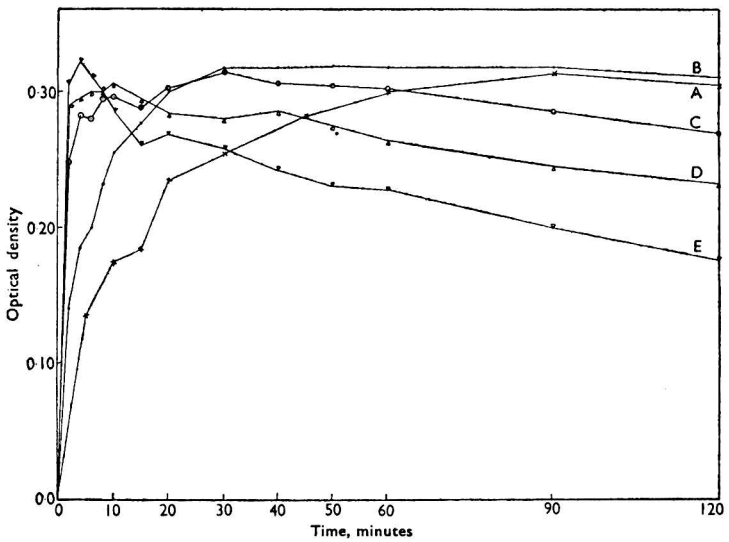


Fig. 1. Effect of concentration of nitrous acid

Curve A, 0·5 ml of 0·01 *N* sodium nitrite; curve B, 1·0 ml of 0·01 *N* sodium nitrite; curve C, 0·5 ml of 0·1 *N* sodium nitrite; curve D, 1·0 ml of 0·1 *N* sodium nitrite; curve E, 2·0 ml of 0·1 *N* sodium nitrite

Effect of temperature—Experiments with 1 ml of 0·01 *N* sodium nitrite solution in the presence of 0·01 *N* hydrochloric acid at 0° and 10° C showed that development of colour was complete after 40 minutes and that there was no significant effect of temperature within this range.

Effect of light—It was found that the purple diazotised solution, prepared under the optimum conditions given above and exposed to direct sunlight or ultra-violet radiation faded rapidly to a fluorescent yellow colour, closely resembling that of unchanged proflavine hemisulphate solution.

COUPLING OF QUINONE-IMINE—

Since the results of the experiments up to this point showed that the quinone-imine alone did not provide a sufficiently stable colour, attention was given to the possibility of coupling with suitable compounds. To this end preliminary experiments were made with R-salt and with N-sulphato-ethyl-*m*-toluidine. The former, in sodium carbonate solution, gave a red colour, but it was found difficult to obtain reproducible results. The latter was even less satisfactory, as only weak colours were given in acid, acetate-buffered or alkaline solutions.

A satisfactory coupling component was, however, found in N-(1-naphthyl)-ethylenediamine.[3] This necessitated first removing from the diazotised solution the excess of nitrous acid by means of sulphamic acid. The components were then coupled to give a purple dye, the optical density of which was found suitable for the colorimetric estimation of quantities of proflavine hemisulphate up to 0·3 mg. In addition the colour from a given amount of proflavine hemisulphate was more intense than that of the corresponding uncoupled quinone-imine.

A typical calibration graph for the Spekker photo-electric absorptiometer with the method described below was a straight line passing through the origin; the reproducibility for a series of test solutions is shown in Table I.

TABLE I

DETERMINATION OF PROFLAVINE HEMISULPHATE IN AQUEOUS SOLUTION BY THE PROPOSED METHOD

Proflavine hemisulphate—								
Added, mg per 100 ml ..	4·60	5·41	4·04	3·12	2·13	1·63	1·27	2·37
Found, mg per 100 ml ..	4·56	5·36	3·96	3·06	2·08	1·60	1·36	2·30

The transmission curves of proflavine hemisulphate, of the quinone-imine form of 2-amino-acridyl diazonium chloride derived from the proflavine hemisulphate after diazotisation and of the dye obtained by coupling the quinone-imine form of 2-aminoacridyl diazonium chloride with N-(1-naphthyl)-ethylenediamine dihydrochloride are shown in Fig. 2.

DISCUSSION

Temperature, pH, light and concentration of nitrous acid have all been shown to affect both the rate of formation of the quinone-imine from 2:8-diaminoacridine and its subsequent stability. To secure maximum stability the excess of nitrous acid and the acidity must each be reduced to the least value consistent with a reasonable rate of reaction. Rise of temperature increases the rate of formation and decreases the stability; light must be excluded. Optimum conditions are therefore somewhat arbitrary and those selected must be rigidly controlled to ensure reproducible results. The observed deterioration in colour with excess of nitrite and low pH may be caused by the partial formation of the yellow acridine-2:8-*bis*-diazonium chloride, which is known to be formed in sulphuric acid solution on treatment with sodium nitrite.[1]

Although Schulte[2] measured the intensity of the colour of the quinone-imine compound itself, it has been found that the inherent instability of the quinone-imine, and particularly its susceptibility to decomposition on exposure to the light required for the measurement of its intensity, make it unsatisfactory for estimation.

A stable red colour was obtained on coupling with R-salt (2-naphthol-3:6-sodium disulphonate) in sodium carbonate solution. Results by this method, however, were erratic, probably because of the decomposition of the diazonium compound by local excesses of carbonate on first mixing the solutions and before complete coupling had taken place.

After removal of the excess of nitrite, the diazonium chloride couples with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a purple dye stable within the pH range 1 to 2. The intensity of the colour produced in this way by a given weight of proflavine hemisulphate is greater than that of the original quinone-imine colour and so gives rise to an over-all increase in sensitivity. The proposed method gives a linear calibration graph within the range 0 to 300 μg of proflavine hemisulphate.

METHOD

REAGENTS—

N-(1-naphthyl)-ethylenediamine dihydrochloride—A 0·1 per cent. w/v solution, which must be stored in a refrigerator and discarded when yellow.

Sulphamic acid (*or ammonium sulphamate*)—A 0·5 per cent. w/v solution, which must be used within 14 days of preparation.

Sodium nitrite—A freshly prepared 0·01 *N* solution.

Proflavine hemisulphate—A standard solution containing 0·02 per cent. w/v of proflavine hemisulphate $(C_{13}H_{11}N_3)_2H_2SO_4$, which must be stored in the dark.

PROCEDURE—

Place in a 100-ml graduated flask an aqueous solution of the material under test containing up to 300 μg of anhydrous proflavine hemisulphate. Add 5·0 ml of 0·1 *N* hydrochloric acid and make up with water to 50 ml. Cool the solution to 10° $\pm$ 2° C, add 1·0 ml of 0·01 *N*

sodium nitrite solution, mix thoroughly and maintain at 10° ± 2° C for 40 minutes in a water-bath, protecting the solution from light throughout this period. Subsequent operations (until coupling has taken place) must be carried out in subdued light.

After 40 minutes add 1·0 ml of sulphamic acid solution, mix thoroughly and allow to stand for 1 minute. Add 2·0 ml of N-(1-naphthyl)-ethylenediamine dihydrochloride solution, mix thoroughly and allow to stand for 5 minutes. Add 5·0 ml of 0·1 *N* hydrochloric acid

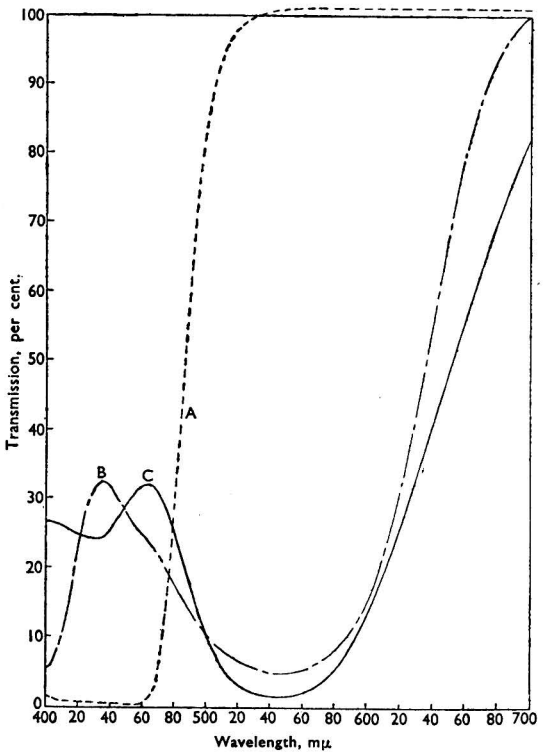


Fig. 2. Transmission curves

Curve A, proflavine hemisulphate, 2 mg in 50 ml of 0·01 *N* hydrochloric acid (1·0-cm cell); curve B, quinone-imine form of 2-aminoacridyl diazonium chloride derived from the proflavine hemisulphate after diazotisation with 1·0 ml of 0·01 *N* sodium nitrite (1·0-cm cell); curve C, dye obtained by coupling 25 ml of the quinone-imine form of 2-aminoacridyl diazonium chloride with 2 ml of 0·1 per cent. N-(1-naphthyl)-ethylenediamine dihydrochloride, diluted to 50 ml (0·5-cm cell).

and make up to 100 ml with water. Prepare similarly a blank solution without the sample under test. Measure the optical density of the solution in a 1-cm cell by comparison with the blank, using a Spekker photo-electric absorptiometer and Ilford 605 filters, or another suitable combination of absorptiometer and filters. By the same method and with suitable dilutions of standard proflavine prepare a calibration graph covering the required range and read from the graph the amount of proflavine hemisulphate contained in the test solution.

Applications

Acriflavine, euflavine—

The method is applicable without modification to both these compounds, which give a linear response with quantities of either up to 300 μg. Small deviations in calibration with different batches of both compounds may be anticipated, owing to the varying proportions of methylated and unmethylated proflavine present.

AMINACRINE HYDROCHLORIDE (5-AMINOACRIDINE)—

Under the conditions of assay no reaction appears to occur between aminacrine and nitrous acid, so that the method is inapplicable.

PREPARATIONS CONTAINING PROFLAVINE HEMISULPHATE—

The method can be applied directly to the following B.P.C. 1949 preparations; eye-drops, pessary, solution and solution tablets.

Typical results on pessaries are shown in Table II.

TABLE II

ESTIMATION OF PROFLAVINE HEMISULPHATE IN A PESSARY MASS CONTAINING 0·209 PER CENT. W/W OF THE ANHYDROUS FLAVINE PREPARED ACCORDING TO B.P.C. 1949

Weight taken, g	Proflavine content: calculated, mg	Proflavine content: found, mg	Anhydrous proflavine, % w/w
0·0486	0·1017	0·100	0·206
0·1334	0·2793	0·270	0·202
0·1259	0·2636	0·260	0·207
0·0675	0·1413	0·139	0·206
0·1219	0·255	0·249	0·204

Difficulty is sometimes experienced in recovering the last traces of proflavine in the presence of fatty matter and extraction with an immiscible solvent may be necessary. Ethylene dichloride is preferable to other common organic solvents and losses of proflavine can be reduced to a minimum by maintaining the aqueous phase at an acidity of about 0·1 *N* with hydrochloric acid.

Table III shows the results obtained, by the method outlined below, for a sample of proflavine hemisulphate cream B.P.C. containing 0·116 per cent. w/w of anhydrous proflavine hemisulphate.

PROCEDURE—

Weigh accurately 0·1 to 0·2 g of the sample into a boiling tube provided with a lip. Warm on a water-bath, dissolve in 2 ml of ethylene dichloride, add 5·0 ml of 0·1 *N* hydrochloric acid and maintain at a temperature of about 50° C for 5 minutes, with occasional

TABLE III

PROFLAVINE HEMISULPHATE IN PROFLAVINE HEMISULPHATE CREAM B.P.C.*

Weight taken, g	Proflavine hemisulphate: calculated, mg	Proflavine hemisulphate: found, mg	Anhydrous proflavine hemisulphate, % w/w
0·1178	0·1367	0·133	0·113
0·1851	0·2146	0·207	0·112
0·1501	0·1741	0·168	0·112
0·1149	0·133	0·131	0·114
0·1543	0·1789	0·174	0·113

* Proflavine hemisulphate cream B.P.C. containing 0·116 per cent. w/w of active agent.

shaking. Transfer the contents to a 15-ml graduated centrifuge tube and add more ethylene dichloride if necessary; wash the boiling tube with two successive 2-ml quantities of warm water, and add the washings to the centrifuge tube. Close the tube by means of a well-fitting rubber stopper and shake vigorously for 30 to 60 seconds. Remove the stopper and rinse any adhering solution back into the original boiling tube. Centrifuge for 5 to 10 minutes at 2500 r.p.m. or until a clear upper layer is obtained. By means of a Pasteur pipette, transfer the bulk of the aqueous layer to a 100-ml volumetric flask, rinse the pipette with two portions of distilled water and add the washings to the flask. Wash the lower layer remaining in the centrifuge tube with the rinsings from the boiling tube, using a total of 8 ml

of warm water. Shake, centrifuge and transfer the upper layer to the flask as before, and repeat the washing procedure.

Dilute the contents of the flask to 50 ml and complete the determinations by the general method, as described on p. 129.

The above procedure is applicable to ointments with a water-miscible base. The mean error, approximately −3 per cent. (see Table III), is small and the method is regarded as sufficiently accurate for routine estimations.

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A Modified Micro-Diffusion Method for the Determination of Ethyl Alcohol in Blood

By F. J. SCANDRETT

A method is described for the micro-determination of ethyl alcohol in blood or urine by means of a new micro-diffusion procedure that gives results in 30 minutes over the range of 80 to 300 μg per 0·1 ml of blood or urine with an accuracy of better than ±3 per cent.

Bahner,[1] working in this laboratory, has devised a convenient micro-diffusion apparatus that permits the separate temperature control of the two chambers. The apparatus, illustrated in Fig. 1, can be applied to many analytical methods in which diffusion at a uniform temperature is slow or incomplete. The lower vessel, which contains the sample for determination, has a flat base with a surface area of 2·5 sq. cm and is attached to the chimney part of the "mushroom" by a B19 standard joint. The "mushroom" is surrounded by a simple form of condenser that allows water at 50° C to circulate over and under it. The flat base of the "mushroom," which has a surface of 10·0 sq. cm, contains the dichromate - sulphuric acid mixture.

The first use made of this apparatus, apart from that by Bahner who designed it for the determination of acetone and "ketone bodies" in blood, was in the determination of ammonia (Scandrett, unpublished). By the boric - hydrochloric acid procedure, full recoveries were regularly obtained in 10 minutes with a solution containing 112 μg of ammonia (NH_3) per 0·5 ml, which demonstrated the efficiency of the micro-diffusion in this apparatus.

Experimental

Preliminary observations indicated that, if the apparatus was to be successfully used for the determination of ethyl alcohol in blood, certain limiting factors would have to be considered and, if necessary, modified to meet the requirements of the unit.

The concentrations of potassium dichromate and sulphuric acid appeared to be critical, especially that of the sulphuric acid, as incomplete absorption and oxidation resulted if the concentration of sulphuric acid fell below 50 per cent.

As the iodine titration depends on the pH of the solution (the optimum value is about pH 1·4), which involves a considerable dilution immediately before titration, and as the capacity of the "mushroom" is limited, only a small quantity, 1·0 ml, of original reagent could be used. This quantity was found just to cover the base of the "mushroom," which from the point of view of absorption is ideal, since the rate of absorption is inversely proportional to the volume (Conway[2]).

Preliminary experiments with 1·0 ml of dichromate - sulphuric acid mixture indicated that the absorption and oxidation is complete in 30 minutes, as against 2 hours by the Widmark method.[3]

A 0·005 *N* solution of sodium thiosulphate is used for alcohol concentrations up to 200 mg per 100 ml; for higher concentrations, *i.e.*, between 200 and 500 mg per 100 ml, 0·01 *N* sodium thiosulphate is used.

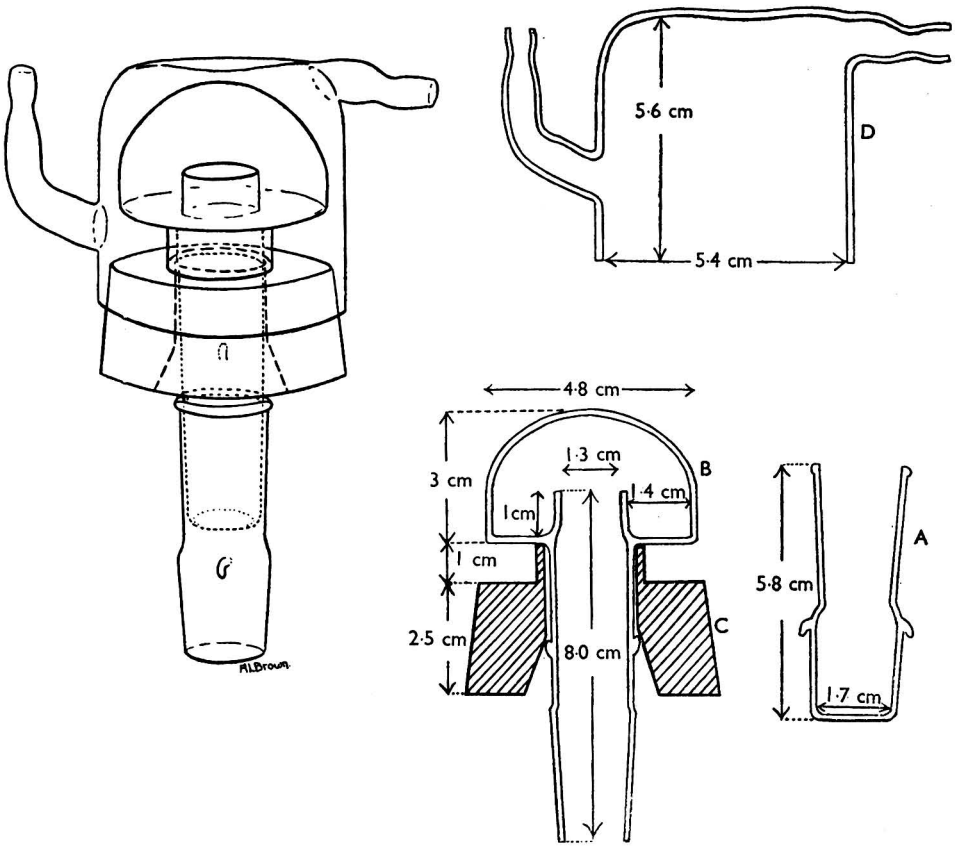

Fig. 1. View and sections of apparatus

A, lower vessel; B, "mushroom" receiver; C, rubber bung; D, condenser jacket

From a consideration of the above conditions and a large number of trials it was evident that the quantities and concentrations of reagents used by Widmark[3] were the most suitable, and this was proved by subsequent experiments.

Method

Reagents—

Potassium dichromate, 0·02 N, in concentrated sulphuric acid—This concentration is used for blood containing less than 200 mg of ethyl alcohol per 100 ml.

Potassium dichromate, 0·05 N, in concentrated sulphuric acid—This concentration is used for blood containing between 200 and 500 mg of ethyl alcohol per 100 ml.

Potassium iodide—A 5 per cent. solution.

Sodium thiosulphate—0·01 *N* and 0·005 *N* solutions.

Starch—A 1 per cent. solution.

All reagents should be of recognised analytical quality. With a Bang burette, which is available in most analytical laboratories, the strengths of the sodium thiosulphate solutions can be reduced. This was not done in the investigation described here.

PROCEDURE—

Transfer 1·0 ml of the dichromate - sulphuric acid mixture to the inverted "mushroom" by means of a 1·0-ml slow-delivery bulb pipette. It is advisable to blow the liquid out and to touch the surface of the dichromate - sulphuric acid mixture with the tip six times, to hold the "mushroom" against a white background and to watch the introduction of the liquid and the withdrawal of the pipette by looking down the chimney. Preliminary experiments with this procedure for delivery of 1·0 ml of dichromate - sulphuric acid mixture showed that the average error of ten such measurements is less than 1 per cent.

Using pipettes, place in the bottom vessel 0·2 ml of water and 0·1 ml of blood, the 0·1-ml blood pipette being rinsed out twice and the water and blood thoroughly mixed. Invert the "mushroom" and, with a cork-screw motion, attach the bottom vessel tightly to the chimney,

TABLE I

RECOVERY OF ETHYL ALCOHOL ADDED TO FRESHLY WITHDRAWN CITRATED BLOOD

Additions made as aldehyde-free ethyl alcohol in distilled water

Alcohol per 0·1 ml of blood			Standard deviation of mean
Added, μg	Found, μg	Mean, μg	
80·0	78·0, 83·0, 80·8, 82·0, 82·0, 84·2, 80·8, 76·8, 77·5, 76·3, 77·5, 84·6	80·3	±2·97
160·0	161·5, 158·0, 161·5, 160·4, 159·2, 162·0	160·4	±1·56
300·0	300·0, 305·0, 300·0, 300·0, 305·0, 305·0	302·5	±2·70

Blanks at each level were: 0 μg added, 0 μg found.

TABLE II

RECOVERY OF ETHYL ALCOHOL ADDED TO FRESHLY VOIDED URINE MADE ALKALINE WITH SODIUM HYDROXIDE

Alcohol per 0·1 ml of urine			Standard deviation of mean
Added, μg	Found, μg	Mean, μg	
300·0	301·7, 299·5, 296·0, 301·7, 304·0, 298·0, 294·0, 296·0, 296·0, 298·4	298·5	±2·80

Blank: 0 μg added, 0 μg found.

taking care to see that the joints are perfectly dry. Suspend the bottom vessel, held in position in this way, in a bath of boiling water almost up to the joint, while the "mushroom," enclosed by the condenser, has water at 50° C flowing over and under it. The rate of flow through the condenser may conveniently be 1 litre every 10 minutes, but this is not critical. After 30 minutes disconnect the unit, drain the condenser of water and take it off. Disconnect the bottom vessel, invert the "mushroom" and cool it under the tap. Add 25·0 ml of water to the "mushroom," again cool (preferably in ice) and add 0·5 ml of 5 per cent. potassium iodide before titrating with sodium thiosulphate in a 5·0-ml micro-burette.

Experiments were also made to determine the effect of added water on the rate of absorption and oxidation by the dichromate - sulphuric acid mixture. The following figures show that additions of water did not affect the acid mixture, as full recoveries in each experiment were obtained at the end of 30 minutes. To 1·0-ml portions of dichromate - sulphuric acid mixture were added 0·1, 0·2 or 0·3 ml of water. The amounts of alcohol found in 0·1-ml portions of blood containing 80·0 μg of ethyl alcohol per 0·1 ml, when acted upon by these solutions, were 78·8, 79·3 and 80·0 μg, respectively. It was not possible to use 0·2 ml of blood, as recoveries were always poor, probably because the heat-coagulated protein formed too great a barrier for the free diffusion of the alcohol vapour through the mixture.

The "mushroom" easily holds 25·0 ml of water and the constricted opening and the chimney are advantageous in iodimetric titrations.

Results and discussion

Accurate results were obtained throughout the range of 80 to 300 μg of alcohol per 0·1 ml of blood and urine, as shown in Tables I and II, the standard deviation being less than $\pm 3{\cdot}0$ μg per 0·1 ml of blood.

The method reduces the time for a single diffusion to 30 minutes, as against 2 hours with the Widmark[3] and Winnick[4,5,6] methods. A large number of determinations can be carried out simultaneously.

Further advantages are that, as a result of the large excess of water in the "mushroom" receiver, there is no possibility of losing the liberated iodine, the end-point is not so abrupt and the subsequent titration and shaking are, therefore, easy to control and manipulate.

By increasing the temperature gradient over which absorption takes place, it is possible to increase the range over which diffusion techniques can be used, so that the versatility of the apparatus is increased.

Experiments made with blood containing 300 mg of alcohol per 100 ml, stored at room temperature for one week, did not show any diminution of alcohol concentration. Normal blood used as a control and kept under the same conditions did not give a "blank."

To Dr. C. P. Stewart and Dr. F. W. R. Bahner, who have given me advice and helpful criticism, I wish to tender my thanks.

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June, 1951

The Isolation, Identification and Determination of Amphetamine in Viscera

By E. RATHENASINKAM*

A scheme is described for the isolation, identification and determination of amphetamine. By combining the Stas-Otto process with a steam distillation method a purer isolation product is obtained than formerly. A number of methods of identification are given and the amount of the alkaloid present is determined by precipitating it as amphetamine oxalate. A comparison of the results obtained by determining the amphetamine by this method with those obtained by a volumetric method is shown.

Method of isolation

Amphetamine can be isolated from viscera either by the Stas-Otto process or by steam-distillation in an alkaline medium. Both amphetamine and its acetate are volatile, so viscera should be extracted with alcohol acidified with tartaric acid. Steam-distillation of viscera in an alkaline medium is not possible as excessive frothing ensues. The isolation of amphetamine from viscera is best effected by a combination of the two methods. Steam-distillation has the advantage of giving a purer product than that obtained by extraction with immiscible solvents in the Stas-Otto process.

Procedure—Extract the minced organs thoroughly with alcohol acidified with tartaric acid. Evaporate the alcohol from the combined alcoholic extracts, take up the residue

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in about 100 ml of water and transfer to a wide-mouthed distillation flask. Add sufficient sodium chloride to saturate the liquid, make alkaline to litmus with sodium hydroxide solution and steam-distil. Distil about 400 ml into a receiver containing 50 ml of 0·5 *N* hydrochloric acid. Evaporate the distillate to a small bulk and make up to a definite volume.

Take an aliquot portion and extract once with ether. Discard the ether extract. Make alkaline to litmus with sodium hydroxide solution and extract twice with chloroform. Wash the combined chloroform extracts with a little water and shake the chloroform solution with 10 ml of 0·1 *N* hydrochloric acid. Draw off the acid layer and evaporate to dryness. The tests for amphetamine are made on the residue obtained.

Methods of identification

Amphetamine gives the general reactions for alkaloids with Kraut's, Wagner's and Mayer's reagents.

Colour tests—

(*a*) With Marquis's reagent amphetamine gives a brick-red precipitate changing to brown and finally to a dirty olive-green. Adrenaline gives a brown to reddish-brown colour changing to a dirty violet. The violet tinge is first observed around the edge of the drop. Ephedrine gives only a light brown colour.

(*b*) In Mohler's test as modified by Illing[1] amphetamine gives a purple colour. Ephedrine and adrenaline under the same conditions give a deep yellow and a slight yellow colour respectively. The test will detect 0·1 mg of amphetamine.

(*c*) Beyer and Skinner[2] devised a colour test to determine amphetamine in urine during their investigation on the detoxication and excretion of amphetamine. On coupling amphetamine with diazotised *p*-nitroaniline a red colour is given that can be intensified by extraction with *n*-butanol.

(*d*) A further test is given by the colour reaction[3] of the nitro-compounds formed by the nitration of amphetamine.

Procedure—Nitrate the alkaloid as in Mohler's test. Dilute the product with water and extract once with chloroform. Discard the chloroform extract. Make the aqueous layer alkaline to litmus with ammonium hydroxide solution and extract once with chloroform. Evaporate the chloroform extract to dryness. Dissolve the residue in 1 to 2 ml of acetone, transfer to a test tube, add 1 to 2 drops of a 10 per cent. solution of sodium hydroxide and mix well. Amphetamine gives a purple colour changing to violet. The purple colour develops slowly. Ephedrine under the same conditions gives an immediate violet colour changing to magenta. Adrenaline gives no colour.

Derivatives—

The benzoyl derivative[4] (m.p. 134° to 135° C) may be prepared and its melting-point determined.

Microchemical tests—

(*a*) In the test described by Cannon[5] a 1 per cent. solution of amphetamine in dilute sulphuric acid was used; more dilute solutions (0·2 per cent.) may be used with a slightly modified technique.

Procedure—Evaporate on a microscope slide a drop of a solution of amphetamine hydrochloride in water. To the residue add half a drop of dilute sulphuric acid and a drop of 5 per cent. platinic chloride solution, mix and set aside for a few minutes. At first, interlaced needles form around the edge of the drop. The crystals gradually spread out in the shape of a holly leaf.

(*b*) In the test described by Keenan[6] about 1 mg of amphetamine sulphate was used. Smaller amounts of amphetamine can be made to react satisfactorily by reducing to a fraction of a drop the amount of gold reagent used.

Procedure—Evaporate a drop of a 0·2 per cent. solution of amphetamine hydrochloride in water on a microscope slide. To the residue, when cold, add a fraction of a drop of 5 per cent. gold chloride solution and stir vigorously with a glass rod till crystals begin to appear. Square yellow crystals of various sizes are formed.

METHODS FOR THE DETERMINATION OF AMPHETAMINE

VOLUMETRIC DETERMINATION—

Ether is used for extracting amphetamine in the B.P. (1948) method for the assay of amphetamine sulphate; Reznek[7] has shown that instead chloroform can be used to advantage. The chloroform extracts are then shaken with an excess of standard sulphuric acid, the excess of acid being titrated, with methyl red as indicator.

1 ml of 0·02 *N* acid ≡ 2·70 mg of amphetamine or 3·68 mg of amphetamine sulphate.

GRAVIMETRIC DETERMINATION—

The determination can be made by benzoylation[5,8] or by precipitation as oxalate.

On adding a solution of oxalic acid in ether to a solution of 0·75 mg of amphetamine in 100 ml of ether, amphetamine oxalate is precipitated after a few hours; the amphetamine oxalate is almost insoluble in ether. This is the basis of a new gravimetric method for the determination of amphetamine.

A number of determinations were made with various amounts of amphetamine and it was found that the weight of the oxalate precipitate was always greater than the theoretical value, assuming that two molecules of amphetamine react with one molecule of oxalic acid. The ratio of the theoretical weight to the weight found was on average 4:4·94. It was thought that the difference between the weight found and the theoretical value was caused by occlusion of oxalic acid by the oxalate precipitate. The oxalate precipitate, after drying and weighing, was dissolved in water; the free acid present was determined by titration with standard sodium hydroxide solution. The weight of free acid, calculated as anhydrous oxalic acid, was always about one-fifth of the weight of the precipitate; the average value obtained was 0·192. From these observations it was inferred that amphetamine is precipitated as the acid oxalate and not as the normal oxalate.

Procedure—To an aliquot portion of the distillate, containing about 50 mg of amphetamine, add sufficient sodium chloride to saturate the liquid, make alkaline to litmus with sodium hydroxide solution and extract three times with 30-ml portions of ether. Wash the combined ether extracts with 15 ml of a saturated salt solution and filter through a layer of anhydrous sodium sulphate placed over a plug of cotton, into a glass-stoppered conical flask. Extract the wash solution twice with ether and pass the ether through the same filter into the flask. Add 10 ml of a saturated solution of oxalic acid in ether, shake occasionally and put the flask and contents aside overnight. Filter the solution rapidly through a sintered-glass crucible with suction, wash the precipitate twice with 25-ml portions of ether, dry at 100° C and weigh the oxalate.

Weight of precipitate × 0·6002 = Weight of amphetamine.
Weight of precipitate × 0·8180 = Weight of amphetamine sulphate.

The ether used to prepare the oxalic acid solution and to wash the precipitate should be dried over anhydrous sodium sulphate.

Visceral matter on steam distillation in an alkaline medium gives small quantities of volatile basic substances that would be estimated as amphetamine in the volumetric method described, which is accordingly not applicable to distillates obtained from viscera. Amphetamine, if present in weighable amounts, can be determined by the benzoylation or oxalate method.

COLORIMETRIC METHODS FOR THE DETERMINATION OF TRACES OF AMPHETAMINE—

Illing's[1] modification of Mohler's test is capable of determining amounts of amphetamine of the order of 1 mg.

The diazotisation method of McNally, Bergman and Polli[9] is based on the colour reaction devised by Beyer and Skinner.[3] Amphetamine is isolated by steam-distilling a sulphuric acid - tungstic acid filtrate of the minced organs in an alkaline medium.

Amphetamine is extracted from the distillate with ether and the ether extract is shaken with three successive portions of 0·5 *N* hydrochloric acid. The combined acid extracts are evaporated to dryness. The residue is taken up in 1 ml of water and coupled with diazotised *p*-nitroaniline. The red colour formed is extracted with *n*-butanol. The transmission is measured on a spectrophotometer, at 530 mμ, and the amount of amphetamine read from a standard graph.

The method is capable of estimating an amount of amphetamine as small as 0·03 mg in 25 g of tissue.

Results

A stock solution of amphetamine sulphate was prepared to contain 49·3 mg of amphetamine sulphate in 25 ml of the solution. Some experimental results are shown in Table I.

Table I

Comparison of results by the proposed method and the volumetric method

Amphetamine found, calculated as amphetamine sulphate		
By volumetric method, mg	By oxalate method, mg	Details of procedure with 25-ml portions of stock solution containing 49·3 mg of amphetamine sulphate in each
49·3	—	Extracted with chloroform
49·3	—	
—	48·5	Extracted with ether and precipitated as oxalate. The oxalate
48·1	48·7	precipitate was dried, weighed and dissolved in water; the amphetamine was re-extracted with chloroform and determined volumetrically
48·1	47·0	Steam distilled and titrated. Amphetamine was re-extracted
48·0	47·0	with ether from the titrated solution and precipitated as the oxalate
48·6	45·4	100 g of liver were extracted with alcohol; the alcohol was evaporated and the residue was taken up in 75 ml of water. Stock solution was added to this and steam distilled. Amphetamine was re-extracted with ether from the titrated solution and precipitated as oxalate
45·8	42·5	Stock solution was added to 100 g of liver, which was cut into
46·9	43·2	small pieces and extracted twice with alcohol (extraction was not complete). Amphetamine was re-extracted with ether from the titrated solution and precipitated as oxalate
2·3	No ppt.	Blank on 100 g of liver calculated as amphetamine sulphate

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An Improved Apparatus for the Vaporimetric Micro-Determination of Molecular Weight

By A. F. COLSON

An apparatus is described for the vaporimetric micro-determination of molecular weight. Full details are given of the application of the apparatus to a typical determination, and results that have been obtained with the vaporimeter are shown. The advantages of the apparatus are detailed.

THE apparatus used by Bratton and Lochte[1] for the vaporimetric micro-determination of molecular weight has been modified and improved. The heating of the vaporisation vessel, particularly over the higher temperature range (200° to 300° C), has been made more efficient. Pressure differences can be measured with greater precision than formerly, and on completion of a determination the vaporised sample can be removed more efficiently. The apparatus can be dismantled more easily than can that of Bratton and Lochte, when the occasion arises.

APPARATUS

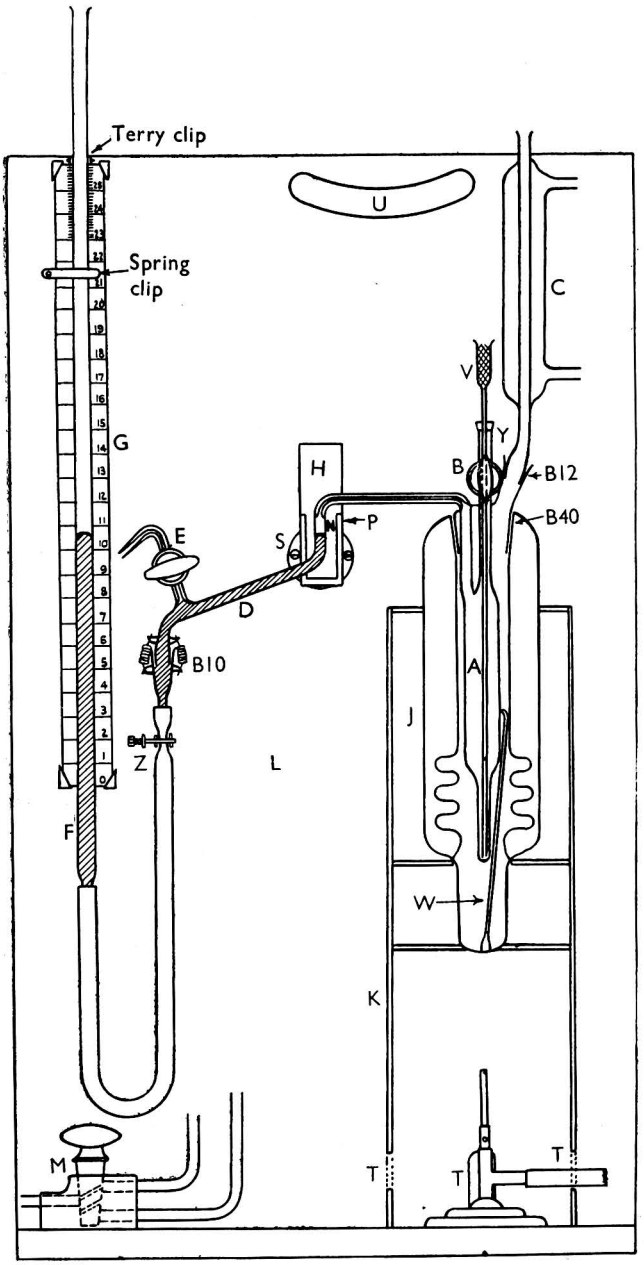
The assembled apparatus is illustrated in Fig. 1. All glass components except the mirrors, G and H, are constructed from Pyrex glass.

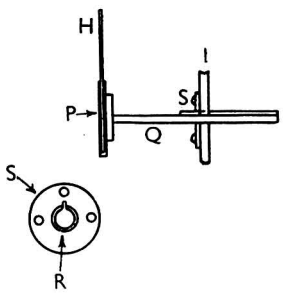
The vaporisation vessel, A, has an internal volume of approximately 30 ml. The main body of this vessel is 10 cm long and has an external diameter of 2 cm. The lower constricted portion is 5·5 cm long with an external diameter of 6·5 mm. The two upper constricted limbs are each of length 5·5 cm and have external diameters of 7 mm. One of these upper limbs connects to a single-bore tap, B, of bore-diameter 3 to 4 mm. The length of the tube from the barrel of the tap to the B40 cone of the vapour jacket is 2 cm. The outlet tube, D, from the vaporisation vessel is in three sections, all of external diameter 7 mm. The part nearest vessel A is a capillary tube of length 9 cm and of internal diameter 0·5 mm. This is followed by a vertical section, 2 cm long, carrying the reference mark, N, and finally by a section 8 cm long inclined downwards at an angle of 45°, that leads to the levelling tube, F, which is 63 cm long and has an internal diameter of 6·5 mm. Another tap, E, of 2 mm bore, is joined by a 15-mm length of tubing to the bottom end of the inclined part of the outlet tube, D. A mirror 35 cm long and 3 cm wide is inset to lie flush with the surface of the support, L, behind the levelling tube. The scale is graduated in millimetres and numbered at each centimetre for 30 cm of its length. The adjustable mirror, H, is made from an ordinary microscope slide and is carried by a metal frame, P. The frame is fastened to a rod, Q, provided with a projecting tongue over 4 cm of its length. The length of the rod is 8 cm and its diameter 5 mm. The rod slides freely without rotation in a slotted tube, R, 8·5 cm long, which projects through the wooden support, L, and is fastened at the front of it by a flat metal collar, S. The mirror facilitates the adjustment of the mercury meniscus in tube D to the reference mark, N, and when not in use is pushed back to the board L. The vapour jacket, J, itself has a vacuum jacket over 22 cm of its length. The internal diameter of this section is 3 cm and the external diameter of the jacket is 7·5 cm.

The vacuum jacket is silvered except for two diametrically opposed strips 1 cm wide through which the vaporisation vessel can be viewed. The unjacketed portion of the vapour jacket is 5·5 cm long and of 4 cm external diameter.

To minimise any risk of fracture at higher temperatures the lower portion of the jacketed section is fashioned in the form of an "expansion bellows" as indicated in Fig. 1. Repeated use of the apparatus at 240° C has not so far resulted in fracture of the vacuum-jacketed section. The Liebig condenser acts as a reflux unit and has the following dimensions: inner tube—length, 21 cm; external diameter, 1 cm; internal diameter, 6·5 mm; condenser jacket—length, 16 cm; external diameter, 2·5 cm.

The support, K, for the vapour jacket is constructed from $\frac{1}{4}$-inch Sindanyo board. It has a height of 15 inches, width of $4\frac{1}{2}$ inches and a depth of 5 inches. The first shelf is at a height of 6 inches above the base. The support is rigidly attached to the vertical board,





Detail of adjustment mirror

Fig. 1. Apparatus for the vaporimetric determination of molecular weight

L. Slots, T T, are provided as shown for the passage of rubber tubing to the bunsen burner. The support, L, for the assembled apparatus is a wooden base board $\frac{3}{4}$ inch thick, 15 inches wide and 10 inches from front to back, carrying a vertical board $\frac{1}{2}$ inch thick, 15 inches wide and 26 inches high.

The vertical board is fixed about 6 inches from the front edge of the base board and is strengthened by a strut (not shown) screwed to the back, and to the base board. A slot, U, is provided for carrying the assembled apparatus.

The diameters of the bores of the double-bore tap, M, are each 2 mm. This tap, securely held in a block of wood screwed to the base board, serves to connect the apparatus to a vacuum pump when required.

METHOD

PROCEDURE FOR DETERMINING MOLECULAR WEIGHT—

Pour into the vapour jacket, J, about 30 ml of a liquid boiling at about 30° C above the boiling-point of the sample. Introduce the boiling-rod, W, lightly grease the top edge of the ground joint on the vaporisation vessel, A, and assemble the apparatus as shown in Fig. 1, omitting the cotton-wool air-filter, V.

Open tap B, lower the levelling tube, F, until the mercury in the outlet tube, D, falls below tap E, and then open tap E.

Boil the liquid in the vapour jacket to obtain a copious reflux in the condenser, raise the levelling tube until mercury fills the bore of tap E, close tap E and adjust the level of mercury in D to coincide with the reference mark N, using mirror H to facilitate the adjustment.

Close tap B and if necessary re-adjust the mercury level to the reference mark N. When no further movement of the mercury in D is observed on opening and closing tap B, open the latter and read the mercury level in the levelling tube against the scale on mirror G.

Weigh out a suitable quantity (5 to 15 mg) of the sample. Liquids should be weighed in thin-walled glass capillary tubes. The tubes should be drawn out at one end to form a solid handle 10 mm long, and at the other end to form a finer capillary about 4 cm long. The middle portion of the tube should be about 2 cm long and 2·5 mm in external diameter. The tip of the finer capillary should be sealed after introduction of the sample.

Solid samples are best weighed as pellets, which can be made in any suitable tablet press, *e.g.*, in the Orthofer tablet press.[2] Introduce the weighed sample into the vaporisation vessel as follows—

If the sample is contained in a capillary tube, insert the tube through tap B, "handle" downwards and break it across the finer capillary end by closing the tap.

If the sample is in the form of a pellet place it in the tube, Y, above tap B, and without delay open and close the tap.

As the sample vaporises, maintain the level of mercury in D at, or a little below, the reference mark N. When no further movement of the meniscus is observed, adjust it exactly to the reference mark and read the mercury level in the levelling tube as before.

Prepare the apparatus for the next determination as follows—

If the sample was added as a pellet, lower the levelling tube, F, until the mercury in D has fallen to a position below the screw-clip, Z. Close the clip and open taps E and B. Insert the tube of the air-filter, V, through tap B as shown in Fig. 1, connect tap E to vacuum via tap M, and draw filtered air through the heated vaporisation vessel for several minutes to remove all vapour of the sample. If a capillary sample tube has been used this must be removed before sweeping out the apparatus with air. This can be done without dismantling the apparatus if a glass tube of suitable internal diameter is attached to vacuum and then inserted through tap B into the lower constricted end of the vaporisation vessel where the sample tube is located. The sample tube will be drawn into the end of the inserted tube and can be removed with it through tap B.

CALCULATION OF THE MOLECULAR WEIGHT—

Bratton and Lochte[1] use the following formula for the calculation of molecular weight—

$$M = \frac{22{,}410 \times 760 \times T \times m}{1000 \times 273 \times V \times P}$$

where M = Molecular weight.
T = Temperature, in degrees Absolute, of the vapour jacket.
m = Weight, in milligrams, of the sample.
P = Pressure, in millimetres of mercury, produced by vaporisation of the sample.
V = Volume, in millilitres, of the vaporisation vessel A.

An alternative method described by Lumsden[3] may be used and is indeed preferable, since it does not involve a knowledge of the values of T and V. In this method the molecular weight is calculated from the formula—

$$M = K \times \frac{m}{P}$$

where M, P and m have the same significance as before and K is a constant (the "molecular pressure") for a particular heating liquid. It is determined as described below.

DETERMINATION OF K—THE "MOLECULAR PRESSURE"—

Take an accurately weighed amount of a suitable pure organic substance and carry out the series of operations already described for the determination of molecular weight,

TABLE I

VAPORIMETRIC MICRO-DETERMINATION OF MOLECULAR WEIGHT

Compound used	Weight (m) of compound taken, mg	Pressure (P) produced by vaporisation, mm	Molecular pressure (K)	Heating liquid	Molecular weight $\left(\frac{K \times m}{P}\right)$ found	Molecular weight, calculated
Methyl alcohol ..	4·611	101·0	701	Water	32·0	32·04
" ..	5·942	131·3	"	"	31·7	
" ..	5·853	126·7	"	"	32·3	
" ..	4·015	87·7	"	"	32·1	
Toluene	8·875	74·8	778	Xylene	92·3	92·13
"	11·001	92·2	"	"	92·8	
"	10·008	84·4	"	"	92·2	
Water	2·062	88·6	"	"	18·1	18·016
"	1·970	82·4	"	"	18·6	
"	2·750	117·9	"	"	18·2	
Bromobenzene ..	15·040	79·3	829	Cymene	157·2	157·02
" ..	15·598	82·6	"	"	156·2	
" ..	15·490	82·2	"	"	156·2	
Ethylbenzene ..	9·356	74·6	"	"	104·0	106·16
" ..	10·435	83·1	"	"	104·1	
" ..	8·520	68·0	"	"	103·9	
Aniline	8·165	77·1	884	Nitrobenzene	93·6	93·12
"	8·081	76·3	"	"	93·6	
Naphthalene ..	10·904	80·7	949	Quinoline	128·2	128·164
" ..	9·601	71·2	"	"	128·0	

with the same heating liquid in the vapour jacket as was used for the vaporisation of the sample. Pure dry benzene is a suitable compound for this determination, over the temperature range 100° to 240° C. Calculate the constant K from the formula—

$$K = \frac{M_1 P_1}{m_1}$$

where K = "Molecular pressure."
M_1 = Molecular weight of substance vaporised.
P_1 = Pressure, in millimetres of mercury, produced by vaporisation of the substance.
m_1 = Weight, in milligrams, of substance.

Results

A series of results obtained in the determination of the molecular weight of compounds with boiling-points ranging from about 66° to 220° C is presented in Table I. The constant K ("molecular pressure") was used in the calculation of these results. The data employed for the calculation of K are given in Table II. For the determinations of the molecular weight of naphthalene the sample was used in pellet form, and "molecular pressure" was determined with pelleted pure camphor.

Table II

Determination of "molecular pressure" constant (K)

Substance vaporised	Molecular weight (M_1)	Weight (m_1) of substance used, mg	Pressure (P_1), mm	"Molecular pressure" (K)	Heating liquid
Benzene	78·108	9·185	82·5	701·0	Water
"	"	8·374	83·4	778·0	Xylene
"	"	7·653	81·2	829·0	Cymene
"	"	6·569	74·3	884·0	Nitrobenzene
Camphor	152·228	7·600	47·4	949·0	Quinoline

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Determination of Small Amounts of Cadmium in Lead by a One-Colour Dithizone Method

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A procedure is described for the colorimetric determination of small amounts, 0·01 to 0·4 per cent., of cadmium in lead or lead alloys without prior removal of lead. The common alloying elements do not interfere. For lower percentages of cadmium a portion of the lead must be removed. Detailed alternative procedures are described and extensions of the method for samples containing more or less cadmium are indicated.

A new interest in the determination of low concentrations of cadmium in lead arises from the particular effect of neutron irradiation on cadmium.[1] Cadmium has also been alloyed with lead metal to facilitate the coating of copper wire by lead.

The cadmium in lead alloys is usually determined gravimetrically or volumetrically after removal of tin, antimony, copper, lead, bismuth and zinc, if these be present.[2] The final determination of the cadmium can then be made as sulphide, sulphate,[2] phosphate, oxalate, quinolate or by electro-deposition.[2,3] Such procedures are not difficult if the proportion of cadmium is large, but they become laborious when the amount of cadmium is small because large samples are required. The colorimetric determination of cadmium as cadmium sulphide[4] and as the 8-hydroxyquinolate also require the separation of cadmium from most elements.

Wichmann's paper[5] on the dithizone system gives information about separations of many of the metallic ions, and shows in particular, that cadmium may be separated from lead at high pH values.

Fischer and Leopoldi[6,7] give detailed experimental results for the separation of cadmium from arsenic, antimony and lead, so that it is only necessary to arrange preliminary separations and then proceed with specified dithizone double washing and extracting techniques.

Certain limiting conditions must be noted. The separation of cadmium from lead must be made at pH 13, with carbon tetrachloride as the organic solvent. The maximum permissible concentration for lead is 0·05 g; otherwise an excessive number of washings will be required. The variations in concentrations of other metals present in the lead must be noted, and the metallic ions of silver, mercury and copper must be removed before dithizone extraction; alternatively these metals can be separated in acid solution when they are not extracted by dithizone. Further, contrary to previous reports,[7] the cadmium - dithizone colour is quite stable in chloroform so that the final readings are made in chloroform solution; the separations are made in carbon tetrachloride.

As the sensitivities of the many colorimetric instruments and filters vary, it is necessary to establish a suitable concentration range of cadmium per 100 ml of chloroform solution (μg per 100 ml of solvent), for each instrument and filter.

Experimental

To ascertain the working limits for cadmium - dithizone solutions, standard cadmium nitrate solutions were extracted according to the procedure and the values of the absorptions of the red extracts were read from a colorimeter. In this manner the number of extractions necessary and volumes for the final readings were established. The values for these red extracts were read at hourly intervals to check the stability of the coloured cadmium complex.

To determine the elements that might interfere, synthetic samples and the Bureau of Standards sample, No. 53c, were analysed. These samples included alloying elements present in amounts that might be expected in normal lead metals.

The three alternative methods, the direct, the plating and the sulphuric acid methods were checked against one another by analysing samples containing identical amounts of cadmium.

Method

Reagents—

Cadmium stock solution—Dissolve 0·100 g of cadmium metal in 50 ml of diluted nitric acid (1 + 3). Boil gently to remove the oxides of nitrogen. Dilute the solution with water to 1 litre in a volumetric flask. Mix well.

1 ml of stock solution ≡ 0·100 mg of cadmium = 100 μg of cadmium.

Cadmium standard solution—By means of a pipette, transfer 100 ml of the cadmium stock solution to a 1-litre volumetric flask and dilute to the mark with water.

1 ml of standard solution ≡ 0·0100 mg of cadmium = 10 μg of cadmium.

Dithizone - carbon tetrachloride reagent—Dissolve 25 mg of dithizone in 250 ml of carbon tetrachloride. Store the reagent in a refrigerator. Discard when a red tint appears in the green solution. The reagent keeps about 3 weeks. Ten millilitres will extract about 60 μg of cadmium.

Dithizone - chloroform reagent—Dissolve 25 mg of dithizone in 250 ml of chloroform. Store the reagent in a refrigerator. Discard when a red tint appears in the green solution. The reagent keeps about 3 weeks. Ten millilitres will extract about 60 μg of cadmium.

Sodium hydroxide—10 per cent. and 2 per cent. aqueous solutions.

Rochelle salt—A 20 per cent. aqueous solution.

Dilute hydrochloric acid—Add 2 ml of concentrated hydrochloric acid, sp.gr. 1·19, to 400 ml of water.

Sulphamic acid.

Direct rapid procedure—

Place a 1·000 g sample of lead alloy in a 250-ml beaker, add 50 ml of diluted nitric acid (1 + 3) and if the tin content is high, add 1 g of tartaric acid solution. Warm until the alloy has disintegrated and the brown fumes have disappeared. Cool and add about 0·1 g of sulphamic acid.[8] Disregard any precipitate.

Size of aliquot—If the cadmium content is between 0·01 and 0·4 per cent., dilute the solution to 500 ml in a volumetric flask and take a 25-ml aliquot. Use smaller aliquots if a higher percentage of cadmium is present; the portion taken must not exceed 25 ml.

Carbon tetrachloride extractions—Transfer the aliquot into a 125-ml separating funnel, A, by means of a pipette. Add 1 ml of Rochelle salt solution. Add an amount of 10 per cent. sodium hydroxide solution equal in volume to the aliquot and mix thoroughly.

(*a*) Add 25 ml of dithizone - carbon tetrachloride solution. Shake for 30 seconds and allow to separate for 2 minutes. Run the lower non-aqueous layer into a second 125-ml separating funnel, B. If this layer is red, proceed to (*b*); if yellow or green, proceed to (*c*).

(*b*) Extract the aqueous solution in funnel A with 15 ml of dithizone - carbon tetrachloride solution and 10 ml of colourless carbon tetrachloride, shake for 30 seconds and allow to separate for 2 minutes. If the lower non-aqueous layer is yellow or green, proceed to (*c*). If the colour is red or pink, add this layer to funnel B. Repeat this extraction until the extract is yellow or green, and drain each extract into funnel B. When the colour is yellow or green in funnel A, drain into funnel B and proceed to the next stage, (*c*).

(*c*) Extract in funnel A with 15 ml of colourless carbon tetrachloride and drain into funnel B. Discard the upper aqueous layer in funnel A.

Acid extractions—To the combined extracts in funnel B add 5 ml of hydrochloric acid solution and 10 ml of water. Mix for 30 seconds and then allow to separate for 2 minutes. Place funnel B above funnel A and drain the lower non-aqueous layer into funnel A. Retain the aqueous solution in funnel B. Again add 2 ml of hydrochloric acid solution and 10 ml of water to funnel A. Mix for 30 seconds and then allow to separate for 2 minutes. Drain the lower, green, non-aqueous layer into a beaker, and discard. Combine the two aqueous acid solutions from funnels A and B in funnel B.

Chloroform extractions—To the aqueous solution in funnel B add 1 ml of Rochelle salt solution and 30 ml of 10 per cent. sodium hydroxide solution.

(*d*) Add 25 ml of dithizone - chloroform solution. Shake for 30 seconds and allow to separate for 2 minutes. Place funnel B above another funnel, C, which has been previously dried. Drain the lower non-aqueous layer from funnel B to funnel C. About as many chloroform extractions will be required as were performed with carbon tetrachloride. If the organic layer is yellow or green, proceed to (*f*); if red or pink, proceed to (*e*).

(*e*) Add 15 ml of dithizone - chloroform solution and 5 ml of chloroform. Shake for 30 seconds and allow to separate for 2 minutes. Drain the lower non-aqueous layer into funnel C. If the lower non-aqueous extract is green or yellow, proceed to (*f*); if red, repeat this extraction. Continue until the lower non-aqueous layer is green or yellow, then discard the aqueous layer in funnel B.

(*f*) Add 15 ml of 2 per cent. sodium hydroxide solution to the combined extracts in funnel C (pH about 13·5). Shake for 30 seconds and allow to stand for 2 minutes. Drain the lower non-aqueous layer into a dry funnel, D. Repeat the extraction with 2 per cent. sodium hydroxide. Drain the red layer into a 100-ml (or appropriate sized) volumetric flask and dilute to volume with colourless chloroform. Mix and allow to settle for 10 minutes. Measure the colour with a photometer; use a green filter (of wavelength 520 mμ). Read the weights of cadmium in milligrams from a standard curve prepared as mentioned below. Calculate the percentage of cadmium taking into account the size of the aliquot and the total dilution of the original sample.

ELECTROPLATING MODIFICATION—

Place a 1·000-g lead alloy sample in a 250-ml beaker, add 50 ml of diluted nitric acid (1 + 3) and warm until the alloy has disintegrated and the brown fumes have disappeared. Cool and add 0·1 g of sulphamic acid. Disregard any precipitate.

Electroplate at 6 volts and 2 amperes between platinum electrodes. Raise the liquid level with water every 10 minutes until no new black deposit appears. Remove the electrolyte in the usual manner. Filter the electrolyte through paper into a 100-ml, 250-ml or 500-ml volumetric flask, depending on the amount of cadmium present. Most of the copper, lead, bismuth, antimony and tin are removed.

Proceed exactly as before beginning with the extractions with carbon tetrachloride.

SULPHURIC ACID MODIFICATION—

Place a 1·000-g lead alloy sample in a 250-ml beaker, add 50 ml of diluted nitric acid (1 + 3) and warm until the alloy has disintegrated and the brown fumes have disappeared. Add 0·1 g of sulphamic acid. Disregard any precipitate. Add, dropwise, 10 ml of 10 per cent. sulphuric acid solution; stir continuously. Cool to below room temperature and filter

through a Whatman No. 40 filter-paper into a volumetric flask of appropriate size. Wash the filter-paper twice with cold 10 per cent. sulphuric acid solution; then discard the paper and precipitate. Dilute the solution in the volumetric flask with water and mix thoroughly.

Proceed exactly as before, beginning with the extractions with carbon tetrachloride.

PREPARATION OF THE STANDARD CURVE—

The standard curve is conveniently prepared from readings obtained by using a series of aliquots of the cadmium standard solution each of which is diluted to 25 ml with water. The procedure from the carbon tetrachloride extractions onwards is then followed. Readings are made with a green filter.

If a photometer or measuring instrument is not available, comparisons can be made with synthetic standards.

RESULTS

The experimental work showed that a convenient volume for final analysis is 100 ml of chloroform for cadmium present in amounts of 0·06 to 0·12 mg. The volume of chloroform may be reduced or increased as required.

After the solution has been set aside for 10 minutes to separate moisture, the red cadmium colour is stable for an indefinite period. For example, the hourly readings for one sample were 288, 288, 288, and next morning, 288. Since there is a convenient stopping-point in the analysis—the acid re-extraction from the carbon tetrachloride—10 minutes to 2 hours could be the usual waiting time before colorimetric readings.

TABLE I

COLORIMETRIC DETERMINATION OF CADMIUM IN 50-mg ALIQUOTS OF LEAD ALLOY

Cadmium found			Cadmium present by manufacturers' analysis	
By direct method, %	By electro-deposition method, %	By sulphuric acid method, %	%	Method
0·17	0·16	—	0·172	By electroplating (average of 3 samples)
0·16	—	0·16	0·172	" " " "
0·16	0·16	—	0·172	" " " "
0·06, 0·06, 0·06	—	—	0·05	—
0·06, 0·06	—	—	0·05	—
0·14, 0·15	—	—	0·16	By mercaptobenzothiazole
0·14, 0·15	—	—	—	—

Blank runs should show little or no colour for cadmium, as it is not an ordinary contaminator of reagents. Zinc has sometimes been found in sodium hydroxide, but no difficulty need be expected in this cadmium procedure.

With Bureau of Standards sample No. 53c, a slight minimum colour was noted in two of the alternative methods, indicating that the elements present in this type of lead alloy do not interfere in the methods outlined.

Table I shows some results on lead alloys. In addition, the method was used for synthetic commercial samples.

A 50-mg aliquot of a Bureau of Standards sample No. 53c was analysed by the direct method and by the electro-deposition method; 0·002 per cent. of cadmium was found to be present by both methods.

A synthetic sample containing 1·01 g of lead, 0·051 g of tin, 0·052 g of bismuth and 0·0503 g of antimony was found by the direct method to contain 0·2 per cent. of cadmium. This figure agreed with the theoretical amount of cadmium in the sample.

Three synthetic samples manufactured to contain approximately 0·15 per cent. of cadmium, 3 per cent. of antimony and lead to 100 per cent. gave the following results for cadmium—

Proposed method, %: 0·15, 0·15, 0·16, 0·16; 0·15, 0·15, 0·16; 0·14, 0·15, 0·15, 0·16
Spectrographic,* %: 0·16, 0·17, 0·17, 0·17; 0·16, 0·16, 0·16; 0·15

* Spectrographic results found by Morris P. Kirk and Sons.

Table II illustrates data used for plotting the cadmium curve.

TABLE II

TYPICAL CALIBRATION CURVE FOR CADMIUM

Cadmium per 100 ml of chloroform, mg	Reading on Klett colorimeter	Cadmium per 100 ml of chloroform, mg	Reading on Klett colorimeter
Distilled water	0	0·10	343
0·005	84	0·16	475
0·01	109	0·17	510
0·02	136	0·18	530
0·04	193	0·19	560
0·06	244	0·20	580
0·08	293	0·21	600

DISCUSSION OF RESULTS

WORKING LIMITS OF CADMIUM—

On the premise that the final colorimetric reading for the cadmium - dithizone complex will be made in 100 ml of chloroform solution, the concentration of cadmium can conveniently vary from 0·06 mg (60 μg) to 0·12 mg (120 μg). If the aliquot of test solution contains more than 0·14 mg of cadmium, an excessive number of extractions must be performed. At the other end of the scale, if the test solution should contain less than 0·06 mg of cadmium, then the final extract may easily be contained in a volume of 50 ml or less of chloroform solution.

COLOUR STABILITY—

In accordance with the procedure the colour concentration of the cadmium dithizone - chloroform solution was experimentally read after it had been set aside for 10 minutes, then hourly and again the next morning. The solutions were stable for at least 3 hours, some for 24 hours. The advantage of a one-colour system for cadmium is apparent, as it is for lead.[9]

BLANKS—

Unlike those for lead or zinc, the several reagents for the cadmium - dithizone system are not likely to contain cadmium. Impurities, such as zinc, nickel or cobalt, will only affect the cadmium results if they are present in the sodium hydroxide reagents. Each new lot of sodium hydroxide should be tested before use.

SIZE OF SAMPLE—

The procedure described here is intended for ordinary lead alloys. For ease of operation, not more than 50 mg of lead are to be used. If more than this amount of lead is taken, an extra series of extractions may be required. It seems more advisable with smaller amounts of cadmium to work in smaller (50-ml, 25-ml) volumes and to make the appropriate calculation.

Lead alloys may also contain various amounts of tin, antimony, copper, bismuth, arsenic and nickel. It is not likely that the size of sample will be dependent on the proportion of any one of these elements. Separations, if necessary, are easily made.

INTERFERENCES: CHOICE OF METHODS—

A 50-mg quantity of the base metal, lead, will not of itself give a colour in the final chloroform solution, nor will it alter the cadmium value. The Bureau of Standards sample No. 53c (0·215 per cent. of copper, 10·19 per cent. of antimony, 5·17 per cent. of tin, 0·095 per cent. of bismuth, 0·042 per cent. of arsenic) gave an equivalent value of 0·002 per cent. of cadmium by both the direct rapid method and the electroplating method, which set the usual working limits for these several elements.

If the amounts of alloying elements will not interfere in the direct rapid method, this procedure may be used; otherwise, the longer plating method is to be preferred. In this way silver, copper, lead and bismuth are removed from solution in one operation. Cobalt

is not likely to be present. Nickel, found in certain babbits, may be removed as dithizonate when the carbon tetrachloride solution is washed with dilute acid. In this event, cadmium, zinc and lead return to the aqueous phase, while nickel, copper and silver are expected to remain in the non-aqueous portion. If abnormal amounts of zinc are present, a sulphide separation may be necessary.

Besides the rapid method (no separations) and the electroplating method (preliminary separation of lead, copper, etc.) a third alternative, the sulphuric acid method (separation of lead sulphate with incidental occlusion of bismuth, antimony, etc.) may be used when the cadmium content is so low that a sample greater than 0·05 g of metal is required.

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The Determination of Cadmium in Cable Sheathing Alloys by a Direct Titration Method

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A rapid method is described for the determination of cadmium in cable sheathing alloys; it makes use of the phase exchange reaction between an amalgam of the alloy and a standardised solution of lead acetate. The cadmium in the amalgam is replaced by the more electropositive lead and the end-point of this replacement reaction can be detected by a sudden change of potential of the amalgam. A lead amalgam electrode is used and the change of potential is shown by means of a galvanometer. The exchange reaction takes place in a stream of oxygen-free nitrogen in a vessel designed specifically for the determination. The method is satisfactory for quantities of cadmium as small as 0·5 mg and is therefore applicable to alloys containing 0·05 per cent. or more of cadmium; alloying constituents such as tin and antimony do not interfere and the quantities of zinc, nickel, cobalt and iron present as impurities are too small to necessitate their removal.

The determination of small quantities of cadmium in lead is a problem to which no completely satisfactory solution has yet been found. The analysis of alloys containing 0·075 to 0·250 per cent. of cadmium, either alone or with tin or antimony, is of importance in the cable industry, and much work has been done to devise a reliable and rapid method for its determination.

Spectrochemical[1] or gravimetric[2,3] methods are generally used. The spectrochemical method necessitates the use of standard alloys for calibration purposes and the gravimetric method involves the estimation of rather small quantities of cadmium sulphate, after a lengthy procedure for separating cadmium from lead. Improved accuracy can be attained

by taking larger quantities of the alloys. This procedure is not always convenient and the quantities of material under test become unwieldy.

The results of a previous study of the properties of amalgams suggested that it might be possible to devise a method for the determination of cadmium by the application of the principles governing the potentials and phase exchange reactions of dilute amalgams. These have recently been summarised by Hohn[4,5]—

(1) The potential of an amalgam is determined solely by its least noble component.

(2) Since each amalgam strives to attain a positive potential, those reactions that produce a more positive potential proceed spontaneously.

(3) An amalgam is unable to take part in a reaction that does not change its potential.

Hence in a system containing an amalgam of lead and cadmium, the amalgam potential will be that of the least electropositive element, namely, cadmium. If this amalgam is brought into contact with a solution containing ions of the more electropositive element, lead, phase exchange takes place, cadmium ions are formed in solution and lead enters the mercury phase. At the end-point of the reaction, all cadmium is removed and the potential of the amalgam rises to that characteristic of lead. These fundamental principles have previously been developed into a technique for the separation and estimation of various metals by Tsimmergakl and Khaymovich,[6] although they did not apparently use this for estimating minor quantities of alloying constituents. Their technique was slightly different, in that they used a solution of a mercuric salt in potassium hydroxide as the titrant and worked at 100° C.

At the beginning of our investigation the work of Tsimmergakl and Khaymovich had not been encountered, so the technique that is to be described was derived from the principles set down by Hohn.[4,5] In these preliminary experiments no attempt was made to exclude air from the reaction vessel, although the necessity for this soon became evident, not only from the ease of oxidation of the amalgams, but also from the low results obtained for cadmium.

Enough mercury was taken to ensure complete solution of the base metals present. The values for the solubilities of some metals in mercury are shown in Table I.

TABLE I

THE SOLUBILITY OF METALS IN MERCURY[4]

Metal	Solubility at 25° C, % w/w	Solubility at 70° C, % w/w
Cadmium	6·0	20·0
Lead	1·3	7·0
Tin	0·6	4·0

Lead acetate solution was chosen as the titrant because the salt was available in a state of sufficient purity to enable a standard solution to be prepared. The early experiments were largely concerned with finding a suitable indicator to detect the end-point of the reaction. Hydrogen sulphide solution was tried at first as an external indicator with the object of detecting the first excess of titrant in the aqueous phase. This method was abandoned in favour of measurement of the amalgam potential with reference to a calomel electrode.

The calomel electrode was incorporated in the system by an agar bridge (ammonium nitrate agar) and the potential between the mercury and the calomel electrode measured with a valve voltmeter; a Cambridge pH meter can be used. The potential of the system was found to be changed when the mercury - water interface was stirred. As stirring facilitates the phase exchange reaction, a thin flat-bladed stirrer is used continuously during the titration. It was decided to use a potentiometric method to detect the end-point, so some attention was paid to the composition of the aqueous phase, and the effect of the addition of various electrolytes was investigated. It was found best to use 10 ml of dilute acetic acid for the aqueous phase. This solution remained clear throughout the reaction and gave good end-point transitions. During the preliminary experiments no attempt had been made to exclude air from the reaction vessel: later this was done by passing a stream of oxygen-free nitrogen or hydrogen through the vessel and the efficiency of this procedure was reflected in the higher values obtained for cadmium. At this stage the apparatus was re-designed to enable air to be excluded more easily and a vessel was constructed to contain

the amalgams during preparation in a similar atmosphere. The plotting of points from readings on a valve voltmeter with repeated standardisations of the instrument was found to be laborious and accordingly the voltmeter was replaced by a galvanometer and resistance. The resistance was approximately 18,000 ohms, so that a deflection of 1 cm was obtained for 0·01 volt. The calomel half-cell was replaced by a lead amalgam electrode, composed of 1 ml of a 1 per cent. w/w lead amalgam in contact with dilute acetic acid and a few millilitres of 0·02 *N* lead acetate solution, which had the effect of reducing the final potential of the system to zero and giving a greater sensitivity to the galvanometer readings. This electrode system became in effect a null-point indicator although, as can be seen from Fig. 1, there was

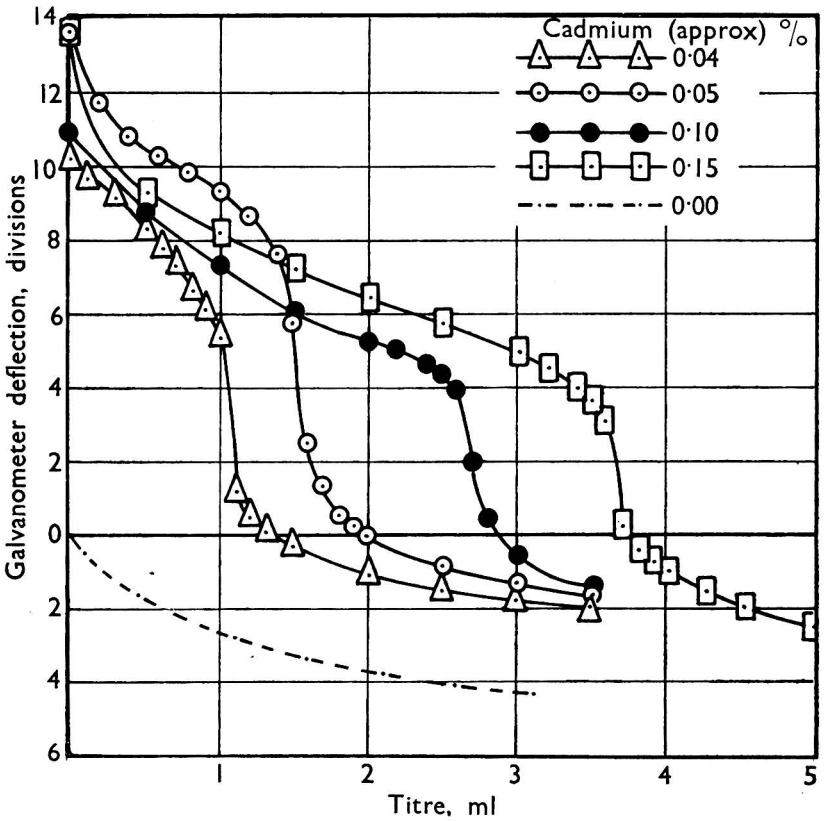

Fig. 1. Typical titration curves for alloys of lead and cadmium

a slight variation of potential between the lead amalgam half-cell and the titration vessel electrode. Variations in temperature of the system are without effect.

It was observed that on the addition of the lead acetate solution the deflection of the galvanometer immediately altered, but slowly recovered until, after about a minute, the galvanometer was steady again and the deflection became slightly less than it was initially. As the end-point was neared, the difference in deflection for, say, subsequent 0·1-ml additions became larger and at the end-point the deflection became appreciable and no stability was attained for some few minutes. A graph was plotted to show the change of potential of the system with time at this point in the titration and it was found, as is shown in Fig. 2, that a maximum rate of change of potential was experienced.

This maximum must be due to a combination of effects. Consider the addition of a small quantity of lead acetate solution to the aqueous phase. This will undergo phase exchange with the cadmium in the amalgam, and as the cadmium content of the amalgam

is reduced the potential of the system can be exactly expressed by the Nernst equation,[7] in the form deduced by Turin[8]—

$$E = E_0 - \frac{RT}{2F}\log_e\frac{C_1}{C_2}$$

where C_1 and C_2 are the concentrations of cadmium in the water and mercury phases respectively, and E_0 the normal potential of the metal. Expressing this equation in natural logarithms and considering only the difference of potential—

$$\Delta E = -\frac{RT}{2F}\log_e\frac{C_1}{C_2}$$

$$= -0{\cdot}029\log_{10}\frac{C_1}{C_2}$$

Fig. 3 shows an experimental curve for a 0·15 per cent. cadmium alloy plotted with the theoretical curve for the same alloy.

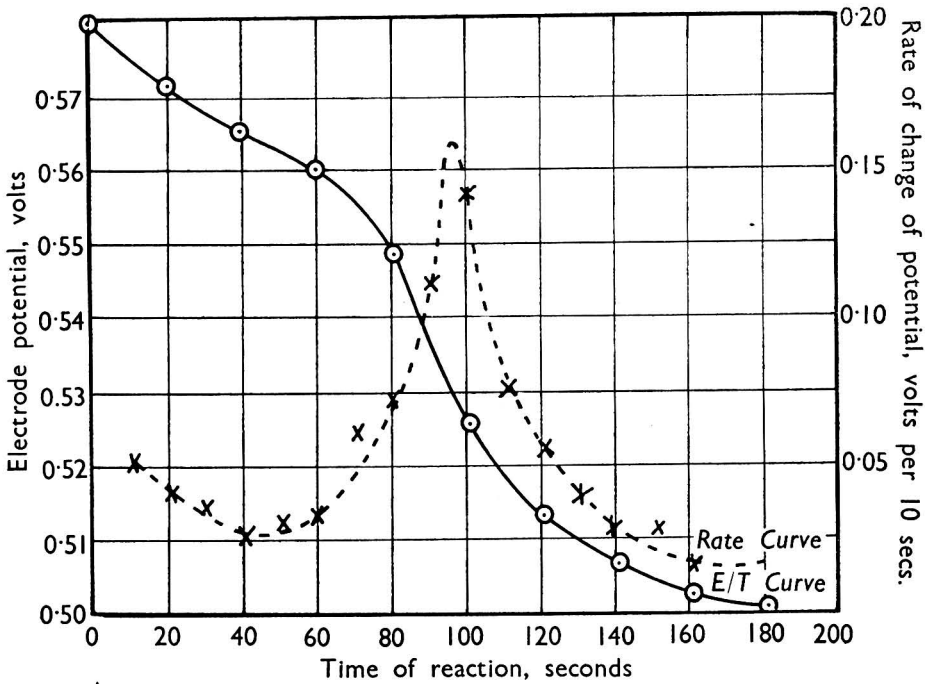

Fig. 2. Rate of the phase exchange reaction at the end-point

The rate of fall of potential of the system increases as C_2 decreases, and therefore ΔE becomes infinitely large at $C_2 = 0$; thus, as the end-point is approached, the last drop of titrant to be added (0·05 ml approximately) causes a very large deflection of the galvanometer needle. The decrease in rate of fall which follows is probably due to the reduction of the concentration of the lead ions, so that diffusion effects become of kinetic importance. The effect of further additions of lead acetate solution can be calculated from a similar equation linking the concentration of lead ions with lead amalgam; the potential remains nearly constant.

Method

Reagents—

Lead acetate solution—Dissolve 7·5870 g of $(CH_3COO)_2Pb.3H_2O$ in distilled water and make up to 1 litre in a graduated flask.

Dilute acetic acid—Add distilled water to 20 ml of glacial acetic acid and make up to 1 litre.

APPARATUS—

The amalgamation vessel—This is a tube designed so that the air it contains can be replaced by a stream of purified nitrogen and the amalgam, when formed, can be transferred to the titration vessel. The amalgamation vessel designed in the course of this investigation was basically a test tube 15 cm in length fitted with a B19 ground-glass joint and stopcock. Half way down the tube a T-arm with another stopcock was blown in. The tube could be evacuated and filled with nitrogen, or a slow stream of nitrogen could be passed through.

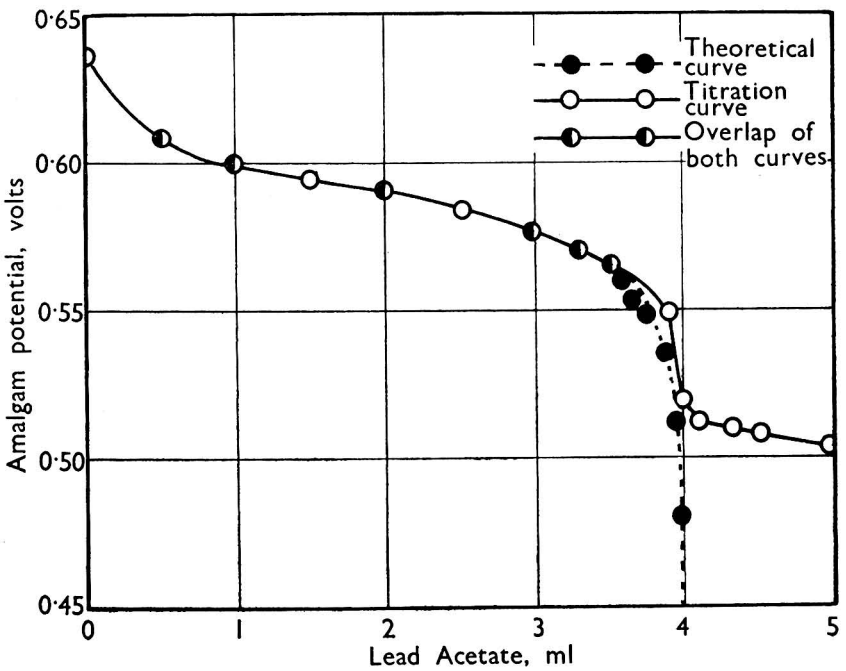

Fig. 3. Agreement between the theoretical curve and one obtained from a typical titration for alloy containing 0·15 per cent. of cadmium

The titration vessel—This is a large test tube 10 cm long and 3·8 cm in diameter, through the bottom of which a platinum wire is sealed. Half way up the tube, on opposite sides, a pair of inclined tubes are sealed in, one for the introduction of an agar bridge to a lead amalgam electrode, and the other for the passage of nitrogen. These tubes are each inclined at approximately 35° to the vertical and are 8 mm in diameter. The top of the tube is a B34 glass joint, through the stopper of which passes a tube for the insertion of the tip of the burette and a collar through which a stirrer can be inserted.

PROCEDURE—

Dissolve about 1 g of the alloy in 7 ml of mercury in the amalgamation vessel by warming gently until the mercury is at a temperature just below its boiling-point. During this operation pass a slow stream of oxygen-free nitrogen through the amalgamation vessel. Allow the apparatus to cool and meanwhile pass a stream of nitrogen through 10 ml of dilute acetic acid contained in the titration vessel. When the amalgam is cool, transfer it to the titration vessel through the tube intended for the insertion of the agar bridge of the lead amalgam half-cell. Insert the bridge, put the galvanometer in circuit and commence the titration with lead acetate solution. Stir throughout the titration. With each small addition of lead acetate read the galvanometer deflection when steady conditions are attained

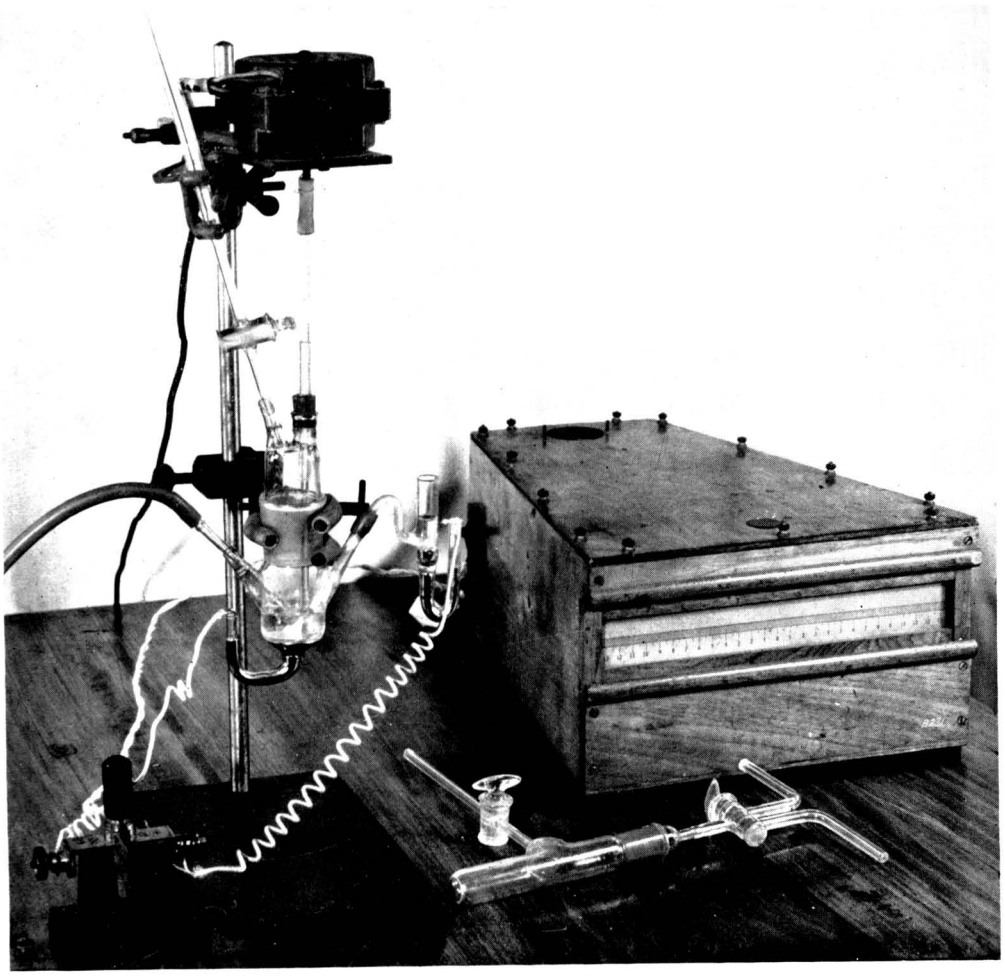

Fig. 4. Apparatus

(after about 1 minute); near the end-point the deflections become larger and the stability of the galvanometer readings decreases, until eventually a large deflection is given to the other side of the zero position. From the graph obtained by plotting the lead acetate content against the galvanometer reading the end-point can be determined easily.

1 ml of 0·02 *N* lead acetate ≡ 0·0012 g of cadmium.

NOTES ON THE DETERMINATION—

The plotting of the deflections of the galvanometer may be omitted as the method becomes familiar, but plotting the graph permits the situation to be visualised and enables the amounts of lead acetate added during the titration to be varied according to the approach to the end-point, and this procedure is, therefore, particularly useful when evaluating the cadmium content of alloys the approximate titres of which are unknown.

The accuracy of the method was assessed in several ways. Known amounts of cadmium metal were added to the lead amalgam, and the cadmium was estimated in the usual manner. It was found that the theoretical and the experimental values for the cadmium agreed to within 2·0 per cent.

A series of analyses was then performed upon standard cable sheathing alloys; the results obtained are shown in Table II.

TABLE II

RESULTS OF ANALYSES ON CABLE SHEATHING ALLOYS

Alloy	Nominal cadmium content, %	Mean cadmium content found, %	Standard deviation	Coefficient of variation, %
Experimental	0·250	0·244	0·0088	3·6
C*	0·150	0·139	0·0040	2·9
½C*	0·075	0·071	0·0042	5·9

* These alloys conform to B.S.S. 801:1938.

From a comparison of these figures with those obtained by other methods for the determination of minor constituents it will be seen that the accuracy of the method is satisfactory. The small error can be accounted for by the uncertainty of interpreting the curves to ±0·05 ml. For example, with 0·02 *N* lead acetate solution and a 1-g sample of 0·15 per cent. cadmium alloy, ±0·05 ml corresponds to an error of 3·73 per cent.

At first sight it might appear that this method would give a simple solution to the often difficult problems involved in the rapid estimation of the alloying constituents in sheathing

TABLE III

HALF-WAVE POTENTIALS OF THE METALS FOUND IN LEAD SHEATHING ALLOYS[9]

Metal	Half-wave potential	Supporting electrolyte
Silver	>+0·04	0·1 *N* KNO_3
Copper	+0·02	0·1 *N* KNO_3
Bismuth	−0·05	1·0 *N* HCl
Antimony	−0·24	1·0 *N* HCl
Lead	−0·435	0·1 *N* KCl or HCl
Tin	−0·49	1·0 *N* HCl
Cadmium	−0·599	0·1 *N* KCl or HCl
Zinc	−0·995	0·1 *N* KCl
Nickel	−1·10	1·0 *N* KCl
Cobalt	−1·20	0·1 *N* KCl
Iron	−1·30	0·1 *N* KCl

and other lead alloys. In fact, the method is limited by the considerations affecting amalgam potentials. These potentials depend upon the most electronegative component, and generally correspond to the half-wave potential of the metal concerned, measured with respect to a calomel electrode. If the half-wave potential of the metal to be determined lies close to that of lead, a clear change of potential will not be detectable, and this precludes the estimation of tin in lead. The fall in potential, in accordance with the Nernst equation, would be greater than that corresponding to a change from tin to lead amalgam. Metals having a more

positive half-wave potential than lead could not, of course, be estimated by this method without prior phase exchange of all the lead in the mercury phase.

The impurities and alloying constituents usually found in lead sheathing alloys are listed in Table III, together with their half-wave potentials, which are referred to the saturated calomel electrode at 25° C.

It can be seen from the table that the metals zinc, nickel, cobalt and iron all possess more negative half-wave potentials than cadmium and would, therefore, be expected to interfere with the method. These metals are, however, with the exception of zinc, completely insoluble in mercury,[4] and even the zinc will not interfere, being usually present as a trace impurity in amounts of 10^{-5} to 10^{-4} per cent. The presence of quantities of zinc comparable with those of cadmium would be expected to show on the graph as a double step, the first, the result of the zinc, the second, of the cadmium, and so could be estimated in this manner.

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The Absorptiometric Determination of Thorium in Magnesium-Base Alloys by 1-(*o*-arsonophenylazo)-2-naphthol-3:6-disulphonic Acid

By A. Mayer and G. Bradshaw

A method for the determination of thorium in magnesium-base alloys is described. A red colour is developed when the reagent is added to a thorium solution. If zirconium is present thorium is separated by precipitation as oxalate. None of the other elements normally present in magnesium alloys interferes. The method gives results correct to within 2 per cent., can be applied to samples with widely differing thorium content and is reasonably rapid. The preparation of the reagent is also described.

The recent introduction of thorium as an important alloying agent in magnesium alloys has made it necessary to determine thorium in amounts ranging from 0·2 to 5 per cent. in the presence of rare earths and zirconium as a routine matter.

After extensive trials of various methods we adopted the procedure detailed below. Thorium is separated from magnesium by an ammonium hydroxide precipitation; separation from zirconium is then effected by precipitating thorium and rare earths as oxalates. The oxalates are converted to perchlorates and thorium is determined absorptiometrically, without removing rare earths, with the sodium salt of 1-(*o*-arsonophenylazo)-2-naphthol-3:6-disulphonic acid (APANS; Thoronol).

APANS and other *o*-arsonic acids of hydroxyazo compounds have been studied by Kuznetsov[1,2] and the analytical chemistry of thorium has been reviewed recently in detail by Rodden and Warf.[3] Most of the gravimetric methods with organic reagents and the

colorimetric procedures based on lake formation necessitate great attention to detail before reliable results can be obtained, and are not suitable as routine methods. The absorptiometric part of the procedure with APANS has been described by Thomason, Perry and Byerly.[4]

Experimental

Preparation of reagent—

Dissolve 11 g of *o*-arsanilic acid (*o*-aminophenylarsonic acid) in 700 ml of water, add 100 ml of 11·3 *N* hydrochloric acid, cool in ice and diazotise by adding 3·5 g of sodium nitrite dissolved in 25 ml of water. Add 16 g of R acid (2-naphthol-3: 6-disulphonic acid) dissolved in 200 ml of water and then about 700 g of sodium acetate. Set this aside for 1 hour and filter off the dye on a large Büchner funnel; dry by suction. Dissolve the dye in 400 ml of hot water, filter off any insoluble matter and add 500 ml of hot alcohol to the boiling solution. Allow to cool, filter, wash with alcohol and dry the dye at 110° C. The yield is about 18 g.

Choice of filters—

APANS solution is orange and the dye - thorium complex is red. The maximum difference in absorption occurs at 555 mμ as determined on a "Uvispek" spectrophotometer (Fig. 1), corresponding to the Ilford No. 605 yellow-green filters. With these filters the dye alone gave an extinction reading of 0·23; this can be reduced to 0·02 with Ilford No. 606 yellow filters at 580 mμ, but the absorption of the thorium - APANS complex is less than one-third of the absorption at 555 mμ. Therefore the No. 605 filters were used for further work and a dye-blank was measured with each batch of solutions. A tungsten filament lamp was used throughout, but the same considerations hold good for a mercury vapour lamp. Thomason, Perry and Byerly[4] state that the absorption maximum is at 545 mμ.

Amount of reagent—

It was found that one atom of thorium combines with two molecules of dye, but the complex is slightly dissociated so that a slight increase in absorption can be obtained by adding an excess of APANS.

For the range 0 to 1·4 mg of thorium per 100 ml, 10 ml of 0·1 per cent. aqueous APANS solution is satisfactory. If 15 ml of dye is used the absorption for 0·8 mg of thorium increases by about 4 per cent. The graph obtained is slightly curved. Concentrations of thorium higher than about 3 mg per 100 ml cannot be tolerated as the complex would not be completely soluble.

Stability of colour and effect of temperature—

The colour is stable for at least 24 hours. Change in temperature from 15° to 25° C causes an increase of about 4 per cent. in absorption.

Effect of pH—

Thomason, Perry and Byerly[4] showed that maximum absorption was obtained between pH 0·2 and 1·0 and decreased rapidly on either side of these limits. From our tests it would seem that the safe pH limits are about 0·5 and 1·2. This corresponds, under the conditions of the test, to a free hydrochloric acid concentration of 1·0 to 5·0 ml of 11·3 *N* acid per 100 ml of solution.

Effect of other ions—

Magnesium^{II} (250 mg), manganese^{II} (55 mg), zinc^{II} (5 mg), aluminium^{III} (11 mg), iron^{III} (0·1 mg), copper^{II} (0·12 mg), Mischmetall (3 mg, equivalent to 1·5 mg of cerium^{III}) and cerium-free Mischmetal (8 mg of a mixture of praseodymium^{III}, neodymium^{III}, lanthanum^{III}, samarium^{III} and so on) per 100 ml cause no interference. Cerium^{IV} and larger amounts of iron^{III} reduce the absorption, but this effect can be overcome by adding hydroxylamine hydrochloride. Zirconium must be absent because it forms a coloured compound with APANS similar to, but much less soluble than the thorium compound. The interference from uranium^{IV} ions can be overcome by their oxidation to uranyl ions by heating with perchloric acid.[4]

One millilitre of 20-volume hydrogen peroxide solution causes no interference. Chloride and perchlorate can be tolerated in solution. Sulphates, phosphates, oxalates and organic hydroxy acids form complexes with thorium and suppress the colour. Fluoride also forms complexes with thorium; the colour decreases as the fluoride concentration increases and this reaction could probably be used for the determination of fluoride. Interference from fluoride can be overcome by fuming with perchloric acid. Large amounts of nitrate interfere.

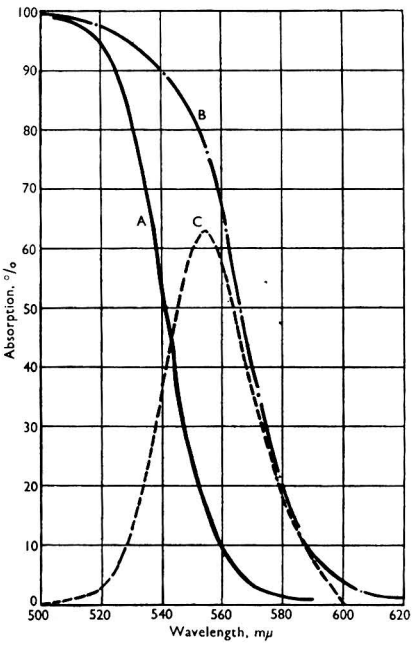

Fig. 1. Absorption spectra of solutions of 1-(*o*-arsonophenylazo)-2-naphthol-3:6-disulphonic acid (APANS).
Curve *A*: 10 ml of 0·1 per cent. solution of APANS + 4 ml of 11·3 *N* hydrochloric acid per 100 ml.
Curve *B*: 10 ml of 0·1 per cent. solution of APANS + 4 ml of 11·3 *N* hydrochloric acid + 0·8 mg of thorium per 100 ml.
Curve *C*: difference, *B*–*A*

The amount of thorium remaining with the acid-insoluble zirconium is extremely small. It was determined in the precipitates from two samples of Zr - Th - Mg alloy and found to be 0·004 and 0·003 per cent. The alloys contained 3 per cent. and 1·75 per cent. of thorium respectively.

The possibility of co-precipitation of zirconium with thorium in the oxalic acid separation (see p. 157) was examined by means of synthetic alloy samples containing 0·5 to 4 per cent. of thorium and 0·7 per cent. of zirconium. The results are shown in Table I.

TABLE I

THE EFFECT OF ZIRCONIUM

All the test solutions contained the equivalent of 0·7 per cent. of zirconium

Thorium added, per cent. ..	4·11	3·07	2·05	1·04	0·51
Thorium found, per cent. ..	4·15	3·12	2·07	1·02	0·51

To test the effect of zirconium under extreme conditions, beyond the range found in magnesium-base alloys, three determinations were made on synthetic solutions corresponding to an alloy containing 0·93 per cent. of thorium and 10 per cent. of zirconium. The results for thorium were 0·94, 0·965 and 0·965 per cent., which showed that under these extreme conditions there is slight interference by zirconium.

TYPICAL RESULTS—

The analytical procedure described below has been in routine use for over a year and has given consistently good results in the hands of junior analysts.

The method has been tested by analysing synthetic samples, and replicate tests by different analysts have been satisfactory. After adding 198 mg of thorium to synthetic samples containing magnesium, rare earths and zirconium, the amounts of thorium found by two analysts were 201, 198, 198, 194, 201 and 198 mg. Duplicate determinations by two analysts, on samples of alloys, gave the following typical results—

Thorium, per cent., 3·02, 3·07; 1·02, 1·04; 13·1, 13·2 (Hardener alloy); 1·85, 1·89.

METHOD

REAGENTS—

Concentrated hydrochloric acid—11·3 *N*.

Diluted hydrochloric acid (1 + 19)—Add 5 ml of 11·3 *N* acid to 95 ml of water.

Ammonium chloride.

Hydrogen peroxide—A 20-volume solution free from phosphate.

Diluted ammonium hydroxide solution (1 + 1)—Add 50 ml of ammonium hydroxide solution, sp.gr. 0·88, to 50 ml of water.

Diluted ammonium hydroxide solution (1 + 49)—Add 2 ml of ammonium hydroxide solution, sp.gr. 0·88, to 98 ml of water.

Oxalic acid—Crystalline.

Wash solution—Add 1 g of oxalic acid to 1 ml of 11·3 *N* hydrochloric acid in 100 ml of water.

Perchloric acid—An approximately 70 per cent. w/v solution.

Potassium permanganate solution—An approximately 0·1 *N* solution. Dissolve 3·25 g of potassium permanganate in 1 litre of water.

Hydroxylamine hydrochloride solution—Dissolve 10 g of the hydrochloride in 100 ml of water.

Dye solution—Dissolve 0·1 g of di-sodium 1-(*o*-arsonophenylazo)-2-naphthol-3:6-di-sulphonate in 100 ml of water.

PROCEDURE—

Weigh out accurately 3 to 5 g of sample, transfer it to a 400-ml beaker, add about 100 ml of water and dissolve by adding 10 ml of concentrated hydrochloric acid for each gram of sample. When the sample has completely dissolved, filter the solution through a paper-pulp pad to remove any insoluble zirconium and wash the pad with hot diluted hydrochloric acid (1 + 19).

Add 5 g of ammonium chloride to the filtrate, bring it to the boil and carefully add 25 ml of hydrogen peroxide; add diluted ammonium hydroxide solution (1 + 1) until the solution is alkaline to litmus paper. Allow the precipitate to settle, filter on a Whatman No. 541 filter-paper and wash 2 or 3 times with diluted ammonium hydroxide solution (1 + 49).

Wash the precipitate back into the beaker from which it came, add 5 ml of concentrated hydrochloric acid and boil until the solution is clear and colourless. Dilute to 150 ml with water, bring to the boil, and dissolve 10 g of oxalic acid in the solution. Set aside for at least 2 hours, or preferably overnight, and filter on a Whatman No. 44 filter-paper. Wash the beaker and precipitate 5 or 6 times with the wash solution and transfer the precipitate back to the beaker. (Rare earths if present will also have been precipitated at this stage but they cause no interference.) Add 10 ml of perchloric acid to the beaker, dilute to about 100 ml with water and bring to the boil. Add 0·1 *N* potassium permanganate slowly to the boiling solution from a burette until the pink colour persists for at least 2 minutes.

Add dropwise hydroxylamine hydrochloride until the permanganate colour is discharged and then add a few drops in excess. Cool the solution, transfer it to a 500-ml graduated flask and dilute to the mark with water. By means of a pipette transfer 50 ml of this solution to a 250-ml graduated flask and dilute to the mark with water. Transfer 25 ml of this diluted solution* by means of a pipette to a 100-ml graduated flask, add 4 ml of concentrated hydrochloric acid, 10 ml of dye solution and make up to the mark with water.

* Take 50-ml portions to cover the range 0·2 to 2·5 per cent. of thorium and 10-ml portions to cover the range 1·0 to 12·5 per cent. of thorium.

Measure the absorption at 555 mμ on a spectrophotometer or other instrument with glass filters corresponding to this wavelength.

Carry out a blank determination with 10 ml of the dye solution and 4 ml of concentrated hydrochloric acid diluted to 100 ml.

Subtract the drum reading of the sample from that of the blank and find the thorium content of the solution from a calibration graph.

STANDARDISATION—

Add 50 ml of water, 4 ml of concentrated hydrochloric acid and 10 ml of APANS reagent to various amounts (0 to 30 ml) of standard thorium solutions (1 ml ≡ 0·05 mg of thorium) prepared from pure Th $(NO_3)_4.4H_2O$; dilute to 100 ml and measure the absorption.

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MANCHESTER

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Notes

TOTAL HARDNESS OF WATER BY THE VERSENATE METHOD—DIRECT TITRATION WITH ETHYLENEDIAMINE TETRA-ACETATE (EDTA)

FOR some years the total hardness of waters has been determined in this laboratory by a soapless method, sometimes referred to as the Pfeifer - Wartha method,[1,2] in which the loss of alkalinity of a hydroxide - carbonate solution, after boiling with the neutralised sample, is expressed as $CaCO_3$ hardness. More recently, we have tried the method introduced by Schwarzenbach and his co-workers and modified by Betz and Noll,[3] in which the calcium and magnesium ions are titrated directly with a solution of an ethylenediamine tetra-acetate salt to produce un-ionised complexes in the presence of Eriochrome black T, an indicator that changes colour when the whole of the ions have been sequestered.

METHOD

REAGENTS—

Titrating solution—Dissolve 4·0 g of the crystalline di-sodium salt of ethylenediamine tetra-acetic acid in 800 ml of distilled water, add 0·86 g of sodium hydroxide and adjust against a standard solution of calcium chloride so that 1 ml is equivalent to 1 mg of $CaCO_3$.

Buffer solution—Dissolve 40·0 g of borax in 800 ml of distilled water. Dissolve 10·0 g of sodium hydroxide and 5·0 g of sodium sulphide in 100 ml of water, cool, mix with the borax solution and make up to 1 litre.

Indicator solution—Add 1·0 ml of *N* sodium carbonate solution to 30 ml of distilled water. Add 1·0 g of Eriochrome black T, mix and make up to 100 ml with 99 per cent. *iso*propyl alcohol.

PROCEDURE—

Put 25 ml (or any other suitable quantity) of sample in a white dish by means of a pipette, add 0·5 ml of buffer solution and 5 drops of indicator solution. Run in the titrating solution from a burette slowly and with constant stirring. The end-point is reached when the colour changes from purple to blue, *i.e.*, the red colour is discharged. It is very important that the last few drops be added slowly with constant stirring. Then—

$$\text{Hardness as } CaCO_3\text{, parts per million} = \frac{\text{ml used for titration} \times 1000}{\text{ml of sample taken}}$$

The colour change is sharp when magnesium ions are present, but may be poor when they are absent, as when standardising with pure calcium chloride solutions. In such circumstances

it is convenient to introduce magnesium ions deliberately by titrating a small quantity of tap water to the end-point before adding the standard hard water.

Over a range of samples differing widely in total hardness, the figures obtained by the two methods compared satisfactorily, so the newer method was adopted for general routine work on account of its directness and simplicity, in contrast to the several time-consuming operations required by the Pfeifer - Wartha method. Although the Schwarzenbach method has been adopted for general work, one sample each week has been examined by both methods. Cooling water for Diesel engines was selected for test; it was taken from a pond that was treated from time to time to remove part of the temporary hardness.

It is interesting to note in Table I that the Schwarzenbach method gives results a little higher than the mixed-alkali method and that, in describing the latter method, Thresh, Beale and Suckling[2] say on p. 751 of their book: "This method gives results which are slightly lower than, but within 5 per cent. of the total hardness calculated as $CaCO_3$ from the calcium and magnesium contents of the water."

Table I shows the figures for hardness as $CaCO_3$ in parts per million obtained by the two methods between September, 1950, and August, 1951; all the determinations were made by the same laboratory assistant.

TABLE I

HARDNESS OF DIESEL COOLING WATER AS $CaCO_3$

Schwarzenbach method, p.p.m.	Pfeifer - Wartha method, p.p.m.	Difference, p.p.m.	Schwarzenbach method, p.p.m.	Pfeifer - Wartha method, p.p.m.	Difference, p.p.m.
162	155	7	220	215	5
108	105	3	228	225	3
122	105	17	224	220	4
116	110	6	240	225	15
128	115	13	248	235	13
135	125	10	236	225	11
136	125	11	228	225	3
144	140	4	224	220	4
142	135	7	220	215	5
156	150	6	220	210	10
148	145	3	220	215	5
176	170	6	216	210	6
180	175	5	116	110	6
160	155	5	156	153	3
176	170	6	84	90	−6
148	140	8	120	125	−5
172	170	2	176	170	6
180	170	10	124	120	4
176	170	6	128	120	8
180	185	−5	116	108	8
180	170	10	124	120	4
180	175	5	124	120	4
220	210	10	128	125	3
220	215	5			

Considering the very different techniques employed and the large number of steps required for the second method the figures are in very good agreement. The solution strengths used were such that 1 drop of titrating solution for the Schwarzenbach method on a 25 ml sample represented 2 p.p.m. of $CaCO_3$, whereas 1 drop for the Pfeifer - Wartha method on a 200-ml sample represented 5 p.p.m.

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NOTE—The original papers by Schwarzenbach and his co-workers are in Swiss journals, but recommended procedures based on their method are given by: Heald, I. A., Coates, K. B., and Edwards, J. E., "New Procedures for the Calcium, Magnesium and Total Hardness of Water," *Industr. Chem. Chem. Mfr.*, 1950, **26**, 428.

CHEMICAL BRANCH
PUBLIC HEALTH DEPARTMENT
L.C.C. SOUTHERN OUTFALL WORKS
CROSSNESS, ABBEY WOOD, LONDON, S.E.2

J. E. HOULIHAN
October, 1951

THE DETERMINATION OF CHLORINATED *o*-CRESOLS WITH GIBBS' REAGENT

SMALL amounts of phenol, particularly in water, are usually determined by coupling the phenol with diazotised sulphanilic acid to form the dye[1] or by Gibbs' reagent.[2] A method was required for the determination of the products of chlorination of *o*-cresol, 4-chloro-*o*-cresol, 4:6-dichloro-*o*-cresol and 6-chloro-*o*-cresol. The sulphanilic acid method is unsatisfactory with *p*-substituted phenols, so attention was directed to the use of Gibbs' reagent (2:6-dibromoquinone-4-chloro-imide). Ettinger and Ruchhoft[3] extracted the blue indophenol colour with *n*-butyl alcohol. They showed that *p*-chlorophenol and phenol gave colours having similar qualitative absorption characteristics. Beshgetoor, Green and Stenger[4] noted that the reaction with *p*-chlorophenol has a quarter of the sensitivity of that with phenol. No reference can be found to the reaction of chlorinated *o*-cresols with Gibbs' reagent although Ettinger and Ruchhoft studied the characteristics of *o*-cresol itself.

Experiments were carried out in this laboratory with a slight modification of the method of Ettinger and Ruchhoft.

METHOD

REAGENTS—

Borate buffer solution—Dissolve 3·1 g of boric acid, 3·5 g of potassium chloride and 30 ml of *N* sodium hydroxide in distilled water and make up to 1 litre. Make 5 ml of this buffer up to 100 ml and measure the pH. Add sodium hydroxide to the concentrated solution until the pH of 5 ml in 100 ml is 9·40.

Gibbs' reagent—Dissolve 0·100 g of 2:6-dibromoquinone-4-chloro-imide in 25 ml of ethyl alcohol. Filter 9 ml of this solution immediately and dilute to 200 ml with water. Use the aqueous solution within 10 minutes of preparation.

n-Butyl alcohol.

PROCEDURE—

Add 10 ml of buffer solution and 4 ml of Gibbs' reagent to a solution containing the appropriate cresol in 200 ml of water. Mix the solution and stand it in the dark for 16 hours; extract the colour with 50 ml of *n*-butyl alcohol and filter the organic layer into a 1-cm or 4-cm glass cell. At the same time carry out a blank determination with the reagents only and measure the absorption of both solutions on an absorptiometer with the appropriate filter (see below).

All colour reactions should take place and extractions should be made at 19° ± 1° C.

RESULTS

Experiments were made with equimolar solutions (25×10^{-8} to 400×10^{-8} *M*) of phenol, *o*-cresol, 4-chloro-*o*-cresol, 4:6-dichloro-*o*-cresol and 6-chloro-*o*-cresol.

It was found that 6-chloro-*o*-cresol and *o*-cresol had a greater speed of reaction than the other two chlorinated cresols, which react at speeds greater than that of phenol itself. The difference in reaction rates, however, is insufficient to enable an analytical method to be devised that makes use of this effect.

Calibration graphs obtained with Ilford No. 607 or No. 608 filters and a tungsten filament lamp were all linear over the range 0 to 300×10^{-8} *M*, with the exception of the graph for phenol. This non-linearity has been deduced[3] to be the result of the slowness of reaction and of the simultaneous decomposition of the reagent.

Experiments were limited to the use of different filters with the Spekker absorptiometer when comparing the absorption characteristics of the compounds studied. Specimen results are shown in Table I.

TABLE I

ABSORPTION CHARACTERISTICS OF THE 50×10^{-8} *M* SOLUTIONS STUDIED

	Absorptiometer reading with a 4-cm cell				
Filter No.	Phenol	*o*-Cresol	4-Chloro-*o*-cresol	6-Chloro-*o*-cresol	4:6-Dichloro-*o*-cresol
607	0·295	0·27	0·27	0·295	0·285
608	0·36	0·26	0·255	0·44	0·43

The following conclusions were drawn from these results and from many others not reproduced.

o-Cresol and 4-chloro-*o*-cresol produce compounds with similar absorption characteristics after reaction with Gibbs' reagent.

6-Chloro-*o*-cresol and 4:6-dichloro-*o*-cresol produce compounds with similar absorption characteristics after reaction with Gibbs' reagent.

o-Chloro-substitution can be detected in *o*-cresols, if phenol itself is absent, by taking two readings, with different filters. The halogen in the *ortho*-position displaces the absorption band towards the red end of the spectrum.

The total molar content of chlorinated cresol plus phenol can be determined within ±10 per cent. by taking a reading with the Ilford No. 607 filter and *o*-cresol as a comparison standard.

The author wishes to thank the Directors of Pest Control Limited for permission to publish this note.

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June, 1951

THE POTENTIOMETRIC DETERMINATION OF SMALL AMOUNTS OF TUNGSTEN WITH CHROMOUS CHLORIDE

The use of chromous chloride solution for the potentiometric determination of tungsten^{VI} at 70° to 90° C has been recently proposed,[1,2,3,4] but the optimum conditions of acid concentration and the limits of the applicability of the method had not been satisfactorily investigated. The object of this investigation was to ascertain (*a*) the optimum conditions to give satisfactory titrations at room temperature and so to simplify the method of determination, and (*b*) the minimum amount of tungsten that can be determined by this method.

An experimental procedure is described in which concentrations of tungsten as small as 5 mg per 100 ml can be determined at room temperature with an accuracy of not less than 1 per cent.

Experimental

Preparation of the chromous chloride solution—

The method of preparation of chromous chloride solutions tested by Zintl and Rienäcker[5] and essentially adopted by Brintzinger and Oschatz[6] was followed. The pure recrystallised chromate was reduced with concentrated hydrochloric acid and pure zinc to blue chromous chloride. This was filtered from the excess of zinc through glass wool and converted into the red acetate. The acetate was washed several times by decantation, dissolved in 2 per cent. hydrochloric acid and transferred to a storage bottle. Filtration, washing and transfer were all done in an atmosphere of pure carbon dixoide. The chromous chloride solution was kept during the titration in a Zintl burette, in an atmosphere of pure carbon dioxide. The solution was standardised before and after each set of experiments against standard copper sulphate solution.

Preparation of standard tungsten solutions—

Two different procedures were used in preparing the standard tungsten solutions. The first was by accurately weighing A.R. sodium tungstate and dissolving it in a known volume of conductivity water; this formed a stock solution. The tungsten content of this stock solution was checked gravimetrically by the benzidine method.[7] The second method was by preparing a standard stock solution by dissolving a known weight of sodium tungstate mixed with sodium carbonate in 36 per cent. hydrochloric acid; this was recommended by Collenberg and Guthe.[8]

The hydrochloric acid solutions—

The titration media used in this investigation were 36, 25, 20, 15, 10 and 5 per cent. w/v solutions of hydrochloric acid. An azeotropic solution of hydrochloric acid was twice distilled and either concentrated with pure gas or diluted with conductivity water, as required.

The potentiometric titration device—

The titrations were done in a tall 500-ml beaker-flask fitted with a rubber stopper that had been previously treated with boiling hydrochloric acid. The stopper carried holes for a burette tip,

a mechanical stirrer, an indicator electrode, a salt-bridge and entrance and exit tubes for pure carbon dioxide. The indicator electrode was a 1 sq. cm platinum sheet sealed in glass, with a mercury contact. The reference half-cell used was the saturated calomel electrode.

The titrations were always carried out at room temperature, 25° ± 1° C, with 100 ml of the hydrochloric acid as the titration medium.

Results and discussion

When 36 per cent. w/v hydrochloric acid was used it was impossible to determine amounts of tungsten smaller than 120 mg per 100 ml of solution, although the titration curves gave rather sharp inflections at the end-point; with lower tungsten contents the experimentally determined amount was found to be less than that actually present. The percentage error increased with decrease of the concentration of tungsten in solution.

When 15 per cent. w/v hydrochloric acid was used the equilibrium, after each addition of the chromous chloride solution, was reached slowly and a continuous drift in potential was observed. The end-point was not sharp and the determined amount of tungsten was again less than the amount actually present.

With 15 to 25 per cent. w/v hydrochloric acids, the equilibrium was reached rapidly, and the titration curves were reproducible with sharp and large inflections at the equivalence points, corresponding to the transformation of tungsten^{VI} into tungsten^{V}. Table I shows some of the results obtained under these conditions.

Table I

Reproducibility of results by the proposed method

Amount of tungsten in 100 ml of hydrochloric acid, g	Hydrochloric acid concentration, % w/v	Molarity of chromous chloride solution	Maximum inflection per 0·1 ml of $CrCl_2$, mv.	Amount of tungsten found, g	Error, %
0·09280	25	0·0925	125	0·09270	−0·10
0·01856	25	0·0622	115	0·01857	+0·05
0·02299	20	0·0622	115	0·02288	−0·48
0·01856	20	0·0622	115	0·01857	+0·05
0·00697	20	0·0622	235	0·00690	−1·0
0·00467	20	0·0622	275	0·00463	−0·92
0·01856	15	0·0622	125	0·01857	+0·05

The fact that the reduction of tungsten^{VI}, as well as molybdenum^{VI}, can take place in comparatively strong solutions of hydrochloric acid probably indicates that the hydrochloric acid forms a complex with the tungsten^{V} or molybdenum^{V}. The quinquivalent form is thus masked, and so prevented from reacting during the process of reduction with unreduced tungsten^{VI} to give the intermediate reduction product, *viz.*, the tungsten blue. Klason[9] showed that $MoOCl_3$ forms green crystalline double salts of the type M_2MoOCl_5; these double salts would not be expected to exist in hydrochloric acid solutions as dilute as 5 or 10 per cent. w/v. Similar behaviour can be expected with tungsten; this explains the low values obtained in these media.

The lower values obtained in the 36 per cent. w/v hydrochloric acid medium when the concentration of tungsten was less than 120 mg per 100 ml of solution, can be explained by the probable instability of the hydrochloric acid complex at such low concentrations. The fact that the percentage error in the estimation is governed by the amount of tungsten present supports this view. If this is so, it would be expected that the addition of a substance that can form a stable complex with the tungsten^{V} would make the titration successful at these low tungsten concentrations.

It is known that molybdenum^{V} and tungsten^{V} have a great tendency to form complexes. Apart from the large variety of compounds formed by interaction of the halides, oxy-halides and other halogen compounds they can be expected to combine with phenols, pyrocatechols, salicylates and many organic acids, *e.g.*, oxalic, formic and citric acids.[4,10,11] The complexes with these organic compounds are known to be very stable.

After several trials with many organic compounds it was found, for tungsten, that the addition of citric acid to the medium (5 mols of acid per mol of tungsten) makes it successful in 36 per cent. w/v hydrochloric acid medium for concentrations as low as 18 mg per 100 ml of hydrochloric acid, which is in accord with the suggested explanation given above. When solutions containing 0·02784 or 0·01856 g of tungsten in 100 ml of 36 per cent. w/v hydrochloric acid were titrated

with 0·0415 *M* chromous chloride solution, the experimentally determined amount of tungsten was found to be less than that actually present. The addition of 0·1 g of citric acid to the medium makes the titration successful, with a sharp inflection (about 125 mv. per 0·1 ml of chromous chloride solution) exactly at the equivalence point.

It is very interesting to note also that the addition of citric acid during the titration makes it successful even in the presence of phosphate. This suggests a very useful experimental procedure for the titration of tungsten in presence of phosphorus, *e.g.*, in the analysis of steel.

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CHEMISTRY DEPARTMENT, FACULTY OF SCIENCE
FOUAD I UNIVERSITY, CAIRO

August, 1951

Official Appointments

PUBLIC ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Food since the last record in *The Analyst* (1951, **76**, 735).

Public Analyst	*Appointments*
ANDREW, Gertrude Garland (Additional) ..	County of Staffordshire.
FLINT, John Walter (Deputy)	Royal Borough of Tunbridge Wells.
MONK, Harold Edward	Royal Borough of Tunbridge Wells.

OFFICIAL AGRICULTURAL APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Agriculture and Fisheries since the last record in *The Analyst* (1951, **76**, 735).

Agricultural Analyst	*Appointments*
ALLEN, Douglas Geoffrey Glenn (Deputy) ..	County Borough of Newcastle upon Tyne.
COOMBES, Alfred Henry (Deputy)	County Borough of Birmingham.
HARRAL, James Charles (Deputy)	County of the West Riding of Yorkshire.
JAFFÉ, Frederick William Moore	County of the West Riding of Yorkshire.

Ministry of Food

STATUTORY INSTRUMENT*

1951—No. 2311. The Milk (Control and Maximum Prices) (Great Britain) (Amendment No. 4) Order, 1951. Price 2d.

This Order, which came into operation on January 1st, 1952, further amends the Milk (Control and Maximum Prices) (Great Britain) Order, 1951, as amended (S.I., 1951, Nos. 1157 and 2065), as follows—

(*a*) by inserting after "Channel Islands milk" in sub-paragraph (*d*) of the definition of Channel Islands milk in paragraph (1) of Article 1 thereof the words "or 'Jersey milk' or 'Guernsey milk', whichever is appropriate."

FOOD STANDARDS COMMITTEE

ARSENIC: SUPPLEMENTARY REPORT

THE Minister of Food has approved for publication a Supplementary Report of the Food Standards Committee's Metallic Contamination Sub-Committee proposing the introduction of statutory limits for arsenic

* Obtainable from H.M. Stationery Office. Italics indicate changed wording.

in foods and beverages in which is recommended some modification of the original proposals made in the Report dated May 26th, 1949.

The limits previously recommended for beverages ready-to-drink, *i.e.*, 0·1 p.p.m., and for all other foods, whether solid or liquid, not otherwise specified, *i.e.*, 1·0 p.p.m., are unchanged.

The limits specifically recommended in the report for arsenic in foods and beverages are as follows—

	Food	Parts per million by weight	
		Arsenic (As)	Arsenious oxide (As_2O_3)
1.	Food colourings other than synthetic colourings	5·0 (on dry colouring matter)	6·6 (on dry colouring matter)
2.	Beverages—		
	Cider	0·2	0·26
	Soft drinks intended for consumption after dilution and "concentrates" used in the manufacture of soft drinks	0·5	0·66
3.	Edible gelatin	2·0*	2·6*
4.	Dried herbs	5·0	6·6
5.	Spices	5·0	6·6
6.	Dried liquorice extract	2·0	2·6
7.	Dried hops	2·0	2·6
8.	Hop concentrates	5·0	6·6
9.	Finings and clearing agents	5·0	6·6
10.	Dehydrated onions	2·0	2·6
11.	Phosphates of ammonia, calcium and sodium	2·0	2·6
12.	Pectin—solid or liquid	2·0	2·6
13.	Chicory—dried or roasted	4·0	5·3

* Already prescribed.

The members of the Metallic Contamination Sub-Committee are: Mr. G. G. Barnes (Chairman), Professor G. R. Cameron, Dr. L. E. Campbell, Professor S. J. Cowell, Dr. J. M. Johnston, Dr. W. P. Kennedy, Dr. G. W. Monier-Williams, Dr. J. R. Nicholls, Dr. G. Roche Lynch, Mr. G. Taylor and Mr. B. W. Smith (Secretary).

The Report is dated June 30th, 1951.

The full Report is published in Ministry of Food Bulletin No. 616, dated September 15th, 1951.

Lead in Food and Drink

The Minister of Food has approved for publication a Report of the Food Standards Committee's Metallic Contamination Sub-Committee recommending the introduction of limits for lead in food and drink. The Sub-Committee recommend that twelve months' notice should be given before any statutory limits are fixed. The limits recommended in the Report for lead in Foods and beverages are as follows—

(1)	*Beverages*—	
	Beverages ready-to-drink and not specified below	0·2 p.p.m.
	Beer and cider—provisionally	1·0 p.p.m.
	to be reduced within twelve months to	0·5 p.p.m.
	Apple juice and concentrated soft drinks	0·5 p.p.m.
	(Note—The limits for concentrates used in the manufacture of soft drinks and not suitable for sale by retail are still under consideration.)	
(2)	*Scheduled Foods*—	
	Colourings	20 p.p.m. (on dry colouring matter)
	Dried herbs, spices, dehydrated onions and flavourings	10 p.p.m.
	Tea	10 p.p.m.
	Phosphates of ammonia, calcium and sodium	5 p.p.m.
	Edible gelatin	7 p.p.m. (already prescribed)
	Liquid pectin	10 p.p.m. (provisional)
	Canned meats and fish	5 p.p.m.
	(Note—The question of the limit for solid pectin is under consideration.)	

(3) *Foods other than those scheduled above—*

Refined sugar and edible oils and fats	0·5 p.p.m.
Other foods except those specified in (2) above	2·0 p.p.m.

The members of the Metallic Contamination Sub-Committee are as given for the Report on arsenic (above). The Report is dated October 31st, 1951.

The full Report is published in Ministry of Food Bulletin No. 628, dated December 8th, 1951.

British Standards Institution

NEW SPECIFICATIONS*

B.S. 642:1951. Calcium Carbide (Graded Sizes). Price 4s.
B.S. 692:1951. Meteorological Thermometers. 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 FFC/4—Solvents and Allied Products.

CN(FCC)8210—Draft B.S. for Methylene Chloride.

CN(FCC)8207—Draft B.S. for Tritolyl Phosphate.

Book Review

A SHORT GUIDE TO CHEMICAL LITERATURE. By G. MALCOLM DYSON, M.A., D.Sc., Ph.D., F.R.I.C., M.I.Chem.E. Pp. vii + 144. London: Longmans, Green & Co. Ltd. 1951. Price 8s. 6d.

This is an admirable introduction to literature-searching for students and beginners in research work. Some information, not exhaustive, is given on the main chemical dictionaries, journals and periodicals, abstract journals, textbooks and special works of reference, including some on medicinal chemicals. An outline of the method of searching the literature is included, and an example is given as Appendix II. Appendix I lists some old and obsolete journals as a reminder that they, too, must be searched. The most useful Appendix is the third, which is a series of tables showing the year and volume number of the main chemical publications. The list could easily be expanded, but Dr. Dyson has not tried to produce an exhaustive and expensive survey and does not intend it to be a substitute for a library.

The book is reproduced photomechanically from a "Varityped" manuscript. This reduces costs and is reputed to obviate proof-reading. But the value of proof-reading is the opportunity it gives of removing small errors and ambiguities; the lack of proof-reading is shown by the presence of at least seven minor misprints in the first 15 pages. Even the index is involved; *Ind. Eng. Chem.* is indexed on p. 69 in error for p. 79. Photomechanical reproduction can be better justified when accuracy or speed of publication is required—in 1942 the D. Van Nostrand Company Inc., of New York, produced an essential textbook on ultra high frequency techniques in 37 days by offset lithography from the typescript.

There is an error in Appendix III, the year - volume number tables, among the journals on analytical chemistry: on p. 129, *Z. anal. Chem.* is listed as having issued volume 128 in 1945; in fact, volume 128, part I, is dated 1947 and parts II and III are dated 1948, volumes 129 and 130 following in 1949 and 131 in 1950. As this appendix is at its most valuable when an otherwise regular series is interrupted, such errors may trap the unwary reader who has no access to scarce volumes. Except for this slip, Dr. Dyson's table has proved valuable in checking reference lists in papers and has already been used by the reviewer to detect and amend incorrectly-copied references.

J. B. ATTRILL

* Obtainable from the British Standards Institution, Sales Department, 24, Victoria Street, London, S.W.1.

THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

FOUNDED 1874. INCORPORATED 1907.

THE objects of the Society are to encourage, assist and extend the knowledge and study of analytical chemistry by holding periodical meetings, by promoting lectures, discussions and conferences, and by the publication of a journal devoted to analytical chemistry; to study questions relating to the adulteration of food, drugs and commercial articles generally, and its detection; and to promote the efficiency and proper administration of the laws concerned with the repression of adulteration.

Every candidate for membership of the Society must be not less than twenty-one years of age and be or have been engaged in analytical, consulting or professional chemistry. Each candidate for election must be proposed by three members of the Society, who must provide written testimony of their personal knowledge of his or her scientific and professional fitness. If the Council of the Society in their discretion think fit, such testimony may be dispensed with for a candidate not residing in the United Kingdom. Every application is placed before the Council and the Council have the power in their absolute discretion to suspend or reject any application, or to elect the candidate to membership.

The Entrance Fee is £1 1s. The Annual Subscription is £2 2s. The Society's official year runs from March to March, but the financial year begins on January 1st and subscriptions are due on that date.

Ordinary Meetings of the Society are held in London, usually on the first Wednesday in October, November, December, February, April and May; the Annual General Meeting is usually held on the first Friday in March. Notices of all meetings are sent to members by post.

The Analyst, the official organ of the Society, is issued monthly to members, and contains reports of the proceedings of the Society, original papers and notes, information about analytical methods, Government reports and reviews of books. All members receive in addition *Abstracts C*, the analytical section of British Abstracts, providing a reliable index to the analytical literature of the world.

Forms of application for membership may be obtained from the Secretary, 7/8, Idol Lane, London, E.C.3.

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THE North of England Section and the Scottish Section were formed to promote the aims and interests of the Society among the members in those areas. Members of the Society residing in England or Wales north of Birmingham may become members of the North of England Section and those resident in Scotland members of the Scottish Section.

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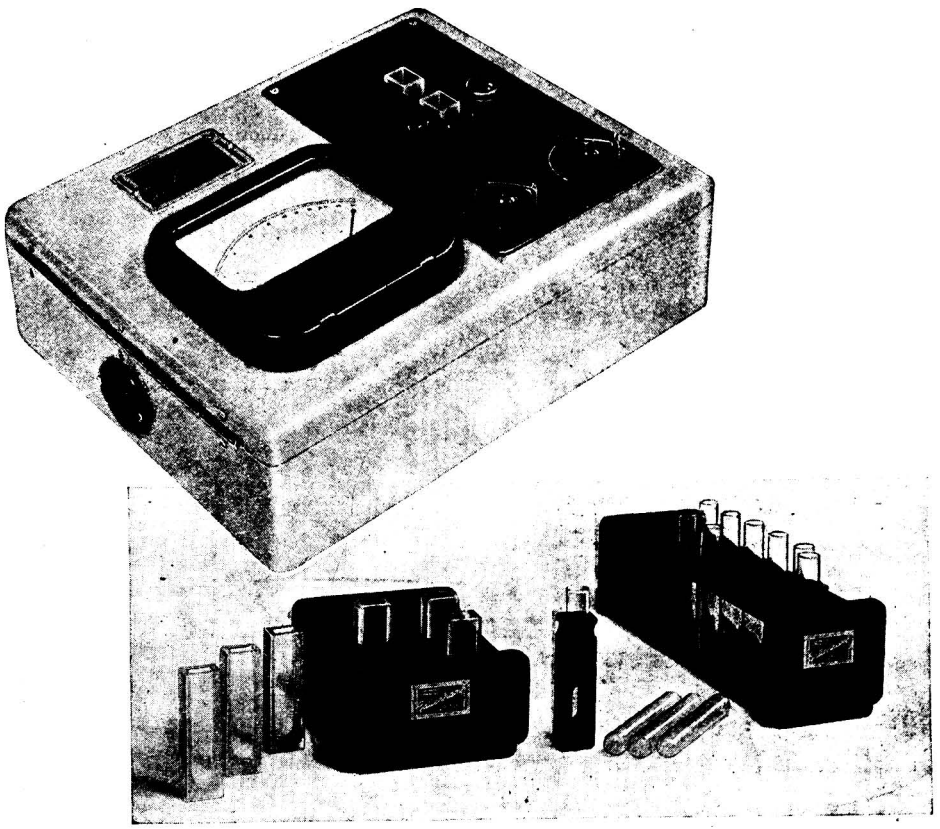

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