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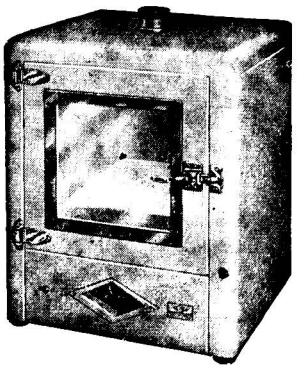

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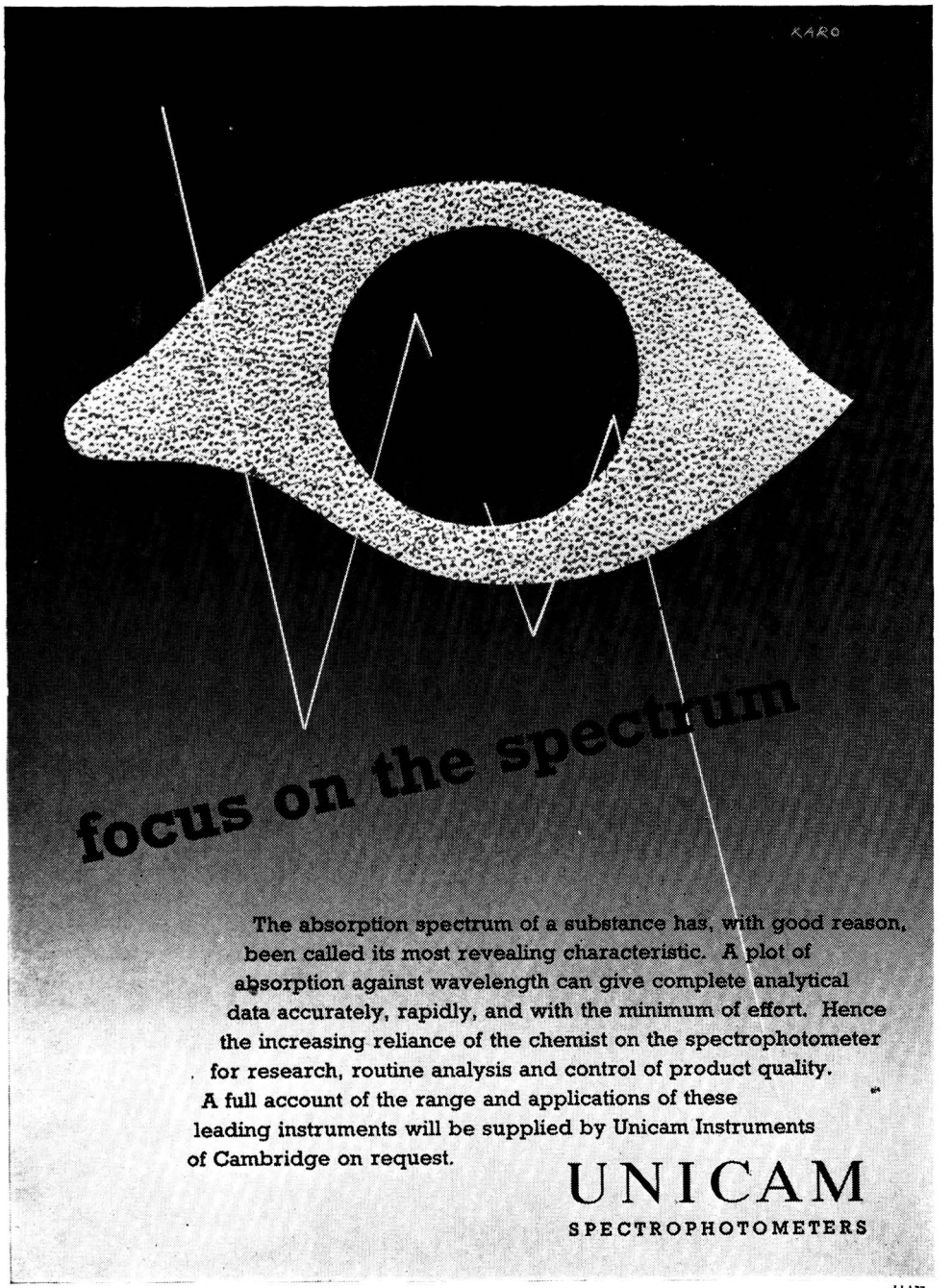
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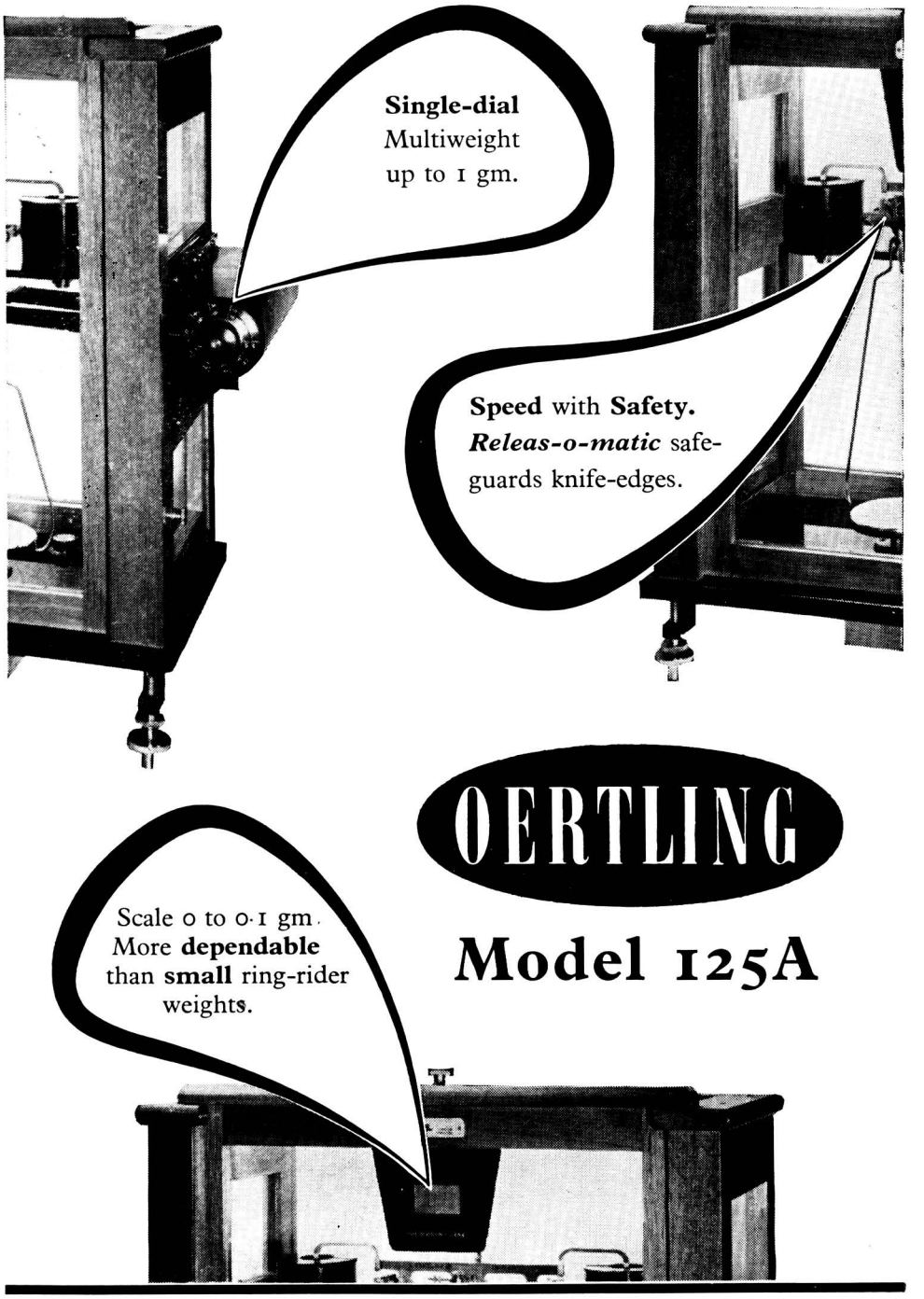

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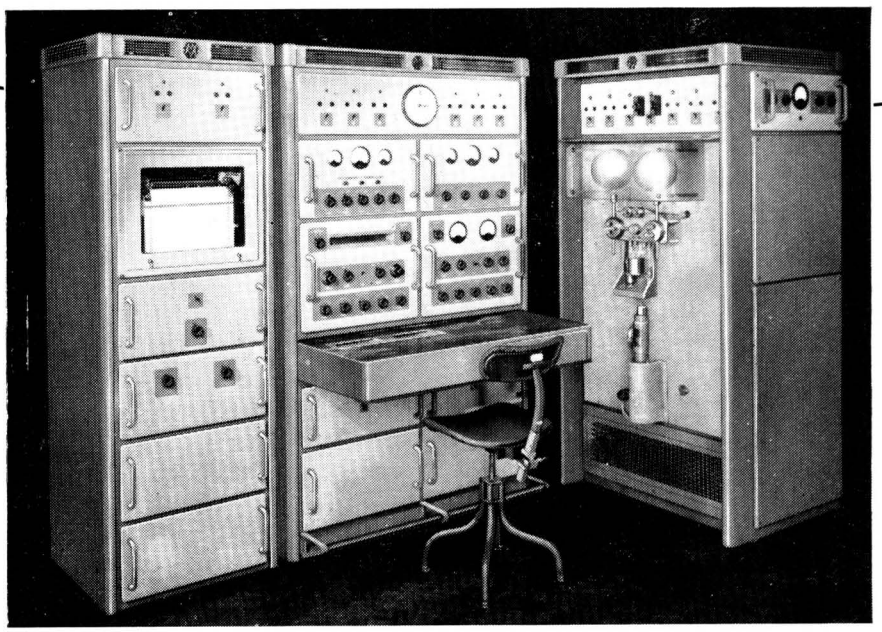

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117	0·56	0·16	0·47	3·2	**69·9**	14·0	2·7	2·9	5·7	0·44
118	0·22	<0·04	<0·04	0·25	0·93	**94·1**	2·95	1·36	0·08	0·10
119	0·33	0·11	<0·04	0·49	0·72	18·5	**71·7**	7·3	0·67	0·22
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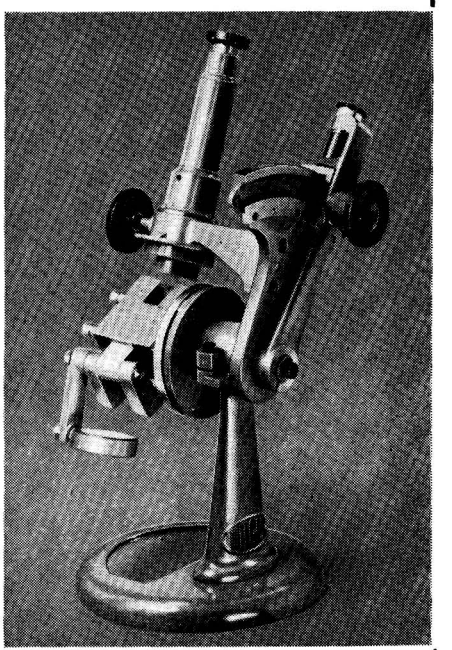

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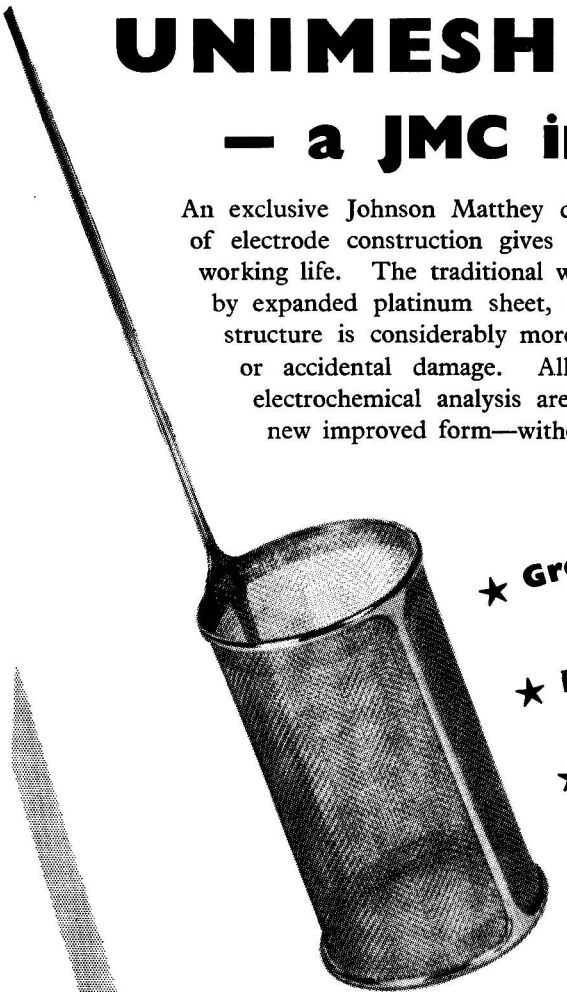

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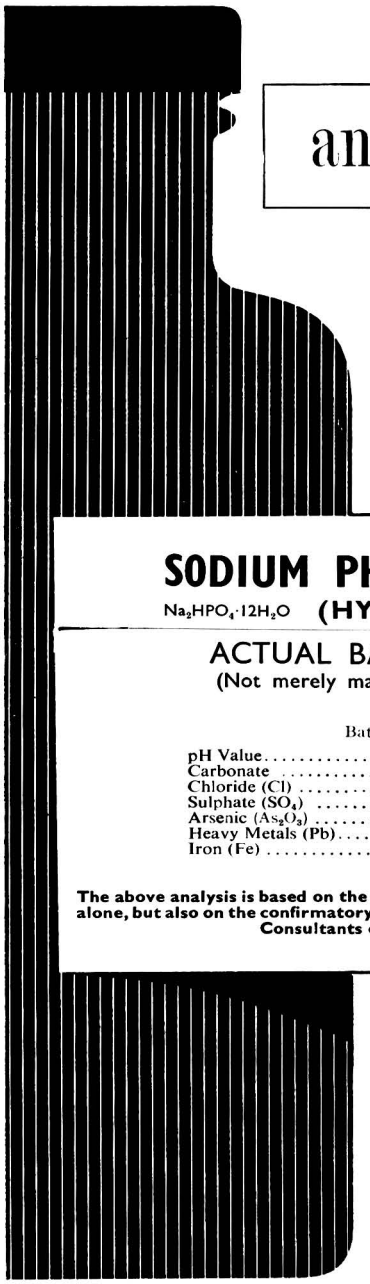

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NORTH OF ENGLAND SECTION

THE Eighteenth Summer Meeting of the Section was held at the Savoy Hotel, Blackpool, from Friday, June 10th, to Monday, June 13th, 1955.

The attendance (including wives and friends) was 30 and amongst those present were Mr. Allport, Dr. and Mrs. Nicholls, Mr. Okell, Mr. Eggleston (Secretary of the Scottish Section) and Mrs. Eggleston. All were welcomed by Mr. J. R. Walmsley, A.M.C.T., F.R.I.C., F.P.S., the Chairman of the Section.

On the Saturday morning a paper entitled "Some Modern Tools of the Analytical Chemist" was presented by Dr. J. R. Nicholls, C.B.E., F.R.I.C., of the Government Laboratory, and a discussion followed.

The party visited the Show on the Pier on the Saturday evening and were taken by coach to Morecambe on the Sunday afternoon.

PHYSICAL METHODS GROUP, MICROCHEMISTRY GROUP AND SCOTTISH SECTION

ABOUT 130 analytical chemists were present at Imperial Chemical Industries' Nobel Division Works at Stevenston, Scotland, on Friday, May 20th, 1955, for the Symposium on Gas Chromatography, arranged jointly by the Physical Methods and Microchemistry Groups with the Scottish Section. All of the visitors were taken on a tour of the Works and Research Laboratories and enjoyed the generous hospitality of I.C.I.

The Symposium comprised two sessions in the Ardeer Recreation Club. In the morning the President of the Society, Dr. K. A. Williams, B.Sc., F.R.I.C., A.Inst.P., M.Inst.Pet., took the Chair, and the following papers were presented and discussed: "Gas - Liquid Chromatography," by A. J. P. Martin, Ph.D., F.R.S., Nobel Laureate; "The Vapour-phase Chromatographic Analysis of Hydrocarbon Mixtures," by D. E. Chalkley, B.A., B.Sc.; "Techniques Used in a Study of the Boron and Silicon Hydrides," by A. B. Littlewood, B.A.

Dr. Traill, B.Sc., F.T.I., F.R.I.C., Research Manager of I.C.I. Nobel Division, occupied the Chair for the evening session, which included the following papers: "Adsorption and Partition Methods," by C. S. G. Phillips, M.A.; "A Rapid Chromatographic Method for the Determination of Bromine-inert Impurities in Ethylene," by N. H. Ray, B.Sc., A.R.I.C.; and a discussion on "Catharometers as Recorders in Gas Chromatography," which was opened by Dr. Keulemans of Koninklijke/Shell-Laboratorium, Amsterdam, Holland. (A fuller account will be published later.)

BIOLOGICAL METHODS GROUP, PHYSICAL METHODS GROUP AND SCOTTISH SECTION

A JOINT Meeting of the Biological Methods Group, the Physical Methods Group and the Scottish Section with the Edinburgh and East of Scotland Sections of the Royal Institute of Chemistry, the Society of Chemical Industry and the Chemical Society was held on Monday and Tuesday, July 11th and 12th, 1955, in the Department of Biochemistry, University New Buildings, Teviot Place, Edinburgh, 8.

The subject of the meeting was "The Use of Radioactive Materials in Biological Assay." On July 11th, under the Chairmanship of Professor G. F. Marrian, F.R.S., of the Department of Biochemistry, Edinburgh University, the following papers were presented and discussed: "The Determination of Radioactive Isotopes in Biological Samples," by R. F. Glascock, B.Sc., Ph.D.; "The Principles of Isotope-dilution Assays with Special Reference to Vitamin B_{12}," by E. Lester Smith, D.Sc., F.R.I.C.; "Bio-assay of Radio-iodinated Plasma Proteins for Clinical Use," by A. S. McFarlane, M.A., B.Sc., M.B., Ch.B.; "Isotope-dilution Assay of Antibiotics in Fermentation Liquors with Particular Reference to Benzylpenicillin and Griseofulvin," by G. C. Ashton, B.Sc.; "The Assay of Aldosterone and Other Adrenal Steroids by the $^{24}Na/^{42}K$ method," by R. N. Jones, B.A., Sylvia A. Simpson, B.Sc., and J. F. Tait, B.Sc., Ph.D.

Dr. L. J. Harris, F.R.I.C., Chairman of the Biological Methods Group, took the Chair on July 12th, when the following papers were presented and discussed: "Assay of T.S.H. Based on the Rate of Discharge of Radioactive Iodine from the Thyroids of Chicks," by Trevor Kinnear, M.B.E., M.B., M.R.C.P., M.R.C.P.E.; "Labelled Metabolic Pools for Studying Quantitatively the Biochemistry of Toxic Action," by F. P. W. Winteringham, F.R.I.C.; "The Use of ^{131}I-labelled Serum Albumin in Determining the Intercellular Plasma in Centrifuged Red Cells," by F. W. Jennings, B.Sc., M.Agr., I. M. Lauder, M.R.C.V.S., and W. Mulligan, M.Sc., Ph.D.; "The Measurement of Health Hazards," by J. F. Loutit, D.M., M.R.C.P. The closing remarks were made by F. J. Elliott, M.Sc., Ph.D., F.R.S.E., F.R.I.C., Chairman of the Scottish Section.

Before the paper-reading sessions on July 11th, there was a visit to the Biochemistry Laboratory, Teviot Place, Edinburgh, where there was an exhibition of counters and equipment. In the afternoon of July 12th a visit was made to the Institute of Seaweed Research, Inveresk, Musselburgh, Midlothian, by courtesy of the Director, F. N. Woodward, B.Sc., Ph.D., F.R.S.E., F.R.I.C. (A fuller account will be published later.)

Zone Electrophoresis on Filter-paper

A Review

By L. F. J. PARKER

Summary of contents

Since 1948 when Wieland and Fischer[1] first published their account of the separation of amino acids by means of electrophoresis on filter-paper, a considerable volume of literature on the subject has accumulated, and the technique has been used for the separation of all types of charged particles, from inorganic ions to complex proteins. The method has a number of advantages over that of electrophoresis in free solution developed by Tiselius.[2]

His method, although very useful for the study of substances of high molecular weight such as blood proteins, is not suitable for the study of smaller charged particles and ions. Further, it is the migration of overlapping zones that is observed in the classical Tiselius apparatus, and complete separation into zones of different mobility cannot be achieved since convection due to gravity would upset the system. Electrophoresis on filter-paper, however, makes possible the complete separation of small ions as well as of proteins, the filter-paper support preventing movement by convection; this also greatly extends the range of temperatures over which the method may be used.

As early as 1907 Field and Teague[3] studied the migration of diphtheria toxin and antitoxin in agar jelly, and Kendall, Jette and West[4] in 1926 utilised the migration of ions in gels to separate the rare earths. The separation of serum proteins into zones was successfully accomplished by Coolidge[5] in 1939 by electrophoresis in a tube packed with ground glass wool, and, more recently, Butler and Stephen[6] separated amino-acid mixtures in a water-cooled polystyrene tube packed with asbestos fibre. Consden, Gordon and Martin[7] in 1946 devised a neat apparatus for the separation of amino acids and peptides in silica jelly and came very near to the use of filter-paper when they added paper pulp to increase the strength of the jelly.

Von Klobusitzky and König[8] investigated snake venom by means of electrophoresis on filter-paper as early as 1939 and indeed refer to an earlier paper by König[9] in which the apparatus is described in more detail. However, these papers appear to have been completely overlooked, and it was not until the paper of Wieland and Fischer[1] appeared in 1948 that the method gained recognition. Although they thus achieved priority in publication, the use of filter-paper soaked in buffer solutions for the separation of charged particles appears to have been developed independently by a number of different workers. Wieland and Fischer's paper was followed by a spate from other authors, including Grassmann and Hannig[10] and Turba and Enenkel[11] in Germany, Cremer and Tiselius[12] in Sweden, McDonald, Urbin and Williamson,[13] Kraus and Smith[14] and Durrum[15,16] in the United States and Biserte[17] in France, describing differing methods and types of apparatus.

A large number of terms such as ionography, ionophoresis, electromigration, electrochromatography and zone electrophoresis have been suggested to describe this technique. The term zone electrophoresis as proposed by Tiselius[18] will be used throughout this review to cover its application not only to proteins, but also to small molecules, such as amino acids and sugars, and even to inorganic ions. The patterns obtained by zone electrophoresis will be referred to as pherograms, as suggested by Bücher, Matzelt and Pette.[19]

Types of apparatus

Numerous descriptions of apparatus for zone electrophoresis have been published, ranging from those made from simple inexpensive laboratory equipment to elaborate instruments incorporating devices for ensuring constant temperature of the paper strip and for preventing evaporation of the buffer solution. In general, however, there are two main types, those in which the filter-paper is freely suspended in a moist chamber and those in which the paper is enclosed between glass plates or immersed in an immiscible solvent to prevent or minimise evaporation.

Suspension in a moist chamber—

The apparatus of Wieland and Fischer[1] was simple and consisted of a strip of filter-paper suspended horizontally between glass plates separated by a Lucite frame, 1 cm in thickness. The two ends of the filter-paper strip were allowed to hang in Petri dishes full of buffer solution and containing the electrodes. A very small drop of amino acid solution was placed on the centre of the strip after it had been soaked with buffer solution. With an acetate buffer at pH 3·7, the application of a potential of 110 volts resulted in the separation of aspartic and glutamic acids or of alanine, glutamic acid and histidine in the course of an hour.

Durrum's[16] simple apparatus consists of two glass tumblers covered with a Lucite plate and each containing an electrode. The paper strip was draped over a glass rod, the ends of the strip passing through slits in the Lucite plate into the buffer solution, a third tumbler being inverted over the apex of the strip. The mixture to be separated was placed on the apex and a potential of 300 to 600 volts was applied.

Flynn and de Mayo[20] describe a modification of Durrum's apparatus in which separations

can be carried out on six strips of paper simultaneously. This has in turn been further modified by Griffiths.[21]

Latner's[22] apparatus is constructed mainly of Perspex and is similar in principle to that of Flynn and de Mayo,[20] though it allows easier handling of the paper strips. It consists of a sheet of Perspex in which are inset two pairs of troughs, one pair at each end. The outer troughs contain the platinum wire or carbon electrodes and are connected by cotton-wool wicks to the inner troughs, which hold the ends of the filter-paper strips. The filter-paper sheet is suspended over a silicone-coated glass rod on a Perspex partition, the whole apparatus being enclosed by a box lid. A slightly modified version of this apparatus is shown in Fig. 1.

A much more elaborate apparatus of the moist-chamber type has been devised by McDonald and his colleagues.[23,24] The paper strips are suspended horizontally over dishes of buffer solution, not over a central rod, and the chamber, in addition to having water-jacketed walls to ensure constant temperature, is filled with moist helium to conduct away the heat produced during electrophoresis.

ENCLOSURE BETWEEN GLASS PLATES OR IMMERSION IN AN IMMISCIBLE SOLVENT—

Among the simplest of the second type of apparatus is that of Kunkel and Tiselius,[25] which is shown in Fig. 2. A strip of filter-paper is enclosed between two glass plates, previously treated with silicone; they are firmly clamped together and their edges sealed with silicone grease. The ends of the strip of filter-paper are pressed against pads of thick filter-paper, which are partly immersed in buffer solution contained in Perspex electrode vessels. The apparatus was used in the cold room, and heavy sheets of copper were placed on top of the glass plates to give additional cooling when high currents were employed. For preparative work as many as ten sheets of paper were piled one on top of the other.

Kunkel and Slater[26] described the separation of proteins by means of this apparatus, but with several refinements of technique. Foster[27] has adapted the apparatus to high potentials of the order of 1200 to 1600 volts, enabling separations to be carried out in as little as an hour. This was done by replacing the copper plates used by Kunkel and Tiselius with a metal sheet in which was incorporated a cooling coil through which cold water could be circulated. Gross[28] has used even higher voltages by enclosing his filter-paper strips between thin polythene sheets resting on aluminium blocks, which conduct away the heat formed.

Cremer and Tiselius,[12] besides placing the sheet of filter-paper between glass plates, immersed the whole apparatus in chlorobenzene. Simpler methods of preventing evaporation by immersion in a solvent have been devised by Consden and Stanier[29] and by Markham and Smith.[30] The former workers describe a simple glass framework that holds the filter-paper horizontally immersed in chlorobenzene. Markham and Smith, however, prefer carbon tetrachloride as a cooling solvent and have the filter-paper in the shape of a V in a tall, narrow vessel.

From the foregoing descriptions, based on only a few of those to be found in the literature, it will be seen that there is no lack of variety in the design of apparatus for carrying out zone electrophoresis and that the type selected may vary with the purpose for which it is to be used. For the analysis of proteins and for the general separation of charged particles the apparatus of Latner[22] is excellent. I have had two of these, the larger one taking sheets of filter-paper 22 inches by 18 inches, in regular use for several years, and they have given very satisfactory results. There is, however, a tendency in this type of apparatus for evaporation of the buffer to occur, especially if a high voltage or a buffer of high ionic concentration is used. This causes concentration of the buffer on the paper, particularly at the apex, with a consequent flow of buffer from the electrode vessels towards the centre, tending to move the zones in a direction opposite to that due to the applied electro-motive force. The movement of charged particles under these conditions does not show a linear relationship with time or with the applied potential.

If it is desired to measure the mobilities or the transport numbers of ions, it is preferable to use an apparatus in which evaporation is prevented by means of glass plates or a solvent, although McDonald[23] claims that his moist-chamber apparatus having a cooled chamber filled with helium can be successfully employed for this purpose.

A source of direct current is clearly necessary for carrying out zone electrophoresis. If the laboratory is not wired for d.c., the simplest source consists of a 120-volt dry battery, or more than one, as recommended by Latner.[22] For those preferring to construct a mains

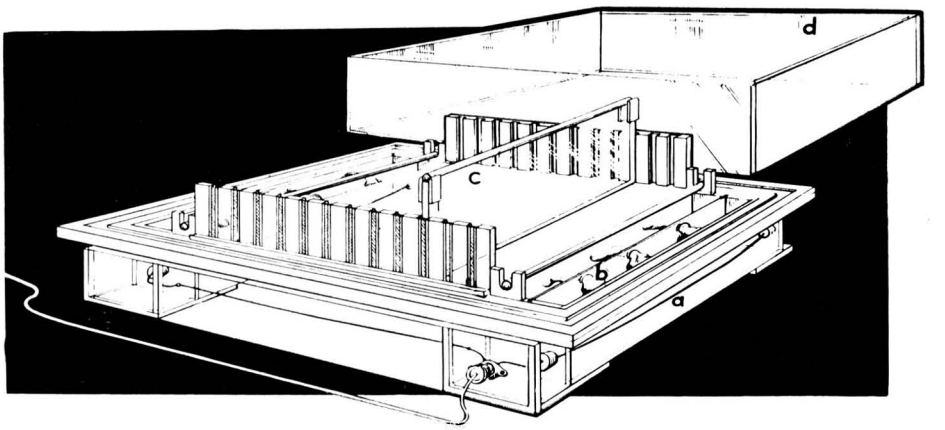

Fig. 1. Moist chamber apparatus. (*a*) Platinum electrodes; (*b*) cotton-wool wicks joining buffer vessels; (*c*) Perspex partition for supporting filter-paper; (*d*) box lid

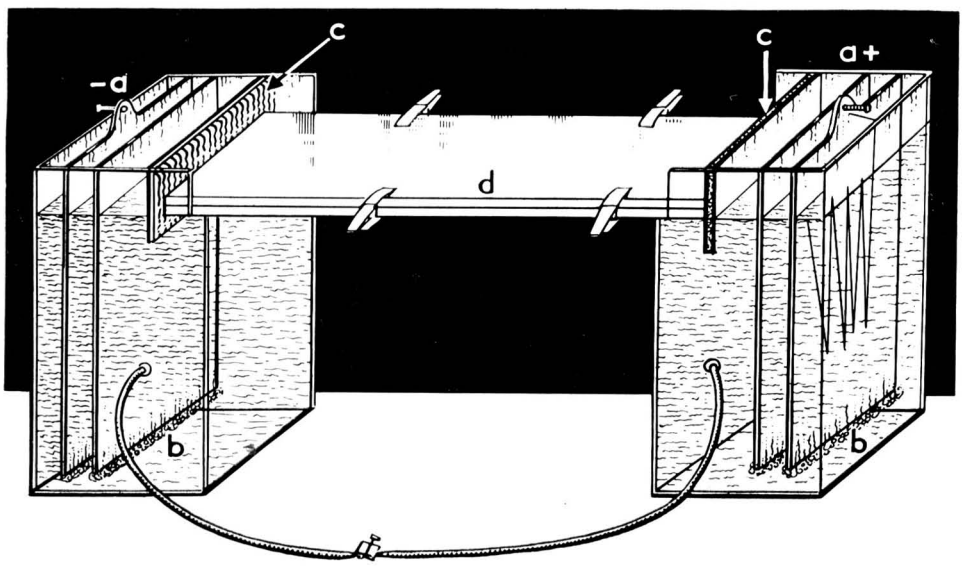

Fig. 2. Apparatus of Kunkel and Tiselius. (*a*) Coiled platinum electrodes; (*b*) glass wool at the junction between electrode chambers; (*c*) highly porous paper carrying liquid from electrode vessels to the filter-paper; (*d*) glass plates surrounding the filter-paper. Reproduced by permission of the Editor from *J. Gen. Physiol.*, **1951**, **35**, 89

[To face p. 640

rectifier, however, suitable circuit diagrams for such an apparatus are given by Markham and Smith[30] and Griffiths.[21]

Factors affecting mobility

The movement of the ions or particles in zone electrophoresis is dependent on a number of factors, most of which are of little importance unless the actual mobilities of the compounds are being measured. Under ideal conditions with no evaporation of water from the buffer solution, there is a linear relationship between the distance moved by the ion and the time of current flow and also between the distance moved and the potential applied across the ends of the paper. This state, however, is difficult to attain. Evaporation almost always occurs, owing to the heat developed in the buffer by the passage of current, although it can be reduced by the addition of glycerol to the buffer.[23] If the paper is hung over a glass rod in the shape of an inverted V, evaporation is greater at the apex than near the buffer compartments, and there is a flow of buffer towards the apex, which is particularly marked with buffers of high ionic concentration. Durrum[31] found that with high currents the mobilities of amino acids fell off sharply after a time and the acids took up equilibrium positions, the electrical transport of the amino acids in one direction being counter-balanced by the movement of buffer in the opposite direction. When mixtures of amino acids were placed at different levels on a number of strips of filter-paper, the individual amino acids arrived at the same final positions in all the strips. McDonald,[23] using his specially designed apparatus, has overcome these difficulties and obtains particle movement that is a linear function of time and the applied potential.

The method of wetting the filter-paper strip has a considerable effect on movement, as also have the levels of the liquids in the buffer troughs. They should be equalised initially by means of a siphon tube. Changes in pH of the buffer solution during the course of an experiment also affect the mobility of particles; to avoid this it is best to have two vessels at each end of the paper, one to contain the electrode and the other to take the end of the paper, the two being connected by cotton-wool or filter-paper wicks.

In addition to these factors, the flow of buffer from anode to cathode due to electro-endosmosis must be taken into account when measuring mobilities. Because of this phenomenon the γ-globulin band appears to travel towards the cathode in the zone electrophoresis of serum, although it is negatively charged. To measure the amount of movement due to this cause, neutral marker compounds have been used. Kunkel and Tiselius[25] employed dextran as their neutral marker; xylose,[32] starch, amylose, sucrose and other polysaccharides have also been utilised.[25] Consden and Stanier[29] found that creatinine and proline were suitable at an appropriate pH, and trinitrobenzene and *o*-nitroaniline have been employed for the same purpose.[33]

The effect of electro-endosmosis is more marked when the filter-paper is sandwiched between glass plates or immersed in a solvent than when it is freely suspended in a moist chamber. McDonald[23] suggests in explanation that the ions travel through the water sheath surrounding the filter-paper when it is freely suspended, whereas this water sheath is eliminated by clamping between glass plates or immersing in a solvent and the ions are then forced to travel through the body of the paper.

The effect of pH—

The pH of the buffer solution has a considerable effect on the migration of a charged particle. In acid buffer, such as 0·5 *N* acetic acid, weak acids, for instance, are un-ionised and behave as neutral compounds. With ampholytes, such as proteins and amino acids, the movement of the particle can be altered in direction by altering the pH of the buffer, the particle being stationary at its isoelectric point.

The effect of the ionic concentration and of the temperature of the buffer—

It is found that the ionic concentration of the buffer has a marked effect on the rate of migration of ions in zone electrophoresis, the rate increasing as the ionic concentration decreases. Fig. 3 shows the effect of ionic concentration on the mobility of leucine in phthalate buffer. Holdsworth[34] also noticed this effect, finding that the rates of migration of vitamin-B_{12}-like compounds were much greater in 0·5 *N* acetic acid than in acetate buffer, although the pH was the same in both solutions.

The rate of migration of particles also increases with increasing temperature. The

effect of temperature on the mobility of bovine serum albumin, as determined by McDonald,[23] is shown in Fig. 4. Thus it will be realised that in mobility determinations great care must be taken to standardise the conditions; in quoting results, the pH and ionic concentration of the buffer and the temperature must all be stated.

The choice of filter-paper

The grade of filter-paper chosen will be governed by the particular kind of separation to be carried out. Thus for quantitative work a high-grade paper, such as Whatman No. 20,

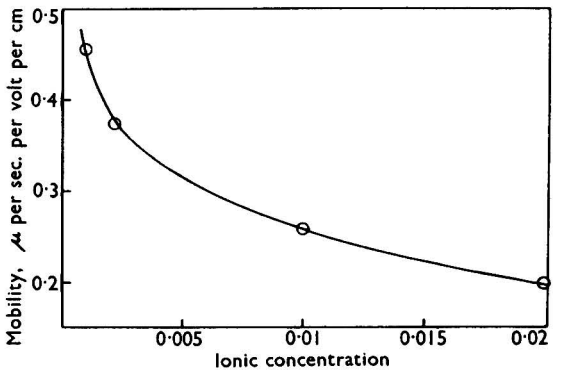

Fig. 3. The mobility of leucine as a function of the ionic concentration of the buffer, after H. J. McDonald. Reproduced by permission of the Editor from *J. Chem. Educ.*, 1952, **29**, 428

must be used. For general diagnostic work I have found Whatman No. 2 paper to be most satisfactory. For preparative work a thicker paper, such as Whatman No. 31 extra thick or Whatman 3MM, is very useful, whereas for comparatively large quantities of materials Whatman seed-test paper has been found highly convenient. A 10-inch wide sheet of this paper will hold up to 200 mg of some materials and can be successfully used in Latner's apparatus[22] or in that described by Cannon and Gilson.[35]

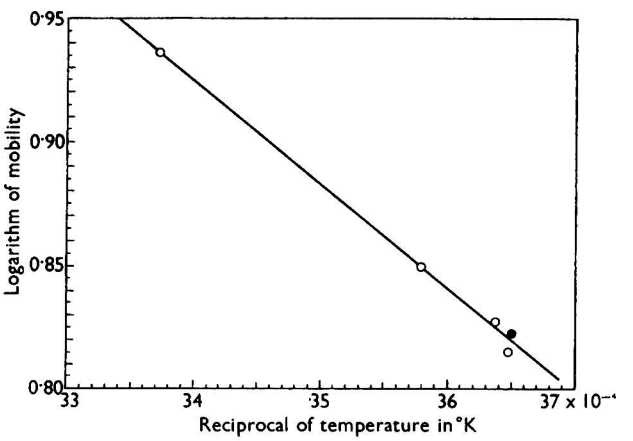

Fig. 4. Effect of temperature on bovine serum albumin (closed circle determined by moving boundary), after H. J. McDonald. Reproduced by permission of the Editor from *J. Chem. Educ.*, 1952, **29**, 428

The degree of electro-endosmosis in any experiment will be affected by the grade of paper when it is pressed between glass plates or under solvent, and Consden and Stanier[29] give a table showing the electro-endosmosis obtained with eleven grades of Whatman filter-paper. McDonald,[23] however, found no difference in the mobility of bovine serum albumin, under his conditions, with six different grades of Eaton and Dikeman filter-paper.

Jermyn and Thomas,[33] using the apparatus of Woods and Gillespie,[36] studied the effect on electro-endosmosis of treating filter-paper with periodic acid. Dialdehyde groups were formed in the paper by this treatment, and these were coupled with hydrazine derivatives to give positive papers. As the paper becomes progressively more basic, the normal cathodic electro-endosmotic flow becomes progressively less, then zero and then increasingly anodic. Polyelectrolytes with a net positive charge, which are adsorbed by the untreated papers, become free to move on positive papers, whereas those with a net negative charge will show the reverse behaviour. Jermyn and Thomas[37] have exploited these effects in separating the components of horse-radish peroxidase. Negative papers were produced by treatment with bromine and alkali or by coupling with phenylhydrazine-*p*-sulphonic acid papers previously oxidised with periodic acid.

The choice of buffer solution

In general, any buffer that can be used for free electrophoresis is suitable for zone electrophoresis, although it is customary to employ buffers of lower ionic concentration in zone electrophoresis, since the mobility of a compound increases with decreasing ionic concentration. The amount of heat to be dissipated is also lower when the ionic concentration is low. The buffer must, however, be of sufficient concentration to maintain a uniform pH throughout the experiment. Buffers of ionic concentration from 0·01 to 0·2 have been found suitable for most systems. As far as possible, the solution to be applied to the paper should be in equilibrium with the buffer solution; thus concentrated solutions of proteins should be dialysed against the buffer to be used, before their application to the filter-paper sheet. When separating vitamin-B_{12} degradation products, I have found that failure to adjust the solution to the pH of the buffer, or to remove high salt concentrations, had a marked effect on the movement of the zones. The material is usually applied as a streak across the paper and the centre portion of the zone is held back, the streak developing an inverted V shape in extreme cases. Prolonged passage of the current will usually correct this trouble, the centre portion of the streak catching up with the two ends.

Non-aqueous solvents can also be used for zone electrophoresis. Paul and Durrum[38] were able to separate dyestuffs with a nitromethane - glacial acetic acid mixture, a pyridine - glacial acetic acid mixture and absolute ethanol and methanol in place of buffer; Marbach and McDonald[39] have successfully used aqueous solutions containing up to 50 per cent. of dioxan.

Continuous electrophoresis

One of the chief limitations of zone electrophoresis in preparative work is the small quantity of material that can be separated in each experiment. A number of workers have attempted to overcome this obstacle by constructing equipment in which buffer solution flows continuously down a sheet of filter-paper, or a rectangular box filled with glass powder, and is allowed to drip from the bottom of the paper or box into a series of collecting vessels. The material to be separated is fed continuously to the top at one point and flows downward with the buffer. Electrodes are fitted to the sides to give a horizontal electric field. With no current flowing, the material passes down the paper as a single narrow band. On passage of the current, however, the charged molecules are drawn towards one or other of the electrodes and this, combined with the buffer flow, causes the material to move diagonally in the manner shown in Fig. 5. Descriptions of such apparatus have been given by Svensson and Brattsten,[40] Durrum,[41] Brattsten and Nilsson,[42] Grassmann and Hannig[43,44,45] and Mould and Synge.[46,47] It is difficult in this type of apparatus to achieve a steady state, and a number of refinements have been suggested by Holdsworth,[48] Bradish and Smart[49] and Karler.[50] Brattsten,[51] in an attempt to achieve this steady state, has constructed an apparatus in which the buffer feed is controlled by a special peristaltic pump, the speed of which is synchronised with the current flowing through the apparatus.

Electrochromatography

When differential adsorption of compounds by the filter-paper occurs, a combination of chromatography and electrophoresis is obtained on passage of an electric current. The technique of electrochromatography has been exploited by Strain and his colleagues[52,53,54] and by a number of other workers.[55,56,57,58,59]

Zone Electrophoresis on media other than filter-paper

Zone electrophoresis has been carried out with other materials as supporting media in place of filter-paper. Glass powder has already been mentioned; agar jelly,[60,61,62] Hyflo Super Cell,[63] washed sea sand,[64] starch,[64] columns of cellulose powder[65] and even string[66] have all been tried with various degrees of success. Of these materials starch, which has a lower rate of electro-endosmotic flow than the others mentioned, appears to be the most promising, particularly in the separation of compounds strongly adsorbed on filter-paper.

The applications of zone electrophoresis

Zone electrophoresis has been employed principally for the simple fractionation of mixtures, and there is now an extensive literature covering this field, particularly that part concerned with the separation of mixtures of proteins, such as those of blood serum.

Proteins—

The quantitative determination of the serum protein pattern by zone electrophoresis is now an established practice in clinical studies. In certain diseases the ratio of the proteins

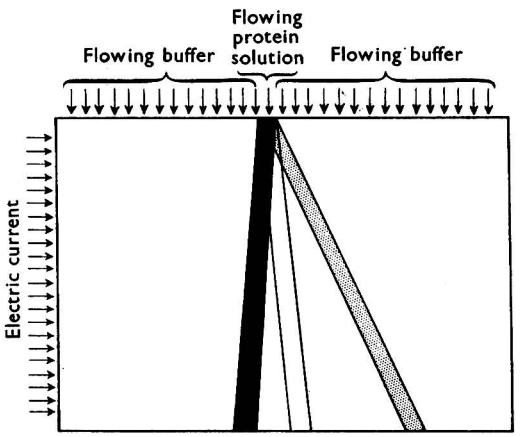

Fig. 5. Diagram showing the continuous zone electrophoresis apparatus of Svensson and Brattsten,[40] after Tiselius and Flodin. Reproduced by permission of the Editor from *Advanc. Protein Chem.*, 1953, **8**, 461

present in the plasma is altered considerably, so that the determination of this ratio is an important aid in diagnosis. Thus in virus hepatitis[67] there is a drop in the albumin fraction of the blood proteins, which can be detected by paper electrophoresis even in cases without laboratory or clinical signs of jaundice. The pattern obtained on electrophoresis of normal blood plasma is shown diagrammatically in Fig. 6.

A number of comparisons have been made between the results obtained by zone electrophoresis and those obtained by the classical Tiselius method[68,69,70,71,72,73,74] and by chemical methods[75]; in general, the results have been found to be in good agreement. Other workers[1,10,12,16,20,21,24,25,26,76–96] have described improved methods of separating proteins or of estimating them when separated.

The proteins in cerebrospinal fluid have been separated by zone electrophoresis[19,97,98,99,100,101] and the technique has been used to investigate the haemoglobinaemias.[102,103,104] Edington and Lehmann[105] were able to isolate a new haemoglobin, haemoglobin G, by this method, and Aggeler, Spaet and Emery[106] successfully employed it for the purification of plasma thromboplastin factor B. Methods for the detection of lipids and lipoproteins in paper pherograms have been devised,[107,108,109,110,111,112] and there have been publications on electrophoresis of the proteins of rat-liver cells,[113,114] of the human and the ox crystalline lenses,[115] of urinary proteins[116] and of plant viruses.[117]

Zone electrophoresis has been widely employed in investigating enzymes and has been found most useful for separating mixtures of enzymes and for purifying single enzymes,

since under the mild conditions prevailing there is little risk of inactivation. Those enzymes investigated include cholinesterase,[118,119] pepsin,[120,121] amylose and trypsin,[122] pancreatin,[123] glucuronidase,[124] peroxidase,[37] chymotrypsin and elastase,[125] hyaluronidase,[126] alkaline phosphatase,[127,128] ribonuclease[129] and deoxyribonucleases.[130]

SMALLER ORGANIC MOLECULES—

Amino acids and peptides were among the first groups of compounds to be separated by zone electrophoresis.[1,16,31,56,131,132,133] Sanger and Thompson,[134] for example, employed the technique for separating the amino acids and peptides obtained by hydrolysis of insulin and were thus able to determine the amino-acid sequence in the glycyl chain of this compound. The method has also been found suitable for study of nucleic acids and their degradation products, and a considerable amount of such work has been carried out by Markham and Smith,[30,135] Smellie and Davidson[136,137] and others.[138,139,140,141]

Carbohydrates, though normally uncharged and therefore not separable by electrophoresis, can be given a charge by allowing them to form complexes with boric acid, as described by Böeseken.[142] A sugar will react with one molecule of boric acid, or several,

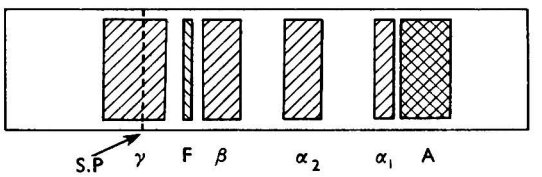

Fig. 6. Diagram showing the separation of blood plasma: A, albumin; α_1, α_2, β, γ, globulins; F, fibrinogen; S.P., starting position

depending upon the number of free hydroxyl groups it possesses and their configuration. Zone electrophoresis in borate buffer has been used for the separation of simple sugars by Consden and Stanier,[29,143] Foster,[144,145,146] Michl[147] and others[28,148,149,150,151,152] and for the separation of polysaccharides by Consden and Bird,[153] Hooghwinkel, Smits and Kroon[154] and Rienits.[155]

The technique of zone electrophoresis has been invaluable in investigations into vitamin B_{12} and related compounds[34,156,157,158,159,160] and into the degradation products obtained therefrom.[65,161] The binding of vitamin B_{12} to serum[162] and to the intrinsic factor of Castle[163,164] has also been studied, and indeed the method has been used by Latner, Ungley, Cox, McEvoy-Bowe and Raine[165] for the isolation of highly purified intrinsic factor.

McDonald and Marbach[166,167,168] and others[169,170,171,172,173] have employed zone electrophoresis for the study of adrenocorticotrophic hormone, and by its means antibiotics have also been investigated.[174,175,176] Other organic compounds that have been separated by zone electrophoresis include phosphoric esters of thiamine,[177] adenosine and related compounds,[178] organic acids,[179,180] steroids,[181,182] polythionates,[183] cytochrome-*c*,[184] animal poisons,[185] alkaloids,[186,187] plant extracts,[188] fluorene derivatives,[189] *iso*nicotinic acid hydrazide and its metabolites[190] and polymers.[191]

INORGANIC IONS—

Besides being used for separating organic compounds, zone electrophoresis has also been employed for the separation of inorganic ions. Lederer,[57,192] for instance, succeeded in separating the metals of the copper group and also iron, nickel and cobalt. These metals and the alkali metals have also been separated by Japanese workers,[193,194,195] and Strain and his colleagues[196] have successfully separated the rare earths, using on some occasions sheets of filter-paper 3 to 6 feet in length. Using dilute hydrochloric acid in place of buffer, Lederer[197] has successfully separated sulphuric from orthophosphoric acid and other pairs of ions, such as selenite from tellurite and ferrocyanide from ferricyanide.

OTHER APPLICATIONS—

Zone electrophoresis has been used for purposes other than the simple separation of compounds and ions. Foster, for example, was able to establish the structure of the carbohydrates bornesitol[198] and sequoyitol[199] by comparison of their mobilities in borate buffer

with those of suitable reference compounds. He has suggested[144] the term $M(g)$ (the mobility of a compound compared with that of D-glucose) for use in the electrophoresis of carbohydrates in a similar manner to that in which R_F is used in paper chromatography. The isoelectric points of proteins and amino acids and the transference numbers of nickel and copper sulphates have been determined by McDonald, Urbin and Williamson[13,24] by means of zone electrophoresis. McDonald and Urbin[200,201] have also suggested the possibility of determining molecular weights by a method involving the use of simultaneous crossed currents. By means of a square sheet of filter-paper an equal potential is applied in both directions at 90° to each other, so that the compounds move at an angle of 45° to the sides of the paper squares. In a study of twelve amino acids and five dinitrophenylamino acids, a linear relationship was noted when the reciprocals of the distances moved by the compounds were plotted against their respective molecular weights.

Localisation, identification and measurement of separated zones

Both the reagents and the spraying techniques developed for paper chromatography[202,203] may be employed in zone electrophoresis for the identification of separated fractions. Thus the presence of amino acids may be shown by spraying the paper with a solution of ninhydrin in *n*-butanol, and reducing sugars may be detected by spraying with aniline hydrogen phthalate. The use of zone electrophoresis for the separation of proteins and compounds of high molecular weight, however, has resulted in the development of a number of new techniques. The proteins, for example, after being fixed to the paper by heating at 110° C, are usually located by their property of adsorbing dyestuffs. A number of these have been suggested: Durrum[16] used an ethanolic solution of bromophenol blue saturated with mercuric chloride, the excess of dye being removed by washing the paper with running tap water, but Kunkel and Tiselius[25] found it preferable to wash it with dilute acetic acid. Azocarmine B in acid methanolic solution was employed by Turba and Enenkel[11] and Plückthun and Götting,[86] whereas Grassmann, Hannig and Knedel[204] used Amidoschwartz 10B. Michl[205] introduced a complex dyeing procedure with Neucoccin and Naphtolblauschwartz to obtain maximum colour responses from all proteins and a number of other dyestuffs have been suggested,[206] particularly by Griffiths.[21] I have found azocarmine B to be the most convenient of these dyes for general purposes, as a clear pattern against an almost white background is obtained, and the dyeing and washing procedure can be carried out in a much shorter time than with bromophenol blue. Naphthalene black is adsorbed by the filter-paper to some extent and consequently gives a rather dark blue background. Lipids and lipoproteins are detected by staining with fat-soluble dyes, such as Sudan III,[109,207] oil red O,[107] Sudan black[112] or Cevol B black.[109] Bon[208] has developed a method whereby the protein bands on a paper electrophoretic strip can be detected without terminating the run. The moist paper is contact-printed on photographic bromide paper. On developing in an ultra-hard developer the protein bands appear as dark spots on a white background. By this means a concentration of 1 μg of protein per μl can be detected.

Several methods have been developed for the quantitative determination of the dyed protein fractions obtained by zone electrophoresis. The simplest consists in cutting out the individual bands and eluting the dye therefrom with dilute alkali. The colour can then be measured photometrically. Another method involves direct estimation of the dyed proteins on the paper strip and avoids the labour of eluting each fraction separately. Latner and Richardson[209] have described a visual matching technique they claim to be suitable for most clinical purposes. More accurate methods have been devised by Crook *et al.*,[80,210] Michl,[205,211] Grassmann and his colleagues,[204,212] Latner, Braithwaite and Nunn,[213] Griffiths,[214] Bassir[215] and Köiw.[216] In these methods the electrophoretic strip is treated with a suitable dye, such as azocarmine, dried and rendered transparent by immersion in an organic solvent having the same refractive index as cellulose. Methyl salicylate[217] or a mixture of 1-bromonaphthalene and paraffin oil[86] has been recommended for this purpose. The treated strip is placed between glass plates and passed over a narrow slit having a light source on one side and a photo-electric cell on the other. The optical density of the strip is thus obtained: on plotting this against the distance from the origin, a graph similar in form to that obtained with the classical Tiselius apparatus results, the area enclosed by each peak being proportional to the amount of the protein it represents. It is important in this method that the filter-paper strip should be thoroughly degassed when treated with the mixture of paraffin and 1-bromonaphthalene or methyl salicylate, as any air bubbles left in the strip will give a

false reading on the galvanometer attached to the photo-electric cell. The degassing is best accomplished by placing the strip, immersed in the solvent mixture, in a desiccator that is then evacuated.

Van Os[218] describes a simple method whereby the stained electrophoresis strip is dried and placed directly between a sodium light and a selenium cell without first making it transparent; Röttger[219] and Grüttner[220] have described reflectance photometers for evaluation of the dyed protein strips. Densitometers for estimation of proteins by these methods can now be obtained commercially; alternatively, Latner[221] has described the conversion of a Spekker absorptiometer for this purpose, and Campbell and Simpson[222] have devised an attachment whereby the colours on protein strips may be measured on a Unicam spectrophotometer.

In any method of estimating proteins involving the adsorption of dyestuffs, considerable care must be exercised, as different proteins may have markedly different binding capacities for the dye used. Cremer and Tiselius[12] found it necessary with bromophenol blue to multiply the globulin fractions by a factor of 1·6, to compensate for the albumin's greater capacity for the dye. Griffiths,[21] on the other hand, found no difference in the absorptive capacities. The difference in results obtained by these workers may be explained by differences in staining technique, as the rate of dye absorption is not the same for all individual proteins.

Substances giving ultra-violet absorption or fluorescence can be determined in a manner similar to that described, without the need for staining with dyes. Similarly, proteins or other compounds tagged with radioactive isotopes are readily detected, and automatic scanning instruments for the measurement of the radioactive zones have been described.[223] The adsorption of radio-iodine by blood proteins has been studied by this method.[224,225]

Levin and Oberholzer[226,227] found that results having the same order of accuracy as those obtained by the classical Tiselius method were got by cutting out the protein bands and determining their nitrogen content by the Kjeldahl method. The determination may be done either on unstained strips or on strips stained with a dye that contains no nitrogen, such as bromophenol blue.

One further method of detection may be mentioned, that of high-frequency papyrography described by Hashimoto and Mori.[228] The pherogram is passed through the guide of a high-frequency oscillator consisting of two metal plates held closely together. The variations in grid current produced by the substances on the paper are automatically recorded.

The future of zone electrophoresis

During the last five years zone electrophoresis has been used in attempts to separate compounds of many kinds, sometimes with considerable success. For the biochemist who wishes to determine blood-protein distribution in large numbers of clinical samples, zone electrophoresis has already established itself as a routine procedure. The research worker faced with the problem of separating and analysing micro-quantities of compounds of high molecular weight, such as proteins and nucleic acids, will find the technique invaluable. Humoller and Zimmermann[229] have described for the analysis of the blood proteins a method that requires only a single drop of blood, and Choisy, Derrien and Jayle[115] were able to compare the protein content of human and ox crystalline lenses in single eyes, a procedure impossible with the Tiselius apparatus. The ultimate refinement of micro technique has been attained by Edström.[230,231] Using single cellulose fibres as supporting medium, he has succeeded in analysing the ribonucleotides present in the nucleic acids derived from single nerve cells.

For the separation of compounds of lower molecular weight, zone electrophoresis will take its place alongside paper chromatography. Some separations readily to be achieved by zone electrophoresis are difficult or impossible by chromatography, though the converse is also true. An example of this nature is to be found in the separation of the dimethyl-L-rhamnopyranoses.[144] Though 2:3-dimethyl-L-rhamnose is readily separated from the 2:4- and 3:4-dimethyl derivatives by chromatography, the latter substances cannot readily be separated from each other. Zone electrophoresis in borate buffer, on the other hand, will easily separate the 2:4- and 3:4-dimethyl derivatives, although it will not separate the 2:4-dimethyl derivative from the 2:3-dimethyl-L-rhamnose. Thus, for complete separation of the three derivatives a combination of the two methods is necessary. When separating vitamin-B_{12}-like compounds obtained from faeces, Brown *et al.*[161] employed electrophoresis on filter-paper for the separation of *pseudo*vitamin B_{12} and factor A, two compounds that run

as a single spot on chromatograms, and Schweitzer and Wittern[160] used the technique coupled with microbiological assay to estimate the vitamin-B_{12} content of liver extracts.

The separation and determination of the higher fatty acids[179] and of hydroxycarboxylic acids[180] by zone electrophoresis have already been described: it should be possible also to separate the lower carboxylic acids by this procedure. Much work remains to be done on the uses of the technique in inorganic analysis, and it is probable that in time zone electrophoresis will be as widely used as paper chromatography is to-day in that field.

For more detailed descriptions of the methods and apparatus mentioned, the reader is referred to the original papers and to the reviews of McDonald and his colleagues,[23,39,232,233] Tiselius and Flodin[234] and others,[235,236,237,238,239] and to the recent book on paper chromatography and paper electrophoresis by Block, Durrum and Zweig.[240]

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GLAXO LABORATORIES LTD.
GREENFORD, MIDDLESEX

April 6th, 1955

The Detection and Determination of Traces of Polynuclear Hydrocarbons in Industrial Effluents and Sewage

Part III.* The Examination of Some Gasworks Effluents

BY PHILIP WEDGWOOD AND RONALD L. COOPER

(*Presented at the meeting of the Society on Wednesday, May 4th, 1955*)

The source of the small concentrations of polynuclear aromatic hydrocarbons in sewage has been traced, in part, to gasworks wastes, and in particular to the aqueous effluent produced in the manufacture of carburetted water gas. A smaller contribution arises from the ammoniacal liquor, a product of the carbonisation of coal. Much smaller quantities may be derived from a smoke-polluted atmosphere by deposition and during periods of rain or snowfalls.

IN Part II of this series of papers[1] it was suggested that the possible sources of the aromatic hydrocarbons found in sewage effluents were the atmosphere, which is known to be polluted,[2,3,4] washings from macadam roads, and industrial effluents, including gaswork effluents, which are likely to make the greatest contribution.

Particular attention has been given to the latter, which includes the waste product called "spent liquor" arising from the steam-distillation of crude ammoniacal liquor for the manufacture of sulphate of ammonia, and also to the aqueous effluent produced in water-gas plants used for the manufacture of towns' gas from coke and petroleum oil. Depending upon the efficiency of the plant, this effluent may contain varying proportions of oil either in suspension or in an emulsified form or both, which can be removed by appropriate treatment before it is discharged to the sewers.[5] In this investigation the de-oiled effluent was examined.

ANALYSIS OF SPENT AMMONIACAL LIQUOR

The chief organic constituents are monohydric phenols and naphthols,[6] dihydric phenols and also their alkyl derivatives.[7] Small amounts of basic and other organic substances are also present. The neutral compounds such as the hydrocarbons probably form the smallest group of the three classes. It has been shown that pyrene and other polynuclear hydrocarbons are each present only to the order of 10 parts per thousand million in the cooled and settled effluent that is discharged to the sewers. The solid matter separating out in the tank provided for the purpose contains a much higher proportion of these compounds.

The method of analysis is particularly interesting from the fact that it has proved successful in spite of the presence in the liquor of a large concentration of interfering material that is also soluble in *cyclo*hexane.

METHOD—

Extract 100 to 1000 ml of effluent made strongly alkaline (10 per cent. in potassium hydroxide), by repeatedly shaking with chloroform. Wash the chloroform solution three times with alkali, then with water, and extract the basic material three times with diluted sulphuric acid, sp.gr. 1·27, and finally with water again. This procedure removes unwanted substances and simplifies the chromatography. Filter the chloroform solution of neutral oils from small globules of water and scum by passing it through a filter-paper which has been previously wetted with chloroform. Carefully evaporate the solution almost to dryness to remove chloroform, and redissolve in warm *cyclo*hexane. Repeat the procedure of evaporation, redissolve in warm *cyclo*hexane and pass the final solution in *cyclo*hexane through a column of alumina.

*For particulars of Part II of this series, see reference list, p. 654.

RESULTS—

The effluent has been shown to contain the following hydrocarbons: pyrene, an alkylpyrene, fluoranthene, 1:2-benzanthracene, chrysene, perylene, 1:2-benzpyrene, 3:4-benzpyrene, anthanthrene and possibly triphenylene. Other compounds, not identified, but giving characteristic spectra, have also been indicated. One appears from its spectrum to be allied to pyrene (Fig. 1) and another to be a strongly adsorbed hydrocarbon probably related to 1:2-benzanthracene (Fig. 2).

In the solid matter deposited from this waste liquor, the following compounds have been found: pyrene, fluoranthene, perylene, 1:2-benzpyrene, 3:4-benzpyrene and anthanthrene.

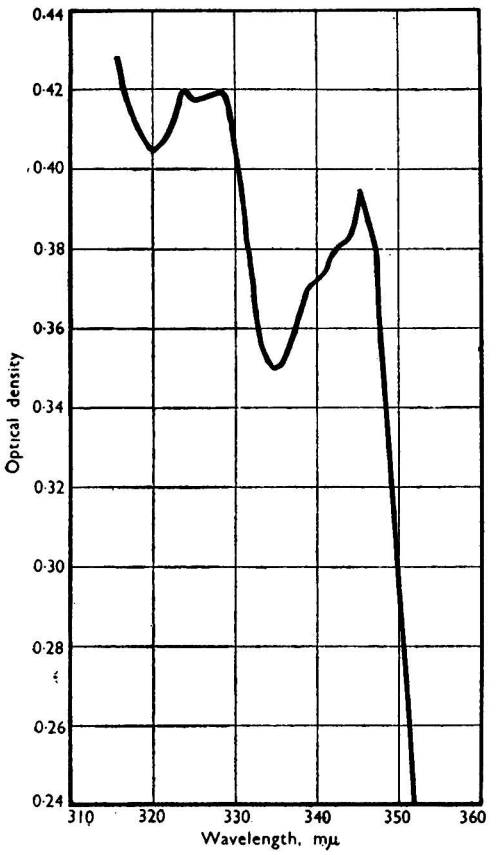

Fig. 1. Absorption spectrum of unidentified compound probably related to pyrene

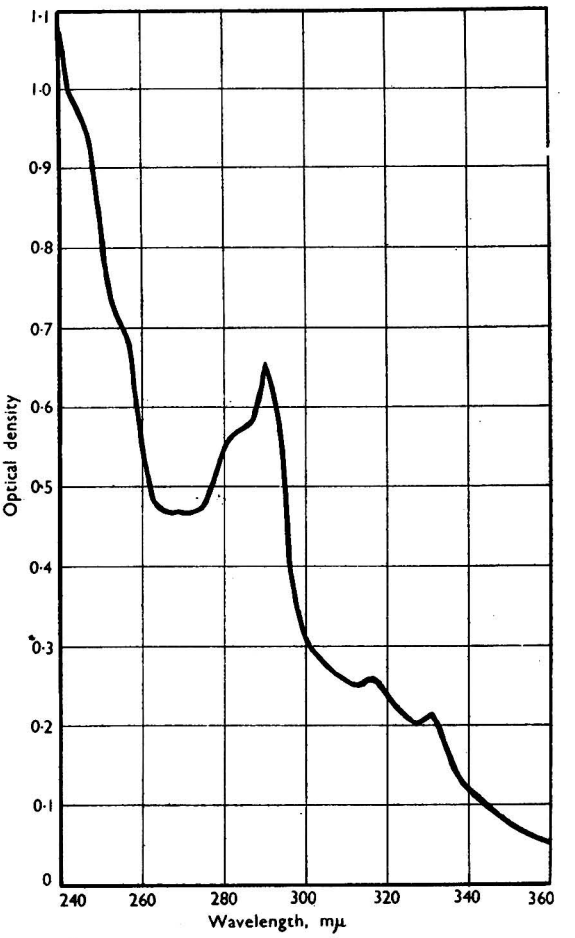

Fig. 2. Absorption spectrum of unidentified compound probably related to 1 : 2-benzanthracene

DISCUSSION OF RESULTS—

The absence of hydrocarbons less complex than pyrene, and especially the absence of anthracene, was surprising, since they had been found in the sewage humus in appreciable amounts. These hydrocarbons may have been removed from the liquor by steam-distillation in the concentrated ammonia-liquor plant and thus would not appear in the spent-liquor effluent.

ANALYSIS OF DE-OILED CARBURETTED WATER-GAS PLANT EFFLUENT

An analysis was conducted on this effluent in an endeavour to find an explanation for the larger amounts of hydrocarbons in sewage humus than could be expected from spent

ammoniacal-liquor effluent and also to find the source of anthracene and other simpler hydrocarbons detected in the humus.

In contrast to the spent liquor this effluent proved to be rich in hydrocarbons and, owing to the fact that it was chiefly neutral in character, preliminary separation of acid and basic substances was not necessary.

METHOD—

Extract the hydrocarbons in about 100 to 500 ml of the effluent into chloroform and from this solution prepare a solution in *cyclo*hexane for chromatographic separation as previously described.

RESULTS—

The following hydrocarbons were found or suspected to be present in the effluent: naphthalene, acenaphthylene, phenanthrene, fluorene, anthracene, pyrene, fluoranthene, perylene, 1:2-benzpyrene, 3:4-benzpyrene, anthanthrene, 1:12-benzperylene and one or two others not yet identified.

Generally speaking, the lower hydrocarbons are present in larger amounts than those having four or more condensed rings.

DISCUSSION OF RESULTS—

From the point of view of hydrocarbon content of sewage this type of effluent is undoubtedly a source of contamination and, particularly when high rates of oil gasification are practised, it may well prove to be a more important potential source of pollution than spent ammoniacal liquor, since the complex hydrocarbons are more resistant to oxidation by bacteria than the major constituents of the liquor produced from coal by carbonisation in retorts. Further, unless de-oiling of the effluent is carried out efficiently, gross contamination may result.

ATMOSPHERIC POLLUTION

Smoke pollution of the atmosphere is important from the public health point of view, but its contribution to the organic matter present in sewage is considered to be relatively unimportant. Samples of humus and effluents taken at different stages of treatment in sewage works receiving little or no industrial effluent have shown the presence of hydrocarbons in trace amounts only. The fact that atmospheric pollution is a possible source of these trace amounts has been demonstrated in an experiment in which freshly fallen snow from different parts of the country has been shown to contain hydrocarbons.[3]

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NOTE—Reference 1 is to Part II of this series.

THE ST. ALBANS LABORATORY
EASTERN GAS BOARD (WATFORD DIVISION)
CLARENDON ROAD, WATFORD
and
THE SIR JOHN CASS COLLEGE
LONDON, E.C.3

October 13th, 1954

DISCUSSION

MR. E. Q. LAWS asked whether separation of the constituents was complete in the chromatographic procedure or whether there was overlapping of the various fractions. If there was overlapping, he wanted to know whether it interfered with subsequent identification.

DR. COOPER replied that complete separation of the constituents was not usually necessary for identification, owing to their highly characteristic spectra. An exceptional example where good separation was important was in the cases of 1:2-benzanthracene and fluoranthene. The latter, which had a sharply defined absorption peak at 287 mμ, was closely followed on the alumina column by the benzanthracene

with a characteristic peak at 288 mμ of similar proportions to the fluoranthene peak. While fluoranthene could be easily identified by further peaks at 342 and 359 mμ, the benzanthracene peak at 288 mμ might be masked, and its peaks at higher wavelengths were of inconveniently low optical density.

MR. A. B. DENSHAM asked what contribution was made by the polynuclear hydrocarbons to the "oxygen absorbed" figure for ammoniacal-liquor effluent.

DR. COOPER replied that the concentration of the polynuclear hydrocarbons in ammoniacal liquor was very low, of the order of a few parts per 100 million; consequently their contribution to the "oxygen absorbed" figure was negligible.

DR. J. H. HAMENCE congratulated the authors on another interesting paper in this valuable series. There were three questions he wanted to ask.

First, he asked if the authors could give him any idea of the identity of a hydrocarbon that he had found in effluents, which gave a brilliant yellow fluorescence in ultra-violet light. His experience was that the literature was not always correct on the subject of the fluorescence of these polynuclear hydrocarbons, and he wondered if the authors had considered identification from this point of view.

Secondly, he enquired whether the authors had examined garage effluents containing mineral oil for polynuclear hydrocarbons, and if these effluents could readily be distinguished from a gasworks effluent.

Thirdly, he referred to the finding of polynuclear hydrocarbons in soils, and enquired if any information was available to show whether or not they were produced by the action of soil bacteria. Some desert soils that he had recently examined showed evidence of the presence of traces of these substances, and quite clearly their presence could not be due to smoke pollution, as the areas concerned were many hundreds of miles from the nearest habitation, which indicated a possibility that these compounds might be produced by soil bacteria.

DR. COOPER replied that the authors had found identification by absorption spectrophotometry to be more useful and certain than fluorescence spectrography, as the identification of the hydrocarbons could be effected while the column was developing. This was not practicable with the fluorescence method. On the other hand, the ultra-violet lamp was very useful for following the development of the zones on the chromatographic column, and fluorescence spectrography was a very useful method for confirming the identity of certain compounds. For example, 3:4-benzpyrene and 1:12-benzperylene had similar absorption spectra but differed in their fluorescence spectra. The hydrocarbon found in effluents by Dr. Hamence, having a brilliant yellow fluorescence in ultra-violet light, might be the somewhat unstable naphthacene or perhaps acenaphthylene. No doubt it could be identified by the spectrophotometric method.

MR. WEDGWOOD added that it was possible from an examination of spectrograms of oil solutions of standard concentration in a suitable solvent to distinguish between mineral oils and different kinds of tar. The method had been used for this purpose in some cases of water pollution that had been investigated. There was no difficulty in differentiating between a garage effluent containing petroleum oil and a gasworks effluent, for example, which might contain some coal tar. A method was now being developed to determine the relative proportions of certain hydrocarbons, taking pyrene as unity, which was expected to be even more useful.

Referring to the third point raised by Dr. Hamence, Mr. Wedgwood said that Kern (*Helv. Chim. Acta*, **1947**, **30**, 1595) had carried out an interesting analysis of garden soil, in which he had found 55 mg of chrysene in 3·5 kg of dry soil, and he had reported the presence of other unidentified hydrocarbons.

MR. A. L. BACHARACH asked whether polynuclear hydrocarbons might be bacterial metabolites derived from steroids.

DR. R. F. MILTON remarked that mention had been made of the work done in South Africa on the determination of cyclic hydrocarbons in sewage sludge before and after composting: it had been suggested that the increase found was due to bacterial action on coprosterol and other steroids.

MR. WEDGWOOD said that the authors were particularly interested in the points made by Mr. Bacharach and Dr. Milton. They had examined fresh cow dung, and the same dung after it had aged in the field for a few days, and had found that the later sample contained higher hydrocarbons not found in the fresh sample. The presence of complex hydrocarbons was confirmed in matured manure taken from a farm stock. They considered it likely that bacterial metabolism resulted in the production of these hydrocarbons. Indeed, this point had been brought out by one of the authors (P.W.) in a paper to the Institute of Sewage Purification in 1953. In the steroids and the polynuclear hydrocarbons found in soil, sewage humus and the final sewage effluent, there was one common characteristic—the phenanthrene ring system. Such bacterial processes in the distant past were probably responsible for the formation of the coal and oil deposits which were being consumed at such a high rate to-day.

The Analysis of Mixtures of Phenols by Partition Chromatography and Ultra-violet Spectrophotometry

By R. M. PEARSON

(*Presented at the meeting of the Society on Wednesday, May 4th, 1955*)

A method is described for the determination of phenol, cresols, xylenols and ethylphenols in their admixture. By partition chromatography the mixture of phenols is separated into groups, which can be analysed by ultra-violet spectroscopy.

The determination of phenol can be made with an accuracy of 0·5 per cent. of the amount present, whilst the accuracy of cresol determinations is about 1·0 per cent.

Zahner and Swann[1] described a method for the separation of phenol from cresylic acid by partition chromatography. From solubility studies they found that the partition coefficient of phenol in the system *cyclo*hexane - water is appreciably different from those of its homologues, and therefore separation of phenol from the isomeric cresols can be achieved. They also suggested that by using longer columns, it might be possible to separate some of the higher homologues of phenol.

This has been realised with mixtures of xylenols, cresols and phenols, *cyclo*hexane - water being used as the solvent system. The separation into groups is such that analysis by ultra-violet spectrophotometry in the region of 2700 A is possible. If ethylphenols are present, the separation achieved is indequate, as the *m*- and *p*-ethylphenols are grouped with 3:4- and 3:5-xylenols and *o*-cresol and no absorption peaks at convenient wavelengths are available for the spectrophotometric analysis of this five-component mixture. However, it has been found that, with a 30 per cent. aqueous solution of methyl Cellosolve as the stationary phase instead of water, this mixture is separated into two groups—

(*i*) *m*- and *p*-ethylphenols and 3:5-xylenol, and
(*ii*) 3:4-xylenol and *o*-cresol,

each of which can be analysed by ultra-violet spectroscopy.

With the system *cyclo*hexane - 30 per cent. aqueous methyl Cellosolve, separation of the C_6, C_7 and C_8 tar acids gives the following groups—

(*a*) 2:6-xylenol,
(*b*) *o*-ethylphenol, 2:3-, 2:4- and 2:5-xylenol,
(*c*) *m*- and *p*-ethylphenols and 3:5-xylenol,
(*d*) 3:4-xylenol and *o*-cresol,
(*e*) *m*- and *p*-cresols, and
(*f*) phenol.

This order of separation agrees closely with the partition coefficients of the phenols in the system *cyclo*hexane - 30 per cent. aqueous methyl Cellosolve.

Phenols with a substituent in the *ortho* position have higher partition coefficients in *cyclo*hexane - water systems than do their *meta* and *para* isomers, doubtless owing to the hindrance of the substituent on the hydroxyl group. Thus, in the case of the cresols a clean separation of the *ortho* isomer from the *meta* and *para* isomers can be achieved, using *cyclo*hexane - water, but no useful separation of the *meta* and *para* isomers is achieved. Indications of slight separation of these two isomers were obtained, as was shown by the spectroscopic examination of fractions on either side of the peak absorption of the combined *m*- and *p*-cresol cut. Any increase in resolution obtainable by increasing the length of the column was not investigated, as resolution of the mixture of the two isomers can be easily and accurately effected by ultra-violet spectroscopy.

Experimental

The column used by Zahner and Swann, which had a diameter of 18 mm and a packing 150 mm in length, was capable of separating phenol from its homologues, but not the individual cresols, as is shown in Table I.

TABLE I

SEPARATION OF PHENOL AND CRESOLS USING 150 mm OF PACKING

Phenol		*m*- and *p*-Cresol		*o*-Cresol	
Present, %	Found, %	Present, %	Found, %	Present, %	Found, %
32·8	33·0	33·8	41·5	33·4	24·7
51·2	50·7	48·8	44·7	—	—
51·7	51·9	48·3	44·6	—	—
51·2	51·4	24·6	28·7	24·2	20·0

To separate the cresols efficiently it is necessary to use a 18-mm diameter column with a 500-mm length of packing, whilst the xylenol separation necessitates a column of similar diameter containing a 900-mm length of packing.

Typical results of these separations are given in Tables II and III, whilst Fig. 1 shows a typical separation of phenol and cresols on a 900-mm column and Fig. 2 the separation

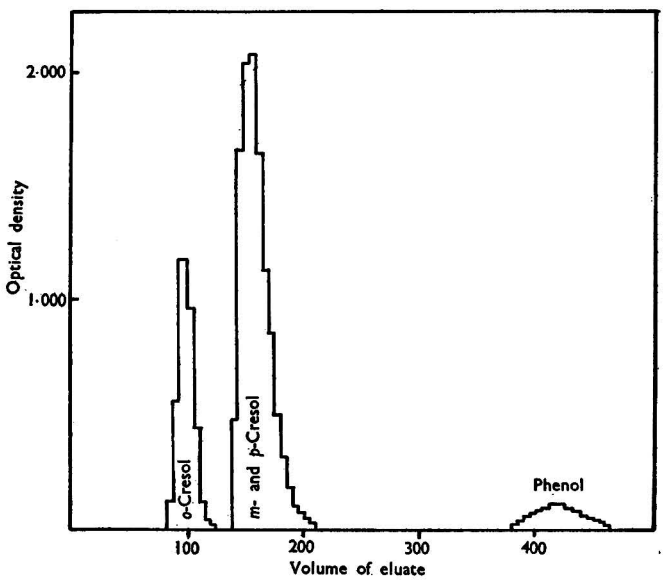

Fig. 1. Separation of cresols and phenol using *cyclo*hexane and water

of the six xylenols on the same column. Comparing the two figures, it will be noted that, in Fig. 1, *o*-cresol leaves the column at the place which is occupied by the 3:4- and 3:5-xylenols in Fig. 2. It can, therefore, be assumed that, in a mixture of cresols and xylenols, *o*-cresol will be grouped with the 3:4- and 3:5-xylenols. This assumption has been verified, as will be seen later.

TABLE II

SEPARATION OF PHENOL - CRESOL MIXTURES USING 500 mm OF PACKING

Phenol		*o*-Cresol		*m*-Cresol		*p*-Cresol	
Present, %	Found, %	Present, %	Found, %	Present, %	Found, %	Present, %	Found, %
0·59	0·59	—	—	—	—	—	—
0·45	0·45	—	—	—	—	—	—
1·0	0·9	57·5	57·5	22·5	22·5	19·0	19·1
2·8	2·5	34·7	34·8	62·5	62·6	—	—
6·9	6·6	18·6	18·7	5·2	5·1	69·3	69·5
9·2	9·0	25·6	26·0	28·5	28·6	36·7	36·0
—	—	—	—	32·4	32·5	67·6	67·7
—	—	—	—	38·6	38·7	61·4	61·4

TABLE III

SEPARATION OF XYLENOLS USING 900 mm OF PACKING

2:3-Xylenol		2:4-Xylenol		2:5-Xylenol		3:4-Xylenol		3:5-Xylenol		2:6-Xylenol	
Present, %	Found, %	Present, %	Found, %	Present, %	Found, %	Present, %	Found, %	Present, %	Found, %	Present, %	Found, %
—	—	83·2	82·5	16·9	17·5	—	—	—	—	—	—
9·2	10·0	70·6	70·0	20·2	20·1	—	—	—	—	—	—
13·3	13·0	67·0	67·4	19·7	19·3	—	—	—	—	—	—
10·3	10·7	30·4	30·3	7·9	7·7	14·3	14·5	11·7	12·0	25·4	25·9
10·3	10·6	30·4	30·7	7·9	8·2	14·3	14·2	11·7	12·0	25·4	25·6

If ethylphenols are present along with cresols and xylenols, it has been found that *o*-ethylphenol leaves the column with 2:3-, 2:4- and 2:5-xylenols. This presents no difficulties, as absorption peaks at convenient wavelengths can be found for the analysis of this four-component mixture. However, a difficulty is presented by the appearance of *m*- and *p*-ethylphenols with 3:4- and 3:5-xylenols and *o*-cresol. No satisfactory absorption peaks can be found for the analysis of this mixture and, even with much longer columns, these five tar acids are not sub-divided when the *cyclo*hexane - water system is used.

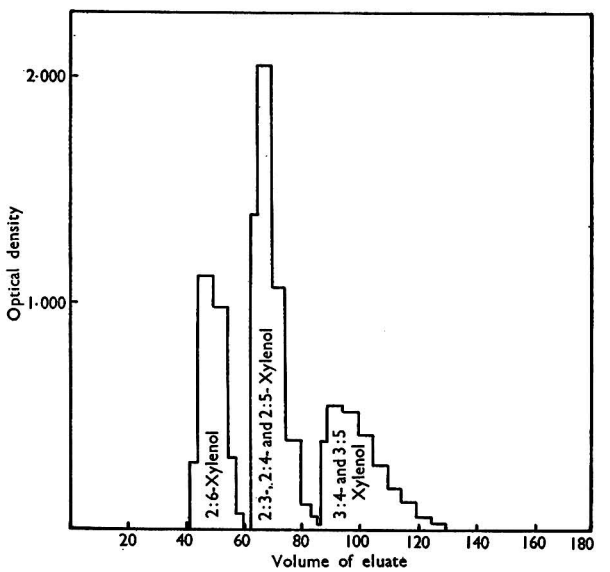

Fig. 2. Separation of xylenols using *cyclo*hexane and water

A search was therefore made for some solvent system which would effect a satisfactory separation of this group of tar acids. Methanol - water mixtures were investigated as the stationary phase without success. However, as will be seen in Fig. 3, sub-division was achieved by using aqueous solutions of methyl Cellosolve as the stationary phase. A 10 per cent. solution of methyl Cellosolve in water gave partial separation of the *m*- and *p*-ethylphenols and 3:5-xylenol from the 3:4-xylenol and *o*-cresol, whilst a 30 per cent. solution gave complete separation, the two groups being capable of analysis by ultra-violet spectroscopy.

It was found that inferior separations of this five-component mixture were obtained with aqueous solutions of ethylene glycol and diethylene glycol.

Typical results of the separations obtained with 30 per cent. aqueous methyl Cellosolve are given in Table IV.

The partition coefficients of the C_6, C_7 and C_8 phenols have been measured for the systems *cyclo*hexane - water and *cyclo*hexane - 30 per cent. aqueous methyl Cellosolve.

A 0·1-g portion of the phenol was dissolved in 100 ml of *cyclo*hexane, and 50 ml of this solution were then shaken with 50 ml of water or 30 per cent. aqueous methyl Cellosolve for

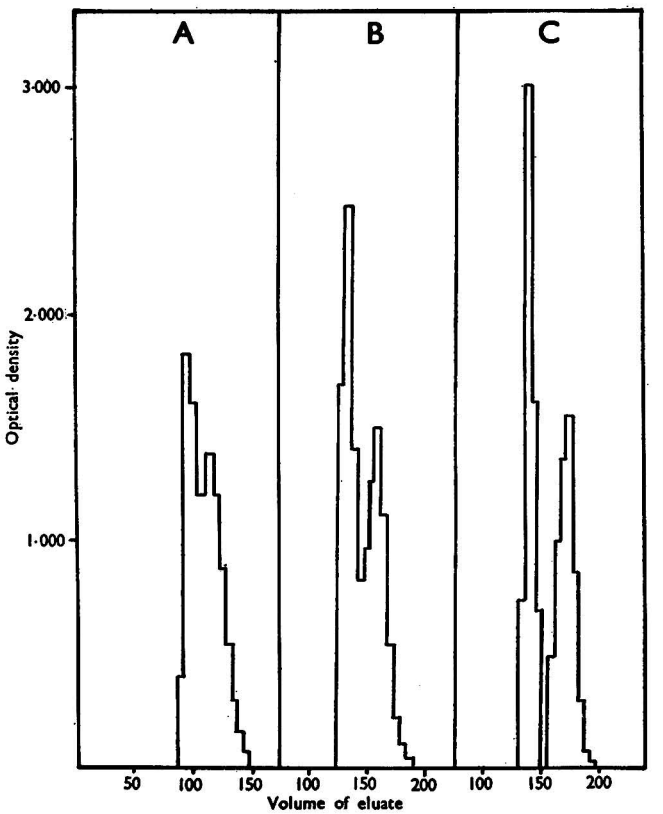

Fig. 3. Effect of methyl Cellosolve on separations of 3- and 4-ethylphenols, 3:4- and 3:5-xylenols and *o*-cresol: A, separation using *cyclo*hexane - water; B, separation using *cyclo*hexane - 10 per cent. aqueous methyl Cellosolve; C, separation using *cyclo*hexane - 30 per cent. aqueous methyl Cellosolve

TABLE IV

SEPARATION OF ETHYLPHENOLS, XYLENOLS AND CRESOLS USING 30 PER CENT. AQUEOUS METHYL CELLOSOLVE

2:3-Xylenol		2:4-Xylenol		2:5-Xylenol		2-Ethylphenol		3-Ethylphenol	
Present, %	Found, %	Present, %	Found, %	Present, %	Found, %	Present, %	Found, %	Present, %	Found, %
16·2	16·8	10·8	10·2	19·4	20·0	53·6	52·9	—	—
—	—	—	—	—	—	13·6	14·0	30·2	30·8
—	—	—	—	—	—	—	—	16·2	16·4
7·4	7·6	8·8	8·2	11·6	11·8	9·2	8·8	14·3	15·0

4-Ethylphenol		3:5-Xylenol		3:4-Xylenol		*o*-Cresol	
Present, %	Found, %	Present, %	Found, %	Present, %	Found, %	Present, %	Found, %
—	—	—	—	—	—	—	—
8·8	8·2	40·6	40·7	6·8	6·5	—	—
10·8	11·2	12·4	12·9	30·6	31·2	30·0	29·6
6·8	6·4	17·2	17·0	12·4	12·4	12·3	12·0

15 minutes. The amount of the phenol remaining in the *cyclo*hexane layer was then determined by ultra-violet spectroscopy. The partition coefficients are given in Table V.

TABLE V

PARTITION COEFFICIENTS OF C_6, C_7 AND C_8 PHENOLS IN *cyclo*HEXANE - WATER AND *cyclo*HEXANE - 30 PER CENT. AQUEOUS METHYL CELLOSOLVE

	Partition coefficient in	
	*cyclo*Hexane - water	*cyclo*Hexane - methyl Cellosolve
2:6-Xylenol	14·4	3·4
2-Ethylphenol	4·2	1·7
2:3-Xylenol	3·5	1·3
2:4-Xylenol	4·6	1·5
2:5-Xylenol	4·0	1·7
3-Ethylphenol	2·5	1·0
4-Ethylphenol	2·7	1·1
3:5-Xylenol	2·2	0·9
3:4-Xylenol	2·0	0·4
o-Cresol	2·2	0·6
m-Cresol	0·6	0·2
p-Cresol	0·6	0·3
Phenol	0·1	0·1

PRECISION OBTAINABLE—

The method described has been in use for over 2 years and has handled more than 1000 samples of tar acids, including normal samples of phenol, cresylic acid and xylenols and mixtures of certain more highly substituted phenols.

The accuracy achieved, based on tests with standard mixtures, is of a high order. For example, phenol, which leaves the chromatographic column as an individual fraction and is easily separated from all other tar acids, can be determined with an accuracy of 0·5 per cent. of the amount present. In Tables VI and VII below are given: (*a*) the results of duplicate determinations of *m*-cresol on standard mixtures done in one laboratory and (*b*) the results of tests on *m*-cresol determinations on commercial cresylic acids carried out in three laboratories, by different personnel and with different apparatus.

TABLE VI

m-CRESOL DETERMINATIONS (WITHIN-LABORATORY VARIATION)

m-Cresol present, %	*m*-Cresol found	
	Test 1, %	Test 2, %
50·0	49·9	49·6
43·0	42·9	43·4
52·0	51·9	51·8
40·0	40·1	39·5

TABLE VII

m-CRESOL DETERMINATIONS (VARIATION BETWEEN LABORATORIES)

Laboratory A	Laboratory B	Laboratory C
44·5	44·0	45·0
42·5	43·0	43·7
53·4	52·7	53·4
52·6	52·6	52·7
50·9	50·8	50·9

METHOD

APPARATUS—

The apparatus is shown in Fig. 4. Attached to the top of the column is a reservoir with a pressure-equalising device. The joints are all spring-loaded. The bottom of the

column terminates in a B7 cone to which can be fitted a drawn-off B7 socket, which acts as a stopper when the column is not in use.

Two lengths of column are used, the 500-mm length, with water as the stationary phase, being suitable for the analysis of phenol and cresols only, whilst the 900-mm column, with 30 per cent. aqueous methyl Cellosolve as the stationary phase, is used when xylenols or ethylphenols or both are also present.

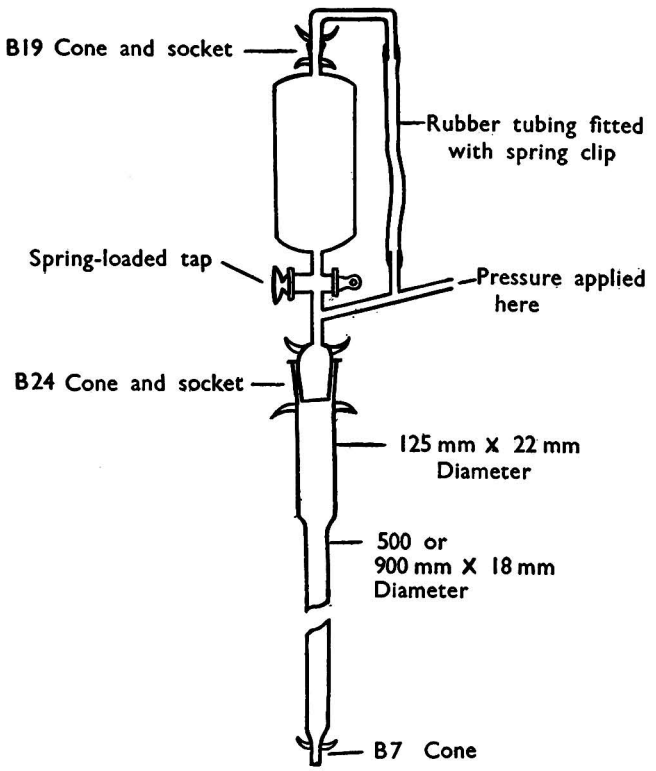

Fig. 4. Chromatographic column and reservoir (all joints are fitted with lugs for spring loading)

The packing in the 500-mm column consists of 56 g of silica gel treated with 42 ml of water, whilst that of the 900-mm column is 100 g of silica gel treated with 76 ml of 30 per cent. aqueous methyl Cellosolve.

REAGENTS—

cyclo*Hexane*—The *cyclo*hexane used must not absorb in the ultra-violet in the 2700 A region.

Methyl Cellosolve—British Drug Houses methyl Cellosolve having no absorption in the ultra-violet region of 2700 A is suitable.

Silica gel—The gel must be able to hold a permanent water layer when *cyclo*hexane is passed through it. It must be of such particle size that an optimum speed of throughput of 120 ml per hour is obtained with a pressure of 15 lb. per square inch. The gel used in the work here described was a mixture of equal parts of material passing a 100 B.S. sieve and retained on a 200 B.S. sieve and material which passed a 200 B.S. sieve. The gel used was supplied by Mallinckrodt Chemical Works, New York.

PREPARATION OF THE COLUMN—

The packing consists of a mixture of silica gel and water, or 30 per cent. aqueous methyl Cellosolve, in equal proportions by weight, the free water in the gel, *i.e.*, that lost when the gel is heated to 100° to 110° C, being taken into account when making up the mixture.

The silica gel is slightly ground in a porcelain mortar while the requisite amount of water is slowly added, the grinding being continued until the mixture has a dry powdery consistency. Enough *cyclo*hexane is added to form a mobile slurry, which is then poured into the column, which contains a wad of cotton-wool at its constricted end. As the mixture is added to the column, it is packed down with a glass rod, excess of *cyclo*hexane being run off at the bottom of the column during the process.

When all the mixture has been added to the column, some *cyclo*hexane is run on to the top of it and an air pressure of approximately 15 lb. per square inch applied, thus forcing the *cyclo*hexane through the column and, at the same time, packing the silica gel more firmly. With such a pressure the rate of flow of *cyclo*hexane through the column should be about 120 ml per hour. After the rate of flow has been checked, *cyclo*hexane is run into the column until the gel is covered to a depth of 1 to 2 cm. Without any further treatment the column is ready for use.

PROCEDURE—

It is advisable to have not more than 2 mg of any one component of the tar acid present in the amount of sample added to the column, otherwise fractions may be collected, the optical densities of which may be too high to measure on the spectrometer. If this does occur, the fractions are diluted with a known volume of *cyclo*hexane and optical density measurements made on an aliquot.

The sample is dissolved in *cyclo*hexane and diluted to a known volume. *cyclo*Hexane is run from the column until the top of the packing is just covered with liquid. An aliquot of the sample solution is then run on to the column, the reservoir replaced and pressure applied to force the sample into the silica gel. As the liquid level reaches the top of the packing, small amounts of *cyclo*hexane, from the reservoir, are allowed to run on to it, thus ensuring that all the sample is quickly washed into the packing.

The *cyclo*hexane that runs from the bottom of the column is collected in 5-ml fractions. Washing of the column with *cyclo*hexane is continued and 5-ml fractions are collected until all the phenols have been washed through the column. On the short column, if phenol itself is present, this will take approximately 450 ml, whilst under similar conditions on the long column about 750 ml will be needed.

The optical densities of the fractions are measured on an ultra-violet spectrometer at 2700 A. If these optical densities, as the ordinate, are plotted against volume of percolate, in ml, as the abcissa, the following groups of phenols will have characteristic peaks, appearing from the bottom of the column in the order given—

(*i*) 2:6-xylenol,
(*ii*) 2-ethylphenol, 2:3-, 2:4- and 2:5-xylenols,
(*iii*) 3- and 4-ethylphenol and 3:5-xylenol,
(*iv*) 3:4-xylenol and *o*-cresol,
(*v*) *m*- and *p*-cresols, and
(*vi*) phenol.

The above groups with more than one component are analysed by using suitable wavelengths, which are determined from an examination of the absorption spectra of the individual phenols. In the work here reported, the following wavelengths were used—

Group (*ii*)	2720, 2790, 2820 and 2860 A,
Group (*iii*)	2700, 2800 and 2840 A, and
Groups (*iv*) and (*v*)	2700 and 2840 A.

However, owing to a variety of reasons, it cannot be assumed that absorption peaks located by one instrument are universally applicable. It is therefore necessary to plot the spectra of the phenols with the instrument in use and locate the absorption peaks. Similarly, it is necessary to determine the extinction coefficient for each phenol at the applicable wavelengths.

CALCULATION OF RESULTS

SINGLE COMPONENT—

When only one phenol leaves the column in an individual fraction, *e.g.*, *o*-cresol in a mixture of cresols and phenol, the concentration of the phenol in the fraction is found by solving the equation—

$$C_a = \frac{D \times V}{E_a} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where C_a = concentration of the component a present in the fraction,
D = total optical density of the fraction,
E_a = extinction coefficient of the component a, and
V = total volume of the fraction

both D and E_a being measured at the same wavelength. Expressed as a percentage, the above equation becomes—

$$a, \text{ per cent.} = \frac{D \times V \times 100}{E_a \times W} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where W = mg of sample added to the column for analysis.

Thus, to determine the percentage of a phenol that leaves the column as an individual peak, the optical densities of the fractions of percolate that make up the peak are measured at the appropriate wavelength, multiplied by the volume of each fraction and totalled. The total optical density obtained is divided by the extinction coefficient of the particular phenol being determined, thus giving the amount of the phenol, in mg, in the amount of sample added to the column.

MULTICOMPONENT MIXTURE—

When a mixture of phenols leaves the column at one and the same time analysis is achieved by a method similar to that described by Brattain, Rasmussen and Cravath[2] in which they show that the total optical density at any one wavelength is equal to the sum of the mole fraction, X_n, multiplied by the extinction coefficient, E_n, of each component, that is—

$$D = E_1X_1 + E_2X_2 + E_3X_3 + \ldots + E_nX_n \quad .. \quad .. \quad .. \quad .. \quad (3)$$

A mixture can then be analysed by the following steps—

(*a*) determination of the E_n values of each pure component at n different wavelengths,
(*b*) measurement of the optical densities of the mixture at these wavelengths, and
(*c*) solution of the n simultaneous linear equations (one for each wavelength) of the type shown above for the mole concentration X.

Consider the four-component mixture 2-ethylphenol, 2:3-, 2:4- and 2:5-xylenols. In a sample containing ethylphenols and xylenols this mixture leaves the column after the 2:6-xylenol and before the 3- and 4-ethylphenols and 3:5-xylenol. By using the above method its analysis is achieved as follows.

The absorption spectrum of each of the four pure compounds is plotted over the range 2600 to 2900 A. Absorption peaks at convenient wavelengths are then chosen, *e.g.*, with a particular photometer, the peaks were 2720, 2790, 2820 and 2860 A. The extinction coefficients of each pure compound at each of the selected wavelengths is then determined, giving a total of 16 values. (These determinations are carried out by first accurately weighing about 0·100 g of a compound and diluting to 100 ml with *cyclo*hexane. Then 5 ml of this solution are diluted to 100 ml with *cyclo*hexane, and the optical density of this dilute solution measured at the four appropriate wavelengths. From the optical densities so measured are calculated the optical densities which would be obtained with a concentration of 1 mg of the phenol per ml of *cyclo*hexane, thus giving the extinction coefficient.)

In this work the values shown in Table VIII for the extinction coefficients of 2-ethylphenol, 2:3-, 2:4- and 2:5-xylenols measured at 2720, 2790, 2820 and 2850 A were obtained.

TABLE VIII

EXTINCTION COEFFICIENTS OF 2-ETHYLPHENOL, 2:3-, 2:4- AND 2:5-XYLENOLS

	Extinction coefficient at			
	2720 A	2790 A	2820 A	2860 A
2-Ethylphenol	16·45	15·25	6·55	1·43
2:3-Xylenol	11·30	12·88	6·08	8·50
2:4-Xylenol	12·35	16·90	15·20	15·85
2:5-Xylenol	14·48	13·78	14·98	4·73

The total optical densities of the four-component mixture at the four wavelengths are then measured. Substitution of these values and the values of the extinction coefficients gives four simultaneous equations as follows—

$$\begin{aligned} D_1 &= 16{\cdot}45X_1 + 11{\cdot}30X_2 + 12{\cdot}35X_3 + 14{\cdot}48X_4 \\ D_2 &= 15{\cdot}25X_1 + 12{\cdot}88X_2 + 16{\cdot}90X_3 + 13{\cdot}78X_4 \\ D_3 &= 6{\cdot}55X_1 + 6{\cdot}08X_2 + 15{\cdot}20X_3 + 14{\cdot}98X_4 \\ D_4 &= 1{\cdot}43X_1 + 8{\cdot}50X_2 + 15{\cdot}85X_3 + 4{\cdot}73X_4, \end{aligned}$$

where X_1, X_2, X_3 and X_4 represent 2-ethylphenol, 2:3-, 2:4- and 2:5-xylenols, respectively, and D_1, D_2, D_3 and D_4 the total optical densities of the sample measured at 2720, 2790, 2820 and 2860 A, respectively.

From these equations the amount of each phenol present, in mg, may be expressed as follows—

$$\begin{aligned} X_1 &= +0{\cdot}2715D_1 - 0{\cdot}1873D_2 - 0{\cdot}1215D_3 + 0{\cdot}1042D_4 \\ X_2 &= -0{\cdot}3202D_1 + 0{\cdot}3485D_2 + 0{\cdot}0369D_3 - 0{\cdot}1549D_4 \\ X_3 &= -0{\cdot}0134D_1 + 0{\cdot}0190D_2 - 0{\cdot}0283D_3 + 0{\cdot}0805D_4 \\ X_4 &= +0{\cdot}0234D_1 - 0{\cdot}0745D_2 + 0{\cdot}1325D_3 - 0{\cdot}0652D_4. \end{aligned}$$

Thus, once the extinction coefficients have been established, it is only necessary to measure the optical densities of an unknown mixture at the four selected wavelengths and substitute them in the above expressions in order to obtain the amount of each component, in mg, present in the weight of sample added to the chromatographic column.

References

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Discussion

Dr. J. E. Page enquired how the technique compared with published infra-red methods.

Mr. Pearson replied that, for major components, infra-red methods were as accurate and convenient as the chromatographic method; for minor components, *e.g.*, those of less than 20 per cent. concentration, the chromatographic method was preferred.

Dr. J. R. Nicholls asked the author if he had had any experience with chlorocresols and chloroxylenols in the presence of cresols and xylenols.

Mr. Pearson answered that he had had no experience with chloro compounds in the presence of cresols and xylenols.

The Determination of Traces of Benzene Hexachloride in Water and Sewage Effluents

By W. HANCOCK and E. Q. LAWS

(*Presented at the meeting of the Society on Wednesday, May 4th*, 1955)

A method is given for the extraction of benzene hexachloride from aqueous solutions by means of activated charcoal and its subsequent determination as *m*-dinitrobenzene. The possibility of concentrating the material from very large volumes of solution is demonstrated.

Although methods are available for the determination of small quantities of benzene hexachloride, there has not previously been a satisfactory method for its removal from contaminated water or sewage effluent. This publication gives a method which is applicable to extremely dilute solutions. It is possible to recover the benzene hexachloride from solutions containing one part in a thousand million parts of water. The quantitative determination is then made by the method of Schechter and Hornstein,[1] with certain modifications. The method is not specific for benzene hexachloride, as it will give positive results with benzene, toluene and so on, or any compound which will yield these under the given experimental conditions; however, the number of such compounds likely to be met with in practice is, fortunately, small. Not the least interesting part of the work is that the extraction of the benzene hexachloride is effected by adsorption, whilst the subsequent determination is by a chemical process either direct on the adsorbent containing the substance or on a solution obtained by washing it from the adsorbent with a solvent.

The advantage of such an approach is that large amounts of very dilute solutions may be extracted either at a single sampling or by continuous extraction over a period. In either case the required compound is concentrated on the adsorbent for the subsequent treatment. The fact that the solution under test and the adsorbent used are the only factors to be reckoned with does away with the difficulties associated with solvent-extraction methods. These difficulties are two-fold and arise from the large-scale separations necessary to deal with, say, 5 to 10 litres of test solution and the fact that most of the industrial solvents supplied today contain benzene or benzene compounds in excess of the amounts we are measuring. The method may be applied to the determination of benzene in air or other gases and should be of general application to a great number of other substances, if there is an available method for their determination after they have been extracted from solution by the solid adsorbent. Here we propose to confine the description to the case of benzene hexachloride, but we believe that the simplicity of the technique will show it to be useful in other determinations.

Experimental

Extraction of benzene hexachloride from aqueous solutions—

The solubility of the gamma isomer of benzene hexachloride in water was measured at room temperature and found to be 7 μg per ml of water. Solvents such as ethyl ether, light petroleum, *n*-hexane, chloroform and carbon tetrachloride are suitable for extracting chlorinated benzene compounds from water, but they are all liable to contain benzene or benzene compounds.

Methods were tried for purifying these solvents, but it was found to be a difficult process. The figures in Table I give an idea of the amounts of impurity involved. Now for the purpose of the colorimetric comparison we choose to work in the region 0 to 50 μg of benzene hexachloride, so that the amounts of impurity shown are very serious and would become more so as more solvent was used to extract larger quantities of water. It was therefore necessary either to obtain a suitable solvent free from benzene and its compounds or to avoid the use of a solvent altogether.

A sample of ether free from interfering impurity was prepared, and it was then found that in the evaporation of an ether solution of benzene hexachloride there is total loss of this

very volatile substance up to 100-μg quantities. This loss occurs whether or not heat is applied during the evaporation. Evaporation with other solvents resulted in similar losses. An attempt was made to minimise the loss by the addition of liquid paraffin to the ether solution before evaporation; this reduced the loss to about 12 per cent. To avoid evaporation losses altogether, adsorption of the benzene hexachloride from the solution on to a solid adsorbent was investigated.

EXTRACTION OF BENZENE HEXACHLORIDE—

Experiment showed that extraction of the benzene hexachloride could be achieved from ether, carbon tetrachloride or chloroform by means of activated charcoal. In addition, the benzene hexachloride could be washed off the charcoal by means of glacial acetic acid. Other solid adsorbents were tried, but were not successful. Alumina and silica were unsuitable, as their alkalinity destroyed the traces of benzene hexachloride by hydrolysis. Activated charcoal of commerce was found to contain a high proportion of benzene or its compounds, and methods had to be devised for ridding it of these interfering impurities.

The possibility of greatly simplifying the procedure by taking the compound directly out of water by means of charcoal was investigated, and it was found that 1 g of charcoal removes the whole of the benzene hexachloride from water irrespective of the volume in which it is contained between the limits of 100 ml and 5 litres. We have no reason to believe that this in any way represents the limit of volume which may be used. The compound may be eluted from the charcoal by means of glacial acetic acid or as an alternative the charcoal with the benzene hexachloride adsorbed on its surface may be placed directly in the reaction flask. It is advisable to prepare the standard curve with charcoal added to the acetic acid solution of the benzene hexachloride. Some water remains attached to the charcoal and to the cotton-wool used for its support. To remove this water, 10 ml of acetic anhydride were added for each 2 g of adsorbed water. It was subsequently found that the complete removal of the water was unnecessary and that 10 ml of a (1 + 1) mixture of glacial acetic acid and acetic anhydride would suffice and was in fact better, as the reaction between acetic anhydride and water, which is slow to start, goes violently when the mixture warms up, but is quite placid in the presence of acetic acid.

A CRITICAL SURVEY OF THE SCHECHTER AND HORNSTEIN METHOD—

This method, on which our work is based, employs an ingenious apparatus in which the benzene hexachloride is dechlorinated to benzene, which is then removed in an internally generated current of carbon dioxide into nitrating acid, whence it is extracted as *m*-dinitrobenzene and assessed quantitatively by means of the reaction between the nitro compound, butanone and alkali, the resulting colour being measured on an absorptiometer or spectrophotometer.

Using the Schechter and Hornstein method and apparatus, we were unable to obtain the 85 per cent. recovery claimed by them. The results were consistent but low, the yield being between 58 and 60 per cent. for the gamma isomer of benzene hexachloride in the range 5 to 100 μg.

We carried out nitration experiments on microgram quantities of both benzene and nitrobenzene with a number of nitric acid - sulphuric acid mixtures. In all cases we found that under our experimental conditions the yield of *m*-dinitrobenzene was 90 per cent., as is the case in the large-scale preparation. This is to be expected, because the nitronium ion is in very great excess relative to the benzene, whilst the water formation, which is of such great importance in macro-scale nitrations,[2,3,4,5] is in this case very small. Nitric acid without sulphuric acid gave nitrobenzene but no *m*-dinitrobenzene. The acid recommended by Schechter and Hornstein was easily decomposed by traces of water, which gave rise to troublesome interference. A mixture of 20 parts of sulphuric acid with 1 part of nitric acid by volume was found to be unaffected by quite considerable amounts of water. There is the additional factor that the nitration mixture is cold in the new apparatus and warm in the Schechter model.

After much work on the method, we concluded that both the dechlorination reaction and the removal of the benzene from the solution occurred with considerable rapidity and that benzene was lost from the nitrating column during the time when the air in the flask expands with heat. Yields were increased to 90 per cent. by carefully controlling the rate

of heating of the reaction mixture. It was also found that any factor tending to steady the rate of flow, such as the use of a nitrating acid of greater density (higher sulphuric acid content) or a second nitrating column in series with the first, resulted in a higher yield. In the latter case no *m*-dinitrobenzene was found in the second column.

Having decided that the low yields were due to the ineffectiveness of the bead column in scrubbing the benzene from the gas stream, we were in a position to approach the problem from the apparatus-design point of view.

The hot jacket of the Schechter and Hornstein apparatus, although highly ingenious, appeared to us to be unnecessary, as the amount of benzene vapour is small relative to the gas stream. In other words, there is enough air and carbon dioxide to remove the benzene vapour even at room temperature.

Fig. 1. Adsorption apparatus

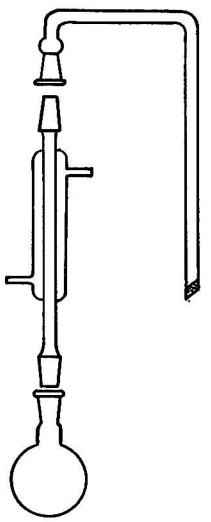

Fig. 2. Dechlorination and nitration apparatus

In consequence, we decided that a simplified apparatus could be used. This is shown in Fig. 2 and is described below. We also used a sintered-glass disc to break up the gas stream into fine bubbles to facilitate absorption of benzene by the nitrating mixture. A condenser was fitted to prevent too great a loss of acetic acid, with consequent dilution of the nitrating acid. With the modified apparatus the time taken for the reaction may safely be cut from 2½ hours to 1 hour.

It was found that the method given by Schechter and Hornstein for mixing the final test solutions gave erroneous results. Shaking for 1 minute followed by standing for 20 minutes was insufficient to produce a maximum or even constant colour. Variations in the depth of liquid gave different intensities; the diameter of the vessel used was a critical factor. A better procedure is to shake the reactants continuously for 1 hour in a glass-stoppered 250-ml Erlenmeyer flask to give a large contact area with the potassium hydroxide solution. Under these conditions a slight turbidity sometimes appears in the test solution and this is removed by centrifuging the solution before making the absorption measurement. It is necessary at all times to keep the reaction mixture in contact with the 40 per cent. potassium hydroxide solution, otherwise rapid fading takes place. Thus a small amount of the potassium hydroxide solution is added to the cell used in the absorption measurements. Comparative figures are shown in Table II.

Method

The new method consists in the removal of the benzene hexachloride from aqueous solution in the chromatographic-adsorption apparatus, the transfer of the charcoal from

this apparatus to the modified apparatus for the dechlorination and nitration reactions, the extraction of the *m*-dinitrobenzene and the absorption measurement.

APPARATUS—

The Hilger Spekker absorptiometer was used for some of the results quoted. Experiments conducted on a Unicam SP600 spectrophotometer gave comparable results.

Adsorption apparatus—This is depicted in Fig. 1. It consists of a funnel having a stem fitted with a B14 ground-glass cone. The socket of the joint is connected to a tube fitted with a tap. The upper part of the cone is cut back so that the end diameter is as big as the stem of the funnel, so that the charcoal column may be extruded. The tap serves to control the flow-rate of the solution. In use a plug of cotton-wool holds the column in position, 1 g of charcoal being placed on the cotton-wool. The total weight of cotton-wool used is 0·25 g.

Dechlorination and nitration apparatus—This is depicted in Fig. 2. It consists of a 150-ml round-bottom flask with a B19 ground-glass socket. The B19 cone is attached to a straight-tube condenser ending in a B14 ground-glass cone. The B14 socket is attached to a tube bent twice at right angles with the downward portion ending in a No. 2 sintered-glass filter-plate, which is placed at an angle so that bubbles do not collect beneath the plate but rise through the nitration mixture. In use, a test tube containing the nitration acid is held in position with the end of the tube about 1 cm from the bottom of the sinter.

REAGENTS—

Activated charcoal—Quality 207 Type B ref. DES/13 Mark 30/40 as supplied by Messrs. Sutcliffe, Speakman of Leigh, Lancashire. The charcoal is freed from impurities first by boiling with concentrated hydrochloric acid to remove sulphides and carbonates, washing with water and drying, and then by heating for 1 hour at 900° C in closed crucibles to remove organic impurities.

Nitration acid—Made from analytical-reagent grade nitric and sulphuric acids mixed in the proportions of 1 to 20 by volume.

Ether—Freed from peroxides and aldehydes by shaking with water followed by saturated ferrous sulphate - 10 per cent. sulphuric acid until a sample of the ether gives no reaction with potassium iodide solution.

Butanone—Commercial samples of this material are liable to be very impure. The substance should be fractionated with a good column and then only the first four-fifths of the fraction boiling between 79° and 81° C should be used. Different commercial samples have been found to give different colorimetric figures, but a satisfactory sample should give a reading for optical density of not less than 0·24 when treated with 20 μg of *m*-dinitrobenzene and potassium hydroxide under the conditions laid down in the procedure. It is emphasised that considerable variations may be found and that a frequent check should be made on the sample selected for use.

In addition the following reagents are required—

Sodium chloride, saturated solution—Prepared from analytical-reagent grade solid.
Sodium hydroxide, 2 per cent. solution—Prepared from analytical-reagent grade solid.
Potassium hydroxide, 40 per cent. solution.
Phosphoric acid—For sealing joints.
Malonic acid.
Zinc dust—Finer than 100 mesh.
Acetic acid, glacial B.P.
Liquid paraffin, B.P.
Ethanol, 95 per cent.

PROCEDURE—

Fill the apparatus (Fig. 1) with water. Push a cotton-wool plug almost to the bottom of the upper tube of the apparatus, taking care not to trap air in it. Add 1 g of the charcoal in portions, allowing enough time between additions for the displaced air to escape. Some dust remains on the surface of the liquid. Tip off about one-half of the water in the wide part of the tube. Push a small plug of cotton-wool down on top of the charcoal in the narrow part of the tube. Some dust will still be sticking to the walls of the tube. Tip out most

of the remaining water and wipe the walls clean with a piece of wet cotton-wool. Most of the water retained after the adsorption is held by the cotton-wool and not by the charcoal. For this reason it is desirable to weigh the cotton-wool used in making the column. A suitable amount is 0·25 g, which, with the charcoal, holds about 2 g of water. The sample is now added. When all the solution has passed the charcoal, allow the latter to drain completely, then remove as much of the remaining water as possible by suction.

Transfer the charcoal together with the cotton-wool plugs to the reaction flask (Fig. 2), and add 10 ml of acetic acid - acetic anhydride mixture (1 + 1). This is sufficient to reduce the amount of water to a satisfactory level. Add 1 g of zinc dust and 2 g of malonic acid and connect to the condenser and distillation tube. Place 5 ml of nitration acid in a test tube about 2 mm larger in diameter than the exit tube of the apparatus, and place it so that the sintered-glass disc is about 1 cm from the bottom of the test tube.

Place the apparatus on a heating bath or in a mantle and apply gentle heat. Turn on the condenser water. Keep the flask heating until the acetic acid is refluxing gently and allow it to continue for 1 hour. The air initially in the flask and the carbon dioxide generated by the malonic acid ensure a sufficient gas stream to sweep out all the benzene. At the end of the run lower the test tube and allow the sintered-glass disc to drain. Turn off the heat, remove the tube with the sintered-glass disc, and wash it inside and out with 50 ml of water, placing the washings in a separating funnel. Pour the acid from the test tube into the separating funnel and complete the washing with a further 50 ml of water. Cool the contents of the separating funnel. Add 50 ml of ether, shake well and separate the layers, transferring the aqueous layer to a second separating funnel. Add 25 ml of ether to the second separating funnel, shake and separate, rejecting the aqueous layer. Wash the ether in the first and second separating funnels successively with 25 ml of 2 per cent. sodium hydroxide solution and 25 ml of saturated salt solution. Filter the ether extract from the first separating funnel into a 250-ml Erlenmeyer flask (fitted with a ground-glass stopper) through a cotton-wool plug in a filter funnel. Rinse the first separating funnel with the ether from the second separating funnel and filter it into the flask through the cotton-wool. Wash with 25 ml of ether. Add 0·5 ml of liquid paraffin and remove the ether by distillation. Alternatively the use of cotton-wool may be omitted if care is taken to see that no water enters the flask with the ether extracts. In the case of some polynitro aromatic compounds we have found that certain samples of cotton-wool remove a high proportion of the solute from the ether solution, but we have not observed this effect with *m*-dinitrobenzene. To the ether-free residue add 10 ml of butanone and 1·0 ml of 40 per cent. potassium hydroxide solution and place the stoppered flask on the shaker. Shake gently for 45 minutes. Spin the solution in a centrifuge at 2000 r.p.m. and 14 cm radius for 1 minute. Transfer as much as is needed to an absorption cell, taking care to have a little of the potassium hydroxide solution in the bottom of the cell, and read the optical density at 565 mμ, or with a No. 6 filter if the Spekker absorptiometer is used.

PREPARATION OF STANDARD CURVE—

Prepare a standard curve by taking a series of known concentrations of pure gamma isomer of benzene hexachloride and putting them through the procedure, adding 1 g of the activated charcoal to the flask. Plot amounts of benzene hexachloride, in μg, against optical density. A straight line which cuts the optical density axis at a value equivalent to the blank is obtained. Prepare the standard solution in glacial acetic acid and make the dilute standard by taking an aliquot and diluting further with glacial acetic acid. Compare the unknown sample with the standard curve.

PROCEDURE FOR ALTERNATIVE COLORIMETRIC COMPARISON—

For a colorimetric comparison when an absorptiometer is not available, it is of course possible to prepare a series of standards by means of *m*-dinitrobenzene and butanone. As, however, the colours of these solutions reach a maximum in 45 to 60 minutes and then fade, it was thought that an alternative, more stable and easily reproducible system might be employed. Such a system is provided by a solution of alizarin in aqueous ethanol at pH 12·5 to 13·5. It happens that this solution shows an absorption maximum at 560 mμ as compared with the *m*-dinitrobenzene and butanone mixture, which gives its maximum at 565 mμ (see Fig. 4). The alizarin shows rather greater absorption in the blue region, but if the pH and concentrations recommended are used the slight difference of tint does not seriously

interfere with a reasonably accurate visual comparison. Curves are given (Fig. 5) for the absorption values of both *m*-dinitrobenzene with one sample of butanone, and alizarin, from which appropriate dilutions may be worked out for the visual comparison. Allowance must be made for the difference between the pure *m*-dinitrobenzene curve and the practical

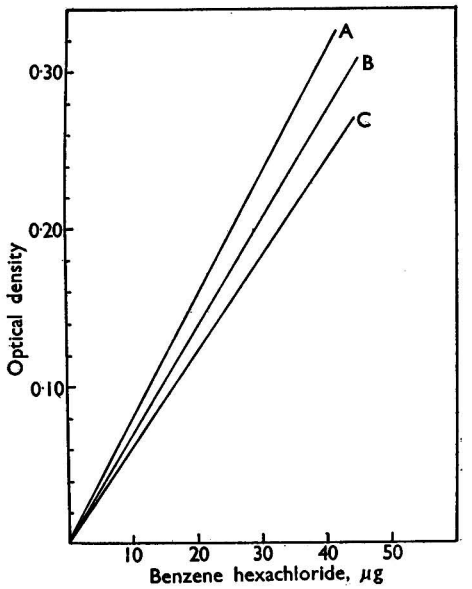

Fig. 3. Optical density at 565 mμ: curve A, benzene hexachloride calculated from the *m*-dinitrobenzene of Fig. 5 corrected for evaporation losses; curve B, standard curve for known amounts of gamma benzene hexachloride in acetic acid, and recovery curves from water and sewage effluent corrected for blanks; curve C, benzene hexachloride recovered from 2·5 litres of tap-water

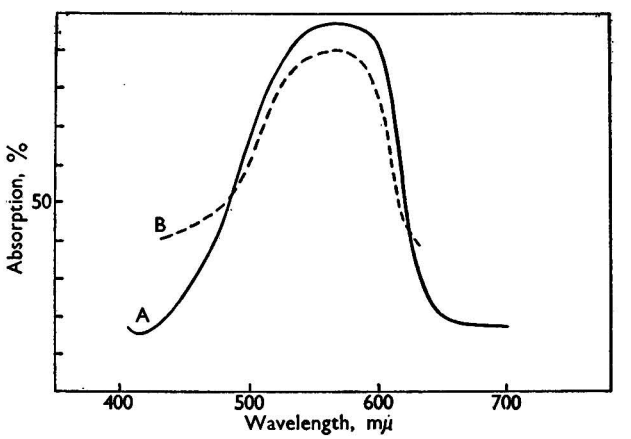

Fig. 4. Absorption curves: curve A, *m*-dinitrobenzene and butanone; curve B, alizarin at pH 13

recovery curve (curve B, Fig. 3) in choosing suitable dilutions if no absorptiometer or spectrophotometer is available. The composition of the mixture is x ml of standard alizarin solution + $(5 - x)$ ml of 95 per cent. ethanol + 0·5 ml of 2 per cent. w/v sodium hydroxide solution. The whole is then diluted to 10 ml with water. The standard alizarin solution is made up in 95 per cent. ethanol and a convenient strength is 50 μg per ml. The volume of 10 ml is chosen because this is the volume of the *m*-dinitrobenzene and butanone mixture

in the standard procedure. Any other suitable volume of the alizarin solution may be prepared and 10 ml used for the colour comparison.

Results

The collected results are given in Figs. 3, 4 and 5 and Tables I, II, III, IV and V. The optical densities were measured on the Unicam SP600 spectrophotometer at 565 mμ or on the Spekker absorptiometer, a No. 6 filter being used. The results obtained are comparable

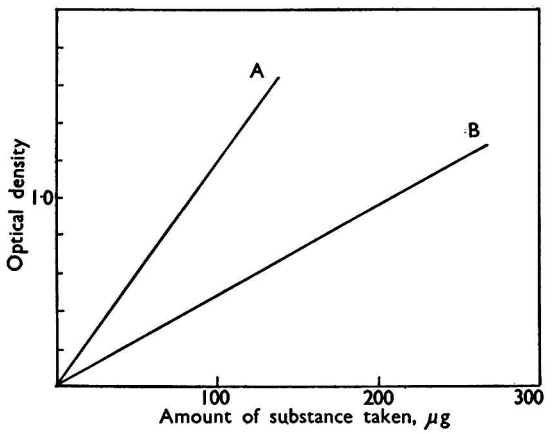

Fig. 5. Optical density - concentration curves: curve A, *m*-dinitrobenzene - butanone - 40 per cent. potassium hydroxide; curve B, alizarin - sodium hydroxide - ethanol - water at pH 13

but not identical. The Unicam instrument is able to deal with both lower and higher concentrations than the Spekker absorptiometer.

Table I

Aromatic impurities in various solvents

Solvent	Aromatic impurities expressed as benzene hexachloride, μg per 100 ml
n-Hexane	Shaken with nitration acid the smell of nitrobenzene was apparent
Light petroleum	
Ether	70
Ether, second sample	Nil
Chloroform	27
Carbon tetrachloride	180
Carbon tetrachloride, second sample	28

Table II

Comparison of methods and times of shaking of *m*-dinitrobenzene with butanone

m-Dinitrobenzene, μg	Optical density: By the Schechter and Hornstein procedure	After shaking for 1 hour	After shaking for 2 hours	After shaking for 6 hours
5	—	—	—	0·058
10	0·066	0·128	0·135	—
15	—	—	—	0·174
20	0·125	0·252	0·252	—
25	—	—	—	0·280
30	0·174	0·377	0·377	—
35	—	—	—	0·398
40	0·276	0·481	0·488	—

TABLE III

COMPARISON OF OPTICAL DENSITY WITH DIFFERENT BATCHES OF BUTANONE

m-Dinitrobenzene, μg	Sample 1 as received	Sample 2			Sample 3		Sample 4	
		As received	After fractionation	After 6 months	As received	After fractionation	As received	After fractionation
5	—	0·036	0·066	0·060	—	—	—	—
10	0·128	—	0·125	0·111	0·076	0·116	0·142	0·131
15	—	0·114	—	0·171	0·119	0·177	—	—
20	0·252	—	0·252	—	—	—	—	0·280
25	—	0·222	—	0·297	0·194	0·305	—	—
30	0·377	—	—	—	—	—	0·434	—
35	—	0·292	—	—	—	—	—	0·495
40	0·481	—	—	—	—	—	—	—
45	—	—	—	—	—	—	—	—
50	—	—	—	0·560	0·310	0·602	0·709	0·700

TABLE IV

RESULTS FOR DIFFERENT ISOMERS OF BENZENE HEXACHLORIDE

Isomer	Amount taken, μg	Equivalent, μg of gamma isomer	Recovery, gamma isomer = 100, %
Alpha	50·0	38·0	76
Beta	50·0	40·2	80
Delta	50·0	37·0	74
P520, a commercial mixture of all isomers	100 = 50 of B.H.C. (6·5 per cent. of alpha isomer)	50·0	100

TABLE V

APPARENT BENZENE HEXACHLORIDE IN SEWAGE EFFLUENTS AND WATER

Description	Apparent benzene hexachloride, p.p.m.
General domestic sewage effluent	0·001
Domestic sewage + gasworks liquor	0·007
Mainly domestic sewage + industrial waste	0·004
Tap-water	0·0004

DISCUSSION OF RESULTS

The results may be regarded as falling under two headings: the general procedure for the estimation of benzene hexachloride and its extraction from water. An attempt has been made to place the determination on a quantitative basis. Whilst this appears to be impossible in the stoicheiometric sense, it is now apparent where the losses occur. There is a loss of 10 per cent. owing to the nitration reaction.[2,3,4,5] A further loss of about 5 per cent. is attributable to the volatility of *m*-dinitrobenzene during the removal of the ether. The mineral oil used governs the extent of this loss and a sample which minimises this factor should be selected. The removal of benzene hexachloride from water is virtually complete up to a total of 5 mg of the substance for 1 g of charcoal. When several litres of water are being extracted, there is a loss of a few per cent. of benzene hexachloride. This can be allowed for by preparing a calibration graph from a similar volume of water. It must be emphasised that there appears to be no satisfactory alternative method for the extraction of this very volatile substance from water. In any event the recovery of 5 μg from 2·5 litres of water is 90 per cent. of the recovery of the same quantity from 100 ml of water. The total blank on all reagents amounts to the equivalent of 3 μg of benzene hexachloride. We consider that 5 μg is the smallest amount that can conveniently be estimated, although 2·5 μg gives a definite reading. The results of a number of determinations give a coefficient of variation of about 10 per cent. between the values of 20 and 50 μg. An advantage of the charcoal-adsorption method is that the sensitivity of the determination can easily be

controlled by regulation of the volume of sample taken for test. By arranging for an automatic feed of the solution, 5 litres may be passed through 1 g of charcoal and if this volume contains 5 μg of benzene hexachloride then the concentration in the original solution is one part in a thousand million and the method will give a value of 4 to 5 μg in 5 litres. For higher amounts the results will be nearer to the true value. The alpha, beta and delta isomers do not appear to differ as widely from the gamma isomer in our apparatus as in the Schechter and Hornstein method. The results are given in Table IV and they are all of the order of 75 to 80 per cent. of the gamma isomer. When they are together in a commercial mixture, as in the case of the powder P520 quoted in Table IV, there is no appreciable difference and the results obtained by the absorptiometric procedure agree with the macro-scale determination of hydrochloric acid formed by hydrolysis, *i.e.*, the method estimates the total benzene hexachloride. In the case quoted the determination of labile chlorine gave 49·5 per cent. total benzene hexachloride in the powder P520 and the absorptiometric result was 50 per cent. The extraction of benzene hexachloride from sewage effluents presents no difficulties except that the blanks are higher than for tap-water. The figures obtained are given in Table V. They all fall below the amount of benzene hexachloride likely to be sought if a question of toxicity is involved. It is most probable that the blanks arise from benzene itself or from some compound other than the hexachloride, particularly as Table V shows that the blank is greatest in the effluent known to contain a high proportion of gasworks liquor (No. 2).

The effluents examined all showed the presence of detergents by excessive frothing of the tanks at the time when samples were taken. A few typical detergent materials and a mixture of the common types of disinfectants were examined by this method. The values obtained are given in Table VI. When it is considered that the concentration of these impurities in the final effluent is a matter of a few parts per million, it is to be expected that the blanks from these sources are likely to be small. This was found to be so in the effluents examined (see Table V).

TABLE VI

APPARENT BENZENE HEXACHLORIDE IN DETERGENTS AND DISINFECTANTS

Description	Apparent benzene hexachloride, p.p.m.
Teepol	200
Alkyl benzenesulphonate	100
Cetrimide	100
Mixed disinfectants	1600

Although this work appears to be of limited application at the present time, it is thought that it may be useful in the future not only in connection with the direct determination of benzene hexachloride in water but possibly in connection with the examination of soils upon which Gammexane sprays have been used.

The authors wish to thank the Government Chemist, Dr. G. M. Bennett, C.B., F.R.S., for permission to publish this paper.

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DEPARTMENT OF THE GOVERNMENT CHEMIST
GOVERNMENT LABORATORY
CLEMENT'S INN PASSAGE
STRAND, LONDON, W.C.2

September 29th, 1954

DISCUSSION

DR. J. HASLAM asked if the authors had a special procedure for colour development to ensure that a stable product resulted in the reaction between the *m*-dinitrobenzene, ethyl methyl ketone and alkali.

MR. HANCOCK replied that the procedure as laid down in the method gave a colour that was constant for a given amount of *m*-dinitrobenzene in a particular sample of ethyl methyl ketone. The colour was

stable for at least half an hour after attaining maximum intensity, which was reached about 20 minutes after the commencement of shaking.

DR. R. F. MILTON enquired if attention has been paid to the conditions of nitration: in his opinion, if this were not rigidly controlled, various proportions of isomeric dinitro derivatives could result, with consequent effect upon the subsequent colour. He also asked if extraction of the dinitro derivative had been attempted by means of butanone. He also wanted to know if the interfering effect of toluene and xylene had been investigated.

MR. HANCOCK agreed that the conditions of nitration were important. That was one of the reasons for the use of the particular nitration acid specified in the method, in preference to Schechter and Hornstein's acid. In Schechter and Hornstein's apparatus the acid became warm, and this was one of the reasons that had led the present authors to re-design the apparatus. Under the conditions of their method, the yield of *m*-dinitrobenzene was found to be constant. They had never attempted to extract the *m*-dinitrobenzene with any solvent other than ether.

MR. LAWS added that the method was designed for benzene hexachloride, but that if benzene, toluene or xylene were present, they would interfere. If the presence of these hydrocarbons was suspected, the determinations could be made without adding zinc to the flask. The colour produced would give an apparent benzene hexachloride figure that could be subtracted from the result obtained when zinc was present. Alternatively, if a relatively large amount of aromatic compounds was present, it would be necessary to carry out the procedure without the zinc and nitration acid. After the specified time, the flask could be cooled, zinc and a further 2 g of malonic acid added, the nitration acid put into the test tube and the determination carried out as usual.

MR. K. F. SPOREK asked why ethyl methyl ketone was used in preference to acetone, and wanted to know if the authors had experienced high blanks similar to those given by soil extracts.

MR. HANCOCK replied that the reason for the use of ethyl methyl ketone, in preference to acetone, was that under these conditions the latter produced a turbid solution, which gave an incorrect optical density figure. The blanks from water and sewage effluent were very small.

DR. J. H. HAMENCE asked whether the reaction was specific for benzene hexachloride. He had found that, in the examination of an effluent, it was often not sufficient to establish the presence of a chlorinated hydrocarbon and that it was in fact necessary to establish its true identity. It occurred to him that a number of chlorinated hydrocarbons, such as DDT and Aldrin, might show similar behaviour in the prescribed test. He asked the authors if this had been found to be so.

Secondly, Dr. Hamence expressed considerable surprise at the very high blanks that the authors had found in a number of different solvents. A modified form of this procedure had been described in American literature and, to the best of his knowledge, no reference had been made to high blanks. He himself was concerned about the high blank found in ether, for which there was no apparent simple explanation.

MR. HANCOCK replied that the method as a whole was specific for benzene hexachloride, with the exception of the hydrocarbons mentioned in Mr. Laws' reply to Dr. Milton. None of the common chlorinated insecticides interfered.

MR. LAWS added that, although the blanks appeared high in comparison with the quantities of benzene hexachloride being determined, they only amounted in fact to a few parts per million and would be unnoticed in ordinary work. The probable explanation of the ether blanks was that some of the commercial ethanol was nowadays dehydrated by azeotropic distillation with benzene, and naturally small quantities were carried over into the ethanol and into ether made from it. These impurities in the ether were fortunately not aromatic nitro compounds, so that the ether could safely be used in the later stage of the method for extracting the *m*-dinitrobenzene.

High-frequency Titrimetry: The Titration of Organic Bases, Phenols and Enols

By E. S. LANE

The use of high-frequency titrimetry in non-aqueous solvents has been extended, and a simple high-frequency titrimetric procedure is described for the determination of equivalent weights of selected classes of organic compounds, including bases, quaternary ammonium salts, phenols and organophosphorus compounds.

The application of titrimetric methods in non-aqueous media to the determination of organic compounds has received considerable attention in the past few years.[1] Potentiometric and visual end-point methods are in common use[2] and a photometric method has been described recently.[3] Wagner and Kauffmann[4] have shown that high-frequency titrations of organic bases in glacial acetic acid with perchloric acid agree well with potentiometric and visual end-point methods. A simple, stable, high-frequency titrimeter designed primarily for the determination of fluoride ion in aqueous systems has recently been described by Dowdall, Sinkinson and Stretch,[5] and it has been possible through the co-operation of these authors to examine the performance of this instrument in non-aqueous systems. This paper extends the pioneer work of Jensen and Parrack,[6] who investigated several organic systems with an instrument of this type for the determination of the equivalent weights of some selected classes of organic compounds, notably bases, quaternary ammonium salts, phenols and organo-phosphorus compounds. In organic solvents, such as glacial acetic acid or ethylenediamine, compounds that are substantially neutral in aqueous solution can be titrated as bases or acids and can, therefore, be regarded as having an equivalent weight.

Method

Apparatus—

Initially, the apparatus used was identical with that used by Dowdall, Sinkinson and Stretch. A modification introduced purely for convenience and economy in the use of the organic solvents was the use of a smaller-capacity titration vessel. Most of the results shown in the Tables were obtained with a 4-inch by 1-inch tube that reduced the volume of solvent from approximately 50 ml to 35 ml. During the course of the work, the backing-out potentiometer was replaced by a helically wound potentiometer of the same value, to give better precision when re-setting the galvanometer needle. With a slight variation in operating procedure, the instrument could now be used as a null-point instrument. Instead of plotting the galvanometer deflection against the incremental addition of titrant, the galvanometer needle could be re-set to zero after each addition and the reading on the greatly extended scale of the helically wound potentiometer (0 to 999) recorded rather than the initial galvanometer deflection.

Reagents—

Perchloric acid, 0·1 N—Cautiously add 8·5 ml of 75 per cent. perchloric acid to 300 ml of glacial acetic acid and dilute the mixture to 1 litre with glacial acetic acid.

Sodium methoxide, 0·1 N—Dissolve 2·5 g of sodium metal, cleaned by immersion in methanol, in 100 ml of absolute methanol. When the reaction is complete, add 200 ml of methanol and dilute the mixture to 1 litre with benzene. Store in such a way that water and carbon dioxide are excluded.

Glacial acetic acid—AnalaR.

Ethylenediamine—The laboratory-reagent grade supplied by The British Drug Houses Ltd. is suitable. Distillation over solid sodium hydroxide before use is recommended to reduce the acid-impurity content. The ethylenediamine may be recovered after use by a preliminary distillation to remove methanol and benzene, followed by treatment of the distillate boiling above 115° C by the method of Putnam and Kobe.[7]

Mercuric acetate reagent (for use with quaternary ammonium halides)—Dissolve 6 g of mercuric acetate in 100 ml of hot glacial acetic acid and cool to room temperature.

Thymol blue indicator—Dissolve 0·3 g of solid indicator in 100 ml of methanol.

o-*Nitroaniline indicator*—Dissolve 0·15 g of *o*-nitroaniline in 100 ml of benzene.

1-*Naphtholbenzein indicator*—Dissolve 0·3 g of solid indicator in 100 ml of glacial acetic acid.

PROCEDURE FOR STANDARDISATION OF REAGENT—

The perchloric acid reagent should be periodically standardised against potassium hydrogen phthalate or diphenylguanidine in glacial acetic acid, 1-naphtholbenzein being used as visual indicator.[2] The sodium methoxide should be periodically standardised against benzoic acid in methanol, thymol blue being used as visual indicator.[2]

METHOD OF USE—

The general method of use was identical with that already described.[5] The method detailed below was used when the function of the instrument was to determine the equivalent weight of an organic compound in solvent medium.

TITRATIONS IN GLACIAL ACETIC ACID—

Weight out the organic compound and dissolve it in the titration vessel by adding 30 ml of glacial acetic acid. The size of the solution should normally be such as to require

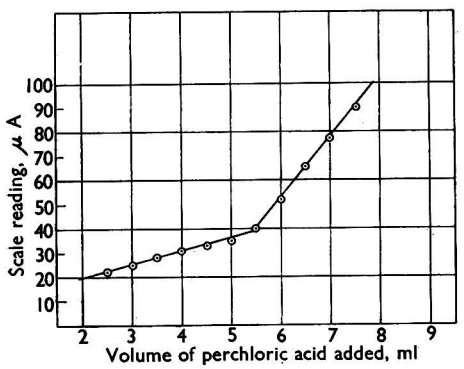


Fig. 1

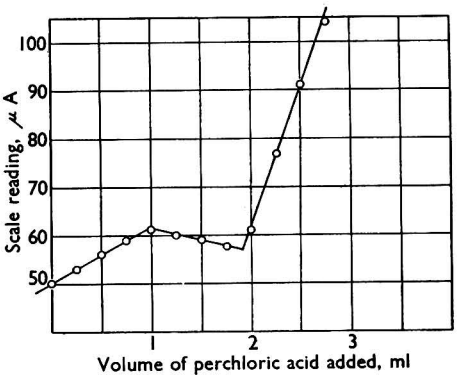
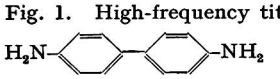


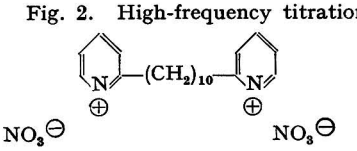
Fig. 2

Fig. 1. High-frequency titration of benzidine



Theoretical equivalent weight ..	184
Found	184

Fig. 2. High-frequency titration of decamethylenebispyridinium nitrate



Theoretical equivalent weight	424 ; 212
Found	426 ; 213

5 to 10 ml of reagent solution for neutralisation. With some compounds, notably quaternary ammonium salts, it may be necessary to dissolve the finely powdered solid in about 1 ml of water before adding the glacial acetic acid. If heat is used to assist the dissolution of the compound, the solution should be allowed to cool before insertion in the titrimeter. When quaternary ammonium halides are encountered, they should be dissolved as above in 20 ml of glacial acetic acid and then 10 ml of mercuric acetate reagent added to ensure complete conversion of the ammonium-type halide to ammonium-type acetate in accordance with the method of Pifer and Wollish.[8] The instrument is set exactly as for use in aqueous systems and the perchloric acid reagent added in suitable increments, and the potentiometer-scale reading (or, with the unmodified instrument, the galvanometer deflection) is plotted against volume of reagent added. The end-point is read as the point of intersection of the plots. Representative plots are shown in Figs. 1, 2, 3 and 4 and in the paper by Dowdall, Sinkinson and Stretch.[5]

With some compounds as the titration proceeded the base perchlorate separated out. Whereas in some titrations this had practically no effect on the stability of the system, in

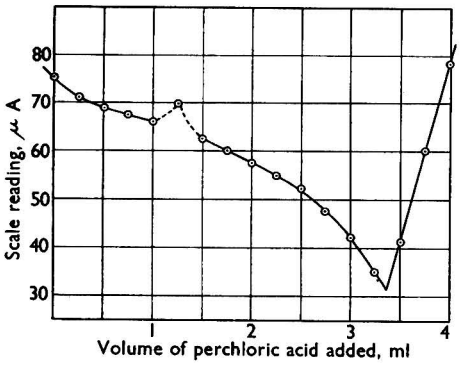


Fig. 3

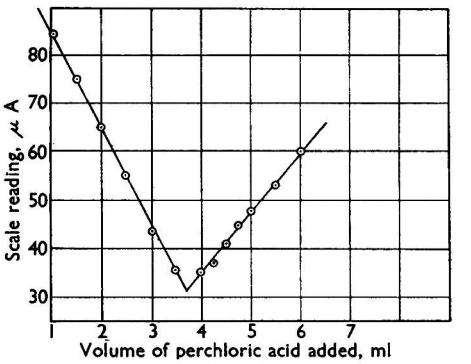
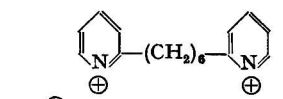


Fig. 4

Fig. 3. High-frequency titration of hexamethylenebispyridinium nitrate



$NO_3^{\ominus}$ $NO_3^{\ominus}$

Theoretical equivalent weight ..	183
Found	182

Fig. 4. High-frequency titration of dianisylmethylphosphine

$(C_6H_4 \cdot OCH_3)_2P \cdot CH_3$

Theoretical equivalent weight ..	260
Found	262

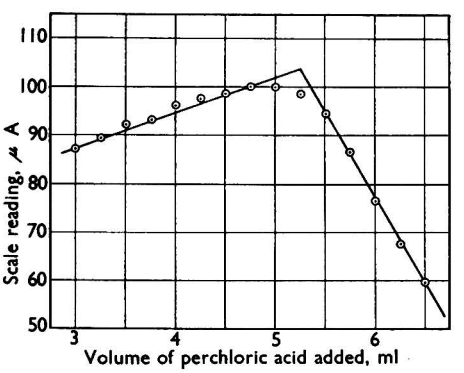


Fig. 5

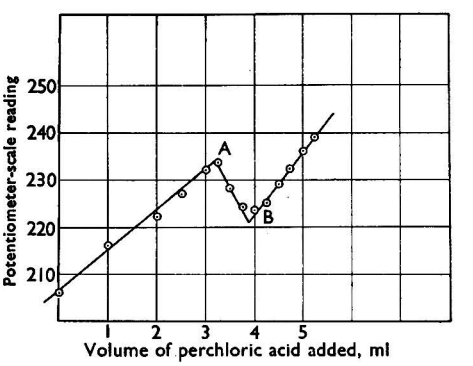
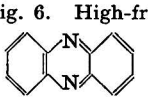


Fig. 6

Fig. 5. High-frequency titration of pyridine

Theoretical equivalent weight ..	79·1
Found	79·5

Fig. 6. High-frequency titration of phenazine



Theoretical equivalent weight ..	180
Found	180

others, notably those of pyridine and phenazine (Figs. 5 and 6), a slow continuous drift of the galvanometer needle was observed. In order to obtain a satisfactory plot it was necessary to work to a time-schedule, and to read the galvanometer 20 to 25 seconds after addition of each aliquot and to add the next aliquot during the next 15 or 20 seconds and so on.

TITRATIONS IN ETHYLENEDIAMINE—

Since ethylenediamine frequently contains acidic impurities (mainly carbon dioxide absorbed from the air), a correction has to be applied to compensate for the extra quantity

of sodium methoxide required to neutralise the solvent used to dissolve the organic compound. This is carried out as follows—

(*a*) Addition of sodium methoxide in benzene - methanol to the solvent before the organic compound is dissolved in it, azo-violet or *o*-nitroaniline being used as visual indicator. For this purpose it is convenient to install a small light source inside the box, enabling the colour of the solvent in the titration vessel to be viewed by transmitted light. This allows a very small amount of indicator to be used and adds to the sensitivity of the end-point determination. After neutralisation, a weighed amount of organic compound is dissolved in the solvent and the mixture is then titrated as before with sodium methoxide reagent.

(*b*) The acid content of a known volume of solvent is neutralised with sodium methoxide reagent, the titrimeter being used for the determination of the end-point. When the acid content per unit volume of the solvent is known, the value of the blank required in subsequent titrations can be deducted from the actual values read from the plots when unneutralised ethylenediamine is used. The latter method is preferred; it uses the same procedure for the determination of the two end-points and eliminates any error due to non-coincidence of the visual and high-frequency end-points. With the same sample of solvent (kept in a large-capacity burette, with carbon dioxide guard tube), the value for the blank remains almost constant during the course of several titrations. The effect of the absorption of carbon dioxide from the air during the actual titration is minimised by covering the top of the titration vessel with a cover provided with holes for the entry of the stirrer and burette tip.

Results

Some results obtained by these general procedures on a selection of organic compounds of widely differing nature are listed in Tables I and II. The accuracy attainable falls mainly within the $\pm$ 2 per cent. range, but with many compounds is considerably better.

Table I

HIGH-FREQUENCY TITRATION OF ORGANIC BASES, QUATERNARY AMMONIUM SALTS AND RELATED COMPOUNDS

Compound	Equivalent weight: Found	Equivalent weight: Theoretical
8-Hydroxyquinoline	146	145
o-Phenanthroline	194	198
Benzidine	97	97
Quinoxaline	130	130
Phenazine	180	180
p-Nitroaniline	139	138
Pyridine	79	79
Hexamethylenetetramine	126 129	140
2-(2-Hydroxyphenyl)benziminazole	209	210
Dianisyldimethylphosphonium iodide	402	402
Dianisylethylmethylphosphonium iodide	418	416
Dianisylmethylphosphine	262	260
Tetraethylammonium bromide	213	210
Tetraethylammonium iodide	254	257
Tetraethylammonium nitrate	191	192
Tetramethylammonium iodide	201	201
Ethyltrimethylammonium iodide	214	215
Phenyltrimethylammonium iodide	262	263
Decamethylenebispyridinium nitrate	416; 213	424; 212
Hexamethylenebispyridinium nitrate	182	183
Tri-(*o*-hydroxyphenyl)sulphonium chloride	344	346

Discussion of results

The results obtained indicate the potentialities of the method, and it is considered that it may prove useful for the quantitative characterisation of a wide range of organic compounds. The method, whilst convenient (the complete determination requires 15 to 20 minutes), is less simple than a visual end-point titration, but the end-point obtained is much more

definite and is devoid of personal error. It is of particular value in those systems for which no visual indicator is practicable and it has been used with satisfactory results on classes of compounds giving highly coloured solutions, which preclude the use of conventional visual indicators. Whilst potentiometric titrations would probably have been suitable in many cases for their determination, the high-frequency titrimetric procedure is regarded as being simple and more convenient and is free from the complications associated with potentiometric measurements in organic solvents, namely poisoning of the electrodes and liquid-junction irregularities.

In glacial acetic acid systems this instrument may possess some advantages over that described by Wagner and Kauffmann. These workers reported difficulty in the titration

TABLE II

HIGH-FREQUENCY TITRATION OF PHENOLS AND RELATED COMPOUNDS

Compound*	Equivalent weight	
	Found	Theoretical
Dimedone	141	140
p-Nitrophenol	139	139
8-Hydroxyquinoline	144	145
2-(2-Hydroxyphenyl)benzoxazole	210	211
4-Hydroxy-2:1:3-benzselenadiazole	204	209
2:3-Dihydroxy-5-methoxyquinoxaline	192	192
5-Hydroxy-2:3-diphenylquinoxaline	302	298
5-Hydroxy-2:3-di-(2-pyridyl)quinoxaline	300	300
5:8-Dihydroxy-2:3-tetramethylenequinoxaline	107·5	108
5:8-Dihydroxy-2-phenylquinoxaline	118	119
5:8-Dihydroxy-2-*iso*propylquinoxaline	104	102
5:8-Dihydroxy-2:3-pentamethylenequinoxaline	248	244
Tri-(*o*-hydroxyphenyl)phosphine oxide	211; 109	213; 107

* The reference compounds used were in many cases research samples of high but unknown purity whose composition has been checked by micro-analysis. Details of the preparation and properties of new compounds will be given elsewhere.

of pyridine and were unable to obtain satisfactory end-points with *p*-nitroaniline, which was considered to be the lowest limit of bases that could be determined by the high-frequency method. With the instrument now described, pyridine was determined with no more inconvenience than the adoption of a timing technique as described above, when precipitation of pyridine perchlorate caused a continuous drift of the galvanometer needle in the vicinity of the end-point. A sharp end-point was obtained with *p*-nitroaniline also. The lower limit with this instrument is probably that of phenazine, which was satisfactorily titrated (Fig. 6) by a timing technique. Here, precipitation of the perchlorate began at A and the true end-point was obtained at B.

The determination of quaternary ammonium salts by a modification of Pifer and Wollish's method has proved particularly successful by the high-frequency method. Perchloric acid is normally used in this method to titrate the quaternary ammonium salt in the presence of mercuric acetate, the end-point being determined potentiometrically or visually with crystal violet. Several quaternary ammonium salts gave satisfactory end-points with glacial acetic acid by this method, and the use of dioxan to obtained sharp end-points, as recommended by Pifer and Wollish, was found to be unnecessary. Pifer and Wollish suggested that phosphonium and sulphonium compounds should also be titratable by this method, and this has been confirmed with the high-frequency technique.

Normally a difunctional compound, *e.g.*, benzidine, is titrated to give only one end-point in glacial acetic acid, but an interesting example was observed when decamethylenebispyridinium nitrate in low concentration was titrated with perchloric acid in glacial acetic acid (see Fig. 2). Two inflexion points were obtained, which corresponded to the separate titration of the quaternary groups. At high concentrations of quaternary ammonium salt the first inflexion point is obscured by a tendency of the system to become unstable in this region, which improves as the titration procedes towards the second end-point. It has been observed in several cases that polyfunctionality is often characterised by some degree of instability in the system until the final or penultimate function is reached, when a sharp end-point is obtained.

The limitations of this method with glacial acetic acid as solvent are those appertaining

to the normal visual or potentiometric titrations in acetic acid. Urea and benzotriazole give straight-line plots on titration in this instrument; both fail to give visual or potentiometric end-points under the same conditions. Hexamethylenetetramine gives a spurious end-point with the titrimeter in the acetic acid system, and this is also met with when crystal violet is used as visual indicator in the same system. Here the acetic acid is believed to enhance the basic strength of a second nitrogen atom in the hexamethylenetetramine molecule to the point where it interferes with the titration of the first.

Summarising, the behaviour in acetic acid this particular high-frequency titrimeter provides a method of obtaining more definite end-points than can be obtained with crystal violet as visual indicator. For these systems where a specific narrow range indicator exists, *e.g.*, 1-naphtholbenzein, the visual indicator method is more convenient.

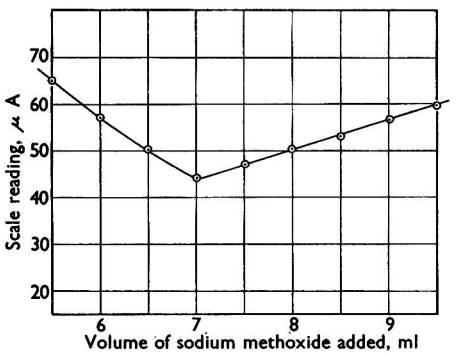


Fig. 7

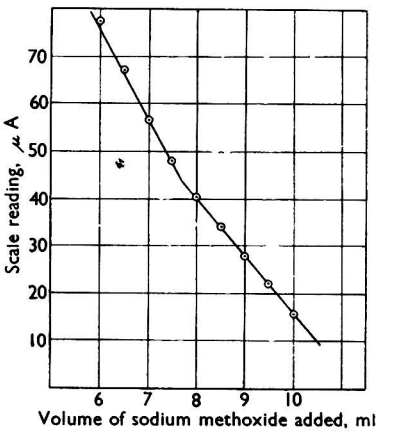
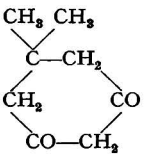


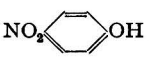
Fig. 8

Fig. 7. High-frequency titration of dimedone



Theoretical equivalent weight ..	140
Found	140

Fig. 8. High-frequency titration of *p*-nitrophenol



Theoretical equivalent weight ..	139
Found	139

Some typical titration curves with sodium methoxide in benzene - methanol and ethylenediamine for the determination of phenolic (and in one case an enolic) compound are shown in Figs. 7, 8, 9 and 10. Curvature of the plots, a common feature in high-frequency titrimetry, tends to be more prevalent in this system, possibly owing to its complexity. Sharp end-points are obtained with a variety of phenols and the method is particularly recommended for the determination of those phenols that give highly coloured solutions in ethylenediamine and preclude the use of conventional visual indicators.

Distillation of the ethylenediamine before use was found to be advantageous in reducing the acidic-impurity content. If this is omitted, the "dilution" of the ethylenediamine with benzene and methanol that necessarily occurs on neutralisation with sodium methoxide may be sufficient to cause a spurious end-point. For the same reason the size of the sample taken should be limited to an amount requiring less than about 10 ml of reagent for the complete titration.

Precipitation of the sodium salt of the phenol occurred during some titrations, but this had less effect on stability than precipitation of perchlorate in the acetic acid - perchloric acid system. An interesting example of the effect of functionality in the ethylenediamine system is shown in Fig. 1, where the second hydroxyl group in tri-*o*-hydroxyplenylphosphine oxide gave a much sharper end-point than the titration of the third hydroxyl group.

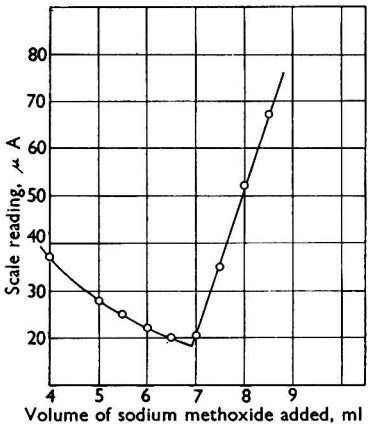


Fig. 9

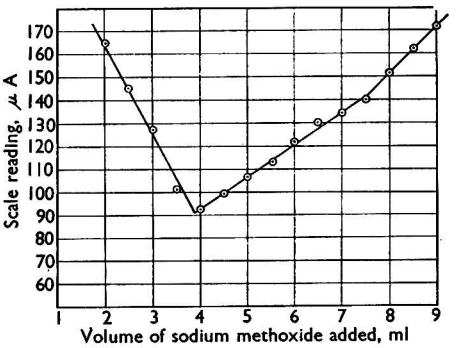
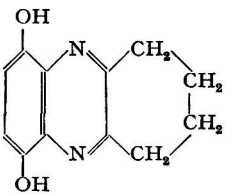


Fig. 10

Fig. 9. High-frequency titration of 5:8-dihydroxy-2:3-tetramethylenequinoxaline



Theoretical equivalent weight ..	108
Found	107·5

Fig. 10. High-frequency titration of tri-*o*-hydroxyphenylphosphine oxide

$(C_6H_4 \cdot OH)_3PO$	Theoretical equivalent weight	213; 107
	Found	211; 109

The author is indebted to the Chief Superintendent, C.D.E.E., Porton, for the loan of the original titrimeter, to Mr. H. Stretch, C.D.E.E., for many helpful discussions, to Mr. C. Lomas, A.E.R.E., for the construction of the modified instrument and to Mr. T. Collier for some experimental assistance.

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The Qualitative Analysis of Surface-active Agents

By V. W. REID, T. ALSTON and B. W. YOUNG

A simple qualitative scheme has been developed for the characterisation of surface-active agents. It is more positive than previous schemes. Present methods depend upon the application of a large number of chemical tests, and this work shows how the number of such tests may be greatly reduced by the ultra-violet spectroscopic examination of the products.

Reference compounds, similar in chemical structure to the various types of commercial product, have been examined in the ultra-violet region to provide data by means of which classification of surface-active agents may be made. About one-hundred commercially available proprietary products have been examined by the scheme, and the classification indicated agreed with data given in the literature for the products examined, when such data were available.

Surface-active agents have a multiplicity of uses and a very large and growing number of products of this type are on the market. Analytical schemes for the qualitative identification of these products have been devised by van der Hoeve,[1] Gilby and Hodgson[2] and many others. These schemes depend upon the application of chemical tests alone, a large number of tests of various reliabilities being required.

The present work shows how considerable simplification of the process of identification of these compounds can be effected if an ultra-violet spectrogram covering the 2100 to 3500 A wavelength region is prepared. The analytical scheme developed deals only with the identification of the separated active ingredient.

Experimental

The excellent paper of van der Hoeve,[1] which describes more than thirty chemical tests, has been examined in detail and found to be generally reliable. Those tests applied to ascertain the nature of the hydrocarbon residue, however, are rather difficult to apply and the test indications are not always clear. The Guerbet test for the identification of the presence of an aromatic nucleus involves nitration, reduction of the nitro compound and subsequent diazotisation and development with 2-naphthol. This test is time-consuming and does not distinguish between benzene, naphthalene, pyridine or *iso*quinoline nuclei. The diazo test for identification of the naphthalene nucleus has not given consistently clear indications over the range of samples tested.

The analytical scheme developed in this work involves the initial preparation of an ultra-violet spectrogram, followed by the application of tests to show the ionic character of the product and the presence or absence of nitrogen. No chemical tests need be applied to ascertain the nature of the hydrocarbon residue, but examination for the presence of sulphur or phosphorus is sometimes necessary. The detailed procedures of van der Hoeve have been used in this work for the few chemical tests applied.

Separation of active ingredient—

To prevent interference from fillers and to allow preparation of solutions of known concentration of active ingredient, it is necessary to separate the active ingredient from the remainder of the product. This can readily be achieved by the methods of Gilby and Hodgson,[2] who describe procedures for dealing with both solid and liquid products. The dried mixture is extracted with ethanol, and the inorganic material, being insoluble, is removed by filtration.

Spectroscopic examination—

The dry separated active ingredient is now brought into solution, a weighed amount being dissolved in water in a calibrated flask to a concentration of some 5 g per litre. During solution, should any turbidity develop, sufficient ethanol of spectroscopic purity is added to produce clarity. This solution is appropriately diluted with distilled water until the

concentration is suitable for preparation of the spectrogram. Any turbidity that appears on dilution is cleared by addition of a suitable volume of ethanol.

A Beckman DU spectrophotometer was used for the absorption measurements in this work, but any similar instrument would be suitable. Distilled water was used in the reference cell.

As the molecular weights of the compounds present are unknown, the absorption values are calculated as $E^{1\%}_{1\,cm}$. With regard to the plotting of such spectrograms, the value for

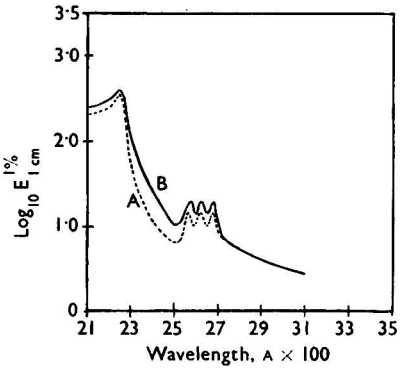

Fig. 1. Alkylbenzenesulphonates. Curve A (reference compound), sodium dodecylbenzenesulphonate; curve B, typical proprietory product

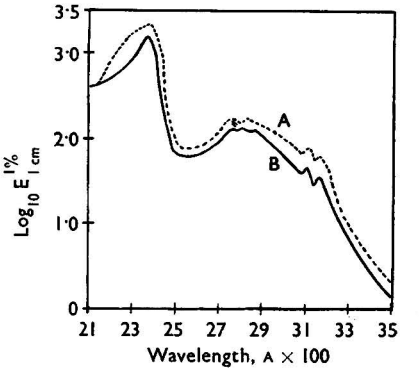

Fig. 2. Alkylnaphthalenesulphonates. Curve A (reference compound), sodium butylnaphthalenesulphonate; curve B, typical proprietory product

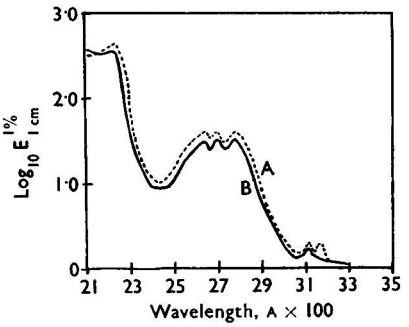

Fig. 3. Curve A (reference compound), sodium tetrahydronaphthalenesulphonate; curve B, typical proprietory product

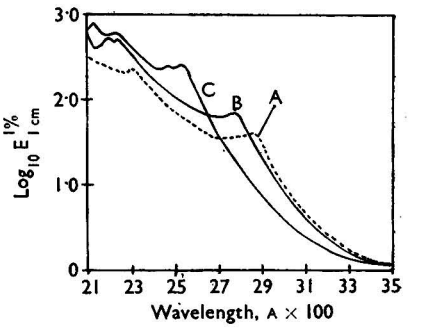

Fig. 4. Arylbenzenesulphonates. Curve A (reference compound), sodium butylphenylphenolsulphonate; curves B and C, typical proprietory products

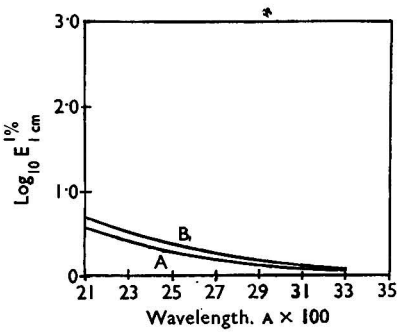

Fig. 5. Aliphatic sulphates and phosphates. Curve A, sodium lauryl sulphate; curve B, sodium di-2-ethylhexyl phosphate (both proprietory products)

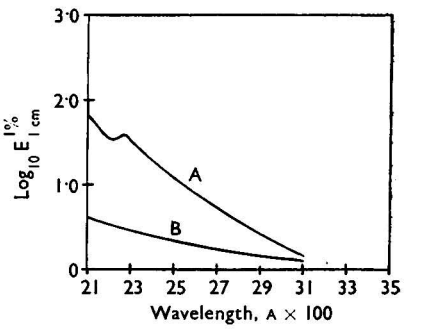

Fig. 6. Conventional soaps. Curve A, sodium oleate; curve B, sodium stearate (both proprietory products)

Figs. 1 to 6. Ultra-violet spectrograms for surface-active agents with anionic nitrogen absent (group I)

$E^{1\%}_{1\,cm}$ often reaches very high numerical values at one absorption maximum, with low values at some other wavelength. For this reason a plot of log $E^{1\%}_{1\,cm}$ against wavelength has been found to be the most satisfactory way of preparing a spectrogram.

ANALYTICAL SCHEME—

An ultra-violet spectrogram covering the 2100 to 3500 A region having been prepared, tests for ionic character and presence of nitrogen are carried out. As cationic compounds that do not contain nitrogen and non-ionic compounds containing nitrogen are manufactured in negligible amounts, four main classifications are thus obtained. Each of these four main groups is now further sub-divided according to the characteristics of the ultra-violet spectrogram.

Reference compounds of known chemical structure, similar in type to the products comprising each of the further sub-groups, have been examined in the ultra-violet region, and the spectrograms obtained are used as a basis for the classification of any given product. Such spectrograms are shown in Figs. 1 to 18, where comparison is made with spectrograms of proprietary commercial products. In no case did data given by the manufacturer conflict with indications given by the reference compounds regarding the constitution of any product. Each of the proprietary products examined was also analysed fully by means of the van der Hoeve scheme of analysis.

The absorption characteristics of each of the product classifications will now be described.

GROUP I: ANIONIC COMPOUNDS WITH NITROGEN ABSENT

This class of surface-active agent comprises the alkylarylsulphonates, alkyl sulphates, arylbenzenesulphonates and conventional-type soaps. The phosphonates are also included in this group, but these will not be dealt with separately as they contribute no individual characteristic to the ultra-violet spectrogram. A check for the presence of sulphur or phosphorous, however, will readily determine the nature of the acid radical. The following sub-divisions of this group are clearly distinguishable by examination of the spectrogram.

ALKYLBENZENESULPHONATES—

In Fig. 1 may be seen the spectrogram of sodium dodecylbenzenesulphonate, together with that of a proprietary product of this type. Twelve such products were examined, all of which showed similar absorption characteristics, with maxima of particularly high intensity in the 2200 A region together with high absorption in the 2500 to 2900 A wavelength region, in which fine structure was always apparent.

ALKYLNAPHTHALENESULPHONATES—

In Fig. 2 may be seen the spectrogram of sodium butylnaphthalenesulphonate together with that of a proprietary product of this type. The particularly high absorption maximum in the 2400 A region, together with the maxima that appear in the 3100 to 3200 A region, distinguish these products from alkylbenzenesulphonates and, indeed, from any other class of product in this group.

TETRALINSULPHONATES—

Tetrahydronaphthalene (tetralin) may be regarded as a substituted benzene, and it is not surprising, therefore, that the spectrograms of a tetralinsulphonate and a commercial product based on tetralin (shown in Fig. 3) should be similar to the spectrograms of the alkylbenzenesulphonates. The reduction of naphthalene to tetralin during manufacture does not appear to be complete, however, as the characteristic absorption maxima of naphthalene in the 3100 to 3200 A region are apparent in both these samples; the absorption intensity suggested a naphthalene nucleus present as impurity.

ARYLBENZENESULPHONATES—

When the substituent on the benzene nucleus consists of a modified phenyl group instead of the more usual alkyl group, spectrograms similar to those shown in Fig. 4 are obtained. Sodium butylphenylphenolsulphonate is shown as reference compound in this group. These spectrograms differ from those of the alkylbenzenesulphonates and ethylnaphthalenesulphonates by the absence of a distinct absorption minimum in the 2400 to 2600 A region.

ALKYL SULPHATES—

Commercial surface-active agents based upon alkyl sulphates, which may be derived from fatty alcohols, olefins and so on, are practically transparent in the ultra-violet region, with a complete absence of absorption maxima. The spectrograms of two such compounds are shown in Fig. 5, where they may be seen to be clearly distinguishable from any of the aromatic sulphonates.

CONVENTIONAL SOAPS—

The spectrogram obtained on a soap of the conventional type will depend on the nature of the fatty acid from which the soap is manufactured. Saturated fatty acids and their soaps are almost transparent in the ultra-violet region, with no absorption maxima, as can be seen from the spectrogram of sodium stearate shown in Fig. 6. They are distinguished from the alkyl sulphates and phosphates by the absence of either sulphur or phosphorus.

Soaps based on unsaturated fatty acids, however, show ultra-violet absorption due to such unsaturation. In Fig. 6 may be seen the spectrogram of sodium oleate, where the absorption maximum in the 2200 to 2300 A region is due to the unsaturation of this compound. This spectrogram is again distinctive.

GROUP II: NON-IONIC COMPOUNDS WITH NITROGEN ABSENT

The non-ionic surface-active agents are formed by the addition of ethylene oxide to phenols, mercaptans, fatty acids, alcohols and so on. Several molecules of ethylene oxide condense on the –OH or –SH group, the ensuing polyether group being transparent in the ultra-violet region. The spectrogram of the resulting products, therefore, will relate to the material upon which the ethylene oxide has been condensed. The various types of spectrogram shown by members of this group, when the following parent compounds are used as starting materials, will now be discussed. Some 7 to 10 molecules of ethylene oxide were condensed with the reference compounds shown.

PHENOLS AND CRESOLS—

Non-ionic compounds based on alkylphenols and alkylcresols show absorption maxima, owing to the benzenoid nucleus, in the 2500 to 2900 A wavelength region. The fine structure of several absorption maxima shown by the alkylbenzenesulphonates in this wavelength region is absent. This is probably due to the presence of a greater number of isomers, because of the increased number of substituents.

The alkylphenols do show a little fine structure in the benzenoid absorption region (see Fig. 7), which may serve to distinguish them from the alkylcresols (Fig. 8), where, with a greater number of substituents, no fine structure is apparent.

When a second benzene nucleus occurs in the molecule, as with the benzylcresol condensate shown in Fig. 9, definite fine structure occurs.

As the transparent polyether group extends, through the addition of further molecules of ethylene oxide, a dilution effect would be expected to occur with respect to intensity of ultra-violet absorption. This effect is clearly shown in Fig. 10, where the absorption maxima at 2800 A steadily fall with increased oxide addition. In certain cases, the extinctions of such condensates at the absorption maxima may be used as an approximate indication of the number of ethylene oxide molecules condensed.

NAPHTHOLS—

The spectrogram of a non-ionic surface-active agent based on 2-naphthol is shown in Fig. 11. The absorption envelope characteristic of the naphthalene nucleus is again apparent and is particularly distinctive.

RESIN ACIDS, FATTY ACIDS AND ALCOHOLS—

Spectrograms obtained on a series of non-ionic surface-active agents based on saturated and unsaturated fatty acids may be seen in Fig. 12. The unsaturated nature of the oleic acid, sperm acids and castor oil results in condensates prepared from these materials showing absorption maxima in the 2300 A region. The saturated fatty acid condensate based on stearic acid provides a spectrogram of low absorption with no maxima.

The fatty alcohol condensates show similar spectrograms to the analogous fatty acid condensates, but may be distinguished from these by the fact that they do not saponify.

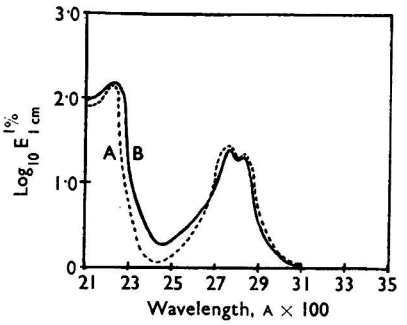

Fig. 7. Curve A (reference compound), nonylphenol condensate; curve B, typical proprietory product

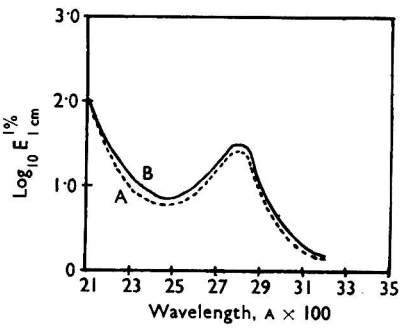

Fig. 8. Curve A (reference compound), octylcresol condensate; curve B, typical proprietory product

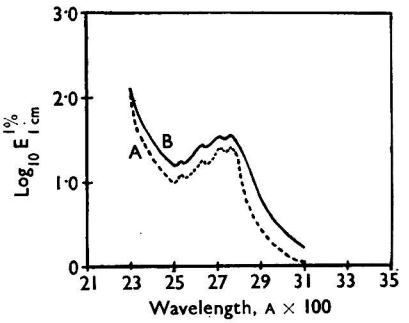

Fig. 9. Curve A (reference compound), benzylcresol condensate; curve B, typical proprietory product

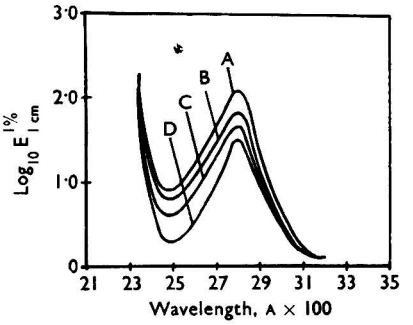

Fig. 10. Ethylene oxide addition. Curve A, octylcresol; curve B, octylcresol + 3 molecules of ethylene oxide; curve C, octylcresol + 5 molecules of ethylene oxide; curve D, octylcresol + 9·2 molecules of ethylene oxide (all proprietory products)

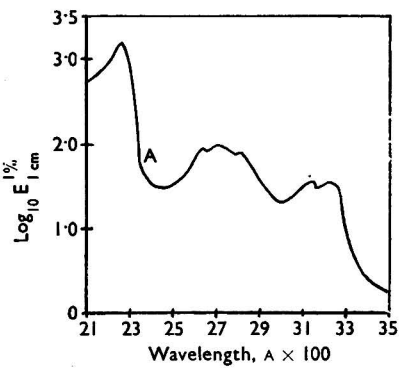

Fig. 11. Curve A, 2-naphthol condensate (proprietory product)

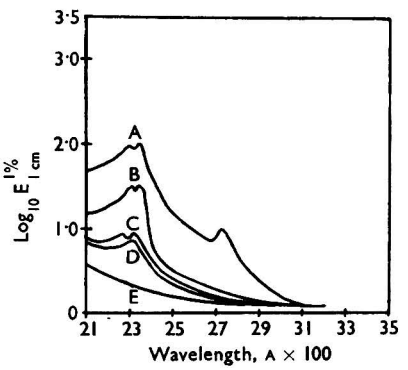

Fig. 12. Fatty acid and resin acid condensates. Curve A, tall oil; curve B, oleic acid; curve C, sperm acids; curve D, castor oil; curve E, stearic acid (all proprietory products)

Figs. 7 to 12. Ultra-violet spectrograms for surface-active agents with non-ionic nitrogen absent (group II)

The saponification value of a fatty acid condensate, indeed, may be used to determine the molecular weight of the condensate. Mercaptans behave in a similar manner to alcohols so far as ultra-violet absorption is concerned, saturated mercaptan condensates being transparent.

The mixed rosin acids present in tall oils contain unsaturated linkages that may be seen, from the spectrogram shown in Fig. 12, to produce greater absorption than that shown by the unsaturated fatty acids. The additional absorption maxima at 2700 A may also be used as a distinguishing feature.

Group III: anionic compounds containing nitrogen

This class of surface-active agent may be divided into two distinct types of product, those based on substituted aromatic bases and alkylolamine soaps.

Aniline derivatives—

In Fig. 13 is shown the spectrogram of sodium dibenzylsulphanilate, together with a commercial product of this class. These spectrograms are quite different from that of any alkylolamine derivative shown in Fig. 14. The presence of the benzene nuclei is shown by the absorption maximum in the 2500 to 2900 A region.

Alkylolamine soaps—

As with conventional sodium soaps, the nature of the fatty acid used in the manufacture of these products will determine the type of spectrogram obtained. In Fig. 14 is shown the spectrogram for triethanolamine oleate and stearate, the absorption maxima shown by the oleate making differentiation straightforward. Two commercial products of this class are also shown, one showing absorption maxima the other not.

Group IV: cationic compounds containing nitrogen

This group may be divided into products derived from quaternary ammonium compounds, quaternary bases and aliphatic amino compounds. The quaternary bases may be derived from pyridine or *iso*quinoline.

Quaternary ammonium compounds—

Quaternary ammonium compounds may contain hydrocarbon substituents of either the aromatic or aliphatic series, the nature of the spectrogram obtained being dependent on the hydrocarbon substituent present. The spectrogram of a quaternary ammonium compound in which one of the substituents is a benzyl group is shown in Fig. 15, the absorption in the benzenoid wavelength region of 2500 to 2900 A showing this clearly. In Fig. 16 may be seen spectrograms of quaternary ammonium compounds in which the substituents are all aliphatic, the low absorptions making this quite clear.

Quaternary base with a pyridine nucleus—

Fig. 17 shows the spectrogram of ethylpyridinium bromide, together with a proprietary product based on pyridine. High absorption in the 2400 to 2800 A wavelength region, associated with some fine structure, occurs. These spectrograms show higher extinctions at the absorption maxima than quaternary ammonium compounds containing benzene nuclei, and the samples may also be quite simply distinguished by the odour of pyridine.

Quaternary base with an *iso*quinoline nucleus—

Spectrograms of ethyl*iso*quinolinium bromide and a commercial product based on *iso*-quinoline are shown in Fig. 18. The spectrograms are very similar and particularly distinctive.

Aliphatic amino compounds—

A small group of surface-active agents manufactured from aliphatic amides and amines is also included in this classification. The fact that these compounds are aliphatic is clear, because of their transparency in the ultra-violet region. They may be distinguished from the aliphatic quaternary ammonium compounds by the van der Hoeves permanganate - chloroform test.

Analysis of mixtures

When mixtures of surface-active agents are being examined, the analysis is complicated, and any scheme of analysis would require modification to suit the mixture under examination. In such cases the indications given by the chemical tests are usually more confusing than indications from the spectroscopic examination, and greater emphasis should be placed on

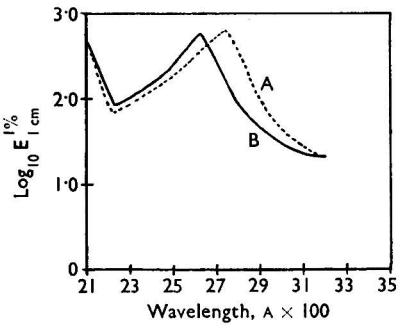

Fig. 13. Arylamines. Curve A (reference compound), sodium dibenzylsulphanilate; curve B, typical proprietory product

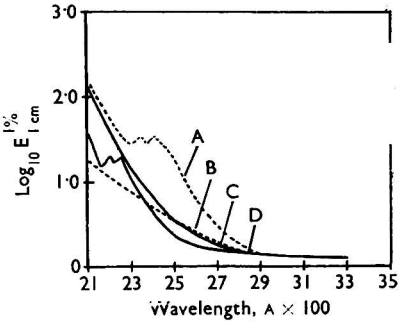

Fig. 14. Alkylolamine soaps. Curve A (reference compound), triethanolamine oleate; curve B (reference compound), triethanolamine stearate; curves C and D, typical proprietory products

Figs. 13 and 14. Ultra-violet spectrograms for surface-active agents with anionic nitrogen present (group III)

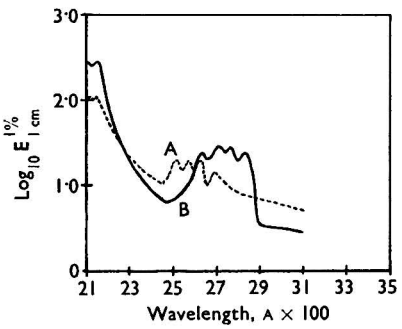

Fig. 15. Quaternary ammonium compounds (aromatic substituent). Curve A (reference compound), stearyltrimethylbenzylammonium chloride; curve B, typical proprietory product

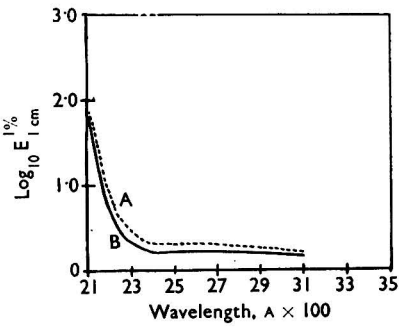

Fig. 16. Quaternary ammonium compounds (aliphatic substituent). Curve A (reference compound), cetyltrimethylammonium bromide; curve B, typical proprietory product

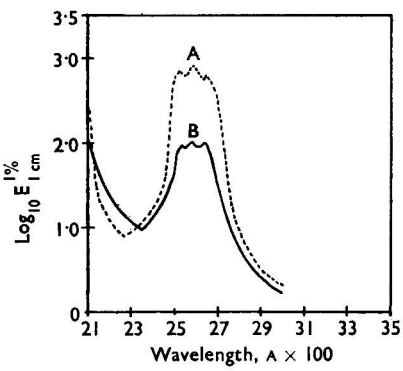

Fig. 17. Quaternary base. Curve A (reference compound), ethylpyridinium bromide; curve B, typical proprietory product

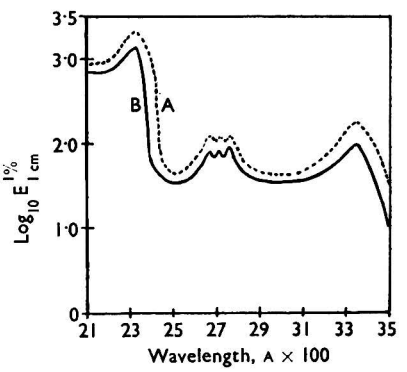

Fig. 18. Quaternary base. Curve A (reference compound), ethyl*iso*quinolinium bromide; curve B, typical proprietory product

Figs. 15 to 18. Ultra-violet spectrograms for surface-active agents with cationic nitrogen present (group IV)

the characteristics of the ultra-violet spectrogram. As the spectrograms obtained may be interpreted on a semi-quantitative basis, it is often possible to discover whether the product identified forms the major active ingredient or is present as an additive. This is a decided advantage over a simple chemical test, which merely indicates the presence or absence of a particular class of product. The spectrogram may also be used to provide most valuable information in a negative sense, as the absence of distinctive types of product may be clearly shown.

With respect to the chemical tests, those indicating ionic character are the most reliable, as anionic compounds are incompatible with cationic compounds and mixtures of these two classes would not normally occur. Non-ionic compounds may occur admixed with anionic or cationic compounds, but chemists experienced in the use of these tests can usually discover which is the major component. A positive test for nitrogen, however, should be treated with reserve, until further confirmation is obtained.

For instance, alkylarylsulphonates are commonly associated with an alkylolamine present in small amount. A positive nitrogen reaction, therefore, would cause an erroneous inference to be made as to the constitution of the major component. Hence, it is sometimes necessary to separate the active components and determine whether nitrogen is present and also the ionic character of each component.

The method of separation employed will depend upon the nature of the mixture of products. In the instance mentioned above, separation of the alkylarylsulphonate may be achieved by heating under reflux with sulphuric acid, which liberates the acid-insoluble sulphonic acids. These sulphonic acids may be washed with light petroleum, in which they are also insoluble, which washing would free the sulphonic acids from any fatty acid or hydrocarbon present. The absence of nitrogen and anionic character can then be shown on the separated sulphonic acids, some idea of yield being also obtained.

Separation may not be necessary, of course, as the ultra-violet spectrogram of the mixture may be quite distinctive. In the instance discussed, the spectrogram of an alkylarylsulphonate is distinctive and would not be confused by the presence of a small quantity of alkylolamine. It is considered preferable to carry out a spectroscopic examination of the active ingredient before any such separation process has been applied, as decomposition of the product may occur during this separation and so obscure the spectroscopic results.

The authors express their thanks to the Management of Petrochemicals Limited for permission to publish this work and to Dr. E. T. Borrows for the encouragement that has been given them to carry out the experimental work.

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March 16th, 1955

A Modified Menzies - Wright Ebulliometer for the Semi-micro and Micro-determination of Molecular Weight

By A. F. COLSON

An improved form of Menzies - Wright ebulliometer suitable for the routine determination of molecular weight on the semi-micro or micro scale is described, and a series of results obtained with a variety of organic compounds as solutes, in benzene and carbon tetrachloride as solvents, is given.

With about 6 ml of solvent and a total sample weight of 40 to 60 mg, the error in the determination of molecular weights in the range 200 to 1000 does not exceed about 3 per cent.

When subsequent recovery of the sample is not required, the same order of accuracy can be obtained in the determination of molecular weights of about 200, a total sample weight of 10 to 15 mg being used,*if a suitable quantity of a non-reactive compound, *e.g.*, anthracene, is dissolved in the solvent before addition of the sample. The apparatus is unsuitable for use with methanol, ethanol or toluene, all of which give unstable readings on the differential thermometer. With the two alcohols this effect is believed to be caused by the presence of aldehydes produced when these solvents are boiled in contact with the internal platinum wire heater incorporated in the ebulliometer.

The effect observed with toluene is attributed to unavoidable excessive superheating of the solvent.

Since the Menzies - Wright ebulliometer was first described,[1] various modifications have been proposed[2,3,4,5,6,7] and micro forms of the apparatus have been devised.[8,9,10,11] In this department experience with one type of micro-ebulliometer has shown that the accuracy obtainable in the determination of molecular weights ranging from 200 to 1000 varies from 5 to 10 per cent. As this order of accuracy is not always acceptable and since the ebulliometer exhibits certain defects in design, the work described in this paper was undertaken with the object of improving both the accuracy and convenience of the apparatus. The satisfactory performance of the macro-ebulliometer designed by Kitson[4] and improved by Ray,[6] suggested that an attempt to adapt it for use on the semi-micro or micro-scale might be profitable.

An apparatus of this type provided with a smaller boiler for use with about 8 ml of solvent proved, however, to be quite unsatisfactory with respect to stability of the thermometer readings.

Some improvement was effected by substituting an internal electric heater for the external type and by varying the design of the Cottrell pump,[12] but sufficiently stable thermometer readings could not be obtained. Since results as accurate as those obtained by Kitson had been reported by Barr and Anhorn,[3] who used an apparatus incorporating the original form of Menzies differential thermometer,[13] attention was next directed to the design of a micro-ebulliometer suitable for use with this thermometer.

The apparatus finally constructed and found to be satisfactory in operation is described in this paper. It resembles an earlier type of micro-ebulliometer described elsewhere,[9,10] but some of the features of the macro apparatus developed by Kitson[4] and improved by Ray[6] have been incorporated in its construction.

Method

Apparatus—

The assembled apparatus, comprising the ebulliometer, A, differential thermometer, B, and Cottrell pump, C, is shown in Figs. 1 and 2, and the components are shown separately in the dimensioned diagrams (Figs. 3, 4, 5, 6). A drying tube (not shown in Fig. 2), loosely packed with calcium chloride, is attached to the tube I. A constant level device is interposed between the tap-water supply and the inlet tube of the condenser, H. The apparatus is supported at the joint, D, by a Terry clip fastened to a Perspex pillar, rigidly attached to

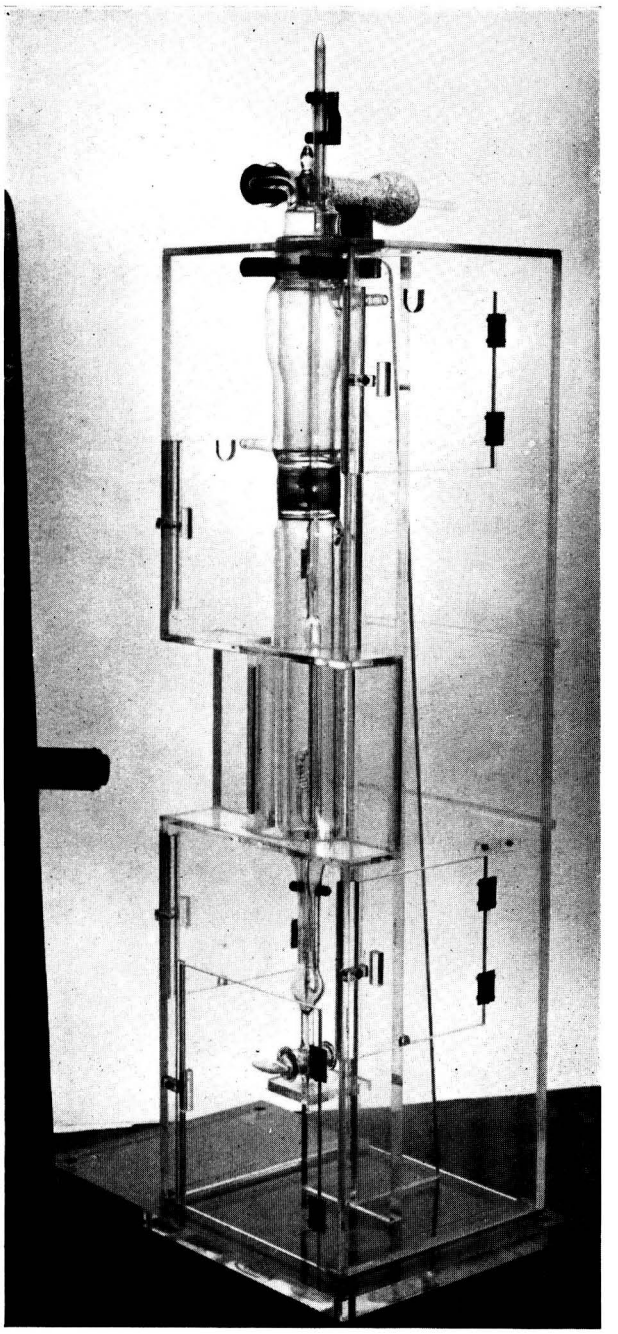

Fig. 1. Assembled micro-ebulliometer

(To face p. 590)

a base plate of the same material. Further support is provided below the tap, E, by a small Perspex shelf with central hole through which the capillary tube, F, is inserted. The apparatus and support are enclosed in a Perspex case provided with a detachable divided lid, the two halves of which fit closely round the top of the condenser at a point slightly below the rim of the joint, D. Hinged doors in the front and sides of the case give access to the tap, E. the heater connections, G_1G_2, and the inlet and outlet tubes of the condenser, H.

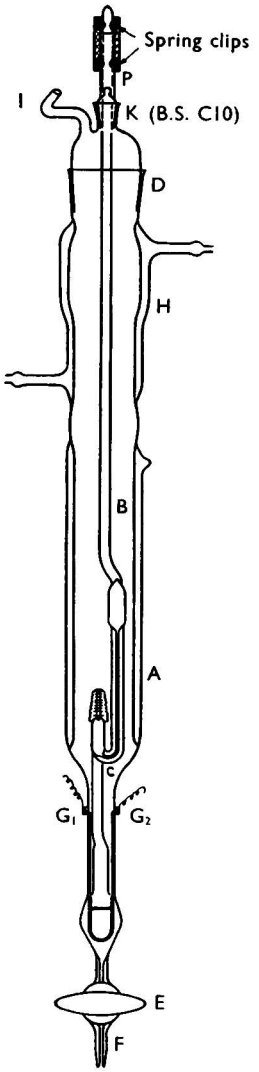

Fig. 2

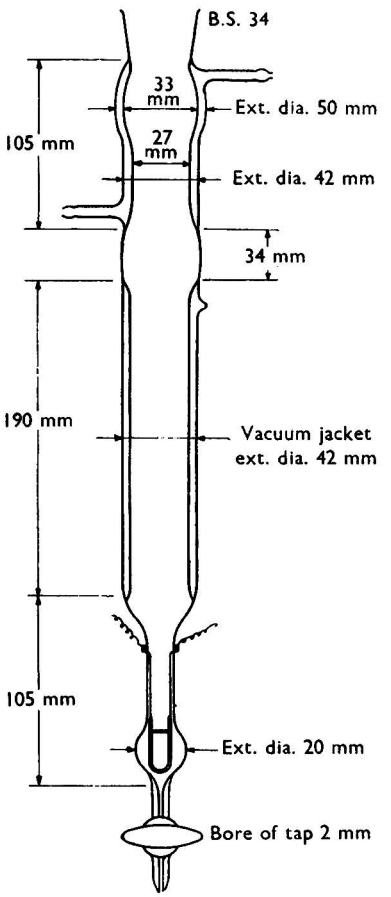

Fig. 3

Fig. 2. Assembled micro-ebulliometer
Fig. 3. Details of condenser assembly

PIPETTE FOR THE DELIVERY OF SOLVENTS—

The one-mark pipette shown in Fig. 7 is suitable for the accurate delivery of a fixed volume (about 6 ml) of solvent. The mouth of the pipette should be closed by a small drying tube (not shown in Fig. 7) to prevent entry of moisture.

TABLET PRESS FOR SOLID SAMPLES—

Solid samples are best transferred to the ebulliometer in the form of pellets, which can conveniently be made in the Orthofer tablet press[14] (obtainable from A. Gallenkamp & Co. Ltd.).

APPARATUS FOR THE TRANSFER OF VISCOUS SAMPLES—

A suitable device is shown in Fig. 8. The sample is taken up on the tip of the weighed tube, J, which is then re-weighed and inserted through the aperture, K, at the top of the ebulliometer, where it is supported by the ground-glass cone, L. The tip of the tube J extends into the region of condensing solvent, where the sample is quantitatively removed within a few minutes.

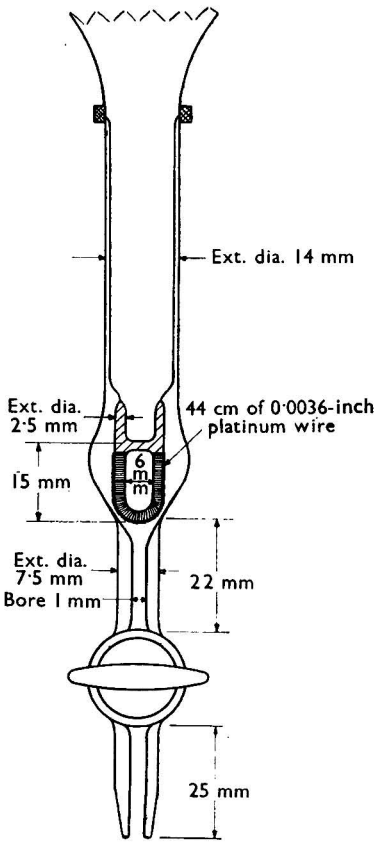

Fig. 4

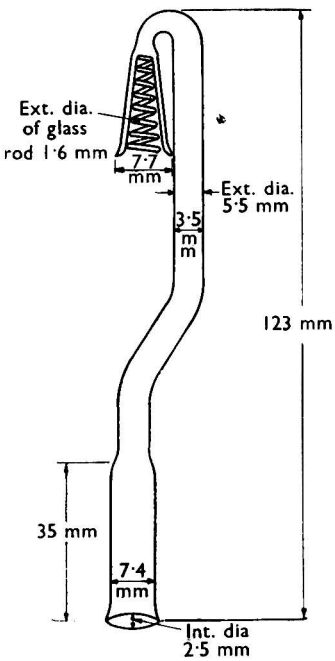

Fig. 5

Fig. 4. Internal heater assembly
Fig. 5. Cottrell pump

APPARATUS FOR THE TRANSFER OF MOBILE LIQUID SAMPLES—

Mobile liquid samples are best transferred to the solvent from the device illustrated in Fig. 9. The open end, M, of the weighed tube, N, is touched on to the surface of the sample and the tube reweighed after wiping off any liquid adhering to the outer surface. If too much sample has been taken up, small amounts may be removed on the end of a narrow strip of filter-paper. Transference of the sample to the ebulliometer is effected as already described for viscous substances.

SOLVENTS—

Benzene—"Benzene for Molecular weight determination," obtainable from The British Drug Houses Ltd., dried and stored over sodium wire.

Carbon tetrachloride—Obtainable from Hopkin and Williams Ltd.; used without further treatment.

PROCEDURE FOR DETERMINATION OF THE CONSTANT, *K*—

Fill the capillary tube above the tap, E (Fig. 2), with clean dry mercury, turn on the water supply to the condenser, and adjust the rate of flow to about 80 ml per minute. Fill

the pipette (Fig. 7) with the selected solvent and transfer the contents to the clean dry ebulliometer. By means of a Variac transformer, adjust the current through the internal heater to such a value (0·8 amp.) that the boiling solvent is pumped slowly over the lower bulb of the differential thermometer, but the solvent does not reach the upper bulb. Maintain the heating current at this value until the thermometer liquid has been expelled from the lower into the upper bulb (10 minutes) and then increase the current to 1·35 amp. (applied voltage, 12 volts). After 10 minutes adjust the position of the thermometer in the sleeve, P (Fig. 2), to give the most stable reading consistent with effective operation of the Cottrell pump.

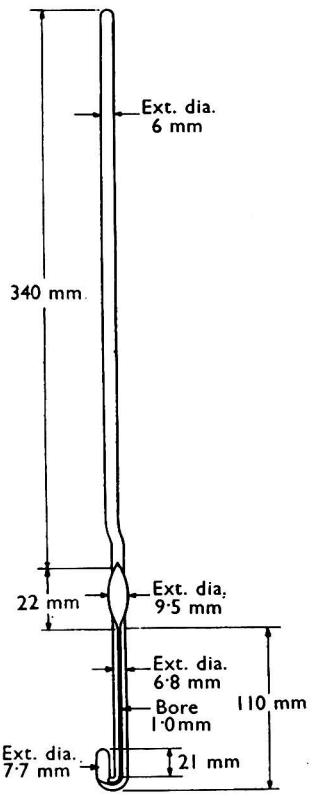

Fig. 6

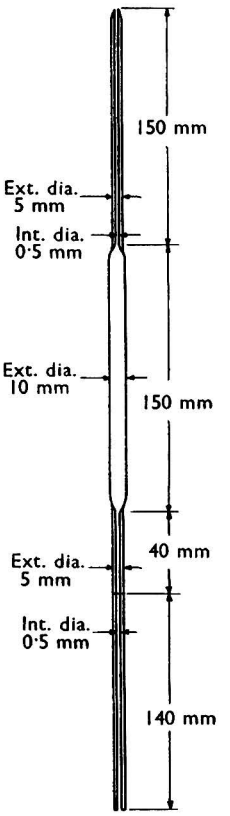

Fig. 7

Fig. 6. Menzies differential thermometer
Fig. 7. Pipette

Using a cathetometer, determine the position of the liquid meniscus in the longer limb of the thermometer, to the nearest 0·05 mm, and repeat the observation at intervals of 5 minutes until a constant reading is obtained.

Add an unweighed pellet of pure dry benzil (about 10 mg) and observe the thermometer reading at intervals of 5 minutes until a constant value is obtained. This value is the "zero" thermometer reading used in the calculation of the constant, K. Finally add three weighed pellets of benzil (about 10 mg each) in succession and record the thermometer reading after each addition.

Calculate the constant, K, from the formula—

$$K = 210{\cdot}22 \times \frac{(R - Z)}{W},$$

where $210{\cdot}22$ = molecular weight of benzil,
Z = "zero" thermometer reading, mm,
R = observed thermometer reading, mm,
W = total weight of benzil, mg, corresponding to the thermometer reading R.

To clean the apparatus, drain off the solution through the tap, E, add about 6 ml of benzene, boil until the vapour reaches about half-way into the uncooled condenser tube, and drain off through the tap as before. Repeat these operations twice more and then draw warm dry air through the ebulliometer for about 30 minutes.

PROCEDURE FOR THE SEMI-MICRO-DETERMINATION OF MOLECULAR WEIGHT—

Determine the zero thermometer reading as described for the determination of K, but in place of the unweighed pellet of benzil add an unweighed amount of the sample (about

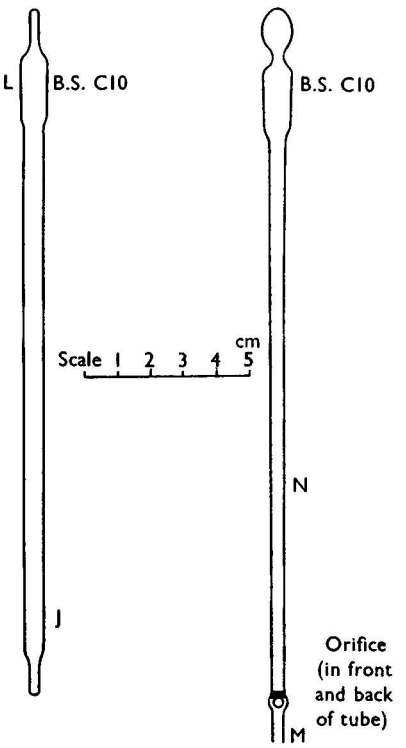

Fig. 8

Fig. 9

Figs. 8 and 9. Devices for adding samples

10 mg). Then add three weighed portions of the sample (about 10 mg each) in succession and record the thermometer reading after each addition as already described.

Calculate the molecular weight, M, of the sample from the formula—

$$M = \frac{K \times W}{R - Z},$$

where K = the constant for the apparatus,
Z = "zero" thermometer reading, mm,
R = observed thermometer reading, mm,
W = total weight of sample, mg, corresponding to the thermometer reading R.

PROCEDURE FOR THE MICRO-DETERMINATION OF MOLECULAR WEIGHT—

Proceed as described for the semi-micro-determination, but in the determination of the zero thermometer reading use about 30 mg of anthracene in place of the unweighed portion of sample, and then complete the molecular weight determination by addition of three successive weighed amounts of sample (3 to 5 mg) each.

RESULTS

The precision obtainable in the determination of the constant, K, with benzil as solute and benzene or carbon tetrachloride as solvent is illustrated by the results recorded in Table I.

For each determination the total weight of benzil present after each addition of a weighed pellet (about 10 mg each) is shown in the second column, and the corresponding total change of the thermometer reading ($R - Z$) is given in the third column. The slight effect produced by the initial addition of anthracene to the solvent is indicated by the results reported for determination number 6.

Some results obtained with benzene as solvent in the semi-micro-determination of molecular weights ranging from about 180 to 1000 are recorded in Table II, and a few results obtained with carbon tetrachloride over the range 200 to 600 are shown in Table III. In these tables only the final total weight of sample is given in the second column.

TABLE I

DETERMINATION OF THE CONSTANT, K

Determination number	With benzene as solvent: Weight of benzil, mg	$(R - Z)$, mm	K	With carbon tetrachloride as solvent: Weight of benzil, mg	$(R - Z)$, mm	K
1	9·709	5·95	128	8·795	5·05	121
	18·722	11·40	128	17·677	10·00	119
	27·231	16·45	127	25·890	14·80	120
2	9·665	6·00	131	8·319	4·75	120
	18·896	11·50	128	18·033	10·20	119
	28·147	17·30	129	27·000	15·17	118
3	9·542	5·90	130	7·778	4·45	120
	19·163	11·80	129	15·414	8·75	120
	28·547	17·40	128	22·317	12·60	119
4	9·460	5·80	129	—	—	—
	18·742	11·40	128	—	—	—
	28·445	17·15	127	—		—
5	9·884	6·10	130	—	—	—
	9·304	11·80	129	—	—	—
	29·199	17·90	129	—	—	—
6*	9·218	5·70	130	—	—	—
	18·841	11·60	130	—	—	—
	27·813	17·15	130	—	—	—
	36·667	22·55	129			
	Mean value of K = 129			Mean value of K = 120		

* In this determination the zero thermometer reading was obtained by the initial addition of about 20 mg of anthracene in place of the 10 mg of benzil used in determinations numbers 1 to 5.

TABLE II

SEMI-MICRO-DETERMINATION OF MOLECULAR WEIGHT WITH BENZENE AS SOLVENT

Compound used	Total weight taken, mg	Molecular weight found (mean value)	Molecular weight calculated	Error, %
Anthracene	29·776	176	178·22	− 1·0
	27·506	177	178·22	− 0·7
Benzophenone	37·684	185	182·21	+ 1·5
*endo*Ethyleneanthracene	37·692	204	204·26	− 0·1
	37·576	203	204·26	− 0·6
Cetane	35·713	224	226·44	− 1·0
Sulphonal	41·531	232	228·33	+ 1·6
*meso*Inositol hexamethyl ether	47·305	271	264·3	+ 2·7
Cholesterol	47·926	377	386·64	− 2·5
Dibromobenzanthrone	30·800	386	388·07	− 0·6
ββ-Bis-(3 : 5-dibromo-4-methoxyphenyl)propane	28·094*	567	571·96	− 0·9
5 : 6 : 7 : 5′ : 6′ : 7′-Hexa-acetoxy-3 : 3 : 3′ : 3′-tetramethylbis-1 : 1′-*spiro*hydrindine	37·171	605	624·60	− 3·0
	28·241*	624	624·60	− 0·1
5 : 6 : 7 : 5′ : 6′ : 7′-Hexabenzoxy-3 : 3 : 3′ : 3′-tetramethylbis-1 : 1′-*spiro*hydrindine	30·66	978	997	− 2·0

* In these determinations the "zero" thermometer reading was obtained by the initial addition of about 20 mg of anthracene instead of from the addition of the first pellet of sample.

TABLE III

SEMI-MICRO-DETERMINATION OF MOLECULAR WEIGHT WITH CARBON TETRACHLORIDE AS SOLVENT

Compound used	Total weight taken, mg	Molecular weight found (mean value)	Molecular weight calculated	Error, %
Anthracene	29·139	174	178·22	− 2·2
	28·084	176	178·22	− 1·3
Benzophenone	35·741	180	182·21	− 1·2
*endo*Ethyleneanthracene	37·378	202	204·26	− 1·1
Sulphonal	40·181	228	228·33	− 0·1
Cholesterol	49·081	377	386·64	− 2·5
ββ-Bis-(3 : 5-dibromo-4-methoxyphenyl)propane	49·144	553	571·96	− 3·3

TABLE IV

MICRO-DETERMINATION OF MOLECULAR WEIGHT WITH BENZENE AS SOLVENT

Compound used	Total weight taken, mg	Molecular weight found (mean value)	Molecular weight calculated	Error, %
Anthracene	10·072	180	178·22	+ 1·0
*endo*Ethyleneanthracene	10·754	204	204·26	− 0·1
	4·140*	215	204·26	+ 5·1
	3·729*	212	204·26	+ 5·0
Phenacetin	13·238	182	179·21	+ 1·7
Benzophenone	8·741	184	182·21	+ 0·8

* In these determinations the "zero" thermometer reading was obtained by the initial addition of about 30 mg of anthracene instead of 20 mg.

In Table IV the results obtained with benzene in the micro-determination of molecular weights in the region of 200 are recorded.

The following compounds used in the work described in this paper were provided by Dr. J. C. McGowan of this department—

*meso*Inositol hexamethyl ether[15];

ββ-Bis-(3 : 5-dibromo-4-methoxyphenyl)propane;

5 : 6 : 7 : 5′ : 6′ : 7′-Hexa-acetoxy-3 : 3 : 3′ : 3′-tetramethylbis-1 : 1′-*spiro*hydrindine[16,17];

5 : 6 : 7 : 5′ : 6′ : 7′- Hexabenzoxy- 3 : 3 : 3′ : 3′- tetramethylbis - 1 : 1′-*spiro*hydrindine. (This compound was prepared from the corresponding acetoxy compound.) There are earlier descriptions[18,19] of the preparation.

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IMPERIAL CHEMICAL INDUSTRIES
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An Indirect Colorimetric Method for the Determination of Beryllium

By M. SUNDERASAN and M. SANKAR DAS

A spectrophotometric method for the determination of beryllium is described; it is based on the precipitation of beryllium ammonium phosphate, which is subsequently determined spectrophotometrically as molybdophosphoric acid. By this method 2 p.p.m. of beryllium in the solution of a sample can be determined. Interference by most elements, including copper and nickel, is avoided by the use of ethylenediaminetetra-acetic acid. The method is particularly useful for ores and alloys of low beryllium content.

Several organic reagents are available for the photometric determination of beryllium. Of these, mention may be made of quinalizarin,[1] sulphosalicylic acid,[2] *p*-nitrobenzeneazo-orcinol[3] and acetylacetone[4] as colorimetric reagents, and quinizarin[5] and morin[6] as fluorimetric reagents. However, copper, iron, aluminium and phosphate, which often occur with beryllium, interfere in these determinations. In the sulphosalicylic acid method, small amounts of beryllium in the presence of large amounts of aluminium can be determined only if an equal amount of aluminium is added to the blank. Iron and copper, even in traces, are highly objectionable and must be removed quantitatively by extraction and electrodeposition. In the absorptiometric method with acetylacetone, the interfering ions are successfully masked by means of ethylenediaminetetra-acetic acid, and beryllium acetylacetonate is preferentially extracted into chloroform. Determination of beryllium is completed in the ultra-violet region after removing excess of acetylacetone, which absorbs highly in this region. Quinizarin is particularly suitable for determining small amounts of beryllium and considerable amounts of the above-mentioned interfering elements can be tolerated, but the complex is highly sensitive to light. The method described in this paper for the determination of beryllium is based on its precipitation as beryllium ammonium phosphate,[7] $BeNH_4PO_4.6H_2O$, in the presence of ethylenediaminetetra-acetic acid and the colorimetric determination of this phosphate precipitate as molybdophosphoric acid. Since the composition of the beryllium ammonium phosphate is known to be rigorous,[8] it was considered worth while to try this method for the determination of beryllium in the concentration range of 2 to 20 p.p.m. in the solution to be examined.

Experimental

All optical density measurements are made with a Beckman DU quartz spectrophotometer, with a tungsten lamp as the source of radiation, 1-cm cells being used.

Reagents—

All reagents should be of recognised analytical grade.

Standard beryllium solution—Prepare a solution containing 0·1 mg of beryllium oxide per ml by dilution from a stock solution of beryllium chloride standardised by the oxide method.

Sodium molybdate—Prepare a 10 per cent. w/v aqueous solution from the dihydrate and keep it in a waxed bottle.

Diammonium hydrogen phosphate—A 10 per cent. aqueous solution.

Ethylenediaminetetra-acetic acid—A 10 per cent. solution of the laboratory-grade disodium salt (obtainable from The British Drug Houses Ltd.).

Sulphuric acid, 2 N—Dilute 27·7 ml of concentrated sulphuric acid to 500 ml with distilled water.

Standard phosphate solution—Dissolve 0·2196 of potassium dihydrogen phosphate in 500 ml of distilled water. Then 1 ml contains 0·1 mg of phosphorus.

Wash solution—A 2 per cent. ammonium nitrate solution adjusted to pH 5·3 with ammonium acetate.

PROCEDURE—

To prepare the calibration curve for phosphorus, add 5 ml each of the sulphuric acid and sodium molybdate solutions to aliquots of the standard phosphate solution to give 1 to 25 p.p.m. of phosphorus in a final volume of 50 ml. Set aside for 15 minutes and then measure the absorption of these solutions gainst a corresponding blank at 390, 400 or 420 mμ. This procedure is identical with that of Baltz and Mellon[9]; the response is linear. Dilute nitric acid unless freshly prepared is not suitable and so dilute sulphuric acid is used.

For the determination of beryllium, put a known aliquot of beryllium solution in a 40-ml centrifuge tube, dilute to about 30 ml, add 1 ml of the 10 per cent. solution of diammonium

TABLE I

COMPARISON OF THE CALCULATED AND DETERMINED AMOUNTS OF PHOSPHORUS

Beryllium oxide taken, mg	Calculated amount of phosphorus, mg	Amount phosphorus found, mg
0·1	0·1255	0·105
0·2	0·25	0·23
0·2	0·25	0·24
0·3	0·37	0·38
0·4	0·50	0·51
0·5	0·62	0·63
0·6	0·74	0·75
1·0	1·24	1·23

hydrogen phosphate, 2 or 3 drops of 0·05 per cent. alcoholic solution of bromocresol green indicator and acidify slightly with diluted hydrochloric acid (1 + 1, v/v). Place the tube in a water-bath and, when hot, add dropwise a 2 *N* solution of ammonium acetate until the colour changes to a distinct blue (pH 5). Leave the tube in the water-bath for 30 minutes for the precipitate to become granular. After the tube has been cooled, spin it in a centrifuge at 2500 r.p.m. for 15 minutes. Discard the supernatant liquid and pour the residue on to a Whatman No. 40 7·0-cm filter-paper. Wash the precipitate three times with the wash

TABLE II

RECOVERY OF BERYLLIUM IN PRESENCE OF IMPURITIES

Beryllium oxide taken, mg	Impurities								Beryllium oxide recovered, mg
	Iron, mg	Aluminium, mg	Calcium, mg	Magnesium, mg	Zinc, mg	Titanium, mg	Nickel, mg	Copper, mg	
0·4	11·1								0·395
0·4								18	0·395
0·6	22·3								0·593
0·4					32				0·395
0·3		14·2							0·298
0·4							58		0·390
0·4		28·4							0·395
0·3	11·1	14·2							0·298
0·5	11·1	14·2							0·500
0·3			12						0·298
0·5			24						0·504
0·5			12	5					0·510
0·3	11·1	14·2	12						0·299
0·5	11·1	14·2	12						0·510
0·5						1			0·470
0·5	11·1					1			0·464
0·4					19		18	18	0·395
0·5					19		18	18	0·475

solution, draining the tube well after each washing. Dissolve the precipitate adhering to the sides of the tube and the filter-paper in hot diluted nitric acid (1 + 4, v/v). Evaporate the solution and washings first on a hot-plate and finally on a water-bath to remove excess of nitric acid. Dissolve the residue in 5 ml of 2 *N* sulphuric acid and 5 ml of molybdate solution, dilute to 50 ml and, after 15 minutes, measure the absorption at 390, 400 or 420 mμ. Prepare a calibration curve by the above procedure on 1, 2, 3, 4, 5 and 10-ml aliquots of

the standard beryllium solution. Straight-line graphs are obtained. The graph at 420 mμ is particularly useful for larger amounts of beryllium. The completeness of the precipitation and quantitative recovery of beryllium is evident from Table I, in which calculated and determined amounts of phosphorus are compared.

Determination in the presence of impurities

In this study the interference of copper, nickel, zinc, iron, aluminium, calcium and magnesium is avoided by taking advantage of the selective complexing property of ethylenediaminetetra-acetic acid.[10,11,12] Adopt the same procedure as for pure beryllium solutions, except that before adjusting the pH of precipitation, add 1 ml of the ethylenediaminetetra-acetic acid solution to the sample. Single precipitations are usually sufficient, but when the impurities are high, a re-precipitation is necessary after decanting off the solution after the first precipitation. Titanium interferes even in the presence of ethylenediaminetetra-acetic acid. The interference can be overcome by adding a few drops of hydrogen peroxide before adjusting the pH. Table II gives a summary of the recovery of beryllium in the presence of various impurities.

Applications of the method

A specially useful application of this method is the determination of beryllium in ores, salts and alloys containing small amounts. A typical analysis of an ore involves bringing into solution a 0·5-g sample by fusion with sodium carbonate and complete removal of silica. Thereafter the procedure is similar to that for beryllium solutions with interferences. Alloys can also be analysed the same way. Results for a few selected samples together with those obtained by other methods are shown in Table III.

Table III

Comparison of results obtained by the present method and by other methods

Nature of sample	By present method		By comparison method	
	Beryllium oxide, %	Beryllium, %	Beryllium oxide, %	Beryllium, %
Apatite, low in beryl	0·64	—	0·50*	—
	0·66	—	0·75†	—
Low-grade beryls containing felspar, quartz and small amounts of mica and apatites	6·30	—	6·40*	—
	10·42	—	10·53*	—
Copper - beryllium alloy	—	1·92	—	2·02†
	—	1·91	—	2·01†

* By gravimetric method. † By gravimetric phosphate method.

Conclusions

The method involves very few operations and practically no separations. By the procedure described 2 p.p.m. of beryllium in the solution of a sample to be analysed can be determined in the presence of very large quantities of impurities with an accuracy of ± 5 per cent. Phosphate, which interferes in other colorimetric methods, is of no consequence.

Thanks are due to Dr. Jagdish Shankar for his keen interest and help during this work.

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Notes

THE DETERMINATION OF TRICHLOROETHYLENE IN AIR BY THE RAYLEIGH INTERFEROMETER

(Presented at the meeting of the Physical Methods Group on Tuesday, March 9th, 1954)

DURING the routine testing of anaesthetic machines, many trichloroethylene - air mixtures had to be analysed in the range 0 to 3 per cent. by volume of trichloroethylene vapour in air. Chemical analysis was considered to be too time-consuming and laborious, so the possibility of using a quicker method was investigated.

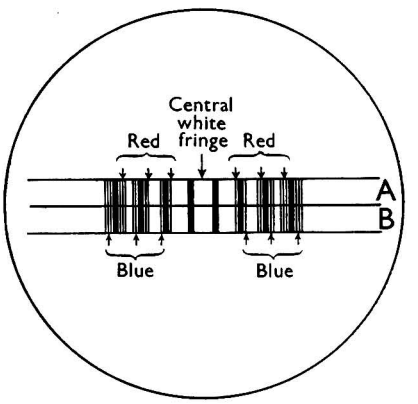

Fig. 1. Appearance of the interference fringes of a Rayleigh interferometer with air in both gas cells. The upper set of fringes, A, are produced by light passing through the gas cells and the lower set, B, by light passing through air

Since a two-component system was being examined, a physical property of the gas mixture such as refractive index, density or thermal conductivity would uniquely determine the mixture. The refractive index was chosen as being the most suitable physical property, as a Rayleigh gas interferometer was available. This instrument was used to measure the difference of refractive index between air and the unknown mixture of air and trichloroethylene.

DESCRIPTION OF THE INSTRUMENT—

The particular instrument used was the Hilger M75 Rayleigh refractometer, fitted with compensating plates 4·7 mm thick and gas cells 1 metre long. With this arrangement the instrument could be used to measure differences of refractivity up to $8{\cdot}0 \times 10^{-5}$. The refractivity change produced by 3 per cent. of trichloroethylene vapour is about 4×10^{-5}, which is about one-half of the maximum reading of the instrument.*

The principle of operation is described in most text books on physical optics and is not, therefore, repeated in this Note. In taking a reading with the instrument the control knob is adjusted so that the two sets of white-light interference fringes are brought into coincidence. The reading on the micrometer head associated with the control knob then corresponds to the difference of refractive index between the gases in the two cells of the instrument. The appearance of the fringes as seen in the eyepiece is shown in Fig. 1.

The corrections described below are applied to the interferometer readings. All readings are corrected for the zero error of the instrument.

Allowance must be made in adjusting the instrument for the effects of dispersion. A white-light source is used, so that the central fringe of each pattern is marked by being the most nearly achromatic or white fringe. If the centres of the two patterns could not be located in this way, the adjustment could be in error by a large number of fringe widths. As the concentration of

* The manufacturers claim that a difference of refractivity of $1{\cdot}5 \times 10^{-8}$ can be detected with the interferometer, corresponding to an accuracy of adjustment of one-fortieth of one interference band. The minimum difference of refractivity that could be detected with our instrument was $3{\cdot}56 \times 10^{-8}$, because the instrument could only be read to the nearest scale division, corresponding to one-fifteenth of a band. This was due to the use of compensating plates 4·7 mm thick instead of plates 1 mm thick, to increase the difference of refractivity range from $1{\cdot}7 \times 10^{-5}$ to $8{\cdot}0 \times 10^{-5}$.

trichloroethylene vapour is gradually increased, the appearance of the upper set of fringes changes, until the fringe to the right of the reference or central fringe becomes the most nearly achromatic or white fringe. This effect is discussed by Edwards.[1]

The position of the reference fringe with respect to the most nearly achromatic fringe was determined as follows. A trichloroethylene - air mixture was introduced into the right-hand gas cell, with air in the left-hand cell, and the fringes were brought into coincidence, the most nearly achromatic fringe in the upper set being used as the central fringe. Air was then slowly passed through the right-hand tube, so that the trichloroethylene mixture was gradually displaced by the air, and the interferometer was kept in continual adjustment. When air was again in both gas cells, the fringes were again brought into coincidence, and the relative movement, measured in terms of fringe widths, was noted. After a number of such tests, the position of the central reference fringe with respect to the most nearly achromatic fringe was established for all possible trichloroethylene concentrations. In all subsequent adjustments of the instrument, the readings were made with respect to the central reference fringe, which was not always the most nearly achromatic fringe.

The interferometer scale readings are not linearly related to the difference of refractivity between the two gases being compared. They can, however, easily be converted to corrected readings that are directly proportional to the difference of refractivity as shown by Adams.[2] The corrected reading r' is directly proportional to the difference in refractivity, where $r' = r - ar^2$, r being the actual scale reading and a a constant.

This constant can be calculated by a method shown by Adams, but a knowledge of the refractive index of the compensating plate is required to make this calculation. If this is not known, it may be easier to find the constant a by experiment than to measure the refractive index of the compensating plate. This can be done as follows. The difference in refractivity between the gas cells is varied in a known way by varying the gas pressure in one of the cells when air is present in both cells, use being made of the fact that the refractivity of a gas is directly proportional to the absolute pressure. From these readings a can be found graphically.

$$r' = r - ar^2 = kM,$$

where r' = corrected reading,

r = actual scale reading,

a = constant,

k = constant of proportionality, and

M = difference of refractivity.

Therefore—

$$\frac{1}{r} = \frac{M}{r^2}k + a.$$

This is in the form of a straight-line equation. When $\frac{1}{r}$ is plotted against $\frac{M}{r^2}$, the slope will be k, and a will be the intercept. The corrected scale reading can now be converted directly to concentration of trichloroethylene by multiplying it by a suitable calibration factor. The calibration factor used was the change in concentration of trichloroethylene that corresponds to a change of one corrected scale division. Determinations of this calibration factor were corrected to the standard temperature and pressure of 25° C and 760 mm of mercury.

Experimental

Four methods of calibration were used. The first is due to Patty,[3] who has already determined the change in refractivity per 1 per cent. of trichloroethylene at 25° C and 760 mm pressure as $13{\cdot}7 \times 10^{-6}$. This can be combined with the physical constant of the instrument, the change in refractivity per corrected division, to give a calibration factor, the percentage of trichloroethylene in air per corrected scale division. The change in refractivity per corrected scale division was determined as $3{\cdot}56 \times 10^{-8}$ by filling both cells of the interferometer with dry air, free from carbon dioxide, increasing the pressure in one cell progressively and noting the corrected scale

reading corresponding to each pressure differential. The difference in refractivity was calculated from the following formula—

$$\Delta R = \frac{273\ (p_1 - p_2)\ R_{air}}{T \times 760},$$

where ΔR = the difference in refractivity,

T = the absolute temperature,

$(p_1 - p_2)$ = the pressure differential in mm of mercury, and

R_{air} = refractivity of dry air, free from carbon dioxide, at 0° C and 760 mm of mercury ($= 2{\cdot}917 \times 10^{-4}$).

In the second method a steady flow of trichloroethylene in air was produced from a suitable vaporiser and passed through a gas-sampling tube and then through the interferometer gas cell, the apparatus shown in Fig. 2 being used. When equilibrium conditions were attained, the interferometer reading was recorded and the gas-sampling tube was sealed.

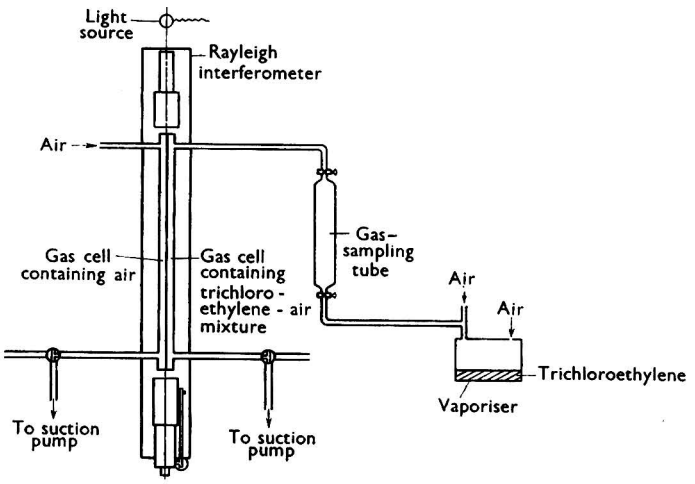

Fig. 2. Apparatus for calibration by direct chemical analysis

The trichloroethylene was determined chemically by determination of the chlorine. The apparatus used was a standard Pregl halogen-combustion train modified to permit the introduction of the sample gas through a 3-way tap into the combustion tube. Alkaline hydrogen peroxide was used as absorbent. A 200-ml sample was passed into the tube from a gas burette at a rate of 10 to 15 ml per minute. Purified air was used to purge the sample through the combustion tube. Determination of the chlorides present after combustion was carried out by acidifying the absorbent and weighing as silver chloride after precipitation with silver nitrate.[4,5]

A graph was plotted of corrected interferometer readings, reduced to 25° C and 760 mm of mercury, against the percentage of trichloroethylene, from which the calibration factor was derived.

In the third method, known mixtures of trichloroethylene in air were prepared and the corresponding interferometer readings determined, the apparatus shown in Fig. 3 being used.

Weighed amounts of trichloroethylene were introduced into a 6-litre glass-stoppered vessel, and allowed to attain equilibrium. The stopper was then removed, a tube inserted to the bottom of the vessel and the mixture drawn slowly through the interferometer cell by means of an aspirator. The interferometer readings rose to a maximum value, indicating that the gas cell was being swept out by the mixture, and after being constant for a time gradually became smaller as air began to mix with the mixture in the vessel. This method gave the most consistent values of the three experimental methods.

In the fourth method a continuous stream of trichloroethylene was allowed to drip into an air stream of measured flow-rate. From the liquid flow-rate and the air flow-rate the concentration of trichloroethylene in the mixture could be calculated. Some of the mixture was then drawn into the interferometer cell and the corresponding scale reading observed. The apparatus used

is shown in Fig. 4. This was the least satisfactory of the three experimental methods, because of the difficulty experienced in maintaining these flows constant.

The error in adjusting the instrument was about $\pm$ 1 per cent.

The calibration factors obtained by the various methods were as follows—

Patty's method	$2{\cdot}60 \times 10^{-3}$
Chemical analysis	$2{\cdot}65 \times 10^{-3}$
Direct static method	$2{\cdot}69 \times 10^{-3}$
Direct dynamic method	$2{\cdot}66 \times 10^{-3}$.

Mean value = $2{\cdot}65 \times 10^{-3}$ per cent. of trichloroethylene per corrected scale division at 25° C and 760 mm of mercury.

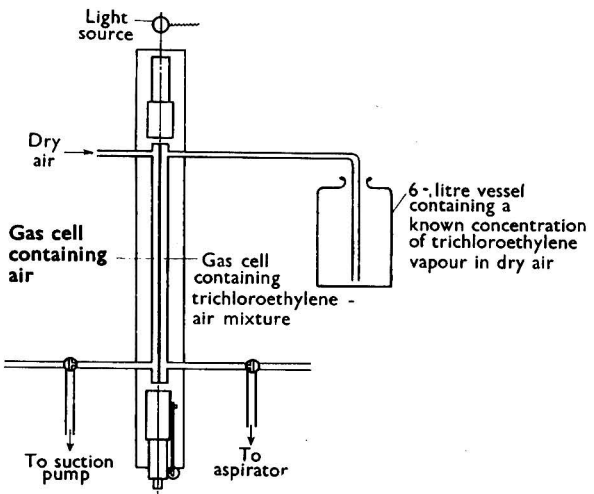

Fig. 3. Apparatus for calibration against a known mixture by a static method

The standard error of the mean is about $\pm$ $0{\cdot}02 \times 10^{-3}$, so that the percentage error due to calibration, as measured by the variation between calibration factors determined by different methods, is about $\pm$ 1 per cent; coupled with the error in adjusting the interferometer, this gives a total error of about $\pm$ 2 per cent. In the analysis of a mixture containing 0·5 per cent of trichloroethylene, this corresponds to a change in concentration of $\pm$ 100 p.p.m.

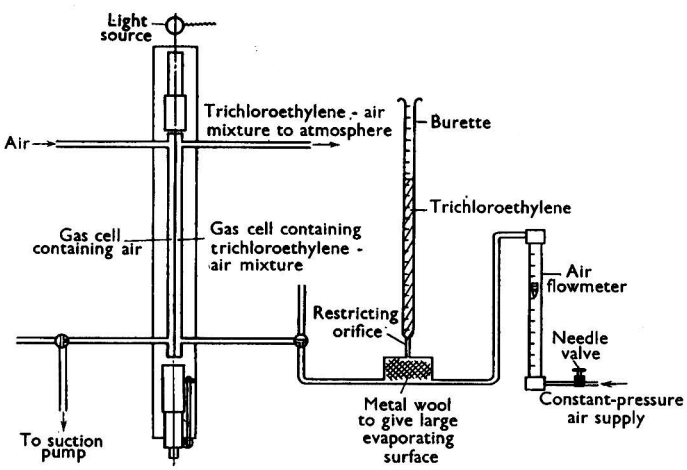

Fig. 4. Apparatus for calibration against a known mixture by a dynamic method

Once the calibration factor had been determined, readings could be taken as fast as the concentration in the cell changed. Atmospheric air was drawn through the standard cell and the mixture to be analysed through the right-hand cell. The pressure in both tubes was brought to atmospheric at the moment of taking the reading, to avoid errors caused by a difference of pressure between the gas cells.

The Rayleigh interferometer proved to be a convenient instrument in this instance, when a large number of analyses had to be performed; the high accuracy of the instrument was not utilised to the full in this particular application.

The author thanks the Directors of The British Oxygen Co. Ltd. for permission to publish this Note.

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December 7th, 1954

THE COLORIMETRIC DETERMINATION OF TRACE AMOUNTS OF ALCOHOLS

THE method of Reid and Truelove[1] for the determination of alcohols in dilute aqueous solution describes the determination of alcohol contents in the 0 to 5·0 per cent. w/w region. The Spekker absorptiometer is employed, with a 1-cm cell and distilled water as the blank.

But as the spectrophotometric conditions under which this colour evaluation is made were chosen to cover the full range of colour development of the reagent, the maximum sensitivity of colour evaluation was not obtained over the range of colours produced by very dilute alcoholic solutions.

This Note deals exclusively with the determination of alcohol contents below 0·1 per cent., results for the determination of methanol, ethanol and *iso*propanol being given.

EXPERIMENTAL

The ceric ammonium nitrate reagent used in this work was prepared and standardised by the method of Reid and Truelove,[1] the same ratio of sample size to reagent volume also being employed in the test procedure.

Several different spectrophotometers were examined in order to discover the most suitable instrument for this work. Although some other spectrophotometers showed a similar order of sensitivity, the Unicam SP 600 was chosen as the most suitable instrument, combining sensitivity with simplicity of operation.

METHOD

REAGENT—

Ceric ammonium nitrate reagent—Prepared and standardised as described by Reid and Truelove.[1]

PROCEDURE—

With a pipette place 10 ml of the aqueous alcoholic solution in a dry 4-cm cell and add, by pipette, 4 ml of ceric ammonium nitrate reagent; mix the two solutions by means of a thin glass rod. Prepare a reference cell in the same manner, using 10 ml of distilled water instead of the alcohol solution.

Exactly 5 minutes after mixing the sample and reagent, evaluate the colour on the Unicam SP 600 spectrophotometer against the reagent - distilled water reference cell, at a wavelength of 486 mμ. Apply the value for optical density obtained to the appropriate calibration curve to determine the alcohol content of the sample under test.

CALIBRATION CURVES—

The calibration curves for each alcohol are most conveniently prepared on a weight - volume basis. Weights of alcohol of about 1 g were weighed into a 50-ml calibrated flask and diluted to the mark with distilled water. Dilution to the appropriate trace concentration was made on a volume basis, accurately calibrated volumetric glassware being used. This technique of sample weighing and subsequent dilution was employed in the preparation of the artificial alcohol solutions, in both aqueous and hydrocarbon solvents, that were employed for accuracy evaluation. The calibration values obtained for methanol, ethanol and *iso*propanol are given in Table I.

TABLE I

CALIBRATION DATA FOR ALCOHOL SOLUTIONS

	Optical density values for solutions of alcohol (% w/w)									
Alcohol tested	0·01	0·02	0·03	0·04	0·05	0·06	0·07	0·08	0·09	0·10
Methanol ..	0·075	0·142	0·210	0·280	0·350	0·417	0·480	0·555	0·622	0·690
Ethanol ..	0·055	0·110	0·162	0·215	0·265	0·315	0·365	0·410	0·455	0·503
*iso*Propanol	0·040	0·077	0·113	0·150	0·185	0·220	0·260	0·290	0·323	0·357

ACCURACY OF METHOD

Assessment of the accuracy of the method was made by preparing artificial aqueous solutions of the various alcohols, the weight of each alcohol dissolved in the 10-ml sample aliquot used in the test being compared with the weight determined by colorimetric analysis. Comparison of the prepared against determined values for the weight of alcohol present in each mixture is made in Table II, from which it can be seen that the recoveries were good. The accuracy obtainable was about $\pm 0{\cdot}2$ mg of alcohol, which corresponds to a concentration of ± 20 p.p.m. w/w for aqueous solutions.

TABLE II

ACCURACY OF THE DETERMINATION IN AQUEOUS SOLUTIONS

Alcohol taken, mg	Methanol found, mg	Ethanol found, mg	*iso*Propanol found, mg
1·0	0·9	0·9, 1·1	1·1, 0·9
2·0	1·9	2·1	2·0, 2·2
5·0	4·8	4·9, 5·1	4·9, 5·1
8·0	8·2	7·8, 8·0	7·9, 8·0
10·0	9·9	9·9, 10·2	9·8, 10·0

DETERMINATION OF ALCOHOLS IN HYDROCARBON SOLVENTS

It is often desirable to determine trace quantities of alcohol in hydrocarbon solvents. This may be effected by water extraction of the alcohol, with colorimetric determination on the aqueous extract. The addition of 20 ml of water has been found suitable for the complete extraction of the alcohol from a 500-ml hydrocarbon sample. Thorough mixing of water and sample is effected by means of mechanical stirring.

As some twentyfold concentration of the alcohol occurs during extraction into the aqueous phase, the degree of accuracy is increased and would be expected to be about $\pm$ 1 p.p.m. That such a degree of accuracy is obtained was checked by the preparation of artificial solutions of methanol in *iso*octane to cover the range 0 to 50 p.p.m. The prepared and the determined alcohol contents were as follows—

Prepared values, p.p.m.	4·8	9·6	9·8	24·5	49·0
Determined values, p.p.m. ..	4·2	9·0	10·0	22·5	49·5

From these results it can be seen that the expected order of accuracy is obtained.

The authors express their thanks to the Management of Petrochemicals Limited for permission to publish this work and to Dr. E. T. Borrows for the encouragement that has been given them to carry out the experimental work.

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THE PRESENCE OF CALCIUM *dl*-TARTRATE IN CANNED CHERRIES

AN interesting and unusual phenomenon not previously reported has been observed in cans of French cherries, containing glass-like crystals, sent to this Station for examination. From the appearance of the cans and their contents, they had been packed at least 2 years previously.

Some of the crystals were quite large, measuring as much as 12 mm in length and 5 mm in width. They were prisms, resembling calcite, very hard and insoluble, and could be mistaken for glass. A peculiar feature of the larger crystals was that they all had a concave spherical surface, corresponding with their having grown on the cherries. On examination of the contents of a newly opened can later in the investigation, it was possible to confirm this observation. Smaller crystals were found free in the liquid at the bottom of the can.

The crystals were found to dissolve without effervescence in dilute hydrochloric acid. On ignition they evolved water, swelled up considerably and charred with a smell of burnt sugar. On further heating, a white lime-like mass remained. The inorganic portion of the compound was identified as calcium by flame and precipitation tests. The combined acid was identified as *dl*-tartaric acid by ascending filter-paper chromatography, with *n*-butanol - acetic acid - water (63:10:27) as solvent and Whatman No. 541 paper ($R_F = 0{\cdot}54$). The spots were located by spraying with bromophenol blue. Confirmation of the presence of tartaric acid was obtained by the colour reactions of Steigmann[1] and Pesez.[2] There was no rotation of the plane of polarised light in a 2 per cent. solution of the crystals in 2 per cent. v/v hydrochloric acid.

Although the calcium *d*- and *l*-tartrates are not normally very insoluble in water, in the presence of both acids a much less soluble *dl*-tartrate is formed. It was concluded from this that the crystals consisted of calcium *dl*-tartrate and that the tartaric acid must have been added to the syrup on canning. The purpose of such an addition was probably to fix the colouring matter (erythrosin). Traces only of tartaric acid have been reported in French cherries,[3] which would presumably be the naturally occurring *d*-form. The source of a calcium content sufficiently high to give approximately 1·5 g of calcium *dl*-tartrate in each can is open to conjecture; a rough estimate of the hardness of the water supply necessary to provide so much calcium is 384°, which seems to rule out the water as a likely source. It is, however, possible that large quantities of calcium could be picked up on storage or treatment in concrete silos.

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THE FRUIT AND VEGETABLE CANNING
AND QUICK FREEZING RESEARCH ASSOCIATION
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H. D. FOWLER

April 5th, 1955

A NEW SPRAY REAGENT FOR PAPER CHROMATOGRAPHY OF BARBITURATES

MANY papers have been published recently about various methods for isolating and identifying barbiturates by paper chromatography. Methods for locating the spots involve colour reactions[1,2,3] or are based on the absorbent qualities of the barbiturates in ultra-violet light.[4,5] A comprehensive review of colour reagents used for the detection of barbiturates on paper has been published by Raventos.[6] We have located the spots in our laboratory by two of these methods. One is based on the fluorescent reaction, with quinine added to a modified Zwicker copper - pyridine reagent; this causes the dark spots of barbiturates to become visible against the fluorescent background. The alternative method is Zwicker's base plus cobalt nitrate reaction, which is based on a colour reaction. We have obtained the best results by combining these two methods.

The barbiturates are separated by ascending-paper chromatography on Whatman No. 1 paper impregnated with *M* potassium nitrate, *n*-butanol - pentanol - ammonia being used as the solvent, as described by Hübner and Pfeil.[3] The paper is kept in a solvent atmosphere for at least 6 hours, the chromatography time usually being 18 hours; mixtures are kept for 12 to 14 hours in a solvent atmosphere.

After development, the dried paper is sprayed with a 1 per cent. solution of cobalt nitrate in absolute ethanol, dried again and then held in ammonia vapour. If the amount of barbiturates is about 50 μg, the spots will be visible as a violet or reddish colour.

In order to achieve an exact definition of the spot limits, especially for small quantities and mixtures, we have sprayed the paper with a solution containing 200 mg of copper sulphate, 2 ml of pyridine and 20 mg of quinine per 100 ml of water. The dried paper is then held in hydrochloric acid vapour. In ultra-violet light, the barbiturates are visible against the fluorescent background either as dark blue spots or dark blue circles, with the centre parts lighter. Pentothal is indicated by a yellow-green colour in visible light. Amounts of barbiturates to 5 to 10 μg can be detected by this means. After this, the paper can be treated with potassium permanganate to indicate the unsaturated barbiturate derivatives.[1,2,3]

Using a solvent of *n*-butanol - pentanol - ammonia not more than 5 days old, at a temperature of 22° C, we have found the following R_F values: Barbitol, 0·33; Phenobarbitol, 0·42; Dial, 0·49; Phanodorm, 0·57 (with additional separation results 1 or 2 spots of R_F values 0·08 and 0·16); Evipal, 0·71; Amytal, 0·80; Nembutal, 0·80; and Pentothal, 0·83.

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April 27th, 1954

Book Reviews

SPECIAL FEDERAL FOOD AND DRUGS LAWS. Annotated by Thomas W. Christopher and Charles Wesley Dunn. Pp. xii + 1334. Chicago: Commerce Clearing House Inc. 1954. Price $17.50.

GENERAL STATE FOOD AND DRUG LAWS. Annotated by David A. Vernon and Franklin M. Depew. Pp. xii + 804. Chicago: Commerce Clearing House Inc. 1955. Price $17.50.

These are the sixth and seventh research volumes in the "Food Law Institute Series" published by the Commerce Clearing House Inc. of Chicago, and conform to the attractive pattern of the earlier works that have been reviewed here.

The Christopher - Dunn work fills an important gap in this up-to-date record of U.S. legislation, for although a working knowledge of the Federal Food Drug and Cosmetic Act is not difficult to obtain, information on allied topics calls for a time-consuming search. Here, in one volume, these miscellaneous and important sections of the Federal law are dealt with in five sections.

The first section deals with meat and gives the text, the regulations, legislative history and leading cases on the Meat Inspection Acts and the Packers and Stockyards Acts. In like manner the second section covers the Food Standards legislation of the Department of Agriculture, the U.S. Grain Standards Act, the Federal Import Milk Act, the Filled Milk Act and the legislation dealing with tea, margarine, renovated butter and certain fruits and perishable agricultural products. Of special interest is the reference to the President's power by proclamation to suspend the importation of articles adulterated to an extent dangerous to the health of the people.

Narcotic drugs are dealt with in the third section, both as regards import and export; the fourth covers drugs and chemicals generally and includes the legislation controlling biological products, the Virus-Serum Toxic Act, the Caustic Poisons Act and the Insecticide, Fungicide and Rodenticide Act.

Finally, a miscellaneous section deals with allied legislation such as that relating to Weights and Measures, Postal Regulations and Warehousing.

It is impracticable to give more than this outline of the scope of the work. As a reference volume for the lawyer or food chemist who meet matters off the beaten track, it will fill a long-felt want.

The Vernon - Depew volume compiles and annotates the general State food and drug laws that complement the Federal Food Drug and Cosmetic Act. The texts of these laws, which were dominant in their legislative areas until the Federal Act of 1906 was passed, are given for every State. Twenty-six States now follow the pattern of the current 1938 Federal Act to a

greater or less extent from the standpoint of product regulation. Clearly there still remains a lack of uniformity between Federal and State legislation, which the authors suggest constitutes a serious defect in the over-all legislative code and calls for correction.

Though this work is not likely to serve the interests of British food chemists to any great extent, it remains a valuable book of reference.

C. A. ADAMS

INDIAN FOOD LAWS. Compiled by Major N. V. R. IYENGAR, M.Sc., A.I.I.Sc., F.R.I.C., B. K. SUR, M.Sc., Ph.D., and G. T. KALE, D.Sc. Pp. viii + 221. Mysore, India: Central Food Technological Research Institute. 1954. Price Rs.4/8.

As the sub-title indicates, this work is a summary of the existing food standards and specifications adopted up to 1954 by the different States of India. The foreword indicates that the first Prevention of Adulteration Act was introduced in Bombay Province in 1899 and that similar legislation has been adopted by other States since. Of the 27 States in the Indian Union, 22 have now either their own Food Adulteration Acts (of which a complete list is given) or are applying those of neighbouring States.

The book is essentially a compilation of tables giving details of the standards adopted by the individual States. The amount of information presented is really remarkable, and the volume should establish itself as an encyclopædia on the composition of Indian foods.

The Government of India established an expert Central Committee for Food Standards in 1944, and its recommendations are bringing greater uniformity into Indian food standards. Further, the Central Government introduced a Food Adulteration Bill in 1952, with the object of enabling further progress to be made in the enforcement of food legislation in the different Provinces. It would seem that India is on the threshold of important advances in this sphere, and the production of this well indexed volume must serve a most useful purpose.

Unfortunately, we are told nothing of the manner in which enforcement is carried out, or indeed anything about the administration of food legislation generally. Clearly this would be a considerable undertaking pending the time when some greater measure of uniformity is achieved throughout the Union. It is hoped, therefore, that this volume may have a successor to complete the picture.

C. A. ADAMS

Publications Received

CHEMICAL AND SPECTROGRAPHIC ANALYSIS OF MAGNESIUM AND ITS ALLOYS. By A. MAYER, B.Sc., F.R.I.C., A.C.T. (Birm.), and W. J. PRICE, B.Sc. Pp. xiv + 250. London and Manchester: Magnesium Elektron Limited. 1954. Price 63s.

HANDBUCH DER MIKROCHEMISCHEN METHODEN. Edited by FRIEDRICH HECHT and MICHAEL K. ZACHERL. Volume II, VERWENDUNG DER RADIOAKTIVITÄT IN DER MIKROCHEMIE: RADIOCHEMISCHE METHODEN DER MIKROCHEMIE, by E. BRODA and T. SCHÖNFELD; MESSUNG RADIOAKTIVER STRAHLEN IN DER MIKROCHEMIE, by T. BERNERT, B. KARLIK and K. LINTNER; PHOTOGRAPHISCHE METHODEN IN DER RADIOCHEMIE, by H. LAUDA. Pp. iii + 423. Vienna: Springer-Verlag. 1955. Price $19.30; 138s. (To subscribers to the whole handbook, $15.45; 110s. 6d.)

GRUNDRISS DER ORGANISCHEN CHEMIE, BY OPPENHEIMER AND NAEGELI. Seventeenth Edition. By Professor Dr. OTTO NEUNHOEFFER and Dr. REINHOLD METZE. Pp. 328. Leipzig: Veb Georg Thieme. 1955. Price DM14.50.

THE PHARMACOPOEIA OF THE UNITED STATES OF AMERICA. Fifteenth Revision. Pp. liii + 1178. Easton, Pennsylvania: Mack Publishing Co. 1955. Price $10.00 in U.S.A.; $10.50 elsewhere.

THE CHEMISTRY OF SYNTHETIC DYES AND PIGMENTS. Edited by H. A. LUBS. Pp. xiv + 734. New York: Reinhold Publishing Corporation; London: Chapman & Hall Ltd. 1955. Price 148s.

Erratum

JANUARY (1955) ISSUE, p. 51, 6th and 7th lines of "REAGENTS." *For* "Dilute 75 ml of 0·1 *N* iodine solution to 250 ml with water" *read* "Dilute 75 ml of 0·1 *N* aqueous iodine solution to 250 ml with *iso*propanol."

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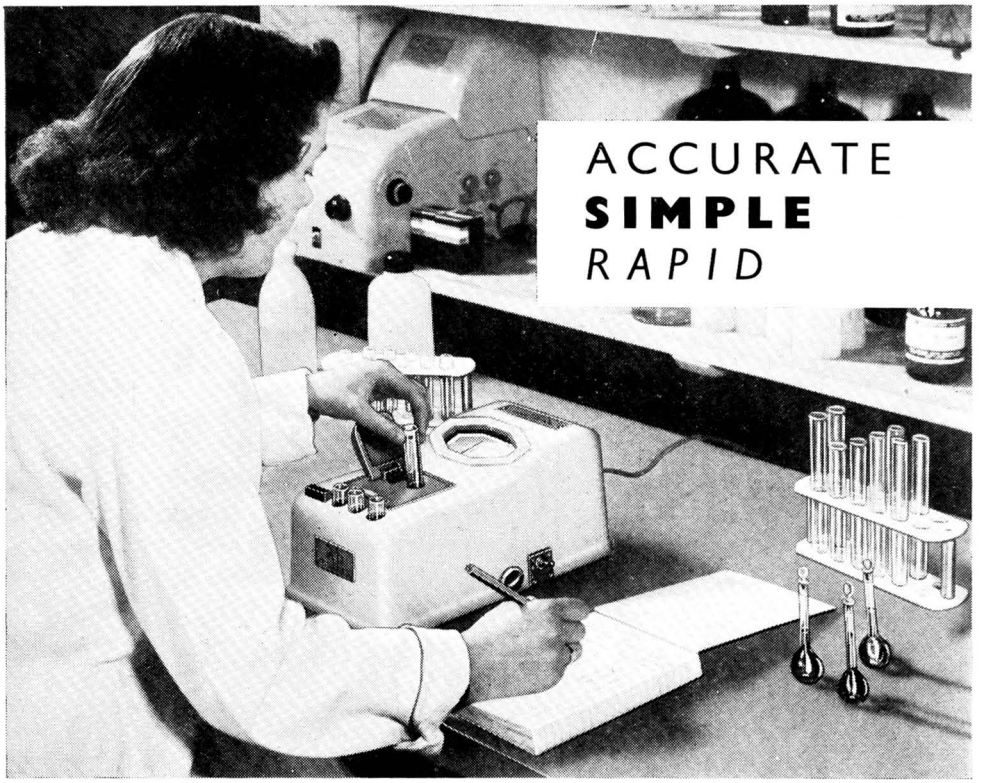

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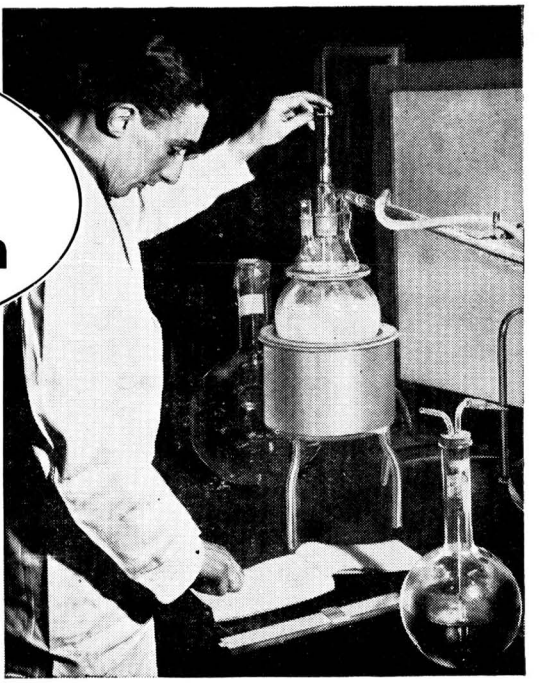

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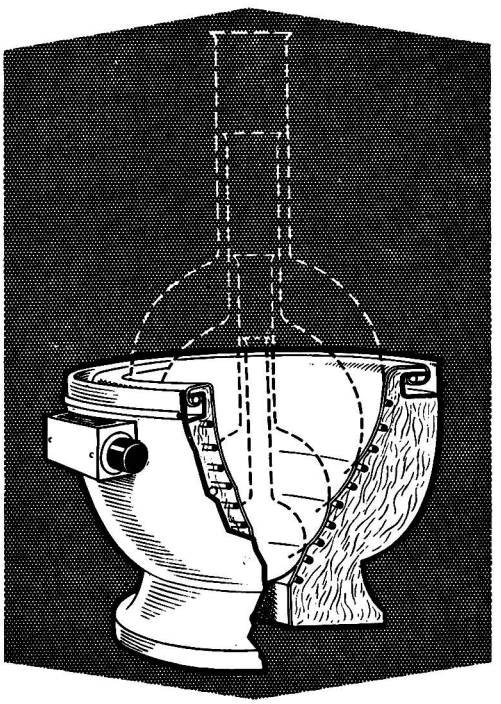

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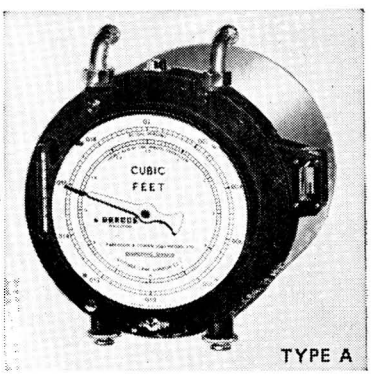

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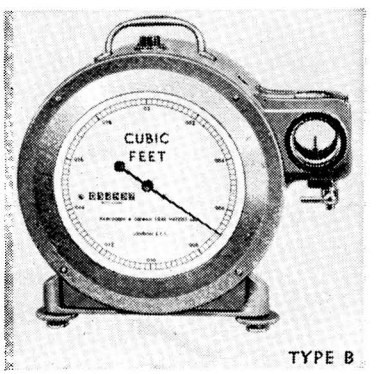

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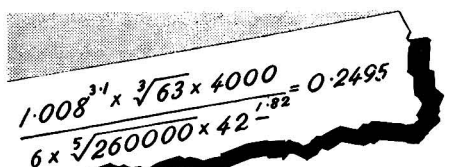

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H. J. Cluley, Determination of K. its application to Silicate analysis. *Analyst*, **80**, *354-364* (May 1955).

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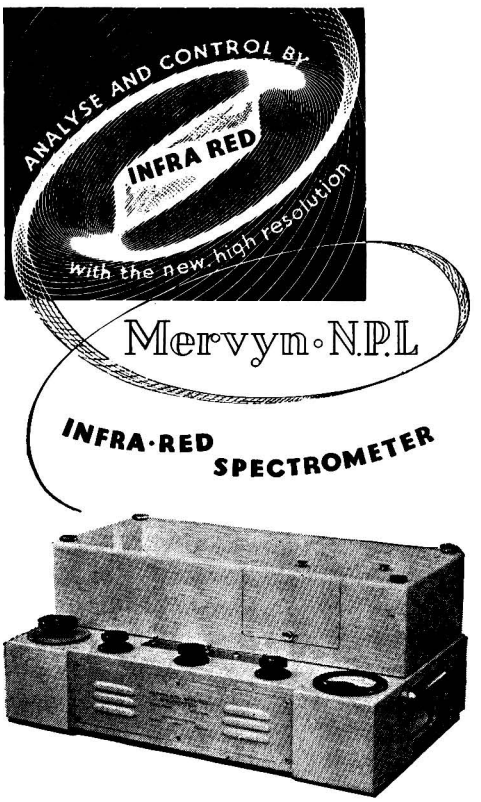

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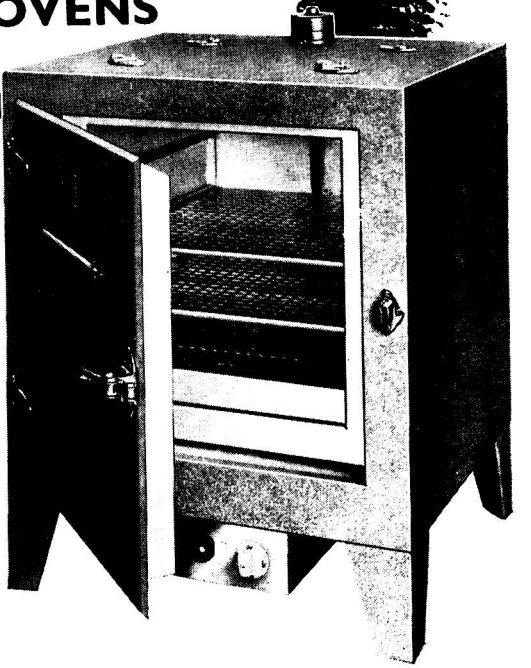

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