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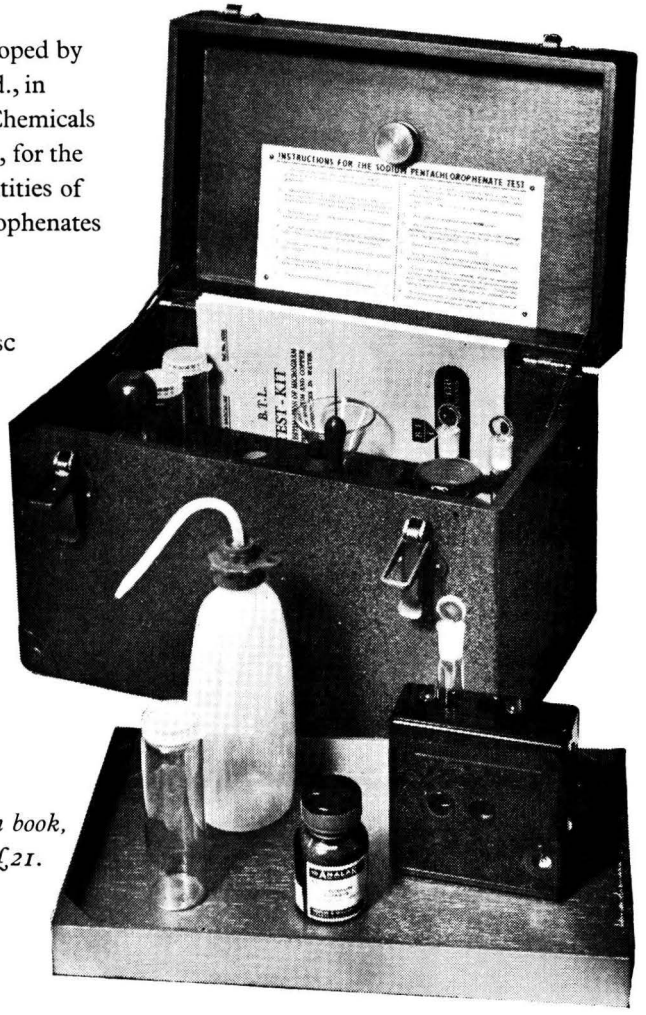

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THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

An Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, October 7th, 1959, in the meeting room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Mr. R. C. Chirnside, F.R.I.C.

The subject of the meeting was "Atmospheric Pollution Analysis" and the following papers were presented and discussed: "Analytical Aspect of the Control of Industrial Atmospheric Contamination," by J. C. Gage, B.Sc., Ph.D., F.R.I.C.; "The Determination of Beryllium Metal in Air," by R. J. Powell, B.Sc., P. J. Phennah and J. E. Still, B.Sc., F.R.I.C.; "The Determination of Atmospheric Polycyclic Hydrocarbons," by B. T. Commins, M.Sc., A.R.I.C.

MICROCHEMISTRY GROUP

A Joint Meeting of the Microchemistry Group with the Thames Valley Section of the Royal Institute of Chemistry and the London Section of the Society of Chemical Industry was held at 6.30 p.m. on Friday, October 2nd, 1959, in the Inorganic Chemistry Lecture Theatre of the University of Oxford. The Chair was taken by Dr. F. M. Brewer, M.B.E., M.A., F.R.I.C.

A lecture on "Quantitative Commonsense and the Chemist" was given by E. C. Wood, B.Sc., Ph.D., A.R.C.S., F.R.I.C. (see summary below).

The meeting was preceded at 1.45 p.m. by a visit to the Chemistry Division of the Atomic Energy Research Establishment, Harwell.

Quantitative Commonsense and the Chemist

Dr. E. C. Wood began by contrasting the attitude of mind of the classical analyst, who regarded precision as an end in itself and experimental "error" as a flaw to be eliminated, with that of the statistician, who accepted the variability of experimental observations as inevitable. No single analytical result could have meaning in itself; there must be knowledge of the variability of that kind of determination, and this could be obtained only by replication. Replicates to be of value must be independent, and there was grave risk of erroneous conclusions when so-called "duplicate" determinations were made by the same analyst on the same sample, perhaps even on the same day. Dr. Wood explained what true independence meant and how this could be achieved by adequate randomisation.

He then demonstrated the merits of the "factorial" type of experimental design by reference to published analytical research papers. Recent developments in design included sequential types in which the results of the first few analyses provide a basis for deciding both how many more observations to make and also the best experimental conditions for making them, so that no unnecessary work is done.

Finally, he dealt with the vexed question of rejecting apparently anomalous results from a series of observations and made the point that the problem was not so much one of statistics as of ethics.

Obituary

THOMAS WHITTAKER LOVETT

THOMAS WHITTAKER LOVETT died on July 6th, 1959, at the age of 66, after a few weeks' illness. He is survived by his widow and a married daughter.

He was a native of Salford, where he worked all his life. In 1907 he joined the firm of Carter Bell and Melling (later Melling and Ardern), Consultant Analysts, as a lab. boy, and through merit and hard work he rose to become head of the firm in 1954. His flair for agricultural analysis earned him locally the unofficial title of "Farmer's Chemist": his formal appointments were Public Analyst and Official Agricultural Analyst for the County Boroughs of Oldham and Rochdale and the Borough of Ashton-under-Lyme.

Tom Lovett joined our Society in 1932 and took a particular interest in the activities of the North of England Section. He was more than once a member of the Committee and became Chairman of the Section in 1953–54, an appointment he held with distinction. Despite his many commitments he still found time for non-professional activities: he was, for example, a member of the Manchester Rifle Club, a Home Guard during the last war, and Chairman of the local Bowling Club.

Throughout his career, Tom Lovett's interests were shared very fairly and happily between his family, his work and his friends. There has never been a more united family, and Mrs. Lovett herself took over much of her husband's burden when his health failed. He never really recovered form the shock of losing his elder daughter in 1957.

As a man, Tom Lovett was utterly without guile, entirely dependable, and kind to a fault. He was especially helpful and patient in his guidance of any youngster joining his firm. One of them writes: "In my early years I had endless help and encouragement from him and I can recall many occasions when he would go out of his way to save my youth and inexperience—and, no doubt, unjustified self-confidence—from near disaster. I don't think he ever refused a helping hand to anyone."

Tom Lovett understood people. We shall all miss him.

J. R. EDISBURY

Gas Chromatography and its Analytical Applications

A Review

BY B. A. ROSE

(Department of Scientific and Industrial Research, Laboratory of the Government Chemist, Clement's Inn Passage, Strand, London, W.C.2)

SUMMARY OF CONTENTS

CHROMATOGRAPHY, like distillation, is a method of separating the components of a mixture by utilising their different migration velocities through a column. In both processes the separation achieved depends on the distribution of the components between a moving phase and a stationary phase. In gas chromatography, the phases consist of an inert gas and a solid, or non-volatile liquid, whereas in distillation they consist of the vapour and liquid phases of the mixture itself.

The extension of chromatography to include systems in which the mobile phase is a gas was first suggested by Martin and Synge in 1941.[1] At that time the detection of small

amounts of foreign vapours in the emerging carrier gas was so difficult that the idea was not then further developed. When the problem of detection had been solved—about 10 years later[2,3,4,5]—the technique was developed rapidly; the number of papers so far published on gas chromatography is well over 500, and complete apparatus for gas chromatography are now marketed by several scientific-instrument manufacturers.

Gas chromatography can be subdivided, according to the nature of the stationary phase, into gas-adsorption (solid stationary phase) and gas - liquid partition (liquid stationary phase) chromatography. The earliest work was of the former type, and two methods of chromatogram development were in use, displacement and elution. The elution technique is analogous to liquid - solid chromatography; the sample, introduced at the beginning of a column of stationary phase, is carried through the column by an inert gas. The ingredients of the sample travel through the column at different rates, according to their respective distribution coefficients between the gas and solid phases, and so emerge at different times after the introduction of the sample. In displacement development, the sample ingredients are displaced from the adsorbent by a displacer vapour, which is carried continuously on to the column at constant concentration in the gas stream. The displacer is chosen so that it is more strongly adsorbed than any of the ingredients of the sample to be analysed. This technique leaves the column saturated with the displacer after an analysis, and it must therefore be re-packed, or desorbed by heating, before being available for another analysis. The elution technique leaves the column clean and ready for immediate re-use; displacement has no compensating advantages over elution and is now seldom, if ever, used.

In gas - liquid partition chromatography the stationary phase is a non-volatile liquid absorbed on an inert powder, such as kieselguhr, as support. The chromatogram is developed by elution, the ingredients of the sample being separated according to their distribution coefficients between the two phases, *i.e.*, each travels along the column at a rate depending on its volatility and solubility in the liquid phase. A gas - liquid column was first described by James and Martin[2] and probably represents the most important advance in gas chromatography, because it enormously increases the scope and versatility of the technique. At present, gas - liquid columns are used in almost all analyses, except those of mixtures of the permanent gases; for these, gas-adsorption columns are often used.

When a mixture has been separated into its ingredients in the column, some means of detecting and timing the emergence of each ingredient with the carrier gas must be provided. Several detection systems have been used and can be divided into two classes, differential and integral.

A differential detector is sensitive to a property of the carrier gas, the value of which is changed by the presence of an impurity; ideally the property chosen should be a linear function of the concentration of impurity, and the detector response should also be linearly related to the operative property of the gas. Properties that have been utilised in detectors are density,[6] thermal conductivity[7,8] heat of combustion[9,10] and ionisation under various conditions.[11,12,13,14,15] A typical chromatogram obtained by using a differential detector is shown in Figure 1.

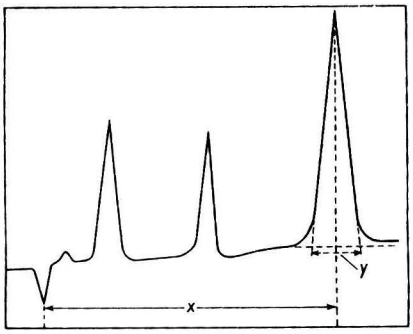

Fig. 1. Typical chromatogram from a differential detector.

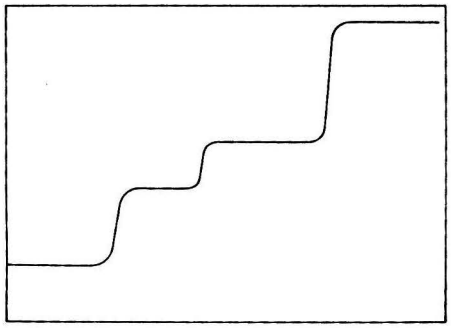

Fig. 2. Typical chromatogram from an integral detector

An integral detector is unaffected by the carrier gas, but collects or reacts with the eluted vapours to give a reading proportional to the total amount of vapour eluted. Examples

are the automatic titration cells used by James and Martin[2,16] and Liberti[17] and the nitrometer used by Sevenster.[18] The last-named is applicable to permanent gases only; carbon dioxide is used as carrier gas and the eluted gases are collected over sodium hydroxide solution, which absorbs the carbon dioxide. Fig. 2 shows how the chromatogram in Fig. 1 would appear if an integral detector were used.

Quantitatively, the total amount of a vapour eluted is measured by the area under a peak when a differential detector is used or the height of the corresponding step when an integral detector is used.

Nearly all gas chromatography is now carried out with differential detectors; integral detectors need cleaning and replenishing after each chromatogram has been obtained, whereas differential detectors are always ready for use. Further, consideration of Figs. 1 and 2 will show that, when an integral detector is used, the sensitivity must be such that all the components of a sample can be accommodated in a single chart-width; with the differential system, the entire width of the chart is available for each peak and higher sensitivities can be used.

In the rest of this review, it will be assumed that a differential-type detector is in use with a gas - liquid column, unless otherwise stated.

Theory

The theoretical plate—

The analogy between gas chromatography and fractional distillation has already been indicated. The behaviour of a chromatographic column can be described theoretically, in a similar way to that of a distillation column, in terms of the theoretical plate; such theoretical treatment was first applied by Martin and Synge[1,19] and by James and Martin.[2] If it is assumed that, when a substance is introduced at the beginning of the column, it dissolves in the stationary phase, then, by the time it has travelled a short distance along the column, its partial pressure in the gas phase will be equal to its vapour pressure over its solution in the stationary phase. The length of column required to establish this equilibrium is analogous to the theoretical plate in a distillation column, and this length is called the height equivalent to a theoretical plate. Martin and Synge treated the column as a series of such consecutive plates; chromatography was imagined to proceed by successive additions of equal volumes (δv) of solvent or gas to the column, which was allowed to rest after each addition until equilibrium was re-established. Glückauf[20] has given a theoretical treatment in which the process is assumed to be continuous rather than stepwise; the final result is essentially the same, but more diffuse bands are predicted by the "continuous" treatment, other things being equal.

It can be shown that any substance obeying Henry's law in relation to the stationary phase will emerge from the column as a band, thereby producing a symmetrical peak on the chromatogram. Also, the ratio of the times taken by two substances to pass through the column—their retention times—will be approximately inversely proportional to the ratio of their vapour pressures over their solutions of equal molar concentration in the stationary phase. Hence, when a stationary phase is used such that solubility and volatility are the only factors involved, the components of a mixture will emerge in order of their boiling-points. In practice, other factors affect the retention time, notably the polarities of the stationary phase and of the sample and, sometimes, specific interaction between a stationary phase and a particular sample ingredient. The order of emergence of the ingredients of a given mixture will therefore be different with different stationary phases; when a highly polar stationary phase, such as polyethylene glycol 400, is used, ingredients will tend to be eluted in order of polarity.

The degree of separation of two ingredients depends on the sharpness of the peaks and their distance apart. The distance apart depends on the retention times of the substances involved, but the sharpness of the peaks is a function of the efficiency of the column. The efficiency can be calculated in terms of the theoretical-plate content of the column by using the formula derived by James and Martin[2] and recommended by the Hydrocarbon Research Group of the Institute of Petroleum.[21] With reference to the last peak in Fig. 1, tangents are drawn to the peak at the points of inflexion. The distance between the points at which these tangents cut the base line, y, is measured and also the distance, x, from the start to the centre of the section y. The number of theoretical plates, N, is given by the equation—

$$N = \frac{16\,x^2}{y}$$

Factors affecting efficiency

Reference has already been made to the mathematical analysis of the phenomena inside a gas-chromatographic column by Martin and Synge and by Glückauf. The analysis has been continued by van Deemter, Zuiderweg and Klinkenberg,[22] who showed that the height equivalent to a theoretical plate, h, was a function of the linear velocity of the carrier gas, the function being of the form—

$$h = A + \frac{B}{u} + C\,u$$

or more fully—

$$h = 2\lambda\, d_p + \frac{2\gamma\, D_{gas}}{u} + \frac{8k\, d_f\, u}{\pi^2 (1 + k)^2\, D_{liq.}}$$

where u is the linear velocity of the carrier gas, λ is a constant depending on the regularity of packing, γ is a constant depending on the tortuosity of paths in the column, d_p is the diameter of the particles of packing, d_f is the thickness of the liquid film on the particles, D_{gas} and $D_{liq.}$ are the diffusion coefficients of the vapour in the gas and liquid phases and k is the column partition coefficient, *i.e.*, the ratio of the number of moles of vapour per unit length in the liquid phase to the number of moles of vapour per unit length in the gas phase.

The height equivalent to a theoretical plate therefore decreases from a high value at low rates of flow to a minimum at $u = \sqrt{(B/C)}$ and then increases more or less linearly with flow rate. Maximum efficiency is attained by keeping the coefficients A, B and C as small as possible.

When the stationary phase is supported on Celite, the value of A can be decreased by reducing the particle size down to about 100 mesh; further reduction in size makes the material difficult to pack evenly, and the value of A tends to increase as a result of an increase in λ.

The value of B can be reduced by decreasing D_{gas} through use of a denser carrier gas, *e.g.*, nitrogen instead of hydrogen or helium; it will be necessary in choosing the gas to consider also the effect of the nature of the gas on the sensitivity of the detector.

The value of C can be reduced by using a low-viscosity liquid phase, provided that the volatility is sufficiently low, or by reducing the film thickness. The latter may be achieved by reducing the particle size—but not below the optimum value for coefficient A—or by reducing the ratio of liquid to solid, in which event the size of sample must also be reduced to avoid overloading. A decrease in the column temperature increases the solubility of vapours in the liquid phase and so reduces the value of C.

These deductions from van Deemter, Zuiderweg and Klinkenberg's equation are confirmed by experimental study of the influence of operational factors on column efficiency.[23,24]

Effect of particle size—

Cheshire and Scott,[24] who used crushed C22 furnace brick as stationary-phase support, found that the particle size can be reduced below 100 mesh, with a further increase in efficiency; this is because the difficulty of achieving regular packing, referred to above, does not arise with this material. They further established that, whatever particle size is chosen, the range must be as narrow as possible. For instance, they found that 100 to 200-mesh material gave a similar efficiency to that of 60 to 100-mesh material at flow rates up to 30 ml per minute in a 5-foot column; at higher flow rates, the efficiency of the 100 to 200-mesh filling decreased more rapidly than that of the 60 to 100-mesh filling, and both gave lower efficiencies than did 100 to 120-mesh material.

Effect of ratio of liquid to solid—

De Wet and Pretorius,[23] who used liquid paraffin on Celite as stationary phase and a mixture of *n*-pentane and *n*-hexane as test sample, found that the optimum liquid content of the stationary phase was 30 per cent. by weight. Cheshire and Scott,[24] who used Apiezon oil A on crushed furnace brick, investigated the effect of the liquid content of the stationary phase in rather more detail. They determined the optimum flow rate for columns containing 2·5, 5, 10, 15, 20, 25 or 30 per cent. w/w of liquid in the stationary phase and then chromatographed a twelve-component mixture on each column at its optimum flow rate. They then calculated the column efficiency in theoretical plates for each component and plotted efficiencies against the corresponding elution times for each column. Their curves show that, for stationary phases containing 10 to 30 per cent. w/w of liquid, the efficiency increases

rapidly with elution time for about the first 5 minutes, with subsequent comparatively slow increases. The maximum efficiency in this range was given by the column containing 20 per cent. of liquid in the stationary phase. When the liquid content was reduced to 5 per cent. or less, however, the rapid rise in efficiency with elution time did not decrease, and after the first 5 minutes it greatly exceeded that of the column containing 20 per cent. of liquid. To obtain the maximum efficiency from any column, overloading with sample must be avoided; the maximum sample load for the column containing 20 per cent. of liquid was about 4 mg, but when the liquid content of the stationary phase was reduced to 5 per cent. the maximum load was 1 mg on a 5-foot column or 0·4 mg on a 25-foot column. Loads in excess of these amounts caused asymmetric peaks and consequent overlapping and low efficiency; the effect is probably due to the sample vapour penetrating the liquid film and becoming adsorbed on the solid support. Cheshire and Scott came to the following conclusions—

(*i*) high-efficiency columns for liquids of high boiling-points should consist in short columns (4 to 5 feet) operated at comparatively low temperature with a low ratio of liquid to support (5 per cent.);

(*ii*) high-efficiency columns for liquids of low boiling-points can be obtained by increasing the length to give a longer chromatogram, the same low ratio of liquid to support being used;

(*iii*) when the stationary phase contains 5 per cent. of liquid, the efficiency is proportional to the length of the column. This is not so when the stationary phase contains 20 or 30 per cent. of liquid.

At present, the tendency in this country is to develop high-efficiency columns along the lines indicated above; 4- or 5-foot columns, either straight or U-shaped, are common, and efficiencies up to 1000 plates per foot have been achieved.

The normal practice in the U.S.A. is to use column fillings containing 20 to 30 per cent. of liquid and lengths between 25 and 50 feet. For obvious reasons, such columns are helical; they are prepared by packing the appropriate straight length of copper tubing and subsequently coiling it on a mandrel. Opinion is divided as to whether coiling a column has any effect on its efficiency. It is possible that particles of packing may be broken during coiling, so extending the range of particle sizes in the column and impairing the regularity of the packing. It is probably advisable to avoid coiling and to build up long columns by mounting shorter (4 to 6-foot) lengths side by side and connecting them in series by means of capillary tubing. The last-named method was used by Evans and Tatlow[25] in constructing preparative-scale columns.

COATED-CAPILLARY COLUMNS—

An ordinary packed column offers several alternative paths for the carrier gas between the particles of packing. If the alternative paths differ in length, the gas-transit times by different paths will vary accordingly, and peaks will tend to spread. This does, in fact, happen if the column packing is not regular. If, therefore, a column were designed with only one possible gas path, sharper peaks and better resolution would be expected. A column consisting of a length of capillary tubing coated on the inside with the stationary-phase constitutes such a one-path column; the tube itself, which may be of glass or metal, is the only support for the liquid phase. The use of coated capillaries has been described by Dijkstra and de Goey[26] and by Desty, Goldup and Swanton.[27] The latter's paper demonstrates that the coated-capillary column gives much better resolution; chromatograms indicating the separation of the xylene isomers on 7:8-benzoquinoline are shown, both a packed column and a capillary column being used.

For the capillary column, resolution was sufficiently sharp for the chromatogram to return to the base line between peaks; resolution of *m*- and *p*-xylene of this order has not been reported on any packed column.

Coated-capillary columns are limited to an even smaller sample load than are packed columns having low liquid contents, the limit being about 1 μg as compared with 0·4 to 1 mg for a packed column containing 5 per cent. of liquid or up to 10 mg for a packed column containing 20 per cent. of liquid.

The choice of a detector for a high-efficiency column will be considered in the section dealing with detectors.

STATIONARY PHASES

Stationary phases, often referred to as "column packing," are of two kinds (*a*) solid adsorbents and (*b*) non-volatile liquids held on an inert solid support.

SOLID ADSORBENTS—

Four adsorbents have so far been found to give satisfactory results—activated alumina, silica gel, activated charcoal and, more recently, Linde molecular sieves. The last-named material includes a range of synthetic zeolites having a uniform pore-size, each catalogued type having a different pore-size[28]; in gas chromatography, vapours are separated by these adsorbents according to molecular size.

In general, the peaks obtained from adsorption columns tend to have sharp fronts and diffuse tails, as shown in Fig. 3, whereas the same vapours chromatographed on liquid columns

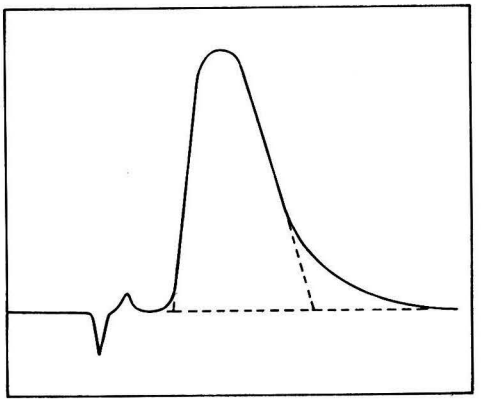

Fig. 3. "Tailing" peak

would give symmetrical peaks. The asymmetric shape of adsorption peaks leads to overlapping and consequent difficulties in their quantitative interpretation; this point is referred to in more detail under "Quantitative Analytical Calculations," p. 587. For this reason, liquid columns are used whenever possible and were in fact used for the analysis of gaseous hydrocarbons in 1956 by Fredericks and Brooks,[29] who appear to have been the first workers to apply gas chromatography to gas analysis.

The only gases that have not been chromatographed satisfactorily on liquid columns are the permanent gases, oxygen, hydrogen, nitrogen, etc. Mixtures containing these gases are usually examined on at least one solid adsorption column. For instance, a 6-foot column of silica gel was used by Szulczewski and Higuchi[30] to separate a mixture of oxygen, nitrogen, nitrous oxide, carbon monoxide and carbon dioxide. The technique was to operate the column in a bath of solid carbon dioxide and acetone until carbon monoxide emerged and then to increase its temperature to that of the room for the elution of nitrous oxide and carbon dioxide. They found it impossible to separate the permanent gases on silica gel at room temperature. Wencke[31] claims to have separated the permanent gases at room temperature on a 2-metre column of activated charcoal, although the results for carbon dioxide are not satisfactory, because this gas is desorbed too slowly and irregularly.

Greene and Roy,[32] who used a charcoal column, found that the retention times of gases depended on the nature of the carrier gas and were reduced by the use of a carrier gas that can be adsorbed by the column packing. This suggests that the column functions partly as an elution column and partly as a displacement column; no such phenomenon has been reported in connection with gas - liquid chromatography. The relative extents to which elution and displacement determine retention times may well vary from batch to batch of charcoal and with the method of activation used.

Adsorption columns have been used for hydrocarbon analysis. Patton, Lewis and Kaye[33] found that silica gel and alumina both gave satisfactory results; substances were eluted from both in the same order, but the retention times were longer when silica gel was used. They also found that the adsorptive properties of alumina depended on its water content; for the most efficient and reproducible separations, the alumina should be dried overnight by passing the carrier gas through it at the temperature at which it is subsequently to be used.

Turkel'taub, Kolyubyakina and Selenkina[34] observed a similar influence of moisture in connection with silica gel. They found that silica gel gave poor separation of gases when thoroughly dry and that the addition of a little moisture improved its performance; the optimum amount of moisture varied from batch to batch of gel.

The use of molecular sieves is a comparatively recent development. Kyryacos and Boord[35] used a 16-foot column of Linde molecular sieve type 5A for separating mixtures containing hydrogen, oxygen, nitrogen, methane and carbon monoxide. Their chromatograms have symmetrical peaks, free from the "tailing" commonly associated with gas-adsorption chromatography.

A molecular-sieve column has also been successfully used by Timms, Konrath and Chirnside[36] for determining impurities in supplies of carbon dioxide for use in gas-cooled nuclear reactors; the impurities normally present are hydrogen, oxygen, nitrogen, argon, methane and carbon monoxide in the range 10 to 3000 p.p.m. Two important points are mentioned by these workers. Molecular sieves are de-activated by moisture because this cannot be desorbed except by ignition (as in the process of manufacture of the sieves); polar compounds generally are strongly adsorbed, and, of these, carbon dioxide in particular is only slowly desorbed. In the analysis referred to, the samples were freed from carbon dioxide and moisture and only the impurities were carried on to the column.

Vizard and Wynne[37] recently reported the use of a molecular-sieve column for determining argon in the presence of oxygen in gas mixtures.

Molecular sieves have been shown by Brenner and Coates[38] and by Whitham[39] to exhibit a selective, irreversible adsorption of *n*-paraffins in mixtures of *n*-paraffins, *iso*paraffins, naphthenes and aromatic hydrocarbons in petrol, kerosene and gas oil. They fitted a short (10-cm) molecular-sieve column immediately before a liquid column in order to remove paraffins before samples reached the liquid column. By chromatographing a sample on the liquid column with and without the preliminary molecular-sieve column, those peaks on the "normal" chromatogram that were suppressed by the sieve column could be identified as being produced by *n*-paraffins.

The phenomenon just mentioned indicates that molecular-sieve columns must be used with caution when samples of unknown composition are being examined. Such samples must be examined on at least one column other than a molecular-sieve column, otherwise there is a risk of some ingredients being missed altogether.

As the properties of adsorbents depend to a considerable extent on their previous treatment, gas-adsorption retention data from different laboratories may not be strictly comparable.

NON-VOLATILE LIQUIDS—

By far the greater part of gas-chromatographic analysis is at present carried out by using columns filled with an inert, *i.e.*, non-adsorbent, powder impregnated with a non-volatile liquid. A survey of the literature reveals a bewildering number and variety of stationary-phase liquids used by various workers. However, all these liquids can be classified by their gas-chromatographic behaviour into three groups (*a*) non-polar, comprising high-boiling paraffins, Apiezon oils and grease and silicones, (*b*) moderately polar, including esters of high-molecular-weight alcohols and high-boiling alcohols themselves, and (*c*) strongly polar, such as the polyglycols and their esters.

The choice of a stationary phase depends on the type of vapours to be separated and the temperature range over which the column is required to operate. When a wide field of analysis has to be covered, involving both identification and quantitative determination of ingredients of mixtures, a battery of columns is needed. The basic requirement, as will be shown under "Analytical Applications," is for a column of at least one liquid from each class just mentioned, to cover the widest possible range of temperature. The lower limit of temperature for any given liquid is its melting-point, which is above room temperature for some of the greases and waxes. The upper limit of temperature depends on the vapour pressure of the liquid and is now regarded[40] as the temperature at which the loss by volatilisation is 1 mg per hour at the rate of gas flow normally used. A useful rule-of-thumb derived from this fixes the upper limit of temperature for any liquid at about 70° C below its high-vacuum boiling-point (*i.e.*, at a pressure of 0·1 to 0·5 mm of mercury). This is more stringent, but safer and more realistic, than the limit suggested by Harvey and Chalkley,[41] *i.e.*, 100° C below the boiling-point at atmospheric pressure.

It is highly desirable that there should be some stationary phases in general use, so that retention data obtained by different workers may be comparable and—most important to the analytical chemist—so that eventually Tables of relative retention volumes (see "Qualitative Analytical Applications," p. 587) of substances on a limited number of generally used stationary phases may be compiled for the identification of unknown compounds. In order that this object may be achieved, it is necessary to reduce the number of stationary-phase liquids in general use; also, when possible, the liquids chosen should be definite chemical compounds, such as squalene (2:6:10:15:19:23-hexamethyltetracosane), rather than mixtures of indeterminate composition, such as liquid paraffin B.P., vacuum-pump oil or lubricating oil. The use of definite compounds should minimise batch-to-batch variations in gas-chromatographic properties.

A useful general-purpose battery of columns would consist of columns containing the substances shown in Table I.

TABLE I

SUBSTANCES FOR USE IN A GENERAL-PURPOSE BATTERY OF COLUMNS

Type of substance	Substance	Range of column temperature, °C
Non-polar	Squalene	20 to 160
	Apiezon L grease	100 to 250
Moderately polar	Dinonyl phthalate	20 to 130
	Dinonyl sebacate	20 to 160
Strongly polar	Polyethylene glycol 400	20 to 150

Squalene would replace the liquid paraffin hitherto favoured by many workers, for the reason already stated. Apiezon L grease is an indeterminate mixture, but its use is suggested for high-temperature work because there is no suitable material of definite composition at present available.

Dinonyl phthalate has already become recognised as a good general purpose liquid and is the stationary phase most frequently described in the literature. It has been used for separating mixtures of hydrocarbons, alcohols, aldehydes, ketones and esters.[42,43] Octyl, decyl and dodecyl phthalates have also been used, but these have practically the same properties as has dinonyl phthalate. Dinonyl sebacate was first used by Haslam and Jeffs[44] in order to separate benzene from methyl*cyclo*hexane, both of which were eluted together from dinonyl phthalate. They pointed out that, although it had essentially similar characteristics, dinonyl sebacate, owing to its lower volatility, should be usable at temperatures 20° to 30° C higher than those for dinonyl phthalate. Haslam, Hamilton and Jeffs subsequently used it in other work on the analysis of acrylic polymers.[45]

A useful investigation of the selective properties of many stationary phases was reported in 1958 by Tenney.[46] In his paper is a list of stationary phases recommended for separating organic compounds according to type within a reasonable boiling range. Of the liquids listed, 2:2′-oxydipropionitrile offers considerable promise as an extra column for "type" separations; it can be used for separating paraffins, olefines, naphthenes and aromatic hydrocarbons from one another and for separating ethers from other oxygen-containing compounds, ketones from esters and ketones from aldehydes.

At times, specific separations require the use of special stationary phases; for example, 7:8-benzoquinoline[27] and di-*n*-propyltetrachlorophthalate[47] have been successfully used in the separation of *n*- from *p*-xylene, and diethylene glycol adipate polyester[48] and 1:4-butanediol succinate polyester[49] in the separation of mixtures of fatty acids.

SOLID SUPPORTS—

It is important that the gas-chromatographic properties of the liquid phase should not be masked or modified by adsorption effects caused by the solid support. As even "inert" solids exhibit some adsorption effects, the particles should be coated with such a thickness of liquid that components do not diffuse through to the support during their residence in any portion of the liquid.

Two support materials are in general use—kieselguhr and crushed furnace brick, made from kieselguhr. Either material must be carefully size-graded for the reasons mentioned earlier. Both materials are readily available in this country and in the U.S.A.; they are marketed, respectively, as Celite 545 and C22 furnace brick and are obtainable in this country from the Johns-Manville Co. Ltd., 20 Albert Embankment, London, S.E.11.

Both materials need preliminary treatment, first with concentrated hydrochloric acid and then with sodium hydroxide solution, to remove alumina and silica, which would give rise to adsorption effects.

Crushed furnace brick has one advantage over Celite in that when impregnated with liquid it is more free-flowing, less susceptible to caking and easier to pack evenly. Columns packed with furnace brick also offer less resistance to gas flow; consequently, a smaller pressure drop along the column is required to maintain a reasonable flow rate. This in turn should lead to a high efficiency for a column of given length, efficiency being a function of the linear flow rate of the carrier gas, which increases as the pressure drops along the column. By reducing the ratio of the inlet and outlet pressures, a greater proportion of the length of the column will operate at or near the optimum linear flow rate.

There is considerable controversy about the relative merits of Celite and furnace brick, apart from the mechanical advantages of the latter. It is interesting to refer to a comparison of the operating characteristics of these materials presented at the 1958 Symposium and the discussion thereon.[50] It appears that there is little to choose between these supports when hydrocarbon samples are being examined, but Celite is to be preferred for the examination of oxygenated or polar substances; it also appears that furnace brick contains active sites on the surface that are not removed by the acid and alkali treatment and may adsorb or even cause catalytic decomposition of oxygenated materials. The result is that peaks become broadened and shortened and sometimes even disappear. For the construction of a general-purpose column it would be safer to use Celite as the support.

Other solid supports have been used. Cropper and Heywood[51] used sodium chloride in columns for work at relatively high temperature. Powdered glass has been used by Liberti, Cortoni and Pallotta,[52] and a low-resistance column was described by Sørensen and Søltoft,[53] in which 1·6-mm × 1·6-mm rings of stainless-steel gauze ("Dixon rings") were used as the support.

PREPARATION OF A COLUMN—

The procedure used in the Government Laboratory for the preparation of a gas - liquid chromatographic column is as follows.

Sift the crushed furnace brick or Celite to a narrow size range, *e.g.*, 60 to 80, 80 to 100 or 100 to 120 mesh. Treat the powder overnight with concentrated hydrochloric acid, separate the powder by filtration, and wash until free from acid. Then treat the powder overnight with 8 per cent. w/v sodium hydroxide solution, separate it by filtration, and wash until free from alkali. Dry the powder for 6 hours at 120° C and then for 2 hours at 300° C. Sift the powder with the finer of the sieves originally used to remove any "fines" produced during the above-mentioned treatment.

Weigh the powder, and then weigh out the appropriate amount of the stationary-phase liquid chosen. Dissolve the liquid in sufficient volatile solvent to cover the powder; recommended solvents are petroleum ether for squalene, liquid paraffin B.P. or Apiezon grease, and acetone for polyethylene glycol or high-boiling esters or alcohols. Add the powder to the solution, and stir the slurry continuously until the solvent has evaporated. Dry the impregnated powder for 3 hours at 100° C, and sift again to remove any "fines" produced during stirring.

To fill U-*shaped glass columns*—Fill each limb by pouring in the impregnated powder, and tap the column vigorously with a "mallet" consisting of a rubber stopper on a glass rod until the powder will not pack down any further. Pour more powder into each limb, and insert a plug of woven-glass wick into one end. Connect the other end of the column to the carrier-gas supply, turn on the gas, and adjust the pressure to 15 lb per sq. inch at the inlet to the column. With the gas flowing under these conditions, tap the column vigorously again until no further packing down occurs. The filling is always found to pack down more closely under pressure, but there is no record of any other workers using this additional packing stage. Finally, disconnect the gas supply and add a ½-inch layer of plain Celite at the inlet end; in use, the sample pipettes will discharge their contents on to the Celite.

To fill metal columns—This procedure is simpler, as the column consists of a straight length of ¼-inch bore copper tubing. Plug one end with woven-glass wick, fill the tube with impregnated powder, and proceed as before. When the column has been filled, coil it on a mandrel or bend it in the centre, according to whether a U-shaped or helical column is required.

Sample-injection systems

Gas samples—

The general method of introducing a gas sample on to a column is to use a U-tube with a by-pass; multi-way taps are fitted so that, while carrier gas is flowing through the by-pass, the U-tube can be filled with the gas to be examined. Then, by turning two taps, the by-pass is isolated and the carrier gas directed through the sample U-tube. A typical arrangement is shown in Fig. 4.

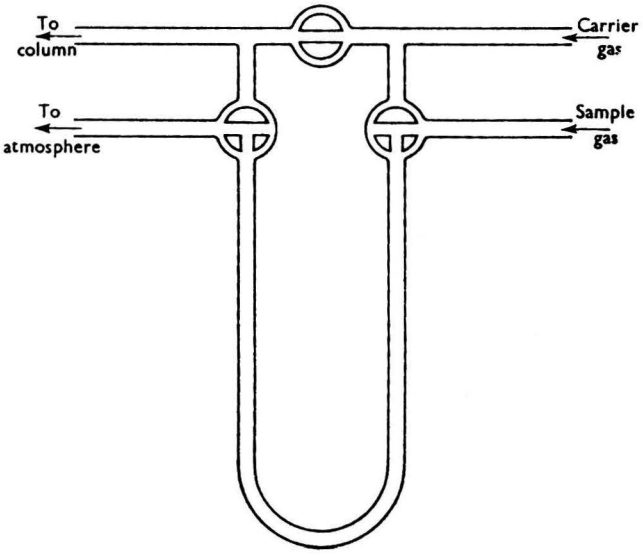

Fig. 4. Gas sample introduction system

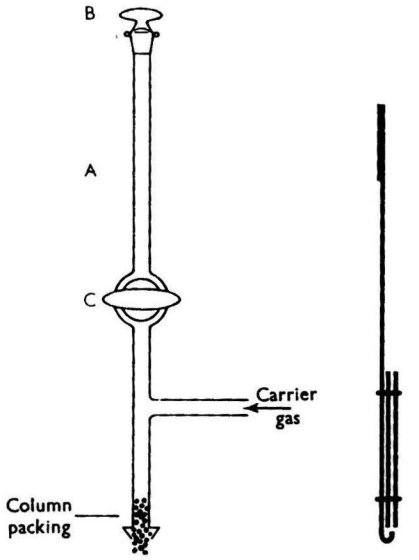

Fig. 5. Capillary-pipette injection system[10]

Liquid samples—

The earliest method of introducing liquid samples involved running the column at an inlet pressure slightly below atmospheric and an outlet pressure considerably below. Just before the beginning of the column packing is a short side-arm closed by a rubber serum cap so placed that a hypodermic needle thrust through the cap just reaches the column packing.[42] Samples are introduced by injection with a micrometer-syringe pipette; the needle holes in the cap are self-closing, and the life of a serum cap is 50 to 100 injections.

Scott[10] devised a simple capillary-pipette system for use at inlet pressures of the order of 40 cm of mercury above atmospheric. The pipette consists of a 2 to 3-cm length of capillary tubing, holding 1 to 2 mg of sample, attached to a length of iron wire. The pipette fills by capillary action when dipped into the sample liquid. It is then dropped into tube A (see Fig. 5), stopper B is replaced and tap C is opened, when the pipette falls on to the column packing and is discharged thereon, again by surface tension effects. It is then withdrawn into A by means of a magnet, tap C is closed, and the pipette is removed.

Probably the best and most positive method of sample introduction was devised by Tenney and Harris[54] (see Fig. 6). Pipette A is filled by capillary action and inserted through the neoprene O-ring, B, which forms a gas-tight seal. Tap C is then opened, and the pipette is pushed home until its tip forms a gas-tight seal on seating D; the carrier gas then passes through the pipette and blows the sample on to the column. The pipette is subsequently withdrawn by reversing the sequence of operations. Pipette E is a modified type having a larger volume, used by the same workers for gas samples. A modified form of this system has been used by Brealey, Elvidge and Proctor for the introduction of samples of viscous liquids.[55]

Flash heater—

Opinion is divided as to whether a flash heater should be fitted to maintain the sample-injection zone of a column at a considerably higher temperature than the rest of the column. If samples do not evaporate almost instantaneously, the result is equivalent to a gradual addition of sample; peaks become widened and may even overlap. It has been suggested

that, if the column temperature is not high enough to volatilise the sample rapidly, any improvement made by a flash heater will be nullified by condensation beyond the heated zone. It should be remembered, however, that it is possible for the column temperature to be high enough to prevent condensation, but not high enough to evaporate the sample sufficiently rapidly. This risk is more serious when some of the components of a mixture have boiling-points above the column temperature or when an extremely large sample has to be injected

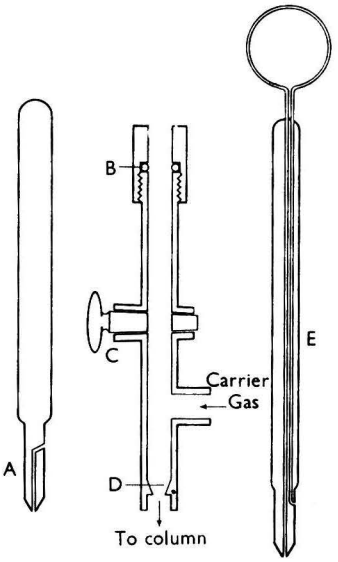

Fig. 6. Capillary-pipette injection system[54]

in order to determine a trace ingredient. Brealey, Elvidge and Proctor found that, in order to evaporate samples of aqueous preparations rapidly, a flash heater at 145° C was necessary.[55] A flash heater was considered essential for petroleum samples by Bradford, Harvey and Chalkley[43]; Pollard and Hardy[56] investigated the effect of sample-injection temperature and concluded that this factor has a profound effect on the sharpness of separations achieved. They consider that a flash heater should always be used. In the interests of efficiency, the column temperature should be as low as possible (see "Factors affecting Efficiency," p. 577); this will tend to make the use of a flash heater more important. The temperature of the flash heater must be chosen to give the most rapid volatilisation possible without causing pyrolysis of the sample.

Detectors

The function of a detector is to respond to a change, caused by the presence of a sample component, in a selected property of the carrier gas, so that the response can be converted into an electrical signal capable of being fed, after amplification, into a recording potentiometer.

Reference has already been made to the existence of integral and differential detectors. James and Martin's[2,16] titration cells are now mainly of historical interest and were soon superseded by Martin's gas-density balance. The titration cell is still used by Liberti and his co-workers,[17,57] the elution of components being detected coulometrically instead of by colour change, as in the earlier cells.

The gas-density balance—

The gas-density balance developed by Martin[6,16] has been fully described by James and Martin.[6] As the name implies, columns of ingoing carrier gas and effluent gas are balanced against each other and changes in the density of the gas, caused by the presence of other substances, can be used to produce an electrical signal. The response of the detector is linear with vapour concentration; it is, however, rather difficult to construct and, mainly for this reason, has not been used as widely as it deserves to be.

THE KATHAROMETER—

The katharometer, or thermal-conductivity cell, has hitherto been the most widely used type of detector. In its simplest form, it consists of two channels in a copper block, each containing a platinum resistance wire mounted axially. Pure carrier gas passes through one channel and effluent gas through the other; the two platinum wires have equal resistances and form two arms of a Wheatstone bridge. The bridge current is adjusted to heat the wires to between 50° and 100° C above the temperature of the copper block, the temperature of which is thermostatically controlled at a value sufficiently near to the column temperature to prevent condensation of eluted vapours. A change in the thermal conductivity of the effluent gas, caused by the presence of a vapour, produces a change in the temperature of the wire in that channel; this changes its resistance and puts the Wheatstone bridge out of balance, and the out-of-balance voltage is fed into the amplifier - recorder system. Many variations of this detector exist; in the U.S.A. it is now common practice to use germanium thermistors instead of platinum wires. These thermistors have a much larger temperature coefficient of resistance than has platinum and consist of a small germanium bead with short leads; thermistor cells are therefore more sensitive[58] and smaller and have a shorter response time. Thermistors are not widely used in this country, although Davis and Howard[59] have obtained good results with little trouble by using a single thermistor in the effluent gas and dispensing with the reference cell. All thermal-conductivity detectors are sensitive to fluctuations in gas-flow rate, and this must be carefully controlled, *e.g.*, by inserting a buffer tank immediately after the gas-supply reducing valve.

The response of a katharometer is not always linearly related to vapour concentration. Operating conditions, such as detector and bridge current, can be adjusted to give a response approximately linear to the thermal conductivity of the gas, but, as pointed out by Keulemans, Kwantes and Rijnders,[60] the conductivity of a gas mixture is not always a linear function of composition. For quantitative work, calibration with known mixtures over the appropriate range is advisable.

THE HYDROGEN-FLAME DETECTOR—

This detector, which is probably the easiest to construct, was introduced by Scott.[9,10] The carrier gas is hydrogen or, better, a mixture containing 75 per cent. v/v of hydrogen and 25 per cent. v/v of nitrogen, and this is burnt at a jet (~0·2-mm bore) at the column exit. Mounted 2 to 4 mm above the jet is a thermo-junction, the output from which is fed into a recorder. When a vapour is eluted, the calorific value of the effluent gas is increased, the flame becomes larger and hotter, and the increased thermocouple voltage produces the appropriate trace on the chromatogram. This detector is rather more sensitive than is a katharometer to substances having reasonably high heats of combustion, but considerably less sensitive to heavily halogenated compounds, which have low heats of combustion.

The sensitivity is increased by reducing the size of the thermocouple bead and the thickness of the wires, in order to reduce the heat capacity and loss by conduction. A recommended thermocouple consists of No. 35 s.w.g. palladium-gold - platinum-iridium wires. The "sensing volume" of this detector is much less than that of a gas-density balance or a katharometer, and hence its response is more rapid.

Stabilisation of the flow rate is essential, as the flame detector is, inevitably, extremely flow-sensitive. The hydrogen - nitrogen mixture is now used as carrier gas instead of hydrogen, because it was found that, when pure hydrogen was used, the flow rate increased as each vapour emerged, as a result of the loss of the more viscous material; in consequence, the chromatogram showed a stepwise shift of base-line after each peak. The use of the mixed carrier gas minimises the effect by increasing the viscosity of the carrier gas itself. Quantitatively, detector response is linear over narrow concentration ranges.

IONISATION DETECTORS—

All the detectors described so far have similar sensitivities, giving a full-scale deflection on the recorder for 1 part of vapour in 10^4 parts of carrier gas when the amplifier conditions are such that the base-line noise is not more than 2 per cent. of full-scale deflection.

In recent years, many detectors have been described in which the effluent gas passes through a cell, where it is ionised; the ionisation current across the cell changes when a

vapour is present in the gas, the change in current constituting the signal fed into the amplifier and recorder. These detectors differ from those described above in that the signal-producing portion has an impedance of the order of 10^6 to 10^{10} ohms, and a more complicated amplifier is needed between the detector and the recorder. Ionisation detectors differ from each other mainly in the means used to ionise the effluent gas.

Boer's ionisation detector was the first to be described.[11] The effluent gas is passed through a metal cell containing a central electrode and a 25-millicurie button of strontium-90; the gas becomes ionised by β-radiation from the strontium, and, when a suitable voltage is applied between the electrode and the cell wall, an ionisation current will flow across the cell. Pure carrier gas is passed through a similar cell, and both cells are electrically connected so that their ionisation currents pass in opposite directions through a common resistance. The voltage developed across this resistance by changes in ionisation current in the "effluent" cell is fed to the amplifier - recorder system. The impedance of the cells is about 10^9 ohms; insulation is therefore extremely important, and Boer used polytetrafluoroethylene insulation throughout. A high input-impedance amplifier is necessary. The advantages claimed by Boer are (*a*) high sensitivity to vapours, (*b*) insensitivity to flow rate, (*c*) linear response to vapour concentration and (*d*) suitability for use with a high-temperature column.

A similar detector, in which argon was used as carrier gas, was developed by Lovelock and fully described in 1958.[15] Advantage is taken of the unique behaviour of argon under ionising radiation; up to 80 per cent. of the argon atoms can become excited although not ionised. The excitation energy of argon is lower than its ionisation energy, but exceeds the ionisation energy of most organic compounds. The excited argon atoms will not give rise to an ionisation current, but, when an organic vapour reaches the detector, its molecules become ionised by collision with excited argon atoms, as well as by the direct effect of the radiation. The use of argon as carrier gas therefore reduces the background current and increases the ionisation current caused by the presence of a vapour. According to Lovelock, about 2×10^{-12} mole of most organic compounds can be detected by the device, and the quantitative response is similar for different molecular species. Calibration is therefore virtually unnecessary. The detector is insensitive to variations in temperature, pressure and flow rate and is suitable for use with high-temperature columns. A single cell only is used.

Lovelock has since modified his detector for use with the extremely small sample loads involved when coated-capillary columns are used.[61]

For both detectors just described, there is no radiation hazard in using the devices, but the ordinary precautions in handling radioactive materials must be observed if the ionisation cell is dismantled.

Ionisation detectors that do not involve the use of radioactive materials have been described. Harley and Pretorius[12] passed their effluent gas through a low-pressure discharge tube, which formed one arm of a Wheatstone bridge supplied at 900 volts d.c. The gas pressure required to give the necessary glow discharge was extremely low, and the column was accordingly operated at a very low over-all pressure. The vacuum system at the detector exit and the 900-volt supply must both be stabilised within close limits. Ryce and Bryce[13,62] used a similar detector, but operated their column with atmospheric pressure at the exit; the low pressure in the discharge tube was achieved by bleeding off a minute proportion of the effluent gas through a controlled leak to the detector and thence to the stabilised vacuum. They also used helium as carrier gas and adjusted the voltage across the tube to a value below the ionisation voltage of helium, which has properties similar to those described for argon. The detector described by Harley and Pretorius has been further developed by Pitkethly,[63] who suggested the use of an ordinary 230-volt 0·5-watt neon lamp as the basis for its construction. He claims to be able to detect 1 part of vapour in 10^7 parts of carrier gas.

The ionising effect of a flame has been utilised. Such a detector resembles Scott's flame detector in construction, but, instead of a thermocouple above the flame, Harley, Nel and Pretorius[14] fitted two platinum electrodes on either side of the flame and in contact with it. McWilliam and Dewar use a metal jet as one electrode and a brass electrode mounted horizontally 1 cm above the flame as the other.

For all ionisation detectors, linearity of response with vapour concentration is claimed so long as the respective workers' recommendations as to the electrical circuits are followed; non-sensitivity to flow rate and vapour sensitivites ranging from 200 times that of a katharometer for the low-pressure discharge tube to 10,000 times for the argon-ionisation detector are also claimed.

EFFECT OF DETECTOR ON COLUMN EFFICIENCY—

As the efficiency of a column is increased, the height equivalent to a theoretical plate, and consequently the volume of gas contained in that length of column is diminished. If the full benefit of a high-efficiency column is to be realised, the volume of the detector should, ideally, not exceed the volume of a theoretical plate; otherwise substances separated in the column may be partly re-mixed in the detector cell.

Also, as the trend in this country is towards the use of short columns having low liquid contents in the stationary phase, or even coated capillaries, the permissible sample size tends to become smaller. Detectors combining high sensitivity with small volume are therefore necessary for use with high-efficiency columns; it is probable that the use of ionisation detectors will become more widespread. In fact, complete assemblies incorporating Lovelock's argon-ionisation detector are now commercially available.

QUALITATIVE ANALYTICAL APPLICATIONS

DEFINITIONS—

The terms used in this section are defined as follows.

Flow rate—The rate of flow of carrier gas, as measured by a flowmeter, in millilitres per minute, at the inlet or exit pressure.

Dead volume—The gas volume of the column, *i.e.*, the volume not occupied by stationary phase and support between the sample-injection point and the detector.

Observed retention time—The time that elapses between injection of a sample and emergence of its peak maximum; it is usually measured in terms of centimetres of chart driven at a constant rate.

Corrected retention time—The observed retention time less the time taken by the carrier gas to travel from injection point to detector. The latter time is usually taken as the time from injection of the sample to the emergence of a peak caused by a small amount of air injected with it. The corrected retention time of a vapour is therefore the time spent by the vapour in the stationary phase.

Corrected retention volume—The volume of carrier gas passed between the injection of a sample and the emergence of its peak maximum.

$$\text{Corrected retention volume} = \text{Corrected retention time} \times \text{Flow rate}$$

Specific retention volume—The volume of carrier gas, measured at 0° C, that would have passed through a hypothetical ideal column containing 1 g of stationary phase when the peak maximum of the particular vapour emerges.

Relative retention volume—The ratio of the corrected retention volume of a vapour to that of a standard substance. If both vapours are chromatographed at the same pressure and flow rate, this ratio is equal to the ratio of the corrected retention times.

The observed retention time of a vapour is a function of the vapour, the stationary phase and the column temperature. It will also depend on the prevailing experimental conditions, *e.g.*, flow rate, inlet and outlet pressures, dead volume and amount of stationary phase in the column. These times must therefore be corrected for the experimental parameters in order to derive results that will be functions of the sample vapour, stationary phase and column temperature only.

Littlewood, Phillips and Price[64] calculated the observed retention volume (observed retention time × flow rate) as a limiting value at 0° C and expressed this as a specific volume per gram of stationary phase; this specific volume is reproducible to within ± 4 per cent. on different columns packed with the same stationary phase and is independent of the experimental conditions.

REPORTING RESULTS—

It is desirable to report the retention volume of a substance as its specific retention volume if it is required for calculating partition coefficients and other theoretical data.

If the retention volume is required primarily for analytical purposes, the relative retention volume is just as accurate and needs far less calculation. Indeed, Littlewood, Phillips and Price found that the relative retention volumes of a substance determined on different

columns of the same stationary phase agreed much more closely than did the specific retention volumes. James and Martin[65] found that the relative retention volume of a substance depends only on the chemical nature of the stationary phase and on the temperature and is closely reproducible. For this reason, they published relative and not absolute figures, as this made it unnecessary to know accurately the amount of stationary phase on the column. It is equally unnecessary to know the flow rate accurately, provided that it is kept constant throughout the examination.

TWO-COLUMN TECHNIQUE—

The relative retention volume of an unknown substance gives a strong clue to its identity, but for unequivocal identification at least two columns are required, containing stationary phases with different characteristics. Although so far no azeotrope formation has been reported in gas chromatography, there are instances of two or more substances having relative retention volumes close together on a given column; on a column of different characteristics, such substances are usually separated. The stationary phases are chosen, as indicated under "Stationary Phases," p. 579, so that one is polar and the other non-polar. Lewis, Patton and Kaye[66] used columns packed with (*a*) tricresyl phosphate and (*b*) vacuum-pump oil, both supported on Celite 545. This combination of columns was satisfactory for identifying paraffins, naphthenes, alcohols, aldehydes, ketones and esters. The two-column technique was also recommended by Brookes and Collins.[67] They showed that a non-polar (liquid paraffin) column gives little or no separation of different types of compound having similar boiling-points, *i.e.*, it behaves like a high-efficiency distillation column, without complications caused by azeotrope formation. As the polarity of the stationary phase is increased, aromatic hydrocarbons are retarded relatively to aliphatics—to such an extent that on a polyethylene glycol column all the aliphatic hydrocarbons boiling below 200° C are eluted before the aromatics appear. A polar column does not give such good separation between homologues as does a non-polar column, although substances having similar polarities are eluted in order of their boiling-points.

For specific problems, it is possible to choose a stationary phase capable of a specific interaction with one or more of the vapours to be separated. James,[3,68] for instance, examined mixtures of amines on three columns containing—

(*i*) liquid paraffin—solubility forces only;

(*ii*) polyoxyethylene—solubility forces *plus* hydrogen bonding (the latter has a strong effect on the retention of primary and secondary amines, but this is not possible with tertiary amines); and

(*iii*) benzyldiphenyl—solubility forces *plus* polar forces.

Another example of specific interaction is the use by Bradford, Harvey and Chalkley[43] of a stationary phase consisting of silver nitrate dissolved in glycol. The silver ion forms weak complexes with unsaturated hydrocarbons, which are thereby markedly retarded relatively to saturated hydrocarbons of comparable boiling-point.

The identification of an unknown substance or mixture involves chromatographing the sample on each of two columns (polar and non-polar); a standard reference substance is also chromatographed under identical operating conditions, and the relative retention volume of each peak obtained from the sample is calculated. The components of the sample are then identified by reference to a Table of relative retention volumes previously compiled for each column by chromatographing pure substances. To cover a comprehensive field, a series of Tables of relative retention volumes for each column, operating at various temperatures, is desirable.

HOMOLOGOUS SERIES—

It was shown by James and Martin[65] and by Desty and Whyman[69] that if log(relative retention volume) is plotted against the number of carbon atoms in the molecule for the members of a homologous series, a straight line is obtained. Also, if the relative retention volumes of members of a homologous series are determined on two columns, A and B, then, if log(relative retention volume) on column A is plotted against log (relative retention volume) on column B, a straight line is again obtained. These graphs can be used in conjunction with Tables of relative retention volumes for identification purposes.

ASYMMETRIC PEAKS—

Reproducible values for specific and relative retention volumes are obtained only if the peaks are symmetrical or nearly so—and in gas - liquid chromatography they usually are symmetrical.

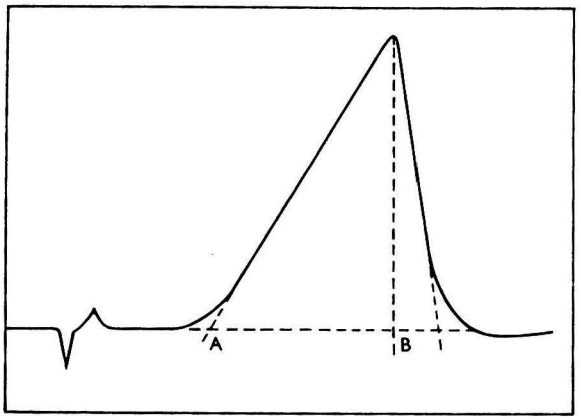

Fig. 7. Asymmetric peak—column temperature too low

If a sample has a boiling-point too high for the prevailing column temperature, asymmetric peaks are produced, which appear to be "leaning backwards," as in Fig. 7. The start and the initial slope are independent of sample size, so that, as the sample size increases, the peak maximum emerges later and later. For such peaks, a reproducible value for the relative retention volume can be obtained by the empirical procedure suggested by Littlewood, Phillips and Price.[64] The straight portions of the peak are produced until they cut the base line at A and B. The peak is then, in effect, turned back to front by adding the time between peak maximum and point B to the retention time corresponding to point A. The retention time so calculated is found to be reproducible and independent of sample size. I have used this method on numerous occasions and found it to be satisfactory; paradoxically, it also gives reliable retention data for peaks that "lean forwards," as in Fig. 3.

EXAMINATION OF GAS-CHROMATOGRAPHIC FRACTIONS—

Gas chromatography is an extremely useful and rapid method for identifying substances; it can often be used when no satisfactory chemical methods exist. Its scope has been extended and the reliability of its identification increased by isolating separated components in traps, cooled in solid carbon dioxide - acetone mixture or liquid air, which are inserted immediately after the detector. The isolated fractions can then be further examined by infra-red spectrography or in a mass spectrometer. Haslam and Jeffs[44] have devised a useful and compact trapping system, comprising four traps and a by-pass, with a suitable arrangement of two-way stopcocks permitting the selection of any one of these five routes for the gas issuing from the detector. Their traps were cooled by a mixture of solid carbon dioxide and methanol. Gas-chromatographic identification was supplemented by the determination of the boiling-point, refractive index and infra-red absorption spectrum of each fraction. The last-named will immediately show whether or not a fraction consists of two or more components having the same relative retention volume and permit them to be identified. As the infra-red spectrum indicates the structure of a compound, it will also decide, when necessary, between two alternative possibilities based on retention data.

QUANTITATIVE ANALYTICAL CALCULATIONS

When the two components of a mixture have been identified by the qualitative procedure, the amount of each component may be assessed by measuring the areas under the peaks on the chromatogram. The area under a peak is a function of the amount of the compound eluted from the column; the peak height is a function of the maximum concentration of that compound in the carrier gas during the elution and will vary with changes of flow rate and temperature, which affect the duration of elution and the width of the peak. Hence, although peak height is an easier quantity to measure than peak area, the latter is independent of the

operating conditions and should always be used for quantitative work,[60] except perhaps for routine work when rapidity is of primary importance.

The factor for converting peak area to weight is a function of the property of the vapour utilised by the detector and may be different for different vapours. In practice, the difference is slight for compounds of similar type; when using Scott's flame detector,[9,10] for instance, it would be permissible, to a first approximation, to use one factor for hydrocarbons, another for alcohols, esters, ketones, etc. and a third for halogenated compounds.

The determination of the conversion factor for a vapour is not a mere matter of calibration with known amounts, because, unless the sample is injected with a micrometer-syringe pipette, it is impossible to know the amount injected with sufficient precision. In practice, direct calibration is not attempted, and two methods of deriving quantitative results are in use.

INTERNAL-NORMALISATION METHOD—

If the conversion factors can be regarded as equal for all the vapours involved, the procedure is simple. According to Dimbat, Porter and Stross,[8] this assumption holds good, when helium is used as carrier gas, with thermal-conductivity detection; it is also valid, to a first approximation, when ionisation detectors are used. The peak areas are measured, the areas are added together and the total area is normalised to 100 per cent., *i.e.*, each peak area is calculated as a percentage of the total.

If the detector response is markedly different for different vapours, each peak area must be corrected for the property of the vapour measured by the detector, and the resulting corrected peak areas are used in the calculation. Browning and Watts[70] who used a katharometer, found that peak areas corrected for the thermal conductivities of the respective vapours were approximately proportional to the percentage by weight of each component in the sample mixture. For high accuracy they recommended calibration with mixtures of known quantitative composition. Morrow and Buckley[71] preferred to determine an empirical factor for each vapour, such that the corrected peak area is proportional to the molar percentage of the compound present.

If the detector response is not linear with vapour concentration, the only satisfactory procedure is direct calibration of the peak-area ratios with mixtures of known quantitative composition approximating to that of the sample being analysed. The composition of the sample may be calculated to a first approximation by simple internal normalisation and the result used as a basis for preparing the calibration mixtures.

It must be remembered that the internal-normalisation method involves the assumption that every ingredient of the sample mixture has been eluted. Occasionally, a sample may contain an ingredient insufficiently volatile to be eluted at the column temperature in use; if this possibility is suspected, the quantitative calculations should be based on an internal standard.

INTERNAL-STANDARD METHOD—

After a chromatogram of the sample has been obtained, a standard reference liquid is added in known concentration to another portion of the sample and the mixture is chromatographed under identical operating conditions. The standard should be a substance that is not an ingredient of the sample and should have a relative retention volume such that there is no risk of its peak overlapping any of the sample peaks.

The peak areas are measured, corrected for detector characteristics, if necessary, and calculated as ratios relative to the area of the peak for the standard substance.

$$\text{Component A, \%} = \frac{\text{Corrected peak area for A}}{\text{Corrected peak area for standard}} \times \text{Standard in mixture, \%}$$

MEASUREMENT OF PEAK AREA—

The area under a peak may be calculated by multiplying the peak height by the width at half-height, *i.e.*, by regarding the peak as a triangle. The most satisfactory method, however, is by means of a planimeter.

Automatic integrators are now available for fitting to most recorders; these indicate at any point the area under the chromatogram from the moment of injection to that point.

OVERLAPPING PEAKS—

Quantitative results can be considered above reproach only when the components concerned are completely separated, so that the chromatogram returns to the base line between

peaks. In practice, occasions arise when quantitative results are required for a mixture containing two ingredients that will not separate completely on any available column. The question arises of how to allocate the overlapping portions of two peaks between the two vapours concerned. The problem was solved empirically by Fredericks and Brooks.[29] They simply drew a vertical line from the lowest point between two overlapping peaks to the base line and treated the vertical line as the boundary between the two peak areas. They found that quantitative results obtained in this way agreed satisfactorily with the percentage by weight of each substance present.

The method fails when one or more of the overlapping peaks is unsymmetrical. In such an instance, quantitative calculations can only be based on peak-height ratios, with careful calibration against known mixtures.

Scope of gas chromatography in analysis

Gas chromatography provides a method of identification and determination that is applicable to all mixtures of organic compounds volatile at temperatures up to about 250° C and to gas mixtures. Columns have been operated at temperatures down to that of a solid carbon dioxide - acetone bath,[30] and column temperatures up to 250° C present no undue difficulties. Higher column temperatures have been reported,[72] but high-temperature gas chromatography is still in the exploratory stage, mainly because of the difficulty of finding stationary phases that neither volatilise nor decompose.

The phenomenal growth of the literature on gas chromatography, especially in the last 3 years, is striking evidence of its usefulness.

Hydrocarbons—

Hydrocarbons are relatively inert and often have such similar properties that chemical methods of separation are difficult and time-consuming. Some gas-chromatographic analyses, which would have been impossible by any other method, are quoted below.

"Cat-cracked" petrol[73] was separated into two fractions by liquid chromatography, each fraction being then examined by gas chromatography.

Eggertsen and Groennings[74] examined "reformer charge stock" by using three columns and determined all but one of its twenty-five component hydrocarbons in the C_5 to C_7 range.

Desty and Whyman[69] used two columns, one containing *n*-hexatriacontane and the other benzyldiphenyl, to determine the relative retention volumes of eighty-eight compounds, including some sulphur compounds, that occur in petroleum fractions. They showed that a chromatogram of a 16-component mixture could be obtained in under 1 hour; composite peaks were resolved by chromatographing the sample on a different column.

In connection with air-pollution studies, Eggertsen and Nelson[75] examined engine-exhaust gas by gas chromatography; they were able to detect therein eighteen unburnt hydrocarbons in the range C_2 to C_5, which amounted to a total of 500 p.p.m. in the exhaust gas.

Boron hydrides—

Separation of the gaseous boron hydrides was effected by Kaufman, Todd and Koski,[76] who used columns filled with liquid paraffin and tricresyl phosphate, respectively, both liquids being supported on Celite. Samples were introduced via a removable calibrated sample-bulb, which was evacuated and filled to the desired pressure with sample gas. This procedure was necessary because the boron hydrides must be handled in absence of air.

Essential oils—

Gas chromatography has been used for the identification of essential oils. Essential oils are consistent in their composition, and an essential oil can be identified, according to Holness,[77] by the shape and peak-height ratios of the chromatogram, although individual peaks may not be identified. Components can be identified if necessary as described under "Qualitative Analytical Applications."

Plastic materials—

Useful as gas chromatography is as a self-contained method of analysis, it should not be regarded as a replacement for other methods. It has been usefully employed in conjunction with chemical methods—as a stage in an analytical procedure—and this is well illustrated by the methods of Haslam and his co-workers for the analysis of plastic materials.

Reference has already been made to the trapping of gas-chromatographic fractions for

further examination. Preliminary treatment of the sample is sometimes required. For instance, poly(methyl methacrylate) was depolymerised by pyrolysis *in vacuo* at 350° C, the depolymerisation products being condensed in a solid carbon dioxide - methanol trap.[78] The condensate from the depolymerisation was chromatographed on a dinonyl phthalate - Celite column, the fractions being trapped for further examination, as already mentioned.

In the determination of poly(ethyl esters) in methyl methacrylate co-polymers, described by Haslam, Hamilton and Jeffs,[45] the alkoxyl groups of the copolymer are converted into the corresponding alkyl iodides by treatment with hydriodic acid and phenol. The iodides are trapped in *n*-heptane cooled with solid carbon dioxide and methanol and examined on a dinonyl sebacate - Celite column. Quantitative calculations are made from peak-height ratios after calibration against known mixtures of methyl and ethyl iodides.

Synthetic methyl methacrylate monomer may contain small amounts of methanol and methyl-α-hydroxy*iso*butyrate, the determination of which by chemical means is difficult and unsatisfactory. These substances are determined by using a dinonyl phthalate column preceded by a 4-inch packing of glycerol on Celite to retard the small amount of moisture normally present in monomers and prevent it from interfering with the methanol peak.

ALCOHOL IN BLOOD—

The use of gas chromatography for the determination of alcohol in blood for forensic purposes has been suggested by Fox.[79] The diluted tungstic acid filtrate from 1 to 3 ml of blood is fractionally distilled and the appropriate fraction is examined by gas chromatography.

FATTY ACIDS—

Fatty acid mixtures produced by the hydrolysis of natural fats were examined early in the history of gas chromatography by James and Martin.[2] Later, these workers[80] recommended preliminary methylation of the fatty acids, the gas-chromatographic examination being carried out on the methyl esters. The esters have lower boiling-points and much lower polarities than the corresponding acids and so pass through the column more rapidly and do not produce "tailing" peaks. By this technique, James and Martin have analysed mixtures of fatty acids containing eighteen carbon atoms at column temperatures up to 200° C. Khan and Whitham[81] have extended the range to acids containing thirty-four carbon atoms; they used the same technique and column temperatures up to 295° C.

PHENOLS—

Phenols, like fatty acids, are difficult to examine by gas chromatography, because of their strongly polar character. Langer, Pantages and Wender[82] found that methylation and then examination of the methyl ethers was more satisfactory. They obtained even better results by converting the phenols to the trimethylsilyl esters by direct reaction with trimethylchlorosilane or hexamethyldisilazane.

CIGARETTE SMOKE—

Cigarette smoke has been examined by gas chromatography; Patton and Touey[83] found that it contained ethane, ethylene, acetylene, propane, propene, *iso*butene and *n*-butane; a silica gel adsorption column was used. Quin and Hobbs[84] found evidence of the presence of ten aliphatic acids and phthalic acid in the condensate from the smoke in a solid carbon dioxide - ethanol trap.

FLUORINE COMPOUNDS—

Gas chromatography was used as early as 1955 for the separation of fluorinated hydrocarbons by Evans and Tatlow.[25] It is the only available method of separating these materials; they are extremely volatile, isomers and closely related compounds often have almost identical boiling-points (much closer than their hydrocarbon analogues) and azeotropes are frequently formed.

PREPARATIVE-SCALE GAS CHROMATOGRAPHY—

The work just referred to was subsequently extended to the use of gas chromatography as a means of separating the fluorocarbons on a preparative scale.[85] A dinonyl phthalate column, 30-mm bore and 16 feet long, was used; an average sample load was about 3 g. The maximum permissible load depends on the ease of separation of the compounds concerned. It is essential in the handling of such large samples to use a flash heater at the injection zone, otherwise volatilisation of the sample is too slow and separations are not

clean-cut. More recently, Evans, Massingham, Stacey and Tatlow[86] have scaled-up the process and now use a dinonyl phthalate column of 75-mm bore and 16 feet long, on which can be placed sample loads of 15 to 50 g, according to the ease with which the components are separated. They claim that, contrary to widely held belief,[8] the increase in column diameter has not caused a decrease in efficiency.

ISOTOPE EFFECTS—

It is reported that replacement of one or more hydrogen atoms in an organic molecule by deuterium or tritium can produce a detectable increase in the relative retention volume. The only examples so far reported are by Wilzbach and Riesz[87] who state that *cyclo*hexane, C_6D_{12}, has a relative retention volume 10 per cent. greater than that of C_6H_{12} and that replacement of three hydrogen atoms in *cyclo*hexane and methyl*cyclo*hexane by tritium increases the relative retention volumes by 5 and 10 per cent., respectively.

SPECIAL TECHNIQUES

Some workers have evolved special techniques to solve their own particular problems. These techniques are interesting, but are generally not likely to be sufficiently reproducible in different laboratories for different workers' results to be comparable.

RISING TEMPERATURE—

One such technique involves increasing the column temperature at a constant and reproducible rate during the elution, in order to accelerate the elution of the long-retention substances and to elute them with sharp narrow peaks resembling those of the earlier components. The technique was suggested by Patton, Lewis and Kaye,[33] who considered that the experimental difficulties of reproducing operating conditions would be excessive and did not pursue the matter.

The method is now used by Dal Nogare and Bennett[88] as their normal procedure for mixtures having wide boiling-point ranges.

The technique is undoubtedly time-saving and useful in a routine control laboratory, but, when it is desirable to compare results with those of other workers or unknown components have to be identified, the orthodox constant-temperature technique is far more reliable and is to be preferred, even if the sample has to be chromatographed on the same column at two or more temperatures.

TWO-STAGE COLUMNS—

This technique consists in the use of two columns containing different stationary phases and connected in series. Simmons and Snyder,[89] for examining petroleum mixtures, use a non-polar column and then a polar column, each column having its own detector. The columns are connected through a sytem of stopcocks such that a fraction emerging from the first column can be either rejected to atmosphere or fed direct to the second column, on which, if composite, it is further resolved according to the polarity of its ingredients.

Madison[90] uses a similar system involving a liquid (dimethylsulpholane) column and then an adsorption (charcoal) column in order to achieve a complete analysis of a mixture of fixed and condensable gases.

COMPOSITE STATIONARY PHASES—

Eggertsen, Knight and Groennings[91] found that with adsorption columns tailing of peaks was virtually prevented by adding about 1·5 per cent. of a high-boiling liquid, such as squalene, to the adsorbent; the order of elution of components was unchanged. As more high-boiling liquid is added to the adsorbent, however, the behaviour of the column changes and gradually approaches that of a column containing the liquid on an inert support; ultimately when the liquid film attains a certain thickness, the effect of the adsorbent is lost. Between the two extremes, these workers found that the relative retention volumes of different types of hydrocarbon could be varied at will by varying the liquid content of the adsorbent.

Although the technique has been used considerably,[73,74] it is difficult to see how retention data from a liquid-modified adsorbent column can be reproducible unless the sample size is carefully controlled. The extent to which the characteristics of the column are influenced by the adsorbent will depend, in effect, on the extent to which the liquid film is overloaded —and this might even vary for ingredients of the same sample if they were present in widely different proportions.

Conclusion

Gas chromatography is available as a method of analysis or as a stage in an analytical procedure whenever mixtures of organic compounds volatile at temperatures up to about 250° C are involved; work is in progress with the object of extending the temperature range.

Compounds can be identified by determining their relative retention volume on at least two stationary phases having different polar or chemical characteristics. For this purpose, Tables showing the relative retention volumes of pure substances on different stationary phases are needed; at present, published Tables of retention data are limited and scattered throughout the literature, although a collection of such Tables has recently been compiled.[92] Further evidence of identity can be obtained by trapping the gas-chromatographic fractions for examination by infra-red absorption spectrography, mass spectrometry or, if sufficient of the fraction is available, refractive-index or boiling-point determination.

High resolving power is best achieved by the use of comparatively short columns (4 to 5 feet) having a low liquid content in the stationary phase. This necessitates a small sample load, which, in turn, requires a highly sensitive detector, preferably an ionisation detector. The small permissible sample size for a high-efficiency column may produce fractions too small for ordinary infra-red spectrographic techniques; however, a vapour-phase infra-red technique recently reported by Anderson and Duncan[93] is effective for microgram amounts of substances having moderate boiling-points. In the future, therefore, it should not be necessary to duplicate a high-efficiency column with a column of lower efficiency, but capable of handling larger samples, in order to provide sufficiently large fractions for infra-red spectrography.

For quantitative work, peak areas rather than heights should be measured whenever possible. If an ionisation detector, for which the response is linear and similar for almost all vapours, is used, accurate results can be obtained without a lengthy and time-consuming calibration.

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The Determination of Small Amounts of Neptunium in Plutonium Metal

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A method is described for determining the neptunium content (10 to 2000 p.p.m.) of plutonium metal. The neptunium is separated from plutonium by solvent extraction with 2-thenoyltrifluoroacetone and then determined with a square-wave polarograph. Results show neptunium contents ranging from 25 to 500 p.p.m. The precision at these two levels is about ± 10 and ± 2 per cent., respectively.

Plutonium metal prepared from irradiated uranium may contain traces of neptunium formed by double neutron-capture in uranium-235 or the $(n, 2n)$ reaction on uranium-238; the neptunium (present as the isotope of mass 237, an alpha-emitter of half-life $2{\cdot}20 \times 10^6$ years) is incompletely removed in the purification process. Metallurgical research on zone melting of plutonium requires sensitive analytical methods for a range of trace impurities including neptunium. In principle, the analytical separation of neptunium from plutonium can be effected by extraction of neptunium^{IV} from plutonium^{III} in dilute mineral acid under reducing conditions, with a solution of 2-thenoyltrifluoroacetone (TTA) in an inert solvent. However, sufficient plutonium is extracted to interfere with an alpha-assay of neptunium-237 (which has a lower specific activity) and a chemical or physico-chemical method is desirable.

Hindman and Kritchevsky[1] report that, in chloride media, the neptunium^{IV} - neptunium^{III} couple is reversible at the dropping-mercury electrode, which indicates the possibility of utilising the high sensitivity of a square-wave polarograph for determining neptunium.

Experimental

For studying neptunium solution chemistry, a standard solution was prepared in hydrochloric acid from a small amount of spectrographically analysed pure neptunium metal, for which the following figures are quoted—

	Ca	U	Ni	Mg	Cr	Pu	Al	Mo	V
Weight, % ..	0·34	0·22	0·06	0·03	0·03	0·03	0·02	0·05	0·06

A 0·2751-g portion of this metal was dissolved in 5 ml of diluted hydrochloric acid (1 + 1 v/v) and diluted to 25 ml in a grade A calibrated flask. Dilutions of this stock solution were used for all subsequent experimental work, including the analyses for unknown neptunium contents of plutonium samples.

Several reducing agents have been suggested[2] for the quantitative reduction of stable neptunium^{V} to the less stable neptunium^{IV}. Hydroxylamine hydrochloride in hot 5 *M* hydrochloric acid and also ferrous chloride in cold dilute hydrochloric acid were used in this work; the latter has the advantage that reduction is complete within a few minutes.

Solvent extraction of neptunium^{IV}—

Neptunium^{IV} shows a similarity to other quadrivalent actinides in the tendency to form strong complexes. This property is used in the solvent extraction of quadrivalent neptunium ions (Np^{4+}) with TTA; the extraction coefficient for the highly charged quadrivalent cation is much greater than those for tervalent neptunium ions (Np^{3+}) and bivalent neptunyl ions (NpO_2^{2+}).

Moore[3] has described an excellent separation, under suitable reducing conditions, of neptunium from plutonium, uranium and other elements with TTA. In general, these conditions have been applied in this work and found to be satisfactory. The extractions were carried out in a glove-box, and a special solvent-extraction apparatus is described for this purpose (see Fig. 1). The extraction vessel is of about 20-ml capacity and is fitted with a swan-neck delivery tube at the bottom and a standard B14 socket at the top. The sample solution and other reagents are added by pipette through the B14 socket, and mixing of the solutions is achieved with the glass paddle driven by the micromotor. The power for the motor is taken from a 6-volt accumulator or dry battery and the speed is controlled by means of 25-ohm wire-wound resistor in series with the motor. The lower phase (aqueous)

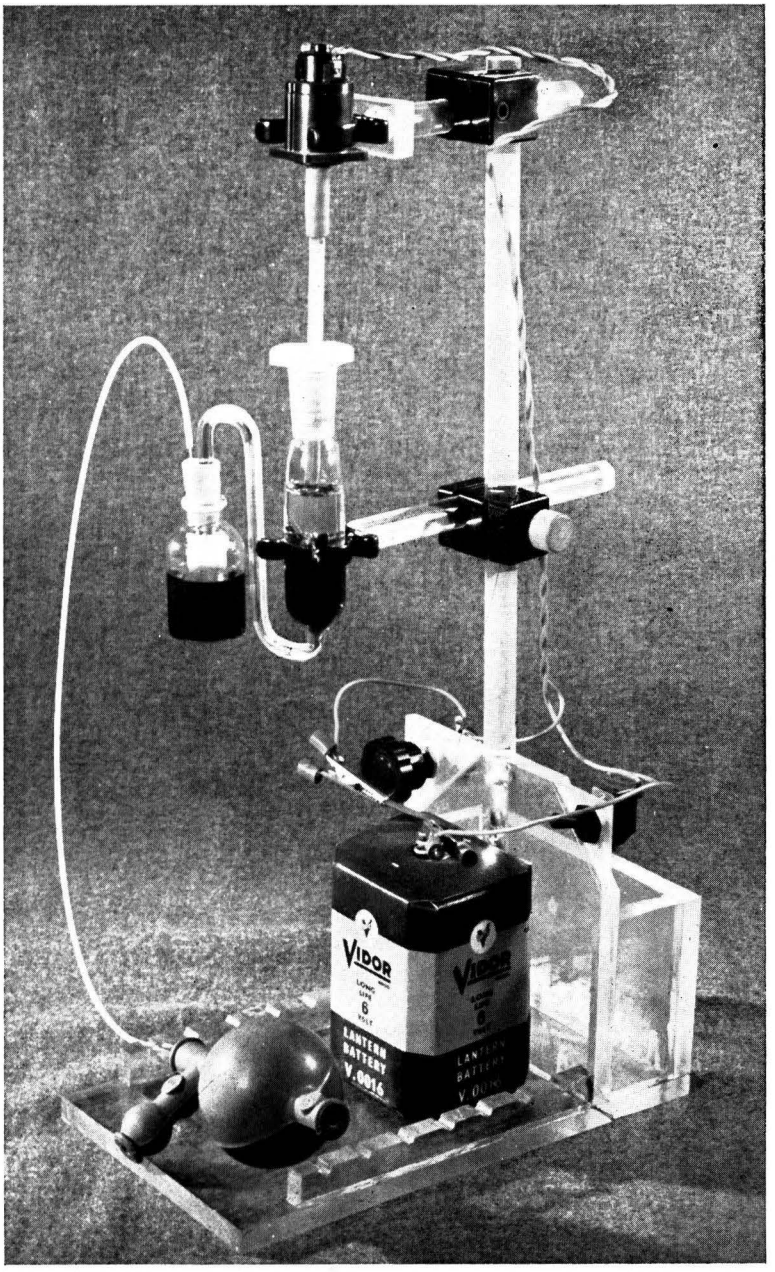

Fig. 2. Solvent-extraction apparatus

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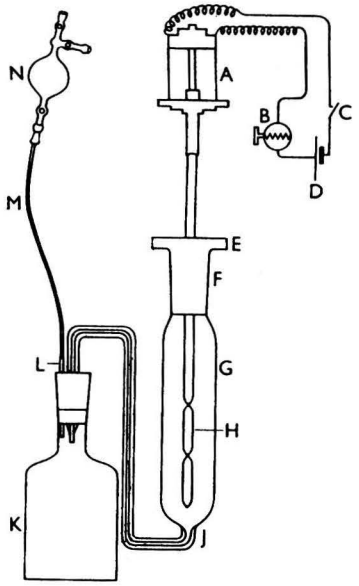

A = Micromotor (Victory Industries, Guildford)
B = 25-ohm wire-wound variable resistor
C = Switch
D = 6-volt battery
E = Polythene stopper
F = B14 socket
G = Glass extraction vessel (20 ml)
H = Glass paddle (with double reversed helix)
J = Swan-neck capillary tube (internal diameter 2 mm)
K = Glass sample bottle (20 ml)
L = Stainless-steel capillary tube
M = Poly(vinyl chloride) tubing
N = Propipette

Fig. 1. Diagram of solvent-extraction apparatus

can be removed from the vessel by attaching a sample bottle to the swan-neck delivery tube and applying suction by means of a propipette (see Fig. 2).

POLAROGRAPHY OF NEPTUNIUM—

In confirmation of Hindman and Kritchevsky's[1] work, well defined waves were obtained for the reduction of neptunium^{IV} at the dropping-mercury electrode. The reduction in chloride media corresponds to a reversible one-electron change ($E_{\frac{1}{2}} = -0{\cdot}11$ volt against the saturated-calomel electrode).

Square-wave polarography—Studies on a square-wave polarograph[4] showed a peak for neptunium in 1 *M* hydrochloric acid that was not completely defined (see Fig. 3), owing to interference from the anodic wave from the oxidation of chloride ions. However, when the extrapolated base line was used, linear calibration graphs were obtained for peak height against neptunium concentration. Identical calibration graphs were obtained with both

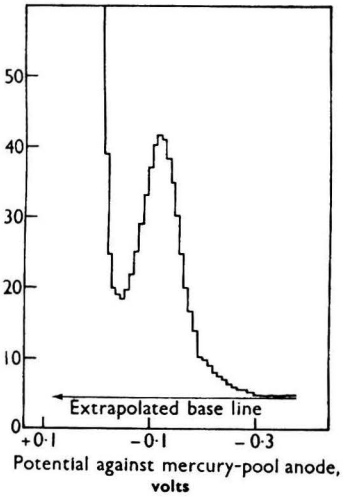

Fig. 3 Square-wave polarogram for neptunium^{IV} in 1 *M* hydrochloric acid - 0·1 *M* hydroxylamine hydrochloride

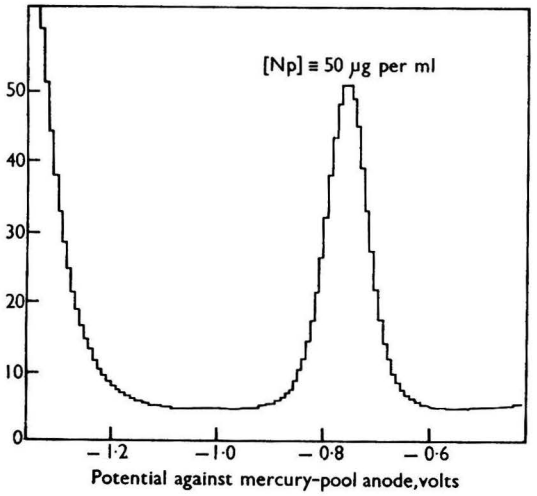

Fig. 4. Square-wave polarogram for neptunium^{IV} in 0·1 *M* EDTA - 1 *M* Cl^- at pH 6

hydroxylamine hydrochloride and ferrous chloride as reductant. Polarograms were measured within hours, since aerial oxidation of neptunium^{IV} slowly took place.

With a view to minimising interferences, the use of a complexing agent to shift the half-wave potential of the neptunium peak was considered. F. Elliot (personal communication) has used a disodium ethylenediaminetetra-acetate (EDTA) base medium and has obtained well defined polarograms in the presence of uranyl and plutonium^{III} ions. It was found, however, that in EDTA solutions containing a reducing agent (hydroxylamine hydrochloride) the plutonium^{III} - EDTA complex was rapidly oxidised by air to the stronger plutonium^{IV} - EDTA complex. This oxidation can be observed by the colour change of the solution from blue to brown; reduction of the plutonium^{IV} - EDTA complex, which is irreversible at the dropping-mercury electrode, caused serious interference with the measurement of the neptunium^{IV} - EDTA peak. However, in the absence of plutonium and with a 0·1 *M* EDTA - 1 *M* hydroxylamine hydrochloride base solution of pH 6, well defined polarograms were obtained on both a conventional and a square-wave polarograph (see Fig. 4). The potential of the reduction of the complex was about −0·8 volt against the mercury-pool anode, *i.e.*, a shift of about 700 mV more negative compared with the reduction of neptunium ions. (Jenkins[5] has since studied the nature and stability of the neptunium^{IV} and neptunium^{III} complexes with EDTA on a manual polarograph.) A comparison of Figs. 3 and 4 shows the improved definition of the square-wave polarogram in the EDTA base medium; the peak is shifted well away from the anodic oxidation of chloride ions and is completely defined. Quantitative reduction of neptunium to the quadrivalent state is not essential before addition of EDTA, since this state is stabilised by complex formation. In practice it is an advantage not to require 100 per cent. reduction efficiency, particularly if hydroxylamine hydrochloride is used.

Linear calibration graphs were obtained for peak heights against concentration in the ranges 20 to 110 μg and 10 to 30 μg of neptunium per ml. Polarograms were well defined and peak heights were reproducible to ± 1 per cent.

If the EDTA concentration exceeds 1 *M*, at pH about 5, the acid is precipitated and peak heights are not reproducible; also the diffusion coefficient of the neptunium^{IV} - EDTA complex is about one-half that in 0·1 *M* EDTA, owing most probably to increased viscosity. For analytical purposes, 0·1 *M* EDTA appears to be the optimum concentration, as shown in Table I.

TABLE I

EFFECT OF EDTA CONCENTRATION ON PEAK HEIGHT

Concentration of EDTA at pH 6, *M*	Peak height, divisions	Remarks
1·0	16·0	Peak shape poor
0·1	24·0*	Well defined peak
0·005	24·6	Well defined peak
0·0005	24·4	Peak shape deteriorating
0·00025	22·7	Peak shape poor

* Peak height from four independently prepared solutions (precision ± 1 per cent.)

TABLE II

EFFECT OF pH ON PEAK HEIGHT

pH	Peak height for 22 μg of neptunium per ml, divisions
1·7	23·1
2·5	24·5
4·8	23·9
6·2	24·0
7·0	23·5

TABLE III

EFFECT OF pH ON HALF-WAVE POTENTIAL

pH	$E_{\frac{1}{2}}$ measured against saturated-calomel electrode at 25° C, volts
0·18	−0·372
0·26	−0·366
0·35	−0·381
0·97	−0·457
1·83	−0·576
4·26	−0·728
4·95	−0·772
5·38	−0·797
6·00	−0·835

Tables II and III show the effect of pH on peak height and half-wave potential. There is no variation in peak height above pH 2 and the half-wave potential is changing as indicated. For this work the base electrolyte conditions selected were 0·1 *M* EDTA - 1 *M* hydroxylamine hydrochloride at pH 6 (measured by narrow-range indicator papers).

METHOD

The sample of plutonium metal is dissolved in a solution of hydrochloric acid and hydroxylamine hydrochloride. Neptunium is reduced to the quadrivalent state with ferrous chloride and extracted quantitatively with a solution of TTA in xylene. The neptunium is then backwashed into nitric acid and finally determined in an EDTA base medium with a square-wave polarograph. The method is suitable for the determination of neptunium from 10 to 2000 p.p.m.

REAGENTS—

Distilled water—Prepare all water by passing distilled water through a mixed-bed ion-exchange resin, and then distil from potassium permanganate solution.

Nitric acid, 10 M—Dilute 625 ml of analytical-reagent grade nitric acid to 1 litre with distilled water.

Hydroxylamine hydrochloride solution, 5 M—Dissolve 175 g of analytical-reagent grade hydroxylamine hydrochloride in distilled water, and dilute to 500 ml.

TTA solution, 0·5 M *in xylene*—Dissolve 28 g of 2-thenoyltrifluoroacetone in 200 ml of pure xylene, filter, and dilute with xylene to 250 ml.

Ferrous chloride—Use solid $FeCl_2.4H_2O$.

EDTA solution, M—Dissolve 37 g of disodium ethylenediaminetetra-acetate in distilled water, adjust to pH 7 by adding ammonia solution, and dilute to 100 ml.

PROCEDURE—

Weigh accurately (to $\pm$0·5 mg) about 300 mg of plutonium metal, and transfer to a 30-ml beaker covered by a watch-glass. Add 5 ml of distilled water and slowly, in drips, 0·5 ml of 10 *M* hydrochloric acid to dissolve the metal. When the reaction subsides add 1 ml of 10 *M* hydrochloric acid and 2 ml of 5 *M* hydroxylamine hydrochloride (to reduce plutonium to the tervalent state). Warm on a hot-plate to obtain complete dissolution. Dilute to 10 ml, allow to cool, add about 250 mg of ferrous chloride (do not exceed this weight of $FeCl_2.4H_2O$, added as the solid), and transfer quantitatively to the extraction vessel. Set aside for about 5 minutes to allow quantitative reduction of neptunium to the quadrivalent state.

Add 5 ml of 0·5 *M* TTA in xylene. Stir rapidly for 10 minutes, allow the phases to separate completely, and remove the upper organic phase quantitatively with a spitzer. Reserve in a 10-ml beaker, and then repeat the extraction with a further 5 ml of 0·5 *M* TTA. With the propipette, suck off the aqueous phase into the sample bottle attached to the swan neck (see Fig. 1). Combine the organic phases in the extraction vessel, add 1 ml of 1 *M* hydrochloric acid wash solution, and stir for about 5 seconds (just long enough to remove entrained plutonium). Suck off and discard the aqueous phase. Add 5 ml of 10 *M* nitric acid, and stir rapidly for 10 minutes to backwash the neptunium. Carefully suck off the aqueous phase into a clean sample bottle, and repeat the back extraction with a further 5 ml of 10 *M* nitric acid. Collect the 10 ml of 10 *M* nitric acid in the sample bottle, and transfer the contents to a 100-ml beaker.

Evaporate the solution on a hot-plate to a volume of about 3 ml, and add 5 ml of analytical-reagent grade nitric acid, sp.gr. 1·42. Evaporate to about 1 ml, and repeat the evaporation with two successive 5-ml portions of nitric acid. Add about 3 ml of analytical-reagent grade perchloric acid, 60 per cent. v/v, and further small additions of nitric acid, sp.gr. 1·42, to complete the wet oxidation of traces of organic matter. Heat to remove perchloric acid fumes until solid perchlorates remain at the bottom of the beaker (do not bake to dryness). Dissolve the residue by warming with 1 ml of analytical-reagent grade hydrochloric acid, sp.gr. 1·18, and 1 ml of water, to obtain a clear solution. Transfer to a 10-ml beaker, add 2 ml of 5 *M* hydroxylamine hydrochloride, and evaporate slowly, without boiling, to reduce the volume to about 0·5 ml. Add 0·5 ml of 1 *M* EDTA solution and from 4 to 7 drops of ammonia solution, sp.gr. 0·880, to adjust the pH to between 5·5 and 6·5; measure the pH by just touching the surface of the liquid with a narrow-range indicator paper. Dilute to 5 ml in an N.P.L. grade A measuring flask, and mix thoroughly. By pipette, transfer 2·00 ml into a polarograph cell, at once, and de-aerate the solution with nitrogen (pre-saturated with water) for about 3 minutes. Record the neptunium peak (see Note) at about —0·8 volt

against the mercury-pool anode, on the square-wave polarograph, at a suitable sensitivity. Add accurately about 0·2 ml (from a blow-out calibrated pipette, E–MIL-type) of a standard neptunium solution, prepared in EDTA as described, such that the original peak height is approximately doubled. Deduce the neptunium concentration of the original solution from the increase in peak height.

NOTE—Polarograms obtained in the EDTA base solution are well defined, provided the method is followed carefully. However, it must be stressed that control of conditions is most important if well defined, reproducible polarograms are required.

RESULTS

RECOVERIES FROM THE SOLVENT-EXTRACTION CYCLE—

First it was necessary to establish the recoveries of neptunium obtained by the TTA solvent-extraction cycle. About 30 μg of neptunium-237 were reduced in 10 ml of 1 *M* hydrochloric acid - 0·1 *M* ferrous chloride and extracted as described under "Procedure." The recoveries, determined by α-counting a small aliquot of the aqueous nitric acid solution obtained from backwashing, were all about 99 per cent. It was observed that, if more than 1 ml of wash liquid (1 *M* hydrochloric acid) were used, small losses might occur.

RECOVERIES FROM PLUTONIUM SOLUTIONS—

A sample of plutonium metal was dissolved and additions of a standard solution of neptunium were made at different levels. No attempt was made to remove neptunium in the sample before these additions. After solvent extraction, recoveries, which included the neptunium originally present in the sample, were determined polarographically in the EDTA base medium by reference to a calibration graph. (A recovery was obtained on the plutonium sample alone to determine the blank value.) The results are shown in Table IV. These

TABLE IV

RECOVERY OF NEPTUNIUM FROM PLUTONIUM SOLUTIONS

Weight of plutonium taken, g	Weight of neptunium added, μg	Neptunium found		Total weight of neptunium present in samples after addition,	Recovery,
		μg	p.p.m.	μg	%
0·4792	—	170	355	0 + 170 = 170	—
0·5095	110	312	612	110 + 181 = 291	107
0·3804	275	395	1,040	275 + 135 = 410	96
0·5503	550	710	1,290	550 + 195 = 745	95

recoveries extend over the range 300 to 1300 p.p.m. Initially, sample requirements will probably be in the range 10 to 1000 p.p.m., so the range 100 to 300 p.p.m. was studied. For this it was necessary to purify a sample of plutonium metal before adding neptunium. Two solvent extractions with TTA solution were sufficient for this purpose. The results are shown in Table V and indicate that losses are usually not greater than 5 per cent. in the range 100 to 300 p.p.m.

TABLE V

RECOVERY OF NEPTUNIUM FROM PURIFIED PLUTONIUM SOLUTIONS

The weight of purified plutonium taken for each test was 500 mg

Neptunium added, μg	Neptunium recovered, μg	Neptunium added, p.p.m.	Neptunium recovered, p.p.m.	Recovery, %
55	52·5	110	105	96
110	110·5	220	221	100
110	102·5	220	205	93
165	161	330	322	98

RECOVERY EXPERIMENT WITH NEPTUNIUM-239 TRACER—

To test recoveries in the 10 to 100 p.p.m. range, some neptunium-239 tracer (half-life 2·3 days) was prepared by irradiating depleted uranium-238 (0·01 per cent. of uranium-235) and extracting the tracer with TTA solution. A known amount of tracer was then added to a plutonium sample containing about 30 p.p.m. of neptunium (previously analysed by the recommended method). An oxidation - reduction step was incorporated to ensure exchange

between added neptunium-239 and neptunium-237 in the sample. The neptunium was extracted, and the recovery was determined by comparing the γ-spectrum with that of a direct aliquot of the original tracer solution. Neptunium-239 gives a well defined peak at about 0·2 MeV. The recovery obtained was 100 $\pm$ 2 per cent. The total mass of neptunium was then determined by square-wave polarography and the result agreed closely with previous figures on the sample.

This result provides an independent check on the solvent-extraction procedure at a lower neptunium level. The reason for the improvement of recoveries by the tracer technique is probably two-fold; first, practical difficulties associated with purifying samples before determining added neptunium, and secondly, inaccuracy in the polarographic method when determining neptunium over a concentration range by reference to one calibration graph.

RESULTS ON SAMPLES—

Several results from two different samples of plutonium metal have been obtained by using the proposed method. The results are shown in Table VI. The letters in column 2

TABLE VI

DETERMINATION OF NEPTUNIUM IN SAMPLES OF PLUTONIUM

Sample No.	Section of sample	Neptunium found by standard-addition method, p.p.m.	Neptunium found by calibration-graph method, p.p.m.	Mean value, p.p.m.
	a	480	485	482 $\pm$ 3
	b	484	479	482 $\pm$ 3
1		485	478	
	c	493	486	496 $\pm$ 8
		507	499	
	a	31	30	29 $\pm$ 3
		31	26	
2	b	24	23	24 $\pm$ 2
			26	
	c	30	25	29 $\pm$ 4
		32	28	

refer to different sections of the sample of plutonium. Each figure in the standard-addition method column refers to an individual analysis. As a check an alternative polarographic finish was used, *i.e.*, the neptunium concentration was deduced by reference to a calibration graph prepared from a standard neptunium solution (results in column 4).

INTERFERING ELEMENTS

An EDTA base solution was used in preference to molar hydrochloric acid by virtue of its freedom from interferences. The half-wave potential of the neptunium^{IV} reduction is shifted well away from—

(*a*) the anodic wave caused by chloride ions;

(*b*) the cathodic reduction waves of uranium^{VI}, plutonium^{IV} and copper^{I}, all occurring in the vicinity of the neptunium peak. Table VII shows values taken from the literature of approximate half-wave potentials of interfering elements compared with that of neptunium.

The extraction of neptunium^{IV} with TTA affords separation from uranyl ions and plutonium^{III} (both these elements in the valency states indicated have low extraction coefficients from dilute mineral acids), but some copper is extracted with neptunium^{IV}; however, it can be seen from Table VII (column 3) that the neptunium peak in EDTA is well separated from copper and also cadmium, lead, uranium, iron and chloride ions. To check these points, a solution of about 50 μg of neptunium^{IV} per ml in EDTA base medium was measured on the square-wave polarograph in the absence and presence of—

(*i*) 50μ g of uranium^{VI} per ml;

(*ii*) 10 μg of copper^{II}, lead^{II} and cadmium^{II} per ml;

(*iii*) 50 μg of plutonium^{IV} per ml; and

(*iv*) 200 μg of iron^{II} per ml.

TABLE VII

HALF-WAVE POTENTIALS OF INTERFERING ELEMENTS

Element	$E_{\frac{1}{2}}$ measured against saturated-calomel electrode,* volts	$E_{\frac{1}{2}}$ measured against saturated-calomel electrode,† volts
$Cd^{2+} \rightleftharpoons Cd$	−0·64	−1·4
$Pb^{2+} \rightleftharpoons Pb$	−0·435	−1·2
$Np^{4+} \rightleftharpoons Np^{3+}$	−0·103	−0·8
$Cu^{2+} \rightleftharpoons Cu$	—	−0·5
$Cu^{+} \rightleftharpoons Cu$	−0·22	—
$UO_2^{2+} \rightleftharpoons UO_2^{+}$	−0·18	−0·3
$Fe^{3+} \rightleftharpoons Fe^{2+}$	+0·04	−0·2
$Cl^- + Hg \rightleftharpoons HgCl + e$ ..	+0·1	+0·1

* Base medium 1 *M* hydrochloric acid - 0·1 *M* hydroxylamine hydrochloride.
† Base medium 0·1 *M* EDTA - 1 *M* hydroxylamine hydrochloride at pH 6.

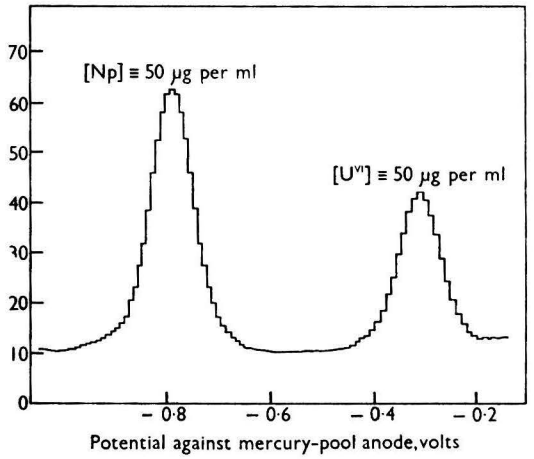

Fig. 5. Square-wave polarogram for neptunium and uranium in 0·1 *M* EDTA - 1 *M* Cl^- at pH 6

Results show that the uranium (see Fig. 5) and copper peaks (see Fig. 6) are well separated from neptunium. Lead and cadmium were not observed at all, as was predicted from their half-wave potentials. No reduction wave was observed for plutonium^{IV} over the range −0·2 to −1·4 volts. Ferrous iron when in a four-fold excess over neptunium did not interfere,

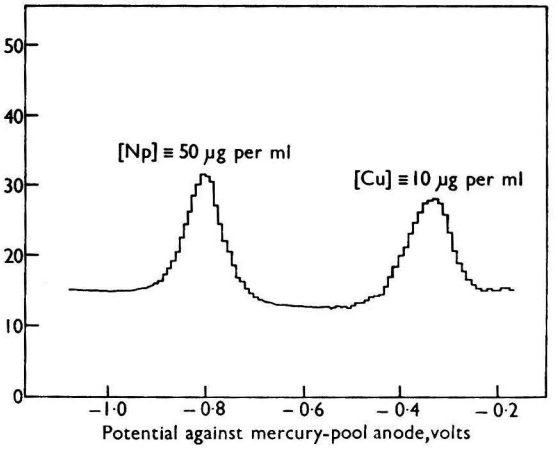

Fig. 6. Square-wave polarogram for neptunium and copper in 0·1 *M* EDTA - 1 *M* Cl^- at pH 6

but at higher levels an interfering peak was evident at the same half-wave potential as the neptunium peak. Moreover, ferrous iron is used to reduce neptunium before separation, and any ferric iron formed at this stage will be extracted with TTA. However, if the neptunium is backwashed with 10 *M* nitric acid rather than hydrochloric acid, the ferric iron remains in the organic phase and no interference is observed when 0·1 *M* ferrous chloride is used as reductant. (It is advisable to control the concentration of reductant to 0·1 *M*.) In the analysis of plutonium samples, no interference should be caused by iron, uranium, copper, lead or cadmium, unless these elements are present in much higher concentrations than anticipated.

Conclusions

The precision (2σ) of the method, given at least 300 mg of sample and a neptunium content of about 500 p.p.m., is estimated to be about ± 2 per cent. The bias is dependent on the purity of the standard and is estimated to be less than $+2$ per cent., since the analysis of the standard shows the neptunium metal to be at least 98·5 per cent. pure. In the work described the over-all accuracy will certainly be better than 5 per cent.

The ultimate sensitivity of the square-wave polarograph is at least 0·4 μg of neptunium per ml, corresponding to 2 p.p.m. in a 1-g sample. At these low levels, the accuracy and precision might be ± 30 per cent., but special attention would have to be paid to the purity or reagents.

We thank Dr. A. J. Fudge for some helpful suggestions.

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Recommended Methods of Assay of Crude Drugs

PREPARED BY THE JOINT COMMITTEE OF THE PHARMACEUTICAL SOCIETY AND THE SOCIETY FOR ANALYTICAL CHEMISTRY ON METHODS OF ASSAY OF CRUDE DRUGS

The Determination of the Capsaicin Content of Capsicum and its Preparations

Introduction

In March, 1956, the Pharmaceutical Society of Great Britain and the Society for Analytical Chemistry set up a Joint Committee on Methods of Assay of Crude Drugs, the purpose of which is to recommend methods of assay for those crude drugs that are widely used in commerce, but for which there are no adequate standard or official methods of assay at present in force.

Capsicum was among the first group of drugs selected for urgent attention, in view of its extensive use in official and non-official preparations. As the generally accepted organoleptic test is unreliable, the Joint Committee considered that an effort should be made to devise a reproducible chemical method for the assay of the capsaicin content. Accordingly, a working Panel was appointed in July, 1956, having the composition—H. B. Heath (Chairman), E. A. Elsbury, C. A. MacDonald, G. R. A. Short and D. O. Singleton, with Miss A. M. Parry as Secretary. Miss B. M. Luckett was co-opted as a member in February, 1957.

The Panel's terms of reference were as follows—

> "To investigate methods of assay of capsicum and capsicum products with particular reference to the determination of the capsaicin content."

This Report of the Panel deals only with the chemical assay of those fruits of the genus *Capsicum* having a relatively high capsaicin content; methods are also given for the oleoresin and for some British Pharmaceutical Codex preparations. Work is proceeding on methods for the evaluation of the capsaicin content of the remaining B.P.C. preparations, of low-potency drugs and of proprietary preparations.

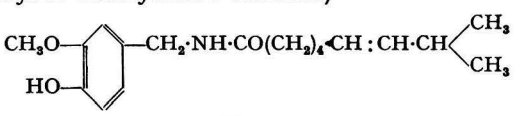
Report

Capsicums are the fruits of various species of the Solanaceous genus *Capsicum*. So many species and varieties have been described that exact classification is difficult, but there appear to be three commercially important species. *Capsicum minimum* Roxb. and *Capsicum frutescens* L. are tropical plants having small, very pungent fruits generally known in commerce as "chillies"; *Capsicum annuum* L., from which "capsicum," "cayenne pepper" and the mid-European "paprika" derive, is a temperate plant having fruit varying widely in size, shape and colour, but generally much less pungent than chillies.

The capsicum of the British Pharmaceutical Codex is described as either the dried ripe fruits of *C. minimum* or small fruited varieties of *C. frutescens*; the U.S. National Formulary X recognises members of two species, namely, the dried ripe fruits of *C. frutescens*, which it calls "African Chillies" and three varieties of *C. annuum*.

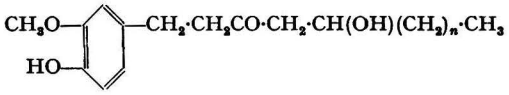
Capsaicin—

It is now established that capsaicin is the only substance responsible for the pungency of capsicum. This pungent principle was isolated in an almost pure state by Thresh in 1876. Its nature and chemical structure were established by Nelson and Dawson,[1] who synthesised it, and subsequently confirmed by Späth and Darling.[2] Capsaicin (**I**) is the vanillylamide of *iso*decenoic acid (8-methyl-N-vanillylnon-6-enamide)—



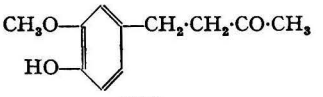
(**I**)

In a review of the chemistry of the constituents of capsicum, Dodge[3] draws attention to the similarity in structure of capsaicin to those of gingerol (**II**) and gingerone (**III**), the pungent principles of ginger (*Zingiber officinale*, Roscoe), and its relation to vanillin (**IV**)—

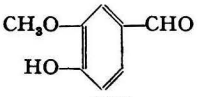


where $n = 3$, 4 or 5

(**II**)



(**III**)



(**IV**)

Synthetic compounds chemically akin to capsaicin, such as the vanillylamide of nonylic acid, also have pungent properties and are used in place of or in addition to naturally occurring capsaicin.

Unlike capsaicin, the pungency of ginger is destroyed by heating with a 2 per cent. solution of sodium hydroxide. The pungency of capsicum can be destroyed by oxidation with potassium permanganate or dichromate, but those synthetic "capsaicins" having a saturated side-chain are not readily oxidised. This property has been made use of in the detection of these synthetic compounds.[4] The determination of these compounds will also be investigated by the Panel.

Capsaicin forms white, pearly leaflets, melting sharply at 64° to 65° C. The crystals are so light that they readily become airborne. Even minute traces in the atmosphere result in unbearable choking, so that extreme care is necessary when handling the substance. Capsaicin is readily soluble in methanol, ethanol, acetone, chloroform or ether and moderately

soluble in hot light petroleum (boiling range 60° to 80° C), from which it crystallises when the solution is cooled; it is almost insoluble in water. With alkalis, capsaicin forms a water-soluble salt from which it is recovered unchanged on acidification.

EVALUATION OF CAPSICUM—

Capsicums are used extensively in food as a spice and in medicine as a rubefacient and carminative. The food industry generally prefers to use large highly coloured capsicums having only a small capsaicin content, whereas for medicinal purposes the more pungent varieties are of greater value.

Berry and Samways[5] examined capsicums, and also oleoresins and tinctures prepared from them, and devised an organoleptic test based on a threshold sensation of pungency in the throat. This test is now the official one in the British Pharmaceutical Codex. As with all such tests, the accuracy is low, since the sensitivity of the human throat varies between individuals and with fatigue, colds, etc. Pure capsaicin can just be detected at a dilution of 1 in 5×10^7 and definitely at 1 in 3×10^7, and with such dilutions it is clearly possible to make only an approximation of the pungency, and hence of the capsaicin content of capsicum. This, and similar methods, despite their shortcomings, are currently in use.

Numerous colorimetric methods have been described for the chemical assay of capsaicin. That developed by Tice,[6] in which use is made of the blue colour produced with vanadium oxytrichloride in carbon tetrachloride solution, was shown by Hayden and Jordan[7] to be unsatisfactory owing to interference by other constituents giving the same colour reaction. Nógrády[8] has suggested a method based on titration with picric acid and fluoresence desorption at the end-point. North[9] evolved a method in which use is made of the blue colour produced by the Folin - Denis reagent, the colour being compared with standard solutions of vanillin, which reacts similarly. Büchi and Hippenmeier[10] used a method based on the reduction of molybdophosphoric acid by capsaicin in alkaline solution. Schenk[11] treated the decolorised eluate from chromatographic separation with ammonium vanadate and hydrochloric acid to give a blue-green colour. More recently, methods (also with chromatographic separation) have been described by Fujita, Furuya and Kawana[12], who used the molybdophosphate reaction, and by Schulte and Krüger,[13] who used the colour produced when capsaicin was coupled with diazobenzenesulphonic acid.

Members of the Panel had found none of the above-mentioned methods completely reliable, but that of Schulte and Krüger was considered to be the most suitable for further investigation and application as a method for routine analysis. This method, which depends upon the formation of an azo dye, is neither original nor specific; any amine or phenol having an unoccupied *ortho-* or *para*-position will couple to give a dye, and the method has been used by Berka and Zýka[14] for the determination of phenols, amines and sulpha drugs. In two recent papers, Spanyár, Kevei and Kiszel[15] and Zitko[16] also report use of the same reaction.

EXPERIMENTAL

Before methods for the assay of capsaicin in compounds could be examined, the isolation of pure capsaicin was the essential first step for the study of its properties and for the provision of an absolute standard.

The method described by Tice[6] was used, with some modification. The melting-point of the pure white crystals of capsaicin was found to be 65° C (Lapworth and Royle[17] give melting-point 64° to 65° C). Details of the procedure are given in Appendix II, p. 615.

EVALUATION OF CAPSAICIN—

The two methods of evaluating capsaicin open to investigation were the direct spectrophotometric method and a colorimetric method based on the coupling reaction between diazobenzenesulphonic acid and capsaicin.

(*a*) *Spectrophotometric characteristics of capsaicin*—

When the Panel began work, no information could be found in the literature about the ultra-violet absorption characteristics of capsaicin. Accordingly, measurements were made of the optical densities of solutions of pure capsaicin in ethanol, in methanol and in 0·1 *N* sodium hydroxide, a spectrophotometer and 1-cm quartz cells being used.

In ethanolic solution the maxima are well defined, although at 229 mμ $E^{1\%}_{1cm}$ values of from 245 to 258 were obtained on the same sample; however, the maximum at 280 mμ shows

a reproducible $E^{1\%}_{1cm}$ value of 102. In methanolic solution the maximum at 229 mμ is masked by the absorption of the solvent, but that at 279 mμ is well defined and of the same value (102) as in ethanolic solution. In 0·1 *N* sodium hydroxide the maxima are displaced, respectively, to 248 mμ ($E^{1\%}_{1cm} = 330$) and 294 mμ ($E^{1\%}_{1cm} = 136$).

Since it was found that the absorptions at these maxima each obey the Beer - Lambert law, it was concluded that any of these solutions could be used satisfactorily for the determination of pure capsaicin.

Early in the investigation it was found that for solutions of pure capsaicin in methanol there is a constant relationship between the optical-density values at 280 mμ and the values at 270 and 290 mμ, the ratios being $\frac{270}{280}$ mμ = 0·60 and $\frac{290}{280}$ mμ = 0·53. If solutions of capsaicin give results differing widely from these, the presence of interfering substances is indicated.

The spectrophotometric characteristics of capsaicin in 0·02 *N* sodium hydroxide and in 0·01 *N* hydrochloric acid, each in 80 per cent. methanol, were then determined. It was found that in the alkaline solution maxima occur at 248 and 294 mμ and minima at 227 and 271 mμ. In the acid solution the maxima occur at 228 and 280 mμ with a minimum at 251 mμ and zero at 305 mμ. The absorption curves are shown in Fig. 1. It was decided to utilise these characteristics in a method of assay.

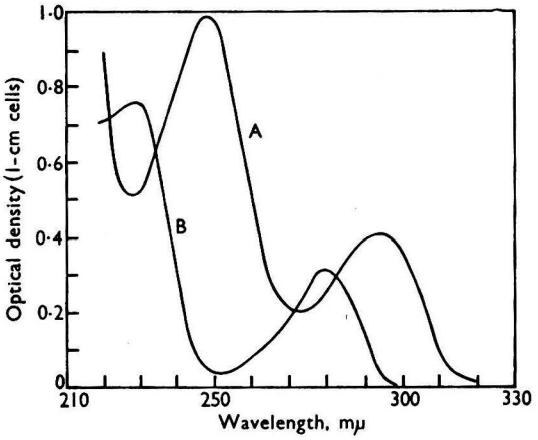


Fig. 1. Absorption spectra of capsaicin: curve A, 0·0033 per cent. solution in 0·02 *N* sodium hydroxide in 80 per cent. methanol; curve B, 0·0033 per cent. solution in 0·01 *N* hydrochloric acid in 80 per cent. methanol

(*b*) *Colorimetric method*—

Appraisal of Schulte and Krüger's method—A uniform sample of Capsicum B.P.C., ground to pass a 40-mesh sieve, was used in collaborative tests by the method as published.[13] This specifies the use of a column of alumina upon which is superimposed a layer of freshly ignited charcoal.

The Panel found that the percolation rate was extremely slow by the method described, but that the rate was increased when the charcoal was intimately mixed with the alumina, giving complete extraction in 250 ml in 2 hours. The course of the extraction supports Fig. 3 of Schulte and Krüger's paper, the bulk of the capsaicin being in the first 100 ml of eluate.

In carrying out the coupling reaction the addition of the diazobenzenesulphonic acid solution is the most critical operation. The authors used 5 ml of eluate (which would contain 160 μg of capsaicin) to which were added 1 ml of 0·2 *N* sodium hydroxide and then 1 ml of diazo reagent drop by drop. When 0·5 ml of the diazo reagent is added, drop by drop with agitation, the full colour develops in about 5 minutes, the remaining 0·5 ml of reagent then being added similarly. When approximately 0·8 ml of reagent has been added the solution becomes acid, as indicated by a total discharge of the colour. With this amount of capsaicin, approximately 100 per cent. excess of reagent is present. When the stated conditions of

heating to remove this excess were used, and then 0·2 *N* sodium hydroxide was added, results varying by ± 3 per cent. were obtained. It was found that, once developed, the azo dye was stable for several hours.

The Panel found, however, that pure capsaicin gave only approximately half the colour intensity indicated in Graph 2 of Schulte and Krüger's paper. It was found, also, that for low concentrations of capsaicin disproportionately low optical-density readings were obtained. At 160 μg per 10 ml, as used in the published method, an optical density of 0·080 was obtained, whereas at 320 μg per 10 ml the optical density was 0·240. Greater concentrations were found to give optical densities more closely rectilinear and it was therefore decided to work within the range 350 to 700 μg of capsaicin per 10 ml in future determinations.

Study of the diazo-coupling reaction—The conditions under which pure capsaicin couples with diazobenzenesulphonic acid to give a red dye were critically examined with a view to the preparation of a permanent calibration graph for the reaction. It was hoped to evaluate and publish the $E^{1\%}_{1cm}$ value of the coloured complex to avoid the necessity of preparing a reference sample of pure capsaicin. In spite of much collaborative work, this has not been found possible. Throughout it has been necessary to prepare a standard graph at the same time as making the determination.

A considerable number of compounds was investigated as possible alternative standards in the diazo reaction; but it was reluctantly agreed that no suitable alternative has yet been found. A detailed account of this work is given in Appendix III, p. 616.

Modification of the technique—The method as published[13] (Method A) gives results varying by ±3 per cent., but the Panel found it to be unduly tedious.

Two alternative methods were then investigated on aliquots of capsaicin solution ranging from 200 to 500 μg per 5 ml, due allowance being made in the concentrations of the reactants so as to maintain the same relative conditions.

Method B

An aliquot of the sample was measured by pipette into a 10-ml calibrated flask, and the volume was adjusted to 5 ml with ethanol as necessary; to this was added 1 ml of diazobenzenesulphonic acid solution (0·4 per cent. in 0·25 *N* hydrochloric acid), and then 1 ml of 0·4 *N* sodium hydroxide. The mixture was shaken and then set aside for 15 minutes; 1 ml of 0·33 per cent. sodium iodide solution was then added, followed by 1 ml of 0·25 *N* hydrochloric acid. The solution was shaken, heated for 15 minutes at 60° to 70° C and then cooled and diluted to the mark with 0·4 *N* sodium hydroxide, and the optical density was read.

Method C

An aliquot of the sample was measured by pipette into a 10-ml calibrated flask, and the volume was adjusted to 5 ml with ethanol as necessary; to this was added 1 ml of diazobenzenesulphonic acid solution (0·4 per cent. in 0·25 *N* hydrochloric acid). The mixture was shaken and diluted to the mark with 0·2 *N* sodium hydroxide. The solution was shaken thoroughly and then maintained at 20° C for 15 minutes and the optical density was read.

Collaborative work showed that by all three of the above-mentioned methods the rectilinear agreement was good, although it was found that the wavelength of maximum absorption was 480 mμ for Methods B and C as against 490 mμ for Method A. Method A gave the most accurate results, but was too tedious for routine application; Method B gave satisfactory results and Method C, although rapid, did not give reproducible results and was not further investigated.

In a method described by Spanyár, Kevei and Kiszel[18] the diazotisation is carried out *in situ*. The results of collaborative tests on this by the Panel were most disappointing, it being observed that the resulting solutions varied considerably both in colour and intensity. Therefore this approach was not considered further, and the Panel decided to proceed with the study of Method B.

This method was then modified so that the sodium hydroxide solution was added *before* the diazobenzenesulphonic acid solution, and it was found that the colour developed immediately and to a greater intensity than previously. Closer agreement between the laboratories was obtained by adding the reagents ice-cold and by allowing a 10-minute interval between the addition of the sodium hydroxide solution and the diazobenzenesulphonic acid solution.

As a result of the investigation into the chromatographic separation, which is discussed later, ethanol was replaced by methanol as a solvent for capsaicin. This also had the effect of producing a brighter and more truly red colour of a slightly greater intensity.

One member of the Panel (E.A.E.) investigated the use of an equimolecular sodium carbonate - sodium bicarbonate buffer solution instead of sodium hydroxide solution, as suggested by Collins.[19] It was found that the wavelength of maximum absorption was at 495 mμ, the results being slightly lower than those obtained with 0·4 *N* sodium hydroxide. The reproducibility was not improved, and, as the method is no more sensitive than Method B, as modified, it was not further considered.

After all modifications had been made, the procedure as recommended (see Appendix I) for carrying out the coupling reaction was found to give results varying by ± 5 per cent. between the five collaborating laboratories (see Table I). In order to obtain this degree of reproducibility the method must be adhered to in detail.

TABLE I

OPTICAL-DENSITY VALUES FOR CAPSAICIN IN METHANOL BY THE DIAZO REACTION

Weight of capsaicin in 10 ml of solution,	Laboratory						
μg	A	B	C	D	E	F	Mean
400	0·435	0·465	0·471	0·460	0·471	0·476	0·463
600	0·729	0·744	0·713	0·712	0·741	0·738	0·730
800	1·012	1·012	0·958	0·965	1·012	1·015	0·997

EXTRACTION OF CAPSAICIN—

Two procedures for the extraction of capsaicin from capsicum and preparations of capsicum were investigated, one by column chromatography and the other by ether - alkali partition extraction.

(*a*) *Chromatographic method*—All the chromatographic methods investigated depended on the removal of impurities by adsorption on charcoal, but differed in the amount of charcoal, in the support for the charcoal and in the eluting solvent. Schenk[11] used a column of alumina intimately mixed with 1 per cent. of charcoal with ethanol as the eluting solvent; Schulte and Krüger[13] used separated layers of alumina and charcoal, the latter amounting to 28 per cent. of the total, and also eluted with ethanol; Suzuki, Tausig and Morse[20] used separated layers of acidic alumina, basic alumina and charcoal mixed with an equal weight of Celite, with 1 per cent. v/v methanol in ethyl acetate as developing solvent and methanol for final elution.

As a result of examining the column materials and solvents used in the above-mentioned methods, the Panel concluded that charcoal was the effective adsorbing material, all other constituents being mainly supporters and diluents. Comparative determinations were carried out to ascertain the optimum amount of charcoal required to give minimum capsaicin retention and maximum removal of interfering substances. Many variations in the composition of the column and of the eluting solvents were tested; they resulted in the recommendations in Appendix I being adopted.

One member of the Panel (E.A.E.) carried out an experiment on this column and recovered 105 per cent. of added capsaicin.

(*b*) *Ether - alkali partition extraction*—During a study of the application of the chromatographic method to the evaluation of highly coloured capsicums, it was found that the column was inadequate to remove all the interfering substances. Since pure capsaicin itself is prepared by a method of partition extraction between aqueous alkaline and organic solvents, it was decided to investigate the application of this method as an assay procedure. As a result of many trial determinations the conditions given in the Recommended Method (Appendix I) were found to give consistent results with capsicum, oleoresin of capsicum and tincture of capsicum.

Since capsicum ointments containing fats and waxes also cannot be assayed chromatographically, the ether - alkali extraction procedure must be used, but with further modifications to avoid the formation of troublesome emulsions not broken by the addition of sodium chloride.

While this investigation was in progress, a similar procedure for the assay of ointments was described by Schülte and Krüger,[21] consideration of which may ultimately lead to a modification of the method given in Appendix I.

Conclusions

The choice of the method of extraction to be used is purely one of convenience, except for highly coloured samples, when the ether - alkali partition procedure must be adopted.

The Panel recommends that the capsaicin content be determined by the spectrophotometric difference method, the results obtained at 294 mμ only being used.

Results of collaborative tests

Results of collaborative tests on Capsicum B.P.C. and some other B.P.C. preparations containing capsaicin are given in Tables II, III, IV and V.

Table II

Assay of capsicum B.P.C.

Laboratory	Capsaicin content, after chromatography, by— direct spectrophotometry,* %	difference method 248 mμ, %	difference method 294 mμ, %	diazo method, %	Capsaicin content, after ether - alkali extraction, by— direct spectrophotometry,* %	difference method 248 mμ, %	difference method 294 mμ, %	diazo method, %	Capsaicin content by organoleptic method,† %
Sample No. 1—									
A 1	1·30	1·08	1·07	1·09	1·45	0·96	1·02	1·20	—
A 2	1·41	0·95	1·05	1·10	1·60	1·05	1·29	1·24	—
B 1	1·32	1·10	1·15	1·25	1·36	0·94	1·04	1·00	—
B 2	1·13	0·99	1·05	1·02	1·09	0·99	1·04	1·09	—
C 1	1·14	1·01	1·03	1·17	1·19	1·0	1·0	—	—
C 2	1·10	1·04	1·04	1·23	1·03	0·84	0·85	0·73	—
D 1	1·3	1·02	1·10	0·98	1·7	1·02	1·13	1·00	—
D 2	1·4	1·08	1·12	1·06	1·5	1·00	1·11	1·00	—
E 1	1·23	0·96	0·96	0·93	1·10	0·97	0·97	0·87	—
E 2	1·27	1·00	1·05	0·95	1·02	0·89	0·87	0·80	—
Sample No. 2—									
A 1	0·83	0·57	0·71	0·56	1·16	0·55	0·59	0·57	0·28
A 2	0·84	0·57	0·63	0·50	1·18	0·46	0·45	0·57	
B 1	0·60	0·51	0·56	0·55	0·81	0·52	0·57	0·56	0·56
B 2	0·60	0·50	0·54	0·54	0·7	0·50	0·55	0·53	
C 1	0·67	0·54	0·58	—	0·91	0·59	0·62	—	—
C 2	0·64	0·54	0·58	—	0·93	0·58	0·61	—	—
D 1	1·07	0·61	0·59	0·57	0·86	0·63	0·56	0·55	0·18
D 2	0·94	0·58	0·64	0·58	0·84	0·57	0·57	0·57	0·17
E 1	1·02	0·47	0·50	0·41	1·04	0·50	0·54	0·50	0·44
E 2	1·04	0·44	0·46	0·40	1·03	0·51	0·55	0·50	

* The specified ratios $\frac{270}{280}$ mμ and $\frac{290}{280}$ mμ were not obtained; therefore the results are inadmissible.

† Threshold for pure capcaisin content = 1 in 3×10^7.

Appendix I

RECOMMENDED METHODS FOR THE DETERMINATION OF CAPSAICIN

Principles of methods—

After preliminary preparation of the sample, the capsaicin is extracted either chromatographically or by an ether - alkali partition extraction procedure. The capsaicin is then determined by a direct spectrophotometric method, by a spectrophotometric difference method or by a colorimetric method based on the coupling reaction between diazobenzenesulphonic acid and capsaicin. Of these, the spectrophotometric difference method gives the most reproducible results.

Applicability—

The methods may be used for the evaluation of Capsicum B.P.C., Oleoresin of Capsicum B.P.C., Tincture of Capsicum B.P.C. and Ointment of Capsicum B.P.C. or similar preparations.

Interfering substances—Capsaicin is accompanied by sterols, fatty acids and colouring matters, which are mostly removed on the column, but a small proportion frequently passes

through the column and renders invalid the results by the direct spectrophotometric method. In the event of a coloured eluate being obtained, this must not be passd through a second column, owing to preferential adsorption of capsaicin from this purer solution.

If the results obtained from this eluate by the spectrophotometric difference method and by the colorimetric method agree, then they may be considered satisfactory. If, however, they disagree, the original sample should be assayed by the ether - alkali partition extraction method.

RANGE—

The ranges of capsaicin content that can be determined with accuracy are—

Capsicum—for contents of 0·5 per cent. w/w and above;
Oleoresin of capsicum—for contents of 2 per cent. w/w and above;
Tincture of capsicum—for contents of 0·01 per cent. w/w and above;
Ointment of capsicum—for contents of 0·05 per cent. w/w and above.

TABLE III

ASSAY OF OLEORESIN OF CAPSICUM B.P.C.

		Capsaicin content, after chromatography, by—				Capsaicin content, after ether - alkali extraction, by—				Capsaicin content by organo-leptic method,† %
		direct spectro-photo-metry,* %	difference method 248 mμ, %	difference method 294 mμ, %	diazo method, %	direct spectro-photo-metry,* %	difference method 248 mμ, %	difference method 294 mμ, %	diazo method, %	
Sample No. 1—										
A	1	12·8	11·3	11·7	10·8	13·9	9·1	9·7	12·0	—
	2	13·0	11·3	11·6	10·7	14·0	11·0	11·6	12·7	—
B	1	12·1	10·1	11·3	10·6	11·4	10·6	11·0	10·3	—
	2	11·7	10·1	10·5	10·4	11·4	10·3	10·6	10·1	—
C	1	11·6	10·3	10·7	9·5	10·7	8·0	7·8	7·5	—
	2	11·5	10·0	10·0	9·4	10·8	8·7	8·5	—	—
D	1	11·6	10·8	11·3	9·6	13·6	10·2	11·9	9·9	—
	2	10·3	10·2	10·8	9·1	13·2	10·2	11·6	10·2	—
E	1	11·3	10·1	9·6	9·6	9·7	9·1	8·9	8·5	—
	2	11·3	10·2	10·2	9·8	11·3	9·4	9·5	9·2	—
Sample No. 2—										
A	1	14·5	12·0	12·9	10·4	16·7	10·6	10·8	11·1	4·7
	2	14·2	12·3	12·9	11·6	16·6	10·9	11·0	11·1	
B	1	12·1	11·1	11·5	9·9	12·4	11·2	11·7	10·4	12·6
	2	12·2	11·1	11·5	9·9	12·0	10·9	11·3	10·3	
C	1	13·6	11·3	11·9	—	12·6	11·3	12·3	—	—
	2	13·7	11·1	11·7	—	12·4	11·0	12·3	—	—
D	1	11·7	12·0	11·1	10·7	14·1	11·8	10·7	11·0	4·2
	2	11·1	12·2	13·4	—	12·4	11·5	10·9	10·5	3·8
E	1	13·4	11·5	11·5	11·3	13·1	11·1	11·2	10·2	6·86
	2	13·2	11·0	11·0	10·3	13·9	11·2	11·4	10·0	

* The specified ratios $\frac{270}{280}$ mμ and $\frac{290}{280}$ mμ were not obtained; therefore the results are inadmissible.

† Threshold for pure capsaicin content = 1 in 3×10^7.

I. CAPSICUM B.P.C., OLEORESIN OF CAPSICUM B.P.C. AND TINCTURE OF CAPSICUM B.P.C.

A. PREPARATION OF SAMPLE—

Capsicum B.P.C.—Macerate an accurately weighed sample (approximately 15 g) in a moderately fine powder (all to pass a 40-mesh sieve) with 80 ml of 96 per cent. v/v ethanol in a closed flask for 24 hours, the flask being mechanically shaken for 6 hours and set aside for 18 hours. Filter the solution through a No. 1 sintered-glass filter into a 100-ml calibrated flask, washing the filter with 20 ml of the ethanol. Dilute to the mark with the ethanol.

TABLE IV

ASSAY OF TINCTURE OF CAPSICUM B.P.C.

Laboratory	Capsaicin content, after chromatography, by— direct spectrophotometry,* %	difference method 248 mμ, %	difference method 294 mμ, %	diazo method, %	Capsaicin content, after ether - alkali extraction, by— direct spectrophotometry,* %	difference method 248 mμ, %	difference method 294 mμ, %	diazo method, %	Capsaicin content by organoleptic method,† %
Sample No 1 —									
A 1	—	0·040	0·044	0·043	—	0·041	0·041	0·036	—
A 2	—	0·041	0·042	0·040	—	0·039	0·041	0·040	—
B 1	0·053	0·037	0·042	0·035	0·047	0·035	0·041	0·033	—
B 2	0·050	0·037	0·039	0·032	0·049	0·034	0·037	0·037	—
C 1	0·049	0·037	0·038	0·039	0·047	0·038	0·046	0·032	—
C 2	0·035	0·036	0·034	0·034	0·045	0·037	0·043	0·030	—
D 1	0·050	0·039	0·041	0·032	0·039	0·035	0·035	0·030	—
D 2	—	—	—	—	0·041	0·037	0·038	0·031	—
E 1	0·048	0·035	0·037	0·034	0·044	0·023	0·023	0·026	—
E 2	0·048	0·035	0·037	0·035	0·042	0·025	0·027	0·025	—
Sample No. 2—									
A 1	0·040	0·023	0·026	0·028	0·048	0·022	0·024	0·027	0·0059
A 2	0·048	0·023	0·026	0·029	0·048	0·030	0·027	0·027	
B 1	0·057	0·024	0·023	0·025	0·027	0·021	0·021	0·022	0·028
B 2	0·055	0·024	0·023	0·024	0·028	0·022	0·021	0·022	
C 1	0·052	0·022	0·023	—	0·033	0·021	0·023	—	—
C 2	0·053	0·023	0·023	—	0·032	0·021	0·023	—	—
D 1	0·047	0·026	0·021	0·020	0·032	0·026	0·022	0·021	0·01
D 2	0·044	0·023	0·024	0·020	0·029	0·022	0·020	0·020	0·01
E 1	0·055	0·023	0·023	0·024	0·040	0·020	0·022	0·022	0·008
E 2	0·051	0·023	0·023	0·023	0·041	0·020	0·023	0·022	

* The specified ratios $\frac{270}{280}$ mμ and $\frac{290}{280}$ mμ were not obtained; therefore the results are inadmissible.

† Threshold for pure capsaicin content = 1 in 3×10^7.

TABLE V

ASSAY OF OINTMENT OF CAPSICUM B.P.C.

Laboratory	Capsaicin content by— direct spectrophotometry,* %	difference method 248 mμ, %	difference method 294 mμ, %	diazo method, %
Sample No. 1—				
A 1	0·130	0·081	0·093	0·063
A 2	0·094	0·082	0·093	0·066
B 1	0·100	0·083	0·089	0·074
B 2	0·090	0·074	0·073	0·074
C 1	0·087	0·076	0·073	0·070
C 2	0·104	0·080	0·077	0·105
D 1	0·089	0·077	0·077	0·068
D 2	0·046	0·026	0·030	0·028
E 1	0·074	0·043	0·040	0·043
E 2	0·066	0·039	0·040	0·040
Sample No. 2—				
A 1	0·118	0·067	0·089	0·078
A 2	0·112	0·071	0·083	0·069
B 1	0·088	0·057	0·059	0·060
B 2	0·087	0·057	0·057	0·058
C 1	0·071	0·072	0·078	—
C 2	0·077	0·079	0·089	—
D 1	0·031	0·028	0·032	0·042
D 2	0·044	0·029	0·032	0·042
E 1	0·080	0·059	0·055	0·052
E 2	0·086	0·057	0·052	0·057

The specified ratios $\frac{270}{280}$ mμ and $\frac{290}{280}$ mμ were not obtained; therefore the results are inadmissable.

(*i*) Proceed to the chromatographic separation by transferring a 10·0-ml aliquot of the solution into the 5-cm layer of methanol above the alumina column:

or

(*ii*) Transfer a 10·0-ml aliquot of the solution into a 150-ml separating funnel, add 15 ml of 96 per cent. v/v ethanol and 15 ml of distilled water, and proceed to the ether - alkali extraction method.

Oleoresin of Capsicum B.P.C.—(*i*) Dissolve an accurately weighed sample of the oleoresin (approximately 2·5 g) in analytical-reagent grade absolute methanol, add if necessary 1 ml of analytical-reagent grade diethyl ether to assist solution (see Note 1), and adjust the volume to 200 ml.

Take an aliquot containing approximately 20 mg of capsaicin, and proceed to the chromatographic separation:

or

(*ii*) Dissolve an accurately weighed sample of the oleoresin (approximately 0·2 g) in 2 ml of diethyl ether, add 25 ml of 96 per cent. v/v ethanol, transfer the solution to a 150-ml separating funnel, add 15 ml of water, and proceed to the ether - alkali extraction method.

Tincture of Capsicum B.P.C.—(*i*) Evaporate to dryness on a water bath about 40 ml, accurately measured, of the tincture (containing approximately 15 mg of capsaicin). Dissolve the residue in about 10 ml of analytical-reagent grade absolute methanol, and add if necessary 1 ml of analytical-reagent grade diethyl ether to assist solution.

Proceed to the chromatographic separation by transferring the solution into the 5-cm layer of methanol above the alumina column:

or

(*ii*) Transfer by pipette a 40·0-ml sample of the tincture into a 150-ml separating funnel, and proceed to the ether - alkali extraction method.

B. SEPARATION OF CAPSAICIN—

Chromatographic method

APPARATUS—

A "Quickfit" No. C.R. 32/20 chromatographic tube, 20 cm long, internal diameter 18 mm, and fitted with a sintered-glass plate.

A "Quickfit" No. D2/42 funnel, 500-ml capacity, fitted with a DA23 adaptor, as a solvent reservoir.

REAGENTS—

Absolute methanol—As alumina removes impurities from methanol, this reagent must be purified by distillation over silver oxide or by passage through an alumina column. The absorption of the purified methanol in a 4-cm cell at 280 mμ against distilled water should not be greater than 0·020.

Aluminium oxide—"Aluminium oxide for chromatographic analysis" (obtainable from the British Drug Houses Ltd., B.D.H. Laboratory Chemicals Division, Poole, Dorset).

Activated carbon—F.W. grade activated carbon (obtainable from Thomas Hill-Jones Ltd., Junction Works, Bow Common Lane, London, E.3).

Kieselguhr—Super-Cel grade kieselguhr (obtainable from Johns-Manville Co. Ltd., 20 Albert Embankment, London, S.E.11).

PROCEDURE—

Preparation of the column—On a glass sinter or a plug of glass-wool prepare first a column of 12 g of aluminium oxide made into a slurry with absolute methanol. (See Note 2.) On top of this add a mixture of 0·9 g of activated carbon and 0·9 g of kieselguhr also made into a slurry with methanol. Drain until a 1-cm layer of methanol remains on top of the column, then place a disc of filter-paper and finally a small plug of cotton-wool on top of the column. Pass absolute methanol from the reservoir through the column, under gravity, until there is no interference at 280 mμ (approximately 100 ml is required). Leave a 5-cm layer of methanol above the cotton-wool plug.

Elution of capsaicin—Transfer the test solution, prepared as described above, to the column, and elute with methanol, under gravity, always keeping a layer of methanol above the cotton-wool plug. Collect 450 ml of the eluate (in about 4 to 5 hours—see Note 3) in a 500-ml calibrated flask, and dilute to the mark with methanol; mix thoroughly. If necessary, filter the solution through a Whatman No. 542 filter-paper, rejecting the first 10 ml of filtrate.

Determine the capsaicin content of this solution by any of the methods given below.

Ether-alkali extraction method

REAGENTS—

Light petroleum, boiling range 80° *to* 100° *C*—Analytical-reagent grade.
Ethanol, 60 per cent. v/v.
Diethyl ether—Analytical-reagent grade.
Absolute methanol—Analytical-reagent grade.
Hydrochloric acid, approximately 0·1 N.
Sodium hydroxide, approximately 0·1 N.
Sodium chloride.
Activated carbon, F.W. grade.

PROCEDURE—

To the ethanolic solution prepared as described above, add 2 g of sodium chloride and 5 ml of sodium hydroxide solution, and shake well. Extract the solution with 3 portions, each of 10 ml, of light petroleum. Wash the combined light petroleum extracts with 10 ml of ethanol, and add the washings to the aqueous fraction. Filter the bulked aqueous fraction through a pledget of cotton-wool into a beaker-flask, washing the filter with 10 ml of ethanol. Remove the ethanol by evaporation on a water bath, dilute the solution to 50 ml with water, and adjust to pH 7·5 with hydrochloric acid (use either a pH meter or phenol red indicator).

Extract the aqueous solution with 6 portions, each of 20 ml, of diethyl ether. Wash the combined ether extracts with 10 ml of distilled water, and discard the washings. To the combined ether extracts, in a beaker-flask, add 20 ml of methanol, and evaporate almost to dryness on a water bath in a fume cupboard. Add methanol to the residue, transfer the solution to a 100-ml calibrated flask, and dilute to the mark with methanol. Add 0·1 g of activated carbon, shake well, and filter the solution through a Whatman No. 542 filter-paper, rejecting the first 5 ml of filtrate.

Determine the capsaicin content of the solution by any of the methods given below, diluting an aliquot with methanol when necessary.

C. DETERMINATION OF CAPSAICIN—

Direct spectrophotometric method

PROCEDURE—

Measure the optical density of the methanolic solution of capsaicin in a 1-cm cell at 270, 280 and 290 mμ, with methanol in the comparison cell.

At 280 mμ, the $E^{1\%}_{1cm}$ value for capsaicin is 102.

The ratios of the optical-density readings for capsaicin at $\frac{270}{280}$ mμ and $\frac{290}{280}$ mμ are 0·60 and 0·53, respectively. If solutions give figures differing widely from these values, the presence of interfering substances is indicated, and the difference method described below should be used.

Spectrophotometric difference method

REAGENTS—

Sodium hydroxide, approximately 0·1 N—Prepare this solution freshly as required.
Hydrochloric acid, approximately 0·05 N.

PROCEDURE—

Transfer by pipette 20·0 ml of the methanolic solution of capsaicin into a 25-ml calibrated flask, add 5·0 ml of sodium hydroxide solution, shake well, and dilute to the mark with methanol (Solution A).

Similarly treat a further 20·0 ml of capsaicin solution with hydrochloric acid (Solution B).

Measure the optical density of Solution A against Solution B at 248 mμ and at 294 mμ. (See Note 4.)

At 248 mμ the $E^{1\%}_{1cm}$ difference is 308.

At 294 mμ the $E^{1\%}_{1cm}$ difference is 116.

Calculate the capsaicin content from the difference obtained at 294 mμ.

Diazo method

REAGENTS—

Absolute methanol—Analytical-reagent grade.

Dilute hydrochloric acid, approximately 0·25 N.

Sodium hydroxide, approximately 0·4 N—Keep this solution ice-cold.

Diazobenzenesulphonic acid—Dissolve 2 g of sulphanilic acid in 15 ml of 8 per cent. w/v sodium hydroxide solution and 4 ml of 20 per cent. w/v sodium nitrite solution; add this solution slowly, with rotation, to 4 ml of ice-cooled hydrochloric acid, sp.gr. 1·18, in a flask immersed in an ice-bath. Wash the precipitated diazobenzenesulphonic acid on a previously-cooled grade 3 sintered-glass filter successively with 100 ml of ice-cold water, 25 ml of methanol and 25 ml of ether. (See Note 5.)

> The diazonium salt when dry is liable to explode, and special care must be exercised when transferring the dry salt from the filter and during weighing. When the salt is in solution it is harmless. Any dry salt remaining after the required weight has been taken should immediately be washed down the sink.

Diazobenzenesulphonic acid solution—A 0·4 per cent. w/v solution of the diazonium salt, prepared as above, in about 0·25 *N* hydrochloric acid. Prepare this solution freshly as required and keep it ice-cold.

Sodium iodide solution—A 0·33 per cent. w/v solution of anhydrous sodium iodide.

Standard solution of capsaicin—Prepare a solution containing 130 μg of pure capsaicin per ml in absolute methanol. Details of the procedure for the preparation of pure capsaicin are given in Appendix II.

PROCEDURE—

Transfer by pipette an aliquot of the methanolic solution of capsaicin (containing between 350 and 700 μg of capsaicin; it may be necessary to concentrate the solution) into a 20-ml calibrated flask immersed in an ice-bath, and adjust the volume to 10·0 ml with methanol as necessary; add 2·0 ml of sodium hydroxide solution, and, after an interval of 10 minutes, add 2·0 ml of diazobenzenesulphonic acid solution. Shake well, and set the mixture aside at room temperature for 15 minutes. Add 2·0 ml of sodium iodide solution and then 2·0 ml of dilute hydrochloric acid, shake well, and heat the contents by immersion of the flask in a water bath at 60° to 70° C for 15 minutes. Cool the solution, and dilute to the mark with sodium hydroxide solution.* If necessary, filter the solution through a Whatman No. 542 filter-paper, rejecting the first 5 ml of filtrate.

Measure the optical density of the solution in a 1-cm cell at 480 mμ with distilled water in the comparison cell.

At the same time, dilute 3, 4 and 5-ml portions of the standard capsaicin solution each to 10 ml with absolute methanol, and carry out duplicate determinations on each solution by the procedure given above. Construct a graph relating the optical densities to the number of micrograms of capsaicin, and from this read the capsaicin content of the test solution.

II. OINTMENT OF CAPSICUM B.P.C.

REAGENTS—

Light petroleum, boiling range 40° *to* 60° *C*—Analytical-reagent grade.

Diethyl ether—Analytical-reagent grade.

Absolute methanol—Analytical-reagent grade.

Hydrochloric acid, diluted (1 + 2).

* If, on cooling, the solution is turbid, add not more than 2 drops of diethyl ether before diluting to the mark.

Barium hydroxide solution, 3 per cent. w/v—Dissolve 5·5 g of barium hydroxide, $Ba(OH)_2.8H_2O$, in 100 ml of distilled water. Prepare this solution freshly as required.

PROCEDURE—

Weigh accurately 4 g of the ointment (containing approximately 2 mg of capsaicin) into a 100-ml beaker. Add 50 ml of light petroleum, and dissolve by warming gently on a steam-bath. Chill the solution to 0° C to precipitate some of the fat. Filter the cold solution through a Whatman No. 41 filter-paper into a separating funnel. Transfer the filter-paper and the residue to the original beaker, and warm with a further 50 ml of light petroleum; repeat the chilling and filtration, and add the filtrate to the bulk.

Extract the light petroleum solution with 5 portions, each of 10 ml, of barium hydroxide solution, washing each extract with the same 50 ml of light petroleum (if emulsions form at this stage they may be broken by the addition of sodium chloride or by centrifugation). Adjust the combined aqueous extracts to pH 7·5 with hydrochloric acid (use either a pH meter or phenol red indicator), and extract with 6 portions, each of 20 ml, of diethyl ether. To the combined ether extracts add 20 ml of methanol, and evaporate almost to dryness on a water bath in a fume cupboard. Add methanol to the residue, transfer the solution to 50-ml calibrated flask, and dilute to the mark with methanol. Add 0·02 g of activated carbon, shake well, and filter the solution through a Whatman No. 542 filter-paper, rejecting the first 5 ml of filtrate.

Determine the capsaicin content of the solution by any of the methods described in I C, diluting an aliquot with methanol when necessary.

NOTES—1. *Solubility of Oleoresin of Capsicum B.P.C.*—A freshly prepared oleoresin of capsicum is normally readily soluble in methanol. This solubility decreases with age, and it may be necessary to use up to 1 per cent. of diethyl ether to assist solution. When difficulty is still experienced in obtaining a clear solution, the insoluble matter should be exhaustively extracted with small portions of methanol. If this procedure has been found necessary, an aliquot of this solution should be evaporated to dryness and the residue transferred to the column with absolute methanol.

2. *Packing of the column*—Since the most convenient method of mixing the activated carbon with kieselguhr is by making a slurry with methanol, the whole column is prepared by this method. If the alumina fines are removed by flotation during this process, more rapid percolation and clearer eluates result.

3. *Speed of percolation*—The conditions for percolation as defined should be adhered to closely, as it is found that hastening the time of percolation by applying positive or negative pressure increases the amount of interfering substances in the eluate.

4. *Spectrophotometric difference method*—When the disparity between results at 248 mμ and 294 mμ is greater than 10 per cent., the wavelengths of maximum absorption should be re-determined for the instrument; if the disparity is confirmed the results are not valid.

5. The diazonium salt is liable to explode when dry, but members of the Panel have never found this to occur unless undue friction was used in handling the salt.

To avoid any possibility of explosion, after washing the precipitated diazobenzenesulphonic acid with 100 ml of ice-cold water and allowing it to drain, dissolve 0·8 g (approximately) of the moist salt in 100 ml of 0·25 *N* hydrochloric acid. This solution should be freshly prepared and kept ice-cold and may be used in place of the 0·4 per cent. solution described in the method.

It is important to note that the intensity of colour obtained by the diazo method increases with increasing concentration of the diazobenzenesulphonic acid solution. The Panel found that a concentration of 0·40 per cent., although not producing maximum colour, gave reproducible results, which were not obtained if larger excesses of reagent were used. It is essential to prepare the standard curve at the same time as the test and to use identical reagents.

Appendix II

PREPARATION OF PURE CAPSAICIN

A procedure recommended for the preparation of pure capsaicin is based on the method of Tice.[6]

Dissolve 100 g of the oleoresin (obtained by extracting capsicum (Mombasa Chillies) with diethyl ether) in 300 ml of light petroleum (boiling range 80° to 100° C), and extract it with 5 portions, each of 100 ml, of 50 per cent. v/v ethanol. Wash the combined ethanolic solutions with 100 ml of ice-cold light petroleum, and filter. Remove the ethanol by distillation under reduced pressure, and extract the resulting aqueous solution with 5 portions, each of 50 ml, of ether. After removal of the ether dissolve the residue in 25 ml of 0·1 *N* sodium hydroxide, dilute with 200 ml of distilled water, and heat under reflux for 10 minutes. Cool, and bubble carbon dioxide through the mixture for 2 hours. Collect the resultant

precipitate on a grade 3 sintered-glass filter, and dry it in an oven at 100° C for 15 minutes. Transfer the crucible and its contents to a wide-necked flask, and heat under reflux with 500 ml of light petroleum (boiling range 60° to 80° C) for 15 minutes. Filter the hot solution, and, after allowing it to cool slowly, chill it in a refrigerator overnight. Collect the crop of capsaicin crystals on a grade 3 sintered-glass filter, wash them with ice-cold light petroleum, and dry them in a desiccator for 4 hours. Recrystallise at least twice from light petroleum until the melting-point of the crystals is 64° to 65° C.

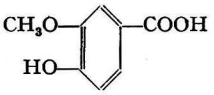
A further crop of pale yellow crude capsaicin may be obtained after concentrating the pale yellow mother liquor.

Appendix III

INVESTIGATION OF SUBSTANCES AS POSSIBLE ALTERNATIVE STANDARDS

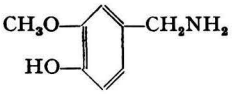
It is admittedly ideal to use pure capsicin as a reference standard in carrying out the diazo coupling reaction. Unfortunately, this material is not generally available, as its preparation is so objectionable. Since experience has shown that a standard curve is necessary each time the assay is conducted, compounds that are non-pungent yet likely to give a colour similar to that obtained with capsaicin were investigated.

As capsaicin has a vanillin-like nucleus, vanillin, vanillin oxime, vanillic acid and methyl vanillate were first investigated. Results showed that only vanillic acid (**I**) produced a colour having an absorption comparable with that of capsaicin, although maximum absorption occurred at 470 instead of 485 mμ. The intensity of the colour produced was approximately double that obtained with capsaicin; from its molecular weight, this is to be expected.



M.W. = 168

(**I**)

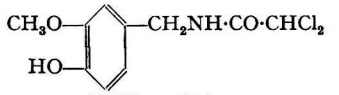


M.W. = 153

(**II**)

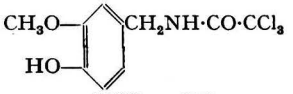
Vanillylamine (**II**) was then prepared from vanillin oxime. Maximum absorption of the resulting complex was at 475 mμ, and again the intensity was approximately double that obtained with capsaicin.

In view of the above findings, it was decided to try compounds having molecular weights nearer to that of capsaicin. Two compounds, the vanillylamide of dichloroacetic acid (**III**) and the vanillylamide of trichloroacetic acid (**IV**), were therefore prepared, the trichloro compound having the same molecular weight as capsaicin.



M.W. = 264

(**II**)



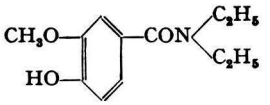
M.W. = 303

(**IV**)

The dichloro compound had ultra-violet absorption characteristics similar to those of capsaicin ($E_{max.}$ at 280 mμ = 115), whereas the ultra-violet absorption of the trichloro compound was somewhat lower than that of capsaicin ($E_{max.}$ at 280 mμ = 94). Although these ultra-violet absorption characteristics looked encouraging, the results with the coupling reaction did not come up to expectation. In each instance the complex had a maximum absorption at 475 mμ, but this could not be read accurately owing to an intense yellow colour masking the normal red of the azo dye. These compounds were, therefore, judged unsuitable as standards.

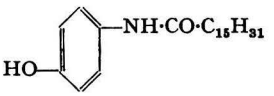
The analeptic "Vandid," vanillic acid diethylamide (**V**), was also investigated. The ultra-violet absorption characteristics of its methanolic solution were similar to those of capsaicin, although the intensity was approximately three times greater ($E_{max.}$ at 280 mμ = 326). In the coupling reaction, however, it was found necessary to use 5000 μg of "Vandid" to be equivalent to 385 μg of capsaicin. The compound was not considered further for a standard.

Two readily prepared phenolic compounds, N-palmitoyl *p*-aminophenol (**VI**) and *p*-acetyl aminophenol (**VII**) were then examined.



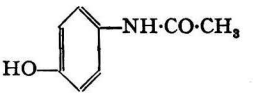
M.W. = 211

(V)



M.W. = 347

(VI)

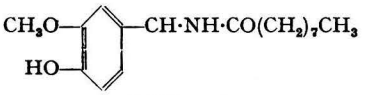


M.W. = 151

(VII)

For neither was there a maximum absorption in the 280 mμ region in methanolic solution. In the coupling reaction, **VI** gave a dye having an intensity about 4·5 times stronger and **VII** a dye about 7·8 times stronger than that of capsaicin. Neither was considered suitable for use as a standard.

As it was known that "synthetic capsaicins" were in use, a commercial sample of the vanillylamide of nonylic acid (**VIII**) was obtained. This compound differed from capsaicin only in the fatty acid portion of the side chain; although it was not suitable as a standard, its characteristics were investigated.



M.W. = 293

(VIII)

The ultra-violet absorption characteristics in acid and alkaline solution were practically identical with those of capsaicin, a methanolic solution having an $E^{1\%}_{1\,cm}$ value of 90 at 280 mμ, calculated on the impure material. In the coupling reaction maximum absorption occurred at 480 mμ; when compared with that of capsaicin the colour intensity ratio was 1·45 to 1. It appears, therefore, that some alternative procedure will have to be found in order to distinguish between this compound and capsaicin. A recent paper[4] covering this aspect is to be investigated.

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Notes

VOLUMETRIC DETERMINATION OF THALLIUM IN SODIUM IODIDE CRYSTALS

THIS method was developed for the routine determination of thallium (about 0·05 per cent.) in single crystals of sodium iodide, grown from a melt, which are used in the measurement of gamma radiation.

A solution containing the thallium, sodium iodide and an excess of free iodine is titrated with a standard solution of sodium thiosulphate until a faint permanent turbidity is produced, owing to the precipitation of thallous iodide (T_1). Starch is added and the titration is completed (T_2). ($T_2 - T_1$) corresponds to the weight of iodine required to keep the thallous iodide in solution, and hence the weight of thallium present is calculated, use being made of the known factor for the titration.

METHOD

Dissolve 5 g of a sodium iodide crystal, containing between 0·01 and 0·2 per cent. of thallium, in 10 ml of approximately 0·02 *N* iodine in 0·6 per cent. w/v sodium iodide solution. Extract with 1·5 ml of *n*-butyl alcohol and then with a further 0·5 ml. To the combined alcohol extracts in a 6-inch × 1-inch boiling tube add 6 ml of 5 per cent. w/v sodium iodide solution, to give the optimum concentration of this salt, and boil gently until no odour of butyl alcohol remains. The boiling should not be prolonged, or too much iodine may be lost, causing precipitation of thallous iodide. Cool, and dilute to 6 ml with water. Shake with 1 ml of chloroform, reject the chloroform layer, which contains any iodoform produced, and boil to remove any residual trace of chloroform. Cool, and dilute to 6 ml with water. Titrate with 0·05 *N* sodium thiosulphate until a faint permanent turbidity is produced (T_1 ml). Add 2 drops of 2 per cent. starch solution, and continue the titration until the blue colour disappears (T_2 ml, total). The weight of thallium in grams = $(T_2 - T_1) \times 0{\cdot}00425$.

This method can be used to determine 0·5 to 10 mg of thallium with an error of $\pm 0{\cdot}2$ mg or less. For smaller amounts of thallium, greater accuracy is obtained with a smaller final volume and the same concentration of sodium iodide. Larger amounts of thallium require more iodine to effect solution.

LIMITING CONDITIONS AND INTERFERENCES

The pH of the solution for titration is not critical, but thallic oxide is precipitated in the presence of excess iodine and strong alkali.[1]

If the concentration of sodium iodide is less than about 2 per cent. w/v, the precipitate formed on titration with sodium thiosulphate is a black, non-stoicheiometric and weakly associated complex of thallous iodide and iodine. The factor for the titration in this instance is slightly reduced. The factor is a maximum (0·00425) when the sodium iodide concentration is 5 per cent. w/v. A two-fold increase or decrease in this concentration lowers the factor by 5 per cent.

No inteference is caused by acetate, sulphate, chlorate or nitrate ions. Precipitates of thallous chloride, bromide, iodide, bromate, iodate, chromate, thiocyanate and sulphide dissolve in an excess of iodine and do not interfere.

Acid solutions of bromates, iodates, chromates, nitrites and other compounds that liberate iodine from sodium iodide cause no interference, as they merely increase the initial concentration of free iodine and do not affect the value of ($T_2 - T_1$). Reducing agents must be completely oxidised before the titration is carried out.

I thank the Directors of E. K. Cole Ltd. for permission to publish this Note.

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EKCO ELECTRONICS LTD.
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(PRESENT ADDRESS: HOPKIN AND WILLIAMS, LTD.,
FRESHWATER ROAD, CHADWELL HEATH, ESSEX)

M. J. TOOGOOD
First received *June 9th*, 1958
Amended, *May 25th*, 1959

Apparatus

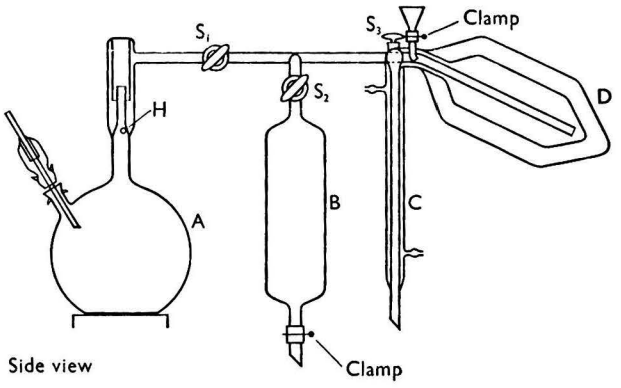
AN ALL-GLASS SELF-CLEANING STEAM-DISTILLATION APPARATUS

THE apparatus described here is well suited to the determination of a wide variety of substances, *e.g.*, the reducing adrenal corticoids by steam-distillation of the formaldehyde, catecholamines by steam-distillation of the volatile amines, and protein-nitrogen by distillation of the volatile ammonia; it can also be used for the steam-distillation of volatile oils, etc.

The apparatus is an all-glass non-jointed single unit; it consists of a 2000-ml flat-bottomed boiling-flask, A, in which steam is generated, fitted with a standard taper entry for replacement of water, a steam trap, a water-cooled condenser and a vacuum-jacketed reaction vessel integrated by a series of stopcocks. The arrangement of the parts is shown in Fig. 1. The steam trap, B,

Vol. 84, 1959: October, p. 619.

Replace the lower part of Fig. 1 by—



Replace the first 5 lines of text under Fig. 1 by—

is 10 inches long and 3 inches in diameter, and the reaction vessel, D, is about 6 inches long and 45 mm outside diameter, the outside diameter of the vacuum jacket being about 60 mm. The main tubing is 10-mm bore, with thin-wall tubing in the condenser. The splash-head, which incorporates a drain-back hole, H, is made up of 10-mm, 18-mm and 28-mm bore tubing, the outer wall of the condenser is of 18-mm tubing with 6-mm water connections, and the stopcock bores are 4 mm. The whole is of Pyrex glass.

is 10 inches long and 3 inches in diameter, and the reaction vessel, D, is about 8 inches long and 3 inches in diameter, the dimensions of the vacuum-jacket wall being about 2 inches greater. The main tubing is 10-mm bore, with thin-wall tubing in the condenser. The splash-head is made up of 10-mm, 18-mm and 28-mm bore tubing, the outer wall of the condenser is of 18-mm tubing with 6-mm water connections, and the stopcock bores are 4 mm. The whole is of Pyrex glass.

The water-replacement unit is made of 6-mm, 10-mm and 18-mm bore tubing and is fitted with a standard 19/38 taper joint; it is connected to a reservoir of triple-distilled water (not shown).

METHOD OF OPERATION

With stopcocks S_1, S_2 and S_3 closed, introduce the sample into the reaction vessel, D, by means of the funnel and the short length of Tygon tubing, which can be clamped tightly with a small Hoffman slip-clamp. Open stopcock S_1, and allow steam to pass into the reaction vessel. Open stopcock S_3 almost immediately, and place the receiver under the tip of condenser C at the beginning of the operation.

When steam-distillation is completed, open stopcock S_2 and close stopcocks S_1 and S_3. Attach a short length of Tygon tubing fitted with a pinch clamp to the outlet of trap B, and direct a stream of cold distilled water at the trap, thereby sucking the spent reaction mixture back into this unit.

Again open stopcocks S_1 and S_3, close stopcock S_2, and allow steam to pass into the reaction vessel, thereby cleaning the unit with distilled water. Remove the wash water formed by condensation of steam in the way just described for the disposal of the spent reaction mixture. Repeat the procedure as many times as is desired.

It is possible to use this apparatus for any number and variety of operations without dismantling it for cleaning purposes. As many as eight to ten determinations of formaldehydogenic corticoids or between ten and twelve protein-nitrogen determinations per hour can be carried out with ease.

Work on this apparatus was aided by a grant from the Josiah Macy, jun., Foundation.

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Book Review

EDTA TITRATIONS: AN INTRODUCTION TO THEORY AND PRACTICE. By H. A. FLASCHKA, Ph.D. Pp. 138. London, New York, Paris and Los Angeles: Pergamon Press Ltd. 1959. Price 42s.

This recent addition to the rapidly increasing number of books on the subject of EDTA is intended primarily as a teaching text for students. Dr. Flaschka develops his theme in a logical manner, assuming no previous knowledge on the part of his reader and interspersing elementary theoretical considerations with well designed practical experiments.

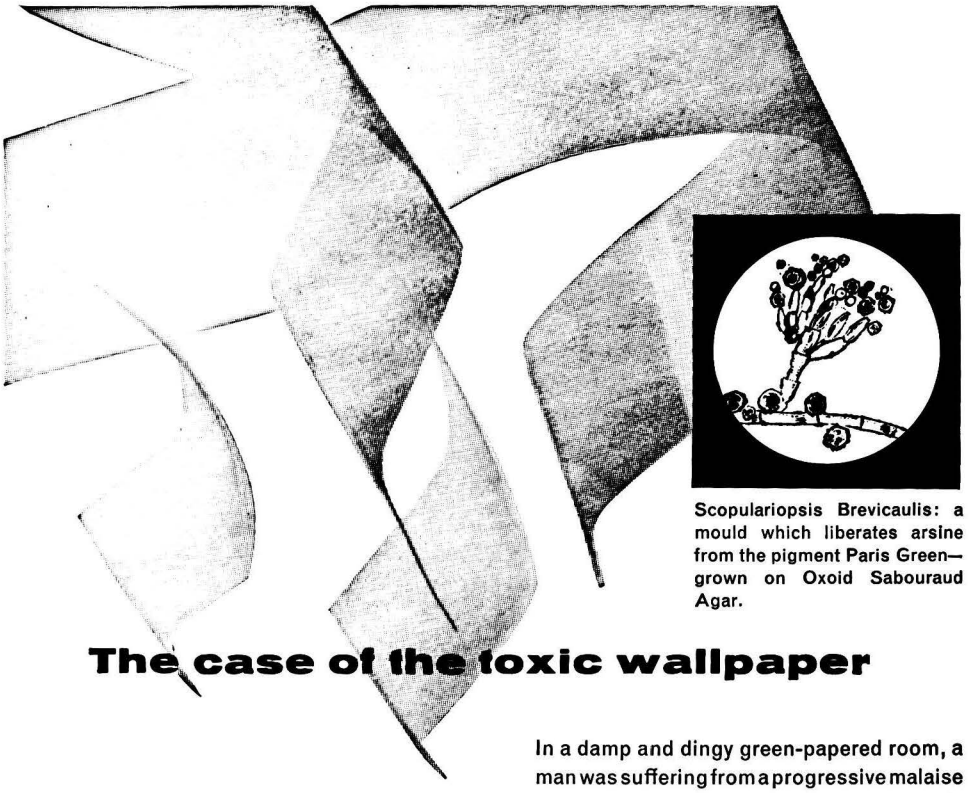
Chapters are devoted to end-point detection, metal indicators, masking reagents, the classification of titration methods and the preparation of standard solutions; then come practical experiments in the various types of titration, including some examples of the analysis of multi-component mixtures. Each method is prefaced by a short but informative discussion of the theoretical considerations underlying the experiment and is followed by remarks on the practical procedure. It is in these comments that the chief value of the book to the practising analyst may lie, for they are clearly based on Dr. Flaschka's very wide experience of his subject. Sections on potentiometric and photometric methods of end-point detection conclude the work, except for appendixes of questions and problems for the student.

In his preface the author makes it clear that the book is intended to cover broad principles rather than to provide a comprehensive survey of the subject. Even so, it is regrettable that only four metal indicators are discussed and that so important and valuable an addition to the range as xylenol orange should have received no comment. This leads the author to make a statement such as "None of the indicators commercially available at the present time can be used in a direct titration of mercury" (p. 89), which would have been so about 2 years ago, but certainly not when this volume was published.

Another point on which one must disagree with the author is in his contention (p. 28) that the ionic strength of a solution can be ignored. The student who is instructed on these lines will suffer an unpleasant surprise when he has to deal with commercial samples rather than the pure metal solutions used for lecture demonstrations and college practical work.

On the whole, however, the book is excellent for its intended purpose. But if it is at students that the work is aimed, why have the publishers seen fit to ask such a high price for a mere 138 pages?

C. A. JOHNSON



Scopulariopsis Brevicaulis: a mould which liberates arsine from the pigment Paris Green—grown on Oxoid Sabouraud Agar.

The case of the toxic wallpaper

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