The ANALYST

The Journal of the Society for Analytical Chemistry

A monthly Publication dealing with all branches of Analytical Chemistry

Published for the Society by
W. HEFFER & SONS LTD., CAMBRIDGE

Volume 90

No. 1072, Pages 379 - 442 July 1965

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

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Volume 90, No. 1072 July 1965

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Electrophoresis in Stabilizing Media

A Review

SUMMARY OF CONTENTS

D. GROSS

Research Laboratory, Tate & Lyle Refineries Ltd., Keston, Kent.

Analyst, 1965, **90**, 380–402.

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The Amperometric Determination of Submillimolar Concentrations of Iron^{III} with Mercury^{I} Perchlorate

The amperometric titration, at a rotating platinum electrode, of iron^{III} at concentrations of from 10^{-5} to 10^{-4} N in potassium thiocyanate - perchloric acid medium has been carried out with mercury^{I} perchlorate solution. Although the titration curve is sharply defined at an iron^{III} concentration of 10^{-4} N, end-point location by the usual method gives high results. The causes of a small anodic current obtained beyond the end-point are discussed.

An end-point method that involves prior addition of an arbitrary amount of iron^{III}, observation of the current, x, injection of the iron^{III} sample and titration to restore the current to x, has been developed. Under proper conditions, the results are precise and accurate to ± 2 and ± 5 per cent. for amounts of approximately 10 and 1 micro-equivalents of iron^{III}, respectively, in 100 ml of titration medium.

JOHN T. STOCK and P. HEATH

Department of Chemistry, The University of Connecticut, Storrs, Connecticut, U.S.A.

Analyst, 1965, **90**, 403–408.

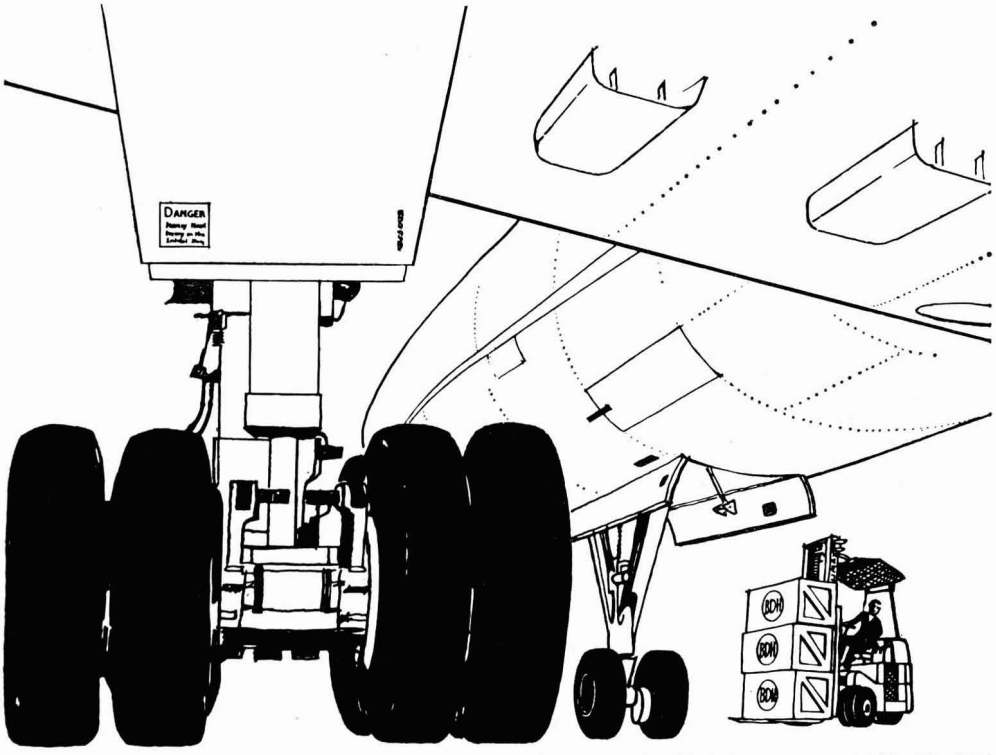
Methylthymol Blue as a Reagent for the Spectrophotometric Determination of Magnesium

A procedure is described for the colorimetric determination of magnesium with methylthymol blue. The complex is formed in an alcohol - water medium buffered to pH 10·8 with ammonia - ammonium chloride solution. The interference of several cations has been examined.

J. METCALFE

Chemical Laboratory, Ferranti Limited, Moston, Manchester, 10.

Analyst, 1965, **90**, 409–412.



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The Analysis of Technical Hexachlorocyclopentadiene by Infrared Spectrophotometric and Gas - Liquid Chromatographic Methods

Infrared spectrophotometric and gas - liquid chromatographic methods were applied in the analysis of hexachlorocyclopentadiene containing about 30 per cent. of various perchloro compounds. Two solvents were used in the spectrophotometric method: (*i*) tetrachloromethane for determination of hexachlorocyclopentadiene (1140, 1242 and 1607 cm^{-1}) and octachlorocyclopentene (1189 cm^{-1}) and (*ii*) carbon disulphide for the determination of octachlorocyclopentene (753 cm^{-1}), tetrachloromethane (764 cm^{-1}), hexachloroethane (780 cm^{-1}), hexachlorobuta-1,3-diene (853 and 980 cm^{-1}), tetrachloroethylene (909 cm^{-1}) and hexachlorobenzene (1347 cm^{-1}). The concentrations of individual components were calculated by the method of successive approximations with an analytical matrix. High reproducibility of results was achieved by the use of several wavenumbers for the determination of main constituents of the mixture. In the gas - liquid chromatographic method, a polyethyleneglycol adipate (with 1 per cent. of orthophosphoric acid) column at 145° C and a flame ionisation detector were used. Precise evaluation of peak areas and suitable choice of internal standard (1,2,3,5-tetrachlorobenzene) resulted in approximately the same reproducibility as for the spectrophotometric method. The analyses of synthetic mixtures and of a series of samples showed good agreement between results of both methods if the samples did not contain more than 5 per cent. of unidentified compounds. The chromatographic method is preferable for analysing samples with more than 5 per cent. of unidentified compounds.

M. LIVAŘ and J. HRIVŇÁK

Research Institute of Agrochemical Technology, Bratislava, Czechoslovakia.

Analyst, 1965, **90**, 413–421.

The Determination of Acetone in Air

Activated silica gel is an efficient adsorption medium for sampling acetone vapour. For the determination, the acetone can be completely desorbed with N sodium hydroxide, but not with water. Complete recovery of acetone can be achieved at atmospheric concentrations up to 4000 mg per cu. metre.

H. BUCHWALD

Division of Industrial Health Services, Department of Public Health, Edmonton, Alberta, Canada.

Analyst, 1965, **90**, 422–428.

The Use of 50 per cent. Hydrogen Peroxide for the Wet Oxidation of Organic Materials

Short Paper

R. P. TAUBINGER and J. R. WILSON

Imperial Chemical Industries Ltd., Plastics Division, Welwyn Garden City, Herts.

Analyst, 1965, **90**, 429-431.

A Simple Micro Colorimetric Method for the Determination of Lactose in Milk

Short Paper

NAGI WAHBA

Biochemistry Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Analyst, 1965, **90**, 432–434.

Micro-determination of Zinc and Copper in a Single Digest of Small Samples of Plant Material

Short Paper

E. R. PAGE
National Vegetable Research Station, Wellesbourne, Warwick.

Analyst, 1965, **90**, 435–436.

The Determination of Sodium in Heavy Fuel Oils by Neutron-activation Analysis

Short Paper

B. D. CADDOCK and J. H. DETERDING
"Shell" Research Ltd., Thornton Research Centre, P.O. Box 1, Chester.

Analyst, 1965, **90**, 437–439.

The Estimation of Tomato Solids by Determination of Lycopene

Short Paper

O. B. DARBISHIRE
Crosse & Blackwell Laboratories, 36 Crimscott Street, London, S.E.1.

Analyst, 1965, **90**, 439–440.

THE ANALYST

Editorial

THE rapid increase in the volume of scientific literature makes it increasingly difficult to keep abreast of the continuous flood of publications. It has become clearly impossible to find, read and usefully digest all the pertinent literature even in a limited field. Review articles and monographs seem to be one of the possible answers to the problem, compressing the available information to narrower and more manageable dimensions, thus keeping the ever-expanding scene within the view of the investigator. However, one finds that reviews often become glorified collections of references, carefully arranged and strung together, but too mechanical and without personal touch. Books, helpful as they may be, are rarely quite up to date. There is always a time lag of about 2 to 3 years between the finishing of the manuscript and the actual publication date. With some books one cannot avoid the impression that the author had concluded his literature survey, or perhaps his interest in the subject, 5 to 10 years before writing the book. There is also the often apparent habit of taking over in good faith, but uncritically, sections from books of well established authors, which sometimes may lead to a certain sterility of style and perpetuation of outdated concepts or outright mistakes. The mechanics of publishing are perhaps too inflexible for errors to be effectively corrected or ideas, once hastily committed to print, to be revised. Scientific writing need not be uniform and dull. Much will depend on the writer, his experience, literary background, critical mind and ability to communicate.

Critical review articles present a more favourable example. Here the writers, specialists in particular fields and up to date with the relevant publications, are in a position to provide a critical assessment of developments by weeding out the mediocre, meaningless and often even misleading publications, while concentrating on the really significant contributions. Reviewers should include a critical evaluation, based possibly on personal experience, of methods, techniques and instruments, because progress in science depends to a great extent on the development of new techniques and instruments. An emphasis, therefore, on methodology and new approaches to well established techniques should prove of great benefit to the reader.

D. GROSS

Electrophoresis in Stabilizing Media

A Review*

By D. GROSS

(*Research Laboratory, Tate & Lyle Refineries Ltd., Keston, Kent*)

Summary of Contents

During the last two decades our chemical knowledge has been greatly enlarged by the introduction and successful application of new analytical tools. Electrophoresis in stabilizing media may be rightfully considered to be one of the most useful of the new techniques, suitable for analytical as well as preparative work in many fields. The number of papers on this subject that have appeared in the last few years is so vast that the selection of references to be quoted in a review article must be rigorous and restricted to a small proportion of them. Under these circumstances a preference for certain contributions may somehow show up, reflecting the reviewer's slight bias, often in the light of his personal experience, in the judgment of their importance. This human weakness seems to be inherent and unavoidable.

Almost 10 years have elapsed since the excellent review article by Parker[1] on zone electrophoresis appeared in the pages of this journal. Since then, great developments have taken place, numerous modifications have been reported, new, and not so new, techniques proposed, theoretical views expounded to help in promoting better understanding of the kinetics and mechanisms of processes, and varied designs of improved apparatus described. To form and give a balanced view of the subject under review, it is essential to take very briefly a second look at the period until 1955 from the present perspective, and integrate it into one continuum.

Theoretical considerations

In moving-boundary or free electrophoresis, charged particles in an electrical field migrate at characteristic rates, ideally each species forming a boundary and being completely separated. For various reasons, the resolution in practice is only partial, and only the fastest and slowest components of a mixture can be separated in a reasonably homogeneous form. Even so, certain conditions must be observed. The contribution of the sample to total conductance should be as low as possible. The ionic strength of the buffer solution should be as high as possible, but the limit is set by excessive generation of Joule's heat. Electrophoresis in dilute solutions leads to instability of boundaries on account of thermal and electro-osmotic convection. This is particularly so for well conducting low-molecular-weight compounds. High-molecular-weight compounds of low conductance and diffusion allow the application of much higher concentrations, resulting in sharper boundaries and better resolution. A certain density increment across the moving boundary is a pre-requisite for a successful separation. Careful control of density, buffer-solution strength and temperature must be maintained to keep the boundaries sharp during the experiment.

Gravitational stability can be ensured by the use of a density gradient, *e.g.*, glycerol, poly(vinyl pyrrolidone) or sucrose, or certain powdered solids. Many materials have been proposed as anti-convection or stabilizing media. The latter, if suitably chosen, permit all components of a mixture to be separated into discrete zones, hence the name "zone electrophoresis," coined by Tiselius[2] to distinguish this technique from moving-boundary electrophoresis.

Stabilizing media include filter-paper, glass-fibre paper, cellulose acetate film, cellulose powder, glass beads, starch powder and gels such as starch, agar, silica and polyacrylamide. The best results are obtained with materials that are (*a*) insoluble in water or the buffer solutions used, (*b*) do not adsorb the sample to a significant degree, (*c*) for gels, form a fairly homogeneous paste with the buffer solution and (*d*) do not develop an excessive electro-

* Reprints of this paper will be available shortly. For details see Summaries in advertisement pages.

osmotic flow. As regards material in sheet form, a reasonable wet strength is important. In spite of thousands of papers that have appeared on the application of zone or stabilized electrophoresis, comparatively little has been written on the fundamental background. The reasons might be that the technique is predominantly used for analytical purposes for which theory is not absolutely essential, and that the lack of precision equipment prevents reliable measurements of electro-kinetic properties. The absence of sufficient theoretical knowledge has proved a stumbling block in its use as a physico-chemical tool similar to the moving-boundary technique. Yet, stabilized electrophoresis has several advantages. Very small amounts of various substances ranging from high-molecular-weight to low-molecular-weight compounds and from organic to inorganic compounds can be determined or, at least, characterised by their mobilities, often simultaneously and within a comparatively short time, by means of relatively simple equipment.

Mobility can give a direct measure of the net charge of the ion, which depends in free electrophoresis on the molecular structure and shape and is influenced by the ionic strength, composition and pH of the buffer solution used. Temperature affects the mobility because of the viscosity and conductance changes in the buffer medium, but has little effect on the charge. Concentration of the test substance exerts a certain influence. In stabilized electrophoresis, the complexity of factors influencing mobility increases. The porous or fibrous structure of the supporting medium causes retardation of migration owing to adsorption, molecular-sieving and physical-barrier effects. The latter makes the migrating ion follow a tortuous path, instead of a straight line. An interesting example of the influence of the machine direction of filter-paper on migration rates was reported recently.[3] This was confirmed indirectly by paper-resistivity measurements.[4] The buffer-solution flow under these conditions is influenced mainly by three factors, *viz.*, capillary flow linked with evaporation due to heat generation, electro-osmosis of varying extent due to the nature of the stabilizing medium and the pH of the buffer solution,[5] and sometimes to hydrodynamic flow due to difference in buffer-solution levels. Peeters[6] summarised the interplay of factors in a neat diagram. The parameters in stabilized electrophoresis are thus dependent on the medium and the type of apparatus. In order to calculate fundamental data, such as mobilities, from migration rates, the effect of the medium used and the flow pattern of the buffer solution must be corrected for. If this is not done, values obtained in different laboratories cannot be compared and results cannot be used for calculating mobility or net charge. In general, too little attention is paid to this point in the publication of results. However, in many instances, relative migration rates under standardised conditions[3,7] provide sufficiently accurate data for characterisation of unknown and identification of known compounds. Standard conditions should include constancy of moisture content and avoidance or minimisation of evaporation. The "tortuous-path" theory[8] was more recently confirmed by new experimental evidence.[9,10] The "barrier" theory[11] appears to have found little support, but a combination of tortuosity and restriction[12,13] was put forward as offering the most plausible explanation for the retardation effect. Pathway restrictions in fibrous material make conductance less uniform and less efficient, decreasing mobilities to a corresponding extent. Biefer and Mason[14] reported on interesting measurements of conductivities of fibre pads, saturated with electrolyte, and others[15,16] published results for paper systems.

Diffusion is not expected to influence mobility, but it causes spreading or blurring of zones and thus counteracts efficient resolution. Since diffusion is time-dependent, being proportional to the square-root of time, its effects can be minimised by applying the highest possible potential gradient.

The influence of ionic strength on the ζ-potential, and thus on mobility, is often disregarded. And yet a simple consideration shows the importance of the choice of buffer solution or background electrolyte. To a first approximation, the mobility is inversely proportional to the square root of the ionic strength—

$$u \text{ (mobility)} = \frac{1}{\sqrt{\frac{\Gamma}{2}}},$$

$$\frac{\Gamma}{2} \text{ (ionic strength)} = \tfrac{1}{2} \Sigma c \; z^2$$

where c_i = concentration and
z_i = valency of buffer-solution ions.

The movement of ions is directly proportional to field strength—

$$X \text{ (field strength)} = \frac{V}{l}$$

$$u = \frac{d/t}{V/l} \text{ or}$$

$$d = \frac{uVt}{l}$$

where d = distance travelled by ion,
l = length of field,
V = voltage and
t = time.

It is obvious from the above that it is not only the concentration, but more so the valency of the ions that can significantly influence the net charge. Particularly with heavy-metal ions, a small increase in concentration can lead to a significant reduction in the ζ-potential and hence mobility. Another of the irregularities often overlooked is the uneven distribution of electrolyte along the migration path. Local concentration due to evaporation or partial drying-out will reduce locally the potential gradient and thus migration rate. Of course, changes in viscosity, conductance or dielectric constant necessarily affect mobility. A theoretical analysis of the effects of stabilizing media on mobility and zone spreading has recently been published.[12] Since the buffer solution provides a certain, though limited, safeguard against some of these changes, electrophoresis is carried out in suitably chosen buffer solutions. Audubert and de Mende[17] dealt extensively with the theoretical principles involved in stabilized electrophoresis in comparison with free electrophoresis, Bermes and McDonald[18] tried to find a conversion formula for mobilities in paper-stabilized systems, whereas others[19] sought to evaluate the optimal conditions based on energy required, or on chromatographic effect.

Techniques

The discontinuous technique may be defined as one in which the separated components are left on the supporting medium, from which they may be individually eluted. In continuous electrophoresis, migration of the separated components is continued along the whole length of the supporting medium with continuous flow of the solution at right angles to the electric field, the individual components being collected separately as they leave the support.

Discontinuous—

The discontinuous-strip or block technique is by far the widest application of the principle of electrophoresis. Several excellent books and reviews of recent date have dealt comprehensively with details of discontinuous methods based on stabilizing media of a great variety, *e.g.*, Bier,[21] Ribeiro, Mitidieri and Affonso,[22] Morris and Morris,[23] Wunderly[24] (for serum proteins in particular), Smith,[25] Bailey,[26] Clotten and Clotten[27] (high-voltage technique), Michl,[28,29] Foster[30] (for carbohydrates) and Heftmann.[31]

Most of the publications that have appeared in the last decade have still dealt predominantly with the analysis of proteins of human, animal or plant origin. Paper was still the stabilizing medium mostly used because of simplicity, cheapness and convenience. However, it is being seriously challenged more recently by cellulose acetate film, starch gel and polyacrylamide. The main disadvantage of paper is its adsorptive affinity for some proteins, particularly albumin, fibrinogen and basic proteins. It was found that the adsorptivity of the paper surface could be reduced by impregnating it with 0·01 per cent. serum solution.[32] This altered the subsequent serum analysis by balancing out some of the adsorption effects. For practical purposes, however, this is too tedious a procedure to adopt. The other difficulty is the staining technique. Although simple enough to carry out, the results are not always quantitatively reproducible, and elution of stained zones for accurate evaluation is often unsatisfactory. This may account for discrepancies in reports from different laboratories on proteins of the same origin. The dyes most commonly used are bromophenol blue, Amido black 10B or Azocarmine B. The mechanism and chemistry of the uptake of dye by protein fractions have been thoroughly investigated and very useful information has been made

available.[33,34,35] Lipoproteins and lipids are usually stained with Sudan black B or Oil red O. From studies[36,37] of uptake of dye and its quantitative relationship, it is quite clear that quantitative analysis of the lipids of blood serum, the most common application, is still far from dependable by this procedure.

The separated zones can either be eluted and measured colorimetrically, or the strip can be rendered transparent and the zones evaluated densitometrically.

Broadly speaking, the elution method is considered to give better reproducibility,[38] whereas the direct densitometric method is speedier, expedient and more suitable for routine analysis.[39,40] The fact that paper electrophoresis permits the serum proteins to be separated into up to 6 fractions, more than free electrophoresis was ever able to accomplish, does not necessarily mean that the components are homogeneous. The application of starch-gel electrophoresis[41] has recently demonstrated the presence of more than 20 fractions in a similar sample, whereas it may be assumed from various sources of evidence that human plasma may contain over 100 protein components. Another fruitful application both of the paper and starch-gel methods is the examination of haemoglobins in serial investigations, which produced an enormous amount of information to enrich the science of haematology.[42,43,44,45] For complicated protein mixtures, starch-gel and polyacrylamide media have provided new tools of extremely high resolving power, based on lack of adsorption and the additional principle of molecular sieving. The analysis of plant proteins has produced some interesting results with polyacrylamide used as stabilizing medium for pea-seed albumins[46] and paper used for proteins from corn leaves.[47] A surprisingly comprehensive analysis of egg-white proteins by means of electrophoresis was reported a few years ago.[48]

The most likely reason why discontinuous electrophoresis has not been used as much for low-molecular-weight as for high-molecular-weight compounds is that paper chromatography, though not suitable for proteins, has established itself widely for analysis of the most diverse groups of low-molecular-weight compounds, and this with comparatively simple equipment. To displace chromatography in routine applications, a new technique has to provide either higher resolution, or different results. Electrophoresis, based on entirely different physico-chemical principles can often reveal the separated components of a mixture in a sequence different from that obtained by chromatography. If high voltage is applied, excellent separations can be achieved in a fraction of the time usually required by paper chromatography, or even by the far more rapid thin-layer chromatography. Quite often such a method may conveniently and usefully replace chromatography, particularly for ionic or complexable molecules. Discontinuous paper-strip techniques, both one-dimensional and two-dimensional, have found widespread use in the field of amino-acids and peptides, often in combination with paper chromatography. Most outstanding examples are the structural analyses of peptides, proteins[49,50] and antibiotics,[51,52,53] yielding results considered to be impossible only a few years ago. Sugars,[54 to 63] organic acids,[64,65,66,67] nucleotides and nucleic acids,[68,69,70,71] phenols,[72 to 76] amines,[77 to 81] inorganic cations,[3,82 to 92] including radioactive elements,[93 to 98] and anions,[99 to 104] including phosphates,[105,106,107] are some of the groups for which paper electrophoresis has found successful application. Starch-gel or other electrophoresis has so far not proved useful for compounds other than proteins. Curiously enough, Pfrunder and co-workers[108] advocated the use of agar gel as stabilizing medium for inorganic separations, claiming several advantages over paper such as self-conductance and smaller surface area of medium, thus eliminating the need for background electrolyte and reducing evaporation.

The structural analysis of proteins relies to a large extent on the reliable resolution of complex mixtures of peptides and amino-acids[109 to 112] produced by hydrolysis or cleavage of the protein molecule. The remarkable suitability of paper electrophoresis, preferably by the two-dimensional high-voltage technique, for this purpose has been proved repeatedly in recent years. This includes the detection and determination of amino-acids and other ninhydrin-reacting compounds in urine.[113,114]

Although there is a multiplicity of variants, basically the differences in technique are small and limited by the type of stabilizing medium. When paper is used, the strip or sheet has to be soaked in background electrolyte or buffer solution and blotted or squeezed so as to leave sufficient moisture, say, 120 to 200 per cent. on the dry weight of paper. The amount depends very much on the nature of compounds to be separated and the type of equipment used. Apart from proteins requiring for some reasons moist chamber conditions, almost all

the other compounds give better results by the methods in which efficiently cooled sandwich-type apparatus is used. In point of fact, the flat surface and the relative thinness of paper make efficient heat dissipation comparatively easy.

It could be said with confidence that the success or failure of a discontinuous technique will depend far more on the type of stabilizing medium used and the degree of control of experimental conditions than on rituals of equilibration and sample application, as often found described in the literature. This point will be discussed in a more detailed way under "Apparatus," p. 385.

CONTINUOUS—

The continuous technique is the target often attempted but rarely reached. A satisfactory method, quick and reliable, would soon supersede most discontinuous methods in current use. The possibility of collecting separated fractions without a tedious, and seldom quantitative, extraction procedure would seem to be the final answer to most analytical and preparative problems. In the latter instance, one's mind marvels at the vast amounts that could be produced continuously, perhaps for several months and with little effort, almost like the water-still in a corner of the laboratory. Alas, the facts and experience over the last 15 years put a damper on these speculations. The main reason for lack of success is usually the inability to control the conditions to a sufficient degree so as to maintain the "steady state," once attained. It is, however, feasible and comparatively easy to run an experiment satisfactorily for a limited period of time, say 1 to 2 days, and achieve useful separations. If a paper sheet or "curtain" is used, irregularities arising after the first day stem mainly from the lack of control over the flow-rate of buffer and sample solutions. This is assumed to be constant, but in practice never is, since the volume sucked into the paper by capillary action per unit of time tends to decrease with length of experiment and vary with temperature, degree of evaporation and, indirectly, changes in conductance affecting electrical conditions. A change in flow-rate or conductance, or both, will necessarily bring about a shift in the separated streams, leading to convergence of neighbouring streams and thus vitiating the incipient separation. Methodical checking of the effluent fractions often reveals a surprisingly high incidence of re-mixing, a fact that might otherwise escape detection. Good temperature control and prevention of significant pH changes caused by diffusion of electrolysis products are helpful in keeping conditions stable, thus avoiding a possible drying out of the paper and non-uniformity of electric field.

Ever since the elegant method, based on the principle of buffer solution and sample moving through an electric field applied at right angles to the flow, was first suggested by Svensson and Brattsten,[115] Brattsten and Nilsson,[116] Grassmann and Hannig[117] and Durrum,[118] there were many attempts to overcome the obvious snags and make the method a reliable tool for preparative work. Hannig's[119,120] persistency in investigation and modifications by stages resulted in a much improved apparatus. The apparatus described by him and that described by Peeters, Vuylsteke and Noë[121] are perhaps the best designs for paper-sheet equipment in recent years. An interesting supporting material was suggested by Stegemann,[122] *viz.*, an acid- and alkali-resistant PVC paper, absorbing little moisture, allowing higher potential gradients and giving improved resolution. Also of interest is the conical paper design published more recently.[123] In another apparatus[124] the rate and pattern of flow of buffer solution is controlled by adjusting the liquid-level heights in the buffer-solution vessels, with the separation area variably tilted from the vertical position. Of the types of apparatus based on powdered substances as supporting media, the most impressive design, in which glass powder was used, was described by Brattsten.[125] However, the apparatus seems to be too elaborate for routine applications. A different, much simpler, design, in which a separating cell packed with cellulose powder was used, was proposed by Dicastro,[126] who had previously introduced several modifications to the paper-sheet technique. A novel design, dispensing altogether with the stabilizing medium, was described by Barrolier, Watzke and Gibian,[127] and later by others.[128,129] It is remarkable because the separation takes place in a thin layer (0·5 mm) of buffer solution, whose flow-rate is regulated chiefly by tilting the water-cooled, enclosing glass plates to a nearly horizontal position. With no possible adsorption and no hydrodynamic obstruction, the easy flow control should be conducive to smooth long runs. Another interesting design[130] for large-scale continuous column operation relies on viscous media to minimise unwanted re-mixing during upward flow and separation. On the theoretical side, excellent

and comprehensive information on theory, technique and equipment for continuous electrophoresis is contained in an article by Pučar,[131] and useful advice on optimal voltages for preparative techniques in a short paper by Caplan.[132]

COLUMN—

Column electrophoresis could be best defined as a discontinuous electrophoretic technique performed in vertical cylindrical tubes, in which various granular or powdered materials are used as stabilizing media and elution is usually relied on for recovery of the separated components. Column electrophoresis has found its main application for preparative work. Development, ever since Flodin and Porath[133] and Porath[134,135] described suitable column equipment, tended to be in the direction of improved capacity and higher cooling efficiency so as to increase load and reproducibility of separation. The procedure itself is neither quick nor simple to carry out. It is a two-stage technique, first separation by electrophoretic migration and then elution of the zones, ideally in a "frozen state." This is very difficult to ensure, and during a lengthy elution some re-mixing is to be expected, thus reducing the efficacy of the separation. The elution technique is similar to that used in column chromatography. There is, however, at least one design that is based on a different principle,[136,137] *viz.*, combining a separating column with an elution strip, having a non-uniform electric field and continuous elution of the separated zones.

Of the stabilizing media used, cellulose powder, poly(vinyl chloride) powder, Pevikon, a copolymer of vinyl chloride and vinyl acetate, granular starch and glass powder are the materials most frequently mentioned.

As in column chromatography, much attention must be paid to the packing of the column, since irregular packing will cause "tailing" and spreading of zones. Temperature control during the electrophoretic run is very important, particularly with columns of large diameter (2 inches and more). The nature of the buffer solution used may also affect adversely the quality of the separation.[135] An excellent review of the technique was recently written by Porath and Hjertén.[138]

The groups of compounds separated by column technique range from amino-acids (on cellulose),[134,139] polysaccharides (glass powder),[140] cholinesterase (poly(vinyl chloride)),[141] lens proteins (cellulose),[142] caseins (cellulose),[143] plasma proteins (Sephadex),[144] serum proteins and microsomes (agarose),[145] to plant pigments (cellulose)[146] and aniline dyes (Pevikon).[147] An interesting high-voltage design for cellulose columns was described by Rentsch,[148] and the design principles for efficient cylindrical columns were theoretically discussed by Hybarger, Tobias and Vermeulen.[149]

APPARATUS

LOW-VOLTAGE APPARATUS—

If low-voltage paper electrophoresis is defined as the technique allowing the application of potential gradients of up to 20 volts per cm, then very few changes in the three basic designs of such apparatus over the last 15 years can be recorded, although many minor, often trivial modifications have been hopefully proposed and described. There is the type consisting of the free-hanging (ridge-pole) paper strip in a moist chamber, first described by Durrum in 1950, recently perfected by him and associates,[150] and made commercially available. The second type consisting of a horizontal strip in a moist chamber, advocated by Grassmann and Hannig in 1950 and also by others has proved to be the most successful and popular design, particularly for clinical routine analysis of serum proteins. Commercial versions of this design with slight variations are now available in many countries. Attempts were made to improve the stability of conditions in the moist chamber and strip for greater accuracy and reproducibility of results.[151,152] Apparatus that could be used with various supporting media was reported by several authors.[153,154] Power-unit designs have been described in detail.[155]

The third basic design, *i.e.*, the strip between glass plates, or sandwich type, first described by Cremer and Tiselius, also in 1950, has, curiously enough, not found such general acceptance. It was, however, chosen by some for development of high-voltage equipment, as it offered possibilities for more effective heat dissipation than any of the other designs.

Perhaps the most rapid advance in the use of low-voltage equipment has been made in the field of block electrophoresis. The instrumental requirements for this "open-column" technique, useful both for preparative and analytical ends, are relatively simple, and so are

the designs of the separation cell, varying from a simple horizontal glass or Perspex plate resting on electrode vessels[156,157] to rectangular open troughs or closed boxes, made of plastic material, such as Perspex, and used in a horizontal or, more latterly, in a vertical position.[158] A polythene sheet is sometimes used for covering the block or the trough. The voltage applied, depending on the current required by the stabilizing medium, the thickness of the layer and the buffer solution used, is never greater than 20 volts per cm, since even with cooling arrangements proper temperature control cannot be maintained. Working in a cold room does not materially help. An average potential gradient of 5 volts per cm with granular starch, cellulose or PVC as supporting media, is about the rule, although separations of lactic acid dehydrogenases[159] and haemoglobins[160] at 20 volts per cm have been reported. The interesting application of even higher voltages, up to 65 volts per cm, for separating haemoglobin derivatives by using either granular starch or gel was described by Künzer and Ambs[161] and Künzer.[162]

The same consideration applies to the equipment used in gel electrophoresis, a technique that has spread considerably in recent years mainly because of its extremely good resolving power for high-molecular-weight compounds, particularly proteins. The horizontal-trough method proposed by Grabar and Williams,[163] Uriel and Grabar[164] and others[165] for agar, and for starch gel by Bernfeld and Nisselbaum,[166] and Smithies,[167,168] in particular, has recently been largely superseded by the vertical-trough method,[169,170] for which several advantages are justly claimed. An apparatus with water-cooled glass walls for starch-gel or polyacrylamide electrophoresis, allowing the application of up to 40 volts per cm was described recently.[171] Excellent monographs by Bloemendal[172] on electrophoresis in starch gel and other media, except paper and agar, and by Wieme[173] on agar-gel electrophoresis, have recently appeared, containing details of the techniques, assessed in the light of each author's considerable personal experience.

High-voltage apparatus—

Easily the most important development in recent years has been the widely adopted use of high-voltage techniques, particularly for complex mixtures of low-molecular-weight compounds for which it is ideally suited. A high-voltage technique can be defined as one allowing the application of a potential gradient of more than 50 volts per cm. With suitably designed equipment, potential gradients of up to 200 volts per cm, or a voltage of 10 kV, can be safely and usefully applied. Since migration rate is proportional to field strength, raising the voltage will shorten the time of the run, which, apart from its expediency, will also minimise diffusion effects. Diffusion is inversely proportional to molecular weight and proportional to the square root of time. It can easily be seen that the application of high voltage will particularly benefit compounds of low molecular weight, producing separations with sharp zones. This point is particularly important for compounds of closely similar mobilities. Some spectacular separations have, indeed, been reported in the last few years. It should be pointed out, however, that the application of high-voltage techniques to high-molecular-weight compounds, such as proteins, has so far met with little success. One explanation often suggested is that the moisture content of paper strips used in high-voltage procedures is much lower, or too low, for the migration of proteins, and that consequently an excessive amount of trailing, or irreversible adsorption, is caused. In the reviewer's experience, potential gradients over 20 volts per cm usually lead to such complications, even with moisture contents of up to 200 per cent. (on dry paper). The greatest field of application for electrophoretic routine analysis, *viz.*, serum proteins, thus seems closed to an otherwise extremely promising technique.

Basically, the application of high voltage can be reduced to one problem, *i.e.*, efficient heat dissipation. Since the generation of Joule's heat is proportional to the square of the current flowing through the strip and the current is proportional to applied voltage, it can be easily realised that a system of low cooling efficiency will soon reach its limits at a relatively low voltage.

It was Michl[174] who as far back as 1951 described a set-up, with an organic solvent as coolant, suitable for high-voltage work. Since then all apparatus described can be reduced to three basic designs, *i.e.*, cooling by organic solvent,[175,176,29] by single plate[177,178] and by double plate.[179] The latter, the sandwich design, is the most elaborate of the three, as it calls for control of uniform pressure exerted on the strip during the run, in order to avoid

irregular migration behaviour. The solvent design is the simplest and easiest to construct, and has proved satisfactory in many laboratories. The limitations are that the cooling capacity is on average no more than 0·2 watt per sq. cm, and that the solvent must be immiscible with the background electrolyte and must not affect the compounds to be separated. There is also the complication of unavoidable evaporation of solvent, with possible toxic and fire hazards. Single-plate cooling, usually in a horizontal position, affords about the same cooling capacity of 0·2 watt per sq. cm, even with use of refrigerated coolant. It does not require a uniform-pressure device, which simplifies the design. Double-plate or sandwich design is the most efficient, capable of a heat dissipation of up to 1 watt per sq. cm when only tap water is used, but is also the most complicated and expensive to construct because of the provision for extremely close contact and uniform pressure. Added to this is the need for a high-capacity power supply of approximately 250 mA for 15-cm wide strips, to utilise the potentialities of the instrument. Most power units offered with commercial instruments are of too low a rating to be used for other than weakly dissociated background electrolytes. This may be a reflection on the limited cooling efficiency of the instruments. Particularly in fundamental investigations in the field of inorganic chemistry, strong background electrolytes such as hydrochloric and hydrobromic acids are often used, causing high current flow even in narrow strips.[180]

Several attempts to improve or simplify the basic designs were made, though with little apparent success, *e.g.*, of Michl's design,[181] Wieland's design[182] and Gross's design.[183,184]

Stabilizing media

Paper and powdered cellulose—

Filter-paper is widely used as a stabilizing medium, because it is convenient, cheap, has sufficient wet strength, and consists of very pure cotton fibres, although it must be mentioned that almost all types of commercially available papers still contain water-soluble impurities. Paper has several advantages, in that it stabilizes a relatively large volume of electrolyte solution per unit weight, is not soluble in commonly used buffer solutions, has not a high ζ-potential, usually negative, and thanks to its thinness can be efficiently cooled. Disadvantages are its fibrous texture, its property of irreversible adsorption of proteins, particularly of basic proteins and polypeptides. Its content of carboxyl, aldehyde and other carbonyl groups may be responsible for certain ion-exchange interaction with some test substances, affecting adversely the performance. An excellent and exhaustive account of the properties of filter-paper used in electrophoresis was given by Grüne.[185] There is also a considerable retardation factor or tortuosity of migration effect.[3,8,13,186]

Cellulose powder, mainly used for column work, has approximately the same properties. Flodin and Kupke[70] have prepared a powdered cellulose, modified by treatment with strong acid or by alcoholysis. This broke up the fibrous structure of the cellulose and rendered it as a fine powder that showed greatly reduced adsorptive capacity for basic proteins.

Cellulose acetate film—

Fine-pore cellulose acetate film was introduced by Kohn[187,188] in 1957 and has since found wide acceptance, particularly in the clinical analysis of human proteins. Its main advantages are complete freedom from adsorptive effects and the ease of evaluation of cleanly separated zones on a colourless background. Further, there is the possibility of dissolving excised zones in acetone before colorimetry. The disadvantages are high electro-osmotic flow, low water uptake and the necessity for low current density to prevent excessive evaporation and drying-out. Separations must be carried out in a completely water-saturated atmosphere to ensure good reproducibility of results. Because of the generally clean separations, the time of the run can be conveniently reduced to a few hours, or a fraction of the time usually required when filter-paper is used.

The use of cellulose acetate film for the separation of a simple mixture of amino-acids was described recently.[189] Determination of proteins by direct photometry was claimed to be made more reliable by the introduction of two new staining procedures with blue dyes,[190] and the separation of soluble liver proteins[191] has been described. There was no lack of attempts to improve the technique by application of higher voltage, in spite of the difficulties inherent in the nature of the medium. Wieland, Pfleiderer and Ortanderl[192] applied up to 60 volts per cm for the separation of lactic acid dehydrogenases of varying activities, obtaining

a much greater number of sharply resolved zones after 90 minutes. Westley[193] applied up to 30 volts per cm for separations of proteins, particularly enzymes such as rhodanese (sulphur transferase), by using ordinary white rayon acetate fabric, thoroughly soaked in buffer solution, as supporting medium. Although electro-osmosis was considerable, the resolution was decidedly better than by paper electrophoresis. A modified material, consisting of cellulose diacetate and triacetate with an "open-pore system," of 3- to 5-μ pore diameter, offering no hindrance to the migration of protein molecules, was proposed by Maier and Voggel.[194] This material is claimed to be particularly suitable for high-voltage separations of proteins, with high resolution and short running times. Detailed information, on the use of cellulose acetate film has recently been published by Friedman.[195]

GLASS-FIBRE PAPER—

The main advantage of this material lies in the fact that it allows the application of drastic spray reagents, containing concentrated strong acids or bases and is suitable for electrophoresis under extreme conditions of temperature, *e.g.*, in fused salts.[196,197] Bourne, Foster and Grant[198] suggested its use for carbohydrates, as did Briggs, Garner and Smith.[199] It was claimed that the new medium eliminated the possibility of a detrimental bonding action between polyhydroxy compounds and paper by means of a common borate ion, and this resulted in less adsorption of amylosaccharides. However, the high and somewhat irregular electro-osmotic effect[200] is an objectionable feature.

The reported separations of a great variety of polysaccharides, ranging from glucans, fructans, pentasans, amylopectins to galactoaraban from beet pulp,[201] seem to show the usefulness of the medium in the attack on a notoriously difficult problem.[202] Relative mobilities of low-molecular-weight carbohydrates on glass-fibre paper are usually not significantly different from those in filter-paper and it thus offers no obvious advantages, except by a possible exploitation of the greater electro-osmotic flow.

AGAR—

The use of agar has been greatly encouraged by the results of work published by Grabar and Williams.[163] Agar can be used as a 3 per cent. solution, the more usual concentration, when it sets easily, or at a concentration of only 0·13 per cent. as "fluid agar," giving a semi-solid film, as described by Ressler and Zak.[165] The separation of serum proteins in the semi-solid film appears to be somewhat superior to that in an agar block of higher concentration, and the lower concentration causes considerably less electro-osmotic flow. A disadvantage is the difficulty in controlling the rigidity of the medium, which, in turn, affects mobilities.

There seems to be little adsorption of proteins in the actual migration, as could be demonstrated by the two-dimensional experiments of Bussard and Perrin,[203] though some protein remains adsorbed in the application slit. The chemical nature of agar is still not quite clear, but the available evidence suggests that it is mainly a galactan sulphate, *i.e.*, a strongly acidic substance. This is a certain disadvantage, and may also be responsible for the high electro-osmotic effect. There is some evidence that agar has a considerable anion-binding capacity, affecting among others the separation of lipoproteins. A more serious complication is the presence of soluble contaminants, such as carbohydrates and acidic compounds, which makes its use for preparative work doubtful. However, for analytical purposes agar offers the overruling advantage of high resolution with minimum distortion of zones and trailing. Although agar found its main application in protein chemistry, it has been used for the analysis of compounds of widely differing nature, *e.g.*, for the quantitative separation of B-vitamins,[204] the quantitative determination by high-voltage technique of nucleic acid components,[205] and the separation of lithium isotopes.[206]

But agar gained really wide application in the new and exciting technique of immuno-electrophoresis. This is based on the combined use of electrophoresis, diffusion of separated proteins, and secondary reaction with antiserum. It provides an extremely useful method for studying various antigen - antibody systems. An up-to-date monograph on immuno-electrophoresis, dealing extensively and most expertly with all aspects of the subject has quite recently been published by Grabar and Burtin.[207] A well illustrated, concise survey of the application and scope of immuno-electrophoresis in clinical chemistry appeared a little earlier.[208]

GRANULAR STARCH—

Granular starch, mainly potato starch, is still widely used for open-block and column techniques, in spite of the increasing preference for starch gel. It has several advantages, such as ease of handling and homogeneous packing, comparative freedom from soluble contaminants after preliminary washing[209] and settling,[156] sharp zones of origins and simplicity of segmentation for recovery of fractions. Washing with buffer solutions minimises any change in conductivity and pH of the medium.[210] Pre-washing the starch with a 0·25 per cent. solution of bovine albumin was reported to have increased the recovery of an enzyme.[211] One serious disadvantage, when unwashed starch is used, is the problem of contaminants such as soluble carbohydrates, ninhydrin-reactive, phosphorus-containing and ultraviolet-absorbing substances. Recovery of lipoproteins from starch presents a great difficulty, and some serum proteins such as albumin and γ-globulin cannot usually be recovered to an extent better than 90 per cent.

But perhaps the most serious limitation is the fact that it is not quite as suitable for smaller molecules as it is for large-sized proteins. Small protein or polypeptide molecules seem to be able to penetrate into the swollen starch granules causing trailing of zones and thus poor separation. The serum proteins and haemoglobins do not penetrate the granules and are therefore not affected, and the white background of the medium can be of great advantage when coloured compounds are dealt with, *e.g.*, facilitating the detection of minor haemoglobin components in normal human blood.[212] Starch-block electrophoresis is also very useful for homogeneity studies. An interesting subfractionation of γ-globulin was achieved,[213] rabbit antisera[214] were separated, and Bloemendal and Ten Cate[215] succeeded in separating α-crystallin from other water-soluble lens proteins. Tuynman, Kwa and Bloemendal[216] used a starch-block technique for partial purification of crude pituitary extracts. Bacterial aspartase[217] was separated from other enzymes and purified. Mandels, Miller and Slater[218] demonstrated the heterogeneity of carbohydrates obtained from fungus cultures. Elson[219,220] applied the technique successfully to the separation of ribonucleoprotein, and so did Cohen and Lichtenstein.[221] Homogeneity studies demonstrated the purity of aspartate transcarbamylase,[222] and the heterogeneity of elastase preparations.[223]

STARCH GEL—

There is no doubt that the introduction of starch gel by Smithies[167] represented a major advance in technique, by significantly widening its scope through greatly enhanced resolving power, particularly for proteins. The medium consists of soluble potato starch of about 15 per cent. concentration in a weak buffer solution, mostly borate. This produces a gel of considerable strength, easy to handle during the staining and recovery procedures. The type of starch, its degree of hydrolysis and its concentration exercise a great influence on the performance as a supporting medium. Strict standardization of the preparation, by control of time and temperature during hydrolysis is a necessity in order to obtain a gel with reproducible properties. The preparation is somewhat laborious and time-consuming, but starch hydrolysed under standardised conditions and ready for use is now commercially available (Connaught Medical Research Laboratories, Toronto, Canada).

Alternatively, a low-concentration gel, prepared from a mixture of 3 per cent. of corn starch, 3 per cent. of Hyflo Supercel and 1·5 per cent. of amylose has been proposed by Bernfeld and Nisselbaum.[166] The addition of amylose allows gel formation at a lower concentration, which in turn significantly reduces retardation of migrating molecules and thus produces relative mobilities of serum proteins close to those obtained by free electrophoresis. The electro-osmotic flow is extremely low, separation good and recovery excellent. The removal of the amylose from the isolated protein fractions presents a certain problem, though.

The reasons for the remarkable resolution of proteins by the starch-gel technique are not quite clear. It is generally accepted that in the interpretation of higher resolving power and more interesting results obtained by gel electrophoresis, the additional effect of molecular sieving must be taken into consideration. Whereas electrophoretic migration in paper or granular-starch systems takes place in an aqueous film around the stabilizing material, in gel system the molecules must penetrate the gel matrix. If the pore diameter of the gel is of the same order of magnitude as the molecular dimensions of the migrating molecules, a considerable impedance is presented by the gel, resulting in a friction effect. This will be specific and relative to the pore size of the gel and the dimensions and shape of the molecule. Starch gels impede the migration of larger proteins far more than that of smaller proteins, *e.g.*,

proteins of molecular weights less than 20,000 are not significantly hindered or sieved. It was found that the separation of such protein molecules in starch gel was not superior to that in agar, which is in agreement with the theory. Comparison with paper electrophoresis, in which proteins of similar mobilities were used, showed that starch gel separated them according to molecular weights.[41,224] A measure of relative sizes of components in complex mixtures can be obtained by determining their retardation coefficients.[225] Stabilization against convection is better in homogeneous gels than in granular media. Molecular-sieving properties of gels depend on porosity of matrix, but the degree of retardation is independent of charge or duration of run. Migration is inversely proportional to starch concentration over a certain range.

Serum proteins can be separated into as many as 12 components in borate buffer solution. Addition of concentrated urea solutions[226,227] and variation of buffer solution[228] may lead to several further subfractions. Poulik and Smithies[41] and Poulik[229] first applied a two-dimensional technique and obtained up to 20 clearly separated components from serum proteins.

The emphasis has so far been on analytical applications, whereas very little preparative work has been reported. Certain difficulties in the recovery of separated fractions, owing to irregular migration and distorted zones between surface and inside of the block, have been encountered. Applications of the starch-gel technique in the field of proteins are too numerous to be mentioned. Bloemendal's book[172] gives a comprehensive and up-to-date account of them.

Among the more striking results obtained quite recently were the study of haemoglobins in cattle,[230] the discovery of new protein components in cerebrospinal fluid,[231] the possible identification of fungi by electrophoretic patterns of mycelic proteins,[232] the use of the electrophoretic pattern for comparison and characterisation of wheat flour[233] and cereal proteins[234] and the discovery of haemoglobin-N in sheep.[235]

POLYACRYLAMIDE—

The use of polyacrylamide gel was first suggested by Raymond and Weintraub,[236] and later described in detail.[237,238] As a synthetic polymer, polyacrylamide (Cyanogum 41, made by American Cyanamid Co.) is chemically better defined than starch, and the pore size can be easily adapted to requirements by varying the proportion of the cross-linking agent. Preparations of gel, based on a mixture of acrylamide and *NN'*-methylenebisacrylamine, with cross-linking agent and catalyst added, were described by Ott[239] and Barka.[240] The gel can be prepared within 1 to 2 hours, depending on the procedure. The advantages over starch gel are smaller electro-osmotic flow, higher resolving power and transparent appearance. It can thus be used for direct densitometry of proteins and nucleic acids in ultraviolet light, or after appropriate staining.[241]

Ott[239] obtained 15 to 20 separated components from normal and abnormal human sera. Chang, Srb and Steward[242] separated the soluble proteins from *Neurospora crassa* and managed to detect 25 components, instead of 9 by paper electrophoresis. Reisfeld, Lewis and Williams[243] reported a modified procedure facilitating the separation of basic proteins and peptides. By using a discontinuous tris - citrate - borate buffer system, Evans and co-workers[244] separated peanut protein into 22 fractions. An interesting two-dimensional technique, in which two different concentrations of gel were used, was applied to serum proteins, enzymes and haptaglobins.[245]

Hjertén[246] found, by a column technique, that the migration rate was not only dependent on charge, but to a large extent on size. Reversal of mobilities could be effected by changes in gel concentration, *i.e.*, pore size. The utilisation of the molecular-sieve effect in combination with a stabilizing medium without such an effect may give extremely good resolution. The effect of buffer solutions on mobility and separation was described by Ferris, Easterling and Budd.[247] A rather instructive article on the use of polyacrylamide appeared recently.[248]

A special technique, in which polyacrylamide is used in small, narrow glass tubes, was introduced and named "disk electrophoresis" by Ornstein and Davis.[249] The technique was later described in detail in a monograph.[250] The name was coined because the separated fractions of a protein mixture, after staining, resemble a stack of flat coloured discs in the transparent gel. The packing consists of three layers of small-pore and large-pore gels in buffer solutions of varying pH, leading to extraordinary zone-sharpening and thereby high resolution over comparably short distances. Over 20 fractions can be easily detected over a distance of 30 mm.

MISCELLANEOUS MATERIALS—

Poly(vinyl chloride), Geon 426, powder was introduced by Kunkel and co-workers[213,251] as a useful alternative to starch systems when certain complications connected with starch have to be avoided. It forms easily a coherent block, does not significantly adsorb proteins, has no soluble impurities, but has the disadvantage of a high and poorly reproducible electro-osmotic flow at high pH values. An improvement on the various Geon-type resins (made by B. F. Goodrich Chemical Company, Cleveland, Ohio) was claimed by Müller-Eberhard[252] and Müller-Eberhard and Nilsson[253] for Pevikon C-870, a mixed polymer of poly(vinyl chloride) and poly(vinyl acetate) (made by Superfosfat Bolaget, Stockholm). It is said to be practically free from electro-osmosis and adsorption, and to offer a possibility of applying potential gradients of 20 to 60 volts per cm for high resolution. Bocci[254] used the medium for separating rabbit-plasma proteins and found it a very mild and useful medium. It can also be combined with starch gel.[255] Bocci developed a special apparatus[256] with water-cooling that allowed good separations of serum proteins in rectangular Pevikon blocks at 60 volts per cm and 160 mA.

Suspensions of agarose,[257,145] a non-acidic agar derivative, were used in column electrophoresis and described as having satisfactory anti-convection properties at concentrations as low as 0·15 per cent. The migration rates in such a medium are similar to those in free electrophoresis, and human-serum proteins and subcellular particles, microsomes, have been well resolved.

Inorganic media such as Hyflo Supercel, after appropriate acid treatment and thorough washing, and Celite have been used in the past, but have given way to the organic or synthetic

Of the most recent media, Sephadex, a cross-linked glucose polymer (made by Pharmacia, Uppsala, Sweden) may be mentioned as having been used in an electrophoretic technique.[144,258] Its molecular-sieving effect, utilised in gel filtration[259] may be found useful as adding another dimension to straightforward electrophoresis.

APPLICATIONS

It is impossible to quote all papers of the last 10 years describing applications of stabilized electrophoresis, and, for reasons of brevity and clarity, a rigorous selection obviously had to be made on the basis of novelty, quality and scope of effort. Since some of the books[21 to 27] and reviews[28,29,30,31,63] mentioned cover adequately the state of knowledge up to a certain date, it is proposed to refer only to some of the more recent contributions in important fields of applications. Quite a number of pertinent publications of this type have already been quoted in earlier sections of this review in conjunction with general aspects of either techniques or properties of media.

INORGANIC—

Paper electrophoresis can be expected to offer advantages over chromatography in certain instances, due to greater speed and higher quality of separation. Surprisingly enough, progress was much slower than expected since the publication of Lederer's[260] excellent book, providing the theoretical background and stimulation of interest in inorganic applications. This was followed by equally useful publications.[86,261,262] Strain and Sato[263] and Strain[264,265] reviewed some theoretical aspects within the framework of differential migration techniques.

A very efficient separation of the alkali and alkaline-earth metals was demonstrated by Gross[83] by means of a suitable high-voltage technique. Complex mixtures of metaphosphates and condensed phosphates were neatly resolved by Sansoni and Baumgartner,[266] again by a high-voltage technique. Gross[83] had also shown that at 100 volts per cm many of the heavy-metal ions could be separated in 30 to 45 minutes as anionic complexes of organic acids, whereas zinc[II] and other ions could be separated in the cationic form[3] in 10 to 15 minutes. Lederer[267] investigated the action of strong acids on rhodium hydroxide, a study continued by Shukla.[268] Pučar[84,85] applied high voltage and current conditions to the separation of chloro-complexes of the copper group, and included later the iodo- and bromo-complexes. He and Jakovac[269,270] studied mobilities of ions in inorganic and organic buffer solutions, and he[131] also wrote a very useful review on theory, technique and equipment of continuous electrophoresis.

Migration rates for a series of anions, particularly halides, were measured by Gross,[99] who used a high-voltage technique, and independently by Lederer,[100] who used a low-voltage

technique, with results in good agreement. Very interesting separations of chloro-, and mixed chloro - bromo-complexes of various members of the platinum group were achieved by Blasius and Preetz[90] and Blasius.[262] Rare-earth separations have been described by Lederer[271] and Langevin-Joliot and Lederer.[272] Schumacher and co-workers[273,274,275,276] described a method of ion focusing by establishing a pH gradient and applying complexing agents and high voltage, which leads to extremely narrow zones within a short time, and thus to high resolution.

In the field of separations of isotopes, several useful applications have already been mentioned.[93,94,95,96] To these may be added the separations described by Van Dijk, Ijsseling and Loman[97] and Adloff and Bertrand.[98] An extensive study of the separability of mixtures of cations in the presence of complexing agents was reported by Wenger, Janstein and Kapetanidis[91,277,278] and others.[279]

Separations of anions,[280] including oxy-acids of sulphur,[281] polythionates,[282,283,284] nitrogen acids,[102] and radioactive-halogen acids[101] were investigated mostly by low-voltage techniques.

The quantitative determination of alkali metals, particularly sodium and potassium, first described by Schier,[82] then briefly mentioned by Gross,[83] was also tried by Pompowski and Grzędzicki[285] on soil extracts, either by titrating the separated metals as carbonates with 0·01 N hydrochloric acid or, better still, by determining them flame photometrically. Recovery was quantitative only for sodium, and the error was given as 2 to 6 per cent. Recovery of the separated metals from paper by extraction is, in the reviewer's opinion, never quite satisfactory, thus reducing the advantages accruing from dealing analytically with cleanly separated ions. A rapid method for alkali metals was proposed by De Vries, Schütz and Van Dalen,[92] and an attempt was made to separate these metals in various organic solvents.[286] The application of high-voltage paper electrophoresis in the field of inorganic chemistry was reviewed by Gross.[287] Several workers reported on electrophoresis in stabilizing media other than paper. Among the media suggested was silica gel[288] or kieselguhr for a thin-layer technique at potential gradients up to 45 volts per cm, resulting in a separation within 1 hour of calcium-45 from barium-133 in lactic acid, and cellulose acetate[289] strip for some transuranium elements in concentrated nitric acid.

On the theoretical side, measurements of migration rates of many cations[3] were reported, a study of mobilities by means of a frontal method[290] proposed, and apparent mobility measurements of radioactive ions[291] described. A very instructive account of some theoretical studies of inorganic electrophoresis was published by Miller and Pickering.[292] Chemla,[293] dealing with the separation of isotopes, described the theoretical principles for choice of conditions in aqueous and non-aqueous (fused salts) media. A paper by Martin[294] dealt mainly with migration and enrichment of isotopes in aqueous media.

ORGANIC (LOW MOLECULAR WEIGHT)—

This is, for reasons stated before, an extremely rewarding area for the application of paper electrophoresis, since groups of compounds like the amino-acids, amines, sugars, carboxylic acids, and nucleic acids are eminently suitable for analysis by electrophoretic techniques. Perhaps the most important development in recent years has been the increasing use of high-voltage techniques with potential gradients up to 200 volts per cm, prompted no doubt by a degree of resolution unmatched by any alternative method.

High-voltage paper electrophoresis proved to be extremely useful in the analysis of peptides and amino-acids in protein hydrolysates and biological fluids, both qualitatively and quantitatively, and either by a one-, or two-dimensional technique. Separations of mixtures of ninhydrin-reacting substances in human plasma, urine and cerebrospinal fluid were described by Efron,[114] in avian plasma by Bell, McIndoe and Gross,[295] arginosuccinic acid in urine was identified,[296] a thorough quantitative method for protein hydrolysates described,[297] and a similar micro-scale method for amino-acids proposed.[298] A quantitative method for urinary amino-acids had been described a little earlier,[299] and a method based on fixing the ninhydrin colour with a zinc salt, instead of the more usual copper or cadmium salts, reported.[300]

Further methods were described by Saint-Blancard and Storck,[301] Braun,[302] and Cook and Luscombe.[303] An improved, stable ninhydrin reagent[304] for quantitative work was recently proposed. The problem of side-reactions occurring when ninhydrin is used on paper, was discussed in several papers.[305,306,307] Most two-dimensional results have been achieved by a combination of chromatographic and electrophoretic techniques. Such a technique was

applied to the separation of peptides produced by the tryptic digestion of human haemoglobins[308] and other proteins.[114] Haemoglobin digests give characteristic patterns of peptides, commonly called "fingerprints." Up to 80 peptides could be separated on one sheet by an improved method.[176] A purely electrophoretic two-dimensional technique for amino-acids and protein hydrolysates was described by Gross.[309] The advantage lies in a much more rapid resolution and a different pattern. An apparatus for two-dimensional work was described very recently.[310] Equipment specially designed for two-dimensional electrophoretic separations on large sheets is now commercially available (Miles Hivolt Ltd., Shoreham, Sussex).

The quantitative analysis of biogenic amines by paper electrophoresis was described in detail by Fischer and Bohn[78] and Ferenčík,[81] and the resolution of aliphatic amines by Gross.[80]

Considerable success was achieved in separations of nucleic acid components,[69,311,312] nucleosides and nucleotides.[68,71,313,314]

Electrophoretic techniques proved useful for phenolic compounds[74,75,76,315,316,317] and, particularly sugars.[54 to 62] Pastuska and Trinks' method[316] is a thin-layer technique, in which silica gel or kieselguhr and a moderate voltage are used. Results from recent research on less common sugars and sugar derivatives demonstrated the superior resolving power of electrophoretic techniques,[63,318 to 325] both for preparative and analytical work. It is rather regrettable that relatively few results on organic acids can be found. This is an area in which the technique could be of real assistance in many problems of separations. In addition to applications[64,65,66,67] already mentioned, more recent results were reported for sugar acids,[326,327] both known and unknown, diaminocarboxylic acids,[328] acids of the citric acid cycle,[329,330,331] known and unknown dicarboxylic acids arising from chemical degradation,[332] mould-produced tetronic acids,[333] the pathway of incorporation of labelled acetate[334] into Cephalosporin C, and for aldehydes.[335,336]

ORGANIC (HIGH MOLECULAR WEIGHT)—

Most routine applications since the introduction of electrophoretic techniques involved proteins, of which the serum proteins formed the greatest part. There were, however, several attempts to resolve mixtures of other high-molecular-weight compounds, such as polysaccharides, the more remarkable of which were the separations of galactan, mannan, inulin, galactogen and glycogen,[337] paper, glass fibre or silk being used as supporting media,[338] the identification by mobilities of two polysaccharides isolated from yeasts,[339] the establishment of heterogeneity of various polysaccharides such as amylopectins, gums, fructans, galactoarabans and pentosans,[201,340] glass fibre being used as medium in preference to paper, and a preparative separation of glycogen from galactogen,[341] with paper as medium.

The vast numbers of papers concerning electrophoresis of proteins in human and animal plasma and other biological fluids, both normal and pathological, enzymes, haemoglobins, mucoproteins, lipoproteins, etc., have been amply and expertly dealt with recently by Bier,[21] Wunderly,[24] Bloemendal,[172] and Wieme.[173]

The important field of plant proteins[342,343] has yielded less spectacular, though quite stimulating results, such as the fractionation of protein-like substances from corn leaves[47] on paper, pea-seed albumins[46] in polyacrylamide gel, and wheat[233] and other cereal proteins[234] in starch gel. An investigation of the interaction between milk proteins[315] and tea polyphenols, with starch or cellulose acetate as medium, produced novel results on a complex problem. Resolution of different types of casein by paper electrophoresis was described by several workers, *e.g.*, fractionation into α-, β- and γ-casein,[344] separation of calcium-sensitive from calcium-insensitive casein,[345] and a quantitative determination of α- and β-casein.[346] The application of the starch-gel technique[228,347] in concentrated urea solutions revealed up to 20 components in casein. The latter technique is extremely helpful in the purification of enzymes. For instance, a preparation of adenosine deaminase,[348] homogeneous in the ultracentrifuge, could be separated into 4 active components, whereas an experiment with crystalline gastricsin,[349] carried out both on paper and starch gel, demonstrated migration and proteolytic activity in starch gel, but not on paper, indicating strong adsorption and possible denaturation by paper. The isolation of pituitary hormones[350] was accomplished by a preparative technique with polyacrylamide gel, and a genetically determined variant of lactic dehydrogenase[351] in red blood cells was discovered by the starch-gel technique, in which the dependence of migration rates on the concentration of medium was utilised. The electrophoretic results of cerebral-tissue proteins on paper, cellulose acetate and in agar have been compared,[352] and

so were results of serum proteins[353] in agar, on paper, and in free solution. Bell and McKenzie[354] separated and compared the β-lactoglobulins of various mammals by using electrophoresis in several media such as starch gel, agar, polyacrylamide and paper.

Critical appraisal of methods, results and potential developments

Stabilized electrophoresis has three main advantages over free electrophoresis, *viz.*, it permits a complete separation, it is suitable for low-molecular-weight compounds and it affords longer distances for mobility determinations. It produces sharp zones of high-molecular-weight compounds, such as proteins, particularly in a stabilizing medium of little adsorptive tendency. This is helped by the fact that high-molecular-weight substances have low diffusion coefficients. With low-molecular-weight compounds, the situation is aggravated by their high diffusion coefficients and conductivities, inorganic compounds in particular, which, during a run lasting 18 to 24 hours and in weak buffer solutions, may more often than not lead to unsatisfactory separations due to blurred and distorted zones. The maintenance of sufficient ionic strength of buffer solution to suppress zone blurring is often not possible because of undue heat generation. An answer to this problem is provided by the high-voltage technique, combining efficient cooling with speed of experiment, thus reducing the effects of diffusion and heat generation to a minimum. Unfortunately the successful application of high voltages to proteins has for various reasons so far not materialised. This, in effect, means that the largest area of application is served mainly by low-voltage methods, in which various stabilizing media were used. Attempts to raise the potential gradients above 20 volts per cm were doomed to failure, with resolution deteriorating rapidly. For low-molecular-weight compounds, however, the technique proved eminently suitable. The main reasons against a drastic change-over from low-voltage to high-voltage techniques appear to be the much higher cost of equipment and the greater risk of serious accidents. These objections can be easily countered by the wide experience with such equipment, which has proved that its far greater speed and separating effectiveness, coupled with suitable safety devices, make it an extremely useful, versatile and safe tool, soon compensating for the initial higher capital outlay. In the reviewer's laboratory, high-voltage equipment has been in almost daily use by various operators for over 12 years without a single accident.

It is interesting to consider in broad outline the historical development of electrophoresis, which can be described briefly as the movement of a dissolved or dispersed substance in an electrical field. This process was actually studied since the middle of the 19th century mainly for the sake of electro-kinetic phenomena. Tiselius[355] in 1937 was the first to realise the great analytical usefulness of a method, based on such a principle, and to propose a suitable technique and equipment for this purpose. Most interesting information on proteins was obtained by the technique of free or moving-boundary electrophoresis, but the instrument required was quite elaborate and expensive, capable of only a partial resolution of constituents, and for various reasons not suitable for low-molecular-weight compounds. In 1948 came a turning point, when the technique of paper electrophoresis was introduced almost simultaneously by Wieland and Fischer[356] and Wieland[357] and Haugaard and Kroner.[358] By 1950 the value of the technique for separating proteins and, to a lesser degree, amino-acids and peptides had been unequivocally demonstrated. Filter-paper became the most acceptable stabilizing medium among the various materials that had been suggested for stabilizing the background electrolyte.

Here, at last, was a simple technique, intrinsically capable of providing a complete resolution of a mixture and potentially suitable for quantitative evaluation. All this could be done with equipment so simple that almost any laboratory could afford it. It is no wonder that the technique has swept the whole scientific world. Then in 1955 Smithies[167] introduced starch gel, followed by Raymond and Weintraub[236] in 1959 with polyacrylamide gel. There is no doubt that these two media have increased the resolving power of the method for proteins to a truly remarkable degree, owing to the molecular-sieve effect of the gel. A further refinement, the addition of urea[359] to the buffer solution made the resolution of sub-units of proteins possible.

Thus, for serum proteins, the advance in electrophoresis has meant the progress in separation and identification from 5 to well over 20 components. There is little doubt that some of the best resolutions of biological mixtures were made possible by the use of starch gel[360] or polyacrylamide, and these media are used increasingly in spite of some technical difficulties involved in their preparation.

Although paper is still the most widely used medium for proteins, cellulose acetate film is increasingly chosen when better resolution and shorter runs are desirable.

A column technique, in which a liquid density gradient is used as stabilizing factor, proposed some years ago[361,362] and developed later,[363,364] offered three main advantages, *viz.*, transparency of medium, absence of adsorption and ease of recovery of separated fractions. This method has theoretically a great potential, particularly for preparative work, but it seems that it is being accepted only slowly and applied successfully to a specific practical problem only rarely.

The great and increasing popularity of thin-layer chromatography has recently prompted several workers to incorporate the essential part of the technique, the thin layer of adsorbent, in an electrophoretic technique. The advantages were claimed to be the possibility of choice of medium with little or no adsorptive properties, greatly reduced diffusion effects and the possibility of the use of aggressive spray reagents. Such varied supporting media as silica gel, alumina, cellulose powder, Plaster of Paris and sintered polymer sheets were tried. Procedures for separating predominantly low-molecular-weight compounds, such as amino-acids and amines,[365,366] phenols,[316] dyes[367] and inorganic ions[368,369] were described. There are two obvious shortcomings attached to thin-layer techniques, *i.e.*, the greatly restricted amount of sample that can be applied and the problem of excessive heat generation, once the energy throughput rises above 0·02 watt per sq. cm. A way of efficient cooling must be found before the technique can make real progress.

The major general problem that still defies a satisfactory solution is the quantitative aspect of stabilized electrophoresis. In contrast to free electrophoresis, the quantitative determination is only possible after the run, the standard error is appreciably higher, and there can be interaction between the medium and the test substance, *e.g.*, irreversible adsorption on paper. If colour reactions are available and the colour formed is proportional to the concentration of test substance, and if the colour thus formed can be either measured densitometrically *in situ* or quantitatively eluted and measured photometrically in solution, then quantitative determination is feasible. Regrettably, these conditions are very rarely fulfilled. Further, there is the difficulty of location of the total substance, particularly with components of closely similar mobilities. Exceptionally good resolution, such as afforded by high-voltage techniques or suitable choice of a supporting medium, allows the use of unspecific but highly sensitive reagents. Bush[370] recently summarised most interesting findings of his thorough investigations into various aspects of quantitative chromatography, and many of his conclusions are valid for corresponding problems in electrophoretic techniques. His experiments on automation of scanning procedures, based on rigorous standardisation and novel instrumentation, are a pointer to future development in quantitative work, particularly for routine analysis of proteins and amino-acids. His results also demonstrate that, with all elaborate precautions taken, an error (95 per cent. fiducial limit) of ± 2 to 3 per cent. for amino-acids determined by paper chromatography is an achievable target rather than a routine result. This statement is refreshingly candid, especially in comparison with the exaggerated claims for so-called quantitative methods so often recorded in the literature. That all is not too well in quantitative analysis and that the stoicheiometric colour reaction is still round the corner, can be deduced from papers that appeared until very recently on improved procedures.

Methods have been described for extracting dyed proteins from paper,[371] from cellulose acetate,[372] from agar,[373] and from starch gel.[374] The dye-binding capacity of proteins has been investigated thoroughly, including the effects of denaturation[375] and the varying affinities between proteins and dyes.[376] Budd[377] reported interesting results from comparative studies on the relative merits of elution and direct densitometry.

Polyacrylamide gel and, to a lesser extent, cellulose acetate film can provide a water-clear medium coupled with high resolution, and may thus be used for direct densitometry of proteins and nucleic acids in ultraviolet light. This may become the method of obvious choice for certain groups of compounds in the future. Starch gel, though of equally high resolving power, cannot be scanned directly, and is hardly suitable for elution because of some possible irregularities in migration behaviour.

With regard to literature surveys becoming increasingly an absolute necessity, special mention should be made of the outstandingly good biennial reviews by Strickland[378,379] and, especially on biochemical analysis, by Hamilton.[380,381] One sometimes speculates how much more useful and time-saving to the research worker such a survey could be if papers with doubtful claims or results were not only ignored, but honestly criticised. The reviewer can

think of at least two papers in which attempts to reproduce the published results were completely unsuccessful.

Finally, a brief remark on terminology. It is gratifying to note that the term "electrophoresis" has been given overwhelming preference by recent authors. "Ionophoresis" and "electrochromatography" have been increasingly abandoned for good reasons. The former term tried to put too much emphasis on the size of the migrating ion, which was historically understandable, but scientifically hardly tenable, the latter term is illogical and somewhat misleading, as the technique resembles only superficially chromatography and is based on entirely different principles. Such odd terms as "electrochromatophoresis," "ionography," "pherography" and "electromigration" have, fortunately, found only infrequent acceptance. "Zone electrophoresis," on the other hand, has been widely used, to define the general character of electrophoresis. This still requires another expression to identify the specific type of technique, *e.g.*, filter-paper.

In the reviewer's opinion, less confusion would be created in identifying the type of electrophoresis by adding some characterising word or prefix to the generic name, denoting the stabilizing medium, *e.g.*, paper, cellulose acetate, starch gel. In some instances, identification can be more specific by also referring to apparatus or to direction of sample migration, or voltage conditions, *e.g.*, column, block, vertical, two-dimensional or high-voltage. "Stabilized" and "free" were the concise and clear terms[382] proposed some time ago for the two main branches of electrophoresis. The description of a technique as, for instance, vertical starch-gel electrophoresis or high-voltage paper electrophoresis should give sufficient information and leave no doubt in the mind of the reader as to what technique is meant by it. A standardisation of the terminology on this basis would be desirable and welcome.

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Received *February 5th*, 1965

The Amperometric Determination of Submillimolar Concentrations of Iron^{III} with Mercury^{I} Perchlorate

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The amperometric titration, at a rotating platinum electrode, of iron^{III} at concentrations of from 10^{-5} to 10^{-4} N in potassium thiocyanate - perchloric acid medium has been carried out with mercury^{I} perchlorate solution. Although the titration curve is sharply defined at an iron^{III} concentration of 10^{-4} N, end-point location by the usual method gives high results. The causes of a small anodic current obtained beyond the end-point are discussed.

An end-point method that involves prior addition of an arbitrary amount of iron^{III}, observation of the current, x, injection of the iron^{III} sample and titration to restore the current to x, has been developed. Under proper conditions, the results are precise and accurate to ± 2 and ± 5 per cent. for amounts of approximately 10 and 1 micro-equivalents of iron^{III}, respectively, in 100 ml of titration medium.

The applications of mercury^{I} nitrate or perchlorate as a stable reducing titrant have been recently reviewed by Berka, Vulterin and Zýka,[1] who point out that the most studied titration is that of iron^{III}. This titration is carried out in the presence of an excess of thiocyanate ion, when complex formation causes the formal potential of the mercury^{II} - mercury^{I} couple to become much less positive than that of the iron^{III} - iron^{II} couple. Belcher and West[2] have shown that the titration is stoicheiometric and have extensively examined its applications. The present work is concerned with the titration of iron^{III} in the concentration range 10^{-5} to 10^{-4} N.

A general problem in low-concentration titrations is the accurate fixing of the end-point. Techniques such as conductometry and amperometry, which permit the end-point to be found from multiple observations made before and after this point, can be valuable in such instances.[3] The necessarily high ionic strength of the medium makes conductometric titration inapplicable to the present problem. Amperometric titration is, however, usually possible when the ionic strength is considerable.

Experimental

Voltammetry—

In a de-oxygenated medium, 0·1 N in both potassium (or ammonium) thiocyanate and perchloric acid ("acid thiocyanate medium"), mercury^{II}, mercury^{I}, and iron^{III} ions all gave reduction currents at a dropping mercury electrode when its potential was more negative than −0·10 volt (unless otherwise stated, all potentials are with respect to the saturated calomel electrode). Iron^{II} did not give a reduction current. Anodic attack by the medium on mercury invalidated observations at potentials less negative than −0·10 volt.

When examined at a rotating platinum electrode, the acid thiocyanate medium sometimes gave an abnormally large residual current (see Fig. 1*a*). This effect, most noticeable in a medium that had aged for several days, cannot be attributed to reagent impurity, since perchloric acid or thiocyanate salt behaved normally when present separately. The curves in Fig. 1(*b*) show that mercury^{II} and iron^{II} ions are not electro-active at a rotating platinum electrode within the potential range 0 to +0·3 volt. The small anodic current caused by the addition of mercury^{I} perchlorate opposes the residual current of the medium. Although the cathodic current of iron^{III} ion is not greatly potential dependent within the range −0·05 to +0·15 volt, there is no true limiting-current plateau. Provided that the current-resistance (iR) drop in the circuit was kept small, the current measured at a potential of +0·1 volt was found to increase linearly with the concentration of iron^{III}.

At the highest concentration, 5×10^{-4} N, of iron^{III} examined, the initial current was 85 μA, measured at a potential of +0·10 volt in 100 ml of solution. During continuous electrolysis for 2 hours, the current fell to 71 μA. About two-thirds of this fall is attributable

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to electrolytic reduction of the active substance, a general source of error in prolonged observations with high-sensitivity electrodes. Photo-reduction is probably a contributor to the remainder of the fall. However, the total current fall at a new electrode was less than at an equally sensitive electrode that had a surface roughened by prolonged alternation of anodic and cathodic treatments.

TITRATION AT A DROPPING MERCURY ELECTRODE—

In the titration of 5×10^{-4} N iron^{III} in de-oxygenated acid thiocyanate medium at a dropping mercury electrode of potential −0·50 volt, the current increased linearly with the volume of mercury^{I} perchlorate solution added. Linear current increase continued, but at a reduced rate, as the titration was continued past the expected end-point. The very obtuse angle between the arms of the titration curve precluded all but approximate end-point location.

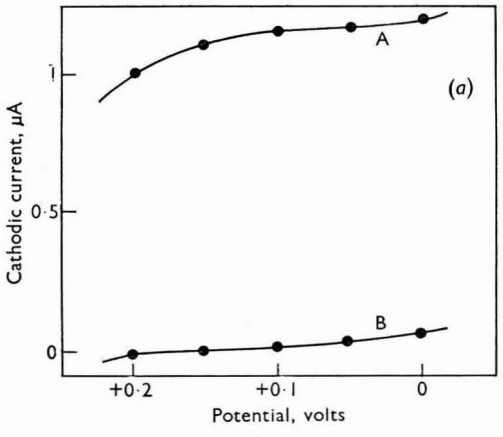

Fig. 1 (*a*). Current - potential curves obtained with a rotating platinum electrode. Curve A, 0·1 N in both perchloric acid and potassium thiocyanate; curve B, 0·1 N perchloric acid or 0·1 N potassium thiocyanate

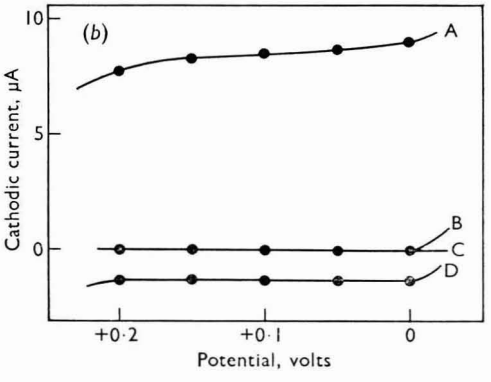

Fig. 1 (*b*). Current - potential curves obtained with a rotating platinum electrode for 5×10^{-5}N metal ions in supporting electrolyte 0·1 N in both perchloric acid and potassium thiocyanate. Curve A, iron^{III}; curve B, mercury^{II}; curve C, iron^{II}; curve D, mercury^{I}

The titration was repeated at a potential of −0·25 volt after the acid thiocyanate medium had been made 0·8 M in zinc sulphate and pre-saturated with zinc mercurithiocyanate. Although the current fell as titrant was added and became essentially zero near the expected end-point, the readings were generally unsteady and the precision was poor.

TITRATION AT A ROTATING PLATINUM ELECTRODE—

Preliminary titrations of 5×10^{-4} N iron^{III} in de-oxygenated acid thiocyanate medium at a rotating platinum electrode were carried out at a potential of +0·10 volt. The current decreased linearly with the volume of mercury^{I} perchlorate solution added, and became zero near the expected end-point. Just beyond this point, the current reversed its direction; further addition of titrant had no significant effect on this small anodic current. Although the shape of the titration curve appeared suitable for end-point location by extrapolation of the arms of the curve, the results thus obtained were about 1 per cent. high. Positive errors of several per cent. were obtained when this method of end-point location was used in the titration of iron^{III} at a concentration of 5×10^{-5} N.

Other preliminary experiments involved titration either to zero current or to the value of the residual current (the current reading obtained in de-oxygenated acid thiocyanate medium alone). Although reasonably satisfactory when the iron^{III} concentration was about 10^{-4} N, both methods gave inaccurate results in the titration of iron^{III} at a concentration of about 10^{-5} N.

A technique that is sometimes useful in low-concentration titrations involves pre-titration of the medium to a mark that is arbitrarily chosen to denote the end-point, introduction of the sample, and continued titration to this same mark.[4] However, attempts to pre-titrate the acid thiocyanate medium to a constant, essentially zero current were unsuccessful. An obvious analogue of pre-titration is the prior addition to the medium of a suitable, but unmeasured, amount of the substance to be titrated. The resulting current reading is taken as an arbitrary end-point mark. After the addition of the sample, titration is carried out to, or to below, this mark. In amperometric titration, the final end-point would usually be found graphically. Applied as described under "Procedure," this method gave uniformly satisfactory results in the desired range of iron^{III} ion concentrations.

Method

Apparatus—

Conventional apparatus for amperometric titration at a rotating platinum electrode is suitable.[7] Exclude strong light from the titration cell. Use a low-resistance potassium nitrate - agar salt bridge to join the cell to the saturated calomel electrode. Store and precondition the platinum electrode as described by Kolthoff and Tanaka.[8]

The platinum electrodes used in the present work were rotated at 600 r.p.m., and had sensitivities of 170 and 175 μA, respectively, per millimole of iron^{III} per litre. These results were obtained at a potential of +0·10 volt in de-oxygenated acid thiocyanate medium at room temperature in the range 24° to 27° C. Most of the work was done with the more sensitive electrode. Voltammetric measurements were made with a Leeds and Northrup type E Electrochemograph, which was operated manually. Mercury^{I} and iron^{III} perchlorate solutions were delivered from syringe-type microburettes.

Reagents—

Use analytical-grade reagents and distilled or de-mineralised water throughout.

Mercury^{I} perchlorate, 0·1 N *in* N *perchloric acid*—Prepare as described by Berka, Vulterin and Zýka,[1] and standardise it by the procedure of McCurdy and Guilbault.[5]

Iron^{III} perchlorate, 0·1 N—Prepare from 97 g of ferric alum, 280 ml of perchloric acid, sp.gr. 1·70, and water to a total volume of 2 litres. (Since this solution was also used for checking the procedure, it was standardised as described by Chalmers.[6])

Perchloric acid, approximately 0·2 N.

Potassium thiocyanate, approximately 0·2 N.

Procedure—

Set the polariser so that a fixed potential of +0·10 volt can be applied to the rotating platinum electrode, but do not close the circuit. Transfer 50 ml of each of 0·2 N perchloric acid and 0·2 N potassium thiocyanate to the titration cell and de-oxygenate with a stream of nitrogen. Maintain this stream, which should not flow over the rotating electrode, throughout the titration. Inject 0·1 N iron^{III} perchlorate so that the amount of iron^{III} introduced into the cell is about 30 per cent. of that contained in the sample solution. After 5 minutes, close the circuit and wait (about 1 minute) until the current remains constant for 10 seconds. Note this current reading, x, at once open the circuit, inject the sample, and leave for 5 minutes. Read the current by temporary closure of the circuit as before, and repeat after adding an amount of 0·1 N mercury^{I} perchlorate equivalent to about one-fifth of the expected titre. Allow 2 minutes between the addition of the titrant and the closure of the circuit. Make similar titrant additions and current readings until the current has fallen to about one half of x. Graphically or otherwise, find the volume of titrant required to restore the current to the level x.

Results and discussion

Sets of ten replicate titrations of iron^{III} ion, at an approximate concentration of 10^{-4} N, were run in 100-ml portions of de-oxygenated acid thiocyanate medium. The observations in a given titration were used for obtaining graphical end-points by four different methods. These end-points were taken as the respective intersections of the descending arm of the titration curve with the excess-titrant line, D, the zero-current line C, the residual-current line B, and the arbitrary-current line A, as indicated in Fig. 2. The mean results of each set

and the corresponding standard deviations are given in Table I. The results for methods D, B and C are of course corrected for the pre-addition of iron^{III} needed for operating

TABLE I

EFFECT OF END-POINT PROCEDURE, POTENTIAL AND COMPOSITION OF THE MEDIUM ON THE TITRE OF 10·46 MICRO-EQUIVALENT AMOUNTS OF IRON^{III}

Potential, V	Thiocyanate concentration, N	Acid concentration, N	Titre, ml of 0·0936 N mercury^{I} perchlorate,* by— method A	method B	method C	method D
+0·10	0·1	0·1	0·112 ± 0·001	0·112 ± 0·002	0·114 ± 0·002	0·116 ± 0·002
+0·10	0·1	0·05	0·112 ± 0·001	0·114 ± 0·001	0·115 ± 0·001	0·117 ± 0·001
+0·10†	0·05	0·1	0·112 ± 0·002	0·112 ± 0·001	0·114 ± 0·001	0·115 ± 0·001
+0·10	0·05	0·05	0·111 ± 0·002	0·114 ± 0·003	0·114 ± 0·002	0·116 ± 0·002
0·00‡	0·1	0·1	0·112 ± 0·002	0·113 ± 0·002	0·116 ± 0·002	0·116 ± 0·002
+0·15‡	0·1	0·1	0·109 ± 0·002	0·109 ± 0·003	0·111 ± 0·003	0·114 ± 0·003

* Calculated titre, 0·1118 ml. † Nine experiments. ‡ Five experiments.

method A. It is obvious that neither the chosen operating potential nor the composition of the medium is critical. A similar set of titrations of iron^{III} at a concentration of approximately 10^{-5} N gave the following results (in ml of 0·0936 N mercury^{I} perchlorate; calculated titre, 0·01118 ml): A, 0·0113 ± 0·0006; B, 0·0121 ± 0·0009; C, 0·0133 ± 0·0009; D, no end-point. End-point location by methods B, C and D is clearly unsuitable in this instance.

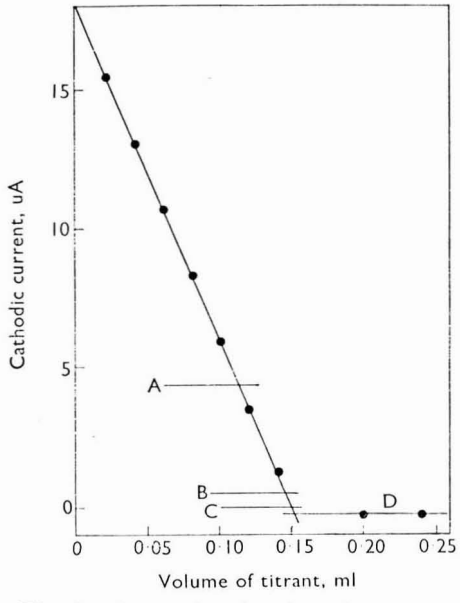

Fig. 2. Curves for titration of 10·46 micro-equivalents of iron^{III} with 0·0936 N mercury^{I} perchlorate after arbitrary addition of 3·20 micro-equivalents of iron^{III}. Curve A, arbitrary current; curve B, residual current; curve C, zero current; current D, excess of titrant

The absence of a true limiting-current plateau causes the descending arm of the titration curve to depart from linearity when the total resistance in the circuit is more than a few hundred ohms. In method A, interpolation between two reasonably close points is used for finding the end-point. Unless quite marked, the departure from linearity has only a small effect upon the accuracy of the titration.

Complexation in 0·1 M thiocyanate lowers the potentials of both the iron^{III} - iron^{II} and the mercury^{II} - mercury^{I} couples. Use of the formation constants of the various iron^{III} - thiocyanate complexes[9] shows that the potential of the iron couple will decrease by less than 0·2 volt. Since the equilibrium constant of the reaction—

$$Hg(SCN)_4^{2-} \rightleftharpoons Hg^{2+} + 4\,SCN^- \quad .. \quad .. \quad .. \quad .. \quad (1)$$

is only 5×10^{-20},[10] the potential of the mercury couple falls by about 1 volt and becomes much more negative than the potential of the iron couple.

Provided that the kinetics are favourable, the titration reaction should be analytically complete. Since the solubility product of mercury^{I} thiocyanate is only 3×10^{-20},[10] any excess of mercury^{I} might be expected to precipitate. The appearance of mercury^{I} thiocyanate is, however, subject to the dismutation—

$$Hg_2(SCN)_2 + 2\,SCN^- \rightleftharpoons Hg + Hg(SCN)_4^{2-} \quad .. \quad .. \quad .. \quad (2)$$

When the solution is saturated with mercury^{I} thiocyanate, the equilibrium constant $K_2 = \dfrac{[Hg(SCN)_4^{2-}]}{[SCN^-]^2}$. From Latimer's[10] values for the standard potentials of the mercury^{II} - mercury^{I} and the mercury^{II} - mercury couples, the equilibrium constant of reaction (1) and the solubility product of mercury^{I} thiocyanate, K_2 is found to be $3{\cdot}5 \times 10^{-3}$. To satisfy this relationship when the thiocyanate ion concentration is 0·1 N, the minimum concentration of mercurithiocyanate ion is about 4×10^{-5} N. The precipitation of mercury^{I} thiocyanate should therefore not occur in the titration of iron^{III} at a concentration of 10^{-5} N, but is just possible when this concentration is 10^{-4} N. Presumably, any tendency to precipitate would be masked by supersaturation phenomena.

An equilibrium that is probably important in the post end-point region of the titration is—

$$Hg_2^{2+} + 4\,SCN^- \rightleftharpoons Hg(SCN)_4^{2-} + Hg \quad .. \quad .. \quad .. \quad (3)$$

Latimer's[10] values for the standard potentials of the mercury^{II} - mercury^{I} and the mercury^{II} - mercury couples and for the equilibrium constant of reaction (1) give a value of 1×10^{17} for K_3, the equilibrium constant of reaction (3). For $[SCN^-] = 0{\cdot}1$ and $[Hg(SCN)_4^{2-}] = 0{\cdot}0001$, the forward reaction of equation (3) should be analytically complete.

The small anodic current indicated by line D in Fig. 2 may be caused by oxidation of (*i*) metallic mercury formed in reaction (3), (*ii*) excess of mercury^{I} ion or (*iii*) mercury^{I} thiocyanate. Reaction schemes and the calculated hydrogen-scale standard potentials are respectively—

$$Hg(SCN)_4^{2-} + 2e \rightleftharpoons Hg + 4\,SCN^- \; (E_4^0 = +0{\cdot}284\ V) \quad .. \quad .. \quad (4)$$

$$2Hg(SCN_4^{2-} + 2e \rightleftharpoons Hg_2^{2+} + 8\,SCN^- \; (E_5^0 = -0{\cdot}221\ V) \quad .. \quad .. \quad (5)$$

$$2Hg(SCN)_4^{2-} + 2e \rightleftharpoons Hg_2(SCN)_2 + 6\,SCN^- \; (E_6^0 = +0{\cdot}356\ V) \quad .. \quad .. \quad (6)$$

For $[SCN^-] = 0{\cdot}1$ and $[Hg(SCN)_4^{2-}] = 0{\cdot}0001$ in a solution that is saturated with mercury^{I} thiocyanate, the hydrogen-scale equilibrium potentials for systems (4), (5) and (6) will be +0·284 V, +0·297 V and +0·297 V, respectively, or approximately +0·05 volt with respect to the saturated calomel electrode. Since the potential of the indicator electrode is more positive than this, all of the oxidation mechanisms are possible. Factors such as electrode motion[11] or reaction kinetics may be deciding ones.

According to these calculations, titration at a potential of zero should cause the anodic current to disappear and give accurate results when the end-point is located by method D. In fact, the results thus obtained are still high (see Table I). Because of (*a*) the numerous species that may exist in a solution containing iron, mercury and thiocyanate, and (*b*) the ignoring of activity and any pH effects due to the supporting electrolyte, the anomaly is not surprising.

This work was carried out with the partial support of the United States Atomic Energy Commission. The authors also thank Mr. R. A. Bailey for carrying out much of the experimental work, and for his assistance in finalising the recommended procedure.

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Received *September 11th*, 1964

Methylthymol Blue as a Reagent for the Spectrophotometric Determination of Magnesium

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A procedure is described for the colorimetric determination of magnesium with methylthymol blue. The complex is formed in an alcohol - water medium buffered to pH 10·8 with ammonia - ammonium chloride solution. The interference of several cations has been examined.

Most spectrophotometric methods for determining magnesium are based on the formation of coloured "lakes." These "lakes" have well known disadvantages, and a recent paper[1] serves to illustrate the necessity of standardised conditions for formation and measurement. However, soluble coloured magnesium complexes are formed by Eriochrome black T,[2] bis-salicylidenediaminoethane[3] and two similar reagents, Magon[4] and Magon sulphonate.[5,6] Methylthymol blue (thymolsulphonphthalein-3,3′-bismethyliminodiacetic acid), first described by Körbl and Přibil[7] as a versatile indicator for use in the complexometric titration of many metals, has been used as a reagent for the spectrophotometric determination of zirconium and hafnium,[8] and was considered to be suitable for the determination of magnesium.

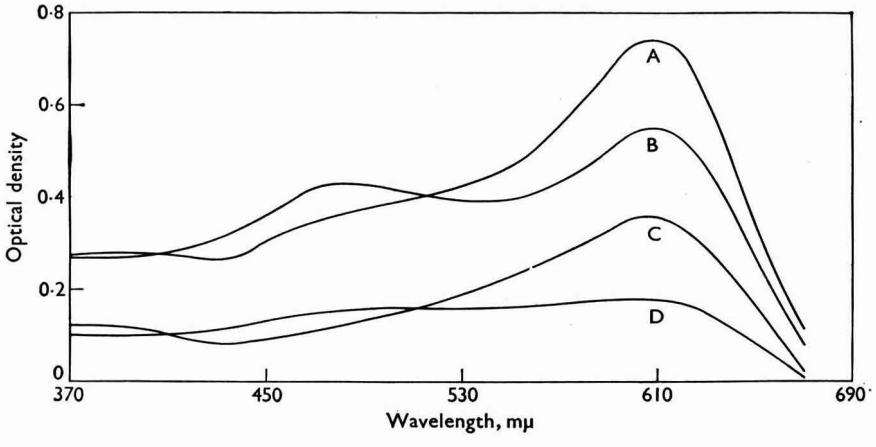

Fig. 1. Absorption spectra for the methylthymol blue - magnesium complex, curves A and C, and for methylthymol blue, curves B and D. Curves A and B, compounds in alcohol - water (50 + 50) mixture; curves C and D, compounds in alcohol - water (85 + 15) mixture

Experimental

Wavelength and optical density—

The variation in optical density with wavlength of both methylthymol blue and the magnesium complex was examined in a medium buffered with ammonia - ammonium chloride solution and the results are shown in Fig. 1. The magnesium complex shows maximum absorption at 610 mμ, and this wavelength was chosen for subsequent measurements.

* Present address: British Acheson Electrodes Limited, Grange Mill Lane, Wincobank, Sheffield.

pH AND COLOUR FORMATION—

Fig. 2 shows that the maximum colour is formed over the range pH 10·4 to 11·3, and the absorption of the reagent itself follows that of the complex. Ammonia - ammonium chloride solution (pH 10·8) was therefore used as a suitable buffer solution to give optimum pH conditions for complex formation.

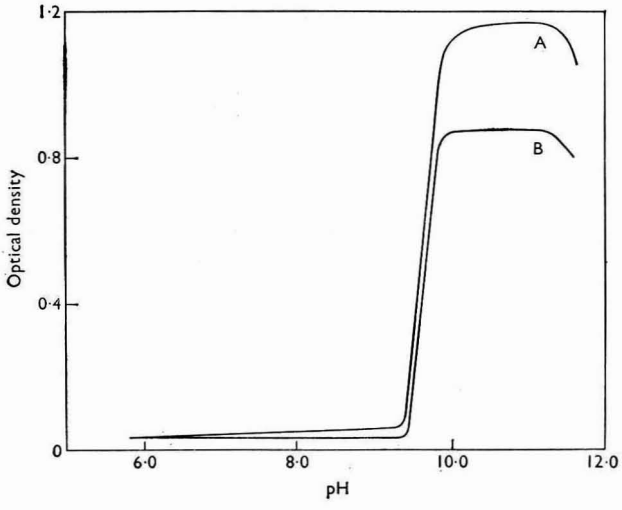

Fig. 2. Curves showing relationship between optical density and pH. Curve A, methylthymol blue - magnesium complex; curve B, methylthymol blue

SENSITIVITY OF THE REAGENT—

The sensitivity of the reaction between methylthymol blue and magnesium, calculated by using Sandell's definition[9] is 0·0016 μg per sq. cm. This corresponds to a molar extinction coefficient, ϵ, of 15,200. These figures may be compared with the sensitivities of Titan yellow (0·017 μg per sq. cm; $\epsilon = 1430$), Eriochrome black T (0·0011 μg per sq. cm; $\epsilon = 22{,}100$) Magon sulphonate (0·0008 μg per sq. cm; $\epsilon = 30{,}400$) and bis-salicylidenediaminoethane (0·0018 μg per sq. cm; $\epsilon = 13{,}500$).

STABILITY OF THE COLOUR—

The complex was formed both in 85 per cent. alcoholic and 10 per cent. alcoholic solutions, and the optical density was measured against a corresponding blank solution over a period of 4 hours. No change in net optical density was recorded, but there was a slight decrease in the optical density of both the blank solution and the magnesium complex when measured against alcohol over this period. It was found that the reagent solution was slightly unstable, and as a precautionary measure, it was freshly prepared each day.

The optical density of the reagent appears to be increased by the presence of increasing amounts of water, but the sensitivity of the reaction with magnesium remains unchanged.

BEER'S LAW AND REAGENT CONCENTRATION—

Calibration results showed that the Beer - Lambert law was obeyed up to about 100 μg of magnesium when 5 ml of 0·1 per cent. w/v reagent solution was used. However, when measurements were made in the 1 + 1 alcohol - water medium, it was found that 50 per cent. more methylthymol blue had to be used to ensure linearity.

In both instances $\epsilon_{610} = 15{,}200$.

STRUCTURE OF THE COMPLEX—

By using the mole-ratio method,[10] the results in Fig. 3 were obtained, and these suggested that in ammonia - ammonium chloride buffered solution, a 1 + 1 metal - reagent complex is formed between magnesium and methylthymol blue.

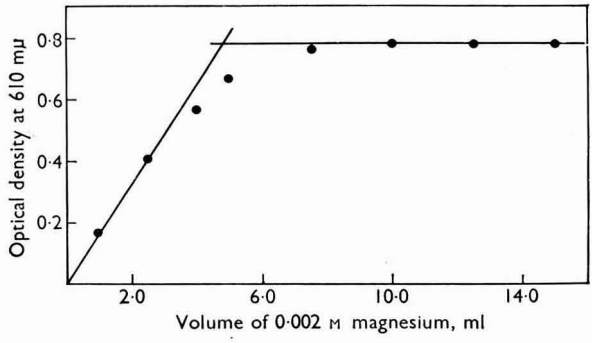

Fig. 3. Empirical formula of magnesium - methylthymol blue complex obtained by the mole-ratio method. The ratio of magnesium to methylthymol blue was 1 to 1 and 5·0 ml of 0·00195 M methylthymol blue was used

INTERFERENCES—

Methylthymol blue can be used as an indicator for the complexometric titration of several metals in ammonia - ammonium chloride buffered solutions, and therefore these metals will interfere in the determination of magnesium. The possibility of using this absorptiometric method for determining magnesium in cast iron was envisaged, and the most likely interfering metals, with the exception of manganese, were complexed with potassium cyanide. Some preliminary work showed that with cast iron, after removal of iron by extraction with isobutyl acetate, manganese interference may be overcome by precipitation as manganese dioxide after oxidation with ammonium persulphate.

TABLE I

RECOVERY OF MAGNESIUM IN THE PRESENCE OF IRON, NICKEL, COBALT, CADMIUM, ZINC AND COPPER

Magnesium added, μg	Iron added,* μg	Nickel added, μg	Cobalt added, μg	Cadmium added, μg	Zinc added, μg	Copper added, μg	Magnesium found, μg
44·0	1000	—	—	—	—	—	44·8
44·0	2500	—	—	—	—	—	46·5
44·0	—	1000	—	—	—	—	43·9
44·0	—	5000	—	—	—	—	43·8
44·0	—	—	1000	—	—	—	44·0
44·0	—	—	5000	—	—	—	61·6
44·0	—	—	—	500	—	—	45·0
44·0	—	—	—	1000	—	—	50·4
44·0	—	—	—	—	1000	—	44·5
44·0	—	—	—	—	5000	—	48·5
44·0	—	—	—	—	—	1000	44·5
44·0	—	—	—	—	—	5000	44·5
52·6	500	500	500	500	600	500	52·5

* Reduced with ascorbic acid.

The recovery of magnesium in the presence of several metals that were complexed with potassium cyanide is shown in Table I. With the exception of cadmium, 1 mg of foreign ions does not cause significant interference. The interference encountered with the larger excess of foreign ions can probably be overcome by increasing the amount of potassium cyanide used.

Method

Reagents—

Reagents should be of analytical-reagent grade whenever possible.

Methylated spirits—68° over proof, industrial grade; filter before use.

Methylthymol blue solution, 0·15 per cent. w/v—Dissolve 0·15 g of methythymol blue (obtainable from Hopkin & Williams Ltd.) in 5 ml of water and dilute the solution to 100 ml with methylated spirits. Prepare the solution freshly each day.

Buffer solution (pH 10·8)—Dissolve 17·5 g of ammonium chloride in 80 ml of water, add 142 ml of ammonia solution, sp.gr. 0·88, and dilute the solution to 250 ml with water.

Ascorbic acid.

Potassium cyanide, 0·1 M—Dissolve 0·65 g of potassium cyanide in 100 ml of water. Prepare the solution freshly each week.

Procedure—

Dilute to 10 ml the slightly acidic sample solution containing magnesium (add 0·1 g of ascorbic acid if any iron is present). Add 5·0 ml of potassium cyanide solution and mix; add 5·0 ml of buffer solution and mix; and add 5·0 ml of methylthymol blue solution and mix. Add 50 ml of methylated spirits and dilute the solution to 100 ml with water. Measure the optical density against the blank solution in a 1-cm cell at 610 mμ.

I thank the Chief Chemist, Ferranti Ltd., for permission to publish this paper.

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Received *October 1st*, 1964

The Analysis of Technical Hexachlorocyclopentadiene by Infrared Spectrophotometric and Gas-Liquid Chromatographic Methods

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Infrared spectrophotometric and gas - liquid chromatographic methods were applied in the analysis of hexachlorocyclopentadiene containing about 30 per cent. of various perchloro compounds. Two solvents were used in the spectrophotometric method: (*i*) tetrachloromethane for determination of hexachlorocyclopentadiene (1140, 1242 and 1607 cm^{-1}) and octachlorocyclopentene (1189 cm^{-1}) and (*ii*) carbon disulphide for the determination of octachlorocyclopentene (753 cm^{-1}), tetrachloromethane (764 cm^{-1}), hexachloroethane (780 cm^{-1}), hexachlorobuta-1,3-diene (853 and 980 cm^{-1}), tetrachloroethylene (909 cm^{-1}) and hexachlorobenzene (1347 cm^{-1}). The concentrations of individual components were calculated by the method of successive approximations with an analytical matrix. High reproducibility of results was achieved by the use of several wavenumbers for the determination of main constituents of the mixture. In the gas - liquid chromatographic method, a polyethyleneglycol adipate (with 1 per cent. of orthophosphoric acid) column at 145° C and a flame ionisation detector were used. Precise evaluation of peak areas and suitable choice of internal standard (1,2,3,5-tetrachlorobenzene) resulted in approximately the same reproducibility as for the spectrophotometric method. The analyses of synthetic mixtures and of a series of samples showed good agreement between results of both methods if the samples did not contain more than 5 per cent. of unidentified compounds. The chromatographic method is preferable for analysing samples with more than 5 per cent. of unidentified compounds.

Hexachlorocyclopentadiene, an important intermediate in the diene synthesis of insecticides (heptachlor, chlordane, aldrin, dieldrin) is usually prepared either (*a*) by chlorination of cyclopentadiene to polychlorocyclopentanes and polychlorocyclopentenes and subsequent de-chlorination of the main intermediate, the octachlorocyclopentene, to hexachlorocyclopentadiene,[1,2] or (*b*) by chlorination of a mixture of C_5 paraffinic and olefinic hydrocarbons to polychloropentanes and polychloropentenes, and then cyclisation and de-chlorination of the resulting octachlorocyclopentene to hexachlorocyclopentadiene.[3,4,5]

The compositions of the reaction mixtures prepared by the two methods differ substantially. Whereas in method (*a*) only hexachlorocyclopentadiene and octachlorocyclopentene are formed, various fission reactions of the aliphatic hydrocarbon chain take place in method (*b*), resulting in a mixture of chlorinated hydrocarbons, namely, tetrachloromethane, hexachloroethane, tetrachloroethylene and hexachlorobuta-1,3-diene. Small amounts of hexachlorobenzene formed are supposed to be the result of a reaction of hexachlorocyclopentadiene with tetrachloromethane.[6]

In the study of the preparation of hexachlorocyclopentadiene by the above methods, an exact knowledge of the reaction-mixture composition was necessary. A simple infrared spectrophotometric method (Livař, unpublished work) provided reliable and accurate results for the reaction mixture from method (*a*), which did not contain more than 5 per cent. of unidentified compounds. A considerably more complicated method had to be used for the multicomponent mixture from method (*b*), which contained, according to a qualitative infrared analysis, all the compounds expected theoretically. In view of the possible effect on spectrophotometric results of the presence of compounds that could not be detected by their absorption peaks, we used an independent gas - liquid chromatographic method. Besides confirming the analytical results we were able to compare the reproducibility and precision of the methods used.

INFRARED SPECTROPHOTOMETRIC METHOD

Selection of analytical wavenumbers

The spectra of the main constituents of the technical hexachlorocyclopentadiene recorded under conditions of the analysis are shown in Fig. 1. Table I contains data on the selected analytical wavenumbers, cell thicknesses, solvents, concentration ranges and sample concentrations used. The selection of the analytical wavenumbers and of the solvent was influenced by the fact that the solutions of hexachlorocyclopentadiene in carbon disulphide are not spectrophotometrically stable. We had found that the optical densities of the 1140- and 1232-cm^{-1} bands decreased slightly, and we could not even obtain perfectly reproducible

Table I

Experimental conditions for the analysis of hexachlorocyclopentadiene

No.	Component	Wave number, cm^{-1}	Cell thickness, mm	Solvent	Concentration range, mg per 10 ml	Sample concentration, mg per 10 ml
1	Hexachlorocyclopentadiene ..	1140	0·161	CCl_4	100 to 260	300
		1232	0·161	CCl_4	100 to 260	300
		1607	0·161	CCl_4	100 to 260	300
2	Octachlorocyclopentene	1189	0·161	CCl_4	0 to 150	300
		753	0·604	CS_2	0 to 30	150
3	Tetrachloromethane	764	0·604	CS_2	0 to 30	150
4	Hexachloroethane	780	0·604	CS_2	0 to 20	150
5	Hexachlorobuta-1,3-diene ..	853	0·604	CS_2	0 to 60	150
		980	0·604	CS_2	0 to 60	150
6	Tetrachloroethylene	909	0·604	CS_2	0 to 30	150
7	Hexachlorobenzene	1347	0·604	CS_2	0 to 30	150

results with freshly prepared solutions. Not being able to explain this observation we were forced to use another solvent in the determination of hexachlorocyclopentadiene. The most suitable was tetrachloromethane, which permitted the use of the 1140-, 1232- and 1607-cm^{-1} bands. Carbon disulphide, which is transparent in the 700- to 800-cm^{-1} region, had to be used as the solvent in the determination of tetrachloromethane and hexachloroethane, two structurally related compounds for which no bands outside this range were available. The overlapping of their bands at 764, 780 and 785 cm^{-1} was the most difficult problem of the spectrophotometric method. We performed the calibration with solutions of standard compounds at 764 cm^{-1} for the determination of tetrachloromethane and at 780 cm^{-1} for hexachloroethane. Having recorded the spectra of binary mixtures containing the two compounds in various proportions, we found only negligible differences between the observed and the calculated values of the optical density at the two wavenumbers. The specific optical densities measured in the spectra of standards could therefore be applied without correction. The interferences at the hexachlorocyclopentadiene 1607-cm^{-1} band and the octachlorocyclopentene 1189- and 753-cm^{-1} bands were evaluated similarly.

Method

Apparatus—

A Zeiss UR-10 double-beam infrared spectrophotometer was used with sodium chloride prism and optical-density cells. The transmission scale was calibrated by means of a rotating sector. The instrument settings were as under—

Slit selector: 4
Recording speed: 12 cm^{-1} per minute
Servo response time: 50 seconds (full-scale deflection)
Chart: 50 mm per 100 cm^{-1}
Amplifier setting: 6
Band-width selector: 2
Time-constant selector: 2
Spectral slit width: 3·0 cm^{-1} at 750 to 900 cm^{-1}
4·0 cm^{-1} at 1000 cm^{-1}
5·0 cm^{-1} at 1300 cm^{-1}

Reagents—

Carbon disulphide—Analytical-reagent grade, without special purification.
Tetrachloromethane—Analytical-reagent grade, without special purification.
Pure samples of individual components—Test the purity by gas - liquid chromatography.

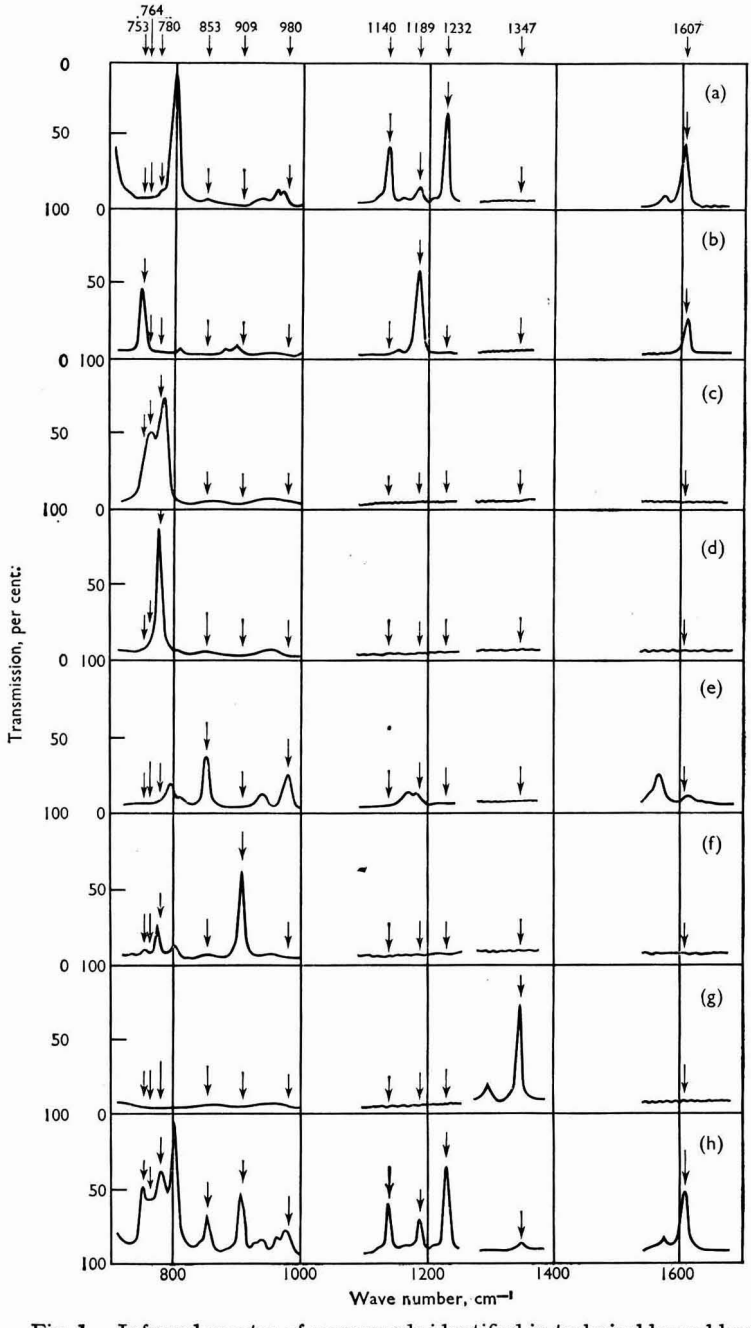

Fig. 1. Infrared spectra of compounds identified in technical hexachlorocyclopentadiene. Solvents and cell thicknesses were as given in Table I. Set (*a*), hexachlorcyclopentadiene, 100 mg per 10 ml of carbon disulphide and 200 mg per 10 ml of tetrachloromethane; set (*b*), octachlorocyclopentene, 20 mg per 10 ml of carbon disulphide and 200 mg per 10 ml of tetrachloromethane; set (*c*), tetrachloromethane; set (*d*), hexachloroethane; set (*e*), hexachlorobuta-1,3-diene; set (*f*), tetrachloroethylene; set (*g*), hexachlorobenzene: concentrations of compounds in solvents for sets (*c*), (*d*), (*e*), (*f*) and (*g*) as for set (*b*); set (*h*), technical hexachlorocyclopentadiene, 150 mg per 10 ml of carbon disulphide and 300 mg per 10 ml of tetrachloromethane

PROCEDURE—

Prepare the solutions of the sample and record their spectra under conditions given in Table I by using reference cells of the same thickness containing the pure solvent. Record also the spectra of pure solvents in the respective pairs of cells and the spectrum of a calcium fluoride filter as a correction for stray radiation. Measure the percentage transmission at all analytical wavenumbers in the spectra of the sample, T, of the solvent, T_0 and of the filter, T_f, and calculate the optical density, A, from the equation—

$$A = \log_{10}\left(\frac{T_0 - T_f}{T - T_f}\right)$$

CALIBRATION—

Record the spectra of solutions of standard compounds in the concentration ranges given in Table I. Evaluate the optical densities at all analytical wavenumbers and calculate the optical densities corresponding to the conveniently chosen 1 per cent. w/v concentration by using the least-squares method. Beer's law is valid for all wavenumbers except 1232 and 1607 cm^{-1}, for which a correction graph must be applied. Check the additivity of optical densities of the overlapping bands at 753, 764, 780, 1189 and 1607 cm^{-1} by using binary mixtures of the respective compounds. Arrange the specific optical-density values into an analytical matrix (see Table II).

TABLE II

ANALYTICAL MATRIX

Experimental conditions used were those given in Table I

Component number	Optical density, $\times 10^3$, of 1 per cent. w/v solution at wavenumber—										
	1140	1232	1607	1189	753	764	780	853	909	980	1347
1	**113**	**237**	**134**	31	15	17	36	5	4	15	3
2	4	—	37	**198**	**1356**	121	13	—	48	—	—
3	—	—	—	—	691	**1557**	1946	6	20	30	3
4	2	—	—	—	25	210	**4470**	—	—	—	—
5	3	—	8	12	9	21	84	**855**	11	**551**	—
6	3	—	—	—	71	41	287	—	**2190**	—	—
7	1	—	—	—	4	2	—	—	4	2	**2430**

Key to components

1. Hexachlorocyclopentadiene.
2. Octachlorocyclopentene.
3. Tetrachloromethane.
4. Hexachloroethane.
5. Hexachlorobuta-1,3-diene.
6. Tetrachloroethylene.
7. Hexachlorobenzene.

CALCULATION—

The method of successive approximations can be used in the calculation of concentrations. Calculate first the concentration of hexachlorocyclopentadiene from the optical density of the sample solution in tetrachloromethane measured at 1232 cm^{-1} by using the analytical matrix. The value obtained is not affected by the presence of the other components. Then compute the concentrations of components numbered 2 to 7 by a step-by-step procedure from the optical densities of the sample solution in carbon disulphide. Take into account the presence of hexachlorocyclopentadiene in constant concentration evaluated in the first step. Divide this value by the ratio of the concentrations of the sample in tetrachloromethane and carbon disulphide; this is approximately $300/150 = 2$. In the second step evaluate the concentration of octachlorocyclopentene from the optical density at 753 cm^{-1}, considering only the presence of hexachlorocyclopentadiene. The concentrations of hexachlorocyclopentadiene and octachlorocyclopentene are then taken into account in the calculation of tetrachloromethane concentration (at 764 cm^{-1}), etc. For hexachlorobutadiene use an average of the concentration values at 853 and 980 cm^{-1} in the further calculation. Obtain the second and third approximation values in a similar way by using the same value of hexachlorocyclopentadiene concentration as in the first approximation. Three approximations are sufficient for the determination of components numbered 2 to 7.

Multiply the computed concentrations by the ratio of sample concentrations (tetrachloromethane to carbon disulphide) and use these values for calculating the hexachlorocyclopentadiene concentration at 1140 and 1607 cm^{-1}. Calculate the octachlorocyclopentene concentration at 1190 cm^{-1} and finally evaluate the average values of the hexachlorocyclopentadiene concentration at 1140, 1232 and 1607 cm^{-1} and of the octachlorocyclopentene concentration at 1190 and 753 cm^{-1}. The individual values do not differ more than by ± 1 per cent. from the average values. In this way great reliability in the determination of the two main constituents of technical hexachlorocyclopentadiene is achieved.

ANALYSIS OF SYNTHETIC MIXTURES—

In order to check the analytical matrix concerning the determination of components numbered 2 to 7 in carbon disulphide solution, six synthetic mixtures were prepared, containing the analysed compounds in various proportions. In the preparation of each mixture, the individual standard compounds were weighed directly into a 10-ml calibrated flask in order of increasing volatility, to minimise the evaporation losses, and the solution was immediately diluted to the mark with carbon disulphide. The results of the analysis of these mixtures are summarised in Table II. The hexachlorocyclopentadiene concentration

TABLE III

ANALYSIS OF SYNTHETIC MIXTURES

Component		Mixture number 1	2	3	4	5	6
Hexachlorocyclopentadiene	taken, mg	60·1	70·0	80·0	90·0	100·1	110·2
	found, mg	Not determined					
Octachlorocyclopentene	taken, mg	30·2	25·3	20·6	15·4	10·4	5·1
	found, mg	29·8	25·5	22·0	15·9	10·3	5·1
Tetrachloromethane	taken, mg	10·0	15·0	20·0	15·0	10·0	5·0
	found, mg	9·7	14·5	19·2	14·3	9·9	4·9
Hexachloroethane	taken, mg	9·0	9·0	6·0	6·0	3·0	3·0
	found, mg	9·2	9·2	6·3	6·2	3·0	3·1
Hexachlorobuta-1,3-diene	taken, mg	20·0	10·0	10·0	20·0	20·0	20·0
	found, mg	21·1	10·2	9·9	20·4	20·5	20·6
Tetrachloroethylene	taken, mg	20·0	20·0	10·0	10·0	10·0	10·0
	found, mg	20·8	20·2	9·9	10·2	10·2	10·2
Hexachlorobenzene	taken, mg	4·0	4·1	4·0	0·0	0·0	0·0
	found, mg	4·1	4·1	4·1	0·0	0·0	0·0
Total	*taken, mg*	*153·3*	*153·4*	*150·6*	*156·4*	*153·5*	*153·3*
	found, mg	*154·8*	*153·7*	*151·4*	*157·0*	*154·0*	*154·1*

was not determined. Although systematic deviations were observed in some instances, the relative average deviation did not exceed the values usually obtained in the analyses of multicomponent mixtures. The total concentration found was in all instances larger than the actual value; nevertheless, it did not differ from it by more than 1·2 per cent.

GAS - LIQUID CHROMATOGRAPHIC METHOD

Gas - liquid chromatography has been previously used in the analysis of chlorinated methane, ethane and ethylene derivatives by Urone, Smith and Katnik.[7] Gas - liquid chromatographic determination of octachlorocyclopentene in a mixture of unidentified chlorinated derivatives of cyclopentadiene was recently described by Lechner.[8] The chromatographic treatment of high-boiling perchloro compounds presents some special difficulties. Some of the compounds studied may undergo thermal degradation on the metallic surface of the chromatographic column. This can be avoided by using a glass column and the lowest possible working temperature. There is also a lack of suitable substrates, poor resolution is usually obtained and "tailing" of chlorinated hydrocarbons on many polar substances takes place. In the example studied, the presence of compounds having a large difference between the minimum and maximum boiling-points required the use of a relatively efficient column. As not all components of the mixture have been evaluated quantitatively, the internal-standard method had to be used, providing the most accurate results.

Method

Apparatus—

A Fractovap Model C (Carlo Erba, Milan) gas chromatograph equipped with a flame ionization detector and a Speedomax G recorder (Leeds and Northrup) was used in establishing the method. A pair of interchangeable gears allowed us to reduce the chart speed in a 2 to 1 ratio as soon as the peak of the internal standard was obtained.

Chromatographic column—

Glass tubing, 1·6 m long and 4 mm i.d., packed with 5 per cent. (by weight) of polyethyleneglycol adipate *plus* 1 per cent. of orthophosphoric acid (85 per cent. aqueous solution) on acid washed 60- to 80-mesh Rysorb BLK (Research Institute of Organic Syntheses, Pardubice-Rybitví) by using an electric vibrator, was used as the chromatographic column. The packing was prepared by evaporating a diethyl ether - polyethyleneglycol adipate - orthophosphoric acid solution made into a slurry with the solid support in a rotary vacuum evaporator immersed in a constant-temperature bath. The packed column was conditioned by a carrier-gas flow at 150° C for 24 hours.

Operating conditions—

The operating conditions of the chromatograph were as under—

Column temperature: 145° C
Injection-block temperature: 190° C
Carrier gas: Nitrogen at flow-rate of 71 ml per minute
Sample volume: 0·2 to 0·4 μl

The column and injection-block temperatures were found to be critical for the best separation of the individual components in the shortest possible time.

Procedure—

Prepare a 10 + 1 mixture of the sample with the internal standard (1,2,3,5-tetrachlorobenzene) by using a microflask closed by a silicone-rubber stopper to avoid evaporation losses. Mix thoroughly, and inject 0·2 to 0·4 μl of the mixture into the column by using a Hamilton microsyringe. The elution time under the conditions given above is 25 minutes. Hexachlorobenzene, because of its relatively high retention time, is not determined.

Results

Qualitative—

A record of a typical hexachlorocyclopentadiene sample is shown in Fig. 2. Different sensitivities were used during the recording of various peaks. The individual peaks were identified by adding standard compounds to the sample. The relative retention times of identified compounds are given in Table IV together with their boiling-points. It is obvious

Table IV

Corrected relative retention times for compounds identified in technical hexachlorocyclopentadiene

Compound	Boiling-point, °C	Corrected relative retention time
Tetrachloromethane	76·7	0·035
Tetrachloroethylene	121·2	0·065
Hexachloroethane	184·4	0·34
Hexachlorobuta-1,3-diene	214	0·46
Hexachlorocyclopentadiene	236	1·00
1,2,3,5-tetrachlorobenzene*	246	1·47
Octachlorocyclopentene	283	4·93

* Internal standard.

from Fig. 2 that the main constituents of technical hexachlorocyclopentadiene are compounds presumed present on theoretical grounds and identified by infrared spectrophotometry. The content of minor unidentified compounds whose peaks are found in Fig. 2 (altogether 10 peaks) does not exceed 8 per cent. according to a quantitative gas - liquid chromatographic analysis. A very important part of the qualitative gas-chromatographic study was the checking of purity of compounds used as standards in the quantitative infrared and gas - liquid chromatographic analysis.

QUANTITATIVE ANALYSIS—

The internal-standard method, which provides most accurate quantitative results, was used in the evaluation of concentrations. The symmetry of elution peaks allowed the measuring of peak areas by multiplying the peak height by the peak width at half the peak height. A previously described[9] simple dial indicator was used for measuring the peak width. Area factors relative to 1,2,3,5-tetrachlorobenzene were determined by analysing five synthetic mixtures with compositions corresponding to the composition of technical samples.

DISCUSSION OF RESULTS

The reproducibility of each method was tested by quintuplicate analyses of a technical hexachlorocyclopentadiene sample. Two samples of similar composition were used in this test. The results are summarised in Table V. The comparison of standard deviations shows the equivalency of both methods. Relatively high reproducibility of the hexachlorocyclopentadiene and octachlorocyclopentene determination by the infrared spectrophotometric method is achieved by using several wavenumbers in the determination of these components. It is obvious from Table V that the reproducibility of the gas - liquid chromatographic method is limited by the accuracy of the measurement of the internal-standard peak area. A comparison of both methods, made by analysing a synthetic mixture, yielded satisfactory results (see Table VI).

Parallel analyses of a series of hexachlorocyclopentadiene samples were carried out (see Table VI). A detailed study of the results seems to indicate a relation between the

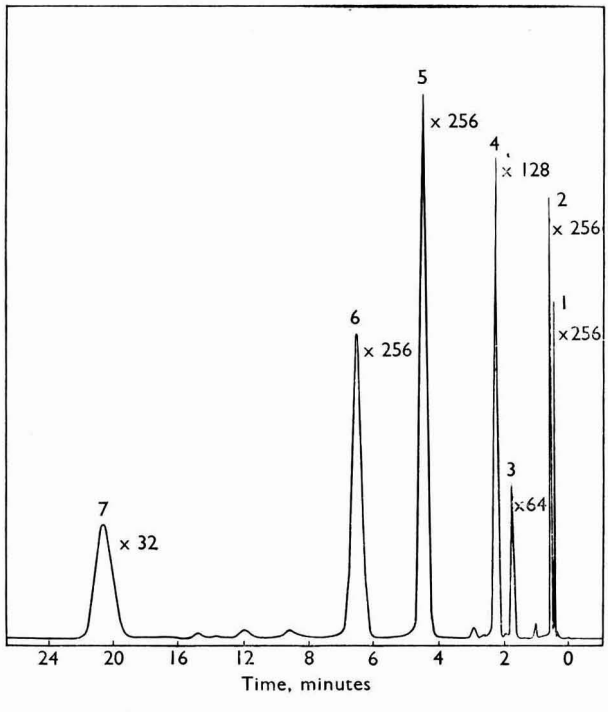

Fig. 2. Chromatogram of technical hexachlorocyclopentadiene with internal standard added. Peak 1, tetrachloromethane; peak 2, tetrachloroethylene; peak 3, hexachloroethane; peak 4, hexachlorobuta-1,3-diene; peak 5, hexachlorocyclopentadiene; peak 6, 1,2,3,5-tetrachlorobenzene (internal standard); peak 7, octachlorocyclopentene. A column of 5 per cent. of polyethyleneglycol adipate *plus* 1 per cent. of orthophosphoric acid was used at a temperature of 145°C. Note the change on time scale above 8 minutes.

TABLE V

REPRODUCIBILITY OF THE SPECTROPHOTOMETRIC AND CHROMATOGRAPHIC METHODS

Found, per cent. by weight, by infrared spectrophotometry, sample B

Component	Determination number 1	2	3	4	5	Average	Standard deviation
Hexachlorocyclopentadiene ..	58·2	57·2	57·0	57·1	57·5	57·4	0·48
Octachlorocyclopentene ..	10·0	10·0	10·0	10·0	9·9	10·0	0·04
Tetrachloromethane	7·7	7·6	7·8	7·5	7·5	7·6	0·13
Hexachloroethane	1·5	1·7	1·5	1·6	1·6	1·6	0·10
Hexachlorobuta-1,3-diene ..	10·2	11·0	11·0	10·7	10·7	10·7	0·33
Tetrachloroethylene	7·7	8·0	8·2	7·9	8·1	8·0	0·19
Hexachlorobenzene	0·7	0·7	0·7	0·7	0·7	0·7	0·00
Total ..	*96·0*	*96·2*	*96·2*	*95·5*	*96·1*	*96·0*	*0·29*

Found, per cent. by weight, by gas - liquid chromatography, sample K

Component	Determination number 1	2	3	4	5	Average	Standard deviation
Hexachlorocyclopentadiene ..	55·9	56·0	56·2	56·8	56·4	56·3	0·36
Octachlorocyclopentene ..	8·0	8·5	8·5	8·7	8·1	8·4	0·30
Tetrachloromethane	5·5	5·9	5·7	6·0	5·7	5·8	0·20
Hexachloroethane	2·7	2·8	2·7	2·6	2·6	2·7	0·08
Hexachlorobuta-1,3-diene ..	10·3	10·5	10·7	10·8	10·3	10·5	0·23
Tetrachloroethylene	8·0	8·1	8·2	8·3	8·1	8·1	0·11
Hexachlorobenzene				Not determined			
Total ..	*90·4*	*91·8*	*92·0*	*93·2*	*91·2*	*91·7*	*1·13*

TABLE VI

COMPARATIVE TEST OF THE INFRARED SPECTROPHOTOMETRIC AND GAS - LIQUID CHROMATOGRAPHIC METHODS

Sample	Method	Found, per cent., by weight— Components 1	2	3	4	5	6	7	Total of components 1 to 6	1 to 7
Synthetic mixture	*Actual composition*	*61·6*	*7·0*	*10·5*	*3·0*	*10·0*	*7·2*	*0·7*	*99·3*	*100·0*
	Infrared	61·1	7·1	9·9	3·1	10·5	7·4	0·7	99·1	99·8
	GLC	61·3	6·8	11·0	3·1	10·4	7·1	—	99·7	—
A	Infrared	56·9	9·4	8·5	1·3	9·3	7·3	0·7	92·7	93·4
	GLC	55·7	8·3	7·4	3·2	10·1	7·2	—	91·9	—
B	Infrared	58·2	10·0	7·7	1·5	10·2	7·7	0·7	95·3	96·0
	GLC	57·3	9·4	7·2	2·4	10·4	7·1	—	93·8	—
C	Infrared	58·0	11·4	7·3	1·6	10·2	8·7	1·0	97·2	98·2
	GLC	57·1	10·1	7·5	2·1	9·4	8·4	—	94·6	—
D	Infrared	65·7	1·9	4·7	0·1	11·7	10·7	1·8	94·8	96·6
	GLC	65·8	1·8	4·7	0·2	11·7	11·0	—	95·2	—
E	Infrared	59·6	10·1	5·7	1·5	9·5	8·5	1·5	94·9	96·4
	GLC	59·7	10·1	6·4	1·5	9·5	8·5	—	95·7	—
F	Infrared	58·3	10·8	5·6	2·8	10·0	8·5	1·5	96·0	97·5
	GLC	58·6	11·3	6·1	2·7	9·8	7·9	—	96·4	—
G	Infrared	61·7	24·1	2·5	0·9	5·0	3·1	0·7	97·3	98·0
	GLC	60·3	25·0	2·3	0·9	4·9	2·6	—	96·0	—
H	Infrared	58·7	23·0	3·6	1·3	5·8	5·1	0·9	97·5	98·4
	GLC	58·6	23·6	3·3	1·2	5·8	4·5	—	97·0	—
J	Infrared	60·7	20·4	4·1	1·1	6·7	5·3	0·9	98·3	99·2
	GLC	59·8	19·8	4·4	1·2	6·4	5·8	—	97·4	—

Key to components

1. Hexachlorocyclopentadiene.
2. Octachlorocyclopentene.
3. Tetrachloromethane.
4. Hexachloroethane.
5. Hexachlorobuta-1,3-diene.
6. Tetrachloroethylene.
7. Hexachlorobenzene.

content of undetermined compounds (determined by the gas - liquid chromatographic method after addition of the hexachlorobenzene content) and the difference of the results of both methods. The best agreement was found for samples D to J, which did not contain more than 4 per cent. of unidentified compounds. The presence of a background in the infrared spectra most probably explains the higher results of the spectrophotometric determination of hexachlorocyclopentadiene in samples A to C and also higher values for the tetrachloromethane content in samples A and B, which affected, in an opposite direction, the hexachloroethane determination.

From the results of the comparative test it is concluded that the infrared spectrophotometric and gas - liquid chromatographic methods may be used alternatively with approximately equal precision and reliability for the analysis of hexachlorocyclopentadiene samples with a low concentration of undetermined compounds. Gas - liquid chromatography is preferable in those instances in which this concentration exceeds 5 per cent. The determination of hexachlorobenzene by the gas - liquid chromatographic mehod would be possible by application of programmed-temperature chromatography.

We thank Mr. T. Magdolen for helpful discussions and for the samples of pure standard compounds, Mrs. V. Marčeková and Mrs. M. Morvayová for assistance in this work and Mrs. T. Kováčiková for translating the paper into English.

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Received *July 6th*, 1964

The Determination of Acetone in Air

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Activated silica gel is an efficient adsorption medium for sampling acetone vapour. For the determination, the acetone can be completely desorbed with N sodium hydroxide, but not with water. Complete recovery of acetone can be achieved at atmospheric concentrations up to 4000 mg per cu. metre.

Acetone vapour acts as a narcotic, and is relatively non-toxic (maximum permissible concentration in air is 1000 p.p.m. by volume, or 2370 mg per cu. metre[1]), but it is used so liberally in industry that it can become hazardous. Various devices are available for determining the concentration in air, including direct-reading indicator tubes and combustimeters.[2] The indicator tubes, which lack in sensitivity and accuracy (Buchwald, unpublished work), only give "spot" readings over short periods of time. Combustimeters, though quite sensitive and accurate, are not specific and suffer considerable interference from other inflammable vapours. The most versatile methods are those in which the contaminated air is passed at a known rate through a device that quantitatively removes the acetone in such a way that it may later be determined. In a recent investigation, the method suggested by Elkins[3] was tried. In this method, the acetone was adsorbed on to silica gel, then desorbed into water and determined. Since low recoveries of acetone were obtained, the method was re-investigated and the results are reported in this paper.

A literature search revealed several methods for determining small amounts of acetone and their application to its determination in air. All of these methods assumed 100 per cent. recovery of acetone from air in the suggested absorption devices, without their being checked against standard concentrations of acetone in air.

The titrimetric method of Messinger,[4] dependent on the iodoform reaction, is an easy and accurate method for determining acetone. Goodwin[5] reviewed the literature up to 1920 and thoroughly investigated Messinger's method. From time to time since then, several variations of the method have appeared.[6] Not less than 0·1 mg of acetone may be readily determined by this method, but other compounds undergoing the iodoform reaction (including those containing the CH_3CO- group) will interfere. Both Jacobs[7] and Elkins[8] recommended Messinger's method for determining acetone in air, the former by using bubblers containing water or N sodium hydroxide for collecting the acetone, the latter by using U-tubes of activated silica gel. A variation of this method by Elliott and Dalton[8] involved the use of bubblers containing sodium hydroxide and standard iodine solution for trapping the acetone; as mentioned later in this paper, this variation gave a poor recovery of acetone.

The Messinger method was criticised by Morasco,[9] who proposed an alternative and somewhat more specific reagent. In his method, acetone was trapped by drawing the air through bubblers containing 0·2 per cent. hydroxylammonium chloride solution. The acetone was determined by titrating the hydrochloric acid liberated, through the formation of a ketoxime, with standard sodium hydroxide solution. This method was considerably less sensitive than the Messinger method (1 ml of 0·1 N sodium hydroxide being equivalent to 6·14 mg of acetone), but more specific to aldehydes and ketones.

The method of Greenburg and Lester[10] was much more sensitive than any of those already mentioned. The method was based on the reaction of acetone with 2,4-dinitrophenylhydrazine to form the hydrazone. The hydrazone was extracted into carbon tetrachloride in the presence of alkali, and determined colorimetrically at 420 mμ. For acetone an absolute sensitivity of 0·1 to 0·2 μg was claimed. For sampling acetone from air, an evacuated vessel containing the reagent was used. If a 1-litre sample of air was taken, the sensitivity was 0·04 to 0·08 p.p.m. of acetone, and 97 to 100 per cent. recoveries from air were claimed. This method is much more sensitive than is normally required for industrial-hygiene studies, and is only suited for analysing samples of air having a volume of less than 1 litre. Use has been made of this method in the determination of acetone in expired air.[11] A slightly less sensitive, though equally selective, colorimetric method was developed by Engelfeldt.[12] This method

was based on the red colour produced by the reaction of acetone with salicylaldehyde in the presence of sodium hydroxide. At a wavelength of 530 mμ, 0·01 mg of acetone per ml could be determined with an accuracy of ±2 per cent.

Preliminary studies

Water was not a very efficient absorption medium for acetone. According to Elkins,[3] a 95 per cent. recovery of acetone from air could be attained with two absorbers in series, each containing 10 ml of water, only if less than 5·4 litres of air were sampled. The factors controlling the efficiency of liquid-based gas absorbers of the bubbler type have been fully discussed by Elkins,[3] Neale and Perry[13] and Gage.[14] The only way of increasing the efficiency was to use an absorption liquid that would react with the acetone to fix it in solution.

The use of N sodium hydroxide, as suggested by Jacobs[7] showed no improvement over water. When approximately 11·6 litres of air containing 1960 mg of acetone per cu. metre were passed through two bubblers in series, each containing 10 ml of water, the mean recovery of acetone was 75 per cent. On repeating the experiment with the same bubblers, each containing 10 ml of N sodium hydroxide, the mean recovery of acetone was only 62·5 per cent. When a sodium hydroxide - iodine reagent[8] was used in a similar manner, only 40 per cent. of the acetone was recovered. The reason for this poor, and rather surprising, recovery was not determined.

Morasco's[9] hydroxylammonium chloride reagent proved to be much more efficient. All the acetone was trapped in the first bubbler and a mean recovery of 94 per cent. was obtained. The reaction is dependent on the equilibrium—

$$(CH_3)_2CO + NH_2OH.HCl \rightleftharpoons (CH_3)_2C:NOH + HCl + H_2O$$

so that the complete formation of the ketoxime is dependent on the removal of the hydrochloric acid also formed. Since the latter is not removed, the reaction never reaches completion under ordinary experimental conditions, and the expected recovery is 94·6 per cent. On this basis, one uses the equivalence of 1 ml 0·1 N sodium hydroxide ≡ 6·14 mg of acetone, which includes the correction for the low recovery.

For the determination of acetone in industrial atmospheres, the use of two or three bubblers in series is not convenient. Even the use of a single bubbler (Morasco's reagent) has its disadvantages, since it involves the handling and transportation of a liquid. Under the circumstances, absorption tubes containing silica gel have certain advantages that will be mentioned later in this paper.

Experimental

Preparation of known concentrations of acetone in air—

These were prepared in a hermetically sealed chamber of stainless steel and glass, having a volume of 1·21 cubic metres. The chamber was provided with a fan for circulating the contents, exhaust ventilation for flushing out and with apertures for sampling and other manipulations. A known volume of acetone was placed by pipette in the chamber and allowed to evaporate, the air being circulated to give a homogeneous mixture. Thus 1 ml of acetone (0·790 g per ml at 20° C) gave rise to a concentration of 653 mg of acetone per cubic metre (or 272 p.p.m. at 20° C and 760 mm of mercury pressure).

Determination of acetone—

Acetone was determined by Messinger's iodimetric method. Full details of the procedure are given in the Appendix, p. 427.

Silica gel—Two batches of silica gel were used, one 20 to 60 mesh, and the other 40 to 60 mesh. The silica gel was activated by heating it in an oven for 4 hours at 150° C and it was then allowed to cool in a desiccator. The time of heating and temperature were not critical, the main purpose of this treatment being to remove adsorbed moisture and other gases (if present).

Procedure—

Five grams of the silica gel were introduced into each arm of a U-tube, being supported by plugs of glass wool. The dimensions of the U-tube were such that 5 g of silica gel just occupied one arm (about 1-cm i.d.). When filled, the U-tube arms were sealed with rubber bungs until used. It was found that when stored in this way, the silica gel remained active

for at least 1 week. Longer periods of storage were not tried. Air samples containing known concentrations of acetone were drawn at a known rate through the U-tubes. After the period of sampling was over, the tubes were sealed again until the desorption step. Several experiments were made in which the acetone concentration, rate of flow, volume sampled and grade of silica gel were varied. Details are given in Tables I and II.

DESORPTION OF ACETONE FROM SILICA GEL—

(*a*) *With water*—The silica gel was poured from the U-tubes into 50 ml of water contained in 70-ml glass bottles. The bottles were stoppered and allowed to stand for 30 minutes with occasional shaking. In some experiments, the silica gel in the arms of the U-tubes was analysed separately, and in others the whole contents of the tube were analysed. Portions (25 ml) of the solutions were withdrawn from the bottles and analysed for acetone by Messinger's method. The results obtained are shown in Table I.

TABLE I

RESULTS FOR ADSORPTION OF ACETONE ON SILICA GEL AND DESORPTION WITH WATER

Acetone concentration, mg per cu. metre	Flow-rate, litres per minute	Volume of air sampled, litres	Contents of U-tube arm analysed	Acetone found, mg	Acetone concentration found, mg per cu. metre	Recovery, per cent.
1170	1·07	21·4	1	17·7	830	71
			2	Nil		
1960	1·00	9·6	1	14·0	1460	74
			2	Nil		
2610	1·05	15·7	1	30·6	1950	75
			2	Nil		
3920	0·92	9·2	1	28·8	3130	80
			2	Nil		
7840	1·05	10·5	1	62·4	5930	76
			2	Nil		
1310	0·72	28·8	both	22·4	780	60
	0·68	27·2	both	22·8	840	64
	1·26	25·2	both	19·5	770	59
	1·10	22·0	both	16·6	760	58
	1·12	22·4	both	16·6	740	57*
	1·05	21·0	both	16·7	800	61*
	1·83	18·3	both	13·7	750	57
	1·85	18·5	both	14·5	780	60
	1·78	8·9	both	6·5	730	56*
	4·8	9·6	both	8·0	830	64
	4·5	9·0	both	7·0	780	59*

* Silica gel of 40 to 60 mesh was used for these experiments.

The low recoveries of acetone were unexplained, and in the first instance the effect of desorption time was studied. Varying the time of desorption between 10 minutes and 24 hours had no effect on the recovery of acetone, which was always between 58 and 60 per cent. The combined silica gel from both arms of the U-tubes was analysed.

The effect of using different amounts of silica gel was tried. Amounts of silica gel from 1 to 10 g were packed into 8-mm i.d. glass tubing, being supported at both ends with glass-wool. After the air - acetone mixture had been drawn through the tubes, the silica gel was poured into 50 ml of water, and allowed to stand for 20 minutes (with occasional shaking) before the solution was analysed for acetone. The results are recorded in Table II.

Since the percentage recovery of acetone was dependent on the amount of silica gel used, a further experiment was carried out to confirm the effect. Portions (50 ml) of a standard acetone solution containing 0·1 mg per ml were introduced into bottles containing varying weights of silica gel. After 15 minutes had been allowed for the solutions to attain equilibrium (with occasional shaking), the acetone contents were determined. The experiment was repeated with a standard solution containing 0·3 mg of acetone per ml. The results are given in Table III.

(*b*) *With sodium hydroxide solution*—The use of an alternative medium for desorbing the acetone was investigated. After a very short search, sodium hydroxide solution was found to be ideal. Mineral acids such as hydrochloric (N) or sulphuric (6 N) yielded poorer recoveries of acetone than water. To determine the correct concentration of sodium hydroxide

needed for complete desorption, 10-g portions of 40- to 60-mesh silica gel were treated with 50 ml of solutions containing a total of 10 mg of acetone and various concentrations of sodium hydroxide. After 15 minutes had been allowed for equilibrium to be attained, the solutions were analysed for acetone. The results are shown below—

Concentration of sodium hydroxide, N	0	0·1	0·25	0·5	1·0	1·25
Acetone recovered, per cent.	61	74·5	84	96	100	100

An air-sampling programme was made with 10 g of silica gel in U-tubes. In each experiment, all the silica gel was treated with 50 ml of N sodium hydroxide for 15 minutes to desorb the acetone. The results are shown in Table IV.

TABLE II

RESULTS FOR ADSORPTION OF ACETONE WITH DIFFERENT WEIGHTS OF SILICA GEL AND DESORPTION WITH WATER

Concentration of acetone was 1310 mg per cu. metre

Flow-rate, litres per minute	Volume of air sampled, litres	Weight of silica gel used, g	Acetone found, mg	Acetone concentration found, mg per cu. metre	Recovery, per cent.
1·28	12·8	1	14·5	1130	87
0·95	9·5	2	10·7	1130	87
0·99	9·9	3	10·0	1010	77
0·96	9·6	4	9·4	980	75
0·90	9·0	5	8·4	930	71
0·94	9·4	10	7·4	790	60

TABLE III

RECOVERY OF ACETONE FROM AQUEOUS SOLUTIONS IN THE PRESENCE OF ACTIVATED SILICA GEL

Weight of 40-to 60-mesh silica gel per 50 ml of solution	Acetone recovered, per cent., from solutions containing—	
	0·1 mg of acetone per ml	0·3 mg of acetone per ml
1	95·5	93·5
2	89	88
3	83	84
4	76·5	78
5	72	73
7	61	62
10	57	58·5

TABLE IV

RESULTS FOR ADSORPTION OF ACETONE ON SILICA GEL AND DESORPTION FOR 15 MINUTES WITH N SODIUM HYDROXIDE

Acetone concentration, mg per cu. metre	Flow-rate, litres per minute	Volume of air sampled, litres	Acetone found in both arms, mg	Acetone concentration found, mg per cu. metre	Recovery, per cent.
1310	1·05	10·5	13·4	1280	98
	0·95	9·5	12·5	1320	101
	1·03	10·3	13·0	1260	96
	1·06	10·6	13·6	1280	98
2610	1·00	10·0	25·5	2550	96
	1·05	10·5	27·1	2580	99
	0·98	9·8	25·1	2560	98
	1·03	10·3	26·9	2610	100
3920	1·05	10·5	40·3	3840	98
	1·06	10·6	41·5	3920	100

NOTE—In some experiments up to four samples were being taken from the calibration chamber at the same time. This represented a loss of 40 litres of air, which had to be replaced with fresh air. The mean loss of concentration caused by this was taken as 1·54 per cent., and the appropriate correction for this has been made in the "Acetone concentration found" column.

The effect of time allowed for desorption was also investigated. After air sampling, the silica gel was treated with N sodium hydroxide and allowed to stand (with occasional shaking) for 10, 20 and 30 minutes before being analysed for acetone. In all experiments, 98 to 100 per cent. recoveries of acetone were obtained.

EFFECT OF OTHER AIR CONTAMINANTS—

Various other solvents are often used in conjunction with acetone. The effect was studied of several of these solvents on the recovery of acetone by the silica gel - sodium hydroxide method. The concentrations of these materials used were about the maximum permissible limits.[1] The results are shown in Table V.

DISCUSSION

The poor recovery of acetone by Elkin's recommended method was surprising (see Table I). When various flow-rates and sampling times were used, the recovery of acetone remained constant at about 60 per cent. When the contents of the two U-tube arms were analysed separately, it was shown that adsorption of the acetone was complete on the first arm. The higher recovery obtained when the contents of the U-tube arms were analysed separately showed that the recovery was dependent on the amount of silica gel present. A separate experiment showed that maximum desorption was obtained in less than 10 minutes.

It became clear that the poor recovery of acetone was due to incomplete desorption in the presence of water. An experiment in which varying amounts of silica gel were used, showed that the recovery of acetone improved as less silica gel was used, though 100 per cent. recovery was not attained (see Table II). It was then shown that silica gel could be used for adsorbing acetone from aqueous solution, the effect being dependent on the amount of silica gel present. The percentage of acetone adsorbed from a given solution appeared to be independent of the initial acetone concentration (see Table III).

Since the preferential adsorption of cations on silica gel is well known,[15] sodium hydroxide solution was the first choice as an alternative desorbent. A short investigation showed that complete desorption of acetone could be achieved with N sodium hydroxide. This solution had the added advantage that it could be used directly for the analysis by Messinger's method. When N sodium hydroxide was used, it was shown that desorption was complete after less than 10 minutes' contact, and that 100 per cent. recovery of acetone from test atmospheres was achieved.

TABLE V

EFFECT OF OTHER AIR CONTAMINANTS ON THE RECOVERY OF ACETONE

Concentration of acetone used was 1310 mg per cu. metre

Air contaminant	Concentration, mg per cu. metre	Maximum permissible concentration,[1] mg per cu. metre	Flow-rate, litres per minute	Volume of air sampled, litres	Acetone found, mg	Acetone concentration found, mg per cu. metre	Recovery, per cent.
Ethanol	1315	1900	0·98	19·6	25·8	1320	101
			1·07	21·4	28·2	1320	101
Toluene	710	750	0·98	14·7	19·3	1310	100
			1·07	16·0	20·8	1300	99
	2130	75	0·98	14·8	19·0	1280	98
			1·08	16·2	20·8	1280	98
Light petroleum, 100° to 120° C ..	1210	~2000	0·99	14·8	19·7	1330	101
			1·08	16·2	21·6	1330	101
Ethyl Cellosolve ..	770	740	0·98	24·5	31·6	1290	99
			1·08	27·0	34·7	1290	99
Trichloroethylene ..	1210	520	0·98	14·8	19·4	1310	100
			1·08	16·2	20·8	1280	98
Ethyl methyl ketone ..	665	500	0·98	14·8	28·2	1910	146
			1·08	16·2	30·5	1880	144

The results in Table V showed that various common volatile solvents did not interfere with the complete recovery of acetone by the silica gel - sodium hydroxide method. This is not surprising, since (*a*) the solvents, except ethanol under certain conditions, do not

undergo the iodoform reaction, and (*b*) acetone is more tenaciously adsorbed. The table of adsorption indices given by Iler[15] places several organic compounds in order of their increasing degree of adsorption. The high result with ethyl methyl ketone as contaminant was fully expected, since it also undergoes the iodoform reaction. If a 100 per cent. recovery of acetone is assumed, then the additional iodine used by the ethyl methyl ketone represented a recovery of 750 mg of ethyl methyl ketone per cu. metre of air (113 per cent.). This high recovery of the ketone by the Messinger method is due to a secondary reaction that has been fully explained.[8,16]

Absorption tubes containing silica gel have several advantages when compared with liquid absorption devices. A single, small U-tube containing up to 10 g of adsorbent is sufficient for complete analytical recovery of acetone from a wide range of atmospheric concentrations. Pre-packed U-tubes are portable and easily set up in the sampling positions. Since no liquids are involved at the sampling stage, the U-tubes may be prepared several days in advance, provided that they are fitted with air-tight stoppers. After use they are easy to pack for return to the laboratory, and may be stored for at least 24 hours without any loss of acetone.

ACCURACY—

If it is assumed that the flow-rate (volume of air sampled) can be measured to within ± 2 per cent., it appears that the recovery of acetone is 99 ± 3 per cent. Since the titration can be judged to $\pm 0{\cdot}03$ ml, it is advisable that for more accurate work not less than 2 ml of 0·1 N iodine should be used. Thus 2 ml of 0·1 N iodine will be used if 10 litres of air containing 485 mg of acetone per cu. metre are sampled, or 1 litre of air containing 4850 mg of acetone per cu. metre. It is assumed that the acetone is desorbed into 50 ml of N sodium hydroxide and that a 20-ml portion of this solution is used for the determination.

The work described in this paper was carried out at The Occupational Hygiene Service, Slough. I thank Mrs. M. A. de Bruin for her help with the experimental work described in this paper.

Appendix

RECOMMENDED METHOD

REAGENTS—

Silica gel, 40 to 60 mesh, chromatographic grade—Activate it by heating for 4 hours at 150° C, and keep it in a desiccator.

Sodium hydroxide, N.

Iodine, 0·1 N—Prepare this in the usual manner, with a potassium iodide base solution.

Iodine indicator or *starch solution*.

Sodium thiosulphate, 0·1 N—Standardised.

Sulphuric acid, 6 N.

PROCEDURE—

Pack 10-g portions of activated silica gel into suitable sampling tubes and support them by plugs of glass-wool. Carefully stopper the tubes with rubber bungs. Remove the bungs when the tubes are at the sampling positions, and draw a volume of contaminated air through the tubes at a rate between 1 and 5 litres per minute. Replace the bungs until the time for analysis.

In the laboratory, transfer the silica gel from the tube into a 70-ml bottle containing exactly 50 ml of N sodium hydroxide. Stopper the bottle and allow it to stand for 15 minutes, with occasional shaking. Transfer 20 ml of the sodium hydroxide solution to a 250-ml conical flask and dilute the solution to 50 ml. (If aqueous solutions only are being analysed, 20 ml of N sodium hydroxide solution should be added before the dilution to 50 ml.) Add 25 ml of 0·1 N iodine and allow the mixture to stand for 15 minutes, with frequent swirling. Frequent swirling is essential for consistent results. Yellow crystals of iodoform will settle out if sufficient acetone is present. Acidify the contents of the flask with 5 ml of 6 N sulphuric acid, and titrate the liberated iodine with standard 0·1 N sodium thiosulphate, the end-point being observed with the help of some form of iodine indicator. Perform a

blank titration on 20 ml of the sodium hydroxide solution, without acetone being present. Each millilitre of 0·1 N iodine used in the reaction is equivalent to 0·97 mg of acetone. A stoppered iodine flask may be used for the analysis, but it was found that if there was sufficient potassium iodide in the iodine solution, the loss of iodine did not exceed 1 per cent. if an open flask was used.

CALCULATION—

If v cu. metres of air are sampled and x ml of 0·1 N iodine are used in the reaction, the concentration of acetone in air is $2{\cdot}43\frac{x}{v}$ mg per cu. metre, provided that the above-mentioned volumes of reagents are used.

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First received *November 5th*, 1963
Amended *September 21st*, 1964

SHORT PAPERS

The Use of 50 Per Cent. Hydrogen Peroxide for the Wet Oxidation of Organic Materials

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One of the most difficult and time-consuming steps in the determination of elements such as lead, iron, cadmium and zinc in organic and especially plastics materials has always been the destruction of the carbonaceous matter in such a way as to leave the element required in a form suitable for determination.

Wet oxidation with hot sulphuric and nitric acids is probably the procedure most used at present. Certain polymers, such as polythene and polypropylene, are, however, very resistant to oxidation, and it may take a long time for these polymers to be destroyed in this way; moreover, the considerable amounts of oxidising acids required may introduce high reagent blank values into the determination. Other materials such as tritolyl phosphate and olive oil tend to froth violently when heated with sulphuric acid, making their oxidation in this way very difficult.

It was suggested by Whalley in a lecture at the Feigl Anniversary Symposium 1962, held in Birmingham in April 1962,[1] that by the use of 50 per cent. hydrogen peroxide in the presence of sulphuric acid, the time for the wet oxidation of, for example, 2 g of polythene could be reduced from several hours to about 20 minutes. The information given here is an extension of that reported by Whalley, and is based on some two years experience with the reagent.

The reagent is prepared by the manufacturer by diluting 85 per cent. hydrogen peroxide with de-mineralised water. The only impurities likely to be present are 0·05 p.p.m. (approximately) of aluminium and silica, possibly 0·1 p.p.m. of iron and a few p.p.m. of phosphate ions added as a stabiliser. Although a stabiliser free from phosphorus is feasible, it is unlikely to be used in the reagent commercially available.

Safety precautions

Fifty per cent. hydrogen peroxide can be stored in a *vented* polythene bottle, which should be kept in a *cool* cupboard with no organic matter near (not a wooden cupboard). Under these conditions it should be quite stable, but should decomposition set in, *e.g.*, induced catalytically by the adventitious addition of trace metals, the reagent should only fizz vigorously. An explosive decomposition is said to occur only with solutions containing more than 65 per cent. of hydrogen peroxide; with such solutions the heat of decomposition initially boils off all the water present.

The reagent must not come in contact with the skin. Being a powerful oxidising agent, it will immediately produce the "white burns" that are obtained with 100-volume hydrogen peroxide, but it is more corrosive than the latter, and any burns must be washed immediately with water or dilute potassium permanganate solution, otherwise they will become painful and may cause blistering.

It is essential that eye protection be worn when the reagent is being handled.

The wet-oxidation procedure described below should be carried out behind a safety screen inside a fume cupboard.

Experimental

The purpose of this work was to assess the possibilities of using 50 per cent. hydrogen peroxide in conjunction with sulphuric acid in preference to a mixture of sulphuric and nitric acids for the wet destruction of plastics and some other materials of current interest.

The general method of oxidation used was to heat 2 g of the sample with 20 ml of sulphuric acid, sp.gr. 1·84, for 5 to 10 minutes to char the sample thoroughly, and then to add 50 per cent. hydrogen peroxide dropwise to the hot solution until all the charred matter had been oxidised. Heating was then continued, with addition of more peroxide when necessary, until the solution remained colourless on heating to white fumes of sulphuric acid. Table I summarises the results of these tests.

As a result of this work it was obvious that the materials so far examined could be classified into four classes.

I. Materials oxidised by hydrogen peroxide without preliminary heating—
   Tritolyl phosphate
   Olive oil.

II. Materials requiring charring before the addition of hydrogen peroxide—

Acrylic materials	Polypropylene
Dinonyl phthalate	Poly(vinyl chloride)
Melinex	Nylon
Polythene	Styrene - acrylonitrile copolymer.

III. Materials whose oxidation with hydrogen peroxide is considered dangerous—
   Liquid paraffin.

IV. Materials not attacked by hydrogen peroxide—
   Carbon black
   Polytetrafluoroethylene.

It was also clear that, because of possible dangers with "unknown" materials, suitable precautions should be written into the method, such as has been done below.

Method

Apparatus—

Kjeldahl flasks—Capacity, 100 ml.

Tap-funnels—Capacity, 100 ml; fitted with polytetrafluoroethylene stopcocks (grease of any kind must *not* be used) and with the stem bent. The tap-funnel must be situated as far away from the mouth of the flask as possible, otherwise the acid fumes given off would condense on the tap and funnel. The fumes would also tend to heat the peroxide in the funnel and to corrode the clamps supporting it. (Alternatively, the apparatus described by Whalley[2] or Bethge[3] could be used, but we have not examined these, finding the above apparatus to be sufficient.)

Safety screen.

Reagents—

Hydrogen peroxide, 50 per cent. w/w.

Sulphuric acid, sp.gr. 1·84.

Procedure—

For unclassified materials—Before attempting to wet oxidise a material not listed in classes I to IV above, make a preliminary wet oxidation with a small sample in order to classify the material under one of the four headings.

For materials in classes I *and* II—Into a 100-ml Kjeldahl flask, weigh 2 g of the sample and add 20 ml of sulphuric acid.

For substances listed in class I above, add hydrogen peroxide dropwise to the cold acidic solution until reaction slows up (the heat of reaction will be sufficient to maintain the reaction) or the solution goes colourless. In either instance, not more than 20 ml of peroxide need be added. Then heat the solution to fumes of sulphuric acid, adding more hydrogen peroxide dropwise as necessary until a colourless solution is obtained.

For substances listed under group II above, heat the sample with the sulphuric acid for 5 to 10 minutes to char the sample thoroughly, then add the hydrogen peroxide dropwise to the hot solution until it goes colourless. Should some un-decomposed material remain, repeat the charring and dropwise addition until the solution remains colourless when heated to white fumes of sulphuric acid.

Discussion

It is clear that 50 per cent. hydrogen peroxide is an excellent oxidising agent for many polymeric materials and doubtless many other organic substances, although the difficulties already noted make it imperative that every new application is considered with care.

The possible loss of metals during this oxidation procedure is something that must be considered, as indeed it must with any such procedure, but so far we have not encountered any such difficulty in our work.

TABLE I

SUMMARY OF TIMES TAKEN AND VOLUMES OF HYDROGEN PEROXIDE USED TO WET OXIDISE 2 g OF MATERIAL HEATED WITH 20 ml OF SULPHURIC ACID, sp.gr. 1·84

Material	Physical form	Volume of H_2O_2 added, ml	Time for complete oxidation, minutes
Nylon 6	Granules	20	40
Nylon 610	Granules	20	45
Nylon 66	Granules	25	45
Poly(vinyl chloride), plasticised	$\frac{1}{4}$-inch tubing	20	20
Poly(vinyl chloride), unplasticised, filled	$\frac{1}{8}$-inch sheet	20	20
Polythene	Film	35	45
Polythene	Granules	35	45
Polythene masterbatch	Granules	See Note 1	—
Polypropylene	Film	30	40
Polypropylene	Granules	45	40
Polypropylene, filled	Granules	20	30
Methyl methacrylate - 30 per cent. methacrylic acid copolymer	Granules	45	60
Acrylic polymer, Plexigum 570	Granules	45	30
Melinex	Film	20	15
Melinex	Granules	20	15
Styrene - acrylonitrile copolymer	Granules	30	15
Polytetrafluoroethylene	Granules	No reaction	—
Dinonyl phthalate	Liquid	40	20
Liquid paraffin	Liquids	(10) See Note 2	(30)
Tritolyl phosphate	Liquids	Violent frothing; see Note 3	—
Olive oil	Liquids		—

NOTES—

1. This sample, which contained 30 per cent. of carbon black, gave a black suspension that could not be oxidised further with this oxidising system. Obviously carbon black cannot be oxidised under these conditions.

2. When hydrogen peroxide was added to the charred liquid paraffin, a flash of flame shot out of the Kjeldahl flask and a cloud of black smoke was produced. The evolution of smoke with occasional flashes of flame continued on further additions of hydrogen peroxide until, after 10 ml had been added, all the paraffin had been oxidised or volatilised.

This experiment was considered dangerous with a risk of explosion. It is recommended that oxidation of liquid paraffin with 50 per cent. hydrogen peroxide should *not* be attempted.

3. Both tritolyl phosphate and olive oil frothed violently when the acid was heated, overflowing the Kjeldahl flasks. These experiments were repeated without initial heating, *i.e.*, by adding the hydrogen peroxide dropwise to the cold sulphuric acid - sample mixture. After a brief induction period, the oxidations proceeded smoothly. When 20 ml of hydrogen peroxide had been added to each sample, the flasks were heated to fumes of sulphuric acid with further additions of hydrogen peroxide, to discolour any charring.

It may be of interest that we have been able to complete wet digestion of 100 ml of olive oil in 1 day. This was done by oxidising 20-ml portions in the presence of 20 ml of sulphuric acid (the volume of sulphuric acid being maintained by further additions as necessary) as a class I material (see classifications below). The total volume of acid used was 100 ml, and the volume of hydrogen peroxide about 500 ml.

We wish to thank Mr. C. Whalley (Laporte Chemicals Ltd., Luton) for much helpful advice and for supplies of the reagent before it became available commercially.

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Received *February* 18*th*, 1965

A Simple Micro Colorimetric Method for the Determination of Lactose in Milk

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The methods most widely used for determining lactose are mainly based on the ability of lactose in hot alkaline solution to reduce certain ions, of which the cupric[1] and ferricyanide ions[2] are most commonly used. The extent of reduction is then established by colorimetric or titrimetric methods. Reducing substances other than lactose, if present, increase considerably the lactose value. A methylamine colour reaction[3] is used in qualitative studies for detecting lactosuria in pregnancy[4] and in determining lactose.[5,6] Interference with the method is caused by the presence of maltose, cellobiose, monosaccharides, sugar phosphates, calcium and barium.[5,7] These methods and other methods hitherto available for determining small amounts of lactose, alone or in the presence of other sugars, are laborious and unsatisfactory. Those normally used for determining lactose in milk suffer from a lack of specificity.

In the hope of obtaining a method that would be more convenient in practice and require the minimum of special equipment, the osazone reaction, used in qualitative studies for detecting lactosuria in pregnancy and the early puerperium, has been developed into a micro colorimetric method for its quantitative determination. I have used this reaction for determining blood glucose[8] and it has given true glucose values; it has also been used for determining the carbohydrate part of collagen after hydrolysis.[9] Its specificity has been also checked and it has been made suitable for routine use.[10] Sugars other than lactose interfere, unless removed by yeast fermentation. The osazone can also be accurately determined by reduction with an excess of standard titanous chloride in the presence of sodium tartrate, and subsequent back-titration with crystal-scarlet to ascertain the amount of reducing agent in excess,[11] but this method is tedious.

The present method is applicable to human milk, and without modification to the milks of other species (cow, buffalo, sheep, goat, ass and camel).

Method

Reagents—

Stock solution—Dissolve 1 g of phenylhydrazine hydrochloride in 80 ml of water, add 5 g of sodium metabisulphite, and dilute the solution with water to 100 ml. Keep the reagent in a brown-glass stoppered bottle. The phenylhydrazine hydrochloride should be re-crystallised from ethanol. This reagent must be renewed once a month.

Working solution—Dilute 10 ml of the stock solution with glacial acetic acid (analytical-reagent grade, free from aldehyde) to 100 ml. This reagent must be prepared freshly every day.

De-proteinising solution—Prepare a 2 per cent. w/v aqueous solution of zinc acetate.

Standard lactose solution—Prepare a stock standard solution by dissolving 1 g of lactose hydrate in 0·2 per cent. w/v benzoic acid solution, and dilute it to 100 ml.

1 ml of solution ≡ 10 mg of lactose hydrate.

Prepare the working standard by diluting 5 ml of the stock solution to 100 ml with 0·2 per cent. w/v benzoic acid solution.

1 ml of solution ≡ 0·5 mg of lactose hydrate.

Procedure—

Introduce 1 ml of milk into a 100-ml calibrated flask, add 1 ml of zinc acetate solution, mix well, and dilute the mixture to the mark with water. Set the mixture aside for 5 minutes and then filter it.

Introduce 0·5 ml of the milk filtrate into a Pyrex-glass test-tube, add 5 ml of the working solution, mix, and heat it for 1 hour in a vigorously boiling water bath. Cool the solution and measure the optical density of the yellow colour at 370 mμ. The full spectrum of lactosazone is shown in Fig. 1.

Measure the optical density of a blank solution of 0·5 ml of water instead of the milk filtrate and subtract this value.

CALIBRATION—

Take standard solutions of lactose hydrate through the same procedure. The optical density increases linearly and the results correspond to Beer's law over the range 50 to 500 μg.

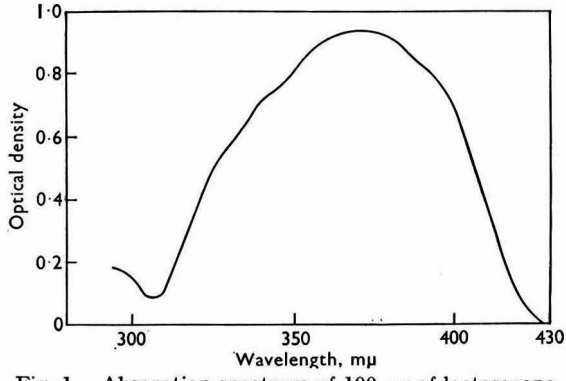

Fig. 1. Absorption spectrum of 100 μg of lactosazone

The heating-time for the development of the colour can be reduced successfully to 15 minutes when heating is carried out in a constant-temperature bath (with glycerine) at 110° C rather than in a boiling-water bath.

RESULTS

STABILITY OF THE YELLOW COLOUR PRODUCED—

The optical density of the yellow colour produced by heating standard lactose hydrate solution, or milk filtrate with the reagents was found to be stable for at least 1 day, even when left exposed to air and light. Further heating does not cause a reduction of the colour.

TABLE I

RECOVERY OF LACTOSE FROM MILK

Milk sample	Amount of lactose added, mg per ml of milk	Lactose found in milk, mg per ml	Lactose recovered, mg per ml	Difference
A	0	48	—	—
	20	68	20	0
	50	99	51	+1
B	0	65	—	—
	10	75	10	0
	40	104	39	−1
C	0	55	—	—
	25	80	25	0
	45	101	46	+1

Mean difference ± standard deviation = +0·17 ± 0·16.

EFFECT OF DE-PROTEINISING AGENTS—

Zinc acetate, in such a concentration as that used, has no effect on the production of the yellow colour or on its optical density, unlike sodium tungstate and sulphuric acid, which are commonly used to prepare a protein-free filtrate of milk and which were found to interfere with the reaction. Also, zinc acetate is more convenient to prepare and use, since it is one solution only, more readily available, more effective and gives a very clear filtrate, and so it was selected as the de-proteinising agent. It is successful with human milk and, without modification, to the milks of other species (cow, buffalo, sheep, goat, ass and camel).

RECOVERY OF LACTOSE FROM MILK—

Standard solutions of lactose hydrate, freshly made in saturated benzoic acid were used in the recovery tests (see Table I). The values given are the means of duplicate determinations and are representative of the many analyses that I have carried out.

COMPARISON OF MILK-LACTOSE VALUES DETERMINED BY THE PROPOSED METHOD WITH THOSE BY THE FOLIN AND WU METHOD[12]—

As procedures involving the use of copper compounds are commonly used in biochemical laboratories, the amounts of lactose in various milk samples were determined by the Folin and Wu method[12] and by the proposed method (see Table II); the values given are the means of duplicate determinations and are representative of the many analyses that I have carried out.

TABLE II

COMPARISON OF RESULTS FOR LACTOSE IN MILK

Lactose found by the Folin and Wu method, mg per ml	Lactose found by proposed method, mg per ml	Difference, mg per ml
76	75	−1
50	50	0
42	41	−1
65	63	−2
55	55	0
48	47	−1
60	59	−1
45	46	+1
52	51	−1
61	60	−1

Mean difference ± standard deviation = −0·7 ± 0·72.

DISCUSSION

Most of the methods reported in the literature for determining lactose in milk are usually unsatisfactory and suffer from lack of specificity, especially those based on the reducing properties of lactose. The colorimetric method described in this paper does not depend on the reducing power of lactose, but on the formation of the yellow lactosazone, which is easily soluble in acetic acid. The colour reaction is known to be specific for the ketoses and aldoses, whereas the reducing substances are not determined by this method.

The yellow colour of lactosazone remains stable over long periods, unlike the blue colour produced in procedures involving the use of copper compounds. Special tubes and many precautions are unnecessary, and so the procedure is easier and simpler to carry out. Determination of lactose in the presence of maltose or glucose or other ketoses and aldoses may be made after preliminary fermentation with brewers yeast as for other specific methods like the methylamine colour reaction.[5,6] The reaction clearly offered the possibility of a specific determination of lactose.

This is a micro-scale method needing only 1 ml of milk and 0·5 ml of the protein filtrate, which is easily clarified by using zinc acetate as the de-proteinising agent. This use has another advantage in the preservative action of zinc. The results conform to Beer's law over the range 50 to 500 μg. Recovery tests on added lactose over the range found in normal milk gave a mean difference ± standard deviation of ±0·17 ± 0·16.

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Received *December 14th*, 1964

Micro-determination of Zinc and Copper in a Single Digest of Small Samples of Plant Material

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When only very small samples of plant material are available, it may be impossible to carry out multiple digestions. In the determination of zinc with dithizone it is necessary to remove interfering copper, and a method is proposed in which the separated copper is conserved and determined, a single digest of plant material thus sufficing for both determinations.

Method

The determination of zinc is based on the method of Cowling and Miller[1] as modified by Verdier, Steyn and Eve[2]; the determination of copper is based on the recommended method of the Society for Analytical Chemistry,[3] in which sodium diethyldithiocarbamate is used.

Reagents—

All reagents must be of analytical-reagent grade.

Nitric acid, sp.gr. 1·42.

Sulphuric acid, sp.gr. 1·84.

Perchloric acid, 60 *per cent. w/v.*

Ammonium hydroxide, approximately 3·5 N—Dilute 200 ml of ammonia solution, sp.gr. 0·88, to 1 litre with water.

Hydrochloric acid, N *and* 0·02 N.

Carbon tetrachloride—Redistilled.

Dithizone solution, 0·01 *per cent. w/v*—Dissolve 0·1 g of dithizone (diphenylthiocarbazone) in 250 ml of carbon tetrachloride, set the solution aside in a refrigerator for a minimum of 12 hours, filter it and dilute it to 1 litre with carbon tetrachloride. Store the solution in a dark-coloured bottle in a refrigerator.

Solution A—Dissolve 52·5 g of nitric acid in water and add ammonia solution, sp.gr. 0·88, until pH 9 is reached, using narrow-range test papers to estimate the pH. Add 75 ml of N ammonium hydroxide and dilute the solution to 1 litre with water.

Solution B—Dissolve 46·6 g of citric acid in water, and add 50 ml of ammonia solution, sp.gr. 0·88, and dilute the mixture to 1 litre with water. (The pH of this solution should be about 9; if it is not, adjust it to this value with ammonia solution.)

Sodium diethyldithiocarbamate—Dissolve 1 g of the solid in 100 ml of water; store the solution in the dark in a refrigerator. Filter it before use; discard it after 7 days.

EDTA - citrate solution—Dissolve 17·28 g of citric acid in water and neutralise it with ammonia solution, sp.gr. 0·88. Add 5 g of EDTA (disodium salt) and dilute the mixture to 1 litre with water.

Standard solutions of zinc and copper—Prepare separate stock solutions of the sulphates to contain 100 μg of the metals per ml, acidifying each solution with 1 ml of sulphuric acid per litre. Dilute the stock solutions as required to give standard solutions containing 10 μg of metal per ml.

Precautions against contamination—

Rigid precautions must be taken at every stage of preparation of the plant material. At harvesting, soil contamination must be avoided; galvanised trays or similar oven fittings must be excluded, and stringent precautions must be taken to prevent contamination from cosmetics.

All glassware must be scrupulously clean, and must be checked by rinsing with dithizone solution until there is no change in the clear green colour of the solution.

Procedure—

Weigh 0·5 g of dried plant material into a 50-ml Kjeldahl flask, and digest the sample with 10 ml of nitric acid, 1 ml of sulphuric acid and 2 ml of perchloric acid, added in the order given. Heat the mixture until a clear digest is obtained, and then continue heating to fuming for another 20 minutes. Cool and wash the contents of the Kjeldahl flask into a 50-ml calibrated flask with about 30 ml of water. Neutralise the solution to methyl red with 3·5 N ammonium hydroxide, add 2 ml of N hydrochloric acid and dilute the solution to the mark.

Transfer a suitable aliquot containing not more than 20 μg of zinc or copper to a 100-ml separating funnel. Add 40 ml of solution *A* and 10 ml of dithizone solution. Shake the funnel for 2 minutes. Run off the lower (carbon tetrachloride) layer into a second separating funnel, and extract the aqueous layer with at least two successive 5-ml portions of dithizone solution, continuing until the extract is a clear green. Reject the aqueous phase.

Add 50 ml of 0·02 N hydrochloric acid to the combined carbon tetrachloride extracts, and shake the mixture for 2 minutes. The zinc passes into the aqueous phase, leaving the copper in the organic phase, which is transferred to a 50-ml Kjeldahl flask and retained for the determination of copper. Extract the aqueous phase twice with consecutive 5-ml portions of carbon tetrachloride, adding these to the contents of the Kjeldahl flask.

To the aqueous phase in the separating funnel, add 15 ml of solution *B* and 10 ml of dithizone solution, and shake the funnel for 2 minutes. Run off the carbon tetrachloride phase and extract the aqueous phase with an additional 5 ml of dithizone solution. Combine the carbon tetrachloride extracts and add 35 ml of carbon tetrachloride (or other suitable volume to dilute the coloured complex appropriately). Read the optical density at 535 mμ in a spectrophotometer with carbon tetrachloride as the reference solution.

Evaporate the carbon tetrachloride solution of the copper - dithizone complex to dryness. Add two drops of sulphuric acid and 5 ml of nitric acid. Heat the mixture to fuming and continue heating for 10 minutes. Cool, add 10 ml of EDTA - citrate solution and two drops of thymol blue indicator. Add 3·5 N ammonium hydroxide until the colour is green (NOT blue), and then add 1 ml of sodium diethyldithiocarbamate and 10 ml of carbon tetrachloride. Shake the flask for 2 minutes and run off the carbon tetrachloride layer into a cuvette and read the optical density at 436 mμ.

PREPARATION OF CALIBRATION GRAPHS—

A blank determination with the same amounts of all reagents is made, and is used together with similar determinations for mixtures of the zinc and copper standards containing 10, 20 and 30 μg of each to obtain calibration curves for both metals. By adhering rigidly to the procedure and by using exactly the same amounts of all reagents, the calibration curve obviates the necessity for elaborate purification procedures for all reagents.

The blank values indicated by the calibration curves show the degree of reagent contamination with copper and zinc. If this approaches an unacceptable level, the reagents should be purified by the usual procedures.[3] A fresh calibration curve must be prepared for each set of determinations, and whenever a new batch of any reagent is used.

Direct exposure of the zinc - dithizone and copper - diethyldithiocarbamate complexes to sunlight is to be avoided. Both are stable, however, to diffuse daylight for short periods, and accurate results can be obtained provided that there is no undue delay in completing the determinations.

RESULTS

Results are given in Table I for 0·5-g samples of oven-dried (105° C) material taken from 6-week-old plants grown at Wellesbourne in a sandy loam of the Newport series.

TABLE I

RESULTS FOR COPPER AND ZINC FOUND IN OVEN-DRIED PLANT MATERIAL

Plant material	Found, p.p.m.		Added,* p.p.m.		Found, p.p.m.		Recovery, per cent.	
	zinc	copper	zinc	copper	zinc	copper	zinc	copper
Red beet	11·2	3·8	20	20	32·8	25·8	108	110
Cabbage	10·2	2·0	20	20	30·2	23·0	100	105
Carrots	14·0	11·8	20	20	34·2	32·4	101	103
Lettuce	17·4	6·0	20	20	36·6	25·8	96	99
Onion	18·0	6·2	20	20	36·8	26·6	94	102

* Added as solutions of the respective sulphates to aliquots of the digest.

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Received *August 10th*, 1964

The Determination of Sodium in Heavy Fuel Oils by Neutron-activation Analysis

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Neutron activation is now a well established technique in the analysis of trace elements.[1] The main advantages of the method are its speed and sensitivity, and there are many examples of its application in the chemical literature.[2] We have used the method for determining sodium in heavy fuels, and have found it to be especially suitable for measurements on large numbers of samples. In this paper we give the procedure, and also describe experimental work done in order to determine the sensitivity and precision of the method and its freedom from interferences.

Method of analysis

The basis of the analysis is the irradiation of small samples with thermal neutrons to generate sodium-24 by the reaction $^{23}Na\ (n,\gamma)\ ^{24}Na$.

The use of small samples is an important feature of our method because it permits many samples to be irradiated at the same time as a set of standards, and greatly reduces the cost of irradiation per sample. A further advantage in the use of small samples is that the possibility of the samples bursting in the reactor, owing to the formation of volatile radiolysis products, is negligible because of the greater relative strength of the small sample containers. In practice we use 10-mg samples sealed in fused silica capillaries. Up to 100 of these capillaries fit into a standard irradiation container and they can be irradiated for 1 hour in a flux of 10^{12} thermal neutrons per sq. cm per second for about £10.

The choice of irradiation time is a compromise between sensitivity and freedom from interference from long-lived isotopes. We chose 1 hour because it gives adequate sensitivity, although it gives only 5 per cent. of the saturation level for sodium-24 (15-hour half-life). After irradiation, the samples are transported to our laboratory, and it is convenient to allow 10 to 15 hours to elapse before the radioactivity measurements are started; this not only gives adequate time for transportation, but also ensures that short-lived interferences decay to negligible amounts.

Sources of error—

Particularly with such small samples it was essential to check that we were not introducing sodium contamination and that the sampling technique was reliable. For determinations down to 1 p.p.m. on 10 mg of sample, we were concerned with 10^{-8} g of sodium. Great care was taken to avoid contamination during the preparation and filling of the capillaries. Freedom from contamination was checked by carrying out analyses on de-mineralised water and medicinal white oil and on empty tubes.

To check the sampling technique, three heavy fuel oils were examined in some detail. Ten separate samples of each fuel were sealed in capillaries and were irradiated and counted in order to determine the overall precision of the procedure. The most likely interferences[3] from other elements result from the reactions $^{24}Mg\ (n,p)\ ^{24}Na$ and $^{27}Al\ (n,\alpha)\ ^{24}Na$, and we assessed these by making measurements on sodium-free aqueous solutions containing 1000 p.p.m. of magnesium and of aluminium.

All the results were calculated with reference to a set of standards (aqueous solutions of sodium chloride) that were irradiated with the samples. The effect of screening was checked in a separate experiment in which sodium standards were irradiated in different positions among the oil samples. The counting rates of these standards did not depend on their position, showing that screening did not occur to a significant extent.

Experimental—

In the evaluation of the method, two irradiation cans were used containing the samples listed below—

> 10 replicate samples of fuel A; 10 replicate samples of fuel B; 10 replicate samples of fuel C; 4 replicate samples of de-mineralised water; 4 replicate samples of medicinal white oil; 3 empty silica replicate tubes; 3 replicate samples of an aqueous solution containing 1000 p.p.m. of magnesium; 3 samples of an aqueous solution containing 1000 p.p.m. of aluminium; and two sets of aqueous sodium standards containing 10, 50, 100, 500 and 1000 p.p.m. of sodium.

One set of the sodium standards was put in each can for calibration purposes. Both cans were irradiated in the BEPO reactor at Harwell for 1 hour in a flux of 10^{12} thermal neutrons per sq. cm per second.

Radioactivity measurements—

Measurement of the 2·75-MeV γ-radiation from sodium-24 was made with a Packard Auto-Gamma scintillation counter fitted with a 3-inch diameter × 3-inch long sodium iodide well-type crystal. This counter has an automatic sample changer capable of holding 100 samples, and gives an output both in printed form and also on punched-paper computer tape. The many advantages of the combined use of automatic-counting and computer-calculation methods have been discussed elsewhere.[4]

Because of the relatively high radioactivity level of the silica after irradiation, sample transfer was necessary. A counting time of 10 minutes was used, and the automatic counter was allowed to accumulate information for five complete cycles, each cycle representing a single measurement on each sample, background and set of standards. In this way, reliable estimates of the decay rates of samples and standards were obtained.

The instrument automatically recorded the accumulated counts and the counting time, from which the clock time of each count could be deduced. These results, together with the weights of the samples and standards, were used for calculating the sodium contents of the samples. The calculation procedure, which was programmed for computer processing, was as described below.

All the counts were corrected for background and then for decay to the mid-point of the complete counting schedule (*i.e.*, to the time corresponding to the middle of the third cycle). The mean value of the corrected counting rate for each sample and standard was determined. All the recorded counts were found to decay with a half-life of 15 hours in agreement with that of sodium-24. A calibration was obtained from the counting rates of the standards. This was linear and yielded a calibration factor in terms of counts per minute per part per million for a 10-mg sample. Each sample counting rate was normalised to a weight of 10 mg and was divided by the calibration factor to yield the final result in parts per million of sodium.

Results and discussion

The results for the standards gave a calibration factor, after corrections for background and decay, of 3·11 ± 0·01 c.p.m. per p.p.m. of sodium for a 10-mg sample. Since the background counting rate is 5 c.p.m., we therefore consider the limiting sensitivity of the method to be 1 p.p.m. The results for the samples are given in Table I.

Table I

Results for determination of sodium

Sample	Sodium found, p.p.m.	Mean and standard deviation of mean, p.p.m. of sodium
Fuel A..	123·5, 130·2, 121·5, 122·5, 117·4 121·9, 126·4, 123·8, 120·9, 122·2	123·0 ± 1·1
Fuel B..	117·7, 118·3, 128·6, 121·9, 123·2 124·1, 123·2, 123·5, 123·2, 123·2	122·7 ± 1·0
Fuel C	80·7, 82·6, 88·1, 90·0, 85·9 79·7, 89·4, 88·7, 82·6, 88·1	85·6 ± 1·2
White oil	−0·3, 0·0, 0·3, 0·3	0·1 ± 0·2
De-mineralised water ..	0·3, 0·7, 0·0, 0·7	0·4 ± 0·3
Empty tube	0·3,* 0·3,* 0·7*	0·4 ± 0·2*
Aqueous solutions containing—		
10 p.p.m. of sodium ..	11	—
50 p.p.m. of sodium ..	51	—
100 p.p.m. of sodium ..	103	—
500 p.p.m. of sodium ..	505	—
1000 p.p.m. of sodium ..	987	—
1000 p.p.m. of magnesium ..	0·7, 1·0, 1·3	1·0 ± 0·2
1000 p.p.m. of aluminium ..	1·3, 1·6, 1·6	1·5 ± 0·1

* Equivalent to parts per million of sodium for a 10-mg sample.

The procedure was designed specifically for the analysis of a single type of petroleum product namely heavy fuel, but it would be equally applicable to other types of hydrocarbon. The range of sodium contents that have been found in heavy fuels is 10 to 1000 p.p.m., and for such samples

the limiting sensitivity of the method of 1 p.p.m. is adequate. The sensitivity of the method could quite easily be extended a hundred-fold by irradiating for longer times in a higher neutron flux, without an appreciable increase in the cost of the analysis.[5]

Although it is usual to quote sensitivity as the main advantage of neutron-activation methods, this study shows how the method can be adapted to handle large numbers of routine samples quickly and economically, especially if automatic-counting and data-processing facilities are available. In our work, when large numbers of samples were analysed in this way, it was found that about 200 measurements could be carried out in 1 week. The most time-consuming part of the procedure was the transfer of the samples from the irradiated silica capillaries to the counting vials. If this could be avoided, *e.g.*, if the capillaries were made from an inert material, the number of measurements per week could be increased considerably.

It is extremely important to ensure that the sample taken is representative of the material to be analysed. This is especially difficult if samples are inhomogeneous, *e.g.*, with brine droplets or solid particles in suspension, and is a well known problem in fuel-oil analysis.[6] In applying our method to the analysis of large numbers of samples, we made the measurements in duplicate. The degree of agreement between these duplicates is a good indication of the reliability of the sampling procedure. From a set of measurements on 100 samples, we found that the duplication was good in 95 of the samples, but was very bad for the remainder. The samples showing bad duplication were all of the same type and it was concluded that for such oils the use of such small samples was not appropriate.

Conclusions

Neutron-activation analysis can be used to determine sodium in heavy fuel oils. Ten-milligram samples are sufficiently representative for reliable measurements to be made, provided that the material to be analysed is homogeneous.

For samples containing about 100 p.p.m. of sodium, the precision is 1 per cent. for 10 replicate measurements, and would be 3 per cent. for duplicate measurements, precision being defined as the standard deviation of the mean of measurements on replicate samples.

The interferences due to magnesium and aluminium are not serious, 1000 p.p.m. of either being equivalent to less than 2 p.p.m. of sodium.

The sensitivity under the conditions of these experiments is such that concentrations of sodium down to 1 p.p.m. can be measured.

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Received *September 29th*, 1964

The Estimation of Tomato Solids by Determination of Lycopene

By O. B. Darbishire

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Stock[1] gave widely differing figures for the lycopene content of tomatoes, but quite a narrow range, 1462 to 1856 p.p.m., for tomato purées. It was concluded that lycopene can be a fairly accurate index of tomato content of products made from tomato purée. In our experience with various commercial purées, we have found considerably more variation.

The extinction coefficient, $E^{1\%}_{1\,cm}$, was first determined on a sample of lycopene extracted from tomato purée and recrystallised from light petroleum. It was found to be 2820 at 505 mμ in light petroleum by using a Unicam SP600 spectrophotometer. (Messrs. Roche Products supply a synthetic lycopene and specify $E^{1\%}_{1\,cm}$ as 2850 at 508 to 511 mμ in cyclohexane.) Stock assumed $E^{1\%}_{1\,cm}$ for lycopene at 505 mμ to be 2000, but the difference can be explained by differences in the

wave-band width of various instruments. At the sharp absorption peaks shown by lycopene, an instrument with a wide wave-band will necessarily give a lower maximum reading than one that covers a narrow band only.

The method of extraction was to shake very vigorously 50 ml of a 0·2 per cent. aqueous suspension of purée with 25 ml of light petroleum, boiling-range 80° to 100° C, after which the mixture was shaken for 15 minutes in a mechanical shaker. With this procedure it is considered that almost complete extraction is achieved. A sufficient portion of the clear extract is drawn off into a 1-cm cell and the extinction coefficient measured at 505 mμ against light petroleum.

Table I gives the results of routine tests on commercial purées received during the past 12 months. Some were samples submitted by our purchasing department, and all samples were of approximately 28 to 30 per cent. concentration.

TABLE I

RESULTS FOR COMMERCIAL TOMATO PURÉES

Lycopene found in dry solids, p.p.m.							
Italian purée			Portuguese purée		Spanish purée		Czech purée
980*	1160	1510	1220	1740	940*	1550	1330
1070	1210	1530	1310	1740	1000*	1580	
1070	1270	1570	1330	1760	1030	1790	
1120	1280	1590	1340	1780	1100*	1800	
1130	1420	1610	1380	1810	1340		
1130	1440	1860	1440	1810	1420		
1150	1480	2000	1620		1550		

Mean value = 1420 p.p.m. Standard deviation = 275 p.p.m.

* Purée rejected as visually unacceptable by our standards.

The inference from these figures is that lycopene determination can give only a rough estimate of the tomato content of a food.

REFERENCE

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Received *December 2nd*, 1964

Book Reviews

METALLURGISCHE ANALYSEN: CHEMISCHE UNTERSUCHUNG VON TECHNISCH WICHTIGEN METALLEN UND ERZEN. By Prof. Dr. JOZSEF MILKA. Pp. 843. Budapest: Akadémiai Kiadó. 1964. Price $12.00.

In this present volume the author's objective has been to bring the earlier volume, also in German, up to date, by presenting a completely revised account of current analytical practice in Hungary for the analysis of certain specified metals, and their closely related alloys and raw materials.

Part 1, Analysis of Metals, is divided into sections under the headings Iron, Aluminium and Aluminium Alloys, Magnesium and Magnesium Alloys, Copper and Copper Alloys, Lead and Lead Alloys, Tin and Tin Alloys, Zinc and Zinc Alloys, and Silver and Silver Alloys. About half of this first part of the book is devoted to the analysis of ferrous materials, the section on Aluminium and Aluminium Alloys is covered in about 100 pages, and the remaining sections take up about 200 pages.

Analytical procedures for the analysis of ores, under the main headings Iron and Manganese, Bauxite, Magnesite and Dolomite, and Copper-, Lead- and Zinc Ores, constitute the second part of the book, and these sections cover some 200 pages. The book also contains an Addendum of about 20 pages in which details are given for the preparation of standard and reagent solutions.

An unusual feature for a publication of this size and coverage is the absence of detailed supporting references, and although at the end of the book, 18 books and 15 journals are listed as having been consulted (and recommended) by the author, these references are in general terms, *e.g.*, *Analyst*, Cambridge, and *Analytical Chemistry*, Washington.

This publication is, essentially, a collection of well established gravimetric, colorimetric and titrimetric procedures for the analysis of the various materials described, with no details, description or discussion of up-to-date instrumental methods that could have an appeal to the specialist.

W. T. ELWELL

SYMPOSIUM ON X-RAY AND ELECTRON PROBE ANALYSIS. Pp. vi + 209. Philadelphia: American Society for Testing and Materials. 1964. Price $10.00.

This little volume comprises papers presented at a Symposium on X-ray and Electron Probe Analysis during a meeting of A.S.T.M. in Atlantic City in June, 1963. A meeting on the same subject was held under similar auspices ten years before, and the 1963 symposium was carefully planned to depict the rapid progress made in the field over the ten-year period. Papers were invited on specific topics from outstanding workers, in an attempt to provide wide coverage of the subject. Obviously the coverage is less than complete, but the organisers are to be commended for the wealth of information that does appear in this publication.

The papers are arranged to develop the subject in a logical order. The two introductory papers are concerned with the fundamentals of the generation of X-ray spectra, and they compare the basic concepts and practices of X-ray fluorescence analysis and microprobe analysis. A paper on X-ray detectors is followed by three papers about X-ray spectrochemical analysis. Of these, one deals with analysis of trace elements, and another discusses the specific advantages and limitations of X-ray analysis of materials in liquid and solution form. In the paper on trace elements, there is a useful summary of the many sources of interference that mitigate against the analytical line-to-background ratio being maintained at the highest possible level.

Of the half-dozen papers dealing with microprobe analysis, the first reviews the electron optical factors important to the design and performance of an equipment. This is followed by a paper on quantitative analysis, in which the author argues that the use of reference calibration curves is of diminishing importance, and that most quantitative results will in future depend upon pure elements alone as reference standards. Later papers describe applications to metallurgical research, to the study of minerals, and to medical and biological specimens.

An adequate list of literature references is included at the end of each paper. There are also two useful short summaries of groups of papers, one of the papers on X-ray spectrochemical analysis on pages 86–87, and the other of the papers on the electron probe on pages 208–209. It is a pity there is no subject index.

The reader will be left in little doubt about the past progress and the potentialities for further development of these two analytical techniques. There will be general agreement with many of the views expressed by L. S. Birks in his contributed paper comparing the two techniques. Birks looks forward to the time when specimens of almost any initial complexity can be handled with ease and precision. The evidence presented in this book provides good support for his claim that the situation in X-ray spectrochemical analysis may be best expressed by the inscription on the National Archives building in Washington, "What is past is prologue." H. P. ROOKSBY

QUALITATIVE ORGANIC MICROANALYSIS: COGNITION AND RECOGNITION OF CARBON COMPOUNDS. By Dr. FRANK L. SCHNEIDER. Pp. xii + 535. Wien: Springer-Verlag. 1964. Price $21.40.

This book, the third, in order of time, to appear in the series, is a worthy companion to the two earlier volumes. Frank Schneider's own contributions to this field are well known and numerous. He is obviously a most suitable choice as the author to produce this truly impressive handbook.

He has served his readers well. The book is composed, essentially, of four sections. In the first, a wide variety of apparatus is described, and its use is detailed. A very considerable amount of critical selection has been applied in recommending the most suitable apparatus for each particular problem, from the multitude that has appeared in the literature. There is a chapter on that so important, but so often neglected matter, the preparation of the sample.

In the second section, the detection of the elements and the determination of the more important physical constants for identification purposes are dealt with. Once again the author steers a difficult course between the Scylla of comprehensiveness and the Charybdis of critical appraisal in a way that cannot be seriously faulted.

The third section could, *mutatis mutandis*, be used as the basis for a systematic analysis on any scale. It is, of course, at its most elegant here, where the amounts of material used for the

classification of the compound in its proper group range from a few milligrams to a few micrograms. I would venture to suggest that few analysts, having mastered some of these techniques, would willingly return to the "ceiling-decoration" methods of macro techniques.

The final section, of some 210 pages, is, in a sense, the cornerstone of the book, though, once again, the scale on which the work is done would be irrelevant. This is a series of tables listing the melting-points or boiling-points, or both, of organic compounds, arranged under functional groups, together with the other criteria (melting-points of derivatives, physical constants) by which the compounds may be identified conclusively.

In my view, no analyst involved in the identification of organic compounds should be without the benefit of this book. As one who has long been associated with micro-analysis, I would, naturally, deplore the attitude of the individual who might make use of the last two sections and ignore the first two. But I would dare to hope (or even to forecast) that the analyst who buys this book for Sections 3 and 4—and this would be completely justifiable—would inevitably be tempted to browse, to experiment, and finally to accept what he will find in Sections 1 and 2; and would end by being a better analyst.

I am asking, I know, for a short, sharp chop on the back of the neck when I say that there is rather more satisfaction (and possibly a considerable saving in time and money) in seeing an identifiable phenylhydrazone—even if only a few crystals under a microscope—rather than observing an infrared band that may or may not be a C=O stretching frequency.

CECIL L. WILSON

CONFERENCE ON THE VIBRATIONAL SPECTRA OF HIGH POLYMERS. Edited by GIULO NATTA and GUISEPPE ZERBI. Pp. vi + 226. New York and London: Interscience Publishers, a division of John Wiley & Sons, Inc. 1964. Price 75s.

Reprinted from Journal of Polymer Science, Part C, Polymer Symposia, No. 7.

This volume contains a set of valuable survey articles concerned with infrared and Raman spectroscopic studies of high polymers, as well as several shorter papers on specific topics in this field. The first survey article deals mainly with the spectral differences that arise from the presence of non-crystalline structures and the identification of these structures. This is followed by articles on Raman spectra, the use of polarised infrared radiation in structural studies, the theory of the normal vibrations of helical polymers and the vibrational assignment of the infrared spectra of isotactic polypropylene. Further reviews deal with normal co-ordinate calculations of large hydrocarbon molecules and polymers, and there is also much interesting discussion on biological polymers, such as nucleic acids and polynucleotides. This book will be welcomed by workers in polymer spectroscopy, both as a key to the literature and a valuable assessment of the present position of this important field.

W. C. PRICE

THE CHEMISTRY OF WHEAT STARCH AND GLUTEN: AND THEIR CONVERSION PRODUCTS. By J. W. KNIGHT, B.Sc., Ph.D., F.R.I.C., F.R.A.C.I. Pp. xii + 156. London: Leonard Hill. 1965. Price 45s.

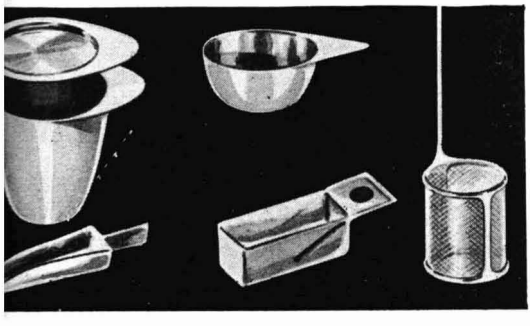
The first part of this book is a description of the technological processes with particular reference to current Australian practice. After a well balanced account of the separation of gluten and starch from flour, the following sections deal with the properties and uses of wheat starch and its modifications, and of gluten and its derivatives. The most recent developments and a mass of otherwise scattered information have been nicely collated.

The second part deals with laboratory methods of testing and analysis. This does not pretend to be comprehensive; most of the methods described are well known, but they have been selected for their proven reliability.

All chemists interested in starch and gluten will find a study of the book rewarding.

J. F. HERRINGSHAW

MACRO or micro



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Volume 90. No. 1072, Pages 379 - 442 July, 1965

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

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Printed and Published for the Society for Analytical Chemistry by W. Heffer & Sons Ltd., Cambridge, England. Communications to be addressed to the Editor, J. B. Attrill, 14 Belgrave Square, London, S.W.1. Enquiries about advertisements should be addressed to Walter Judd Ltd., 47 Gresham Street, London, E.C.2.

Entered as Second Class at New York, U.S.A., Post Office