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PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

AN Ordinary Meeting of the Society was held at 6.45 p.m. on Wednesday, October 6th, 1954, in the meeting room of the Royal Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. D. W. Kent-Jones, F.R.I.C.

The following papers were presented and discussed: "The Theoretical Basis of 'Sensitivity Tests' and their Application to some Potential Organic Reagents for Metals," by H. M. N. H. Irving, M.A., D.Phil., F.R.I.C., L.R.A.M., and Mrs. H. S. Rossotti, B.A., B.Sc.; "An Investigation of 5-Nitroso-oxine as an Analytical Reagent," by H. M. N. H. Irving, M.A., D.Phil., F.R.I.C., L.R.A.M., and R. G. W. Hollingshead, M.A.

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DEATHS

WE regret to record the deaths of

Harry Hurst
James Waywell.

The Mass Spectrometer as an Analytical Instrument

By G. P. BARNARD

(*Presented at the meeting of the Physical Methods Group on Tuesday, April 6th*, 1954)

A brief description of some modern instruments, techniques in operation and analytical procedures is given. Some examples of quantitative organic and inorganic analysis are considered. In respect of accuracy and speed in these analytical applications, mass spectrometry is shown to compare favourably with other physical methods of analysis. The use of the isotope dilution method for the determination of trace elements in solids is described. Other uses of the mass spectrometer for the determination of impurities are noted briefly.

During the last decade the mass spectrometer has become an indispensable analytical instrument in much technological progress in many industrial spheres. However, many physical methods laboratories in this country are not yet equipped with mass spectrometers; and for analytical chemists in these laboratories it may be difficult, without direct experience, to assess the value to them in their work of this new, apparently complex and rather costly machine. Solely on grounds of simplicity and initial cost, the mass spectrometer must compare unfavourably with radiation spectrometers, so that a careful scrutiny is required of the claims of experienced mass spectrometrists as to accuracy, speed, versatility and saving of man-hours in a wide range of analytical work. In this paper an attempt is made to survey, without a great deal of detail, the principles and modern techniques of operation and some recent applications of mass spectrometers.

The principles and modern techniques of operation

The treatment of the theme is defined throughout by the requirements of an analytical chemist who has no practical experience of mass spectrometry, but who seeks a general notion of this modern instrument and its capabilities in applications to some analytical problems. It is assumed that, for this purpose, much technical detail would be unwelcome.

It may be self-evident, but it is, nevertheless, worth emphasising here, that mass is the key to the identity of any particle under observation. Hence, if a complex mixture is introduced into the apparatus, the mass spectrometer must carry out automatically a sorting process, so that identification according to mass number of all the different mass species is possible. The first problem in design is how to achieve the required degree of mass separation without losing in the process too many particles of all mass numbers. There is bound to be a considerable loss, however good the design; the more so, if a high mass resolution is required. Moreover, imperfections in this respect signify more than just some loss of sensitivity, because the percentage lost at each mass number is not the same. This preferential treatment by the machine of each mass species is known as mass discrimination, and all instruments suffer from this defect to various extents. It follows that when the mass sorting process is completed, a simple counting procedure cannot, without reference to known standards, give a precise indication of the true relative amounts of the various mass species. Hence, with mass spectrometry, as with so many other laboratory methods, comparative measurements are essential for quantitative analysis.

Although an absolute first-order single-focusing instrument must be regarded as practically unattainable, it is important for work to continue so that a better understanding can be gained of the various sources of mass discrimination, the causes of instabilities in the system and of aberrations in the particle trajectories through the apparatus. New knowledge gained from such studies makes improved instrumentation possible for new and more difficult applications. The larger manufacturing firms maintain substantial research staffs for this purpose.

It has been emphasised that accurate measurements depend mainly on the operator's ability to calibrate the mass spectrometer with known reference standards. In routine studies, calibration with reference standards is accepted as a normal feature of a planned programme of work; but in studies of a non-routine character, the need for calibration can

sometimes be overlooked, particularly in new applications. Some confusion may then arise, because the mass spectrometer always provides an answer without calibration. If calibration is not undertaken, it is up to the laboratory workers to decide what the answer means. In isotopic work, calibration has been achieved by the use of known synthetic mixtures of previously separated pure isotopes. By this means, Nier[1] has been able to determine for 10 common elements isotopic abundance ratios that are believed to be accurate; probably the work will be extended in due course to many other elements.

In all analytical applications, pure elements or compounds or known synthetic mixtures of compounds and elements are used for calibration purposes. However, in spite of a need to undertake, sometimes at intervals of only a few weeks, the extra operations involved in calibration, the mass spectrometer compares favourably in respect of speed with all other existing methods in analytical work. For example, the analysis of a C_1 to C_5 hydrocarbon gas stream, containing paraffins, olefins, hydrogen, oxygen, nitrogen, carbon monoxide and carbon dioxide, by means of Podbielniak low-temperature distillation methods in conjunction with chemical methods and infra-red spectroscopy (and perhaps Raman and ultra-violet spectroscopy) might take up to 12 hours, whereas with the mass spectrometer a complete analysis of the same mixture could be undertaken in less than 2 hours with modern computing aids. This gain in speed of analysis has been used to great advantage in many large oil refineries throughout the world. For refinery control, both gas and liquid samples are fully examined for an average of 16 components at an average rate of about 700 samples per month per machine. Apart, however, from purely routine work of this nature, machines are used as extensively for analyses of samples arising from process research studies. It is interesting to note that with the experience gained in the operation of standard commercial machines, it has been found possible in at least one organisation to build up a library of calibration data in association with known settings of the instrument controls, and from this information to adjust the output characteristics of the machine to coincide with a previously determined set of calibration data. In this way full recalibration was found unnecessary for a period of 2 years. During this period only spot checks with a specific mixture were made. In the petroleum industry, the mass spectrometer has become, therefore, the first choice as a routine gas-analysis instrument, although it cannot supplant entirely the older equipment.

In spite of the great speed attained in the use of the mass spectrometer, the results are not appreciably less accurate than those of the best of the older methods. For example, with certain exceptions, determinations of the concentration of a component in a complex mixture to 0·3 mole per cent. would be regarded as a good average performance for routine work.

Modern commercial machines can be divided roughly into three classes. In the first class, analytical and isotope-ratio mass spectrometers are combined in one machine for a mass range of about 2 to 100 mass units. Analyses are limited to specimens that exert a minimum vapour pressure at room temperature of about 200 microns absolute pressure. Within these limitations in respect of mass number and vapour pressure, inorganic or organic compounds of almost any class or chemical nature can be analysed. As an isotope-ratio instrument, the ratio of the concentration of two isotopes in a gas, from 0 to 1·0, can be measured directly by the use of dual collectors for simultaneous collection of the two isotopic beams and a balancing electronic network. For isotopic ratios above 0·1 the ratio measurement can be made to six decimal places, with a useful limit in sensitivity of a few parts in 10^6. For example, successive determinations of carbon-13 at the level of natural abundance (ratio of ^{13}C to $^{12}C = 0{\cdot}0110$) on a routine basis would show reproducibility to well within $\pm 0{\cdot}3$ per cent. of the ratio. With well stabilised controls, high repetition accuracy is thus a satisfactory feature of these machines. However, as has been emphasised, absolute accuracy depends on the operator's ability to calibrate with known standards. Finally, it may be added that for a single sample introduction with a molecular flow leak, only about 150 litre-microns of gas are required. The normal gas consumption of a continuously pumped spectrometer is about 10^{-2} lusecs (litre-microns per second), so that measurements should be conducted on a rigid time schedule to avoid errors arising from gradual fractionation of the sample with time.

The second class of machine is used mainly for quantitative or qualitative analyses of mixtures of inorganic or organic gases and liquids, and again the scope of the work that can be undertaken is limited mainly by sample volatility. Components for analysis must be

such as to provide at room temperature a vapour-phase sample at about 50 microns absolute pressure. The maximum mass resolution available lies in the range of 250 to 400 mass units, depending on the variation of design from one manufacturer to another. On one American instrument,[2] it is possible, with maximum resolution adjustment, to separate mass 44·004 of carbon dioxide from mass 44·076 of propane. The high sensitivity available is such that, in favourable mixtures, 0·001 mole per cent. could be detected with certainty, as for example, in the detection of argon in neon.

The third class of machine, which has been developed recently by modification of the second class, permits accurate scanning of the mass scale for molecular ranges of up to 1000 mass units. This high-mass instrument was designed primarily for studies of lubricating oils, waxes and greases containing up to 31 carbon atoms per molecule. The very low vapour pressures exerted at room temperature by these materials prohibit normal handling, so that provision must be made for heating the sample inlet system. The inlet system[3] used is shown in Fig. 1; this is continuously heated at temperatures up to 400° C to get these solids into the vapour phase. The secret lies in the use of molten gallium, which boils at 2000° C, as a valve and as a seal over a sintered-glass disc.

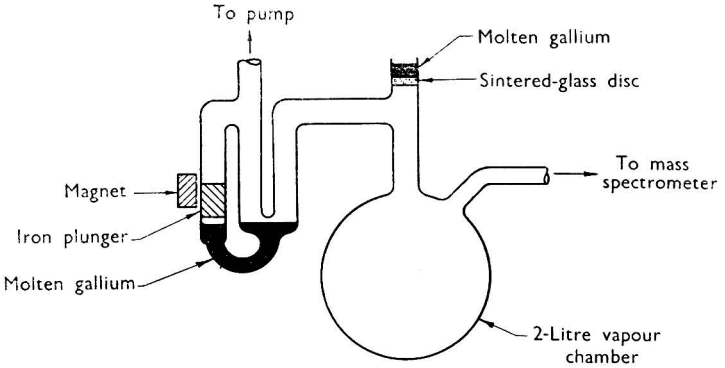


Fig. 1. Heated inlet system for sample introduction

So far, little has been said about the internal working of the orthodox mass spectrometer. To a large extent this is mainly the province of the physicist and instrument designer; but the analytical chemist should have at least a general notion of the mechanism, if only so that he is able to tell the physicist in no uncertain terms what new modifications are required to meet special needs in further development. Operational training is normally given to an intending purchaser, and superficial experience for routine working is quickly gained.

It is apparent from earlier comment that the sample for analysis is introduced in the gaseous or vapour phase into an evacuated enclosure. At the point of entry the gas or vapour enters an ionisation chamber in which a group of positive ions representative of the original material is produced. Normally the ions are produced by bombarding the gas with a stable beam of electrons of closely controlled energy. Some of these ions are withdrawn from the chamber and then accelerated as an ion beam of narrow divergence by a system of collimating electrodes. The ion beam is projected into a magnetic field in a direction at right angles to the magnetic lines of force. The mass sorting process occurs in the magnetic field.

The term "mass spectrometer" is really a misnomer, for, essentially, the instrument produces a momentum spectrum, and it is only by the application of various artifices and devices that the momentum spectrum becomes, to all intents and purposes, a mass spectrum. If the group of ions produced in the ionisation chamber varied in random fashion in both mass and energy, so that no constant relationship existed between the velocity of a particle and its mass, the arrangement would, without additional devices, be useless as a mass spectrometer.

The ion-source system in a conventional spectrometer is arranged, therefore, to produce a group of practically mono-energetic ions. Suppose now these ions acquire their velocity by falling through an electrostatic potential difference, V. We can write—

$$\tfrac{1}{2}Mv^2 = eV \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where M represents the mass of a particle, v its velocity, and the charge on an ion is measured in terms of a single electronic charge, e. As Mv^2 is constant by arrangement, it is apparent that a means of mass selection is presented immediately because a characteristic velocity is associated with each mass number. This is the basis of the radio-frequency mass spectrometer, in which radio-frequency fields are used for velocity selection and hence mass selection. However, these instruments have not yet been applied industrially, and much development work is still needed. In a conventional machine the beam of mono-energetic particles is sent into a magnetic field, H, in a direction at right angles to the magnetic lines of force. From elementary mechanics, it is seen that a particle experiences a centrifugal force Mv^2/r, where r is the radius of curvature of the particle. For equilibrium this must be balanced by the force, Hev, due to the field, or—

$$Mv^2/r = Hev,$$

so that

$$r = Mv/eH \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

It is seen that the radius of the trajectory of each particle is proportional to Mv, and the instrument yields a momentum spectrum. However, by the selected terms of reference

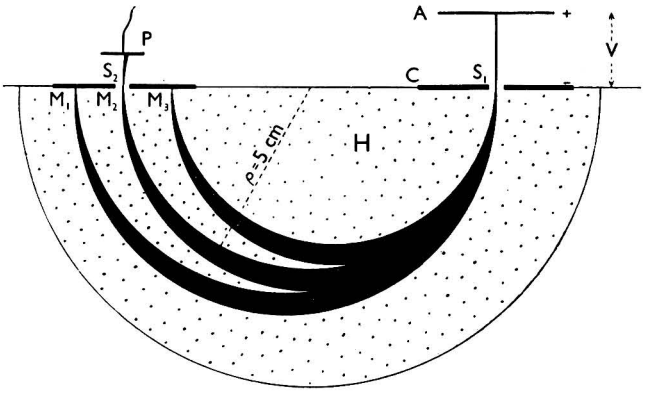


Fig. 2. Schematic diagram of Dempster's mass spectrometer. The dimensions are those given in Dempster's paper[4]

(Mv^2 constant), r becomes proportional to $M^{\frac{1}{2}}$, and the momentum spectrum is converted into a mass spectrum with a definite velocity associated with each mass. Equations (1) and (2) can be combined and v eliminated to give the mass spectrometer equation—

$$M/e = r^2H^2/2V \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

The mass spectrometer differs from the mass spectrograph in two main respects. First, it is a constant deviation spectrometer in that it has fixed entrance and exit slits and all particles have essentially the same trajectories. Either V or H or both are adjusted to bring the beams to the fixed exit slit. Secondly, the beam intensities are measured electrically. There are also other differences. From the three equations given, it is apparent that others can be derived to give expressions for dispersion and mass resolution; but it should not be forgotten that there are many sources of aberration and discrimination in the instrument, so that such expressions really only represent design ideals. For all applications the mass spectrometer should be used as a comparator and should be calibrated in some suitable way.

The various masses describing paths that have different radii in accordance with equation (3) are shown in Fig. 2. It will be noticed that refocusing of the initially diverging beams occurs in the plane of the exit slit, S_2. It is apparent that the modern mass spectrometer depends upon the existence of this refocusing property of magnetic fields. However, for a first-order direction-focusing machine, this refocusing is not perfect, and the focusing error or spherical aberration is found to be $r\alpha^2$, where α represents the semi-angle of divergence of the beam from the source. Fig. 3 shows the ion paths for a mass spectrometer having a 60° sector field. Studies of such trajectories provide working rules for positioning the entrance and exit slits with respect to the magnetic field so as to secure maximum mass resolution. In sector-field instruments, some compensation for fringing flux disturbances

is necessary; usually the set-up is made in accordance with ideal field theory and simply withdrawn, as shown, from the field to compensate for the effect of fringing flux.

The analytical chemist will want to know the relative advantages and disadvantages of the sector-field instrument and the 180° instrument, apart from obvious considerations relating to size and cost of magnet for a particular design. Theoretically, the angle of deviation in the magnetic field in these first-order focusing machines is immaterial from the point of view of resolution. In practice it is found that a certain minimum and by no means inconsiderable magnetic field is required in the region of the source, not only to align the electron beam for impact ionisation, but also to produce satisfactory collimation (small α) of the ion beam. It would be expected, therefore, that in the 180° instrument, in which the region of the source is almost fully immersed in the main magnetic field, greater mass discrimination would occur, but at the same time there would be better collimation of the ion beam with a gain in resolution; this appears to be so.

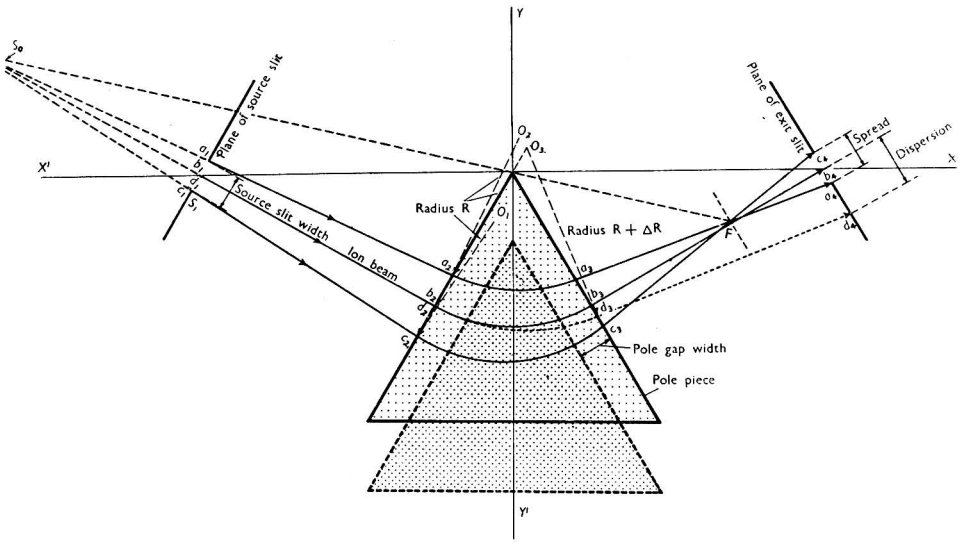


Fig. 3. Magnetic mass selection and focusing of ion beam

It may be helpful at this stage to consider the various component parts to be assembled and correctly inter-related as a complete mass spectrometer (see Fig. 4). It is seen that the complete machine houses a considerable amount of auxiliary apparatus: gas-inlet plant, high-vacuum pumping equipment, stabilised supplies for electron and ion-beam acceleration and for magnet current control, and a sensitive d.c. amplifier, together with automatic recording equipment. A large analytical machine of the 90° sector type, made by Metropolitan-Vickers, is shown in Fig. 5.

Reference was made earlier to the introduction of the sample into an evacuated enclosure. It is apparent that high-vacuum conditions must be established to avoid scattering of the ion beam by many collisions between gas molecules and ions. The working pressure sought in the main vacuum in the analyser is 10^{-6} mm of mercury or less. In the region of the ion source, with its relatively small dimensions, a total gas pressure of up to 10^{-4} mm of mercury on introduction of the sample can be permitted. Many requirements have to be met in the introduction of a complex gas mixture consisting of many components. Perhaps the most important of these are the ensuring of (*a*) a known relationship between the partial pressures of the components in the sample and the corresponding partial pressures of the components in the ionisation chamber, (*b*) a constant composition of the mixture in the gas reservoir during the analysis and (*c*) no interference between components in transit from sample reservoir to ionisation chamber. The last requirement is essential to ensure that a mixture ion beam, consisting of contributions of ionised mass fragments all having the same mass number but arising from different components, should be made up of linear superpositions of individual ion-current intensities, *i.e.*, at any mass number the machine should be linearly additive.

As an ion beam of a particular mass number is swept across the exit slit of the spectrometer, the current to the collector behind the exit slit will first rise, then attain a maximum, and finally fall as the beam leaves the slit. The shape of the curve depends on the relationship between exit-slit width and ion-beam width. A mercury isotope spectrum is shown in Fig. 6.

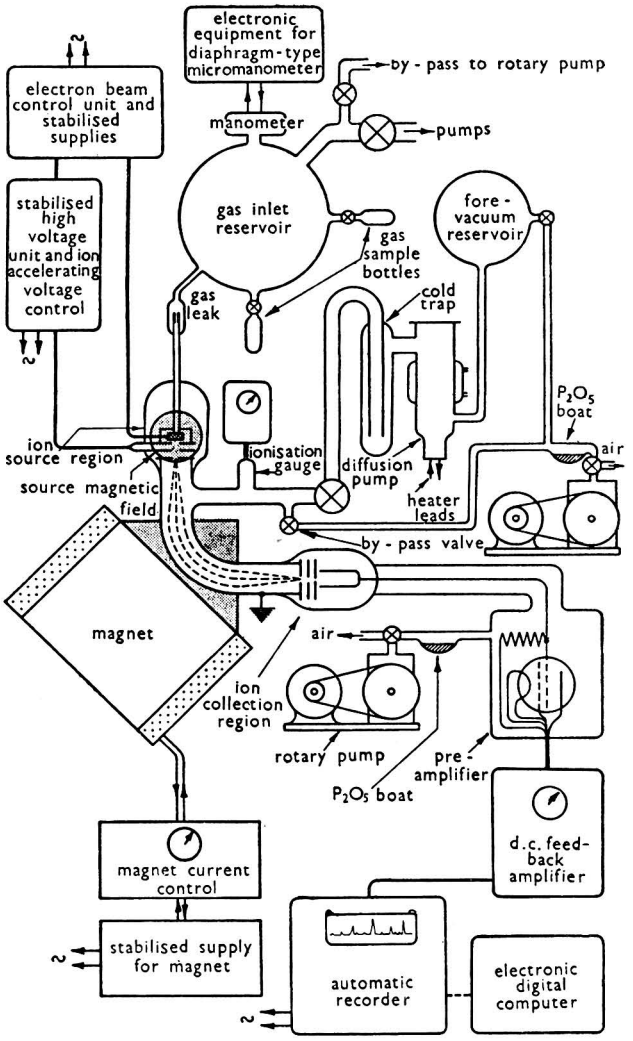


Fig. 4. Schematic relationship of component parts of mass spectrometer assembly

It can be seen that if there are as many peaks as this to deal with, it can be very laborious to determine them all by individual measurements. A considerable strain is put also on the stability of all the controls. The next stage is clearly to have an automatic recorder with automatic range-changing to deal with peaks covering a wide range of heights. This is shown in Fig. 7.[5] With a complex sample, many measurements of peak height may have to be made, and the most recent development is a machine that can make a direct conversion of analogue information—in the form of varying voltages—to digital information, *i.e.*, to numerical form. The recorder then becomes unnecessary. An analytical mass spectrometer made by the Consolidated Engineering Corporation, U.S.A., with analogue-to-digital converter, is shown in Fig. 8.

When a complex molecule is bombarded by electrons of energies of from 50 to 100 eV, the molecule is found to disintegrate into all conceivable fragments, with simultaneous ionisation of the fragments. No theoretical predictions can be made as to the abundances of the fragments. To some extent the cracking pattern depends on the operating conditions in the instrument, owing to mass discrimination. However, for practical analytical work with a chosen set of experimental conditions, it is most important to establish the disintegration scheme for each molecular species and to ensure that the intensities of the

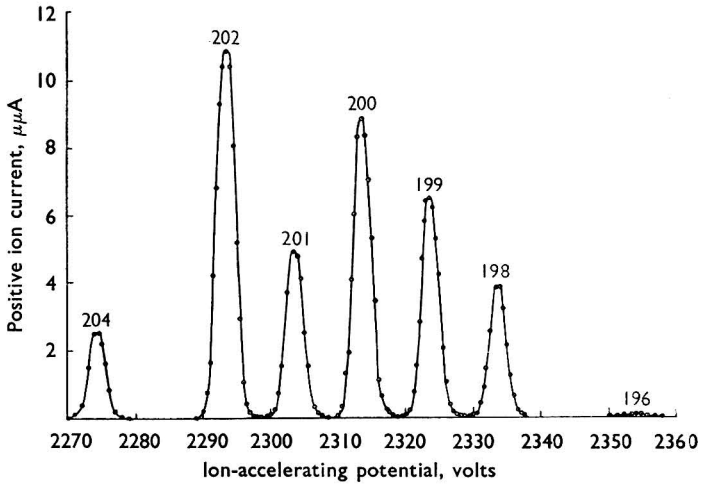


Fig. 6. Mercury isotope spectrum with narrow slits. Pressure $1{\cdot}4 \times 10^{-6}$ mm of mercury

ion fragments relative to the intensity of the parent molecule ion, or to the intensity of the most abundant ion fragment, or to the total ion current summed for all mass numbers, are constants that are characteristic of the species. Characteristic fragmentation for each molecular species permits identification of different molecular species having almost identical mass numbers. This is shown in Table I, in which are given the cracking patterns for nitrogen, carbon monoxide and ethylene.

TABLE I

CRACKING PATTERNS OF NITROGEN, CARBON MONOXIDE AND ETHYLENE

M/e	Nitrogen	Carbon monoxide	Ethylene
1	—	—	4·11
2	—	—	0·51
12	—	4·71	2·14
13	—	0·05	3·52
13·5	—	—	0·42*d*
14	4·32*d*	0·75*d*	6·31
15	0·02*i*	—	0·56
16	—	1·67	—
24	—	—	3·71
25	—	—	11·7
26	—	—	62·3
27	—	—	64·8
28	**100·0**	**100·0**	**100·0**
29	0·74*i*	1·16*i*	2·22*i*
30	0·005*i*	0·22*i*	0·04*i*

d = doubly-charged ion. *i* = isotope peak.

Generally, the way in which a complex molecule disintegrates under electron bombardment is that expected from general chemical experience, although some particular aspects

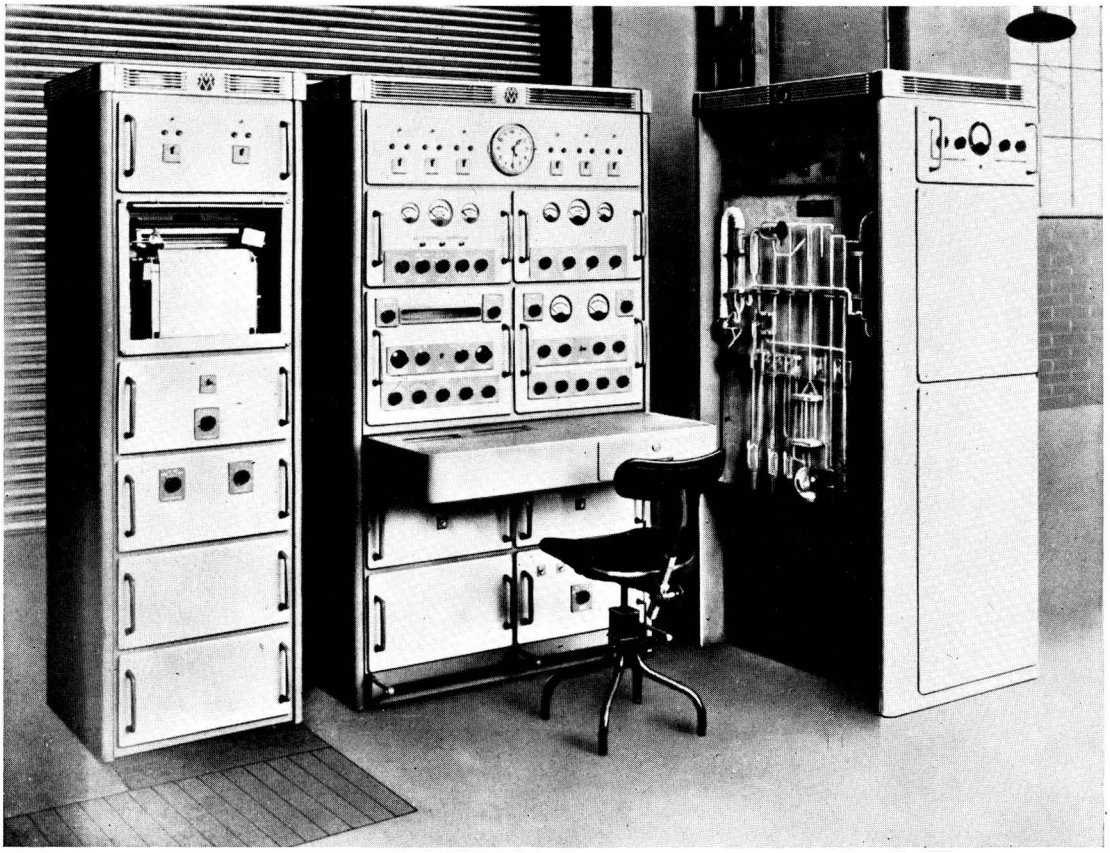


Fig. 5. Metropolitan-Vickers general purpose mass spectrometer, M.S.2. Sample inlet system on right; control panel in centre; recorder on left

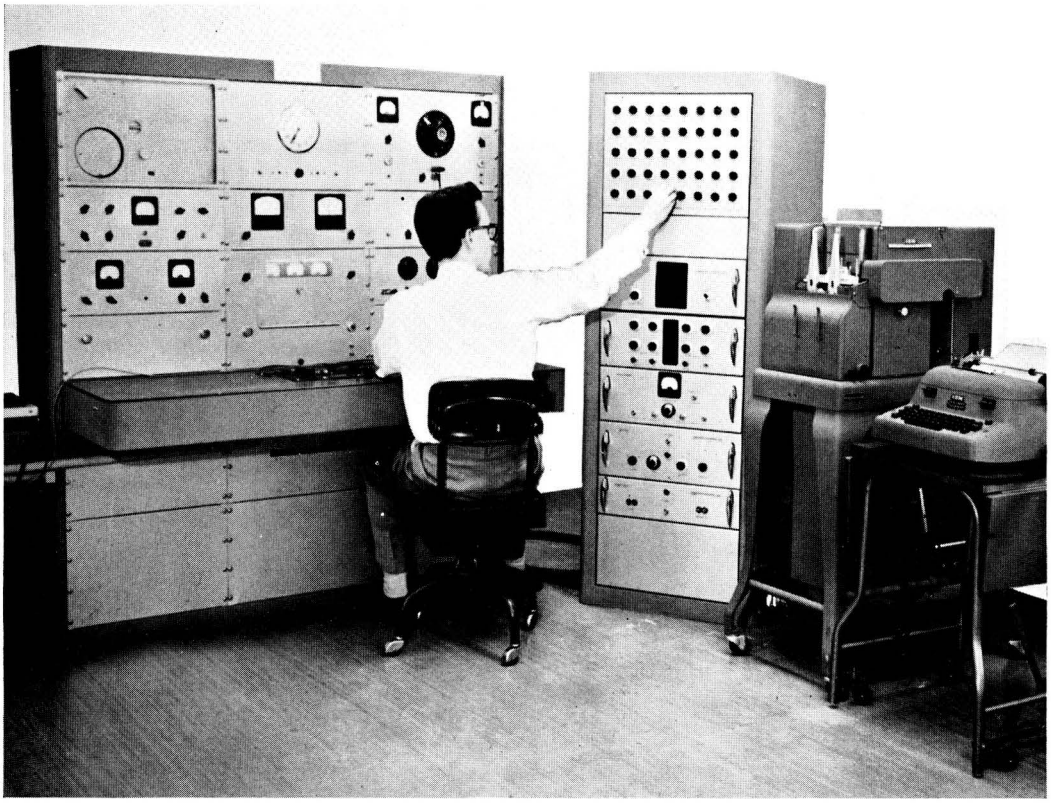
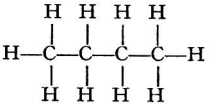


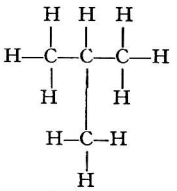
Fig. 8. General purpose analytical mass spectrometer with automatic analogue-to-digital converter, made by the Consolidated Engineering Corporation, U.S.A. A peak selector is also incorporated for pre-programming a run. Measurement of individual peaks on a record is unnecessary

of bond rupture are not clearly understood. Consider, for example, the structural formulae of—



n-butane

and



*iso*butane

It would be expected that these isomers would disintegrate in different ways. In *n*-butane, by the rupture of a single carbon-to-carbon bond, the molecule can split into two equal parts, forming two C_2H_5 fragments of mass 29. This cannot happen in *iso*butane. Hence, in the cracking patterns for *n*-butane and *iso*butane (see Table II), the mass 29 should be more abundant for *n*-butane than for *iso*butane.

However, some isomers have similar cracking patterns and present a large number of ill-conditioned equations for computation. This is particularly true for the individual

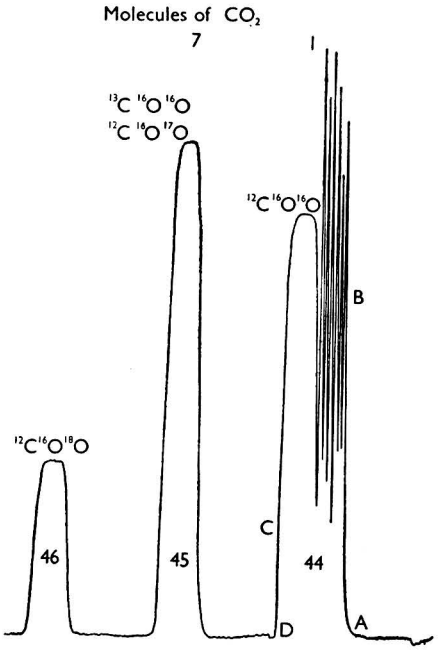


Fig. 7. Automatic record of analysis of carbon dioxide with mass spectrometer, M.S.2

butenes, which generally cannot be separated accurately. This is not a serious limitation, because often knowledge of the total butene content only is wanted.

Table II has been included to give some indication of the computations involved. In the calibration cracking patterns for these five compounds, the relative peak intensities are referred to the most abundant ion fragment as 100. At the bottom of the table, sensitivity factors, *s*, are given. The sensitivity of the reference peak is expressed as the height of the peak in so many divisions for unit pressure of calibration gas in the inlet sample bottle. The requirements for satisfactory analysis are (*a*) constant cracking patterns and *s* values over a reasonably long period and (*b*) linear superposition of the spectral contribution of each component in the composite spectrum for any mixture of compounds.

Suppose now these five compounds are proportioned in some way in a mixture and it is required to find how much of each is present. Let the partial pressures of each be p_1, p_2, p_3, p_4 and p_5, respectively, and s_1, s_2, s_3, s_4 and s_5 be the corresponding sensitivity

coefficients. Then a set of simultaneous equations can be written down directly. There is one such equation for each mass number. For mass 15, for example, the equation is—

$$5{\cdot}30\ s_1 p_1 + 6{\cdot}41\ s_2 p_2 + 6{\cdot}19\ s_3 p_3 + 4{\cdot}57\ s_4 p_4 + 85{\cdot}6\ s_5 p_5 = 167{\cdot}3$$

The five unknowns, p_1 to p_5, are to be found. This is a particularly simple example. It will be observed that increasing numbers of gaps occur in the rows as the mass number increases. It is possible to start at the high-mass end and eliminate the high-mass components first, by choosing for the solution the set of five equations for M/e = 58, 57, 44, 30 and 16. There are some problems in procedure here. For example, is the chosen set the best? Which set of five equations should be chosen? Or would it be better to use all the available data and obtain a solution by a weighted least-squares method? There is a slightly different answer each time. How should the best solution be defined? These are some of the problems studied in recent publications.[6,7] The computations can become quite complicated in some circumstances, and modern computational aids are necessary to reduce the labour involved. If a typical mixture is presented many times for analysis, *i.e.*, if the same components recur in various concentrations and if the calibration data can be taken as constant, then it is not necessary to undertake the full computation each time. The procedure is to set up an inverse matrix, valid for the duration of the work, and to substitute each time the new mixture peak-heights.

TABLE II

CALIBRATION DATA AND MIXTURE PEAK-HEIGHTS FOR DEPROPANISER OVERHEAD SAMPLE

M/e	*n*-Butane	*iso*Butane	Propane	Ethane	Methane	Mixture, peak heights
15	5·30	6·41	6·19	4·57	85·6	167·3
16	0·12	0·18	0·15	0·08	100·0	134·2
26	6·17	2·36	8·59	23·0	—	146·1
27	37·1	27·8	39·4	33·3	—	365·6
28	32·6	2·62	59·1	100·0	—	757·8
29	44·2	6·16	100·0	21·7	—	613·1
30	0·98	0·13	2·20	26·2	—	130·1
31	—	—	—	0·55	—	2·4
38	1·89	2·77	5·29	—	—	29·0
39	12·5	16·5	17·0	—	—	98·0
40	1·63	2·37	2·52	—	—	14·7
41	27·8	38·1	12·7	—	—	94·1
42	12·2	33·5	5·82	—	—	49·9
43	100·0	100·0	22·8	—	—	207·4
44	3·33	3·33	29·0	—	—	145·4
45	0·05	0·03	0·88	—	—	4·6
50	1·29	0·89	—	—	—	0·6
51	1·05	0·74	—	—	—	0·5
52	0·26	0·15	—	—	—	0·1
53	0·74	0·50	—	—	—	0·6
54	0·19	0·07	—	—	—	0·1
55	0·93	0·42	—	—	—	0·7
56	0·72	0·34	—	—	—	0·5
57	2·42	3·00	—	—	—	2·5
58	12·3	2·73	—	—	—	5·0
59	0·54	0·11	—	—	—	0·2
s	0·396	0·464	0·335	0·392	0·246	—

APPLICATIONS OF THE MASS SPECTROMETER

The mass spectrometer has been applied successfully to a wide range of work, including the determination not only of hydrocarbons, but also of oxygenated compounds, for example, alcohols, ethers and esters, of halogenated compounds—alkyl chlorides and iodides—and of many types of sulphur compounds. It has been suggested also that gas analysers of this type could be used as primary elements in closed feed-back loops to give, with servo-operated equipment, complete automatic control of a process. No gas analyser has been used as yet

for automatic process control. But as a first step towards this goal, mass spectrometers have been used for continuous monitoring of a process stream to provide information whereby an operator can detect off-specification conditions and then take manual corrective action to restore the plant to the specified state.[8] The mass spectrometer has no rival for this purpose. In the chemical industry, for example, mass spectrometers have been used for furnace control in the production of acetylene by the Wulff process, the first step in producing the newest synthetic fibres, Dacron (Terylene) and Dynel.[9]

A number of recent publications[6,10,11] give much wider surveys of routine work than is possible here. It is proposed, therefore, to deal only with a few recent applications with special research instruments to indicate the trend of future development.

Analysis of Stainless Steels

An application of the mass spectrometer in a different sphere is its use for the analysis of stainless steels.[12] The method has not yet been applied on a routine basis for the analysis of alloys, such as steels, but it is already apparent that the mass spectra of metals and their alloys are much less difficult to interpret than, say, the emission spectra of the metals. There is, therefore, considerable industrial interest in this development for routine work.

The best method of producing the required positive ions from a solid material of this nature is by use of the high-frequency vacuum-spark source developed by Dempster in 1934. The material to be analysed forms one electrode of a high-voltage spark gap and is usually in the form of a rod of 0·025 inch diameter. When the necessary potential is applied across the gap to cause electrical break-down, some of the electrode material is evaporated into the space between the electrodes, where it is ionised by the electrons in the discharge. The discharge is maintained between the sample rod and a tantalum disc by a pulsed radio-frequency supply, obtained either from a Tesla coil or pulsed electronic oscillator. This type of source has the advantage that it has no blind spots; that is, all elements present are ionised with roughly the same efficiency. There are several disadvantages with a source of this type, and these for a long time restricted its use to special researches with mass spectrographs. Quite recently, however, it has been demonstrated that, by suitable design, the source can be used for the analysis of solids by mass-spectrometric methods. Analyses of six samples of stainless steel by mass-spectrometric methods and by existing established analytical methods revealed excellent agreement. This is shown in Table III.

Table III

Analyses of Samples of Stainless Steel

	Chromium		Nickel		Iron		
Sample	Chemical, %	Mass spectrometer,* %	Chemical, %	Mass spectrometer,* %	Chemical, %	Mass spectrometer,* %	Other elements by chemical methods, %
X3534	23·7	23·4 ± 0·4	13·6	13·7 ± 0·5	61·6	61·8 ± 0·4	1·1
X3380	18·2	19·3 ± 0·3	8·6	8·7 ± 0·2	69·8	68·6 ± 0·3	3·4
X3275	13·4	13·7 ± 0·2	0·37	0·25 ± 0·01	83·0	82·8 ± 0·3	3·2
X3532	9·1	9·1 ± 0·2	0·57	0·56 ± 0·02	85·5	85·5 ± 0·3	4·8
X3533	5·5	5·5 ± 0·1	6·8	7·1 ± 0·1	84·3	84·0 ± 0·1	3·4
X3522	2·95	2·97 ± 0·05	25·7	25·6 ± 0·3	70·0	70·1 ± 0·1	1·3

* Mean value and average deviation of 4 or 5 determinations.

The main disadvantages to be overcome in the use of the vacuum-spark source in mass spectrometry are that (*a*) ions are produced with a very large energy spread, so that a conventional single-focusing mass spectrometer cannot be used, (*b*) the intensities of the ion beams fluctuate so wildly that electrical measurement by normal methods becomes impossible and (*c*) electrical interference between the spark source and sensitive electrometer amplifiers necessitates elaborate screening precautions. The instrument used is shown in Fig. 9. The large energy spread makes the source suitable for use only with the rather more complex double-focusing mass spectrometer of, say, the Mattauch or Dempster type, in which the beam dispersion in the magnetic field caused by velocity spread is compensated exactly by an equal and opposite dispersion in a preceding electrostatic field. Double focusing

in respect of velocity and direction is thus achieved. However, the source is still erratic and subject to wild fluctuations; and this calls for some ingenuity in method. Here slit S_4 is used not only as a slit but also in part as a monitoring collector. A constant fraction of the total ion current emitted by the source is collected on the edges of S_4 and fed into a d.c. amplifier. In the usual way an ion beam of any particular mass number is focused on to S_5 and collected. The current collected by the plate behind S_5 is fed into a second amplifier, and the outputs of the two amplifiers are compared on an automatic recorder. Hence, although the ion beam from the spark source is fluctuating, the outputs of the two amplifiers fluctuate in unison, so that the ratio of the two signals is independent of source fluctuations. When the magnetic field is scanned, the ratio of the selected beam current to the total beam

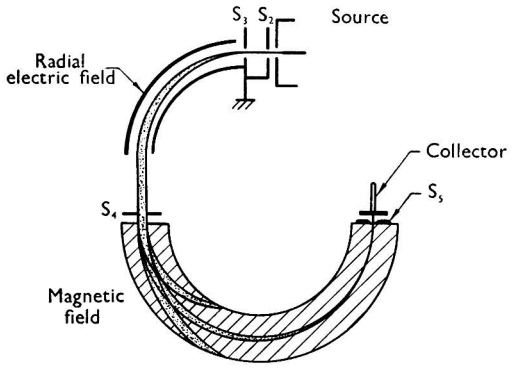


Fig. 9. Dempster-type double-focusing mass spectrometer

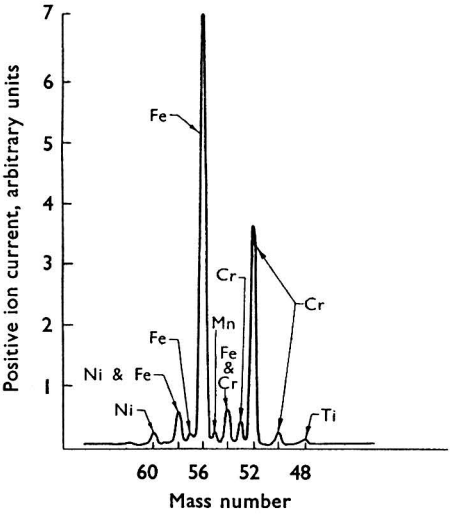


Fig. 10. Mass spectrum of stainless steel sample No. X3380, 7/24/50

current varies according to the intensity of the selected mass peak, and the recorder chart plots a mass spectrum similar to that obtained with a conventional instrument (Fig. 10). Here again the mass spectrometer is used as comparator and is first calibrated with known synthetic samples to obviate errors due to mass discrimination in the instrument. To determine the chemical composition, corrections have to be made for the known isotopic distributions, and the analysis shows the relative distribution of the components in the original material. In the results shown in Table III everything was referred to iron, which was used, in effect, as an internal standard.

Determination of trace elements in solids

In orthodox mass spectrometry with the best mass analysers now in use, the limit of sensitivity is about 10^{-6}. But there are to-day many analytical problems in which much higher sensitivities are required. For example, consider the germanium rectifier and transistor, in which more than 1 part per million of arsenic as impurity is sufficient to reduce the maximum back-voltage for negligible current flow (or turnover voltage) from about 100 volts for the purest specimens to less than a few volts. Therefore much more sensitive methods of analysis are required.

The isotope dilution method has been known for a number of years, but has been used only comparatively recently for impurity work.[13] The method was developed originally by Rittenberg[14] for the quantitative analysis of complex mixtures (particularly amino-acids) encountered in protein studies, and it depends on the fact that the usual chemical procedures do not result in significant fractionation of isotopes.

It is not proposed to discuss its use in biological work, but to indicate how it has been applied, with essentially similar principles, to trace element determinations in solids. For this work it has become a practical analytical method with sensitivities for many elements

far surpassing anything previously known. This may, perhaps, be illustrated best by considering a hypothetical example.

Suppose it is required to determine the amount of an element, E_0, present as a trace impurity in a solid, D. Suppose also that element E_0 has two stable isotopes, I_1 and I_2, with a normal abundance ratio of $R_0 = I_2/I_1$. Now the atom per cent. concentration of isotope I_1 in element E_0 can be defined as—

$$\frac{\text{Number of } I_1 \text{ atoms} \times 100}{\text{Number of } I_2 \text{ atoms} + \text{Number of } I_1 \text{ atoms}},$$

or

$$\text{Atom per cent. of } I_1 = \frac{100}{R_0 + 1}.$$

Suppose now a small quantity of the pure element E is available enriched in respect of isotope I_1 to give an isotopic abundance ratio of $R = I_2/I_1$. Then the atom per cent. excess concentration of I_1 over the normal is given by—

$$C \equiv \text{Atom per cent. excess of } I_1 = \frac{100}{R + 1} - \frac{100}{R_0 + 1}.$$

The experimental procedure is as follows. To a measured weight, Mg, of solid, D, add a small quantity, Wg, of the pure element E enriched in respect of isotope I_1. These are now brought into solution, so that the tracer element E mixes with any normal element E_0 present in solid D to form an inseparable mixture so far as the usual chemical procedures are concerned. From the mixture isolate a small quantity of pure element E' and measure the isotopic abundance ratio, I_2/I_1. Suppose that this ratio is R'.

Clearly, if R' is not equal to R, the normal element E_0 is present in solid D, and dilution of the tracer element E has occurred. The new atom per cent. excess concentration of isotope I_1 over the normal is given by—

$$C' = \frac{100}{R' + 1} - \frac{100}{R_0 + 1}.$$

Let W' g be the weight of the normal element E_0 present in weight Mg of solid D, and let A_0 be the atomic weight of the normal element E_0 and A the atomic weight of the enriched element E. The relation between C and C' is given by—

$$C \times \frac{W}{A} = C'\left[\frac{W}{A} + \frac{W'}{A_0}\right],$$

or

$$W' = \frac{C - C'}{C'} \times \frac{A_0}{A} \times W$$

or

$$W' = \frac{R' - R}{R_0 - R'} \times \frac{R_0 + 1}{R + 1} \times \frac{A_0}{A} \times W,$$

on substitution for C and C'. In practice, except when the tracer element E is very appreciably enriched in respect of isotope I_1, A_0/A may be taken as unity to a first approximation.

A hypothetical example can now be considered. For the normal element E_0, let the atom per cent. of I_1 be 20·0, and for the tracer element E, let it be 40·0. Then $R_0 = 4{\cdot}0000$ and $R' = 1{\cdot}5000$. As a difference of 0·0001 in relative abundance ratio can be detected with certainty, it is apparent that an amount W' as low as $0{\cdot}0001W$ can be detected. If initially $M = 1$ g and $W = 1$ μg, then an amount W' of 10^{-10} g can be detected. In fact, it has been found that many of the elements can be detected with as little as 10^{-12} g of material present, while some elements, for example, potassium, can be detected easily at a concentration of about 10^{-14} g. These high sensitivities have been attained so far only with special research instruments; but it should be remembered that the method was developed originally for use with an ordinary gas machine. Trace element determinations in a lower sensitivity range, say 0·01 to 10 p.p.m., by use of this method would appear to be possible with conventional machines.

The advantages of this method are that (*a*) the final result does not depend on the complete quantitative recovery of the element; for, in the example considered, if a certain percentage of I_1 had been lost in the processing, the same percentage of I_2 would also have been lost, (*b*) the method is absolute and (*c*) the method is extremely sensitive to trace

impurities. There are, of course, some disadvantages. The first is that only one element can be dealt with at a time. Secondly, the separated isotopic tracers must have isotopic compositions considerably different from normal, and some restrictions exist on their use by intending purchasers. However, sometimes high sensitivity can be attained with quite a small degree of enrichment, as indicated by the above example. If, therefore, an analytical laboratory is solely interested in a particular impurity determination, it may well be possible for such a laboratory with its own facilities to build a small plant[15] to provide the tracer material. A third disadvantage is that some elements do not have a second stable isotope that can be used as the diluent. However, sixty-seven elements can be determined in this way. A fourth disadvantage results from problems of contamination. If, for example, 1 milligram of ordinary dust became mixed with the sample at any time during the chemical processing, considerable errors might arise.

Other impurity problems

The detrimental effect of 1 p.p.m. of arsenic as impurity in germanium has already been noted. Unfortunately, arsenic has no second stable isotope, so that the isotope dilution method cannot be used. It may be noted that the chemist is also interested in trace quantities of boron in germanium, and for its determination the isotope dilution method can be applied.

The hope of using the mass spectrometer for determinations of traces of arsenic has not, however, been abandoned, and attention has been directed to physical enrichment processes,[16] whereby the impurities can be drawn out of the sample and concentrated in very small zones, so that the mass spectrometer has a more concentrated sample for analysis. This has been carried out in a gradient furnace. The raw material is melted in a carbon crucible of high purity and is then permitted to cool gradually while the crucible is lowered slowly out of the furnace. Impurities that lower the melting point of germanium concentrate in the top zones of the ingot, which are the last to solidify; the main body is purified, and impurities that raise the melting point of germanium are concentrated in the bottom. It has been found that the concentration of impurities in the top may be increased a hundred-fold in a single operation, and it is believed that routine analyses of arsenic in germanium should soon be possible to within 1 part in 10^8.

Another impurity problem of quite a different character arises in water pollution abatement work, particularly in the determination of minute quantities of contaminants—mainly hydrocarbons—in drinking water. Recently a method for applying the mass spectrometer to this work was described.[17] Briefly it consists in taking a 1·5-litre sample of water and stripping the volatile compounds from the boiling water by passing purified hydrogen gas through it. Hydrocarbons boiling below 400° F are carried over and condensed in a liquid-nitrogen trap. The condensate is then analysed in the mass spectrometer. The method is now being used to determine gaseous and liquid hydrocarbons, boiling up to at least 400° F, in the range 0·01 to 100 p.p.m. in water. The sensitivity depends, of course, on the volume of sample-water taken.

Conclusion

It is apparent that, in respect of speed, accuracy and versatility, the mass spectrometer has rapidly become a powerful analytical instrument. Much development work continues and new applications are being reported frequently. The main deterrent in acquiring such an instrument is often one of cost in comparison with the older physical instruments for chemical analysis. For a particular analytical laboratory, the main basis of assessment must be on the saving of man-hours without loss of accuracy and the greater facilities afforded for a wider range of work.

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TEDDINGTON, MIDDLESEX

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An Automatic Coulometric Titrimeter

BY N. BETT, W. NOCK AND G. MORRIS

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

Coulometric titrimetry in which the titrant was generated externally was examined by manual methods, and the experimental error was shown to be about 0·1 per cent. for titrations of 25 ml of 0·01 *N* acid or alkali. For the titration of 0·1 *N* solutions, a modified form of electrolysis cell and a specially stabilised current supply were devised.

As coulometric analysis depends on the flow of an electric current, it is particularly suitable for automatic operation. An automatic titrimeter was constructed and by its use 25 ml of 0·1 *N* acid or alkali were titrated in about $4\frac{1}{2}$ minutes; the standard deviation was less than 0·2 per cent.

When an instrument capable of recording a quantity of electricity by integration is available, there is no need to stabilise the electrolysis current, and a different speed from that of the titration can be used in the approach to the end-point. An automatic titrimeter incorporating such an integrator is described in detail.

Titrations involving the generation of iodine are described, and the results were satisfactory.

When the automatic titrimeter was used in a routine analytical laboratory, the results were found to be reproducible to within $\pm 0{\cdot}2$ per cent.

IN recent years an analytical technique has been developed in which titrations are performed with an electrolytically generated titrant. This procedure is known as coulometric analysis and has been used as a manual method, particularly when a few micro-equivalents of material had to be determined. This paper records an attempt to apply coulometric analysis in routine factory analytical work. Coulometric analysis has certain advantages over conventional volumetric analysis, especially if it is required that the titration result be recorded. In addition there is no burette to be filled and adjusted, and standard solutions are unnecessary.

To compete with volumetric methods the electrolytic generation must be sufficiently rapid to titrate 25 ml of decinormal acid or alkali in 5 minutes or less.

One of the earliest uses of coulometric methods was made by Szebelledy and Somogyi,[1] who standardised volumetric solutions by means of a silver coulometer. More recently Furman, Cooke and Reilley[2] have shown how to produce ceric ions quantitatively, and micro-determinations have been made of vanadium,[3] thallium,[4] iron,[5] arsenic,[5] manganese,[6] silver[7] and many other substances. In addition to ceric ions, iodine,[8] chlorine,[4,9,10,11,12] bromine,[4,9,10,11,12] ferrous ions,[6,13] hydrogen ions[14] and hydroxyl ions[14,15] have been produced in small quantities and in measured amounts by an electric current. The development of sensitive end-point procedures[16] has made coulometric titration a valuable asset in micro analysis. Internal generation was used exclusively in the above processes, but DeFord,

Pitts and Johns[17] have demonstrated the production of acid, alkali and iodine in quantitative amounts in an external cell and have shown that automatic titrations[18] can be performed on this basis. This paper is solely concerned with external generation.

Preliminary experiments

As the quantities of titrant to be handled were much greater than usual, preliminary experiments were made to ascertain whether the necessary reproducibility would be maintained.

A cell similar to that used by DeFord, Pitts and Johns[17] was fed with a 1 per cent. solution of sodium sulphate in distilled water, the pH being adjusted to 4·0 (that of the chosen end-point); the solution was conveyed to the cell by a short length of rubber tubing provided with spring and screw clips for flow control. The sample beaker was supported by a Baird and Tatlock magnetic stirrer in such a way that it received the discharge from one of the delivery arms of the cell, the contents of the other arm being drained to the sink. It was essential that the delivery tip of the cell should not come into contact with the sample in the beaker, as this would affect the pH-electrode indication.

The progress of the titration was indicated by measurement of the pH of the sample with a glass electrode.

The electrolysis current was taken from a current-stabilised source and was measured by the potential drop across a standard resistor.

The time was measured with a stopwatch.

Manual titrations at a concentration of 0·01 *N*—

It was found that, when the efficiency of the cell was 99·6 per cent., 25 ml of 0·01 *N* sulphuric acid could be titrated to the end-point at pH 4·0 in 174 seconds by a current of 139 mA. The standard deviation of 12 determinations was 0·08 per cent. The efficiency was remarkably high.

In all the work reported here the instrument was standardised against chemical standards so that the absolute value of its efficiency is not important; its reproducibility is essential for accurate titration.

Development of a cell for titrations with 0·1 *N* solutions—

In order to titrate decinormal samples in a reasonable time, it was necessary to increase the generating capacity of the cell. A current stabiliser that could supply current over the range from 200 mA to 1 amp. was constructed. Several designs of cell were tried in a attempt to increase the conductivity and improve the arrangements for disposal of the gas evolved during electrolysis. These cells were no better than the cell of DeFord, Pitts and Johns. With this cell, the electrolysis current was increased until the efficiency of the cell began to drop; the highest useful current was found to be about 300 mA. A cell to carry 1 amp. was then made by joining three of these cells together.

When the cells were operated with a current of 300 mA each, the concentration of the electrolyte had to be increased to reduce the cell resistance and so reduce heat generation. The concentration of the electrolyte was finally increased to 100 g of sodium sulphate per litre of water. The heat generated during a titration was the greatest obstacle in cell design, as it determined the minimum rate of electrolyte flow permissible if a serious rise in temperature was to be avoided. It was also important that the space around the platinum electrodes should be kept small to prevent large bubbles of gas accumulating and to reduce the amount of "dead liquid" to be washed out at the end of a titration.

It was found that the conductivity of the cell was almost independent of the rate of flow of electrolyte and therefore, while this could not be reduced much below 5 ml per minute at each delivery arm, there was no great advantage in increasing it.

Automatic titration—

Many workers have described automatic devices for volumetric titrations. Some of these use calibrated motor-driven syringes, while others use conventional burettes with electrically operated taps. Titrations with the former take an unacceptably long time, while of the great variety of taps proposed, not all are acceptable. In addition, the use of a conventional burette requires it to be set up initially and the remaining volume read, an operation that requires some skill and cannot, at present, be recorded at a distance.

From the point of view of automatic titration, coulometric methods have the advantage that the titre is recorded as a quantity of electricity; this is easier to control and record than a volume of solution.

A first model automatic titrimeter was built in which the current from a stabilised source was passed through the triple cell until the pH, measured by a glass electrode and calomel cell, reached a value preset so that the final pH of the titration when the cell was flushed with electrolyte after the current was cut off was the required end-point.

The indicator of the pH meter was fitted with a modified form of a Fielden Tektor so that the electrolysis current was started manually and was stopped by the Tektor relay. The time of current flow was recorded by a stopwatch electrically operated by the electrolysis current.

In this form the titrimeter was used in a routine analytical laboratory for some time, and it was exhibited at the International Congress on Analytical Chemistry at Oxford in September, 1952.

When a current of 923 mA was passed, 25 ml of 0·1 *N* sulphuric acid, which had been standardised with bromophenol blue as indicator, were titrated automatically in 275 seconds.

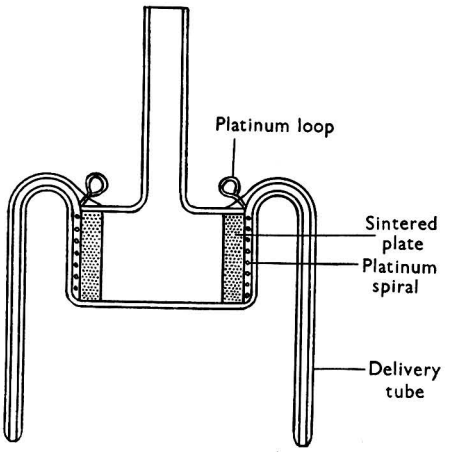


Fig. 1. Section of cell in its final form

The standard deviation of the results from a typical set of 11 determinations was 0·13 per cent. This was not quite so small as for the manual experiments, but was fully satisfactory for routine laboratory tests. The efficiency of the cell was only 95·6 per cent., and the rise in temperature in the titration sample averaged 14° C.

The electrolysis cell—Considerable trouble was experienced owing to clogging of the electrolysis cell by small particles of glass-wool and so on. The temperature rise in this cell was excessive. A cell was constructed in which the glass-wool used in previous models was replaced by two sintered-glass discs of porosity 1. The platinum electrodes were flat spirals, $\frac{1}{2}$ inch in diameter, that lay on the outside of the partitions. The cell in its final form, which was made to our specification by Messrs. Mullard Ltd., is shown in Fig. 1. It was large enough to pass a current of 1 amp. without a serious rise in temperature, the heat dissipation being about 60 watts. The essential features of the design were the small end-volumes, *i.e.*, hold-up volumes on the outside of the sintered plates, and the elevation of the exit tubes from the electrode chambers. The latter feature caused the gas bubbles to move away rapidly into the narrow delivery arms where the speed of the flow prevented large gas bubbles from forming. It also allowed the electrodes to be completely surrounded by electrolyte at all times.

The integrating motor—A device that can accurately integrate the generating current in a coulometric titration will greatly simplify the design of a titrimeter. A short time after the first titrimeter was constructed, an integrating motor made by Electro Methods Ltd. became available.

This apparatus consists essentially of a d.c. motor in which friction and heat loss have been reduced to a minimum. The rotation of the armature shaft is geared to drive a light

mechanical counter. The integrating property of the motor depends on the linear relationship between the speed of rotation of the shaft and the applied voltage. If this voltage is derived from the potential drop across a standard resistor through which the electrolysis current is passing, the counter reading will be proportional to the quantity of electricity that has passed through the resistor and hence through the cell. The resistor, being only about 1 ohm, provides adequate damping for the motor and ensures good response to rapid changes of input voltage.

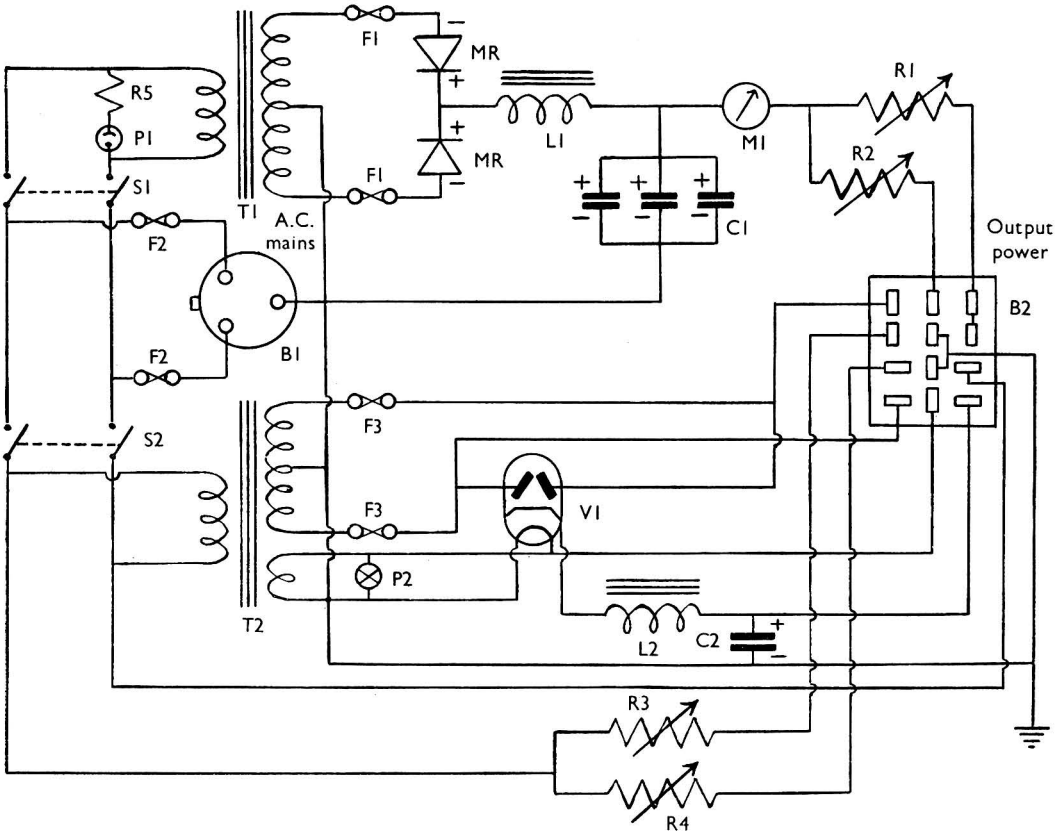


Fig. 3. Power supply

An integrating motor for 1·5-volt operation was obtained and its performance was investigated. The accuracy of integration proved to be $\pm 0{\cdot}1$ per cent. over the range 0·25 to 1·5 volts. When tested for a continuous run of 400 hours, the motor behaved satisfactorily.

Two major improvements in design could now be made: (*i*) the electrolysis current no longer required to be stabilised; and (*ii*) the electrolysis current could be decreased near the end of a titration to allow a slower approach to the end-point.

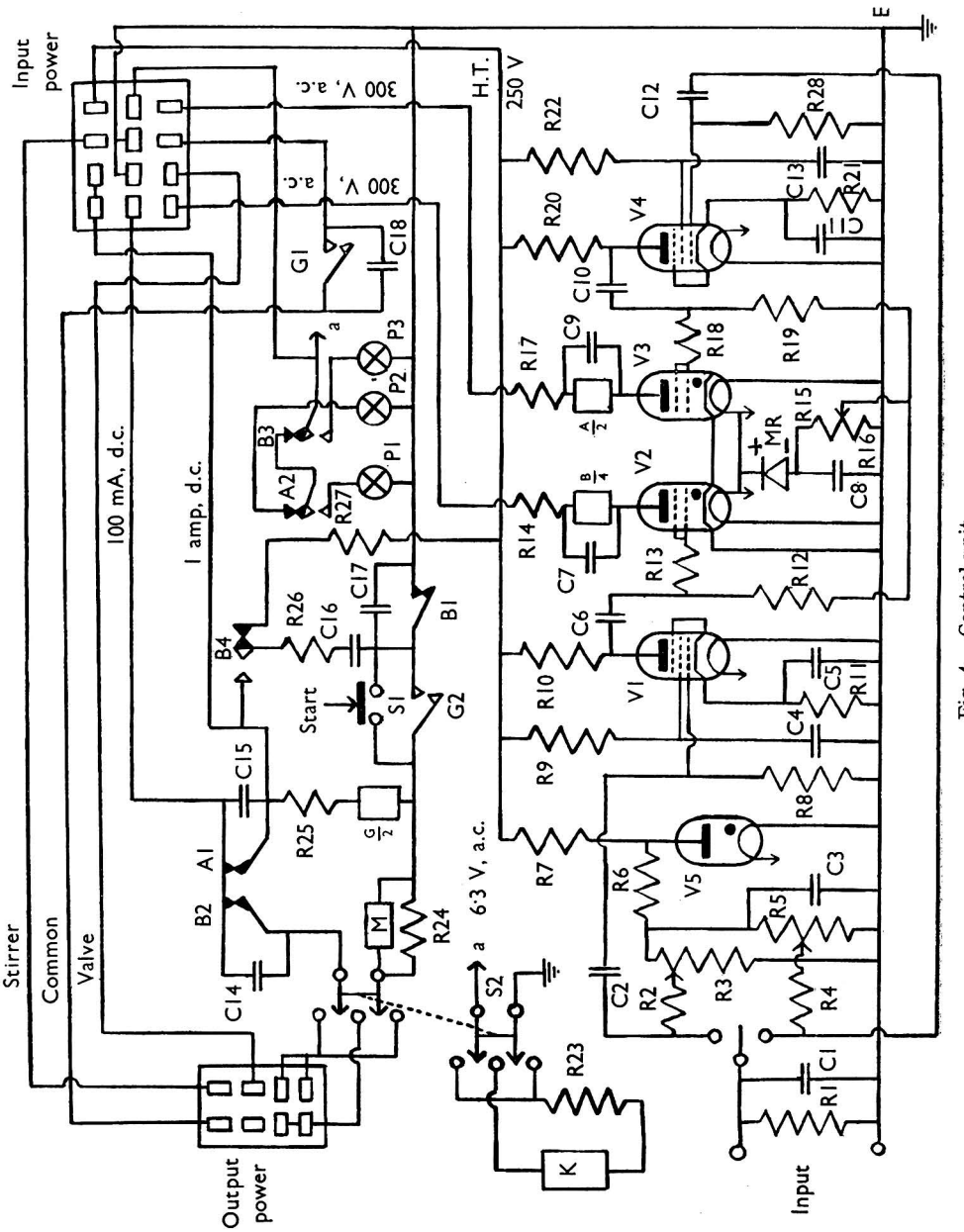
The automatic coulometric titrimeter

The automatic titrimeter will now be described in detail. A commercial pH meter was used, as it was considered to be an added advantage to have the pH meter as a separate unit. Fig. 2 is a photograph of the complete apparatus, which is seen to consist of four parts: the power supply, control unit, pH meter and titration stand.

The power supply—The power supply, the circuit diagram of which is shown in Fig. 3, consists of two independent units, each with its own mains switch. The smaller unit provides high-tension current at 250 volts d.c., two supplies of 300 volts a.c. in antiphase, 230 volts a.c. from the mains and 6·3 volts a.c. for the valve heaters. The larger unit supplies the titration

Fig. 2. The complete automatic coulometric titrimeter

current at two levels, each level being capable of independent variation by means of the toroidal rheostats, R_1 and R_2. The circuit is designed to deliver a maximum electrolysis current of 1·5 amp. at 100 volts.



The control unit (*Fig.* 4)—This instrument is designed to monitor the input voltage from the pH meter (derived from the socket marked RECORDER) and to close the relays A and B in turn at two pre-determined voltage levels. This is achieved by comparing the input voltage with the voltages on the sliders of two helical potentiometers, R_3 and R_5, making use of a Carpenter relay operating at 50 cycles per second. Hence from each side contact

of the Carpenter relay a square wave is obtained, the amplitude and phase of which is determined by the input voltage and the setting of the appropriate helical potentiometer. The two waveforms are fed into two separate but identical phase-sensitive amplifiers that operate their respective relays when the input waveform changes phase. To ensure that the response of the thyratrons, V_2 and V_3, during a phase change in the input signal is sharp, it is necessary to provide from 4 to 6 volts negative bias (obtained across R_{16}). The circuit will detect changes in the input voltage of about 1·5 mV; this represents a change in pH value of 0·05 in the input to the pH meter.

Relay A selects the low value of titration current when energised, while relay B in closing shuts off the titration current altogether. These operations are indicated by three dial lamps, green, yellow and red. A third relay, G, serves to prolong the stirring and flow of

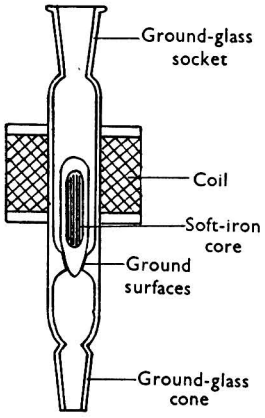


Fig. 5. Solenoid valve

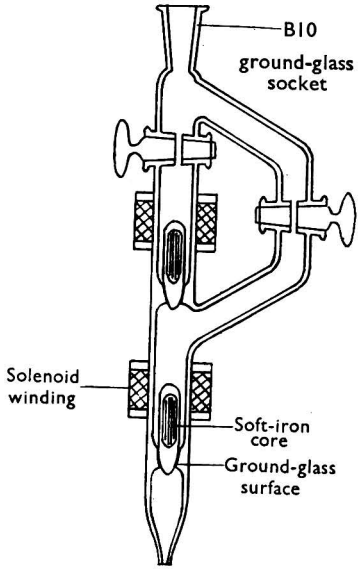


Fig. 6. Automatic burette tap

electrolyte for about 8 seconds after the titration current has ceased, for reasons previously explained. The delay network, R_{26} and C_{16}, provides the necessary time lag. This relay also breaks the titration current line so that, once stopped, the titration can only be started again by pressing the push-button marked START.

The switch, S_2, reverses the polarity of the electrolysis cell so that samples of alkali can be titrated as well as acid; S_2 also reverses the phase of the Carpenter relay so that the switching sequence of the electrolysis current is reversed.

The front panel of the unit carries three lamps. When the instrument is ready for use or is running at full current, the green lamp is lit. When the instrument is operating at reduced current, the yellow lamp shines, while the red light indicates that titration is complete.

The pH meter—This is of the direct-reading type made by Electronic Instruments Ltd., having a scale with pH values from 0 to 14. A constant current output of 20 μA per pH unit is available at the socket marked RECORDER; this is an adequate signal with which to operate the control unit.

Provision is made for temperature compensation because of the large rise in temperature observed in a sample during titration. The ordinary type of temperature compensation is unsuitable because it does not compensate for zero errors but merely provides scale correction. A special compensating unit was obtained from the makers, and it could provide temperature compensation at a fixed pH.

The titration stand—A double-posted retort stand is used to support the various components, the uppermost of which is a sintered-glass filter of porosity 2, introduced to protect the sinters of the cell from clogging. The magnetic flow valve is a glass type of simple

construction, as shown in Fig. 5. The coil consists of 5000 turns of 36 S.W.G. enamelled copper wire and requires a potential of about 40 volts a.c. for operation. Below the valve and joined to it by a ground-glass socket is the electrolysis cell, which is supported on a light brass platform. The electrical connections to the valve and cell terminate in Belling-Lee plugs and sockets on the base of the stand and these are wired underneath to a Jones connector that receives the output from the control unit. The apparatus is easily dismantled for cleaning purposes.

OPERATION OF THE TITRIMETER—

Setting up—To set up the end-point controls of the titrimeter, the pH meter reading is first adjusted to the end-point pH by means of the knobs marked SET BUFFER, and this provides the control unit with a signal equal to that at the end-point. The knob marked END-POINT (R_3, Fig. 4) on the front panel of the control unit is adjusted until the light just changes from yellow to red. The pH meter reading is then adjusted to the pH at which

TABLE I

AUTOMATIC TITRATIONS WITH THE COULOMETRIC TITRIMETER

In each titration, the sample was 25 ml of 0·1 *N* sulphuric acid (factor 1·002)

Counter reading	Deviations from mean	End pH	Initial temperature, °C	Final temperature, °C
110·20	+0·15	4·00	19	34·5
110·20	+0·15	4·05	20	37·5
110·30	+0·25	4·05	21	37·0
110·25	+0·20	4·02	20	36·5
110·20	+0·15	4·01	21	36·5
110·15	+0·10	4·01	21	37·5
110·10	+0·05	4·00	21	37·0
109·90	−0·15	4·00	20	37·0
109·90	−0·15	4·01	21	37·5
109·90	−0·15	4·00	20	37·0
110·10	+0·05	4·01	20	37·5
110·10	+0·05	4·02	21	38·0
110·05	0·00	4·01	21	37·0
109·70	−0·35	4·01	21	37·0
109·90	−0·15	4·01	21	37·0

Standard deviation = 0·15 per cent.

the titration rate is desired to slow down and an adjustment is made to the LOW-CURRENT START knob (R_5, Fig. 4) until the light just changes from green to yellow. The pH meter is then standardised with a buffer solution and the instrument is ready for use.

Titration—To carry out a titration, the beaker containing the sample is placed in the operating position, the counter reading is noted, and then the START button (S_1) is pressed. With the cell described, a titration current of 1·3 amp. can be used and this is reduced to 250 mA near the end-point. The minimum electrolyte flow that should be used is 10 ml per minute.

When the red lamp is illuminated, the titration is complete, and the reading on the counter is noted. The change in the counter reading is a measure of the titre. The counter reading is calibrated by titrating a known amount of acid or alkali and the concentration of an unknown sample is found by proportion. By this procedure the counter indication is directly related to the chemical standards of the laboratory: absolute efficiency of electrolysis does not affect the performance of the titrimeter.

PERFORMANCE OF THE AUTOMATIC TITRIMETER—

The instrument was tested in the routine analytical laboratory where titrations were performed coulometrically for several days. Some of the results are shown in Table I. The pH of the sample after titration has been recorded to show the efficacy of the apparatus in detecting the end-point. The initial and final temperatures of the sample are also recorded.

It was found that standardisation once daily was adequate and the control settings required only occasional checking. Rapid operation requiring a minimum of skill consistently gave results that were reproducible to within ±0·2 per cent. The efficiency of the

cell was found to be 97·5 per cent. and was measured by performing some titrations with an accurately stabilised current and measuring the titration time.

IODINE TITRATIONS WITH THE AUTOMATIC TITRIMETER—

Some experiments were carried out to determine whether iodine could be produced coulometrically in the cell. The electrolyte was a 5 per cent. aqueous solution of potassium iodide, which dissolved the iodine as it was produced at the anode of the cell. Titrations

TABLE II

TITRATION OF SODIUM THIOSULPHATE BY COULOMETRICALLY GENERATED IODINE

In each titration the sample was 10 ml of 0·1 *N* sodium thiosulphate and starch solution was used as indicator

	Counter reading	Deviation from mean
	36·75	+0·05
	37·00	+0·30
	36·70	0·00
	36·95	+0·25
	37·20	+0·50
	36·90	+0·20
	36·80	+0·10
	36·80	+0·10
	36·30	−0·40
	36·80	+0·10
	36·30	−0·40
	36·50	−0·20
	36·60	−0·10
	36·40	−0·30
	36·40	−0·30
Mean	36·70	

Standard deviation = 0·26 per cent.

were performed in which the sample taken was 10 ml of 0·1 *N* sodium thiosulphate solution, and the results are shown in Table II. The end-point indication was based on an amperometric principle described by Knowles and Lowden,[19] who used a platinum indicator and calomel reference electrode. The electrodes could be connected directly to the terminals of the control unit provided that the 1500-ohm resistor (R_1 in Fig. 4) was removed.

In the presence of excess of sodium thiosulphate, there was practically no e.m.f. across the electrodes, but an excess of iodine corresponding to 0·2 mg in 100 ml was sufficient to produce an e.m.f. of 250 mV. The control unit was so adjusted that an input of 200 mV terminated the titration, and 100 mV was a sufficient signal to start titration at the slow rate. For successful titration, two modifications had to be made in the control unit—

(*i*) the relay A in Fig. 4 was made to seal down when energised so that a transient signal of 100 mV caused the titration rate to drop permanently until the end-point; and

(*ii*) the flow and titration current were stopped simultaneously so that if, after the end-point has been reached, continued stirring shows that more iodine is needed, it will be added dropwise.

The titrimeter with these alterations was found to switch on and off repeatedly near the end-point until equilibrium was reached. It was unnecessary to wash out the cell between successive titrations, as there was practically no tendency for the iodine in the delivery arm to diffuse through the sinter.

As the main source of error in these titrations lies in the determination of the end-point, the standard deviation in the titration of 10-ml samples of 0·1 *N* sodium thiosulphate (Table II) should not be very different from that found in titrating larger samples. There may, however, be a slight and inconsistent loss of efficiency in the cell owing to small amounts of oxygen being liberated with the iodine.

RECENT DEVELOPMENTS

Recent experience has shown that the electronic end-point detecting circuits of the automatic titrimeter can be usefully applied to volumetric titrations wherever electrodes can

be made to yield a sufficiently sharp voltage change at the end-point. For example, with a glass electrode and conventional pH meter or metal electrode, *e.g.*, tungsten or antimony, directly coupled to the apparatus, it can record changes of pH, whilst with the circuit described above for use in the determination of iodine it can be made to detect an amperometric end-point.

As the signals for "approach to" and "arrival at" the end-point take the form of sharp changes of coloured lamps, these changes are more easily and accurately detected than with either a meter reading or with a coloured indicator. Several special instruments have been built for routine applications of this kind.

There are many analytical reagents that cannot be quantitatively generated by an electric current. For these an electrically controlled burette having two speeds of flow has been made; this is shown in Fig. 6, and it consists of a conventional burette to which is attached by means of a ground-glass joint a double valve, each part of which is identical with the electromagnetic valve described on p. 612. When both valves are open, liquid flows from the burette at a rate determined by the upper tap. When the upper magnetic valve is shut, the rate of delivery of the burette is determined by the glass tap in the by-pass arm. The rate of dropping should be such that between any two successive drops equilibrium is established in the titrating vessel.

Trials of this apparatus have so far been limited to a few conventional titrations in which it has been successful, but there is no reason why it should not be used to perform any titration for which a suitable electrode system for the detection of the end-point can be found. For rapid titration there must be adequate warning of the approach of the end-point; good stirring is also necessary to ensure an accurate end-point.

The authors thank those of their colleagues in I.C.I. Ltd., who have helped with the analytical applications of the apparatus described above.

APPENDIX

List of components used in the construction of the coulometric titrimeter

Control unit (fig. 4)—

R_1	= 1500-ohm, wire-wound resistance.
R_2, R_4, R_{27}	= 100,000-ohm, 0·5-watt, carbon resistance.
R_3, R_5	= 5000-ohm, helical potentiometer (Colvern Ltd.).
R_6	= 100,000-ohm, wire-wound resistance.
R_7	= 33,000-ohm, 1-watt, carbon resistance.
R_8, R_{10}, R_{20}, R_{28}	= 220,000-ohm, 0·5-watt, carbon resistance.
R_9, R_{22}	= 1·5-megohm, 0·5-watt, carbon resistance.
R_{11}, R_{21}	= 1000-ohm, 0·5-watt, carbon resistance.
R_{12}, R_{13}, R_{18}, R_{19}	= 0·47-megohm, 0·5-watt, carbon resistance.
R_{14}, R_{17}	= 20,000-ohm, wire-wound resistance.
R_{15}, R_{26}	= 47,000-ohm, 0·5-watt, carbon resistance.
R_{16}	= 100,000-ohm, wire-wound potentiometer.
R_{23}	= 3300-ohm, 0·5-watt, carbon resistance.
R_{24}	= 1·2-ohm, precision resistor, 1·5 amp. rating (Croydon Precision Instruments Ltd.).
R_{25}	= 10,000-ohm, 0·5-watt, carbon resistance.
C_1, C_3, C_8	= 100-μF, 12-volt working, electrolytic condenser.
C_2, C_{12}	= 0·1-μF, 350-volt working, paper condenser.
C_4, C_7, C_9, C_{13}	= 1-μF, 350-volt working, paper condenser.
C_5, C_{11}	= 50-μF, 50-volt working, electrolytic condenser.
C_6, C_{10}	= 0·01-μF, 350-volt working, paper condenser.
C_{14}, C_{15}, C_{17}, C_{18}	= 0·05-μF, 350-volt working, paper condenser.
C_{16}	= 64-μF, 450-volt working, electrolytic condenser.
V_1, V_4	= EF91 valve.
V_2, V_3	= 2D21 thyratron valve.
V_5	= 85A2 neon stabiliser valve.
P_1, P_2, P_3	= Pilot lights, 6-volt, 0·2 amp. (Arcolectric).
S_1	= Push button switch (Lucas).
S_2	= Double-pole, two-way, wafer switch.
M	= Integrating motor, 1·5 volts (Electro Methods Ltd.).
K	= Carpenter polarised relay, Type 3.
A	= Post Office relay 10,000 ohm.
B	= Post Office relay 10,000 ohm.
G	= Post Office relay 10,000 ohm.
MR	= Metal rectifier, Type RM1 (Standard Telephone Co. Ltd.).

POWER SUPPLY (FIG. 3)—

R_1 = 50-ohm, 100-watt, Toroidal potentiometer.
R_2 = 500-ohm, 50-watt, Toroidal potentiometer.
R_3 = 2000-ohm, 50-watt, Toroidal potentiometer.
R_4 = 1500-ohm, 50-watt, Toroidal potentiometer.
R_5 = 100,000-ohm, 0·5-watt, carbon resistor.
C_1 = 96-μF, 500-volt working, 1500 mA ripple current, electrolytic condenser.
C_2 = 32-μF, 450-volt working, electrolytic condenser.
T_1 = Mains transformer: primary windings, 10–0–220–240 volts; secondary windings, 220–0–220 volts at 1·5 amp.
T_2 = Mains transformer: primary windings, 10–0–220–240 volts; secondary windings, 300–0–300 volts at 100 mA, 6·3 volts at 3 amp.
L_1 = Low-frequency choke, 5 henries, d.c. resistance 20 ohms, rating 1·5 amp.
L_2 = Low-frequency choke, 20 henries, 20 mA.
MR = Selenium rectifier, 220 volts, full-wave, rating 1·5 amp.
M1 = Ammeter, moving coil, 0 to 5 amp.
P1 = Neon indicator, Type F (G.E.C.).
P2 = Pilot light, 6-volt, 0·2 amp. (Arcolectric).
B1 = 3-pin plug (Bulgin).
B2 = 12-pin Jones connector (Painton).
F1 = Fuses, 3 amp.
F2 = Fuses, 5 amp.
F3 = Fuses, 250 mA.
V_1 = EZ40, rectifier valve.
S_1 = Double-pole switch (Painton).
S_2 = Double-pole switch (Painton).

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The Determination of Total Tocopherol

By J. R. EDISBURY, Miss JEAN GILLOW and R. J. TAYLOR

A modified Emmerie and Engel test has been evolved to avoid non-linearity, high or variable blanks and the use of corrosive solvents. A solution of the saponified material in light petroleum is purified by chromatography, the tocopherol being adsorbed on mildly alkaline alumina, washed with diethyl ether - light petroleum solvent and subsequently eluted with chloroform. The eluate is concentrated by evaporation and treated with chloroform solutions of ferric chloride and 2:2′-dipyridyl. After the solution has stood for 5 minutes, a measured volume of water is added and the optical density of the aqueous extract is determined at 520 mμ; alternatively the depth of colour, which is stable for some hours, may be assessed visually, *e.g.*, by comparison with a standard cobalt sulphate solution. The only interference encountered has been from highly oxidised carotenoids, for which correction is possible.

Sensitivity is five times that of the original Emmerie and Engel test and precision is about ±5 per cent.

Tocopherol (fat-soluble vitamin E) is credited with a number of valuable physiological functions ranging from the relief of cardiac distress and the cure of "Crazy chick" disease to the general maintenance of fertility, and it is important chemically as an anti-oxidant in oils and fats. Four distinct tocopherols and their esters have been identified in the natural state and have since been synthesised. The commonest and physiologically most active form, α-tocopherol, is the one naturally chosen for large-scale synthesis, the other three forms being much less active, β-tocopherol about one-third, γ-tocopherol one-fifth and δ-tocopherol one-hundredth. They can be separated by paper chromatography (Brown,[1] Russel Eggitt and Ward[2]) and if a sensitive colour test is used, this may lead to a convenient method of individual assay of the four types of tocopherol. Meanwhile there is a need for a simple method of determining total tocopherol, particularly when a series of assays on similar materials is required. The method described in this paper was developed for the determination of tocopherol in feeding stuffs, with which the difficulties of isolating tocopherol effectively are probably greater than with any other substance. It is, however, suitable for general application and has the particular advantage of substantially increased stability of the 2:2′-dipyridyl complex with tocopherol.

The magnitude of the general problem is indicated by the number and variety of published tests that have arisen through dissatisfaction with previous methods. Competent reviews of these are given by Bacharach[3] and Eden and Booth,[4] and useful information is provided by Tosic and Moore.[5]

Emmerie and Engel[6] described the first quantitative approach. Our method is a development of their original technique, which was based on the fact that in the presence of 2:2′-dipyridyl one molecule of free tocopherol reduces two atoms of ferric iron and the resulting ferrous iron forms a 3 to 1 chelated complex ion with the 2:2′-dipyridyl. Hence each molecule of tocopherol is stoicheiometrically equivalent to six molecules of 2:2′-dipyridyl. The complex is red and under ideal conditions gives a pink solution owing to absorption of light in the green region of the spectrum.

Absorption spectrum of the complex—

The pink solution is conventionally described as showing a broad absorption maximum at 515 mμ. Investigation with a visual spectrophotometer showed that this broad region of absorption results from two overlapping bands, one at 484 mμ and the other at 529 mμ (see Fig. 1). Measurements of optical density can be made with almost equal validity at any point within this range; we have selected 520 mμ as a suitable value with any commercial instrument (we have used Beckman DU, Unicam SP500 with tungsten or hydrogen lamps and SP600), and a spectral slit-width of up to 20 mμ can be used without introduction of error.

MODIFICATION OF THE EMMERIE AND ENGEL METHOD—

Of the several published modifications of the Emmerie and Engel test that we have tried, only those in which glacial acetic acid is used as solvent[7,8,9,10] have given a reaction colour that is both stable and in linear relation to the amount of tocopherol present; but the corrosive acid vapour could seriously damage a photo-electric instrument and we cannot recommend the routine use of these methods. Ethanol has been widely used as solvent, but it has certain limitations: the 2:2′-dipyridyl solution is relatively unstable and the intensity of colour

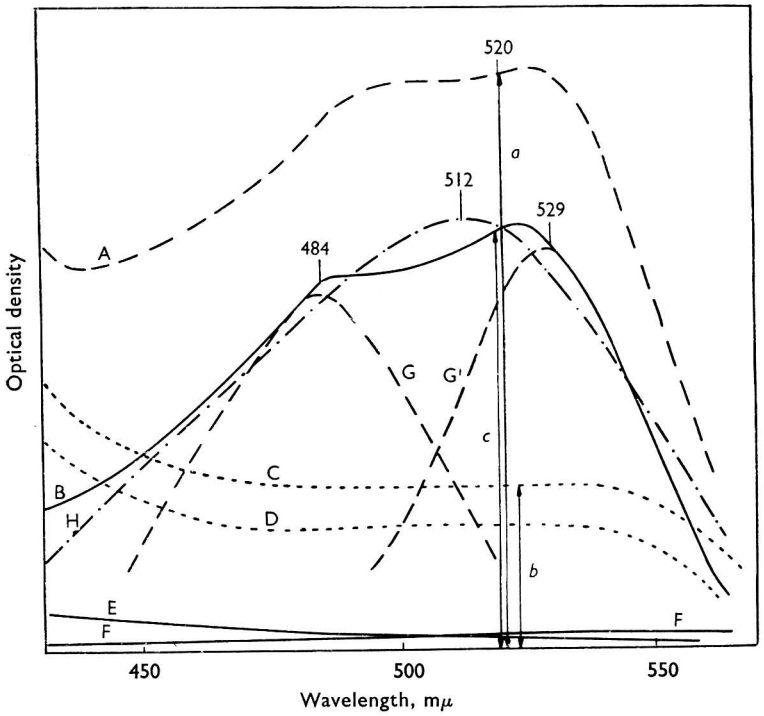

Fig. 1. Spectra of relevant absorption for the determination of tocopherol: curve A, uncompensated test solution; curve B, test solution after allowance for blank; curve C, uncompensated blank; curve D, blank compensated against distilled water; curve E, contribution of excess of ferric chloride to curves A, C and D; curve F, contribution of excess of 2:2′-dipyridyl to curves A, C and D; curves G and G′, spectroscopic components of curve B; curve H, cobalt sulphate. Length *a*, optical density of uncompensated test solution at 520 mμ; length *b*, optical density of uncompensated blank at 520 mμ; and length $c = a - b$

development is non-linear; the blanks are high and the precision is not always certain. The interest that other workers have shown in the use of acetic acid is indicative of the difficulties with ethanolic solutions. Chloroform, with its faintly acidic bias, has proved to be as controllable as acetic acid, but the blanks were again high and the reaction colour was seldom pink. It was found, however, that the colour was transferable to water, and further investigation showed that the transfer of the complex was quantitative. The aqueous solution had a clear pink colour, stable for some hours and suitable for spectrophotometric or other measurement, and the blanks were reasonably low (see curves C and D in Fig. 1).

Before the reaction is attempted, the tocopherol must be freed from other reducing substances, otherwise any substance that reduces ferric iron will be recorded as tocopherol. Emmerie and Engel themselves recognised this, but of the adsorbents suggested by them or by subsequent investigators, none that we have tried has been wholly satisfactory when dealing specifically with feeding stuffs. In the double column chromatographic assay of vitamin A (Boldingh and Drost[11]), the lower column of alkaline alumina retains the tocopherol and similar substances, but while tocopherol is undoubtedly retained it cannot afterwards

be recovered; as tocopherol is markedly unstable in strong alkali, it is probably destroyed. Trials with alumina of different degrees of alkalinity led us to select alumina containing 0·7 to 0·8 per cent. w/w of sodium hydroxide, and at this concentration free tocopherol is quantitatively adsorbed from light petroleum solution and is firmly retained while most carotenoids, vitamin A and probably some other contaminants are removed by washing with diethyl ether - light petroleum solvent; it can then be eluted with chloroform so that the impurities remain in a tenacious grey zone at the top of the column. It is recommended that all tocopherol preparations, even concentrates, should be treated in this way before test. Pure α-tocopherol is recovered quantitatively and so can be used to assess the suitability of each batch of alkaline alumina.

Some of the more highly oxidised carotenoids may also be retained with the tocopherol, and in such circumstances allowance can be made by following the Tosic and Moore[5] technique of correction for β-carotene. They multiplied the amount of β-carotene, in μg per g, by a factor lying between 2·3 and 2·5 and subtracted the resultant value from the total apparent amount of tocopherol, in μg per g; Kaunitz and Beaver[8] used a factor of 4·8 under different conditions; for oxidised carotenoids we use the value 3·0. Although the correction is only approximate, it is generally small. Ideally the chloroform eluate is practically colourless and no correction is required, but if a correction is necessary, then—

$$\text{carotenoid content, in } \mu g \text{ per ml} = \text{E at } 450\ m\mu \times 4,$$

where E at 450 mμ is the optical density measured in a 1-cm cuvette. This can be calculated directly to concentration of carotene, in μg per g, in the original material and so, by the factor of 3, to the required tocopherol correction.

Method

Reagents—

Potassium hydroxide solution—Dissolve 60 g of potassium hydroxide pellets of analytical-reagent grade in distilled water and dilute to 100 ml.

Ferric chloride solution—Dissolve 0·2 g of crystalline ferric chloride of analytical-reagent grade in chloroform and dilute to 100 ml.

2:2′-*Dipyridyl*—Dissolve 1·0 g of 2:2′-dipyridyl of analytical-reagent grade in chloroform and dilute to 100 ml.

α-*Tocopherol, pure.*

Gallic acid or *pyrogallol.*

Chloroform, pure.

Ethanol, absolute.

Light petroleum, boiling range 40° *to* 60° *C.*

Diethyl ether—Freshly distilled over sodium hydroxide pellets.

Acetone—Analytical-reagent grade.

Supply of carbon dioxide.

Active alumina, standard type—Prepare it by heating crystalline alumina trihydrate at 800° C for 7 hours, and use the fraction that passes through a 150-mesh B.S. sieve.

Active alkaline alumina—To 10 g of activated alumina in a 50-ml round-bottomed flask, add 10 ml of a 0·75 per cent. w/v aqueous solution of sodium hydroxide, shake the mixture well, attach the flask to a water pump and leave it under reduced pressure (3 to 4 cm) for 1 hour. While the flask is still under reduced pressure, place it for a further 45 minutes in an oil-bath maintained at 135° C. Cool the alumina under reduced pressure and when cold transfer it to a small well-corked bottle.

Procedure for extraction of unsaponifiable fatty matter—

Samples containing more than 40 *μg per g of tocopherol*—Weigh up to 5 g of sample into a 200-ml flat-bottomed flask, add 5 ml of potassium hydroxide solution, 25 ml of ethanol and 40 to 50 mg of gallic acid or pyrogallol, and boil the mixture under a reflux condenser for 30 minutes. Transfer it to a 500-ml separator, using two 25-ml quantities of distilled water, and extract it once with 100 ml and then three times with 50-ml portions of diethyl ether. Wash the combined extracts successively with four 50-ml portions of lukewarm distilled water. Remove the ether by distillation in an inert atmosphere, add 2 ml of ethanol and heat the flask while passing a stream of carbon dioxide through it to dehydrate the

extract and to free it from solvent. Dissolve the residue in light petroleum and dilute to 5 ml.

Samples containing less than 40 *μg per g of tocopherol*—Weigh 10 to 20 g of sample, add 100 mg of gallic acid or pyrogallol and extract the mixture for 2 hours in a Tait thimble with peroxide-free diethyl ether or acetone. Remove the solvent by distillation in an inert atmosphere and immediately saponify the treated sample as described above.

CHROMATOGRAPHY—

Set up the chromatographic tube, shown in Fig. 2, fit a small wad of cotton-wool in the lower tip and place the tip on a piece of rubber to prevent the liquid from running through.

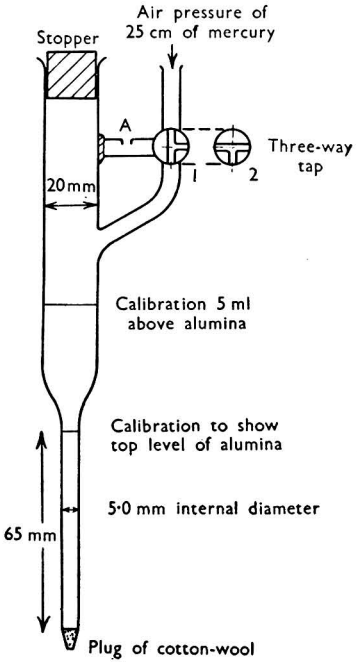

Fig. 2. Apparatus used for isolation of tocopherol by chromatography. The column contains 1 g of alumina; when the three-way tap is in position 1, the air pressure is applied, but in position 2 the pressure is released through the side-arm, which is open to the atmosphere at A and is attached to the column for support

Fill the narrow section of the tube with light petroleum and add 1 g of alkaline alumina, tapping the side of the tube to make the column compact. Remove the tip of the tube from the piece of rubber and allow the solvent to run through under a slight air pressure. When the upper solvent level is just above the alumina, run in 1 ml of the solution of unsaponifiable matter and repeat the procedure. Then add 5 ml of a 36 per cent. v/v solution of diethyl ether in light petroleum. Now replace the receiver by a small clean flask and pass 5 ml of chloroform through the column.

COLOUR REACTION—

Evaporate the chloroform solution to approximately 0·5 ml in an inert atmosphere. Cool it and add 0·2 ml of ferric chloride solution and then 0·5 ml of 2:2′-dipyridyl solution. Allow the solution to stand for 5 minutes, then add 3·6 ml of distilled water and shake the mixture vigorously. Transfer it to a centrifuge tube and spin it at a moderate speed in a small hand centrifuge for 10 seconds. With a pipette, remove the lower (chloroform) layer and spin it again more vigorously.

Make a blank determination with the reagents.

MEASUREMENTS—

Spectrophotometric—Transfer the clear pink aqueous solution to a 1-cm cuvette and measure its optical density by a spectrophotometer at 520 mμ. Repeat this with the blank solution. Then if E_s is the optical density of the test solution and E_b that of the blank—

$$100(E_s - E_b) = \text{tocopherol, in } \mu g, \text{ in aliquot taken for test.}$$

The tocopherol content, in μg per g, of the meal may now be calculated directly.

Absorptiometric—An absorptiometer fitted with a 520-mμ filter is first calibrated in terms of amount of tocopherol, in μg, by means of tests with a series of solutions of pure α-tocopherol in chloroform.

Then if R_s and R_b are the values from calibration results—

$$R_s - R_b = \text{tocopherol, in } \mu g, \text{ in aliquot taken for test.}$$

From this value the total amount of tocopherol, in μg per g, can be calculated.

CORRECTION FOR CAROTENOIDS—

Most carotenoids are eliminated during the chromatographic stage, but occasionally oxidised products that remain with the tocopherol are present; this is indicated by the orange-yellow colour of the chloroform solution. Treat an independent aliquot of the solution by passing it through a column of alkaline alumina as before, then remove the chloroform by evaporation in carbon dioxide, dilute the residue to a suitable volume with light petroleum and measure the optical density of this solution at 450 mμ.

Let E at 450 mμ = optical density.
V_c = volume of light petroleum solution, in ml.
E at 450 mμ × 4 = carotenoid content of solution, in μg per ml.
E at 450 mμ × 12 = equivalent tocopherol content of solution, in μg per ml.
E at 450 mμ × V_c × 12 = equivalent tocopherol content of aliquot taken for test, in μg.

This gives the value of the equivalent tocopherol, in μg per g, of the interfering carotenoids in the meal, and so the necessary correction can be made.

EXAMPLES—

(*a*) A 10-g sample of a meal was extracted and the extract was saponified. The unsaponifiable matter was diluted to 5 ml with light petroleum and 1-ml aliquots were taken for test; the mean E at 520 mμ reading of the 2:2′-dipyridyl complex was 0·44. The correction from a blank test was 0·16.

Then nett E at 520 mμ value attributable to tocopherol = 0·28.
Tocopherol per ml of aliquot = 100 × 0·28 = 28 μg.
Tocopherol content of meal = 14 μg per g.

(*b*) The same meal as in (*a*) was fortified with 40 μg per g of pure tocopherol. A 3-g sample was saponified directly and the unsaponifiable matter was diluted to 3 ml with light petroleum; 0·5-ml aliquots were taken for test. The mean E at 520 mμ reading was 0·44.

The nett E at 520 mμ reading attributable to tocopherol was 0·28.
Tocopherol per 0·5-ml aliquot = 100 × 0·28 = 28 μg.
Total tocopherol content of meal = 56 μg per g (compared with a nominal 54 μg per g).

(*c*) A 5-g sample of another meal was saponified directly and the unsaponifiable matter was diluted to 5 ml with light petroleum; 0·75-ml aliquots were taken for test. Carotenoids were present in the chloroform eluate. The mean E at 520 mμ reading was 0·56. The correction from a blank test was 0·18.

Then nett E at 520 mμ reading = 0·38.
Apparent tocopherol content of meal = 50·6 μg per g.

Carotene correction—A 0·75 ml aliquot of the eluate was treated as described above, and the residue was dissolved in 3·6 ml of light petroleum. The E at 450 mμ was 0·168.

$$\text{Equivalent of tocopherol in meal} = \frac{0{\cdot}168 \times 3{\cdot}6 \times 12}{0{\cdot}75} = 9{\cdot}6 \ \mu g \text{ per g.}$$

True tocopherol content of meal = 41 μg per g.

Discussion

This modification of the Emmerie and Engel test requires only about one-fifth of the amount of tocopherol per reaction as the original test. It may be that this low level is one of the reasons for closer adherence to linearity. There are, however, probably several other factors involved in what is undoubtedly a complex reaction.

In the reaction of tocopherol with ferric chloride and 2:2′-dipyridyl, the results have been best when sufficient iron was present to oxidise, theoretically, 320 μg of tocopherol and sufficient 2:2′-dipyridyl was present to oxidise 2400 μg, although only 20 to 70 μg of tocopherol are present in a typical reaction mixture; unless all these amounts of reagent are present in a total of 1·5 ml or less of solution, the reaction is not complete. (This is similar to the reaction of vitamin A with antimony trichloride, when a molecular ratio of 10^5 to 1 is required, but is of no avail unless the antimony trichloride solution is nearly saturated.) As it is the tocopherol that is being determined and not the iron or 2:2′-dipyridyl, it is to be expected that both reagents should be in excess; but the general behaviour is unusual. Below a molecular ratio of iron to tocopherol of 10 to 1, *i.e.*, an excess of five times the nominal stoicheiometric requirements, there is a marked diminution of colour even when 2:2′-dipyridyl is present in excess, and the diminution can be partly arrested by further additions of 2:2′-dipyridyl. With about 20 times the stoicheiometric amount of iron, there is again a diminution of colour no matter how much 2:2′-dipyridyl is present. In the presence of a reasonable excess of iron (*e.g.*, 10 to 1), 2:2′-dipyridyl requires to be in much greater excess for full colour development. With amounts of iron up to about 50 times the nominal quantity, the effect of progressively increasing the amount of 2:2′-dipyridyl can be discerned; thereafter the rate of increase of colour is undetectable. Under our conditions of test there is an excess of iron of between 4·5 and 16 to 1 and of 2:2′-dipyridyl of between 34 and 120 to 1 over the stoicheiometric requirements, with usual values of 10 to 1 and 75 to 1, respectively, representing molecular ratios of tocopherol to iron to 2:2′-dipyridyl of 1 to 20 to 450, in place of the theoretical 1 to 2 to 6.

The experimental requirement that 20 to 70 μg of tocopherol should be present in the reaction mixture makes it generally expedient to try one or two pilot tests on the solution of the extract before proceeding to the chromatographic stage. For these tests an aliquot of the test solution is taken and freed from light petroleum, and it is then treated directly with ferric chloride and 2:2′-dipyridyl in the prescribed manner. The complex is transferred to water and its absorption at 520 mμ is determined. This will yield a result for the tocopherol that may be 100 per cent. too high, but the test is quickly made and it will enable a correct choice of aliquot to be made for the more exact analysis.

The time factor is important but not critical. Under our conditions development of colour for less than 3 minutes usually gives low results; over 7 minutes may give slightly high results with a correspondingly high but less predictable blank. The choice of 5 minutes is a workable compromise. Measurement of the time of development has been used by Stern and Baxter[10] to distinguish between the various forms of tocopherol; in glacial acetic acid the α-form produces colours most rapidly and the δ-form least rapidly. The method is not likely to be reliable, but should not be overlooked. More promising as a means of differentiation is paper chromatography of a novel kind devised by Brown[1] and modified by Russell Eggitt and Ward.[2] Filter-paper thinly pre-treated with petroleum jelly or liquid paraffin, B.P., is used, with 75 per cent. aqueous ethanol as the developing solvent. The developed spots are identified on one strip and eluted from corresponding locations on a parallel strip. This method might well be linked with our own test.

The factor of 100, which we use to calculate the amount of tocopherol in the test solution, in μg, is applicable only when the total volume of the aqueous solution is 3·6 ml and a 1-cm cuvette is used for measurement. It is based on an $E^{1\%}_{1cm}$ value of 360 at 520 mμ for the 2:2′-dipyridyl complex in terms of tocopherol. As the complex is really one of reduced iron, this value is conventional, but it can be used for the purpose of calculation when volumetric conditions of test other than those here described are chosen.

If a tocopherol standard is not available but the alkali-treated alumina is known to be efficient, the colour due to the tocopherol can be visually matched against an aqueous solution of cobalt sulphate. In Fig. 1 the absorption spectrum of the iron - 2:2′-dipyridyl complex is compared with that of cobalt sulphate, which shows a maximum at about 512 mμ and, apart from divergence below 450 mμ, follows the over-all shape of the complex curve more

closely than most of the standards that find ready acceptance in abridged spectrophotometry. This divergence imparts a blue tinge that can be corrected by addition of a small amount of ferric chloride to the cobalt solution, so securing an almost perfect visual match. As a first approximation, a 1 per cent. w/v solution of recrystallised cobalt sulphate can be taken as visually equivalent to 5 μg per ml of tocopherol.

A series of six narrow test tubes containing graded concentrations of cobalt sulphate from about 3 to 1 per cent. w/v at intervals of about 0·4 per cent., and a constant 0·2 per cent. w/v of crystalline ferric chloride irrespective of cobalt concentration, covers a useful range suited to the conditions of test already outlined. Aqueous extracts of measured volume from known reaction mixtures can be directly assessed by visual comparison to within probably 10 to 15 per cent.; alternatively, a galvanometer instrument can be roughly calibrated with cobalt sulphate, or cobalt sulphate can be used on one side of a dip-tube colorimeter.

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Volumetric Determination of Pectin as Calcium Pectate

By R. Holt

A method is described for the direct determination of the calcium content of precipitated calcium pectate by titration with ethylenediaminetetra-acetic acid. The pectate ions do not interfere. The values so determined are useful as a measure of the calcium pectate yield of the sample from which the precipitate is derived. The calcium contents of precipitates containing 6 to 24 mg of calcium (as calcium carbonate) were proportional to the quantities of citrus pectin from which the precipitates came.

The procedure described for saponification and precipitation of pectin can be completed within 2 hours.

Gravimetric determination as calcium pectate has been widely used for measuring pectin.[1,2,3,4,5] Hinton[1] used the phrase "calcium pectate yield" to denote the weight of calcium pectate produced from the sample expressed as a percentage of the original sample. The same author found the criterion useful in assessing the purity of pectins extracted from fruits. Both he and Branfoot[6] found the precipitate to be of fairly constant composition. Despite its empiricism, the method is widely used and is particularly valuable for purposes of comparison. The significance of the results and the numerous modifications that the method has undergone are discussed by Kertesz.[2]

The essential stages in all methods for the determination of calcium pectate yield are extraction, purification, saponification, acidification, precipitation, filtration, washing, drying and weighing. It is not proposed to consider in this paper the extraction and purification stages, and it is assumed throughout that a suitably purified solution of pectin is being examined. For details of the methods of purification and extraction, reference should be made to the work of Emmet and Carré[3] and to that of Kertesz.[2] Table I illustrates the conditions used in some typical procedures recommended for the determination of the calcium pectate yield.

TABLE I

CONDITIONS USED FOR THE DETERMINATION OF CALCIUM PECTATE YIELD

Method	Normality of NaOH used for saponification	Time of saponification, hours	Interval between precipitation and filtration, hours	Time of drying, hours
Carré and Haynes[4]	0·025	Overnight	1	12
King[5]	0·02	1	1	Not specified
Hinton[1] ("rapid method") ..	0·2	0·5	1	2
Kertesz[2]	0·1	1	None	Overnight

The total time taken for the shortest procedure, that of Hinton, would be at least 4 hours.

The object of the present work was to replace the drying and weighing of the calcium pectate precipitate by a titration of the calcium, and also to reduce the time of saponification and the interval between precipitation and filtration to a minimum. The method evolved by Schwarzenbach, Biedermann and Bangerter[7,8] was adopted as a basis for the determination of calcium.

EXPERIMENTAL

An outline of the methods used will be given first to explain the various conditions that were studied. The full recommended procedure is described below.

MODIFIED CARRÉ AND HAYNES PROCEDURE FOR PRECIPITATION—

An aliquot of the citrus pectin solution was measured into a conical flask and made up to 300 ml with water, 100 ml of 0·1 *N* sodium hydroxide were added, and the solution was left to stand overnight; 50 ml of *M* calcium chloride were then added and, after 1 hour, the contents of the flask were boiled for a few minutes and then filtered. The flask and filter-paper were washed twice with boiling water. The precipitate was washed back into the original flask and boiled for 5 minutes with 200 to 300 ml of water. The contents of the flask were filtered through the same filter-paper as before and washed with boiling water until chloride was absent from the filtrate, as shown by testing it with silver nitrate.

It should be noted that the washing technique is somewhat different from that originally proposed by Carré and Haynes. It is shorter and has been found satisfactory by later workers, *e.g.*, Kertesz.[2]

SHORTENED PROCEDURE FOR PRECIPITATION—

An aliquot of the citrus pectin solution was measured into a conical flask and a quarter of its volume of 0·1 *N* sodium hydroxide was added. After 5 minutes, 50 ml of *N* acetic acid and sufficient water to make the total volume about 350 ml were added. The addition of calcium chloride, filtration and washing were as in the modified Carré and Haynes procedure. The interval between precipitation and filtration was varied as described below.

DETERMINATION OF CALCIUM IN THE ASHED PRECIPITATE—

The ash was dissolved in 1 ml of concentrated hydrochloric acid and this solution was evaporated to dryness. The residue was dissolved in water and the calcium in this solution was titrated with sodium ethylenediaminetetra-acetate solution (EDTA solution), Eriochrome Black T being used as indicator.

DIRECT DETERMINATION OF CALCIUM IN THE PRECIPITATE—

The precipitate was taken up in excess of buffered EDTA solution and its calcium content was evaluated by titrating the excess of EDTA with standard calcium solution.

By means of these methods, the following experiments were performed in an attempt to shorten the over-all procedure. All the results are calculated for 100 ml of pectin solution and expressed as mg of calcium carbonate.

COMPARISON OF DETERMINATION OF CALCIUM IN THE PRECIPITATE DIRECTLY AND AFTER ASHING—

It was necessary first to determine whether the titration of calcium by EDTA was applicable in the presence of pectate ions. Four 100-ml aliquots of pectin solution were

treated by the modified Carré and Haynes procedure, and 12·2 and 12·3 mg of calcium were found in the ashes from two of the precipitates. The calcium contents determined by direct titration of the other two precipitates were 12·1 and 12·2 mg. This agreement was satisfactory.

TIME OF SAPONIFICATION—

Four 100-ml aliquots of pectin solution were treated by the shortened procedure. The interval between precipitation and filtration was 1 hour. The calcium found after ashing two of the precipitates was 12·3 mg in each instance. By direct determination the calcium contents of the other two precipitates were found to be 12·1 and 12·4 mg. The shortened saponification procedure appeared to be suitable.

When the means are considered, the agreement in these experiments between titration of calcium directly and after ashing is good, but the duplicate results of the direct determinations do not agree with each other. Eight more determinations by the direct method over the range 50 to 200 ml of pectin solution were therefore made. The results, together with the two previously determined, are shown in Table II.

TABLE II

REPRODUCIBILITY OF RESULTS BY SHORTENED PROCEDURE

Pectin solution taken, ml	$CaCO_3$ per 100 ml of pectin solution, mg
50	12·2, 12·1
100	12·1, 12·4, 12·3, 12·3
150	12·4, 12·3
200	12·2, 12·1

INTERVAL BETWEEN PRECIPITATION AND FILTRATION—

The time of standing between precipitation and filtration was first reduced to 10 minutes without any apparent effect on the filtration or the results. A series of results determined with filtration immediately after precipitation were also similar. In Table III these results are summarised and compared with those in Table II. Neither the time of saponification nor the interval between precipitation and filtration had any effect on the quantity of water or the time required to wash the precipitate free from chloride.

TABLE III

EFFECT OF TIME INTERVAL BETWEEN PRECIPITATION AND FILTRATION

Time of standing, minutes	Number of tests	Mean, mg per 100 ml	Standard deviation, mg per 100 ml
0	6	12·1	0·18
10	6	12·3	0·12
60	10	12·2	0·12

The means of the samples allowed to stand 0, 10 and 60 minutes were not significantly different.

METHOD

REAGENTS—

Sodium hydroxide, 0·5 N.

Acetic acid, 1·0 N.

Calcium chloride, 1·0 M.

Buffer solution—Dissolve 40 g of borax, 10 g of sodium hydroxide and 5 g of sodium sulphide in distilled water and make up to 1 litre.

Standard calcium solution—To 0·5 g of pure calcium carbonate, add 2·5 ml of diluted hydrochloric acid (1 + 1). Allow the solution to stand until the calcium carbonate is dissolved. Make up to 1 litre in a calibrated flask.

Ethylenediaminetetra-acetate solution—Dissolve 4·0 g of the disodium salt in 43 ml of 0·5 *N* sodium hydroxide, make up to 1 litre and standardise this solution periodically,[9] as described below, against the standard calcium solution.

Eriochrome Black T indicator solution—To 1·0 ml of *N* sodium carbonate and 30 ml of distilled water add 1·0 g of solid indicator. Shake the solution and make it up to 100 ml with *iso*propanol.

PROCEDURE—

Standardisation of the EDTA solution—To 130 ml of water and 20 ml of EDTA solution, add 10 ml of buffer solution and 3 or 4 drops of indicator. Titrate with the standard calcium solution until the first pink tinge appears in the solution, *i.e.*, the colour changes from blue to purple.

Determination of pectin—Measure into a 500-ml conical flask an aliquot part of the solution under test. It should be less than 200 ml in volume and contain preferably 30 to 120 mg of pectin (calculated as calcium pectate). Add from a graduated cylinder one-quarter of the volume of 0·5 *N* sodium hydroxide and rotate the flask. Set the mixture aside for 5 minutes, add 50 ml of *N* acetic acid, mix the contents of the flask, add water to make the total volume about 350 ml and again mix. From a burette or otherwise in a thin stream, add 50 ml of *M* calcium chloride solution while vigorously rotating the flask. Allow the flask to stand for 10 minutes.

Boil the contents of the flask for 2 minutes and filter through a hard, fast, 15-cm filter-paper (Whatman No. 541 is suitable). Wash the flask and the filter-paper twice with boiling water. Return the precipitate to the original flask and boil it for 10 minutes with 200 to 300 ml of water. Meanwhile wash the filter-paper once or twice with boiling water. Pour the contents of the flask through the same filter-paper. Filter and continue to wash with very hot water until the filtrate shows no reaction for chloride when tested with silver nitrate. Wash the whole of the filtrate back into the original flask with boiling water, making the volume roughly 150 ml. Add 10 ml of buffer solution and an excess of EDTA solution. Heat the flask until the contents are boiling or almost boiling, then cork it and shake it, cautiously at first, until the precipitate dissolves. Cool the flask until it can be handled comfortably. Add 3 or 4 drops of indicator and titrate with standard calcium solution to the same colour change as in standardising the EDTA solution.

The quantity of EDTA to be added to the suspension of calcium pectate is problematical. It is convenient to have an excess of about 10 ml, but the result does not depend on the exact amount. If the approximate pectin content of the solution under test is known, use can be made of the fact that 1 ml of EDTA solution is approximately equivalent to 5 mg of pectin. Even with a completely unknown solution, an estimate of the amount present can, with a little practice, be made from the appearance of the precipitate. Failing these devices it will be necessary to add the EDTA solution in, say, 10-ml increments, shaking between additions until the precipitate dissolves.

Calculation and expression of results—The theoretical calcium content of the calcium salt of pectic acid is 10·2 per cent. The average calcium content of the precipitates, as determined by most workers, is about 7·6 per cent. On this basis 1 mg of calcium carbonate is equivalent to 5·3 mg of calcium pectate. For such purposes as comparing a series of samples or extracts from similar sources, it will probably be most convenient to express the results as mg of calcium carbonate per g of material or per 100 ml of extract.

DISCUSSION AND CONCLUSIONS

The factor that is most likely to affect the reliability of the gravimetric yield is co-precipitation of araban and galactan. Various quantities of these substances are present in extracted pectins, and they are extremely difficult to remove either by precipitation with miscible solvents or by precipitation of pectic acid as its salts.[2,10] In determining calcium pectate yields, Peynaud[11] found arabans present in his precipitates. The results were 8 to 10 per cent. higher than those determined by titrating the pectic acid with an alkali.

Calcium pectate yields determined by titration of the calcium in the precipitate will probably not be affected to the same extent as the gravimetric results by co-precipitation of araban and galactan. No appreciable amount of calcium would be expected to combine with these substances, although they might hinder the combination of calcium with the pectic acid. There are other factors that might affect the ratio of calcium to pectic acid in the precipitate, but in extracts from similar sources, at least, these are not likely to detract seriously from the utility of the method for rapid comparative purposes.

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The Biological Assay of Adrenaline with the Hexamethonium-treated Cat

BY G. F. SOMERS

A method is described for the assay of adrenaline by means of the hexamethonium-treated cat. It obviates the need for spinalising and the results by this method compare favourably with those by other methods. Hexamethonium is a potent hypotensive drug that blocks the sympathetic and parasympathetic ganglia, so providing a simple alternative procedure for preparing the cat, and the results by this method are compared with those by other methods.

NUMEROUS methods have been described for the biological estimation of adrenaline, but few of them are suitable for routine assay purposes. They have been reviewed by West,[1] Gaddum[2] and Burn, Finney and Goodwin.[3] The method used should be amenable to suitable design and statistical analysis, so as to permit the precision of the estimates to be evaluated and to provide evidence that the actions of the preparation under test and of the standard preparation do not differ qualitatively (Emmens[4]).

Methods based on the vasopressor action of adrenaline on mammalian blood pressure are preferred to those utilising isolated tissue preparations, because the responses are more regular and the preparation is not so susceptible to the presence of interfering substances such as preservatives. Elliott's[5] method, involving use of the "spinal" cat, is the commonest in this country; the official method in the United States[6] uses the atropinised dog. The technique of preparing a spinal cat is relatively simple in experienced hands, but it does involve a radical surgical procedure, haemorrhage may be severe and casualties can occur. The cat is spinalised in order to give a low blood pressure and to destroy the parasympathetic compensatory mechanisms, so enhancing its sensitivity to adrenaline.

The use of hexamethonium,[7] a potent hypotensive drug that blocks the sympathetic and parasympathetic ganglia, provides a simple alternative procedure for preparing the cat. The use of this drug in adrenaline assays is described and the results by this method are compared with those by other methods.

METHOD

The assay procedure is as follows. The cat is anaethetised by direct intravenous injection of chloralose (60 mg per kg), with the addition of pentobarbitone sodium (6 mg per kg).* The blood pressure is recorded from the carotid artery and injections are made into the femoral vein. A dose of 50 mg per kg of hexamethonium bromide is given subcutaneously, and the blood pressure is allowed to fall to a steady base-line, usually about 70 mm of mercury.

* As the proprietary preparation Nembutal.

Occasionally cats have been found to be refractory to even larger doses of hexamethonium, and they have had to be spinalised. If the base-line is unsteady, the injection of 1 mg per kg of atropine often stabilises it. The standard is a sample of adrenaline as a 1 in 1000 w/v solution prepared according to the directions of the British Pharmacopoeia for Solution of Adrenaline Hydrochloride.[8] Dilutions of this (1 ml in 40 ml) are prepared in normal saline immediately before the assay and stabilised by the addition of 100 mg of ascorbic acid. Fresh dilutions are prepared each hour. Similar dilutions of the test preparation are prepared. A dose of the standard that produces a submaximal effect is determined, and this is designated the high dose of the standard, S_1. A dose of the test is then determined with about the same effect, T_1. The low doses of the standard, S_2, and of the test, T_2, are usually half the high doses, and it is important that the ratios should be the same. Suitable doses having been selected, they are given at 5-minute intervals in a randomised order according to a Latin Square design. Each assay therefore consists of sixteen responses, but additional responses may be obtained to suit the accuracy required.

Results

The results of a typical assay are shown in Table I and the statistical analysis in Table II. The calculation of potency ratio and limits of error were made by the methods described by Schild[9] and Holton.[10] The test solution was an 80 per cent. dilution of the standard, the strength of which was unknown to the analyst. The calculated potency was 81·6 per cent. with fiducial limits of error (P = 0·05) from 75·7 to 87·9 per cent. The slope b was 56·4 and the index of precision s/b or λ, 0·027. Hence the method is accurate. From 18 assays the mean values for b, s and s/b were: $b = 53{\cdot}3$ with a standard deviation of 13·0; $s = 2{\cdot}07$ with a standard deviation of 0·70; and $s/b = 0{\cdot}04$ with a standard deviation of 0·019.

Table I

Increase in blood pressure, in millimetres of mercury, when adrenaline is administered to a hexamethonium-treated cat

Preparation and dose	Dose,	Groups 1	2	3	4	Sum
S_1	0·4	60	62	60	62	244
S_2	0·2	40	42	47	46	175
T_1	0·4	55	55	56	57	223
T_2	0·2	38	38	39	41	156
	sum	193	197	202	206	798

Table II

Statistical analysis of results in Table I

Source of variation	Degrees of freedom	Sum of square	Variance	F	P
Groups	3	24	8	3·31	>0·05
Standard and unknown	1	100	100	41·39	<0·001
Regression	1	1156	1156	478	<0·001
Deviation from parallelism ..	1	0·25	0·25	0·1	>0·05
Residual error	9	21·75	2·416	—	—
Total	15	1302			

$S_m = 0{\cdot}0143$. $b = 56{\cdot}4$. $s = 1{\cdot}55$. $s/b = 0{\cdot}027$.

For 9 degrees of freedom, $t = 2{\cdot}262$ (P = 0·05). The fiducial limits for $M = \pm S_m \times t = 0{\cdot}0323$. As $M = \bar{1}{\cdot}9115$, fiducial limits are $\bar{1}{\cdot}9438$ and $\bar{1}{\cdot}8792$.

The potency of T is therefore 81·6 per cent. of S with fiducial limits (P = 0·05) from 75·7 to 87·86 per cent.

These results compare favourably with those published by Noel[11] for the atropinised dog (see Table III), but the slope, b, is not quite so steep. A limited number of comparative assays were carried out on the spinal cat; their results (see Table III) suggest that this is the most accurate method of all. The use of hexamethonium can also be applied to the dog, with results similar to those for the hexamethonium-treated cat. The cat method has also

been compared with the better known isolated organ preparations in which the same experimental design was used. The results, shown in Table III, confirm the superiority of the blood pressure methods.

TABLE III

COMPARISON OF METHODS OF ASSAYING ADRENALINE

Method	Author	*b*	*s*	*s/b*	Number of assays
Dog blood pressure	Noel[11]	73	2·44	0·034	27
Cat blood pressure, hexamethonium	Somers	53	2·09	0·042	19
Cat blood pressure, spinal	Somers	51	1·42	0·028	4
Dog blood pressure, hexamethonium	Somers	44	1·77	0·041	2
Rabbit gut	Somers	79	5·00	0·063	7
Rat colon, acetylcholine	Somers	128	7·55	0·059	4
Rat colon, Carbachol	Gaddum and Lembick[12]	—	—	0·13	9
Rat uterus, Carbachol	Gaddum and Lembick[12]	47*	2·06*	0·062	9

* One experiment only.

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SCHOOL OF PHARMACY
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The Determination of Small Amounts of Lithium

BY C. F. FORSTER

A method has been devised for quantitatively separating microgram quantities of lithium from an excess of sodium and potassium salts by precipitating the lithium potassium ferricyanide hexamethylenetetramine complex from an acetone - water solution.

The yellow complex can be separated by filtration and weighed, or redissolved in water and the lithium determined absorptiometrically.

THE determination of small amounts of lithium by chemical methods is difficult owing to the small number of lithium compounds that are sufficiently insoluble to be of use for its isolation from large quantities of other elements. For this reason the most reliable modern methods are physical in character. For example, flame photometry[1] and emission spectrography[2] are frequently used, and polarographic[3] and fluorimetric[4] methods are also used.

Before the introduction of modern physical methods of chemical analysis, most methods for the determination of lithium depended on the solubility of lithium chloride in various organic solvents and its separation thereby from the comparatively insoluble sodium and potassium salts. The solvent was then removed by distillation, and the lithium chloride was weighed directly or after conversion to the sulphate. These methods, as well as the recent ion-exchange separation,[5] permit reasonable results to be attained when the amount of lithium in the sample is fairly large, and the determination can be performed on the macro

scale. They are still useful for effecting a preliminary concentration of small quantities of lithium from much larger quantities of sodium and potassium salts. The amount of sodium and potassium chlorides soluble in the organic solvents used is largely dependent on the amount of water present in the solvent. The complete dehydration of some of the solvents is not easy, as most of them have a considerable affinity for water and some are hygroscopic.

Some of the organic liquids that have been used to effect the separation of lithium chloride from other alkali metal chlorides are shown in Table I.

TABLE I

HISTORICAL SURVEY OF SOLVENTS USED FOR THE SEPARATION OF LITHIUM CHLORIDE

Solvent	Method	Date
Ethyl ether - ethanol	Rammelsberg[6]	1845
Amyl alcohol	Gooch[7]	1886
Pyridine	Kahlenberg and Krauskopf[8]	1908
*iso*Butanol	Winkler[9]	1913
n-Butanol - ethyl acetate	Smith and Ross[10]	1925
Dioxan	Sinka[11]	1930
Acetone	Brown and Reedy[12]	1930
2-Ethylhexanol	Caley and Axilrod[13]	1942
n-Propanol	Plyuschev and Shakhno[14]	1953

In 1929, Moser and Schutt[15] made a thorough investigation of the then known methods and declared that most of them were unreliable. They approved Winkler's *iso*butanol method and improved it by the use of a new technique for drying the solvent with barium oxide. Sinka[11] and Brown and Reedy[12] claimed that sodium and potassium chlorides were nearly insoluble in the completely dry solvents. In 1953, Plyuschev and Shakhno proposed the use of *n*-propanol saturated with dry hydrogen chloride gas. They found that sodium and potassium chlorides were nearly insoluble in this solvent, but the method was only of use for macro amounts of lithium salts.

Among the more novel procedures is one described by Brauner[16] in which lithium was precipitated as lithium phosphate, which was centrifuged in a standard capillary tube. The height of the precipitate was used as a measure of the amount of lithium precipitated.

A method for removing the small amounts of potassium and sodium chlorides remaining after a separation with an organic solvent was devised by Caley,[17] who precipitated the lithium as stearate and measured the resultant turbidity. By this method he claimed to be able to detect 0·1 mg of lithium in 1 g of mixed chlorides.

Various lithium compounds have been proposed as suitable for the precipitation and determination of lithium, and their solubilities are shown in Table II.

TABLE II

SOLUBILITIES OF VARIOUS LITHIUM COMPOUNDS IN WATER AT 15° C

Compound	Solubility in 100 ml of water at 15° C, g
Lithium carbonate	1·33
Lithium fluoride	0·27
Lithium phosphate	0·04
Lithium arsenate	Very slightly soluble
Lithium silicate	Insoluble
Lithium palmitate	0·01
Lithium stearate	0·01
Lithium aluminate, $2Li_2O.5Al_2O_3$	Insoluble

The use of sodium arsenate was proposed by T. Gaspar,[18] who claimed that the resulting precipitate of lithium arsenate could be ignited and weighed.

Grothe and Savelsberg[19] severely criticised the use of lithium fluoride and lithium phosphate, and they recommended the use of potassium aluminium sulphate, $KAl(SO_4)_2.12H_2O$, as a precipitant. The resulting lithium compound could then be ignited to lithium aluminate, $2Li_2O.5Al_2O_3$.

In 1938, Korenman and Kursina[20] published details of a spot test for the detection of small amounts of lithium in the presence of sodium, potassium, caesium and rubidium.

The lithium salt was precipitated from a concentrated aqueous solution by the addition of 15 per cent. potassium ferricyanide and 15 per cent. hexamethylenetetramine (hexamine) solutions in water. The formation of characteristic octahedral crystals, visible under the microscope, was indicative of the presence of lithium. Ammonium, potassium, sodium, rubidium and caesium did not interfere, and as little as 0·6 μg of lithium could be detected at a concentration of 1 in 50,000.

Poluektoff[21] described a spot test that was specific for lithium. It involved the use of a ferric periodate complex dissolved in excess of potassium hydroxide, which formed a yellowish-white precipitate with lithium. There was no interference from potassium, sodium, rubidium or caesium. This reaction forms the basis of a method devised by Sandell[22] in which the precipitate was removed by filtration, washed with potassium hydroxide solution, then dissolved in acid and the iron determined. The sodium compound was only sparingly soluble and was co-precipitated, so that it was first necessary to remove most of the sodium by extraction of the dry chlorides with ethyl ether - ethanol mixture.

Rogers and Caley's method[23] was similar, but their periodate complex was of uncertain composition and it had to be precipitated under rigid conditions to ensure uniformity of results. The final determination was made by liberation of the iodine followed by titration with standard sodium thiosulphate.

Experimental

The lithium potassium ferricyanide hexamine complex is extremely soluble in water and in an attempt to precipitate it quantitatively, some method of decreasing this solubility was sought, but this must not cause the precipitation of the reagents. Closely allied to this problem is that of washing the precipitate free from the excess of potassium ferricyanide, which is yellow like the lithium complex. This can be done by keeping the volume of the solutions as small as possible and by adding a miscible organic liquid, such as ethanol or acetone, to decrease the solubility of the complex.

The reagent was prepared by mixing the solutions described by Korenman and Kursina[20] in equal proportions and adding acetone until a slight precipitate was formed. It was also found to be necessary to add acetone to the test solution. This was most conveniently done by evaporating the solution to dryness and dissolving the residue in the minimum amount of the solvent mixture. A similar mixture containing slightly different proportions was used for washing the precipitate, followed by acetone - water mixture to remove the hexamine present in the first washing mixture, if the determination was to be completed by the gravimetric method. The precipitate can be dried and weighed, or it can be dissolved in water to form a stable yellow solution, the optical density of which can be measured with a Spekker absorptiometer. The optical density method is more reliable owing to the difficulty of removing the excess of hexamine from the lithium complex.

A colour 20 times as sensitive can be developed from the colourless leuco base of malachite green, which is quantitatively oxidised to the green dye by ferricyanide. This reaction can be used to determine ferricyanide ion provided that no other oxidising agent is present in the solution.

Method

Apparatus—

Thermostatically controlled water-bath—To maintain reagents and test solutions at 20° C.

Equilibrium chamber—This consists of an ordinary glass desiccator containing an acetone - water (1 + 1) mixture in place of the normal desiccant. The beakers of test solution are placed in it during the standing period after precipitation so that there is no loss of acetone by evaporation, as this would increase the solubility of the lithium complex.

Micro filtration apparatus—This consists of a filter-stick connected by rubber tubing and fine-bore glass tubing to a suction flask or Witt's filtering apparatus. The rubber connections of the filter-stick should have sufficient flexibility to permit the filter-stick to be lowered on to the surface of the liquid in the beaker and to follow it down as the liquid is removed. If the head of the filter-stick is immersed, the droplets on top of it are not sucked in when the beaker becomes dry and extra washing is required.

REAGENTS—

Hexamine solution, 30 per cent.—Dissolve 30 g of hexamine in 80 ml of water and dilute to 100 ml.

Potassium ferricyanide, 15 per cent. solution.

Ferricyanide reagent—Mix 50 ml of 30 per cent. hexamine solution, 50 ml of water and 100 ml of 15 per cent. potassium ferricyanide solution. Warm the mixture to about 35° C and slowly add 150 ml of acetone with stirring. Place the reagent solution in a stoppered bottle and put the bottle in a thermostatically controlled water-bath at 20° C for 24 hours before use so that the solution can reach equilibrium; a small amount of solid will settle on the bottom of the bottle.

Solvent solution—Mix 100 ml of 30 per cent. hexamine solution, 105 ml of water and 200 ml of acetone and store the solution at 20° C.

Washing solution—Mix 100 ml of 30 per cent. hexamine solution with 110 ml of acetone and store the solution at 20° C.

Malachite green leuco base—Warm 0·4 g of malachite green with granulated zinc and dilute hydrochloric acid until the solution is colourless and then dilute it to 500 ml. Decant the clear solution into a bottle.

MACRO PROCEDURE—

Concentration of the lithium—(This procedure is unnecessary if the total alkali salts present are less than 50 mg.) Evaporate the solution containing only chlorides of the alkali-metal group to dryness in a porcelain dish and bake the dish gently to remove ammonium salts. Scrape out the solid into a mortar and crush the aggregates. Transfer it to a Soxhlet thimble that has been dried in an oven at 105° C. Insert a plug of glass-wool into the top of the thimble and return it to the oven for a further half hour. As rapidly as possible insert the thimble into a continuous-extraction apparatus and extract it for 6 hours with 40 ml of dry *n*-propanol.

At the end of this time cool the flask and filter the contents through a filter-paper into a clean flask, washing the original flask with dry *n*-propanol. Remove the solvent by distillation, and transfer the salts to a 10-ml tared beaker with the aid of a few millilitres of distilled water. Evaporate the solution to dryness, cool the residue in a desiccator and weigh it.

Precipitation—Dissolve the dry residue in solvent solution at 20° C, using 1 ml of solvent for each 5 mg of solid. When all the residue is dissolved, transfer 1 ml of the solution by pipette to a 5-ml beaker and add 2 ml of ferricyanide reagent. Mix the solution carefully, avoiding wetting or splashing the sides of the beaker. Place lids on the weighing-bottles and maintain them at 20° C for a quarter of an hour. If beakers are used, place them in the equilibrium chamber at 20° C for a quarter of an hour.

Filtration—Filter the solution through a No. 3 sintered-glass crucible under suction. Allow the precipitate to drain and, without delay, wash out the beaker with 0·5 ml of solvent solution followed by successive 0·5-ml portions of washing solution delivered from a pipette, pouring each portion through the sinter so that eventually the whole precipitate is transferred to the sinter and washed free from the excess of ferricyanide reagent. Four or five such washes are generally sufficient. For large precipitates 1-ml portions may be used.

Gravimetric determination of the complex—If a gravimetric determination is desired, the precipitate must be further washed with a mixture of acetone containing 6 per cent. of water to remove the hexamine that is present in the normal washing solution. About five 1-ml portions of aqueous acetone are sufficient for this. The crucible is finally washed with pure acetone, dried at 50° C and weighed.

The weight of precipitate divided by 48·905 = weight of lithium.

Absorptiometric determination of the complex—Dissolve the precipitate through the filter into a clean flask with water and dilute the solution to a suitable volume (the use of 100 ml of water for each 1500 μg of lithium ensures a suitable concentration). Measure the absorption of the solution, making use of the Spekker absorptiometer with a 1-cm cell and Ilford No. 601 violet filters and a water setting of 1·00.

Blank determination—As a check on the purity of the reagents, a standard of known lithium content should be tested with each batch of determinations.

High results are caused by an excess of acetone in one of the solutions leading to precipitation of the ferricyanide reagent and the co-precipitation of other salts.

Low results are usually due to one or more of the following—

(*i*) a rise of temperature during precipitation;
(*ii*) loss of acetone by evaporation causing the water to acetone ratio to be too great;
(*iii*) absorption of water from the atmosphere; and
(*iv*) incomplete drying of the salts when the solution is evaporated to dryness.

Calibration—Accurately measure aliquots of a solution of pure lithium chloride in water into 10-ml beakers. Aliquots containing 0, 200, 400, 600, 800, 1000, 1200 and 1400 μg of lithium are suitable. Evaporate the solutions to dryness and proceed as described in the method above, using 1 ml of solvent solution and 1 ml of reagent. Plot the optical densities against concentration of lithium in μg per 100 ml of solution.

MICRO PROCEDURE—

For the determination of less than 50 μg of lithium, some refinement of the above method is necessary.

Precipitation—Transfer the final solution containing the lithium salt, freed from other metals, to a 5-ml beaker or weighing-bottle and evaporate it to dryness. The total residue should not exceed 2000 μg. Dissolve it in 0·2 ml of solvent solution at 20° C. Add 0·5 ml of reagent from a pipette without splashing the sides of the beaker and place the beaker in the equilibrium chamber at 20° C for 1 hour.

Filtration—After applying the suction, gently lower the filter-stick on to the surface of the liquid and continue to remove the liquid until the beaker is dry. Wash the beaker with 0·5 ml of solvent solution followed by three successive 0·5-ml portions of washing solution from a pipette so that all the excess of reagent solution is removed. The final washings should be quite colourless as they pass through the capillary tube. Replace the receiver by a clean flask. Dissolve the precipitate in a few drops of water and suck the solution through the filter-stick into the flask. Wash the beaker and filter-stick with further small portions of water and suck them through into the flask. Transfer the solution to a 100-ml calibrated flask and add 40 ml of ethanol and 8 ml of malachite green leuco base solution. Dilute the solution to the mark with water and mix it thoroughly. Allow it to stand for 15 minutes before measuring the optical density.

Transfer a portion of the solution to a 2-cm cell and measure its optical density, making use of the Spekker absorptiometer, with an Ilford No. 607 orange filter and a water setting of 1·00.

Calibration—With a pipette, place 2 ml of each of the yellow solutions used to calibrate the graph for the macro method and a blank into 100-ml calibrated flasks. To each solution add 40 ml of ethanol and 8 ml of malachite green leuco base solution. Dilute the solution to the mark and mix it. Allow it to stand for 15 minutes and measure the optical densities with a Spekker absorptiometer, Ilford No. 607 orange filters and a water setting of 1·00. Plot the optical densities against concentration of lithium in μg per 100 ml of solution.

Alternatively a calibration graph can be prepared from a standard solution of potassium ferricyanide on the assumption that 2 atoms of lithium are equivalent to 1 molecule of potassium ferricyanide.

RESULTS

Some typical results are shown in Tables III, IV, V and VI.

INTERFERING RADICLES

The heavy metals interfere and must be removed by normal analytical procedures.

Calcium can be removed by precipitation as oxalate and magnesium by precipitation with 8-hydroxyquinoline. The use of ammonium phosphate to precipitate magnesium should be avoided as the excess of phosphate is difficult to remove. Calcium and magnesium can be removed simultaneously by precipitation with 8-hydroxyquinoline.[24]

The alkali metals do not interfere but, if they are present in greater quantities than the limits stated below, they are co-precipitated and lead to high results for lithium.

The amounts of alkali metals as chlorides that can be tolerated in 1 ml of solvent solution at 20° C are as follows: potassium, 30 mg; ammonium, 5 mg; sodium, 5 mg; rubidium, 50 mg; and caesium, 30 mg.

TABLE III

DETERMINATION OF KNOWN AMOUNTS OF LITHIUM BY THE MACRO METHOD

Lithium taken, μg	Lithium found, μg	Error, μg	Lithium taken, μg	Lithium found, μg	Error, μg
50	48	−2	150	150	Nil
55	58	+3	150	157	+7
60	58	−2	160	167	+7
70	65	−5	180	178	−2
70	72	+2	190	200	+10
83	86	+3	220	230	+10
90	88	−2	250	257	+7
100	100	Nil	280	275	−5
100	102	+2	310	315	+5
110	115	−5	500	488	−12
120	119	−1	1000	1010	+10
130	128	−2	1500	1515	+15
138	145	+7	2000	2000	Nil

TABLE IV

DETERMINATION OF LITHIUM AFTER SEPARATION FROM A LARGE EXCESS OF SODIUM CHLORIDE BY EXTRACTION WITH *n*-PROPANOL

Lithium taken, μg	Lithium found,* μg	Error, μg
1 g of sodium chloride present—		
60	65	+5
90	93	+3
120	124	+4
150	147	−3
180	190	+10
20 g of sodium chloride present—		
400	415	+15
500	510	+10
600	620	+20
1000	970	−30
2000	1970	−30

* Corrected for blank due to impurities in the sodium chloride.

TABLE V

DETERMINATION OF LITHIUM IN THE PRESENCE OF OTHER SALTS

Lithium taken, μg	Lithium found, μg	Error, μg
4·5 mg of sodium chloride present—		
50	47	−3
50	53	+3
60	60	Nil
60	65	+5
70	73	+3
80	84	+4
80	79	−1
90	88	−2
100	104	+4
100	104	+4
150	155	+5
200	195	−5
30 mg of potassium chloride present—		
500	497	−3
1000	990	−10

TABLE VI

DETERMINATION OF LITHIUM BY THE MICRO METHOD

Lithium taken, μg	Lithium found, μg	Error, μg
5	7	+2
10	11	+1
15	16	+1
20	20	Nil
30	33	+3
40	43	+3
50	49	−1

COMPOSITION OF THE COMPLEX

The complex was originally considered to be lithium ferricyanide plus an unknown number of hexamine molecules. However, the graph constructed for the determination of lithium by malachite green based on potassium ferricyanide showed that there were only two lithium atoms equivalent to each molecule of ferricyanide.

To establish the probable formula of the complex the following analysis was made. The complex (68,100 μg) made by precipitating 1400 μg of lithium was dissolved in water. Silver sulphate was added to the hot solution to precipitate silver ferricyanide. This was removed by filtration, washed with water, dissolved in concentrated sulphuric acid, and the silver was determined by precipitation as chloride.

This showed a ratio of 3Ag to 2Li, which confirmed the result from the calibration graph.

Nitrogen was determined in the filtrate from the silver ferricyanide precipitation by means of Nessler's reagent after distillation, and 9400 μg of nitrogen were found.

From these results a probable formula for the complex is—

$$2Li_3Fe(CN)_6.K_3Fe(CN)_6.5[(CH_2)_6N_4.6H_2O].$$

Hence 48·905 g of the complex contain 1 g of lithium.

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The Separation of Zinc from Other Elements by the Use of Activated Copper

By ALEXANDER BRYSON and S. LENZER-LOWY

Zinc can be separated from other metals by the addition of activated copper powder to a solution containing cyanide and tartrate. Lead, bismuth, tin, cadmium, silver and mercury are precipitated by the copper, whereas zinc, cobalt, nickel, copper, iron and aluminium remain in solution. Zinc is precipitated from the filtrate by sodium sulphide, and the sulphide is redissolved in acid and determined either volumetrically or gravimetrically. Manganese, if present, is removed before the copper powder is added. The method is applied to the analysis of several ores.

A METHOD is presented for the separation of zinc from other elements in alloys such as brasses, bronzes and zinc ores. Activated metallic copper powder is added to a solution of the alloy containing cyanide and tartrate. It displaces lead, bismuth, tin, cadmium, silver and mercury so that only zinc, copper, cobalt, nickel, iron and aluminium remain in the solution. When sodium sulphide and sodium hydroxide are added to this solution, zinc sulphide free from other metallic sulphides is precipitated; the zinc in the precipitate is determined volumetrically or gravimetrically. Antimony interferes and should be absent except for small amounts. Manganese, if present, is removed as manganese dioxide before the reduction with copper.

The only method hitherto described that ensures in two operations a reasonably complete separation of zinc from most other metals consists in precipitating the metals of the copper and arsenic group with hydrogen sulphide in fairly strong acid solutions, and then precipitating zinc after buffering to pH 2 to 3. This method, however, has some undesirable features. Besides requiring large amounts of hydrogen sulphide, it is well established that copper, cadmium and mercury sulphides adsorb considerable amounts of zinc sulphide.[1,2,3] Caldwell and Moyer[4] recommend the addition of small amounts of crotonaldehyde to reduce co-precipitation.

For routine analysis by the acid ferrocyanide titration, it is usually unnecessary to separate zinc as sulphide, as of the common interfering elements, copper, cadmium, manganese, aluminium and iron, all but cadmium are readily removed. The usual methods of separating these metals, however, lead to unsatisfactory conditions for the ferrocyanide titration.[5] In the alkaline ferrocyanide method frequently used in mining laboratories, copper and cadmium must be removed, while ferric ion, if absent, is added together with citrate and ammonium hydroxide. The end-point of the titration with potassium ferrocyanide is indicated by the appearance of Prussian blue on a spot-plate in the presence of acetic acid. The method is very sensitive to operating conditions, but apparently the results are consistent when it is used by experienced workers. The figures for zinc quoted by the laboratories supplying the samples of zinc ore used by us were determined by this method.

In a previous paper[6] we described a method for the separation of cadmium and zinc based on the fact that copper will displace cadmium but not zinc from a solution containing cyanide and tartrate. This principle has now been extended, and it has been found that from solutions containing cyanide and tartrate, activated copper will also displace bismuth, tin, lead, mercury and silver, while zinc, cobalt, nickel, antimony, manganese, iron and aluminium remain in solution. When sodium sulphide and sodium hydroxide are added to the solution, zinc sulphide free from all elements except antimony and manganese is precipitated. Although antimony trisulphide is not precipitated on the addition of sodium sulphide and sodium hydroxide, appreciable amounts are co-precipitated with zinc sulphide. As it is insoluble in the acid used to dissolve the zinc sulphide, a small amount of antimony trisulphide can be tolerated, but manganese must be removed.

It is possible to determine approximate values for the standard electrode potentials, E_o', for the following reactions—

$$\begin{aligned}
Cu + 4CN' &\rightleftharpoons Cu(CN)_4''' + e \\
Zn + 4CN' &\rightleftharpoons Zn(CN)_4'' + 2e \\
Cd + 4CN' &\rightleftharpoons Cd(CN)_4'' + 2e \\
Hg + 4CN' &\rightleftharpoons Hg(CN)_4'' + 2e \\
Ag + 2CN' &\rightleftharpoons Ag(CN)_2' + e \\
Ni + 4CN' &\rightleftharpoons Ni(CN)_4'' + 2e
\end{aligned}$$

from the standard electrode potentials of the metals in non-complex forming solutions and the instability constants[7,8] of the complex ions. The calculated values refer to solutions of unit activity for the ions concerned and therefore do not apply to the conditions used in practice. However, they may be taken as indicative of the relative ease of replacement of the metal. Calculated values for E_o' together with recorded instability constants for the complex ions are as follows—

	Copper	Zinc	Cadmium	Mercury	Silver	Nickel
Instability constant ..	2×10^{-27}*	$1{\cdot}3\times10^{-17}$†	$1{\cdot}4\times10^{-17}$†	$2{\cdot}5\times10^{-41}$†	$3{\cdot}8\times10^{-19}$†	3×10^{-29}‡
E_o'	1·09	1·26	0·90	0·37	0·29	1·09

* From Sidgwick.[7]
† From Latimer.[8]
‡ Value from spectrophotometric measurements by B. Morris.

These figures indicate that copper should displace silver, mercury and cadmium from a solution containing cyanide.

There is little known about the instability constants of cobalt and manganous complex cyanides and the published figures for the $Ni(CN)_4''$ complex are unreliable owing to the uncertain behaviour of nickel as a metal electrode; but qualitative tests show that these metals are not displaced from solution by activated copper. Cobalt and nickel do not interfere with the precipitation of the zinc sulphide by sodium sulphide from complex cyanide solution.[9] Manganese, however, must be removed because it is partly precipitated under these conditions.

Experimental

Qualitative tests were made initially to determine the metals, other than cadmium, precipitated by copper. This was done by forming the complex cyanide - tartrate of the metal being tested, boiling the solution with copper powder and observing whether or not a deposit appeared on the copper. The filtrate was tested for quantitative removal of the precipitated metal. It was found that bismuth, tin, lead, silver and mercury were precipitated by copper powder, while iron, copper, cobalt, manganese and nickel were converted to stable complex cyanides that remained in solution. Arsenic remained in solution as arsenate and antimony and aluminium as complex tartrates. Only manganese was precipitated by the addition of sodium sulphide, but in presence of zinc and cyanide ions, orange antimony trisulphide was co-precipitated with zinc sulphide and so antimony should be absent except for traces. Arsenic was not precipitated under these conditions.

If manganese is present, it is removed by precipitation as manganese dioxide with hydrogen peroxide from the complex cyanide solution. This method is only suitable with small amounts of manganese, because in the presence of zinc the precipitate may contain manganites. Also, if tartrates are present, the manganese is so strongly complexed that it is not precipitated by hydrogen peroxide, or if partly precipitated, as sometimes happens, it usually redissolves on boiling. Therefore the manganese must be removed before the tartrate is added. If manganese is absent, it has been found preferable to add sodium potassium tartrate to the acid solution after heating to fumes with sulphuric acid rather than after neutralisation of the acid solution with sodium carbonate and addition of potassium cyanide. In this way it is easier to form the soluble complex tartrates, particularly those of metals that do not form complex cyanides. The concentrations of potassium cyanide and sodium potassium tartrate for the cadmium - zinc separation[6] were found to be suitable for the general separation also. The preparation of an active copper powder is described in the same paper.

To determine the efficiency of the method, two sets of quantitative tests were made. In the first series, solutions were prepared containing 1 mg per ml each of arsenic, tin, cadmium,

bismuth and lead; 25 ml of this mixture were added to various amounts of zinc from 6 to 160 mg. After separation of the interfering elements, the zinc was determined. The results were as follows—

Weight of zinc added, mg	166·0	66·4	33·2	6·6
Weight of zinc found, mg	165·5	66·0	33·2	6·2

In the first two experiments the zinc was estimated volumetrically and in the last two gravimetrically.

In a second series of experiments, zinc was separated from mixtures containing various amounts of silver and mercury. The zinc was determined volumetrically and the results are shown in Tables I and II.

TABLE I

SEPARATION OF ZINC FROM MIXTURES CONTAINING VARIOUS AMOUNTS OF SILVER

Weight of silver added, mg	104·0	42·0	104·0	42·0
Weight of zinc added, mg	130·5	130·5	65·3	65·3
Weight of zinc found, mg	130·0	130·1	65·3	65·3

TABLE II

SEPARATION OF ZINC FROM MIXTURES CONTAINING VARIOUS AMOUNTS OF MERCURY

Weight of mercury added, mg	98·0	39·0	98·0	39·0	98·0	39·0
Weight of zinc added, mg..	163·1	163·1	130·5	130·5	65·3	65·3
Weight of zinc found, mg..	162·5	162·5	130·0	130·1	64·9	65·0

The method was then applied to five zinc ores supplied together with their analyses by Broken Hill South Limited and North Broken Hill Limited. The zinc in the ores was determined by separating it as described above and titrating it with potassium ferrocyanide according to the procedure of Richardson and Bryson.[5] Check analyses on two ores were made by the classical method of removing the copper and arsenic group with hydrogen sulphide from a moderately strong acid solution, followed by the precipitation of the zinc from a solution containing formic acid and sodium formate at a pH of 2 to 3. The zinc was subsequently determined gravimetrically as zinc pyrophosphate. The results of the analyses are recorded in Table III.

NOTES TO TABLE III—

In ores B and D the copper treatment was omitted, as the only interfering metal present in appreciable amounts was lead and this was removed during the initial stages by precipitation with sulphuric acid. In ore C a small amount of free sulphur remained after dissolution in acid. It must be removed by filtration before neutralising with sodium carbonate as it will dissolve in the alkaline solution and precipitate the metals as sulphides.

The results by the present method and the check analyses are in satisfactory agreement, but all the figures are lower than those supplied. These latter, however, were determined by the alkaline ferrocyanide method, which is generally considered to be less accurate than other procedures.

METHOD

Weigh a sample of ore or alloy containing not more than 200 mg of zinc into a 250-ml conical beaker, dissolve it in 10 ml of aqua regia, cool the solution and add 2 ml of concentrated sulphuric acid. Evaporate the mixture to fumes of sulphur trioxide, cool it and wash the sides of the beaker with 5 to 10 ml of water, and then add 5 g of sodium potassium tartrate and boil for several minutes. Cool the solution and neutralise it by adding small amounts of solid sodium carbonate until effervescence ceases; keep the beaker covered with a watch-glass during the operation. Wash the sides of beaker and add 10 per cent. potassium cyanide solution so that the weight of potassium cyanide is equal to about ten times the weight of metals in the solution. For instance, to 0·3 g of alloy add 30 ml of 10 per cent. potassium cyanide solution. When an unknown ore is analysed, assume a metal content of 50 per cent., *e.g.*, for 0·5 g of ore, add 25 ml of 10 per cent. potassium cyanide solution. Heat the alloy solution, which should have a volume of 70 to 80 ml. Add freshly prepared copper powder[6] in small amounts at a time, boiling the solution after each addition until after the last addition it remains red after boiling for several minutes. The reaction takes 15 to 30 minutes, according

to the amount of metals to be removed. Separate the copper powder and precipitate by immediate filtration through Whatman No. 30 filter-paper and wash the solid with water. To the filtrate add 20 ml of 5 *N* sodium hydroxide, dilute to about 200 ml, heat to boiling and add 25 ml of 10 per cent. sodium sulphide solution. Leave the white flocculent zinc sulphide to settle for several hours or overnight. Remove it by filtration through a Whatman No. 42 filter-paper and wash it with 2 per cent. sodium chloride solution. No difficulty should be experienced with the filtration if the filter-paper is carefully fitted to the funnel

TABLE III

ANALYSES OF ZINC ORES

Sample	A	B	C	D	E	
Insoluble matter, %	3·3	32·33	—	—	—	Analyses supplied
SiO_2, %	2·5	26·63	—	2·46	3·96	
Fe, %	8·79	5·70	13·8	5·67	9·30	
Al_2O_3, %	0·67	3·27	—	0·39	0·38	
MnO, %	1·96	4·47	—	1·11	1·72	
CaO, %	0·33	1·75	—	0·28	0·44	
S, %	31·50	10·71	29·3	16·66	31·17	
SO_3, %	0·075	2·66	—	—	—	
Pb, %	1·00	22·40	11·5	62·80	0·96	
Cu, %	0·09	0·18	23·5	1·26	0·075	
As, %	0·083	0·128	0·04	0·10	0·02	
Sb, %	0·40	0·055	—	0·29	0·036	
Cd, %	0·217	—	—	—	0·19	
Sn, %	—	—	14·7	—	—	
Ag	20 oz per ton	18·8 oz per ton	121·4 oz per ton	0·14%	0·004%	
Zn, %	52·00	15·90	5·6	8·30	51·10	
Zinc found by present method, %	51·41 (51·50, 51·30, 51·42)	15·55 (15·56, 15·53)	5·42 (5·51, 5·38, 5·36)	8·20 (8·14, 8·30, 8·14)	49·14 (49·17, 49·11)	
Zinc found by pyrophosphate method, %	51·64 (51·53, 51·75)	—	—	—	48·84 (48·95, 48·72)	
Copper treatment	yes	no	yes	no	yes	

Key to zinc ores:

A South Mine zinc concentrate from Broken Hill South Ltd.
B South Mine oxidised crude ore from Broken Hill South Ltd.
C South Mine Conrad ore from Broken Hill South Ltd.
D Flotation lead concentrate from North Broken Hill Ltd.
E Flotation zinc concentrate from North Broken Hill Ltd.

and washing is carried out as recommended. Of the electrolytes tried, sodium chloride was found to be the most satisfactory for preventing peptisation. Return the zinc sulphide precipitate and filter-paper to the beaker used for precipitation, dissolve it in dilute acid and determine the zinc in the filtrate either volumetrically or gravimetrically.

For amounts of lead exceeding 50 mg, it is often more convenient after the evaporation with sulphuric acid and before sodium potassium tartrate is added, to remove the lead sulphate by filtration, as lead sulphate is partly soluble in the tartrate. This also reduces the amount of copper powder required.

If manganese is present, it can be removed in the following way. After heating to fumes with sulphuric acid, dilute the residue with a small amount of water and neutralise the acid with solid sodium carbonate, and then add the calculated amount of 10 per cent. potassium cyanide solution and a few millilitres of 30 per cent. hydrogen peroxide and heat slowly to boiling. Continue to boil the solution for several minutes, filter it while hot through a Whatman No. 31 filter-paper and wash the residue with hot water. To the filtrate add sodium potassium tartrate and proceed as above.

Sometimes, particularly when much tin or bismuth is present, small amounts of the interfering metals remain in solution and are subsequently precipitated by sodium sulphide. On solution of the zinc sulphide in dilute acid, the impurities either remain insoluble or may be precipitated by passing hydrogen sulphide through the solution.

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Polarographic Determination of Free Sulphur in Petroleum Fractions

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A method is described for the polarographic determination of free sulphur in petroleum fractions. The solvent - electrolyte medium used is a 0·2 *M* solution of ammonium acetate in glacial acetic acid. A reduction wave of half-wave potential −0·39 volt is produced with a mercury-pool anode. The method is shown to be satisfactory for the direct determination of concentrations of free sulphur ranging from 0·5 to 10 p.p.m.

The non-interference of a number of organic sulphur compounds is demonstrated.

OWING to the corrosive effect of free sulphur on engine parts, the careful control of the free sulphur content of petrol and other hydrocarbons is considered essential in their production. Until recently no entirely satisfactory method had been proposed for the analytical determination of free sulphur. Proske[1,2] was the first to publish a polarographic procedure for the determination of sulphur extracted by pyridine from rubber. The polarographic reduction of the sulphur was carried out in an aqueous acetate buffer solution, which was an unsuitable solvent for petroleum fractions. With methanol - pyridine - hydrogen chloride as the electrolyte - solvent, Hall[3] reported successful determinations of free sulphur in petrol at concentrations of from 1 to 100 p.p.m. In the same year Gerber[4] published details of a polarographic method for determining free sulphur in kerosene and petrol, with a mixture of ethanol and benzene as the solvent and acetic acid - sodium acetate as the electrolyte. A more recent contribution on this topic is a paper by Eccleston, Morrison and Smith[5] on free sulphur in crude oil. Difficulties of solubility led these workers to use benzene - methanol - pyridine - hydrogen chloride as the solvent - electrolyte medium.

Bergman and James,[6] who investigated non-aqueous solvent - electrolyte media, reported on the use of glacial acetic acid containing ammonium acetate in polarography. Preliminary investigations indicated that this solvent - electrolyte might be used in the determination of free sulphur in petrol. The method has now been successfully developed and applied to petrols containing as little as 0·5 p.p.m. of free sulphur.

We have investigated the possibility that several organic sulphur compounds might interfere in these polarographic procedures, and have concluded that the polarographic method should be of wide application to petroleum products.

DISCUSSION AND RESULTS

With this method, in which a solution of ammonium acetate in acetic acid is used, the solubility of the petrol in the solvent - electrolyte was limited by the concentration of electrolyte in the solvent. The reduction waves were satisfactory in 0·2 *M* electrolyte solution. The waves became less distinct in more dilute solutions.

When dealing with petrols containing not more than 10 p.p.m. of free sulphur, a mercury-pool cell was found to be entirely satisfactory. Gerber[4] suggested, however, that for higher concentrations of free sulphur (20 p.p.m.), the use of a mercury pool was unsuitable owing to formation of a film of mercury sulphide on it.

This determination of free sulphur is based upon the measurement of the diffusion current produced when sulphur is reduced at a dropping-mercury cathode, the reduction occurring at a potential of −0·39 volt. Hall[3] suggested that the sulphur was reduced according to the following equation—

$$S + 2H^+ + 2e^- = H_2S$$

To establish the reliability of the quantitative determination of free sulphur, a number of standard solutions of sulphur of different types and sources in various solvents were prepared, and these standards were checked one against the other. A standard of 10 p.p.m. of sulphur in the electrolyte - solvent was finally used for calibration.

TABLE I

DETERMINATION OF FREE SULPHUR IN SYNTHETIC SOLUTIONS

Sulphur added, p.p.m.	0·40	0·50	0·80	1·00	1·20	1·60	2·00	2·00	2·40
Sulphur found, p.p.m.	0·40	0·45	0·80	1·00	1·20	1·60	2·00	1·90	2·40
Sulphur added, p.p.m.	2·80	3·00	3·20	3·60	4·00	5·00	5·00	6·00	
Sulphur found, p.p.m.	2·84	2·90	3·20	3·66	4·00	5·05	5·00	6·10	

It was observed that a maximum developed in the reduction wave of free sulphur in petrol at concentrations greater than 6 p.p.m. It has not been possible to find a suitable maximum suppressor. Despite its very low solubility in glacial acetic acid, gelatin may be used as a maximum suppressor, but in this application the gelatin was found to give a significant sulphur wave. In the medium used, the half-wave potential of the sulphur wave is −0·39 volt when a mercury-pool anode is used.

A number of synthetic samples of petrol containing from 0·4 to 6·0 p.p.m. of free sulphur were examined, and the results are shown in Table I; the polarograms are shown in Fig. 1.

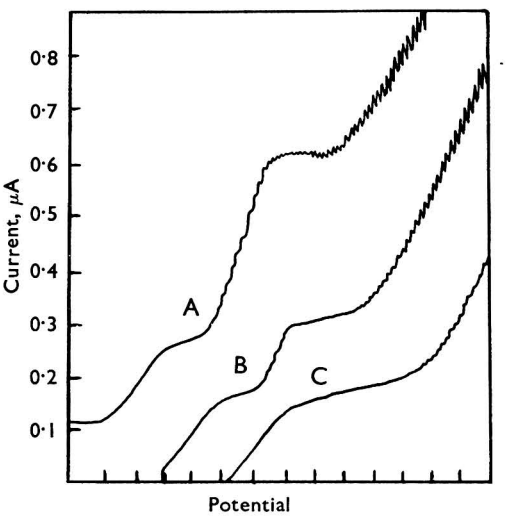

Fig. 1. Polarograms of free sulphur in petrol in glacial acetic acid solvent: curve A, 6·4 p.p.m. of sulphur; curve B, 2·4 p.p.m. of sulphur; and curve C, 0·4 p.p.m. of sulphur

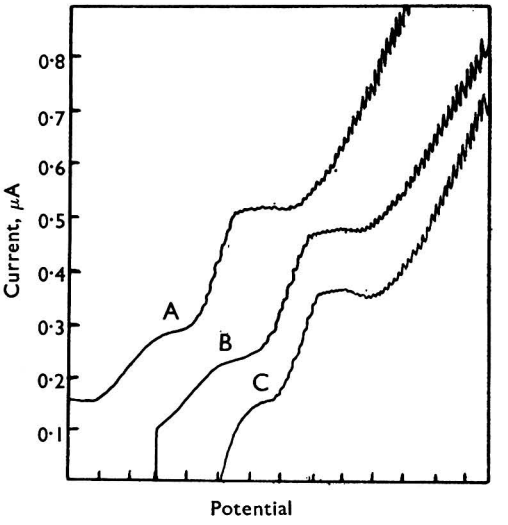

Fig. 2. Polarograms of free sulphur in petrol with added sulphur compounds: curve A, 4·4 p.p.m. of sulphur; curve B, 4·4 p.p.m. of sulphur + 100 p.p.m. of thiophene; and curve C, 4·4 p.p.m. of sulphur + 100 p.p.m. of butyl mercaptan

The diffusion currents were plotted against concentration and gave a straight line.

The results indicate satisfactory precision. The applicability of the method, however, depends on the non-interference of organic compounds, particularly those containing sulphur.

The examination of a number of such compounds was undertaken. Solutions of the compounds at concentrations of 100 to 500 p.p.m. in petrol were examined polarographically to determine whether there were reduction waves at the reduction potential of free sulphur (−0·39 volt). The same solutions were then examined after a standard amount of sulphur (4 p.p.m.) had been added to each; no change in the diffusion current or reduction potential

TABLE II

DETERMINATION OF FREE SULPHUR IN THE PRESENCE OF ADDED SULPHUR-CONTAINING COMPOUNDS

Sample*	*iso*Pentyl sulphide added, p.p.m.	Butyl mercaptan added, p.p.m.	Diphenyl sulphide added, p.p.m.	Sulphur added, p.p.m.	Sulphur found, p.p.m.
1	8·0	1500	1500	2·00	1·80
2	8·0	1500	1500	0·40	nil
3	nil	nil	nil	0·80	0·65
4	nil	nil	nil	4·00	3·65
5	8·0	1500	1500	1·60	1·40
6	8·0	1500	1500	10·0	10·0
7	nil	nil	4000	2·00	1·85
8	nil	nil	4000	4·00	3·65
9	nil	1000	2000	0·80	0·60
10	nil	1000	2000	nil	nil

* Samples 5 and 6 were made from refined petrol; all other samples were made from crude petrol mixtures.

of the sulphur was observed. The effects of the following sulphur compounds were examined: *iso*pentyl disulphide, carbon disulphide, thiophenol, thio-*p*-cresol, dimethylaniline sulphide, *iso*pentyl sulphide, β-phenyl-β-ethylmercaptopropiophenone, 4-phenylmercapto-2-butanone, phenol monosulphide, tetra-ethylthiuram disulphide, thiophene and butyl mercaptan (see Fig. 2).

It was observed that in the presence of thiophenol and thio-*p*-cresol the reduction wave of sulphur was shifted to a slightly less negative potential, making measurement of the wave-height slightly less accurate. It should be noted, however, that the proportion of free sulphur to "added impurity" was 1 to 25. Such a high ratio is not likely to be encountered in petroleum fractions.

TABLE III

DETERMINATIONS OF FREE SULPHUR IN PETROLS AND BENZENE

Sample	Petrol 1	Petrol 2	Petrol 3	Petrol 4	Benzene, A.R.	Crude benzene
Sulphur found, p.p.m.	0·10	0·40	0·50	0·30	nil	1·50

Hall[3] suggested that when a mercaptan was added to a petrol containing free sulphur the sulphur was "consumed," being converted into disulphide, and that the rate of consumption could be followed polarographically. A sample of petrol containing 4 p.p.m. of free sulphur and 100 p.p.m. of added butyl mercaptan was repeatedly examined polarographically over a period of 8 hours. No change in the diffusion current occurred over this period, which indicated the absence of any reaction of free sulphur with the butyl mercaptan.

A number of samples were prepared from petrol to which were added large amounts of organic sulphur compounds and small amounts of free sulphur. These samples were examined polarographically for free sulphur, and the results are shown in Table II.

The method described is of wide applicability to petrol fractions. Many samples of petrol and benzene have been examined and small amounts of free sulphur have been determined. Some typical results are shown in Table III. The reagents, glacial acetic acid and ammonium acetate, are easily obtained pure, and the mixture is an ideal solvent for many non-aqueous materials. The sensitivity of the method (0·5 p.p.m.) is such that it is not necessary to make polarograms for solutions containing more than 1 volume of petrol to

4 volumes of solvent - electrolyte, so difficulties due to limited miscibility of the hydrocarbons with the solvent - electrolyte do not arise.

Method

Apparatus—

A Tinsley pen-recording industrial polarograph, with mercury-pool cell. Capillary characteristics: drop time, 6·6 seconds in distilled water; rate of mercury flow, 1·8 mg per second; height of mercury column, 70 cm.

Reagents—

Acetic acid, glacial.
Ammonium acetate.
Sulphur, sublimed or monoclinic.

All reagents were of recognised analytical purity. Samples of sulphur from various sources were used to prepare standard solutions, monoclinic sulphur being the most soluble form.

A simple mercury-pool cell was used in this work. It was designed with a mercury trap to ensure an efficient seal to prevent interference from atmospheric oxygen.

For purposes of dilution a standard petrol containing less than 0·5 p.p.m. of free sulphur was used throughout the work.

The electrolyte - solvent used was 0·2 *M* ammonium acetate in glacial acetic acid.

Procedure—

Prepare the samples for analysis in 25-ml calibrated flasks. Into the flask place 5 ml of petrol sample and make it up to 25 ml with 0·2 *M* ammonium acetate in glacial acetic acid. If the sample contains more than 10 p.p.m. of free sulphur, dilute it with a standard petrol of known sulphur content.

Transfer the petrol solution to the polarographic cell, lower the anode dome, and bubble nitrogen through the solution for 5 minutes. As it is not certain that all the oxygen is removed when nitrogen is bubbled through the solution, it is important that a standard procedure is adopted. The seal prevents further exposure of the petrol solution to the atmosphere. Lower the anode dome further, until the anode lead is in contact with the mercury pool, and then raise the mercury reservoir to a standard height so that the mercury flows through the cathode capillary at a rate of 1·8 mg per second. Set the sensitivity of the instrument to 1·0 μA and the potentiometer panel indicator to 0·0 volt. Adjust the zero in the usual way and switch on the automatic recorder.

Switch on the auto-potentiometer and record the polarogram between 0·0 and −1·2 volts. Finally measure the height of the reduction wave at a potential of −0·39 volt.

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Polarographic Determination of 2:4:6-Trinitrotoluene and *cyclo*Trimethylenetrinitramine in Explosive Mixtures

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A polarographic method is described for the micro-determination of 2:4:6-trinitrotoluene and *cyclo*trimethylenetrinitramine.

Samples of less than a milligram of explosive are qualitatively identified by observing their characteristic half-wave potentials in an acetone solution of alkaline sulphite. The Ilkovič equation is shown to apply for the quantitative determination of the nitro compounds in such media.

The general composition of explosive mixtures containing ammonium nitrate, barium nitrate, nitrocellulose, pentaerythritol tetranitrate, 2:4:6-trinitrotoluene, *cyclo*trimethylenetrinitramine, and so on, has been described broadly by Thorpe and Whiteley.[1]

No reliable method exists for the accurate micro-determination of some of these constituents when present in small samples of mixed explosives, *e.g.*, explosives of the amatol or baratol type.

Solvent extraction or titrimetric methods are unduly cumbersome and inaccurate when only milligram quantities of sample are available and this necessitates the use of colorimetric or polarographic methods of analysis.

Colorimetric methods for the micro-determination of 2:4:6-trinitrotoluene are generally based on measurements of the intensity of the red colour that is given by this explosive in certain alkaline solutions (*cf.* Halfter and Winkler[2]).

The polarographic method has been found to give a reliable quantitative analytical procedure for cyclonite and T.N.T. explosives, and samples weighing about a milligram can be quickly and accurately assayed. But it should be remembered that the errors inherent in such polarographic micro-determinations are generally about ± 2 per cent. Where macro quantities of explosives are available, the classical methods of gravimetric analysis would be more accurate.

Pentaerythritol tetranitrate and nitrocellulose are irreducible at the dropping-mercury cathode, and their presence in the mixtures to be assayed introduces no particular complication. The sensitive explosive 2:4:6-trinitrophenylmethylnitramine does, however, give an ill-defined reduction wave at $-0{\cdot}3$ to $-0{\cdot}6$ volt with respect to the saturated-calomel electrode, and the presence of this substance would interfere with the polarographic determination of related nitro compounds. Organic nitro derivatives can generally be removed from mixtures containing inorganic nitrates by extraction with suitable solvents.

The mechanism of the polarographic reduction of nitro compounds has been studied by several investigators, and an excellent review of these researches has been published by Page,[3] who gave a comprehensive bibliography of work on the organic nitro derivatives.

Pearson[4] has carried out a detailed study of the reduction of nitrobenzenes and nitrotoluenes in aqueous ethanolic solution, and he has postulated that the irreversible reduction of the nitro group in these compounds consists in the reversible deposition of hydrogen ions at the cathode, which is a reaction determining the cathode potential and producing the observed current.

Few explosives are satisfactorily soluble in aqueous ethanol, and aqueous acetone is probably the only suitable medium for the polarography of insoluble *cyclo*trimethylenetrinitramine. The deoxygenation of the acetone solutions by means of a stream of nitrogen gas, with consequent loss of volatile solvent, may be rendered unnecessary by the use of a base solution that is $0{\cdot}05\ M$ with respect to sodium sulphite and borax. This procedure avoids the wave due to dissolved oxygen and considerably expedites the routine determinations of numerous small samples.

Experimental

2:4:6-Trinitrotoluene—

Pearson's values for the half-wave potentials of trinitrotoluene in alkaline ethanolic solutions have been confirmed. In alkaline acetone solutions of pH 9·98, the reduction

potentials at 25° C against the saturated-calomel electrode were markedly increased and were found to be −0·51, −0·65 and −1·05 volts, respectively, three separate reduction waves being readily discernible, as shown in Fig. 1.

Maxima were frequently developed at higher reduction potentials and constituted examples of what Heyrovský[5] has called "negative maxima." Gelatin, gum arabic and various dyestuffs were tried as suppressors, the results being best with 0·01 per cent. w/v solution of gentian violet or basic fuchsin. These dyes were strongly adsorbed on glass surfaces, but the use of a suppressor when only the first wave of trinitrotoluene was being recorded was considered unnecessary, as this wave appeared to be of a consistently stable character. The present analytical method is based on an examination of this first wave, and so no suppressors are used in the quantitative determinations.

Cambridge pen-recording and photographic polarographs were used throughout the investigation, the cells being maintained at 25° ± 0·2° C. Drop times were noted at the half-wave potentials, as they were found to vary appreciably with the applied voltage (*cf.* Lingane and Kolthoff[6]).

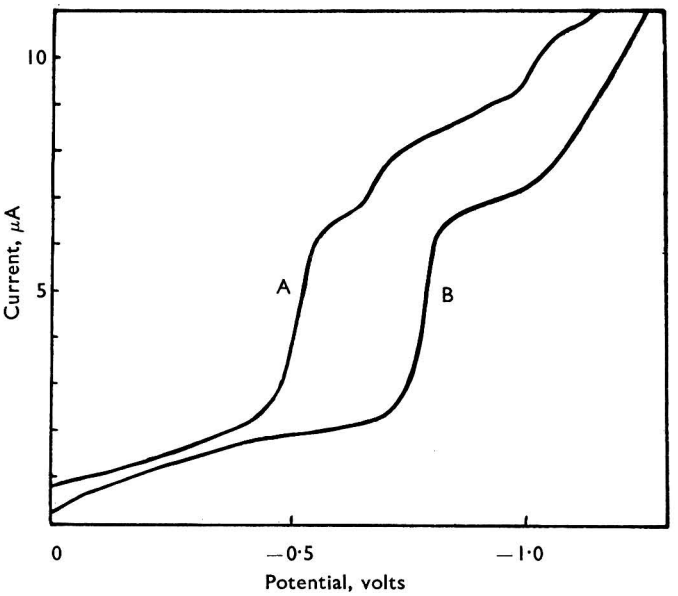

Fig. 1. Polarograms of T.N.T. and cyclonite: curve A, 0·1 g of T.N.T. per litre; curve B, 0·2 g of cyclonite per litre

ILKOVIČ CALIBRATION CURVE FOR 2:4:6-TRINITROTOLUENE—

For the preparation of a calibration curve, 2:4:6-trinitrotoluene (setting point 80·7° C) was dissolved in AnalaR acetone. Then 0·05 *M* sodium sulphite and 0·05 *M* borax solutions were prepared, and all solutions were placed in a thermostatically controlled water-bath maintained at 25° C. Just before the polarographic readings were taken, 1 volume of the 2:4:6-trinitrotoluene solution was intimately mixed with 2 volumes of the sodium sulphite solution and 2 volumes of the borax solution. Results for the diffusion constant are shown in Table I.

DETERIORATION OF 2:4:6-TRINITROTOLUENE SOLUTIONS—

Alkaline sulphite solutions of 2:4:6-trinitrotoluene undergo a slow decomposition on standing, and the recorded diffusion currents show a small, but progressive, decrease in magnitude. This reaction begins when the supporting electrolyte is mixed with the acetone solution of the explosive, and the rate of decomposition is of the same order at concentrations in the range 0·01 to 0·1 g of T.N.T. per litre. As a polarographic measurement can readily be made in 10 minutes by the method described above, the error in observation is less than 2 per cent., and for most purposes it may be regarded as negligible. The decomposition

is almost certainly due to the sulphite additive and, if the reaction is analysed according to the unimolecular law, the velocity constant decreases with increasing time, as shown by the results in Table II.

TABLE I

ILKOVIČ CALIBRATION CURVE FOR 2:4:6-TRINITROTOLUENE

$m = 1{\cdot}708$ mg per second $t = 3{\cdot}22$ seconds $m^{\frac{2}{3}}t^{\frac{1}{6}} = 1{\cdot}736$

Temperature = 25° C

Concentration of 2:4:6-trinitrotoluene, g per litre (C)	Current, μA	Diffusion constant, i/C
0·1129	4·25	37·8
0·0904	3·44	38·0
0·0600	2·28	38·0
0·0540	2·05	37·9
0·0452	1·75	38·7
0·0225	0·92	40·8

TABLE II

POLAROGRAPHIC DETERMINATION OF THE DETERIORATION OF A SOLUTION CONTAINING 0·01608 g OF 2:4:6-TRINITROTOLUENE PER LITRE

Time, hours	Current, μA	Loss, %	$1/t \log_{10} i/(i - x)$*
0·00	0·60	—	—
0·92	0·53	11·7	0·059
1·59	0·50	16·3	0·049
19·17	0·30	50·0	0·015
43·67	0·00	100·0	—

* x = amount of T.N.T. consumed.

The alkaline ethanolic solutions of pH = 9·20 that were used by Pearson[4] have also been examined for this effect, and the decrease in diffusion current was found to be negligible during the first day, but it occurred slowly thereafter. If the explosive mixture can be satisfactorily extracted with ethanol, then the use of an ethanol supporting electrolyte is to be recommended. It is considered, however, that the use of acetone as a solvent considerably simplifies the experimental procedure, and that the results are within the polarographic errors of measurement. Typical results for the recovery of known amounts of explosive are shown in Table III.

The explosive samples were weighed directly on a semimicro balance having a sensitivity of 9 μg per division, then dissolved in 1 ml of cold acetone and intimately mixed with 4 ml of the sulphite - borate base solution.

TABLE III

RECOVERY OF KNOWN MILLIGRAM AMOUNTS OF 2:4:6-TRINITROTOLUENE (DIFFUSION CONSTANT, $i/C = 37{\cdot}9$)

T.N.T. used, mg	Current, μA	T.N.T. recovered, mg	T.N.T. recovered, %	Mean deviation
2·58	19·12	2·522	97·7	2·6
1·78	14·00	1·847	103·8	
1·10	8·62	1·137	103·4	
0·72	5·39	0·711	98·7	

The average recovery from the four samples was 100·9 per cent., which gave a value of 2·6 per cent. for the relative mean deviation. Quantities of trinitrotoluene outside the range of the average microbalance can be determined quite satisfactorily by making polarograms for suitable solutions of small particles of explosives and determining the unknown concentration by interpolation of the Ilkovič calibration curve.

*cyclo*TRIMETHYLENETRINITRAMINE (CYCLONITE, HEXOGEN)—

No polarographic investigations on this explosive are recorded in the literature. The general chemistry of this substance has been described in detail by Davis,[7] who recommends acetone as a solvent for its recrystallisation, and this is the solvent used in the present investigation. One of the complications attending the analysis of this trinitramine is its insolubility in common organic solvents. The acetone base solution recommended for T.N.T. is suitable as a supporting electrolyte provided that the concentrations of the explosive are below 0·2 g per litre. Solutions in 50 per cent. acetone and in acetone - dioxan mixtures have been examined, but the inorganic salts tend to crystallise from these solvents. In a 20 per cent. acetone base solution, *cyclo*trimethylenetrinitramine is reducible at the mercury cathode to give a single, well-defined wave with a half-wave potential of −0·77 volt against the S.C.E. at 25° C. Thereafter, the polarographic curve rises steeply, the molecule being presumably unstable at the higher reduction potentials.

The diffusion current is found to vary linearly with concentration, the current magnitude for equivalent concentrations being approximately half that recorded for 2:4:6-trinitrotoluene at the same drop electrode. Typical results are shown in Table IV.

TABLE IV

ILKOVIČ CALIBRATION CURVE FOR *cyclo*TRIMETHYLENETRINITRAMINE

$m^{\frac{2}{3}}t^{\frac{1}{6}} = 1{\cdot}736$

Concentration, g per litre	Current, μA	Diffusion constant, i/C
0·1668	3·20	19·18
0·1334	2·55	19·11
0·1001	1·90	18·99
0·0667	1·29	19·34
0·0335	0·71	21·26
0·0167	0·28	16·89

MECHANISM OF THE REDUCTION OF CYCLONITE—

In the application of the Ilkovič equation to the determination of the electrode process, the diffusion coefficient, D, of the reducible substance must be known with reasonable accuracy. For the nitrophenols and nitrotoluenes, Pearson suggested that the molecular sizes of these materials were about the same as the benzoate ion, and he used a value for the diffusion coefficient of this ion that is calculable from conductivity data as $8{\cdot}28 \times 10^{-6}$ sq. cm per second at 25° C.

The Stokes - Einstein[8] equation suggests that D is given with fair accuracy by the expression—

$$D = \frac{RT}{N} \times \frac{1}{6\pi\eta r},$$

where r is the molecular radius and η is the viscosity. This expression has been used extensively[9] to determine the diffusion velocity of large ions at 25° C, and it is stated by Kolthoff and Lingane[10] in the form—

$$D = \frac{2{\cdot}96 \times 10^{-7}}{\eta V^{\frac{1}{3}}} \text{ sq. cm per second,}$$

where V is the molar volume of the pure substance in the solid state and D is considered to be at infinite dilution.

The viscosity of the 20 per cent. acetone base solution used in this investigation was determined at 25° C by means of a conventional B.S.S. Ostwaldt viscometer and was found to be 1·281 centipoises. Thorpe and Whiteley[11] quote a figure of 1·83 g per c.c. for the absolute density of cyclonite, and from these figures the diffusion coefficient at 25° C was computed to be $4{\cdot}67 \times 10^{-6}$ sq. cm per second.

Similarly, Lewis[12] gives a figure of 1·65 per g per c.c. for the absolute X-ray density of T.N.T., whence $D = 4{\cdot}48 \times 10^{-6}$ sq. cm per second for this substance in the present supporting electrolyte solution.

Accepting the conventional Ilkovič symbols, values of n, the number of Faradays required per mole of the electrode reaction, are as follows—

Substance	$m^{\frac{2}{3}}t^{\frac{1}{6}}$	Concentration, millimoles per litre	Current, μA	n
Cyclonite	1·901	0·5403	2·70	2·01
Cyclonite	1·736	0·7512	3·20	1·88
T.N.T.	1·736	0·4973	4·25	3·85

Conclusions

It is apparent that under the stated conditions, cyclonite undergoes a two-electron reduction according to the scheme—

$$2e + RNO_2 + 2H^+ = RNO + H_2O$$

Similarly, the first polarographic wave for 2:4:6-trinitrotoluene is due to a hydroxylamine reduction of the following type—

$$4e + RNO_2 + 4H^+ = RNH{\cdot}OH + 2H_2O$$

The author is indebted to the Division of Atomic Energy, Ministry of Supply, for permission to publish this paper.

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May 14th, 1954

Notes

THE DETERMINATION OF METALLIC LEAD IN PIGMENTS

ALTHOUGH metallic lead has been used in reductors of the Jones type by Cooke, Hazel and McNabb[1] and by McNabb, Hazel and Dantro,[2] the determination of metallic lead by a volumetric reduction method does not appear to have found practical application.

Light and Russel[3] used a solution of ferric sulphate in diluted sulphuric acid for the assay of aluminium powder. As there was need for a method of determining finely divided lead, it seemed probable to the author that, by the use of a similar but modified reagent, a sufficiently accurate method for technical consultative work might be evolved.

METHOD

REAGENTS—

Ferric sulphate reagent—Heat 250 g of anhydrous ferric sulphate with 135 g of 95 per cent. w/w sulphuric acid and 800 ml of distilled water until the solution is clear and light brown in colour; this usually entails heating for 5 minutes at 80° C.

Ferric sulphate - mixed acid reagent—To 300 ml of ferric sulphate reagent add 100 ml of 32 per cent. w/w hydrochloric acid and 50 ml of 85 per cent. w/w phosphoric acid.

Sodium acetate reagent—A saturated solution of sodium acetate containing 1 per cent. v/v of acetic acid.

Sodium thiosulphate solution, 0·1 N.

Iodine solution, 0·1 N.

Potassium permanganate solution, 0·1 N.

Ferrous sulphate solution, 0·1 N.

PROCEDURE FOR METALLIC LEAD—

Place a weighed amount of powder or pigment containing approximately 0·2 g of lead in a conical flask and add 45 ml of ferric sulphate - mixed acid reagent. Displace the air from the flask with carbon dioxide, fit a bunsen valve to the flask and heat the contents at 90° to 95° C for ½ to 2 hours. Cool the flask to 20° C, dilute the contents to 200 ml with cold freshly distilled water and titrate with 0·1 *N* potassium permanganate. Make a blank determination concurrently.

1 ml of 0·1 *N* potassium permanganate ≡ 0·01036 g of lead.

The precision approaches ±0·25 per cent., depending on the weight of lead taken.

PROCEDURE FOR METALLIC LEAD - RED LEAD MIXTURE—

Triturate a weighed amount of the powder with 30 ml of the sodium acetate reagent and add a trace of wetting agent if necessary. Add 50 ml of 0·1 *N* sodium thiosulphate and stir the mixture until the red lead disappears. Filter the mixture through a No. 2 porosity thimble, and wash the residue, the original flask and the thimble with water. Titrate the excess of sodium thiosulphate in the combined filtrate and rinsings with 0·1 *N* iodine. Make a blank determination in a similar manner.

1 ml of 0·1 *N* sodium thiosulphate ≡ 0·0343 g of red lead.

Wash the residue, mainly metallic lead, in the thimble with water and then with acetone. Dry it at 105° C, weigh it and then determine the metallic lead by the procedure described above.

PROCEDURE FOR ALUMINIUM AND LEAD IN ADMIXTURE—

Treat a weighed amount of the powder with the ferric sulphate reagent and heat the mixture at 60° to 70° C for 15 minutes. Cool the flask to 20° C and separate the lead and insoluble matter by filtration on a No. 2 porosity thimble. Dry the solid, weigh it and determine the lead as described above. Dilute the filtrate to a suitable volume and titrate aliquots with 0·1 *N* potassium permanganate.

1 ml of 0·1 *N* potassium permanganate ≡ 0·00090 g of aluminium.

It has been found possible to determine the lead remaining in the flask after the titration by adding hydrochloric and phosphoric acids as in the ferric sulphate - mixed acid reagent, and

then reducing the ferric salt by heating at 90° to 95° C. When lead chromate or zinc chromate is present in the pigment, alternative methods of analysis must be used.

PROCEDURE FOR ZINC AND LEAD IN ADMIXTURE—

Treat a weighed amount of the powder (containing about 0·1 g of zinc) with a mixture of 10 ml of the ferric sulphate reagent and 20 ml of 0·1 *N* potassium permanganate at 20° C for 10 minutes. Separate the lead and insoluble matter by filtration on a No. 2 porosity thimble. Dry the solid, weigh it and determine the lead as described above. Titrate the filtrate with either 0·1 *N* potassium permanganate or 0·1 *N* ferrous sulphate solution, whichever is necessary.

1 ml of 0·1 *N* potassium permanganate ≡ 0·00327 g of zinc.

The zinc should be free from aluminium, iron and magnesium, as these metals would affect the result.

RESULTS

The results of the determination of lead in various mixtures by the methods described above are shown in Table I.

TABLE I

DETERMINATION OF LEAD IN VARIOUS MIXTURES

	Lead taken, g	Lead found, g
Metallic lead pigment powder (99·1 per cent. of Pb)	0·2318	0·230
Metallic lead powder	0·193	0·191
White lead	0·308	0·307
Metallic lead powder	0·1672	0·166
Red lead	0·318	0·312
Sample containing 0·5 per cent. of organic matter—		
Metallic lead	0·307	0·306
Metallic aluminium	0·0342	0·0340
Sample free from organic matter—		
Metallic lead	0·2975	0·294
Metallic aluminium	0·0326	0·0322
Metallic zinc	0·0732	0·0730
Metallic lead	0·2198	0·2170

DISCUSSION

Sampling requires special care on account of the wide differences in specific gravity and other physical properties of the components of heterogenous mixtures containing metallic powders.

It may be necessary to add to the sample a weighed amount of finely divided inert inorganic material of high specific gravity, such as barium sulphate, on which the metallic powders will disperse themselves uniformly. Sometimes the method in which the entire sample is dissolved and aliquots are used for the assay is useful, especially for pigments extracted from paints. The above remarks are more important for samples that are free from organic matter. For samples containing organic matter, it has been found satisfactory to boil the extracted pigments twice with pure dioxan followed by one wash with cold acetone; great care is then needed when the final sample is prepared. On occasion, heating the sample in a vacuum oven at 250° to 300° C is useful for the removal of organic matter.

The author thanks Mr. F. Fancutt for permission to publish this Note and Mr. F. G. Dunkley and Mr. W. J. Hair for helpful advice.

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First submitted, *January 19th*, 1954
Amended, *April 27th*, 1954

MANGANESE DIOXIDE - ASBESTOS IN STEEL ANALYSIS

IN a combustion train for determining carbon in high-sulphur steel, the disadvantages of an acid silver nitrate solution for absorbing the main bulk of the sulphur oxides, caused by (*a*) dilution of the chromic acid trap by aqueous vapour and (*b*) by the necessity for frequent renewal of the silver solution, can be overcome by substituting for it a dry manganese dioxide absorbant.

A suitable reagent that does not impede the flow of oxygen can be prepared as follows.

Shake 25 g of ignited asbestos fibre with 400 ml of saturated potassium permanganate solution in a 2-litre flask, add 400 ml of saturated manganous sulphate solution and shake the flask thoroughly. Separate the manganese dioxide - asbestos by filtration under slightly diminished pressure, through a 6-inch funnel and filter-cone. Wash the fibre twice with hot water and dry it at 100° C.

A U-tube, $\frac{3}{4}$ inch in diameter and $5\frac{1}{2}$ inches high, filled with the reagent and placed in the combustion train immediately in front of the usual chromic acid bubbler, will absorb the bulk of the sulphur oxides from about 1100 g of steel containing sulphur in amounts varying between 0·03 and 0·50 per cent. before the filling needs renewal.

THE PARK GATE IRON AND STEEL CO., LTD.
ROTHERHAM, YORKSHIRE

A. P. LUNT
November 5th, 1953

Apparatus

A TWIN-BEAM NULL-POINT FLUORIMETER FOR THE ANALYSIS OF LIQUID SAMPLES

(*Presented at a meeting of the Physical Methods Group on Friday, May 28th*, 1954)

THE instrument described was constructed to measure the fluorescence produced at an intermediate stage in a particular reaction. Peak fluorescence is produced in 30 to 60 seconds after the reaction begins and then it rapidly diminishes in value. The exciting radiation is 3600 A and the wavelength of the fluorescent light is approximately 4000 A.

An attempt was first made to use a Hilger absorptiometer with barrier-layer cells and a fluorimeter attachment to measure the fluorescence developed, but this instrument was insufficiently sensitive. No high-amplification instrument for the measurement of fluorescence was commercially available in this country at the time the investigation of the reaction began, so it was decided to construct an instrument to make use of a photomultiplier as the light-detecting device.

A photomultiplier was chosen rather than a photo-cell and valve amplifier combination, because it offered a much more compact amplification system and at least as good a signal-to-noise ratio as could be attained when a photo-cell followed by a broad-band amplifier was used.

The first instrument made utilised a single light beam and one photomultiplier in which the unknown fluorescent solution was compared with a reference standard. A series of Ayrton shunts were connected as the final dynode load and operated with a Yaxley switch. A suitable shunt was switched into the circuit and the fluorescence value was indicated by the swing on a galvanometer connected across one arm of the shunt.

This instrument was very sensitive to the variations of the input voltage of both the lamp and the photomultiplier. The graph (Fig. 1) shows the magnitude of this error, even when the power supply was regulated through a stabilising transformer. Another disturbing feature of high-amplification single-ended instruments is that it becomes very difficult to remove the final traces of ripple in the power supply by smoothing, and this is indicated by a gentle oscillatory motion of the galvanometer needle when the instrument is used on a lightly shunted range. For these reasons it was decided to build a balanced light fluorimeter, of which the essential parts are described below.

SOURCE OF LIGHT—

A 12-volt 24-watt bulb having a single vertical spiral filament was used as the source of illumination. The single-ended fluorimeter was originally fitted with a mercury-vapour lamp as the light source, as this gave peak excitation at the required wavelength. In practice, however, it suffered from several defects, which were as follows—

(*i*) The apparent intensity fluctuated erratically owing to changes of path in the mercury arc. Other types of mercury lamp, in which attempts had been made to confine the arc to a precise path, still suffered from the same fault, although perhaps to a lesser extent.

(*ii*) It generated considerable heat, and thermal effects in the instrument became difficult to obviate.
(*iii*) The intensity of illumination depended on the length of time the lamp had been in use.
(*iv*) It was necessary to screen the lamp to avoid harmful effects to the operator.
(*v*) The current-limiting choke or resistance was bulky, so the final instrument lacked portability.

The small tungsten lamp replaced the mercury-vapour lamp with little loss of sensitivity. No attempt has been made to use a parallel beam of exciting light through the test solution.

DETECTOR SYSTEM—

Two photomultipliers, one on each side of the lamp, are connected together with the final dynode load resistors, one of which is variable, in a bridge circuit. A galvanometer is joined between the final dynodes. Under normal conditions a maximum current of 8 μA is permitted

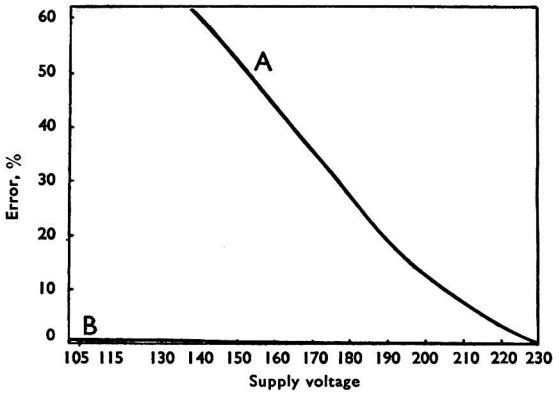

Fig. 1. Graph to illustrate the error in fluorimetric readings caused by variations in mains voltage: curve A, single photomultiplier, single-beam instrument; curve B, twin photomultiplier, twin-beam instrument

on each photomultiplier final dynode. It seems likely that both photomultipliers are operated under fatigue conditions, and this appears to give more stable long-term operating conditions with only a slight loss of amplification under the conditions of use.

(*a*) The photomultipliers are chosen with low dark-current, high gain and maximum response in the blue spectral region; 25,000-ohm, 1-watt resistors are used in the dynode chain.
(*b*) The variable load resistor is a 25,000-ohm Colvern or Fox helical-wound potentiometer fitted with a slow motion dial graduated in one thousand divisions. This is the final dynode load resistor in the control photomultiplier circuit.

The value of the fixed load resistor is determined experimentally as follows. With no light on either photomultiplier and the variable resistance set at maximum value, the apparatus is switched on. Beginning with a 25,000-ohm resistor as the test-photomultiplier final-dynode load resistor, resistances are inserted until the nearest point to current balance is found. If the value of resistance is markedly different from 25,000 ohms, say 15,000 or 35,000 ohms, one of the photomultipliers should be replaced, the test photomultiplier if the balance is at 15,000 ohms, the control if it is at 35,000 ohms.

(*c*) A Cambridge spot galvanometer, 1-μA full-scale deflection, is used to indicate the null-point. A shorting switch is fitted to protect the galvanometer.

TEST CELLS—

An optical-glass cell with internal dimensions of 4·5 cm high, 3·5 cm long and 1·5 cm wide was chosen because it had a capacity of 22 ml and the total amount of solution for examination was 25 ml. Light enters through a narrow face of the cell, and the photomultiplier measuring the developed fluorescence is placed as near as possible to a wide face.

A second cell, 3·5 cm high, 1·1 cm long and 1·1 cm wide, holding 2 ml of solution, was obtained, and retained in position in a suitably machined wooden block.

FILTERS—

(*a*) Wood's-glass filters are placed between the test cell and the lamp and between the control photomultiplier and the lamp. Originally, a 7·5 per cent. copper sulphate solution was also used to cut out unwanted red radiation, but it was later omitted without the accuracy of measurement being affected.

(*b*) An Ilford No. 106 filter is inserted between the test cell and the photomultiplier to eliminate stray light reflected or refracted from the test solution.

The filters described are used in our particular analytical procedure; alternative filters may be used in other determinations.

(*c*) A variable-density neutral filter is placed between the control photomultiplier and the light source, and is arranged to be readily adjustable. The range of this filter will determine

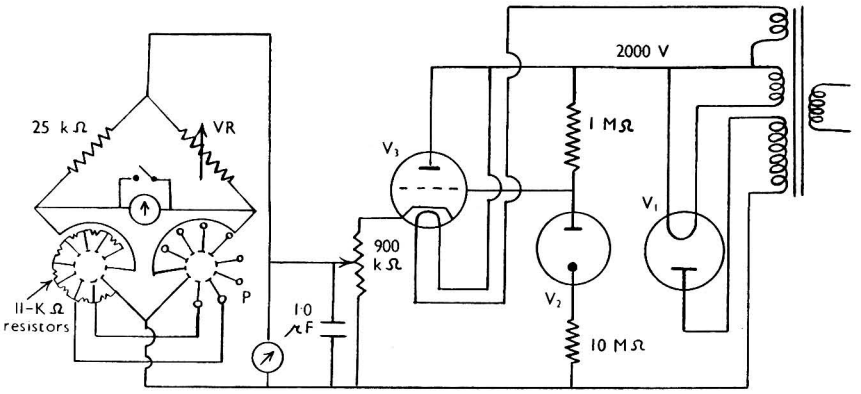

Fig. 2. Circuit diagram of twin-beam fluorimeter

the range of the instrument; the lowest density will give the largest range, say 0 to 100 μg of material, read over 1000 divisions on the graduated potentiometer dial, and the highest density gives the smallest range, say 0 to 10 μg.

POWER SUPPLY—

Many types of power supply have been tried, principally with the single-ended fluorimeter.

Large accumulators to supply the lamp current were tried and discarded. In use considerable time was required before they settled down to a reasonably steady e.m.f., and even then the voltage gradually decreased and this necessitated continual adjustment of the fluorimeter against a standard reference solution throughout the day. Bubbles of gas in the cells caused spurious flicks on the galvanometer, and a final objection to the use of accumulators was that they were bulky and troublesome.

High-tension batteries to power the photomultipliers were tried. They proved to have a poor shelf life, they were bulky and they were liable to be highly dangerous to operators.

The following system has proved highly satisfactory. A constant-voltage transformer is used to supply a lamp transformer at 12 volts and 2 to 3 amps., and a stabilised 1000-volt, 2 to 3-mA supply.

The most successful circuit, with very few components, but having a high ratio of output-to-input stability, makes use of a corona stabiliser controlling a high-gain triode connected as a cathode follower. The power supply to the photomultipliers is controlled by a potentiometer fitted on the fluorimeter panel and connected between the cathode and earth of the cathode follower (see circuit diagram, Fig. 2). The instrument is normally used at 900 volts, and little or no adjustment of the voltage is required under widely different mains input conditions.

The graph (Fig. 1) shows the remarkable stability of the instrument over a wide range of mains input voltages. The apparatus has also been successfully run from a carbon-pile-regulated rotary convertor, which makes it independent of mains supply.

SCREENING—

The test cell, photomultipliers and filters are screened to prevent the ingress of stray light. Light falls on the control photomultiplier through a $\frac{1}{8}$-inch diameter hole in the cylindrical screening can and on the test-cell photomultiplier through a series of $\frac{1}{2}$-inch $\times$ $\frac{1}{4}$-inch holes cut in the screening boxes. The photomultiplier is arranged so that the maximum photocathode surface is presented to the fluorescent light.

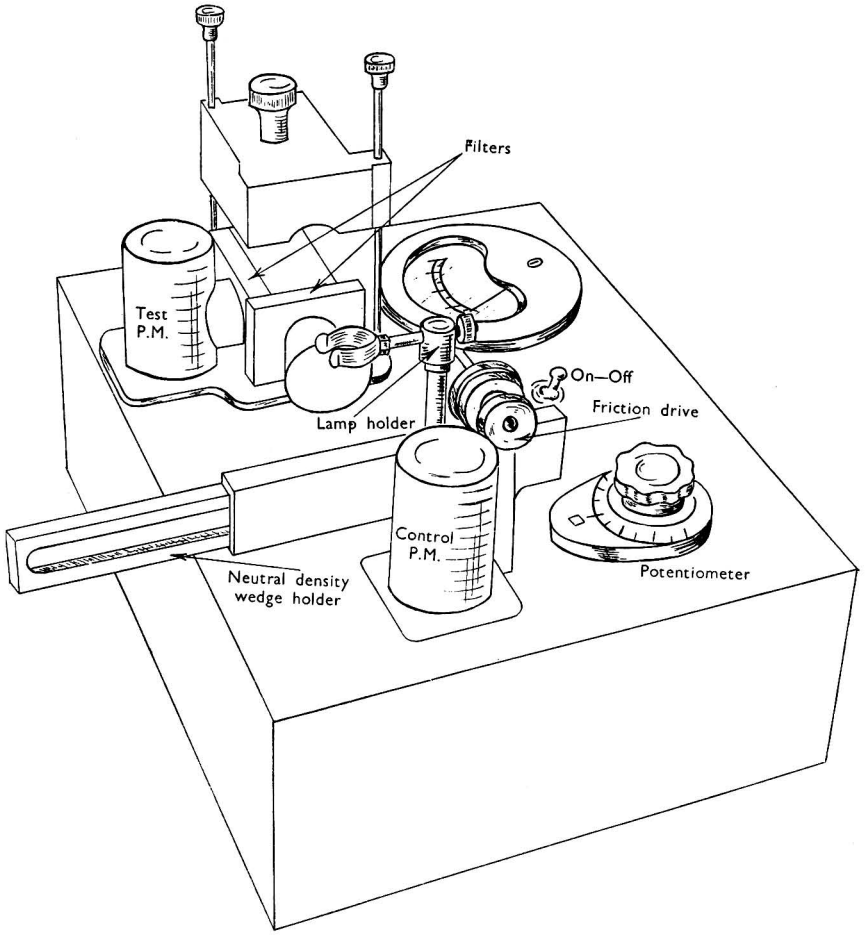

Fig. 3. General view of apparatus

A light shutter, made from a strip of bakelite in which is cut a suitably placed $\frac{9}{16}$-inch $\times$ $\frac{5}{16}$-inch hole, is fastened to the lid of the test-cell screening box, which is positioned by four vertical rods, and is used to protect the photomultiplier from an excess of light when the test cell is being removed or placed in position.

The instrument should be constructed of sheet brass or copper. An aluminium fluorimeter was made, but it was found that control readings depended on the temperature of the laboratory.

The apparatus is normally used in a wooden box, open at the front, in order to obviate the effect of draughts and bright sunlight.

A general view of the apparatus is shown in Fig. 3.

USE OF THE INSTRUMENT—

The galvanometer is set to zero and then short-circuited. The fluorimeter is switched on and 5 minutes is allowed for it to warm to operating temperature.

At the end of this time a known strength of solution of a fluorescent substance, which should have a similar fluorescent emission to that of the substance under examination, is placed in the cell. The reading should correspond approximately to the maximum expected for the samples under examination.

The dial of the helical-wound potentiometer is adjusted to a precise figure (say 900), the galvanometer is switched into the circuit, and the variable-density neutral filter is moved until a null-point is indicated on the galvanometer.

The instrument is now ready for use. It is advisable to check it at intervals throughout the day against the standard solution. Variation seems to be mainly caused by the deposition of a film of moisture on the filters and test cell, and is most noticeable on a cold wet day. Normally, however, little adjustment has to be made.

The solution under examination is placed in the cell with the galvanometer short-circuited. The potentiometer is turned to the expected reading, the galvanometer shorting switch is opened, and the dial of the potentiometer is rotated until a null reading is indicated on the galvanometer. The reading on the dial is noted; it corresponds to the fluorescent value of the unknown solution.

A reagent blank is placed in the cell and the reading on the dial recorded as described above. A calibration curve is prepared with known weights of the substance under examination, and the amount of the unknown substance is found by referring the reading, corrected for the blank, to the curve.

SENSITIVITY—

With the appropriate filter combinations, 0·01 μg of riboflavine and sodium naphthionate in 2 ml of solution can be determined, and 0·001 μg of fluorescein can be measured. These values are given with some reserve, as the chemical stability of the compounds at this level of concentration is unknown; but fluorescein, for example, appears to be readily decomposed by light in very dilute alkaline solution. The results for a fluorescent substance having a similar response to sodium naphthionate show a standard deviation of ± 2 per cent. on amounts between 0·5 and 40 μg.

The instrument has been in use for about a year, and has been in almost daily use. Reference curves made over 12 months indicate that the long-term stability of the instrument is reasonably satisfactory.

The advice of Prof. E. J. Bowen, F.R.S., in the early stages of development of the instrument is gratefully acknowledged. This paper is published by permission of the Chief Scientist, Ministry of Supply.

CHEMICAL DEFENCE EXPERIMENTAL ESTABLISHMENT
MINISTRY OF SUPPLY
PORTON, WILTS.

J. P. DOWDALL
H. STRETCH
May 5th, 1954

TWO IMPROVED CONTROL SYSTEMS FOR HORIZONTAL MICRO-BURETTES

RECENT work in these laboratories has necessitated the use of a simple micro-burette of 1 to 2-ml capacity. Of the many micro-burettes described in the literature, that of Hybbinette and Benedetti-Pichler[1] is the simplest, but it has the disadvantage that the jet is difficult to adjust, and cleaning, filling and emptying of the burette are consequently rather tedious. These difficulties were overcome to some extent by Stock and Fill,[2] who used a screw-valve device embodying a capillary brake similar to that mentioned by Benedetti-Pichler.[3] With a mechanism of this type, efflux of liquid from the burette continues after the air inlet is closed, and ceases only when the air pressure in the burette is reduced sufficiently to compensate for the hydrostatic head in the jet. Unless the rate of efflux is extremely slow, therefore, the burette is difficult to control. Experience has shown that the permissible rate of efflux is limited more by this factor than by drainage errors, or by the necessity for preventing diffusion of the titrated liquid into the tip of the burette, which is normally immersed. Attention was therefore concentrated on the construction of a micro-burette that would combine reasonably rapid delivery with adequate control near the end-point of the titration. Two control systems that have proved satisfactory are shown in Figs. 1 and 2.

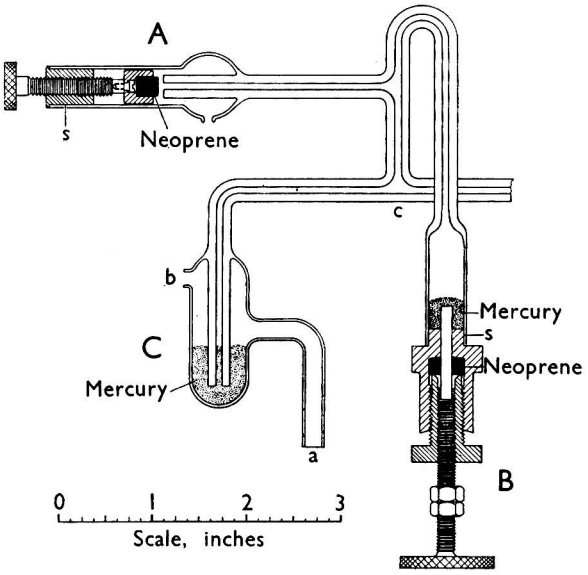

Fig. 1

In both systems the main tube of the burette terminates in the mercury trap, C, the purpose of which is described below, and the control valves are connected to a side tube attached at *c*.

In the system of Fig. 1, valve A is similar in principle to that described by Stock and Fill, the open end of the tube being closed by a resilient pad under pressure from the screw. It has been found desirable, however, to fuse the glass housing of the valve to the capillary tube, to have the pad-holder attached to the screw, although not rotated by it, and to replace the rubber pad by one of Neoprene, which has a greater resistance to wear. Valve B is a screw-operated piston. It was not found possible to make a mercury-tight joint simply by passing the screw

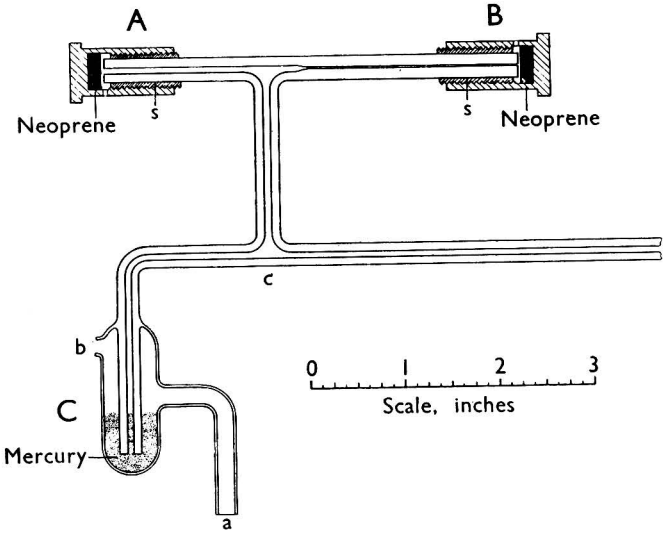

Fig. 2

through a suitably tapped plug into the mercury. The end of the screw was therefore turned on a lathe to give a smooth plunger, which was passed through a Neoprene washer in the simple gland assembly shown. By tightening the gland nut, the joint was easily made mercury-tight. It is important that the mercury chamber should be long enough to contain the plunger when it is

screwed up fully. If this is not so, lock-nuts should be fitted, as shown, to limit the travel of the plunger. It does not matter if the plunger emerges from the mercury, as the main purpose of the latter is to provide an air-tight joint. Valve A is conveniently made of brass, but valve B must be made of steel to avoid amalgamation with the mercury. The threaded block containing the screw of valve A and the gland box of valve B are cemented to the glass tubing with sealing wax.

The greater part of the solution used in the titration is added fairly rapidly by manipulation of valve A, but when the end-point is approached this valve is closed and the titration is completed by screwing in the plunger of valve B. This arrangement permits very accurate control, but it must be possible to complete the titration within the capacity of the plunger and the plunger must be reset before each titration.

To overcome this difficulty the simpler but slightly less precise assembly shown in Fig. 2 was devised. Valve A is similar in function to valve A in Fig. 1. Valve B is made of fine capillary tubing, the bore of which is further obstructed by the insertion of a length of platinum wire. This obstruction is adjusted so that, when valve B is open, liquid will flow only very slowly out of the burette. As a result, the flow stops almost instantaneously when valve B is closed. There is some risk that foreign matter entering this obstructed tube may choke it completely, and therefore the wider tube of valve A is made co-axial with it, so that the wire and foreign matter can be ejected by a suitable wire inserted through valve A. In addition, the valve mechanism has been modified. Threaded brass sleeves are cemented over the capillary tubes by means of sealing wax, and they carry threaded brass cups containing the Neoprene closing pads. These cups are easily removed for the purpose of cleaning the capillary tubes. The titration is nearly completed by the use of valve A and is then completed under the more sensitive control of valve B.

The mercury trap, C, enables the burette to be filled and cleaned rapidly. This is done by closing valve A (Fig. 1) or both valves (Fig. 2), applying gentle suction at *a* and closing hole *b* with the finger while the jet is immersed in the appropriate standard solution. This solution then flushes the burette and runs to waste at *a*. When the burette is judged to have been flushed sufficiently, valve A is cautiously opened, when the column of liquid breaks at *c* and the excess runs to waste through the trap. Hole *b* is then promptly uncovered and valve A is closed. For refilling, it is necessary to close *b* only until sufficient solution has been drawn into the burette.

The constant hydrostatic head characteristic of horizontal burettes ensures that the meniscus of the mercury in C is stable throughout a titration and no errors attributable to movement of the mercury have been detected.

References

1. Hybbinette, A., and Benedetti-Pichler, A. A., *Mikrochemie*, 1942, **30**, 15.
2. Stock, J. T., and Fill, M. A., *Metallurgia*, 1944, **31**, 103.
3. Benedetti-Pichler, A. A., *Z. anal. Chem.*, 1928, **73**, 200.

The British Cotton Industry Research Association
Shirley Institute, Didsbury
Manchester, 20

A. G. Hamlin
J. M. Bather
April 6th, 1954

Ministry of Food

STATUTORY INSTRUMENT*

1954—No. 1089. The Food Standards (Soft Drinks) (Amendment) Order, 1954. Price 2d.

*This Order, which came into operation on August 22nd, 1954, amends the Food Standards (Soft Drinks) Order, 1953 (S.I., 1953, No. 1828; Analyst, 1954, **79**, 56), by extending the exemption of fruit juice from the provisions of the principal order to include undiluted fruit juice, with or without added sugar, and any such juice in concentrated (or frozen) form.*

FOOD STANDARDS COMMITTEE

Antioxidants

The Minister of Food has approved for publication a Revised Report, presented to the Food Standards Committee by its Preservatives Sub-Committee, making recommendations about the use of antioxidants in foods. An earlier report on this subject was published in June, 1953 (see *Analyst*, 1953, **78**, 504). Since then the Sub-Committee has reviewed representations from trade and other interests and the Revised Report replaces the earlier Report.

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

The present Report proposes that the Public Health (Preservatives, etc., in Food) Regulations should be amended to give effect to the Sub-Committee's revised recommendations. Any further representations from interested parties should be made to the Assistant Secretary, Food Standards and Labelling Division, Ministry of Food, Great Westminster House, Horseferry Road, London, S.W.1, before November 30th, 1954.

Copies of the report can be obtained from H.M. Stationery Office, price 6d. (plus postage).

British Standards Institution

NEW SPECIFICATIONS*

B.S. 593:1954. Laboratory Thermometers. Price 4s.
B.S. 619:1954. Incubator, Water Bath and Oven Thermometers for Laboratory Use. Price 2s. 6d.
B.S. 1428: Part G1:1954. Heating and Cooling Blocks for Microchemical Purposes. (Microchemical Apparatus: Group G: Heating, Cooling and Drying Accessories.) Price 2s. 6d.
B.S. 2070:1954. Lunge Nitrometers. Price 2s. 6d.
B.S. 2455:1954. Methods of Sampling and Testing Boiler Water Deposits. Price 6s.
B.S. 2486:1954. The Treatment of Water for Land Boilers. Price 6s.

Book Reviews

METHODS OF BIOCHEMICAL ANALYSIS. Volume I. Edited by DAVID GLICK. Pp. x + 521. New York and London: Interscience Publishers Inc. 1954. Price $9.50; 75s.

This is the first volume of a series intended to provide authoritative information on methods for the determination of enzymes, vitamins, hormones, lipids, carbohydrates, proteins and minerals. The techniques and instruments used in chemical, physical, microbiological and perhaps animal assays will be described, and the Editor is assisted by an Advisory Board of well-known workers, including, from this country, Consden, Marrian and J. K. N. Jones. The declared policy is to give preference to subjects of current importance and to "essentially new approaches which bear promise of great usefulness." Each chapter is planned not only to include a critical evaluation of previous work but also to "furnish the laboratory worker with the complete information required to carry out the analysis. The series . . . will become an encyclopaedic treatment of the complete methodology of Biochemical Analysis." This is an ambitious project, and the 17 articles in the first volume give it a good start.

The first chapter (by Chinard and Hellerman) is on the determination of "sulfydryl" groups, and the second chapter (by Bray and Thorpe, of Birmingham) is on the analysis of phenolic compounds; both are clear and balanced accounts. The next two chapters deal with microbiological assays of antibiotics and of vitamin B_{12}. Then comes a concise account (by J. H. Roe) of the chemical determination of ascorbic, dehydroascorbic and diketogulonic acids. Kunkel, under the title zone electrophoresis, deals with preparative electrophoresis, the use of filter-paper, starch blocks and purified cellulose fibres, as well as column electrophoresis.

The chromatographic separation of the steroids of the adrenal gland (discussed by Haines and Karnemaat) is topical, and the authors themselves regard the chapter as a review of work in progress rather than a set of definitive procedures. Chromatography is also considered (by L. Hough) in relation to the analysis of mixtures of sugars. Roche and his colleagues discuss the chromatography of ^{131}I-labelled substances for studying the biochemistry of iodine and thyroid hormones. Useful chapters follow on choline, nucleic acids, and raffinose and kestose (the latter by de Whalley and Gross, based on work carried out in this country). Strehler and Totter, of the Oak Ridge Laboratory, describe the determination of ATP and related compounds. Various methods are discussed, but the firefly luminescence system is preferred; it is sensitive and specific and has proved "consistent and reproducible to about 5 per cent. accuracy for as little as 10^{-10} g of labile ATP phosphorus. Fireflies are available during early summer in the entire eastern United States and because of the economy of the test involved, the collection of a few evenings will be sufficient for a year's investigation in the average laboratory." The flies (*Photinus pyralis*) are vacuum dried and stored for use.

The assay of catalase and peroxidases is very fully covered in two sections, general assay methods by Maehly and special methods by B. Chance. The *in vitro* determination of hyaluronidase (Tolksdorf) is followed by ultracentrifugal analysis of serum proteins (de Lalla and Gofman) and the assay of urinary neutral 17-ketosteroids (L. L. Engel).

* Obtainable from the British Standards Institution, Sales Department, 2, Park Street, London, W.1.

This book is a first rate example of the secondary literature of chemistry that is flourishing in the form of annual series published in the U.S.A. These books are expensive, particularly outside America, and each new series needs to be carefully assessed before a library is advised to add it to the list. This new series will clearly be worth while in libraries serving biochemical laboratories.

R. A. MORTON

BIOCHEMICAL PREPARATIONS. Volume III. Editor-in-Chief, E. E. SNELL. Pp. viii + 128. London: Chapman & Hall Ltd.; New York: John Wiley & Sons Inc. 1953. Price 28s.

With Volume III of the series, "Biochemical Preparations" may well be said to have become an established institution, and biochemists engaged in preparative work can now look forward with pleasurable expectation to the publication of each succeeding volume in the certain knowledge that in due course one or other of his tasks will be made easier by the publication of these excellent monographs on the preparation of substances of biochemical importance. If "Organic Syntheses" has become essential to the organic chemist, how much more so will its counterpart become to his biochemical colleague, in a field in which even greater attention to detail is necessary in order to achieve success and where there appear to be so many different ways of making irrevocable mistakes.

In the volume under review, for instance, the preparation of two crystalline enzymes—muscle phosphorylase and ribonuclease—is described. The methods are extremely complicated, and only a detailed monograph compiled and checked by experts would be of any use in such instances. This is just what "Biochemical Preparations" provides. Two other methods, only slightly less complicated, are the preparation of the two coenzymes, di- and bi-phosphopyridine nucleotides, from yeast and liver, respectively. The preparation of two other coenzymes, pyridoxamine phosphate and pyridoxal phosphate, of a group of six organic acids and related compounds that are used as substrates in enzymic reactions, of several amino-acids and of the amino-acid reagent, dinitrofluorobenzene, are described in other monographs.

Several of the preparations involve the use of conventional organic chemical reactions: some, such as those already referred to above, involve the extraction of animal tissues: and some of the compounds described are made by microbiological synthesis; L(+)- and D(−)-lactic acids, for instance, by the fermentation of glucose with certain *Lactobacilli*, and L-citrulline by the action of *Streptococcus faecalis* on L-arginine. Enzyme reactions are also used: sodium keto*iso*-caproate is made from L-leucine by the action of rattlesnake venom, although British biochemists will presumably prefer to make it by the alternative process in which D-amino-acid oxidase from hog kidney is allowed to act on D- or DL-leucine. *d-iso*Citric acid is made by extracting the leaves of a plant belonging to the *Crassulaceae*, and it is recommended that the leaves be picked early in the afternoon because then their content of other organic acids is at a minimum.

This brief summary will indicate what a wide variety of methods is described by those responsible for the monographs, but it makes no mention of points of detail throughout the book that will be found of great value to those interested in biochemical preparations. Modern biochemical techniques are used extensively in those preparations where they are appropriate, for example, in the crystallisation of enzymes and in lyophilisation and ion-exchange chromatography for purification and isolation. As usual, each monograph has a section on properties and purity, and in addition to the use of the more conventional constants for characterisation, such as optical rotation and ultra-violet absorption, reference is made when necessary to appropriate biochemical techniques for assaying the substances made.

The printing is up to the usual high standard of the earlier volumes of the series and its counterpart, "Organic Syntheses," and the binding has been designed to stand up to the hard wear this book will receive in the laboratory, where it will undoubtedly be kept.

F. A. ROBINSON

STRUCTURE AND MECHANISM IN ORGANIC CHEMISTRY. By C. K. INGOLD, D.Sc., F.R.S. Pp. vii + 828, with many tables. London: G. Bell & Sons Ltd. 1953. Price 77s. 6d.

Some practising analysts may be surprised to see more than a passing reference in this journal to a book on what might appear essentially to be theoretical problems of organic chemistry. But the analyst dealing with organic materials has always been aware of the need to allow adequate time for his reactions to go to completion, and can often base analytical procedures on the different rates at which components of a mixture react. The increasing use of organic reagents in inorganic analysis as complexing reagents, and in the determination of microgram quantities by solvent extraction or absorptiometry, has provided innumerable examples of procedures in which the speed of formation or decomposition of some intermediate or final product is of vital importance.

Such kinetic phenomena have generally been studied only to the extent demanded by the solution of the analytical problem at issue, but there is no question that more detailed studies would strengthen one of the weakest sections of present-day fundamental analytical chemistry and should help to reduce time and effort now spent on purely empirical solutions of similar problems.

Professor Ingold deals first with problems of valency and molecular structure, the types of interactions possible between and within molecules, and their physical properties. After a discussion of the aromatic nucleus and a clear account of ways of formally classifying reagents and reactions, he elaborates his theme with reference in turn to electrophilic and nucleophilic substitutions, olefin-forming eliminations, and saturated-, unsaturated- and aromatic-rearrangements. The work concludes with a chapter on addition reactions and their reversal, and a critical account of contemporary acid - base theory, carboxyl reactions and nucleophilic aromatic substitutions. This is a hard book to read and demands the closest attention, but it is packed with authoritative information and shows on every page the impact of a logical and penetrating mind. It is indispensable to anyone seriously studying the kinetics of reactions (inorganic as well as organic) and its perusal cannot fail to stimulate others, even though—and perhaps even because—it makes no direct reference to problems of chemical analysis. H. IRVING

INDEX TO THE LITERATURE ON SPECTROCHEMICAL ANALYSIS. Part III, 1946–50. By B. F. SCRIBNER and W. F. MEGGERS. A.S.T.M. Special Technical Publication No. 41–C. Pp. iv + 226. Philadelphia, Pa.: American Society for Testing Materials. 1954. Price $4.50.

Part III closely resembles Part II (published in 1947) in form of presentation and contains 1264 references with abstracts, listed for each year in alphabetical order of authors' names. A detailed subject index is provided as before, and an author index is now included. The abstracts are truly informative and of varying length according to the subject-matter. References to published reviews are given for books. This compilation is more than an index; it is a comprehensive bibliographical survey. D. M. SMITH

Publications Received

REPORTS ON THE PROGRESS OF APPLIED CHEMISTRY. Volume XXXVIII: 1953. Edited by F. CLARK, B.A., B.Sc., A.R.I.C. Pp. 989. London: The Society of Chemical Industry. 1954. Price 60s.

WOOL: ITS CHEMISTRY AND PHYSICS. By P. ALEXANDER, Ph.D., D.I.C., A.R.C.S., and R. F. HUDSON, Ph.D., D.I.C., A.R.C.S. Pp. x + 404. London: Chapman & Hall Ltd. 1954. Price 45s.

THE STRENGTHS OF CHEMICAL BONDS. By T. L. COTTRELL. Pp. viii + 310. London: Butterworths Scientific Publications; New York: Academic Press Inc. 1954. Price 30s.; $5.50.

PETROLEUM MICROBIOLOGY. By E. BEERSTECHER, JUN. Pp. xvi + 375. New York and Amsterdam: Elsevier Publishing Co. Ltd.; London: Cleaver-Hume Press Ltd. 1954. Price 55s.

VOLUMETRIC GLASSWARE: SCIENTIFIC ASPECTS OF DESIGN AND ACCURACY. National Physical Laboratory Notes on Applied Science No. 6. Pp. iv + 21. London: H.M. Stationery Office. 1954. Price 1s. 6d.

BALANCES, WEIGHTS AND PRECISE LABORATORY WEIGHING. National Physical Laboratory Notes on Applied Science No. 7. Pp. vi + 46. London: H.M. Stationery Office. 1954. Price 2s.

SIGNIFICANCE OF PROPERTIES OF PETROLEUM PRODUCTS. Edited by G. SELL, F.Inst.Pet. Pp. iv + 74. London: The Institute of Petroleum. 1954. Price 7s. 6d.

MICROANALYST. May & Baker Limited, Dagenham, Essex, have a vacancy for an analyst of B.Sc. or A.R.I.C. standard in the Microanalytical Department of their Chemical Research Organisation. The duties will include not only supervision of quantitative microanalysis of organic compounds by established procedures, but also experimental work designed to introduce new and improved methods and to widen the scope of the laboratory. Applicants must have a good knowledge of organic chemistry and experience in microanalysis or at least a real aptitude and liking for microscale techniques, so as to take advantage of the opportunity for acquiring skill in this specialised branch of analysis which is vital to research. Apply initially in writing to the Personnel Officer.

ASSISTANT ANALYST (age 19–27) required for British Welding Research Association. O.N.C. level or equivalent Several years' experie nce of metallurgical analysis required Must be careful and accurate. Good prospects. Appy Secretary, B.W.R.A., 29, Park Crescent, London, W.1.

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Write stating age, qualifications, experience and salary required to Personnel Officer, Smith & Nephew Research Ltd., Hunsdon Laboratories, Ware, Herts. quoting BWM/TA.

LARGE FOOD FACTORY, South-West of Manchester, requires the services of two Assistant Chemists for analytical work. One up to B.Sc. standard and one with Ordinary National Certificate. Pension scheme. Canteen facilities. Salary according to qualifications. Applications to Box No. 3870, The Analyst, 47, Gresham Street, London, E.C.2.

ANALYTICAL ASSISTANT, aged 25 to 30, Military Service completed reqd for general analytical work by important industrial concern. Experience in analysis of organic/inorganic materials desirable. Work interesting and varied. Good prospects of advancement. Pension Scheme. Canteen facilities, position permanent, W. London. Write stating age, qualifications, experience and salary reqd to Box No. 3872, The Analyst, 47, Gresham Street, London, E.C.2.

ANALYST required for large laboratories engaged in food and agriculture. Applicants must have wide experience in general analytical technique and be able to assume full responsibility. Excellent salary with prospects of future development. Box 3871, The Analyst, 47, Gresham Street, London, E.C.2.

RESEARCH CHEMIST—A well-qualified chemist, experienced in research methods, is required by London firm of manufacturing chemists to carry out fundamental and applied research in the pharmaceutical field. This is a responsible and well paid position of which the Company's staff have been notified. Contributory pension scheme. Applications will be treated in strictest confidence, and should give full details of candidate's age, qualifications experience, etc. Please address to Box No. 3873, The Analyst, 47, Gresham Street, London, E.C.2.

ALBRIGHT & WILSON LIMITED have a further number of vacancies for Junior Shift Chemists at their factory at Barry, Glam., which is engaged in the manufacture of Silicone products. Applications are invited from persons, with at least Inter B.Sc., or equivalent, under 30 years of age and who have completed their National Service. A number of these posts may be filled by science graduates without previous industrial experience.

The posts are of two types:—

(*a*) *For Plant Work.*
Duties involve assisting in all aspects of plant operation and control.

(*b*) *For Laboratory Work.*
Duties consist of quality control and laboratory scale production of new products.

The Company possesses a non-contributory pension scheme. Commencing salary according to age, qualifications and experience.

Applications to Appointments Officer, Albright & Wilson Limited, Oldbury, Birmingham.

ALBRIGHT & WILSON LTD., Chemical Manufacturers, invite applications for the post of Research Analyst in the Laboratory of their factory at Barry (Glam.). The duties involve the investigation into and the development of new methods for the analysis of organo-silicon compounds and intermediates associated with the manufacture of silicones. Candidates, aged under 30, must have an Honours Degree in Chemistry or A.R.I.C. Experience of classical and modern analytical techniques together with the ability to control an analytical research section is essential. Commencing Salary according to age, experience and qualifications. There is a non-contributory pension scheme. Apply to the Appointments Officer, Albright & Wilson Ltd., Oldbury, Birmingham.

Volume 79. No. 943, Pages 593-660 October, 1954

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

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