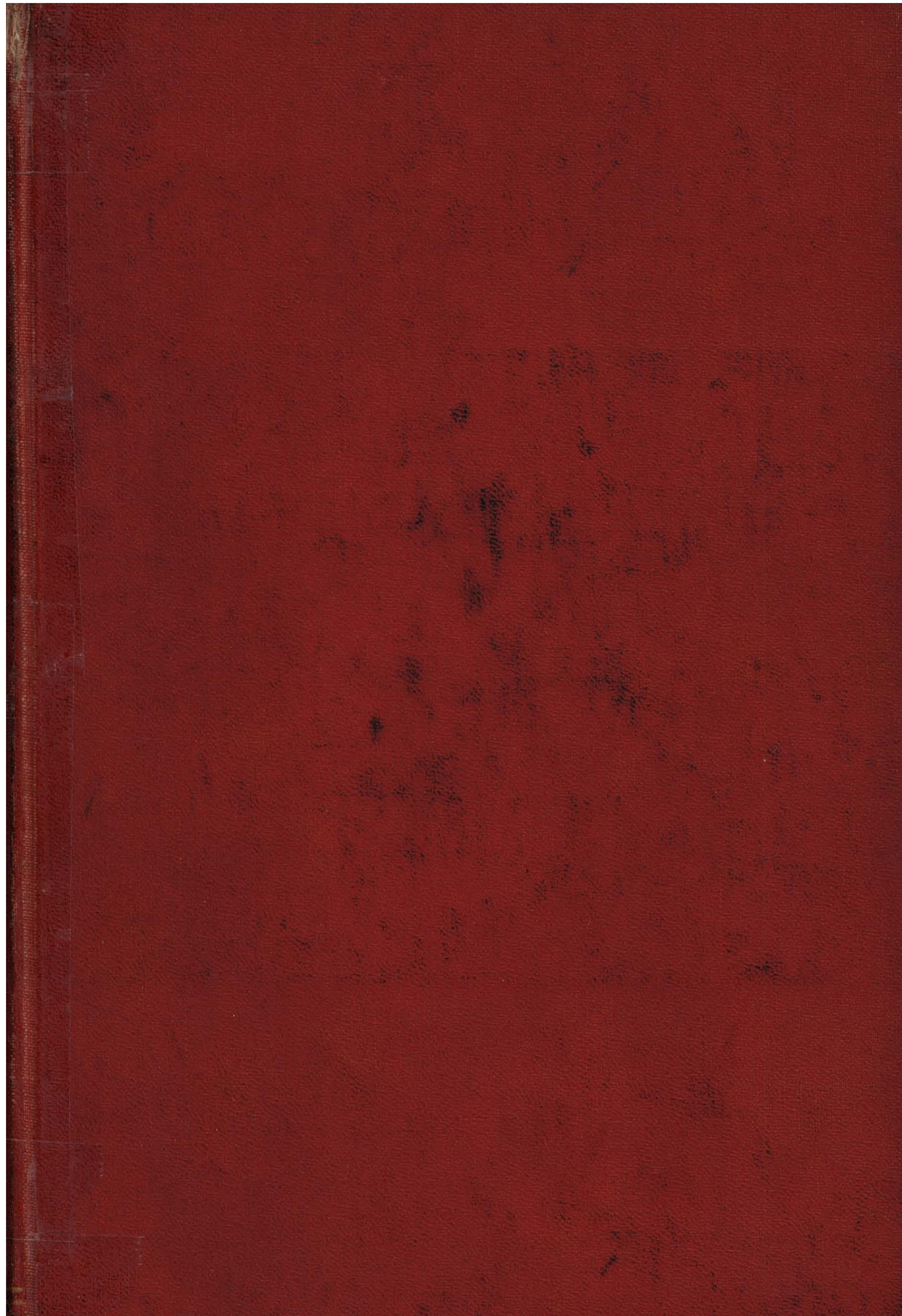

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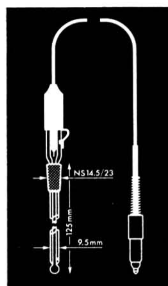

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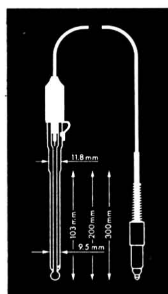

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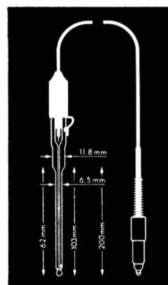

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Mass Spectrometry for the Analysis of Organic Compounds

A Review

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A. E. WILLIAMS and H. E. STAGG

Imperial Chemical Industries Limited, Dyestuffs Division, P.O. Box 42, Hexagon House, Blackley, Manchester, M9 3DA.

Analyst, 1971, **96**, 1–25.

Some Observations on Oxidation - Reduction Indicators of the Benzidine, Naphthidine and Diarylamine Types

A spectrophotometric investigation has been made of representative compounds of the benzidine class of indicators in an attempt to resolve fundamental problems concerning their oxidation mechanism in sulphuric acid media. In all instances the oxidation to the coloured compound is a single-step two-electron process. There is no detectable evidence for the formation of an intermediate, either of a benzidine from arylamines or of a semiquinone from a benzidine or naphthidine. Reduction or spontaneous decay of oxidised arylamines stops at the benzidine stage. All oxidised indicators are unstable both intrinsically and in the presence of excess of oxidant: unsubstituted benzidine and naphthidine are, additionally, photosensitive in the oxidised state. The spontaneous decomposition of the oxidised form regenerates the reduced form and is a disproportionation, probably of 2:1 stoicheiometry, but a kinetic study shows that the rate-controlling step is a unimolecular precursive reaction, which may be the relaxation of the triplet state of the coloured dication diradical oxidised form. Stability decreases on sulphonation and with increasing temperature, and increases with increasing sulphuric acid concentration and with progressive substitution of the amino hydrogen atoms.

E. BISHOP and Mrs. L. G. HARTSHORN

Chemistry Department, University of Exeter, Stocker Road, Exeter, Devon.

Analyst, 1971, **96**, 26–36.

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The Determination of Carbon in Steel by Coulometric Titration in Partially Aqueous Medium

A method that involves coulometric titration of carbon dioxide absorbed in a partially aqueous medium is described for the precise determination of carbon in steel. It does not depend on empirical standardisation and the use of high-vacuum systems and inflammable titrants is avoided. The apparatus used is inexpensive and the technique is simple. The application of appropriate instrumentation could provide a routine control method of high precision.

The analytical performance of the method compares well with that of other techniques considered suitable for reference analysis; 95 per cent. confidence limits of $\pm 0{\cdot}0007$ per cent. of carbon at the 0·05 per cent. of carbon level to 0·008 per cent. of carbon at the 0·9 per cent. of carbon level were obtained in this laboratory.

H. J. BONIFACE and R. H. JENKINS

British Steel Corporation, Strip Mills Division Research Centre, Port Talbot, Glamorgan.

Analyst, 1971, **96**, 37–46.

The Determination of Yttrium, Europium, Terbium, Dysprosium, Holmium, Erbium, Thulium, Ytterbium and Lutetium in Minerals by Atomic-absorption Spectrophotometry

A procedure is described for determining yttrium, europium, terbium, dysprosium, holmium, erbium, thulium, ytterbium and lutetium in zirconium and calcium rare earth silicates by atomic-absorption spectroscopy, which involves the use of a lanthanum suppressor and has potential applications to other minerals containing these interferences. Although many of the associated ingredients are found to interfere, the addition of lanthanum overcomes these problems in many instances. Sensitivities and detection limits found with the proposed procedure are given for each element.

J. C. VAN LOON, J. H. GALBRAITH and H. M. AARDEN

Department of Geology, University of Toronto, Toronto 5, Canada.

Analyst, 1971, **96**, 47–50.

The Determination of Fluorine in Rocks and Minerals by a Pyrohydrolytic Method

A rapid pyrohydrolytic method, with simple apparatus, is described for the determination of fluorine in rocks and minerals. The sample is heated with a three-component flux in a stream of moist air, and the liberated hydrogen fluoride is absorbed into an alkaline solution. The recovered fluorine is determined either colorimetrically or by means of a fluoride-specific electrode. The method is suitable for determining fluorine at concentrations down to about 50 p.p.m.

R. L. CLEMENTS, G. A. SERGEANT and P. J. WEBB

Department of Trade and Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1.

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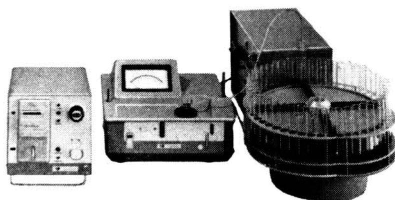

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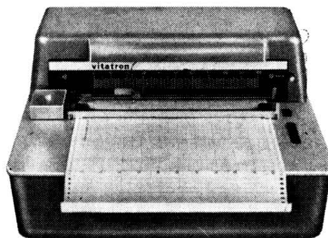

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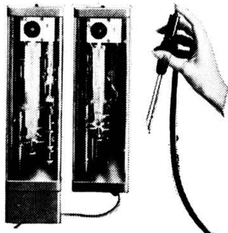

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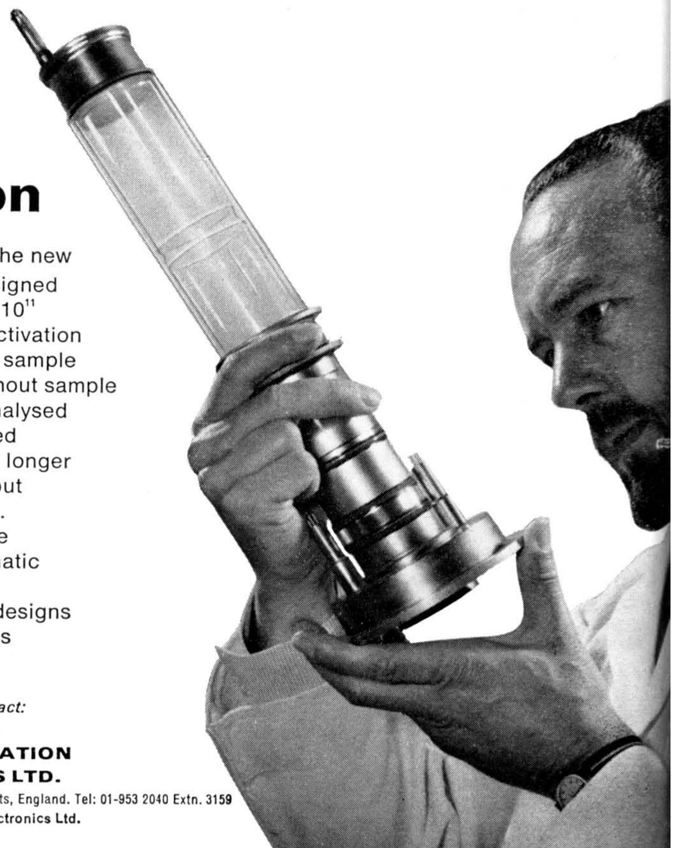

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JANUARY, 1971 Vol. 96, No. 1138

THE ANALYST

Mass Spectrometry for the Analysis of Organic Compounds

A Review*

By A. E. WILLIAMS and H. E. STAGG

(*Imperial Chemical Industries Limited, Dyestuffs Division, P.O. Box 42, Hexagon House, Blackley, Manchester, M9 3DA*)

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Current developments and future trends

Early history

In 1886 a German physicist, Eugen Goldstein, who was experimenting with electrical discharges at low pressures, observed that when he used a perforated cathode, diverging streamers of light could be seen emanating from the holes in the cathode. He surmised that these luminous streamers were associated with rays or particles travelling in the opposite direction to the usual cathode rays. Twelve years later, in 1898, Wien showed that these rays could be deflected by a magnetic field, and that they were composed of positively charged particles.

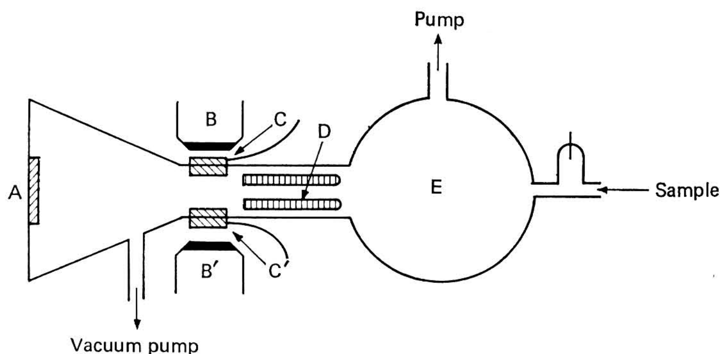

Fig. 1. Thomson's positive-ray analyser

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In 1911 J. J. Thomson[1] built his positive ray analyser that was the forerunner of the modern mass spectrograph (Fig. 1). Positive rays produced in a gas discharge tube (E) were accelerated towards a cylindrical cathode (D) with a fine hole bored along the axis. Some of the positive rays passed through this hole and entered a highly evacuated region where very few gas collisions could occur. The positive rays were then deflected by parallel electrostatic and magnetic fields and detected on a fluorescent screen or photographic plate. The locus of the points formed by positive rays (or ions) of the same mass but different velocities should be a parabola, and with hydrogen alone in the discharge tube a single parabola was observed; the charge-to-mass ratio was calculated to be 9571 e.m.u. This value was the same as that found for the hydrogen atom produced by electrolysis, and confirmed that the positive rays formed in the hydrogen discharge were hydrogen atoms from which one electron had been removed.

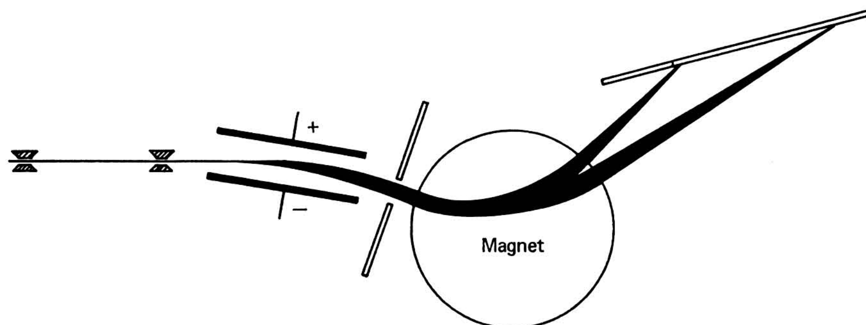


Fig. 2. Aston's mass spectrograph

Refinements made to the instrument resulted in improved resolution that enabled adjacent parabolae, corresponding to mass differences of less than 10 per cent., to be distinguished, and in November 1912 a sample of liquid-air residues from J. Dewar's low-temperature experiments was examined in the instrument. An intense parabola was detected at mass 20 and ascribed to neon (atomic weight by chemical determination 20·2) and a parabola of weak intensity was also detected at mass 22. No element of this atomic weight was known to exist. The intensity ratio of the two parabolae remained stubbornly constant for a wide range of experimental conditions, and Thomson concluded from these observations that neon probably existed in two forms that were chemically indistinguishable but which had different atomic weights. This conclusion gave independent support to Soddy's evidence for the existence of isotopes, based on experiments with radioactive isotopes. When polyatomic molecules were introduced into the discharge tube many parabolae were obtained. In the case of methane, parabolae that could only be interpreted as being due to the combination CH_3, CH_2 and CH were detected. Phosgene produced parabolae due to the ions C^+, O^+, Cl^+, CO^+, Cl_2^+, $COCl^+$ and $COCl_2^+$. These elegant experiments were the foundation upon which a new form of critical spectroscopy, the spectroscopy of "mass," was to be built.

The photographic plate used by Thomson in his early experiments had a non-linear response to ions of different masses, so that a measure of the relative number of ions detected was difficult to obtain. Thomson therefore modified his instrument by inserting a metal plate with a parabolic slit in the path of the deflected positive rays. By altering the magnetic field, the parabolae produced by the various ions were brought in turn on to the slit, through which they passed on to an electroscope; a plot of electroscope current against magnetic field resulted in a series of peaks that corresponded to the different ions detected, and in which the height of the peak was proportional to the number (or as it is now called, the abundance) of ions detected. Therefore, the basic principle of mass spectrometry had been established and the first "mass spectrum" produced.

Thomson gave an accurate prediction of the future in this statement. "I feel sure that there are many problems in chemistry which could be solved with far greater ease by this than by any other method. The method is surprisingly sensitive, more so than spectrum analysis (optical), requires an infinitesimal amount of material, and does not require this to

be specially purified. The technique is not difficult to practise if appliances for producing a high vacuum are available."

Although Thomson's original parabola method had been considerably refined and improved, there was a limit to the performance of the instrument and F. W. Aston, a contemporary of Thomson at Cambridge, and A. J. Dempster, of the University of Chicago, initiated investigations to improve the resolution and sensitivity of the apparatus so that it could resolve ions of adjacent mass numbers.

Aston's mass spectrograph,[2] shown in Fig. 2, was completed in 1919 and involved the use of a discharge tube with collimating slits as a source of positive ions. The collimated beam of ions was deflected in tandem electrostatic and magnetic fields and was brought to an energy focus on a photographic plate. All particles of the same mass-to-charge ratio were focused together irrespective of their velocities, and as a result masses could be measured with an accuracy of 1 part in 1000. In 1919 Aston proved conclusively the existence of two neon isotopes of masses 20 and 22.[3]

In 1918 Dempster[4] built his first mass spectrometer. In this instrument ions were formed with low kinetic energy and accelerated through a large electrostatic field. A fraction of this accelerated-ion beam was then admitted into a magnetic analyser, deflected through an angle of 180°, and focused on to an exit slit. Thus in Dempster's design all ions of the same mass and the same kinetic energy are brought to a focus, *i.e.*, the instrument has direction-focusing, whereas Aston's instrument had energy-focusing, properties. By using this instrument Dempster was able to announce his discovery of the magnesium isotopes in 1920.

Atomic mass

In spite of the great interest that existed in the determination of atomic mass, Aston maintained a virtual monopoly in this type of investigation for a decade or more, and the isotopic constitution of a large number of elements was determined. Aston's earlier measurements had, at long last, firmly established the whole-number rule. By 1923 he had also observed that there were small divergences from this whole-number rule. Costa[5] (in Paris), by using a modified Aston instrument with an increased accuracy of measurement of 1 part in 3000, investigated these divergences. He obtained an accurate value for hydrogen-1 and made useful comparisons involving helium-4, lithium-6, lithium-7, carbon-12 and nitrogen-14.

Aston chose oxygen-16 to be the standard of atomic mass because oxygen was considered to be free of isotopes. By this choice Aston intended to achieve complete identity of the physical and chemical mass scales, but with the discovery in 1929 by optical spectroscopy of naturally occurring oxygen-17 and oxygen-18 isotopes this intended identity was no longer absolute. On the physical scale, therefore, the masses of isotopes were measured relative of the mass of the oxygen-16 isotope, to which a mass of 16·000000 atomic mass units (a.m.u.) was assigned. On the chemical scale the mass of an element was measured relative to the mass of the element oxygen, comprising a mixture of the three stable isotopes oxygen-16, oxygen-17 and oxygen-18. Both Aston and the Committee of the International Commission on Atomic Weights decided in 1931 that it was unnecessary to change either the physical or chemical scales to bring the two into exact agreement because the conversion factor was so small. Although no conversion factor was defined, by general usage it was accepted that oxygen, for the purposes of the chemical atomic-weight scale, has become that mixture of the isotopes of this element whose atomic weight is 1·000275 times the mass of oxygen–16.[6] It is interesting to note that the extence of deuterium was not suspected by the early mass spectrographers until its existence was demonstrated by optical spectroscopy in 1931 by Professor Urey.

Aston realised the importance of these divergences in the study of nuclear structure and in 1925, constructed a second instrument of improved performance with a resolution of 1 part in 600 and an accuracy of mass determination of 1 part in 10000. With this instrument he measured accurately the divergences of twenty elements, and invented the term "packing fraction" to indicate the extent of these divergences, *i.e.*,

$$\text{packing fraction} = \frac{M - A}{A}$$

where M is the exact atomic mass and A the nearest whole number.

In 1927 Aston published his famous packing fraction curve that depicts the general manner in which nuclear stability varies as a function of mass number. Aston, in retrospect,[3] stated that "the packing fraction curve depicted in a general way the changes of energy to be expected from transmutations of nuclei, not only by the aggregation of light atoms to form heavier ones, but also the prodigious release of energy to be expected from the fission of uranium by neutron bombardment, a phenomenon entirely undreamt of when the curve was first drawn."

During the period 1927 to 1938 the masses of the lighter elements were intensively studied by Aston, Dempster, Bainbridge and Jordan, and Mattauch. A new generation of double-focusing instruments was in operation with accuracies of measurement of 1 part in 100000. The secondary standards of mass, hydrogen-1, deuterium-2 and carbon-12, were rigorously examined. Dempster concentrated on the remaining involatile elements platinum, palladium, gold and iridium, and in 1935, by using a novel ionisation source in which a high frequency spark passed between metal electrodes in a very low pressure enclosure, succeeded in producing ions directly from the electrodes. As a result, in 1938 Dempster was able to produce a greatly improved version of Aston's packing fraction curve.

Organic analysis

Although the potential of the mass spectrograph or mass spectrometer was realised by Thomson, difficulties of instrumentation and manipulation deterred the chemist from applying the technique to his problems. The instrument therefore remained mainly in the domain of the physicist or physical chemist until about 1935. By this time, vacuum techniques had improved, the unreliable discharge tube had been replaced by the electron bombardment source, and ion currents could now be detected by means of sensitive electronic circuits. In 1935 Taylor[7] produced complete mass spectra of complex organic molecules from a modified Aston-type instrument. The electron bombarding energy was variable from 30 to 120 eV, and the ion currents produced were measured with a sensitive electronic amplifier.

Ironically, it was the war that created new applications for the use of mass spectrometers in the United States; Professor Nier's 60°, 6-inch radius instrument[8] was used as a prototype for monitoring the production of uranium to provide accurate ratios of uranium-235 to uranium-238. Mass spectrometers also found application in the development of synthetic rubbers to replace the loss of natural rubber supplies from the Far East, and in the petroleum industry, in which analyses of hydrocarbon mixtures were urgently required for accurate blending of high grade aviation fuel.

Although in this last case the instrument was used primarily to provide rapid and accurate quantitative analyses of hydrocarbon mixtures containing known components, the developed instrument was capable of obtaining the mass spectrum of any organic compound that could produce a vapour pressure of about 1 torr at room temperature.

The basic design features of the type of instrument used are shown in Fig. 3. The instrument and the sample systems are dynamically pumped, a normal operating pressure in the analyser section being 10^{-6} to 10^{-7} torr before introduction of the sample. This low pressure is required for two reasons. First, the bombarding electrons are produced from a heated filament (about 2000 °K), so that any appreciable air pressure would cause rapid filament failure. Second, after their formation the ions have to traverse an appreciable distance in the instrument before detection. A long mean free path is therefore required so that any loss of ions by collision with neutral gas molecules is of very low probability.

The sample vapour is introduced into a reservoir and allowed to enter the ionisation chamber through a pressure-reducing leak. The electrons emitted from the heated tungsten filament interact with the sample vapour molecules. The energy of these bombarding electrons is maintained at a steady value in the range 50 to 70 eV; this energy is more than sufficient to ionise any known molecule so that there will be a sufficient excess of energy to produce fragmentation of the molecule at the weaker bonds. It requires an energy of the order of 10 to 12 eV to ionise an organic molecule or to break a covalent bond. A range of fragment ions is therefore formed in the ionisation chamber. By means of a repeller electrode mounted in the ionisation chamber these ions are impelled towards an exit slit situated in the side of the ionisation chamber, which is maintained at a high positive electrostatic potential (typically 1 to 8 kV). As soon as the ions emerge from the exit slit they are accelerated by

the electrostatic field towards an earthed defining slit. The equation governing their motion will be—

$$\tfrac{1}{2} m v^2 = e V \quad \ldots \quad (1)$$

where m is the mass of the ion, v is the velocity of the ion, V is the accelerating potential and e is the electronic charge.

The ions transmitted by the earthed defining slit drift at constant momentum until they enter the mass analyser, which consists of a permanent or electro-magnet mounted with the field perpendicular to the flight path of the ions. The ions will be deflected in a circular path in this field, and the radius of the path traversed will be proportional to the mass of the ion. The angle of the arc through which the ions are deflected varies from the 180° type, based on Dempster's original design, to Nier's 60° instrument.[8] The instrument shown in Fig. 3 is defined as a 90° magnetic-sector instrument. The equation of motion for the accelerated ions in the magnetic field is—

$$\frac{m v^2}{r} = B e v \quad \ldots \quad (2)$$

where m, v and e are as defined in equation (1) B is the magnetic field intensity and r is the radius of the ion path.

By combining equations (1) and (2) and eliminating v it follows that

$$m/e = \frac{B^2 r^2}{2V} \quad \ldots \quad (3)$$

As most of the ions carry a single positive charge and r is a constant defined by the geometry of the instrument, usually 6 inches (or 25 cm) for the type of instrument shown in Fig. 3, the mass of a specific ion being transmitted by the instrument will be directly proportional to the square of the magnetic field intensity (B) and inversely proportional to the accelerating potential (V). Progressive variation of either B or V (the alternate field being held constant) will allow sequential transmission of the various ion species through the final collector defining

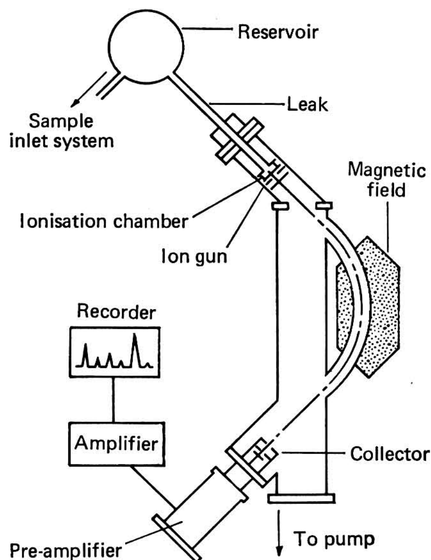


Fig. 3. A single-focusing mass spectrometer

slit. The small current generated at a collector electrode by the arrival of the various ion species is detected, amplified and displayed on a suitable recording system as the "mass spectrum." Most mass spectrometers used for analysis scan the mass spectrum by variation of the magnetic field.

The separated ion species should arrive at the collector as sharply defined groups of ions. It is important, therefore, to know the practical ability of the instrument to distinguish between adjacent ion species, *i.e.*, the resolving power. The resolving power is more stringently defined than in optical spectroscopy, as any interference from adjacent ion species will produce incorrect measurement of the mass and the abundance (or intensity) of the ion. Fig. 4 shows two adjacent groups of ions (or peaks). The distribution of the ions in the peak

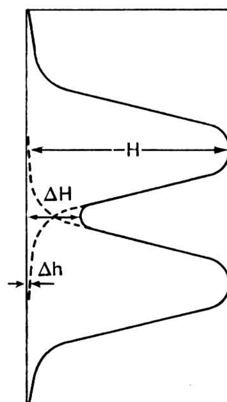


Fig. 4. Illustration of definition of resolving power

is approximately Gaussian and, for simplicity, the abundances (H) of the two ion species are assumed to be equal. There will be interference between the two peaks as shown by the dotted portion of the envelopes, and there will be a trough of height ΔH between the two peaks, where this interference is additive and at a maximum. The resolution is usually defined as a ratio of ΔH to H, *i.e.*, two peaks are said to be resolved if ΔH is less than 0·01H,

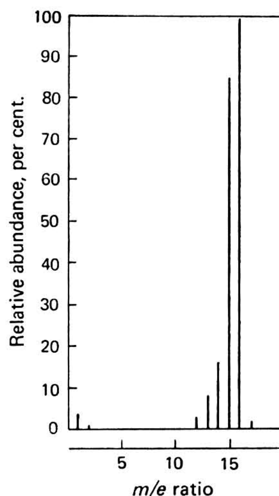


Fig. 5. The mass spectrum of methane

0·1H or 0·5H. It is usual in analytical work to choose the value where $\Delta H/H$ is 0·1; for this condition there will be a 5 per cent. contribution from each component at the cross-over point and the contribution Δh at the centroid will be negligible.

If the mass spectrometer has stable electronically controlled supplies to the source, accelerating potential and magnetic field, and the position of the magnet is adjusted for optimum direction focusing, then sharply defined peaks will be obtained. The resolving power will then be determined by the aperture of the source and exit slits and the radius of curvature of the ion path. In the case of the instrument shown in Fig. 3, where r is 6 inches and fixed source (S_1) and exit slits (S_2) are 0·012 inch wide, the resolving power (RP) is approximately defined by the equation—

$$\text{RP} = \frac{r}{S_1 + S_2} = \frac{6}{0{\cdot}024} = 250 \qquad (4)$$

i.e., two adjacent ions of masses 250 and 251 would be completely separated. Volatile compounds with a molecular weight of up to 250 can be examined completely with the instrument; every ion in the mass spectrum will be completely resolved from the adjacent ions, and the integral mass and relative abundance of all ions can be accurately established. Some information (without complete resolution) can also be obtained up to mass 500, but its value falls rapidly once the mass exceeds the resolving power.

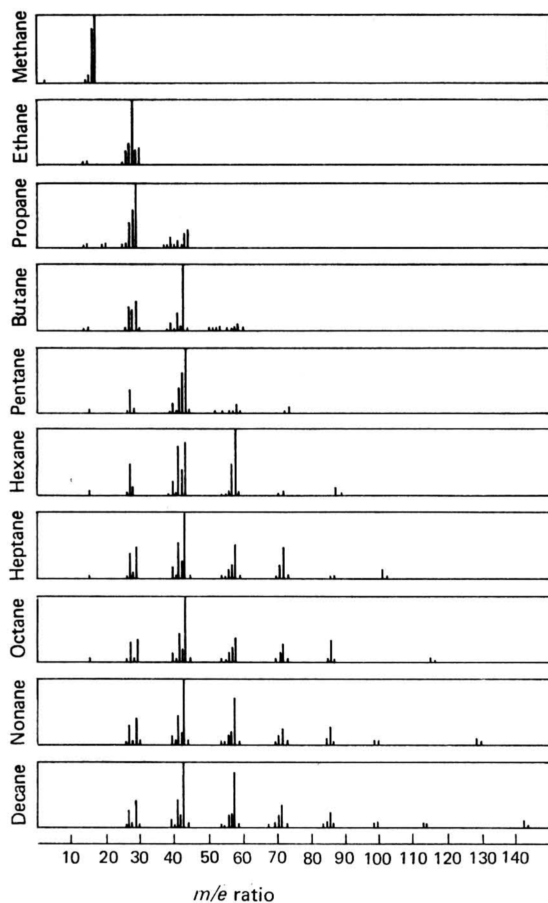


Fig. 6. The mass spectra of alkanes from methane to decane

In the early 1940s instruments with this typical performance were in routine use for the quantitative analysis of complex hydrocarbon mixtures.[9] However, before routine methods of analysis could be established, pure samples of the components expected to be present had to be examined rigorously in the mass spectrometer to establish their exact fragmentation patterns.

A number of the earlier workers in the field collected the mass spectra of various classes of simple hydrocarbons and made some generalisations about the way in which these molecules fragmented under electron impact. Washburn, Wiley, Rock and Berry, in 1945,[10] examined the mass spectra of C_5, C_6, C_7 and C_8 paraffins. Mass spectra of pure reference compounds were accumulated from laboratories by the American Petroleum Institute Research Project 44A,[11] and by 1950 some 436 reference spectra (mainly hydrocarbons) were available. A group of review papers was published in *Analytical Chemistry* in 1949 and in 1950. The 1950 mass spectrometry review[12] discussed seventy-nine articles published during the previous twelve months, and of these twenty-four were related to the analysis of organic compounds, again mainly hydrocarbons. However, Thomas and Seyfried[13] reported the qualitative analysis of mixtures of oxygen-containing compounds including alcohols, aldehydes, esters, ethers and acids. The stage was now set for an explosive interest in correlation studies of a wide range of volatile organic compounds.

The mass spectrometer can be used to obtain three important and different kinds of information about positive ions. The mass-to-charge ratio (m/e) of the ion can be measured relative to that of ions of known m/e, the abundance of the ion can be measured relative to that of other ions in the spectrum, and detailed information on the mode of formation of the ion from the compound under investigation can be deduced. All three measurements

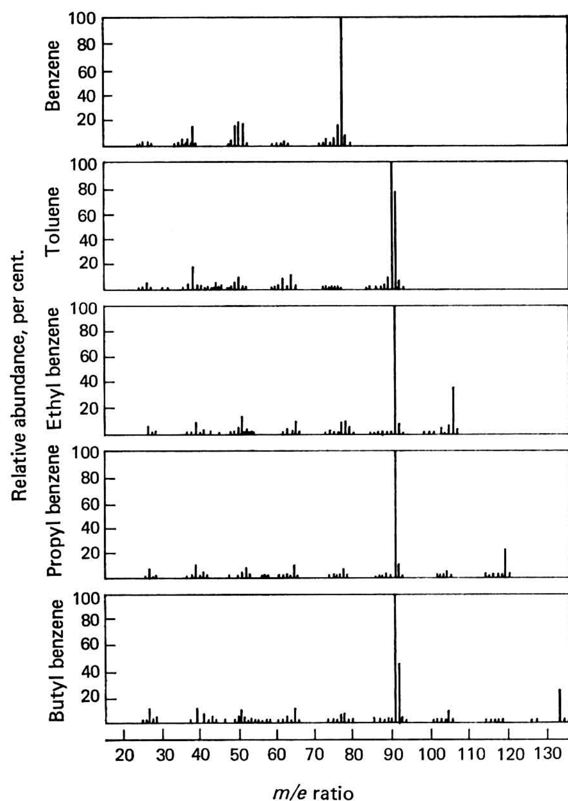


Fig. 7. The mass spectra of benzene and some alkyl benzenes

are important in the determination of structural formulae of unknown organic compounds. For example, in the mass spectrum of methane, shown in Fig. 5, it can be seen that the ions of *m/e* 16 (base peak) and 15 are abundant, with lesser amounts of ions of *m/e* 1, 2, 12, 13, 14 and 17. If we assume that we are dealing with a compound of molecular weight 16, then the ions at masses 15, 14, 13 and 12 indicate successive loss of 1, 2, 3 and 4 mass units that can only be hydrogen atoms. The residual fragment of mass 12 can only be carbon, and the compound must therefore be methane. The ion of *m/e* 17 is due to the presence of naturally occurring heavy isotopes, in this case either carbon-13 or deuterium, *i.e.*, *m/e* 17 is made up of either $^{13}CH_4$ or $^{12}CH_3D$. The fragmentation pattern shown in Fig. 5 is unique for methane.

Methane is the simplest member of the alkane series; if the spectra of a group of n-alkanes, such as those shown in Fig. 6 for methane to n-decane, are examined, it is obvious that as the chain length increases a pattern of behaviour emerges. In all cases the molecular ion (although of low abundance above propane) is readily detected. The ions in the C_3 or C_4 regions dominate the spectrum, and there is an almost exponential decay of intensity towards the molecular ion. This characteristic behaviour of n-alkanes is true even for chain lengths greater than forty carbon atoms. Correlations can also be made within a series of isomers; one of the earliest studies of this type was made by Bloom, Mohler, Lengel and Wise[14] on the eighteen isomers of octane. From their observations they proposed a number of empirical rules of fragmentation for aliphatic hydrocarbons.

(*i*) The relative height (or abundance) of the parent peak (or ion) is greatest for the straight-chain compound and decreases as the degree of branching increases.

(*ii*) The loss of a fragment containing a single carbon atom (see Fig. 6) is unlikely unless the compound contains methyl side chains.

(*iii*) Fragmentation is most likely at highly branched carbon atoms.

(*iv*) Ions of odd mass tend to be more abundant than those of even mass, and secondary fragmentation involving loss of hydrogen atoms or larger fragments also tends to produce ions of odd mass, especially in the case of straight-chain compounds. (As will be seen later, the reverse is true for molecules containing an odd number of nitrogen atoms.) Most of the ions can be formed by fragmentation of a single C—C bond in the parent ion.

(*v*) Prominent peaks at even mass numbers would indicate fragmentation of two separate side chains and therefore a high degree of branching.

(*vi*) In the spectra of paraffins peaks corresponding to C_3 and C_4 ions are always large.

A detailed discussion of the fragmentation pattern of the eighteen isomers of octane is given by Beynon, Saunders and Williams.[15] The introduction of a benzene ring into a structure produces a marked difference in the fragmentation pattern, *e.g.*, Fig. 7, which shows the mass spectra of a series of n-alkylbenzenes. The alkyl fragmentation pattern shown in Fig. 6 is no longer apparent. The influence of the stable benzene ring is reflected in the much greater abundance of the molecular ions. In benzene itself, for example, the molecular ion is by far the most intense ion in the spectrum. In the remaining four spectra the base peak is *m/e* 91 and originally was thought to be $C_6H_5.CH_2$—. It has been elegantly shown by Grubb and Meyerson[16] that *m/e* 91 is in fact the cyclic seven-membered tropylium ion—

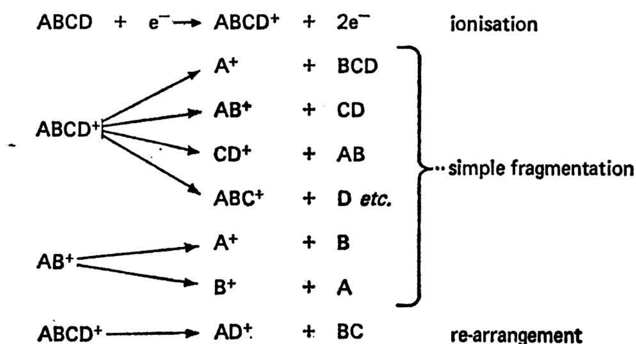
and that when ring expansion occurs, the additional carbon atom can be any of the carbon atoms in the side chain. Empirically, therefore, the presence of *m/e* 91 usually indicates that a compound contains an alkyl chain attached to a benzene nucleus. Empirically, also, if we designate the bond between the ring and the first carbon atom as α, then in the cases of the four substituted benzenes shown in Fig. 7, *m/e* 91 is formed by β-fragmentation of the side chain. It can be seen from the examples given above of the detailed examination of a homologous and an isomeric series that correlations of characteristic behaviour can be made and, even if the mass spectrum of an unknown compound cannot be matched against a reference spectrum for identity, it may still be possible to relate it to a known series of

compounds. Once a relationship is established, the fragmentation pattern of the unknown can be interpreted to give at least a partial structure.

FRAGMENTATION PROCESSES—

From the behaviour of hydrocarbons it seemed that fragmentation of molecules under electron impact was a fairly straightforward process. However, when the technique was applied to more complex molecules containing elements other than carbon and hydrogen, it became obvious that this process was not as simple as was at first thought. An energy of the order of 10 eV is required to ionise an organic molecule or to break a covalent bond. When the sample vapour is bombarded with 50-V electrons, there is considerable energy in excess of that required for ionisation. This excess of energy enables the molecule to undergo considerable fragmentation and, sometimes, rearrangement. Many of the fragments are positively charged and will be detected in the mass spectrometer.

With considerable simplification, the various ions in a mass spectrum may be pictured as arising by removal of an electron from the molecule to form the molecular ion. This ion subsequently dissociates by breaking at weakened bonds. Thus in the hypothetical molecule ABCD the following simplified processes may be found to occur—



It can be seen that the last of these dissociations involves an intramolecular rearrangement of the atoms.

REARRANGEMENT IONS—

Rearrangement ions are quite common and can register a high abundance in the mass spectra of a wide range of compounds. The interpretation of a mass spectrum may be complicated by the presence of rearrangement ions, although in many cases they enable one to gain a useful insight into some of the intramolecular forces that might otherwise remain obscure. It is perhaps sufficient to note at this stage, as an example of rearrangement, that compounds such as isobutane and neopentane produce a $C_2H_5^+$ ion even though neither compound contains an ethyl group. For a more detailed description of rearrangements the review paper by Brown and Djerassi[17] should be read.

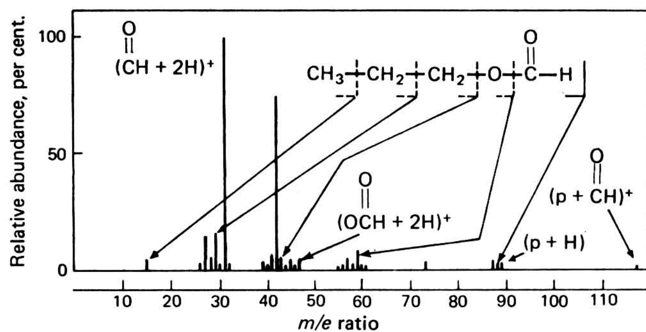


Fig. 8. The mass spectrum of propyl formate

Ion - molecule reactions—

Provided the pressure in the ionisation chamber is low enough for the mean free path to be high compared with the dimensions of the source, no intermolecular reactions can occur and the spectrum recorded will be the unimolecular decomposition spectrum of the sample. At higher pressures, however, ion - molecule collision-induced processes of the following type sometimes occur—

$$\underset{\text{ion}}{ABCD^+} + \underset{\text{molecule}}{ABCD} \rightarrow (ABCD.ABCD)^+ \rightarrow ABCDA^+ + BCD.$$

The most probable reaction is the addition of a proton to the original ion, and an ion M + 1 will often be detected. Fortunately, the formation of such ions depends on pressure and, if the sample pressure is doubled, then all normal ions will be twice as abundant, whereas all ions formed in ion - molecule reactions will increase in intensity by a factor of 3 or 4.

The mass spectrum of propyl formate, shown in Fig. 8, illustrates many of the possible fragmentation processes discussed above. The ions of masses 15, 29, 43 and 59 are formed by fragmentation at a single bond as indicated by the arrows. Other ions, including those of masses 27, 41 and 42, are formed by multiple fragmentations. The largest peak (base peak) in the spectrum, of mass 31, is due to the rearrangement ion formed by fragmentation of the bond between the carbonyl group and the ether oxygen atom followed by the transfer of two hydrogen atoms. Another rearrangement ion due to $(HCOO + 2H)^+$ is observed at mass 47. Both of these particular rearrangements are characteristic of formates, and homologous ions are detected in other esters, *e.g.*, acetates contain a prominent and unusual ion of mass 61 due to $(CH_3COO + 2H)$. The molecular ion of mass 88 is of very low abundance whereas M + 1 ion of mass 89, formed by an ion - molecule reaction, is more intense. A further ion at mass 117 is due to $(M + CHO)^+$, which is also formed in an ion - molecule reaction.

Metastable ions—

Some of the ions formed in the ionisation chamber by electron bombardment are metastable.[18] These are sufficiently stable to be withdrawn in large numbers from the ionisation chamber, but their half-life is only of the order of 1 μs and many will dissociate to an ion of lower mass and a neutral fragment during their passage between the ionisation chamber and the collector. If the dissociation occurs after acceleration and before entry into the magnetic analyser, a diffuse peak centred at a non-integral mass will be detected in the mass spectrum. Such peaks are loosely referred to as "metastable" peaks and an example is shown in Fig. 9; there, the main metastable peak centred at mass 46·8 is caused by the

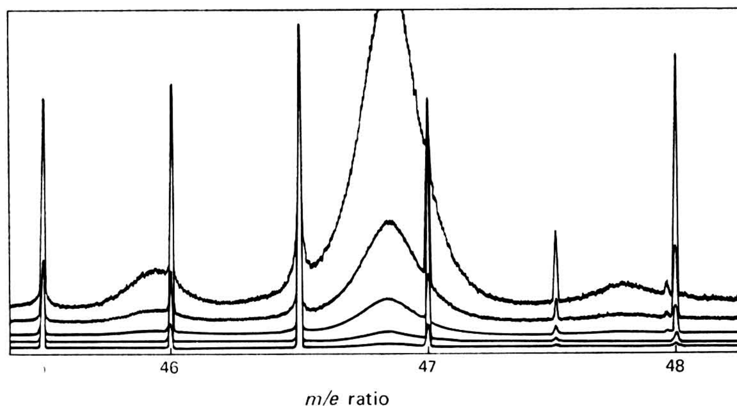
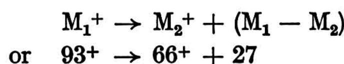


Fig. 9. The metastable peak in aniline from the dissociation $93^+ (C_6H_7N) \rightarrow 66^+ (C_5H_6) + 27 (HCN)$

dissociation of the aniline molecular ion of mass 93 to an ion of mass 66 with loss of a neutral fragment of mass 27, *i.e.*—



$$M_1^+ \rightarrow M_2^+ + (M_1 - M_2)$$
$$\text{or} \quad 93^+ \rightarrow 66^+ + 27$$

It can be proved[19] mathematically that

$$\frac{M_2^2}{M_1} = M^* \qquad \text{.. .. ..} \qquad (5)$$

where M* is the position of the metastable peak on the mass scale.

Valuable structural information can be obtained about the mode of fragmentation of a compound from the presence of metastable peaks. The masses of the ions M_1 and M_2 can be established unequivocally (they are usually quite abundant) either by trial substitution in equation (5) or by reference to a published table of metastable transitions.[20]

MOLECULAR ION AND THE NITROGEN RULE—

The molecular ion, if it can be recognised, is, of course, the most important ion in the mass spectrum, as it immediately defines the molecular weight of an unknown compound; unfortunately only about 90 per cent. of compounds produce molecular ions. If the molecular ion is odd in mass, then the compound must contain an odd number of nitrogen atoms. This is the so-called "Nitrogen rule," and it is true because all elements except nitrogen are either even in mass and have an even valency or are odd in mass and have an odd valency, so that any combination under normal valency conditions will yield even molecular weights. Nitrogen is the exception, with an even molecular weight (14) and an odd valency (3 or 5). Chlorine and bromine, with apparent molecular weights of 35·5 and 80, appear at first glance to be exceptions to the rule, but in fact they are not, because in even the simplest mass spectrometers chlorine and bromine will be separated discretely into the naturally occurring isotopes chlorine-35 and chlorine-37, and bromine-79 and bromine-81, respectively.

NATURAL ISOTOPES—

The presence of naturally occurring stable isotopes provides the mass spectroscopist with a valuable tool to extract further useful information about the composition of unknown compounds.

The elements carbon, hydrogen, nitrogen and oxygen all have small but measurable amounts of heavy, stable isotopes, *viz.*, carbon consists of carbon-12 and carbon-13 in the ratio of $1:1{\cdot}0806 \times 10^{-2}$, hydrogen of hydrogen-1 and hydrogen-2 or deuterium in the ratio of $1:1{\cdot}6003 \times 10^{-4}$, nitrogen of nitrogen-14 and nitrogen-15 in the ratio of $1:3{\cdot}8145 \times 10^{-3}$ and oxygen of oxygen-16, oxygen-17 and oxygen-18 in the ratio of $1:3{\cdot}9093 \times 10^{-4}: 2{\cdot}0048 \times 10^{-3}$. For any compound of molecular formula $^{12}C_a{}^{1}H_b{}^{14}N_c{}^{16}O_d = M$, the isotopic contribution one mass heavier than the molecular ion (M + 1) caused by the probability of the molecule containing carbon-13, hydrogen-2, nitrogen-15 or oxygen-17 can be calculated precisely. Similarly, the contribution of succeeding masses M + 2, M + 3 and so on, caused by various combinations of the isotopes, can be calculated. Obviously these higher combinations will soon become negligible, but in most compounds with a molecular ion of reasonable abundance M + 1 and M + 2 ions will be detected, so that their abundance relative to the molecular ion can be measured with reasonable accuracy. For a given combination of carbon, hydrogen, nitrogen and oxygen the relative abundance of M:M + 1:M + 2 will be unique, so if these masses are free of interference from impurity or fragment ions they can then be measured with sufficient accuracy to give a probable molecular formula. Tables listing isotope-abundance values for all possible combinations of carbon, hydrogen, nitrogen and oxygen in which nitrogen and oxygen are equal to or less than 6 up to a molecular weight of 500 are available.[21] As an example, a few of the possible combinations of these four elements of mass 78 are tabulated below—

	M	M + 1	M + 2
C_6H_6	100	6·580	0·181
$C_5H_2O_2$	100	5·474	0·321
$C_4H_2N_2$	100	5·117	0·106
$C_2H_6O_3$	100	2·374	0·618
$CH_6N_2O_2$	100	2·018	0·414

It can be seen that there is considerable variation in the ratios for the various formulae, and that careful measurement of M, M + 1 and M + 2 should yield the correct formula. The presence of other hetero atoms in the molecule is often readily detected if they are composed of a mixture of stable isotopes. Elements with a useful isotopic distribution include chlorine, bromine, sulphur, silicon and boron; many metals such as nickel, iron, copper, zinc, tin and lead also have a distinctive isotope pattern. The isotopic patterns of some elements are shown in Fig. 10.

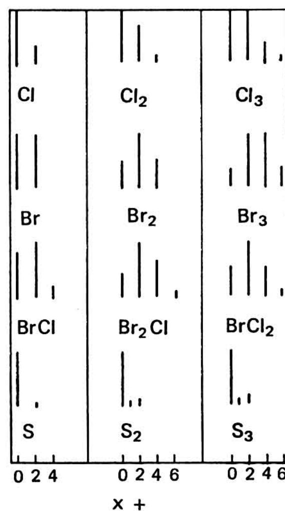


Fig. 10. Isotopic distribution patterns

HIGH RESOLUTION—

During the period from 1950 to 1960 there was an increasing interest in the application of mass spectrometry to organic analysis. Sampling systems were developed by the petroleum industry for analysing the less volatile and higher molecular weight products from crude oil. Paradoxically, this was because of the introduction of the new technique of gas - liquid chromatography in the early 1950s. Gas - liquid chromatographs could now be used in the routine quantitative analysis of hydrocarbon mixtures at a much reduced running cost, therefore releasing the mass spectrometers from routine quantitative work and enabling them to be applied to the field of qualitative organic analysis. One development of special importance arose from the realisation that if the masses of parent and fragment ions could be measured with sufficient accuracy to distinguish between different elemental combinations with the same nominal mass, then the degree of certainty with which unknown compounds could be identified would be greatly increased.

Aston used the oxygen-16 isotope as his standard of mass[22] and this standard was retained in 1931 by Aston and the Committee of the Commission on Atomic Weights.[23] On this mass scale oxygen-16 = 16·000000 atomic mass units (a.m.u.), hydrogen-1 = 1·008145 a.m.u., carbon-12 = 12·003844 a.m.u. and nitrogen-14 = 14·007550 a.m.u. By comparing the unknown ion with a reference ion of known composition, and hence known exact mass, the exact mass of the unknown ion could be established to within 1 part in 20000.[24,25]

With this accuracy, the elemental composition of a molecular ion in the mass range up to 200 could be established unequivocally, providing there was no interference from impurity ions of the same nominal mass number. In favourable cases mass spectrometry could therefore establish the correct molecular formula (not empirical formula) of an unknown compound. This formula could be established in the presence of other components if they did not interfere with the molecular ion under consideration.

There were, however, two problems to be solved. The first involved the mechanism of fragmentation, in which it was considered possible that some ions could be formed with kinetic energy. If kinetic energy was present it would cause the fragment ion to be displaced on the mass scale; measurements on fragment ions were, therefore, treated with caution, although they did support logical combinations of elements. The second problem was more practical and involved the resolution available. With a single-focusing instrument of the type used in the original measurements,[24,26] a maximum resolving power of about 700 could be attained, *i.e.*, *m/e* 700 could be discretely separated from *m/e* 701 with a considerable decrease in sensitivity. When this resolution is considered in relation to the mass differences present between ions of the same nominal mass but of different elemental composition, it is found to be completely inadequate except in one or two special cases. For example, the ion OH^+ has the exact mass 17·0081 a.m.u. and NH_3^+ has the exact mass 17·0320 a.m.u.; the mass difference is 1 part in about 700 so that these two ions could be resolved. It would therefore be possible to distinguish between NH_3 and OH. On the other hand, a resolving power of 1 part in about 2300 is necessary for separating cyclohexane (C_6H_{12} = 84·1208 a.m.u.) from cyclopentanone (C_5H_8O = 84·0844 a.m.u.) so if the technique was to be applied to all ions in the mass spectrum a new type of instrument capable of overcoming the two problems of resolution and possible energy variation was required.

In 1953 Metropolitan-Vickers (now A.E.I. Instrumentation), who were the principal manufacturers of mass spectrometers in Europe, were considering designs for a new instrument with an increased resolving power; a design based on the Nier - Roberts geometry was selected.[27] This design, with tandem electrostatic (90°, 7½-inch radius) and magnetic (90°, 6-inch radius) sectors, has first-order energy-focusing and second-order direction-focusing properties. Therefore, ions emerging from the source with small variations in energy and angular dispersion can be brought to a focus at the collector slit.

The energy-focusing property of the design is very important: ions formed with a variation of energy will be brought to a sharply defined focus at the correct position on the mass scale. Consequently, the exact mass, and therefore the elemental composition of all fragment ions as well as the molecular ion, can be uniquely identified. For ease of identification of ions of unknown elemental composition, a tabulated list of elemental composition with the exact calculated mass is necessary. Such a table has been compiled[28] for all possible ions containing carbon, hydrogen, nitrogen and oxygen up to mass 250. Certain restrictions were imposed: that the formula must not violate normal valency requirements; that the number of nitrogen or oxygen atoms must not exceed 4; and that the number of nitrogen *plus* oxygen atoms must not exceed 6. Even with these restrictions there are 47 different atomic combinations listed at *m/e* 200. Such a table is internally self-consistent and if the presence of a hetero atom such as sulphur, chlorine or phosphorus in a measured ion is suspected, the exact mass of the suspected hetero atom should be subtracted from the measured value and the composition of the remainder of the ion is established by consulting the mass table with the residual accurate mass.

A prototype instrument designated the MS8 was constructed and commissioned in 1957.[29] The projected resolving power was 3000, and a multiple sample system enabled a wide variety of samples, from gases to involatile compounds with a vapour pressure of less than 1 mm of mercury at 350 °C, to be examined. The design was extremely flexible and by narrowing the entrance and exit defining slits a resolving power in excess of 15000 could be obtained, although at the expense of sensitivity. This prototype instrument was applied in the authors' laboratories over a period of 5 years to the analysis of complex organic molecules, structural correlation studies, rearrangement processes, analyses at high mass and ion - molecule reaction studies.[30,31] Other mass spectrometric laboratories throughout the world showed considerable interest in the performance of the new instrument and the unique results obtained.

From 1955 to the early 1960's a number of other important developments occurred. The first concerned the detection and presentation of the ion current. A conventional d.c. amplifier will detect ion currents in the range 10^{-15} to 10^{-10} A. At the lower currents the response of the system is relatively slow, so that the output display could be a pen recorder, plotting a mass spectrum over a period of minutes. For example, a mass scan from *m/e* 10 to 200 at a resolving power of 250 could take 5 minutes. With the advent of high resolution such a mass spectrum scanned at a resolving power of 2500 would require a 50-minute scan time.

In 1939 Allen[32] first used an electron multiplier to detect ion currents in a mass spectrometer. Here the ion beam is allowed to strike a cathode, which emits electrons. These electrons cascade and multiply down a series of electrodes arranged in a potential ladder. If the potential between each electrode is high enough, the final electron current is larger in magnitude (but opposite in sign, of course) than the original ion current. This magnification or gain is obviously important and by the late 1950s electron multipliers with an over-all gain of 10^6 were available. If run at an over-all gain of 1 000 over the conventional d.c. amplifier, the speed of response can be increased by a factor of 1 000, and the output can be fed to high-speed photographic recorders. A complete mass spectrum can be recorded in a few seconds and with ultraviolet-sensitive recording paper, an "instant" spectrum can be obtained. (It is perhaps presumptuous to say that we have reached the absolute limit of detection sensitivity; however, with the presently available high-gain multipliers it is possible to detect a current of 10^{-19}A so that, as one ampere is a flow of 10^{19} electrons per second, it is possible to detect the discrete arrival of a single positive ion at the collector.)

The second improvement was an increase in the ease and accuracy of mass measurement by superimposition of the known and unknown ions on an oscilloscope screen[33]; one peak is moved relative to the other by means of an accurate six-dial decade box. When the peaks are accurately superimposed, the mass ratio of the two peaks is read directly from the decade dial readings. If the exact mass of one ion is known, the mass of the unknown ion can be rapidly calculated by simple division or multiplication to an accuracy of one or two parts in 10^6. The third major improvement lay in the field of sample handling. Most sample systems had been evolved for quantitative analysis, for which the samples are completely volatilised into a reservoir (usually of about 1-litre capacity) and fed to the ionisation chamber through a leak. However, for qualitative analysis the leak can be dispensed with. A number of advantages are gained: in the case of a mixture careful temperature programming of the sample will yield spectra of the components as the sample is fractionated into the ionisation chamber; and, if the sample is thermally unstable, a mass spectrum can be obtained at a much lower temperature than with conventional systems.

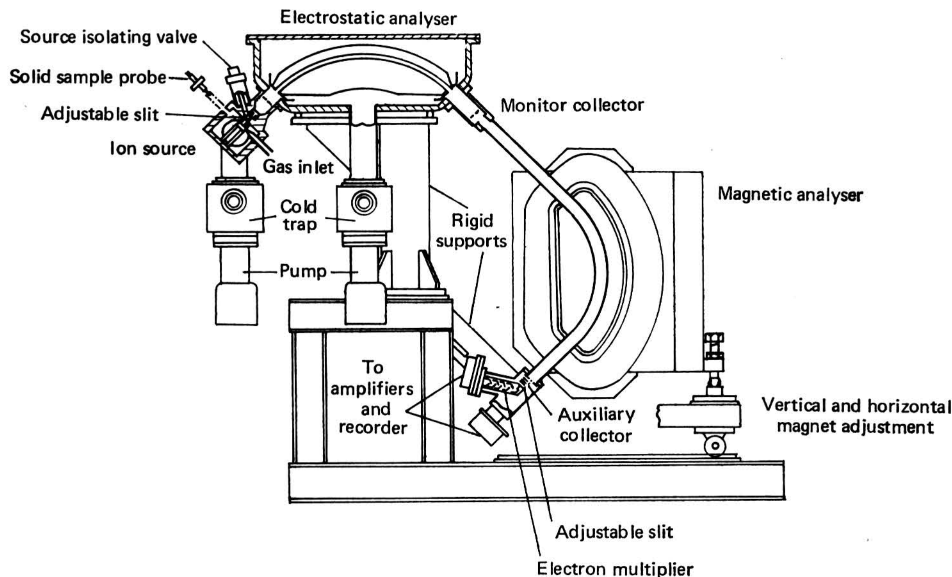


Fig. 11. The MS9 high-resolution double-focusing mass spectrometer

In modern instruments the sample is introduced directly into the ionisation chamber through a vacuum lock. The amount of sample required for this method is extremely small. We have, under normal operating conditions, loaded 10^{-8} g of sample on to the probe, inserted the probe through the vacuum lock, obtained a mass at a resolution of 2 500, measured the

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accurate mass of the molecular ion, established the molecular formula and withdrawn the probe and sample, all within a period of 15 minutes. Not all samples are pure, however, and a mixture may require successive scans as the temperature of the sample is programmed upwards. Some samples adsorb and give rise to instrumental background. Accurate mass measurements are invaluable but do take time, usually 15 minutes for the initial measurement and 5 to 10 minutes for each succeeding measurement by an experienced operator.

In 1961 the 10th International Union of Pure and Applied Physics recommended that the new standard of mass should be based on carbon-12 = 12·000000 u (where u is the symbol of atomic mass). Then, hydrogen-1 = 1·007825 u, nitrogen-14 = 14·003074 u and oxygen-16 = 15·994914 u. The exact masses of all known nuclides on the new scale have been tabulated.[34] Although the existing accurate mass table[5] was self-consistent, the opportunity was taken to calculate a new table of the exact masses of all possible combinations of carbon, hydrogen, nitrogen and oxygen (the number of nitrogen and oxygen atoms being equal to, or less than, 6) up to m/e 500, and to include isotopic-abundance data.[21] A list has also been compiled for combinations that include sulphur and chlorine.[35] Inclusion of one extra hetero atom in the calculations will approximately double the number of entries. Other alternative methods for the determination of ionic formulae are available.[36,37,38]

Thus, in the early 1960's the stage was set for the application of mass spectrometry as a qualitative analytical tool alongside infrared, ultraviolet and nuclear magnetic resonance spectroscopy. The production instrument, based on the MS8 prototype, was the first of a new generation of high resolution double-focusing mass spectrometers. Designated the MS9,[39] it had a 90°, 15-inch radius electrostatic sector followed by a 90°, 12-inch radius magnetic sector. It was probably the first commercial instrument specifically designed for the routine analysis of organic compounds. Multiple-sample systems, electron multiplier, and a mass measurement display system were fitted as standard. A schematic diagram of the MS9 is shown in Fig. 11. A routine resolving power of about 17000 was guaranteed; Fig. 12 is a slow scan of m/e 28 showing five separate components at a resolving power of 20000.

If a compound can be volatilised into the mass spectrometer without thermal decomposition, a unique fragmentation pattern (except in the case of close isomers) will be obtained. If the molecular ion is detected, the integral molecular weight is known exactly and it can be measured accurately, the exact atomic composition of the compound can be established. Isotopic abundances will identify the presence and exact numbers of hetero atoms such as chlorine, bromine, sulphur and silicon (see Fig. 10). It may be possible to identify the compound by matching against known reference spectra[11,40,41] and reference spectra can be readily tabulated into search indexes.[42,43,44] If the spectrum cannot be matched, consideration of the fragmentation pattern will yield useful structural information, *e.g.*, at—

m/e	
29, 43, 57, *etc.*	Alkyl system or group
30	Amine ($CH_2{=}NH_2^+$)
31, 45, 59	Alcohols, ethers, R–O or $(CH_2)_nOH$
41	Nitriles
44	Aldehydes
59	Amides

Lists of dominant ions related to structural groups have been published.[45,46] Metastable peaks should also be made use of to follow competing fragmentation processes.[47] Rearrangement ions can also provide useful structural information[48,49] and McLafferty has considered

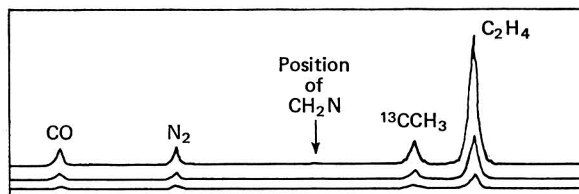
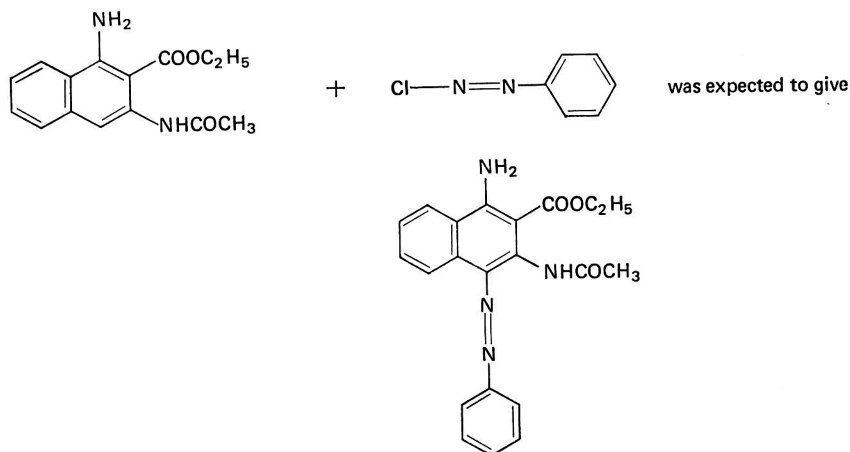


Fig. 12. High resolution scan of m/e 28 showing five separate components: CO, N_2, CH_2N, $^{13}C^{12}CH_3$ and C_2H_4

decompositions and rearrangements of organic ions.[50,51] There are a number of source books dealing exclusively with the interpretation of the mass spectra of known classes of compounds.[52 to 57]

The technique can be applied to a wide variety of problems, *e.g.*, confirmation of structures, complete structural determinations, detection and identification of small amounts of impurities or additives, especially after they have been separated by chromatographic techniques, trace-solvent analysis, and identification of odours collected on absorbent media.

It is of particular value when used in combination with other analytical techniques, *e.g.*, the reaction between



Although the expected product was isolated, a significant amount of impurity was also present in the reaction mixture. This impurity was isolated and submitted for analysis. The following complementary information was submitted by the chemist—

Microanalysis	$C_{15}H_{12}N_4O$ was assumed
Nuclear magnetic resonance analysis ..	–N=N–; 1 × CH_3; about 10 aromatic protons; No $-OC_2H_5$ group detected
Infrared analysis	CO band not due to ester; no CN group present.

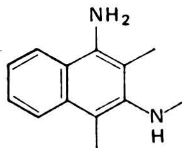
The mass spectrum obtained by using a vacuum-lock probe is shown in Fig. 13. The molecular ion is obviously *m/e* 329, *i.e.*, the compound must contain an odd number of nitrogen atoms, so the assumed formula based on microanalysis figures is incorrect. The molecular ion was measured accurately and the formula was discovered to be $C_{19}H_{15}N_5O$. (If we convert the original formula to include N_5 it becomes $C_{18\cdot25}H_{15}N_5O_{1\cdot25}$.) A check of the microanalysis figures gave better agreement with the formula established by accurate mass measurement.

The six major ions detected in the mass spectrum were established by the presence of metastable peaks to be linked together in a sequential fragmentation pattern. The masses of these six ions were measured accurately and their formulae were established—

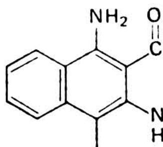
m/e	Measured formula	Fragment lost
329	$C_{19}H_{15}N_5O$	Molecular ion
224	$C_{13}H_{10}N_3O$	$C_6H_5N_2$
183	$C_{11}H_7N_2O$	C_2H_3N
155	$C_{10}H_7N_2$	CO
128	C_9H_6N	HCN
101	C_8H_5	HCN

It is possible in this case to rebuild the structure of the original molecule in the following sequence.

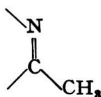
A value for *m/e* of 101 is typically produced by naphthalene derivatives, and the measured formula (C_8H_5) would support the presence of a naphthalene system. The loss of the HCN molecules is characteristic of aromatic amines such as aniline or naphthylamines. The ion of *m/e* 155 ($C_{10}H_7N_2$) can therefore be allocated the tentative structure



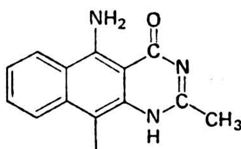
This structure is directly related to the naphthalene derivative present in the reaction. The ion of *m/e* 183 ($C_{11}H_7N_2O$) can also be directly related to the starting compound—



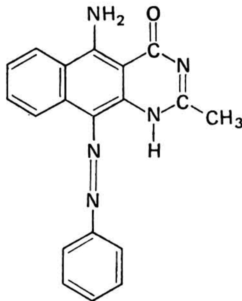
The next stage involves the addition of the unit C_2H_3N to *m/e* 183. Now C_2H_3N is the formula of acetonitrile, a stable, neutral molecule. The original molecule may therefore include the elements of acetonitrile, *e.g.*, an arrangement such as—



could readily be eliminated as acetonitrile and *m/e* 224 could have the structure



The difference between *m/e* 224 and the molecular ion is $C_6H_5N_2$, *i.e.*, the phenylazo group and because there is only one unsubstituted position remaining, the following complete structure for the unknown compound was proposed—



This structure will satisfy the exact molecular formula $C_{19}H_{15}N_5O$, unequivocally established by high-resolution mass measurement, and the observed fragmentation pattern. The infrared and nuclear magnetic resonance structural evidence also supports the suggested structure.

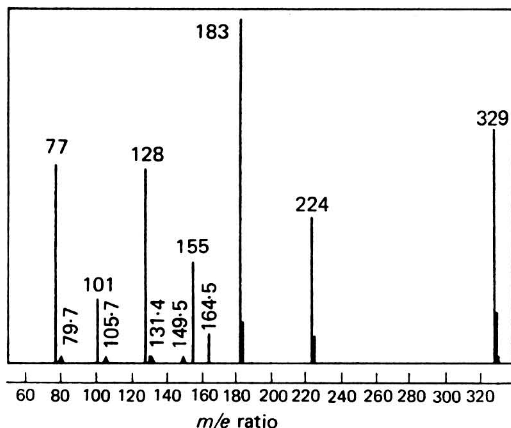
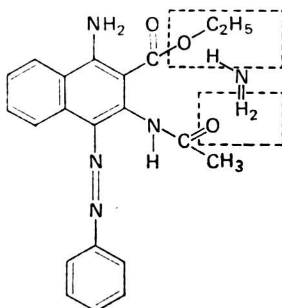


Fig. 13. Mass spectrum of unknown reaction product

It was subsequently found that ammonia had been present during the process, and the impurity was formed by reaction of 1 mole of NH_3 with 1 mole of the expected product, with elimination of 1 mole of H_2O and 1 mole of C_2H_5OH—



OTHER TYPES OF MASS SPECTROMETER—

The magnetic-sector mass spectrometer was for many years the most commonly used type of instrument. The subsequent extension into high-resolution double-sector instruments was a natural development. Since then, other types of mass spectrometer based on completely different principles have been postulated and developed. Some of these instruments have special features that make them suitable for special analytical applications. Most will, within their capabilities, produce adequate mass spectra similar to those obtained from magnetic-sector instruments.

The cycloidal mass spectrometer—This design is based on a scheme reported by Hipple and Bleakney[58] in 1938. It was shown that if a homogeneous electrostatic field is superimposed upon a uniform magnetic field, double focusing can be achieved in a plane normal to the magnetic field. The ions follow a cycloidal trajectory, and the mass spectrum can be scanned by variation of either the electrostatic or electromagnetic fields.

The coincident fields produce a mass analyser of small dimensions. Commercial instruments with small permanent magnets can be used for residual-gas analysis and monitoring of low molecular weight components in chemical plant streams. A commercial instrument of larger dimensions and a resolving power of 2500 is available.[59]

The time-of-flight mass spectrometer—The prototype of the present day time-of-flight spectrometer, based on a proposal by Stephens,[60] was constructed by Wiley and McLaren.[61] The instrument consists of a conventional ion source, an ion-accelerating grid system, a drift

space and an ion collector - detector - display system. The ions formed in the source are accelerated to a constant momentum and, when these ions enter the drift region, the lighter ions will have a higher velocity than heavier ions and will arrive first at the collector. If the ion beam is pulsed and the drift space is long enough, the ions of different mass will arrive sequentially at the collector, thus producing a mass spectrum. The mass spectrum is usually displayed on a cathode-ray oscilloscope. With a suitable fast-response detector 10000 mass spectra can be scanned per second. The time-of-flight system is therefore unique and extremely useful for monitoring fast-reaction products, reaction rates and reaction profiles. The resolution will depend basically upon the length of the drift space. Small commercial instruments are available with a resolution of about 100. At the other end of the scale instruments with 170-cm drift space can scan up to m/e 900 in 1·5 s.

Quadrupole mass spectrometers—These are non-magnetic instruments[62] consisting of four parallel and symmetrically disposed rods or electrodes. A conventional ion source generates a beam of ions that are accelerated along the axis of the electrode system. A combination of d.c. potentials and a radiofrequency potential is applied to the electrode system and control of these potentials can cause an ion of selected mass to be transmitted through the system, detected and displayed. Unwanted masses undergo an oscillating trajectory of increasing amplitude until they strike one of the electrodes and are lost from the system. The resolution of the instrument will depend to a considerable extent on the length of the electrode system. The mass spectrum can be scanned rapidly so that this type of instrument is useful for monitoring rapidly changing systems.

A number of commercial instruments based on this design are available, from small, simple designs for residual gas analysis to large sophisticated systems suitable for the qualitative organic analysis of complex molecules. One of the advantages of the quadrupole lens system is the linear mass scan produced, *i.e.*, all ions will be equally spaced on the mass scale in contrast to the usually exponential scan of magnetic-sector instruments. This feature is particularly important when a weak mass spectrum containing gaps in the fragmentation pattern is produced. The masses of isolated ions can be established by linear interpolation or extrapolation.

Instruments with Mattauch - Hertzog geometry—When Mattauch and Hertzog developed their general equations for double-focusing mass spectrometers[63] they chose to construct a double-focusing system with a 30° 50′ electrostatic sector followed by a 90° magnetic sector. This design has the property of possessing double focusing for all masses. These masses are brought successively to a focus along a line parallel to the magnetic field boundary. If a photographic plate is placed along this line of focus, ions will be detected and recorded simultaneously. Instruments based on this design were manufactured for the analysis of metals. The metal to be analysed is formed into a pair of electrodes and inserted inside the evacuated source, after which a high frequency spark is generated between the metal electrodes. Ions formed in the discharge are then accelerated and mass analysed. In suitable cases concentrations of the order of 1 part in 10^9 can be detected and accurate quantitative analysis of metal alloys and semiconductors can be carried out.

A conventional electron-impact source can be installed and a fragmentation pattern detected on the photographic plate. A conventional mass spectrum can be obtained from the photographic plate by using an automatic densitometer. The abundance of the ions detected will not be accurately displayed because of the non-linear response of the photographic emulsion; the exact mass of the ions detected can, however, be accurately determined from the plate. This system has the advantage that all the ions in the spectrum are recorded instantaneously and integrated with time. Some instruments of this type can also be fitted with an electron multiplier - detector and magnetic scanning to bring the ions sequentially across the detector.

Gas - Liquid Chromatography Combined with Mass Spectrometry

Mass spectrometry is a very powerful technique for the structural identification of unknown organic compounds and generally requires only minute amounts of sample. It is therefore ideally suited to the identification of components separated from mixtures or technical products by gas - liquid chromatography. A number of authors[64,65,66,67] have discussed methods of collecting individual samples from gas - liquid chromatographic columns

and transferring them to the mass spectrometer for identification. One disadvantage is that a complicated collector system is required and minor components could be lost during collection and transfer.

An alternative method[68] is to pass the gas - liquid chromatographic effluent directly through the isolated mass spectrometer sample system. When a peak is detected eluting from the column, the pumping exit from the sample system is closed and the eluting component *plus* carrier gas builds up pressure in the system. When sufficient pressure is obtained, the column or effluent is by-passed to waste or the column flow is stopped. If the eluted component is in high concentration, sample and carrier gas can be admitted directly to the instrument for analysis. If the fraction is a minor component, a portion of the sample system is frozen in liquid nitrogen, the sample is condensed, and the carrier gas is pumped away. The condensed sample is then re-evaporated and admitted into the mass spectrometer for analysis. This system is extremely sensitive and no loss of sample occurs. If the elution times between components emerging from the gas - liquid chromatographic column is greater than about 3 minutes, then the eluted components can be examined in succession. If the elution gap is less than 3 minutes, alternate components, say 1, 3 and 5, can be analysed on one run of the chromatograph and a second run will enable components 2, 4 and 6 to be analysed. If two components eluting from the column are only partially resolved, then cuts on the leading and trailing edges of the composite peak will usually yield usable mass spectra of the individual components. Scott, Fowlis, Welti and Wilkins[69] have devised an automated version of the interrupted elution system for simultaneous recording of infrared and mass spectra by using a small, relatively cheap, slow-scanning mass spectrometer.

All of the systems outlined above are aimed at the production of low-resolution fragmentation patterns only. If high-resolution information is required this normally takes more time. Frequently, too, the identification of an unknown component in a "sick" chemical process requires the application of more than one physical technique. Some system of collection followed by distribution to the techniques will yield the maximum amount of analytical information. Even if the amount of sample is extremely small, a potassium bromide disc will provide an adequate infrared spectrum. The component can then be extracted from the disc with a suitable solvent for nuclear magnetic resonance analysis, and the solution can be evaporated to provide a sample for the mass spectrometer. A sample obtained in this manner or by trapping on a suitable absorbent followed by regeneration[70] can be exhaustively examined in the mass spectrometer. Accurate mass measurements can be carried out to give valuable structural information.

The ultimate solution to the combination of gas - liquid chromatography and mass spectrometry is obviously to feed the column effluent directly into the mass spectrometer. There is, however, one major problem: a gas - liquid chromatographic column usually elutes at atmospheric pressure, but a mass spectrometer usually cannot tolerate a pressure greater than 10^{-5} mm of mercury in the ionisation chamber. There is also the additional problem that a gas - liquid chromatograph peak is usually of small duration and gaussian in shape. The mass spectrum should therefore be started just before the period of maximum concentration of sample and completed within a few seconds. The problem of the pressure differential was solved in earlier systems by accepting only a small fraction of the column effluent (typically 5 per cent.). This small fraction was drawn into the mass spectrometer by a pressure reducing system. The scan time of the conventional sector instrument then available was too long for the entire examination of gas - liquid chromatograph peaks; however the time-of-flight system already described had suitable scan times. These earlier systems[71,72,73] had a low sensitivity because of the short time constants necessary for the rapid-scanning detection circuits and dilution by the carrier gas. However, the effluent from a gas - liquid chromatographic column was analysed continuously.

At present there is a wide variety of instruments available with scan rates of a few seconds with magnetic scanning and fractions of a second with voltage scanning on a conventional magnetic-sector instrument. Other types, such as the time-of-flight and quadrupole systems with their fast scanning rates, are eminently suitable for combination with a gas - liquid chromatographic column. The availability of multiplier detectors gave a real increase in sensitivity and scanning rates.

The major problem to be solved was the high pressure and diluting effect of the carrier gas. If the carrier gas can be removed preferentially after elution of the component from the

gas - liquid chromatographic column, then a much higher concentration of sample vapour at a reduced total pressure can be admitted into the mass spectrometer for analysis. A number of separators have been designed in which the transfer of either the carrier gas or the sample across an interface is preferential. For example, the following are systems for preferential carrier gas transfer: a fritted glass tube surrounded by a glass envelope[74] (Watson and Biemann); a supersonic jet separator[75] (Ryhage); metallic permeable membranes (silver, stainless steel); and plastic permeable membranes.[76]

Combined gas - liquid chromatograph - mass spectrophotometer systems can be purchased and most standard analytical instruments can be readily adapted for interfacing to most commercial gas - liquid chromatography equipment with a suitable separator. There are a number of combinations available for less than £5000. There is no doubt that in the field of natural products, essences, flavours and perfumes, combined gas - liquid chromatography - mass spectrometry can produce mass spectra of most of the components present even at very low concentrations. The major problem at this stage is the interpretation of the forty or more mass spectra that can be obtained in an hour or so. The Mattauch - Hertzog design described earlier is fitted with a photographic plate detector. Here all ions are detected simultaneously, and this is an advantage when minor components are to be detected.[74]

Although gas - liquid chromatography is the chromatographic technique most compatible with the mass spectrometer, any separation technique that will provide discrete volatile components from a mixture could be utilised. For example, thin-layer chromatography can be used to separate components on a plate. The substrate containing a specific component can be scraped off, immersed in a suitable solvent, and centrifuged to separate out the substrate particles, after which the solution containing the component can be removed and the solvent evaporated to leave the unknown component ready for examination. For minor components strict cleanliness is essential, and the quality of the substrate is also important, otherwise impurities present in the solvent or the substrate will be present in higher concentration than that of the unknown component. The authors have found, for example, that a substrate such as silica, after storage in plastic containers, will contain a significant amount of the plasticiser used in the manufacture of the plastic. Column chromatography and high-pressure liquid chromatography can also be used to produce samples for mass-spectrometric examination.

CURRENT DEVELOPMENTS AND FUTURE TRENDS

Within a decade, mass spectrometry as an analytical tool in organic chemistry has expanded from an expensive technique available only to a few large industrial research laboratories to a versatile and necessary instrument wherever organic chemistry is practised. There is a wide range of types, sizes and prices of instruments readily available.[77] In the two-year period ending December 1967 several thousand papers were published in the field of mass spectrometry. There are two journals currently devoted exclusively to mass spectrometry: *Organic Mass Spectrometry* (Heyden) and *The International Journal of Mass Spectrometry and Ion Physics* (Elsevier). *The Mass Spectrometry Bulletin*, published monthly by the Mass Spectrometry Data Centre (M.S.D.C.) at A.W.R.E., Aldermaston, efficiently abstracts most of the literature and presents the information in a form that is easy to retrieve. The M.S.D.C. also supplies reference mass spectra of organic compounds.

Small, inexpensive instruments with good performance are available for laboratory teaching and demonstration. These instruments are portable and can be used for manufacturing process control.

New methods of ionisation such as photo-ionisation,[78] field-ionisation[79] and chemical ionisation[80] are available. These three techniques give enhanced fragmentation of molecules in the higher-mass regions of the spectrum, and in particular produce M + 1 ions (M + 1 or M − 1 for chemical ionisation) in compounds that do not produce a molecular ion under electron impact. Additional structural information is therefore obtained, although routine use will require the examination of a large number of reference compounds to provide fragmentation data directly related to structure.

The theory of breakdown of organic molecules under electron bombardment is still not clearly understood, and there is much fundamental work to be carried out before the fragmentation pattern of a known structure can be accurately predicted. Studies on metastable transitions, rearrangement processes, etc., are being carried out in many laboratories throughout the world.

In the field of instrumentation large double-focusing instruments with resolutions of 500000 to greater than 1000000 have been constructed; however, these are more suitable for academic investigation, although the performance of existing commercial instruments is continually being uprated by the manufacturers. The MS9 double-focusing mass spectrometer (see Fig. 11), originally rated at 20000, will now attain a resolution of 200000. The non-linear scan (usually exponential) of a magnetic scanning instrument poses problems in allocating *m/e* values to some of the peaks detected. Accurate and reliable mass markers are now available as optional accessories for most commercial instruments. It has been mentioned earlier that combined gas - liquid chromatography - mass spectrometry is an increasingly used analytical tool, particularly in the field of natural products, and that package instruments of varying degrees of sophistication are readily available.

Perhaps the most important trend in mass spectrometry is the increasing use of the computer for data processing. The technique was originally pioneered by Biemann and his co-workers.[81] Today, experimental systems are operating on a routine basis,[82] and "package deals" are available at various levels of sophistication for small dedicated computers directly on line to the signal output of the mass spectrometer. For low-resolution instruments a fairly simple system will convert the mass spectrometer output as it scans into a tabulated mass spectrum in a matter of seconds. A gas - liquid chromatograph - mass spectrometer - computer system can process a multi-component mixture as rapidly as the individual components are eluted.

For high-resolution instruments, more sophisticated systems are available with which a complete mass spectrum can be processed to provide exact formulae for every ion in the mass spectrum. With systems increasing in cost and complexity it is refreshing to note that one manufacturer has recently introduced a small double-focusing instrument capable of transmitting twin parallel beams of ions side by side for independent detection and processing. Push-button controls are available for a fixed resolving power of 1000, 3000 or 10000 with push-button selection of scanning speeds. One half of the instrument can provide a low-resolution fragmentation pattern complete with metastable peaks while the other half of the instrument can transmit a high-resolution spectrum that can be processed on a computer. One can envisage in the future a memory bank containing a library of mass spectra being matched against the computerised output of a mass spectrometer to identify, or at least (if no exact match can be found), to place the unknown within a known group of compounds.

Mass spectrometry and other complementary physical techniques such as nuclear magnetic resonance have superseded many aspects of conventional wet chemical analysis. Even if compounds are too involatile to be examined by mass spectrometry, a suitable derivative can be prepared (*e.g.*, acids can be converted to esters and involatile hydroxyl compounds can be acetylated, benzoylated or converted into the trimethylsilyl derivatives). When a specific isomer is to be identified, synthesis of compounds specifically labelled with carbon-13 or deuterium atoms becomes necessary. In the case of very large molecules selective chemical degradation will yield units that can then be identified specifically for ultimate integration to reconstruct the original basic structure.

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Some Observations on Oxidation - Reduction Indicators of the Benzidine, Naphthidine and Diarylamine Types

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A spectrophotometric investigation has been made of representative compounds of the benzidine class of indicators in an attempt to resolve fundamental problems concerning their oxidation mechanism in sulphuric acid media. In all instances the oxidation to the coloured compound is a single-step two-electron process. There is no detectable evidence for the formation of an intermediate, either of a benzidine from arylamines or of a semiquinone from a benzidine or naphthidine. Reduction or spontaneous decay of oxidised arylamines stops at the benzidine stage. All oxidised indicators are unstable both intrinsically and in the presence of excess of oxidant: unsubstituted benzidine and naphthidine are, additionally, photosensitive in the oxidised state. The spontaneous decomposition of the oxidised form regenerates the reduced form and is a disproportionation, probably of 2:1 stoicheiometry, but a kinetic study shows that the rate-controlling step is a unimolecular precursive reaction, which may be the relaxation of the triplet state of the coloured dication diradical oxidised form. Stability decreases on sulphonation and with increasing temperature, and increases with increasing sulphuric acid concentration and with progressive substitution of the amino hydrogen atoms.

For many reasons, such as sparing solubility, instability of oxidised forms, side and induced reactions, low exchange currents, photosensitisation and the need with certain oxidants of providing an inductor, potentiometric measurements on di- and triarylamines and alkyldiarylamines (hereafter collectively termed arylamines), benzidine, naphthidine and their derivatives are attended by severe difficulties. Interpretation of such results as can be obtained is often uncertain and equivocal. In few instances is it known beyond doubt how many electrons are involved in the oxidation of the indicator, and little beyond the fact of its occurrence is known about the decomposition or further oxidation of the oxidised form of the indicator. None of the indicators is sufficiently stable in the oxidised form to permit accurate determination of its formal potential by direct potentiometric titration, and few allow approximate estimation of either formal potential or number of electrons by this method. Instead, recourse must be made to the method of conducting a potentiometric titration of, say, iron(II) with dichromate or cerium(IV) in the presence of a substantial amount of indicator that involves passing through the end-point region with a 10 or 100-fold diluted titrant while recording potentials and visual observations of the indicator colour. Rapid titration to avoid error from decomposition of the indicator produces inaccurate potentials; slow titration to achieve accurate potential measurements permits significant decomposition of the indicator. Consequently it is more usual to report "transition potentials," *i.e.*, the potential at which a discernible colour first appears (all indicators of these classes are colourless in the reduced form), and "transition ranges," *i.e.*, the potential range from the first appearance of colour to its full development. Potentials and ranges are both dependent on experimental conditions, and are valid when the indicator is used under the same conditions. But the ranges do not unequivocally define the number of electrons involved in the indicator reaction, either by the slope of the potentiometric curve and the magnitude of the range, or by the amount of oxidant consumed.

Manual spectrophotometric examination of a decomposing species is obviously difficult, and only when the decomposition is slow can reasonably accurate values of wavelengths of maximum absorption ($\lambda_{max.}$) and of molar absorptivities (ϵ) be obtained. Mechanistic

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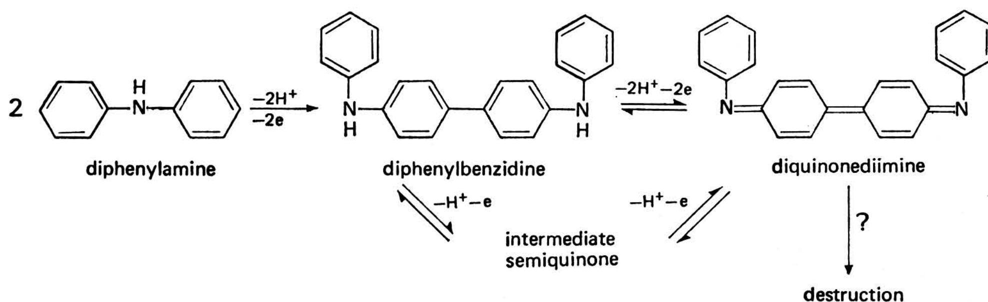
investigations have been few, and are empirical and largely speculative. It is much easier to rationalise the oxidation of benzidines on a basis of a one-electron mechanism than on a basis of a two-electron mechanism. The little unequivocal evidence available supports a two-electron mechanism; the remainder favours a one-electron mechanism. Kolthoff and Sarver[1] postulated that arylamines were first oxidised by an irreversible two-electron bimolecular process to the colourless diarylbenzidine, and that this benzidine was then reversibly oxidised by a two-electron unimolecular reaction to a diquinonediimine. The blue or violet oxidised form is unstable and is destroyed by an irreversible process. Further, frequent reports suggest that an intermediate product occurs in the oxidation of the benzidine, and this is described as a molecular complex of the benzidine and the diquinonediimine. For the unsubstituted parent compound, and ignoring protonation—



This theory has since been tacitly accepted as applying generally to all arylamines and benzidines and, by analogy, to naphthidines. However, the intermediate diphenylbenzidine has not been isolated and characterised, except by reduction of the purple oxidation product, nor have the intermediate semiquinone and the destruction product been examined. Further, the oxidation product formulated as a diquinonediimine could not be expected to have an intense blue colour. It has been reported, but in only one instance, that the original reduced form of the indicator is regenerated during the decomposition reaction,[2] thus suggesting a disproportionation. Many questions, even one so fundamental as how many electrons are involved in the oxidations, remain to be answered, therefore, before the behaviour of these indicators can be rationalised. This brief introduction could be annotated by 200 or more references to the literature, and be extended to include a detailed argument of the possibilities, but this has been done in a recent monograph[3] and need not be repeated here. Earlier work was carried out without the benefit of modern instrumentation, and it was thought that fast scanning spectrophotometry might be used to define the number of electrons involved in the oxidations and to give some information on the formation of intermediates and on the decomposition reactions. As it seemed likely that free radicals would be involved, electron spin resonance spectrometry might also yield some useful information.

EXPERIMENTAL

REAGENTS—

Indicators—These were purified by crystallisation from water or dilute sulphuric acid, or by precipitation from concentrated sulphuric acid by dilution and cooling, under a carbon dioxide atmosphere.[2] They were examined for impurities by appropriate thin-layer chromatographic methods. Commercial diphenylbenzidine was found to contain about 10^{-6} mole per cent. of dichromate, otherwise the compounds appeared to be pure individual authentic species: commercial samples contained traces of oxidised species. Determinate stock solutions, usually 10^{-2} M, were prepared in water or concentrated sulphuric acid and diluted as required.

Sulphuric acid—Aristar or AnalaR grades, free from traces of oxidants, were used concentrated, or after dilution with distilled water. Concentrations were determined by precise density measurements.

Cerium(IV) solutions—A stock 0·1 M solution of cerium(IV) in M sulphuric acid was prepared from AnalaR ammonium hexanitratocerate(IV) by the usual method,[4] and standardised against freshly prepared primary standard 0·05 M arsenic(III) by using osmic acid as the catalyst and tris(1,10-phenanthroline)iron(II) as the indicator. More dilute solutions were prepared by diluting the stock solution with 1·0 M sulphuric acid.

Dichromate solutions—Stock 0·01667 M aqueous solutions were prepared determinately from AnalaR potassium dichromate and diluted with water as required.

INSTRUMENTS—

Spectral scanning was carried out over a pre-set wavelength range on a Unicam SP800B double-beam recording spectrophotometer, with the appropriate solvent in the blank beam. The repetitive scan mode of the instrument was used in preliminary kinetic exploration of decomposition rates, the time of passing the wavelength of maximum absorption being measured by stop-watch for each scan. PTFE-stoppered 10-mm Spectrosil cells were used. A Perkin-Elmer 137 UV double-beam recording spectrophotometer was also used in a similar fashion as a check. Didymium and holmium glass wavelength standards were used for calibration.

Precise absorbance measurements at fixed wavelength were made with a Hilger H700 single-beam spectrophotometer, with blank and sample cells mounted in a constant-temperature housing fed from a thermostat tank maintained to within $\pm 0{\cdot}05$ °C of the required temperature. Molar absorptivities were thus determined and precise values of $\lambda_{max.}$ checked on this instrument.

Electron spin resonance measurements were made by courtesy of the Physics Department on a Decca electron spin resonance spectrometer, operating in the Q band, but were makeshift as proper solution handling components were not available.

METHODS

DETERMINATION OF NUMBER OF ELECTRONS AND OF APPROXIMATE VALUES FOR $\lambda_{max.}$ AND ϵ—

A series of solutions in 100-ml calibrated flasks was prepared, each containing the same amount of diluted indicator solution, so that the absorbance at the wavelength of maximum absorption would be between 1·0 and 1·5 (2×10^{-5} to 10^{-4} M after making up to volume), and the required amount of sulphuric acid to give the desired concentration after making up to volume. To these were then added diluted cerium(IV) or dichromate solution in amounts corresponding to 0, 0·5, 1·0, 1·5, 2·0, 3·0 and 4·0 equivalents with respect to the amount of indicator present. After addition of the oxidant, the solution was made up to volume with water, quickly mixed and immediately scanned through the appropriate wavelength range to include both ultraviolet and visible peaks. The series of spectra was recorded on the same chart. The spectra over the range from 200 to 850 nm were carefully examined for evidence of intermediate or other products formed during the oxidation process and also with excess of oxidant. Approximate values for $\lambda_{max.}$ for reduced and oxidised forms of the indicator were noted for precise checking later, and approximate values of ϵ were calculated from the absorbances at the wavelength of maximum absorption. A graph of absorbance against number of equivalents of oxidant added gave the number of electrons involved.

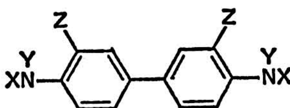
Rate of decay of oxidised form—The rate of decay of the oxidised form was explored by repetitive scanning of the samples at timed intervals. The main examination was carried out on the samples treated with two equivalents of oxidant, but other ratios were also examined. During the decay process the spectra were again examined over the range 200 to 850 nm for evidence of other products. More precise studies of decay were made at constant temperature and at a fixed wavelength of $\lambda_{max.}$ in the manual spectrophotometer. From the results thus obtained, rate plots for orders of reaction from zero to fourth order were made.

Precise measurement of $\lambda_{max.}$ and ϵ values—By using the $\lambda_{max.}$ values from spectral scans as a guide, and taking advantage of the small induction period usually allowed by a purified indicator, the region ± 5 to 10 nm about the approximate $\lambda_{max.}$ value was quickly surveyed on the manual instrument with a solution containing two equivalents of oxidant. The molar absorptivity at the wavelength of maximum absorption was then calculated. A similar check was made on the reduced form of the indicator.

Determination of the amount of reduced form regenerated—A solution containing the stoicheiometric amount of oxidant was allowed to decay completely, after its absorbance at the wavelength of maximum absorption had been determined immediately after mixing. After decay the spectrum was again scanned and showed no more than a slight elevation over the base-line in the visible region. The decayed solution was then divided into aliquots, which were treated one after the other with successively larger increments of oxidant, the required amount of sulphuric acid was added and the solution made up to volume and immediately scanned. From a graph of absorbance against amount of oxidant added, the amount of the latter required fully to oxidise the regenerated reduced indicator was found. This was checked against the maximum absorbance reached which, from Beer's law, also gave the amount of regenerated reduced form. As will be explained, the second method is the more reliable.

Results and discussion

The primary questions concerned the oxidation of benzidines of the form



When X=Y=Z=hydrogen, the parent compound benzidine (4,4′-diamino-1,1′-biphenyl) appears; when X=phenyl and Y=Z=hydrogen, the putative intermediate diphenylbenzidine formed from diphenylamine appears; when X=Y=hydrogen, the compound is a nuclear-substituted benzidine, X or Y, or both, being other than hydrogen give *N*-substituted benzidines. Addition of a fused benzene ring on the side opposite to Z gives the corresponding naphthidines (4,4′-diamino-1,1′-binaphthyl). As many compounds as could be readily obtained were examined, and gave a reasonable cross-section of the whole. These were X=Y=hydrogen and Z=hydrogen, benzidine; Z=methyl, 3,3′-dimethylbenzidine (*o*-tolidine); Z=methoxyl, 3,3′-dimethoxybenzidine (*o*-dianisidine); Z=hydrogen, X=hydrogen and Y=phenyl, diphenylbenzidine; and X=methyl and Y=phenyl, *NN*′-dimethyl-*NN*′-diphenylbenzidinedisulphonic acid. Naphthidine (X=Y=Z=hydrogen) and 3,3′-dimethylnaphthidine (X=Y=hydrogen and Z=methyl) provided examples of the naphthyl analogues. Of the presumptive precursor diarylamines, diphenylamine, diphenylamine-4-sulphonic acid, *N*-methyldiphenylamine-4-sulphonic acid and 2-carboxydiphenylamine (*N*-phenylanthranilic acid) were also examined; the last should give a benzidine with X=hydrogen, Y=phenyl and Z=carboxyl, but the location of the carboxyl groups is indeterminate.

Number of electrons—

In the indicator reaction,

$$\mathrm{Ind_{ox}} + ne \rightleftharpoons \mathrm{Ind_{red}}$$

the number of electrons, n, is in all instances 2. Graphs of absorbance of $\mathrm{Ind_{ox}}$ against equivalents of oxidant added are linear for all compounds examined up to $2 \pm 0{\cdot}02$ equivalents per mole of $\mathrm{Ind_{red}}$, whether this be a benzidine, a naphthidine, an arylamine or an alkylarylamine: the absorbance remains constant when oxidant in excess of the two equivalents is added.

Spectra—

Immediately after oxidation—All spectra show a cut-off in the far ultraviolet region because of high sulphate concentrations and aromatic ring currents. Usually $\mathrm{Ind_{red}}$ shows a peak in the mid or near ultraviolet region; the wavelength is longer for the benzidine than for the arylamine, exceptions being for Z=alkoxyl, when a double peak appears, and the naphthidines, when there is a double hump or a complex spectrum: none shows any other absorption above the vibrational frequencies. The $\mathrm{Ind_{ox}}$ spectrum shows a single peak in the visible region, except for Z=alkoxyl, when the peak is again split, and naphthidine, which shows an additional weaker higher frequency absorption. Starting with pure $\mathrm{Ind_{red}}$ and scanning

solutions containing progressively larger increments of oxidant, the ultraviolet absorbance of Ind_{red} decreases proportionately, usually to zero, and the visible absorbance of Ind_{ox} appears and increases proportionately until two equivalents of oxidant have been added; thereafter there is no further change. At the same time the absorbance of solvated cerium(III) appears in the ultraviolet (λ_{max} = 252 mm, ϵ = 905 l mol^{-1} cm^{-1}) and increases proportionately, reaching a maximum when two equivalents of cerium(IV) have been added. The changes in these absorbances are precisely linear with the number of equivalents of oxidant added, terminating at 2 ± 0·02 equivalents. These peaks show no further change when more than two equivalents of cerium(IV) are added, but the absorbance of the latter ($\lambda_{max.}$ = 317 nm, ϵ = 5 900 l mol^{-1} cm^{-1}) appears and increases in proportion; there is no evidence whatever of a further oxidation product of the indicator. The series of Ind_{red} — Ind_{ox} spectra shows a characteristic isosbestic point pattern (Fig. 1). The shape of the Ind_{ox} spectrum is the same

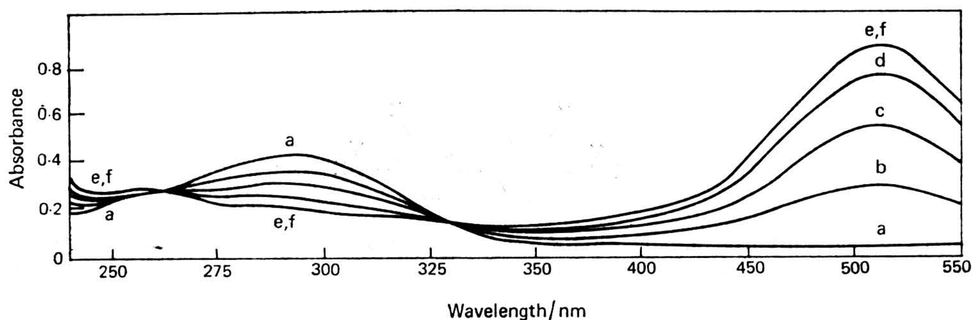


Fig. 1. Illustration of the isosbestic pattern on oxidation. Solutions 4 × 10^{-5} M in *N*-methyldiphenylamine-4-sulphonic acid and 2·0 M in sulphuric acid treated with a, 0; b, 0·60; c, 1·21; d, 1·72; e, 2·0; and f, 2·2 equivalents of cerium(IV): e and f are coincident

as that of Ind_{red}, unless the latter is an arylamine, with merely a bathochromic shift and an increase in molar absorptivity, which are nearly constant for a given type of compound. This indicates that there is no basic change in structure but merely a decrease in mean energy of the electronic transitions. Spectral measurements are collected in Table I.

TABLE I

SPECTRAL CHARACTERISTICS OF SOME BENZIDINE AND NAPHTHIDINE DERIVATIVES AND SOME ARYLAMINES IN 2·0 M SULPHURIC ACID AT 25 °C

	Ind_{red}		Ind_{ox}			
Compound	λ_{max}/nm	ϵ/l mol^{-1} cm^{-1}	λ_{max}/nm	ϵ/l mol^{-1} cm^{-1}	Spectral shift/kcal mol^{-1}	$\epsilon_{ox}/\epsilon_{red}$
Biphenyl	252	20000 (in ethanol)				
Benzidine	248	20300	426	69500	50·8	3·3
3,3′-Dimethyl-	248	18000	438	—	49	
benzidine	250*	18000*	435*	65000	48·6	3·6
3,3′-Dimethoxy-	252	12800	454	31000	49	2·4
benzidine	287	8100	510	23000	41·5	2·8
NN′-Diphenylbenzidine	253†	31200†	560	50000	61·9	1·6
Diphenylamine	220‡	Cut-off	565	45000	—	—
N-Phenyl-anthranilic acid	256‡	—	524	30000	—	—
N-Methyldiphenyl-amine-4-sulphonic acid	320§	24000§	511	44000	62	1·7
	294‡	14000‡				
Naphthidine	282	—	400	7000	30	
	294	11000	527	18000	43	1·6
3,3′-Dimethyl-naphthidine	Complex spectrum		543	35000	—	—

* In 10^{-3} M sulphuric acid.
† In concentrated sulphuric acid.
‡ Arylamine form.
§ In benzidine form; oxidised with two equivalents of oxidant and immediately reduced with zinc dust.

When Ind_{red} is an arylamine, a slight change in shape occurs for the Ind_{ox} spectrum, but when Ind_{ox} is reduced to the presumptive benzidine and the new Ind_{red} spectrum compared with that of the arylamine, the change is seen to be minor. Arylamine oxidation occurs in a single step: no evidence exists for the intermediate formation of the benzidine. Lest this be due to use of a very strong oxidant [cerium(IV)], dichromate, vanadate, iron(III) and other oxidants were tested: either no oxidation occurred, or the single-step two-electron oxidation took place. If the arylamine is oxidised with two equivalents of oxidant and then immediately reduced with zinc dust, a scan shows that the ultraviolet peak is shifted to a longer wavelength, thus indicating the benzidine form. Subjecting this solution to the oxidation and scanning process shows that the oxidised form is identical in spectral characteristics with the oxidation product of the original arylamine, but the maximum absorbance is now reached at one equivalent of oxidant per mole of original arylamine, which corresponds to two equivalents per mole of the presumptive benzidine produced by the zinc dust reduction. The example shown in Fig. 1 is an alkylarylamine; reduction with zinc dust and repetition of the process gives a similar set of spectra with the ultraviolet peak shifted to 320 nm and a corresponding shift in the isosbestic point.

As the sulphuric acid concentration of the medium is increased, a considerable bathochromic shift occurs in $\lambda_{max.}$, which is caused by the solvent effect, as shown in Table II. Belcher has observed this shift with naphthidine derivatives.[5] In sulphuric acid media there is no detectable evidence in any of the spectra for the formation of an intermediate, even transiently, either of the semiquinone type from a benzidine, or of a benzidine from an arylamine. In acetic acid some evidence exists that a different reaction may occur, and that this is specific to the presence of acetic acid or acetate ion. (Kolthoff's solutions contained acetic acid.[1]) For example, a double-humped peak in the region 300 to 420 nm was observed in the oxidation of diphenylbenzidine in a mixture of concentrated sulphuric acid, glacial acetic acid and water (2 + 78 + 20 v/v after correction for the addition of aqueous acid cerate); peculiarities have also been noted with *o*-dianisidine in acetate media.[2] This, however, opens up a large field of subsidiary study, which will not be pursued at present.

On decomposition—On standing, all oxidised solutions, whether containing a deficiency, the stoicheiometric amount or an excess of oxidant, faded more or less rapidly, eventually becoming colourless and occasionally depositing a precipitate. In scanning the spectrum at intervals during this decay, as the absorbance of Ind_{ox} decreased, the absorbance of Ind_{red} appeared and increased with time, again giving an isosbestic pattern as illustrated in Fig. 2. The absorbance of Ind_{ox} fell virtually to zero, but the absorbance of Ind_{red} increased to only about one half of its original value. Apart from *N*-phenylanthranilic acid, no other peaks appeared and there was no evidence for the formation of a semiquinone or any other compound, except that the base-line of the whole spectrum rose by a small but detectable and fairly uniform amount, such as may be expected from the formation of an insoluble but disperse phase. There was no change in shape or in the λ_{max} values of either Ind_{ox} or Ind_{red} peaks during the decay process, except that an arylamine decayed to the benzidine giving the same Ind_{red} spectrum as that after zinc dust reduction of the freshly oxidised arylamine. Even in solutions containing one equivalent of oxidant, which might be expected to encourage

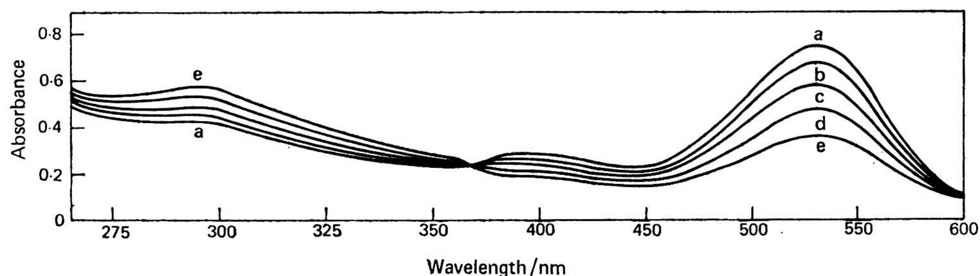


Fig. 2. Illustration of the isosbestic pattern on spontaneous decomposition. A solution 4×10^{-5} M in naphthidine and 2·0 M in sulphuric acid treated with 2·0 equivalents of cerium(IV) and allowed to decay. Interval between scans a to e, about 12 minutes

formation of the semiquinone, no spectral shift was observed, although decay was accelerated. Evidence for autocatalysis was found in many instances. The cerium(III) and cerium(IV) peaks, when present, complicate interpretation of the spectra, but their resolution is possible from the law of additive absorbances.

Unsubstituted benzidine and naphthidine oxidised forms decay rapidly in daylight, but in darkness, or in the cell housing of the spectrophotometer, the decay is drastically retarded, thus indicating that the process is photosensitive. Belcher, Lyle and Stephen[5] have reported that 3,3'-dimethoxynaphthidine is photosensitive at the wavelength of maximum absorption and they were therefore unable to determine the $\lambda_{max.}$ or ϵ value for this compound. The curious behaviour of *N*-phenylanthranilic acid is noteworthy. It is directly oxidised, without any intermediate benzidine formation, to the bluish red Ind_{ox}. The Ind_{ox} band at 524 nm immediately begins to decay and a new band at 436 nm grows and the colour changes to green. This decay is rapid with a half-life of 10 minutes, and the set of spectra scanned at 3-minute intervals shows a good isosbestic point, smeared only slightly by the over-all slower decay of both species.

Re-oxidation of the decayed solutions gave an identical Ind_{ox} peak and the same Ind_{red} - Ind_{ox} isosbestic behaviour. When the decay of a solution treated with excess of oxidant was followed by the scanning method, the decay was slower to begin with because of re-oxidation of the regenerated Ind_{red} by the excess of oxidant, but the cerium(IV) absorbance decayed more quickly than could be accounted for by this reaction: the cerium(IV) absorbance is therefore useless in assessing the extent of reaction.

ELECTRON SPIN RESONANCE SPECTRA—

Without the proper components for work with solutions the electron spin resonance spectra have only a qualitative value. Solutions of diphenylbenzidine with a range of sulphuric acid concentrations and amounts of oxidant were examined. Cerium(III) and cerium(IV) gave no signals at the operating frequencies. A half-oxidised solution showed a signal that grew gradually to a maximum, and showed a Landé splitting g factor of almost exactly 2, indicative of a free radical with a single unpaired electron, and then decayed at a rate similar to the rate of decay of absorbance at 560 nm previously observed spectrophotometrically. The band was unusually broad, probably on account of solvent effects, and may arise from an averaging effect, so that it cannot be regarded as evidence of a semiquinone, particularly in view of the absence of optical evidence. As the amount of oxidant was increased, finally to two equivalents, this band disappeared and was replaced by a weak complex signal that can be related to, but does not with any certainty identify, a diradical with two separated electrons of parallel spin.

KINETICS OF DECOMPOSITION—

The spectral scanning method gave a clue to the pattern of decomposition, there being some indication of a small induction period with pure indicators and strong evidence of autocatalysis. Precise kinetic measurements were made by following the change in absorbance at the wavelength of maximum absorption of Ind_{ox} with time, at controlled temperature. The decay rate was found to increase with rising temperature, to decrease with increasing sulphuric acid concentration (as shown in Table II), and to depend on the nature of the substituents X, Y and Z. The stabilising effect of high sulphuric acid concentrations is implicit in some earlier work,[3] and may be caused by the decrease in water concentration, or by increased protonation of the various basic species. The decay of the oxidation products of the unsubstituted benzidine and naphthidine is greatly accelerated by exposure to daylight as has been noted. Sulphonation of the indicator de-stabilises the oxidised form, as has several times been observed before,[3,5] and could well result from a shift in the charge density

TABLE II

SOLVENT SHIFT AND EFFECT OF SULPHURIC ACID CONCENTRATION ON DECAY OF OXIDISED DIPHENYLBENZIDINE AT 25 °C

Sulphuric acid, per cent. . .	18	22	36	63
$\lambda_{max.}$ Ind_{ox}/nm	560	565	570	585
Half-life/minutes	188	200	445	1300

distribution. Partially oxidised solutions decay faster than stoicheiometrically oxidised solutions, and addition of Ind_{red} to a decaying solution accelerates the process markedly, as with diphenylbenzidine, giving support to the hypothesis that the autocatalysis is caused by the growth of regenerated Ind_{red} in the solution.

The kinetics of the decay process do not fit any reaction order, but come closest to first order; indeed, some show an almost perfect first-order plot. It is possible to fit the decay process for diphenylbenzidine to an equation of the form—

$$-\frac{d\,[Ind_{ox}]}{dt} = k_1\,[Ind_{ox}] + k_2\,[product]$$

in 2 M sulphuric acid ($k_1 = 2{\cdot}3 \times 10^{-3}$ minute^{-1} and $k_2 = 8{\cdot}2 \times 10^{-3}$ minute^{-1}). Rather than present artificially fitted rate constants, the speed of the decay process is represented by the half-life in Table III under the conditions specified. As Ind_{red} in the benzidine form

TABLE III

HALF-LIVES OF IND_{ox} IN THE ABSENCE OF EXCESS OF OXIDANT, AND IN THE INITIAL ABSENCE OF IND_{red}, IN 2 M SULPHURIC ACID AT 26 °C; AND THE RECOVERY OF IND_{red} AFTER COMPLETE DECAY

Compound	Half-life/minutes	Ind_{red} regenerated, per cent.
Benzidine	500*	80†
3,3′-Dimethylbenzidine	264	50
	200‡	46‡
3,3′-Dimethoxybenzidine	190	60
NN′-Diphenylbenzidine	188	50, 51, 46
Diphenylamine	96	—
N-Phenylanthranilic acid	6 to 10§	100§
N-Methyldiphenylamine-4-sulphonic acid‖	170	55
Naphthidine	23¶	40
3,3′-Dimethylnaphthidine	80	35, 40

* Measured in darkness. Decay accelerated in daylight to about 50 minutes.
† Value very high.
‡ In 10^{-3} M sulphuric acid.
§ Initial fast decay to green form.
‖ Presumptive benzidine derivative: Ind_{red} oxidised with two equivalents of oxidant, immediately reduced with zinc dust, then re-oxidised.
¶ At 30 °C. Decay much faster in daylight, or under continuous illumination at wavelength of maximum absorption (about 2·5 minutes).

is regenerated in the decay process, the latter must be of the nature of a disproportionation with a minimum molecularity of two, and therefore the rate-controlling step must be a preceding unimolecular reaction that could be accelerated by energy exchange with Ind_{red}. Substituents in the Z position appear to protect the amino group, as Belcher, Lyle and Stephen suggest,[5] but this is perhaps more a matter of preventing the photochemical reaction, with the exception noted,[5] than of hindering further oxidation. It is notable that the half-life of oxidised diphenylamine is about half that of diphenylbenzidine. Substituents in the X or Y position stabilise the oxidised form, and maximum stability is reached when both X and Y are substituted. The instance of *NNN*′*N*′-tetramethyl-3,3′-dimethylbenzidine (tetramethyl-*o*-tolidine) is strongly relevant.[6]

RECOVERY OF IND_{red}—

Determination of the amount of Ind_{red} regenerated would define the stoicheiometry of the decomposition reaction. Re-oxidation of the regenerated Ind_{red} gives precisely the same spectrum and decay pattern as the original indicator. Measurement of the absorbance at the appropriate wavelength of the regenerated Ind_{red} and calculation from Beer's law will give the amount of Ind_{red}, provided proper correction for other absorbances such as cerium(III) and decomposition products is made. Measurement of the absorbance of Ind_{ox} for a series of solutions to which successively larger amounts of cerium(IV) have been added and a plot of absorbance against equivalents of oxidant do not give reliable results, because cerium(IV) is consumed in other reactions, as noted before. However, addition of a slight excess of

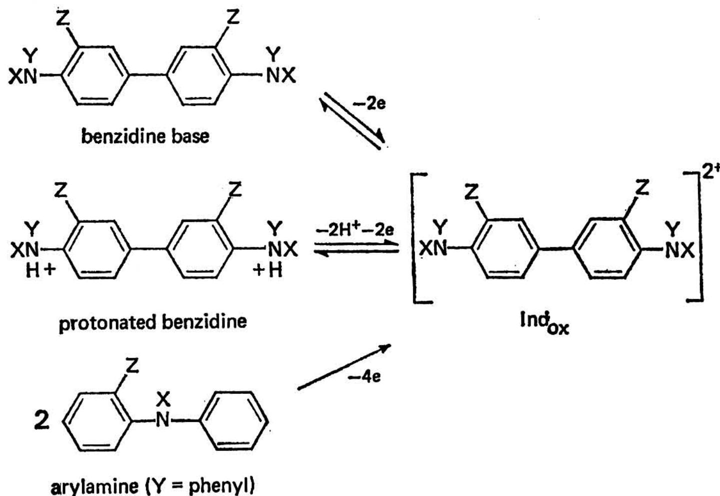
oxidant [checked by the appearance of a small cerium(IV) peak], measurement of the absorbance of Ind_{ox} and application of Beer's law give reasonably reliable results, provided the measurement is made quickly enough to avoid significant decay and yet allow sufficient time for the full development of colour. This latter time is no more than a few seconds except for benzidine, which takes up to 30 minutes. It is not possible to check the stoicheiometry by adding the integrated number of equivalents of cerium(IV) (*e.g.*, four for a 2:1 disproportionation) so that there is no residual Ind_{red} left after decay. Ind_{red} is regenerated even in the presence of a large excess of oxidant, and this phenomenon persists until about twenty-two equivalents of oxidant have been added, so clearly oxidant is attacking a decomposition product by ring fission faster than Ind_{red} is regenerated. If the disproportionation is formulated as

$$m\ Ind_{ox} \rightarrow D + n\ Ind_{red}$$

where D is the primary decomposition (disproportionation) product, then excess of oxidant is consumed by three processes: (*a*) re-oxidation of regenerated Ind_{red} to Ind_{ox}, (*b*) direct oxidation of Ind_{ox} to D, and (*c*) attack of D to give fission products, and of these reaction (*c*) is the major process. Results are included in Table III and, on balance, favour a 2:1 disproportionation (50 per cent. regeneration), although other stoicheiometries such as 5:2 (40 per cent. regeneration) are not excluded, and there are notable exceptions.

Conclusions

In sulphuric acid media all indicators are oxidised directly to Ind_{ox} by a single two-electron step. Reduction of oxidised arylamine stops at the benzidine stage. Oxidation of arylamines does not proceed by a benzidine transformation,[3] and is not, therefore, an intramolecular reaction but an intermolecular reaction. Starting from an arylamine there are five possible products depending on which carbon atoms provide the bridge, and if the arylamine is substituted in the nucleus the possible products are multiplied in number according to the rings in which the substituents finally appear: there are fifteen possible products, for instance, for *N*-phenylanthranilic acid, although it is probable that a single product will preponderate in a given instance. Presupposing that the product is a *p,p'*-benzidine and the substituent Z appears in the benzidine nucleus, the main reaction can be formulated as—



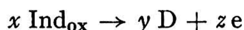
where Ind_{ox} is a triplet diradical dication. It should be emphasised that benzidine has not been isolated except after zinc dust reduction of Ind_{ox} for the case when X = Z = hydrogen and Y = phenyl. The identity in shape of the benzidine and Ind_{ox} spectra and the isosbestic pattern suggest that no gross change occurs on oxidation, and the bathochromic shift indicates

an elevation of the mean ground-state energy of the molecular orbitals, so that electronic transitions require a lower energy; this is supported by the increase in molar absorptivity.

Except for the curious instance of *N*-phenylanthranilic acid, Ind_{red} is invariably regenerated on decomposition (as the benzidine in the case of arylamines), together with a weakly coloured sparingly soluble decomposition product, D. Regeneration of Ind_{red} is clearly the reverse of the main indicator reaction. Neglecting the possible participation of hydrogen ion, this is—

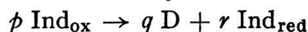
$$\text{Ind}_{\text{ox}} + 2\text{e} \rightleftharpoons \text{Ind}_{\text{red}}$$

The necessary electrons must come from somewhere, and as Ind_{ox} will itself consume oxidant, it is the obvious source—



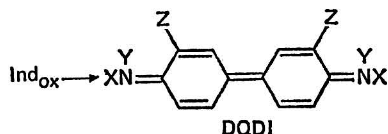
$$x\,\text{Ind}_{\text{ox}} \rightarrow y\,\text{D} + z\,\text{e}$$

The spontaneous decomposition of Ind_{ox} is therefore a disproportionation, several examples of which are known in this field of chemistry.[3]



$$p\,\text{Ind}_{\text{ox}} \rightarrow q\,\text{D} + r\,\text{Ind}_{\text{red}}$$

However, the minimum value of p for a disproportionation would be 2, and the decay does not show second-order kinetics. A monomolecular rate-controlling step must therefore precede the disproportionation, and it is not unlikely that this should be the relaxation of the triplet state, catalysed by energy exchange with Ind_{red}, to the



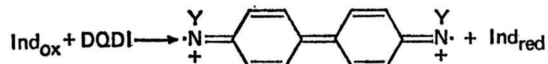
diquinonediimine; it could also be the deprotonation of DQDI to give the free base. The final reaction could then be formulated as

$$a\,\text{Ind}_{\text{ox}} + b\,\text{DQDI} \rightarrow \text{D} + c\,\text{Ind}_{\text{red}}$$

for which the over-all stoicheiometry would be $(a + b)/c$. The product D has been neither isolated nor examined, and even if it had it would be difficult to be sure that it had not been changed in the process. Discussion of the nature of D is necessarily speculative, but there is obviously a connection between the substituents X, Y and Z and the stability of Ind_{ox}. X and Y are those which matter in the decay process, and it is worth recalling that stability increases with increasing sulphuric acid concentration, so that protonation may be influential. Moreover, biphenyl is itself unreactive so that the activity must reside in the nitrogen atoms.

When neither X nor Y is a hydrogen atom, then the oxidation product, whether formulated as the diradical dication or the diquinonediimine, has no lone pairs available for protonation, and it is not possible to formulate a further oxidation stage without breaking bonds or ejecting substituents. That the tetra-*N*-substituted derivatives are stable in the oxidised form is well known,[3,6] but a quantitative definition of stability is lacking. That the oxidised form of *N*-methyldiphenylamine-4-sulphonic acid does decompose and regenerate a benzidine may be the result of the de-stabilising effect of the sulphonic acid group but is still difficult to understand.

When either X or Y is a hydrogen atom, then protonation is possible and the diquinonediimine does offer electrons accessible to oxidation. A 2:1 disproportionation can be formulated as—



where the primary decomposition product, D, is the diradical dication of the DQDI. This unlikely looking species would be very reactive and may polymerise by nuclear attack. A 3:1 disproportionation is easier to formulate but is contrary to the 50 per cent. recovery of Ind_{red}.

When X=Y=hydrogen, the 2:1 disproportionation would again require formation of the diradical dication DQDI, but with hydrogen in place of Y, which opens up further possibilities. A 3:1 disproportionation would allow the formation of a hydrazine that is capable of being oxidised to an azo compound, which could isomerise to a dimeric diquinonediimine or could be oxidised to an azoxy compound. The formation of a linear trimeric bis-hydrazine would give a 5:2 stoicheiometry and a 40 per cent. recovery of Ind_{red}. There is also the possibility of a phenazine type of polymerisation.

It is not profitable to enumerate further all the possibilities because the matter remains speculative until accurate information is available on the nature of D. However, the stability of Ind_{ox} increases as Z, Y and X are successively substituted and as the sulphuric acid concentration increases, and the recovery of Ind_{red} suggests that the oxidation of Ind_{ox} to D is a two-electron process.

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The Determination of Carbon in Steel by Coulometric Titration in Partially Aqueous Medium

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A method that involves coulometric titration of carbon dioxide absorbed in a partially aqueous medium is described for the precise determination of carbon in steel. It does not depend on empirical standardisation and the use of high-vacuum systems and inflammable titrants is avoided. The apparatus used is inexpensive and the technique is simple. The application of appropriate instrumentation could provide a routine control method of high precision.

The analytical performance of the method compares well with that of other techniques considered suitable for reference analysis; 95 per cent. confidence limits of ±0·0007 per cent. of carbon at the 0·05 per cent. of carbon level of 0·008 per cent. of carbon at the 0·9 per cent. of carbon level were obtained in this laboratory.

RECENT trends in methods for determining carbon in metals, particularly in steel, have been towards rapid routine techniques, which have also provided an increase in precision at low levels. Many physical techniques have been successfully used over the past 10 years and commercial equipment based on the following principles are available: infrared gas analysis,[1,2] thermal conductivity and electrical conductivity. Invariably, the capital cost of the equipment has been relatively high. Rapidity coupled with good accuracy, however, was achieved at relatively low cost by Jones, Gale, Hopkins and Powell[3] in their non-aqueous titration method, and although not as robust as those with the physical instruments it has found wide application in works' laboratories.

Most of the recently developed methods depend on empirical standardisation and are unsuitable for reference purposes. The gravimetric methods, which have been largely replaced, were unreliable at low carbon levels, although Bagshawe and Pinder[4] improved the sensitivity of the method by using large sample weights. For many years the low-pressure volumetric method used by Wells,[5] and later modified by Cook and Speight,[6] was generally accepted as a reference method as it was based on fundamental gas laws. It has lost favour because of the fragile nature of the equipment, the skill required in its operation and the need for a supply of liquid oxygen. Dunnill and Kent[7] recently proposed a modification of the method that overcomes some of these objections.

The need arose in this laboratory to replace the low-pressure volumetric method by a simpler technique suitable for reference analysis. In 1959, Abresch and Lemm[8] proposed a coulometric technique for the determination of oxygen in steel. The application of Faraday's laws and the measurement of quantities such as current and time, which can be made with high accuracy, were the attractive features. An investigation was made into the possibility of determining carbon in steel by coulometric titration. Conditions similar to those of Abresch and Lemm were used with aqueous barium perchlorate solution as the absorbent for carbon dioxide in an oxygen stream. Promising results were obtained, but difficulties experienced in achieving efficient absorption of carbon dioxide caused the method to be unreliable. However, commercial instruments that can be successfully used for coulometric titration in aqueous media have since been marketed in Germany.

Jones and his co-workers[3] and Braid, Hunter, Massie, Nicholson and Pearce[9] had demonstrated the high efficiency of dimethylformamide containing monoethanolamine in absorbing carbon dioxide. Consequently a study was made of the coulometric titration of carbon dioxide absorbed in non-aqueous and partially aqueous solvents in which various electrolytes were used. During this investigation Whymark and Ottaway[10] reported their work on coulometric titration in semi-aqueous media; they found that the presence of 20 to 30 per

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cent. of water was necessary for 100 per cent. current efficiency in pyridine and dimethylformamide, but no other electrolyte was used.

The titration of acids, including carbon dioxide, by generating a base in non-aqueous solution, has been described by several authors.[11,12,13,14,15] End-points were determined by either potentiometric or visual methods. Previous experience indicated that serious difficulties could arise because of the disturbing influence of the generating current on the indicating electrodes. It was therefore decided to use a photometric end-point detection method.[16]

The primary criteria for a successful procedure were recognised as (*i*) complete current efficiency and (*ii*) complete absorption of carbon dioxide. These considerations form the basis of the investigation.

It is emphasised that this paper describes only the basic conditions that have been found suitable for the determination of carbon dioxide evolved by ignition of steel samples in oxygen. The apparatus and technique could well be developed further to provide automatic operation.

EXPERIMENTAL

APPARATUS AND CONDITIONS—

The apparatus used is shown diagrammatically in Figs. 1 to 3.

The cell for the initial experiments consisted of a 150-ml squat beaker containing a strip of 0·6-cm wide platinum foil formed into a circle lying around the inside wall at the base of the beaker; this strip acted as cathode. A Perspex lid carried a glass cylinder with sealed-in sinter disc at its lower end containing the anolyte and a graphite rod anode. A gas delivery tube also passed through the lid; its tip was diverted off-centre of the beaker section. The current source (see circuit diagram, Fig. 3) provided a 20-mA constant current, which was measured with a milliammeter. End-point detection was provided by an Evans Electroselenium Limited (EEL) Quantitrator with Ilford filter No. 607, the cell being positioned on the magnetic stirrer stand of the titrator.

Later, an all-glass cell with a side-arm for the anode chamber was constructed (Fig. 1), and a silver rod was substituted for the graphite anode. The lid was made of glass and fitted to the cell body by a ground-glass joint.

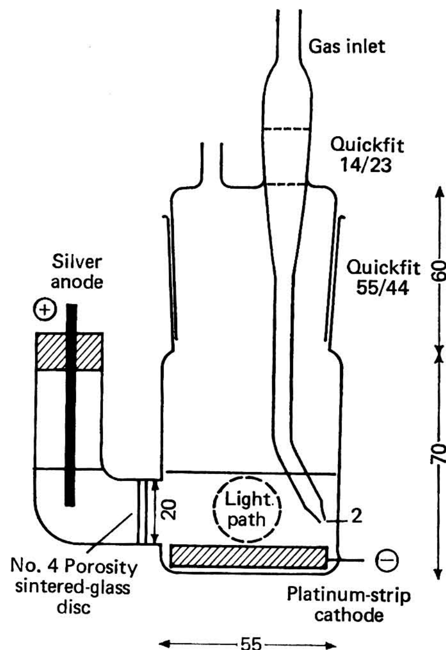
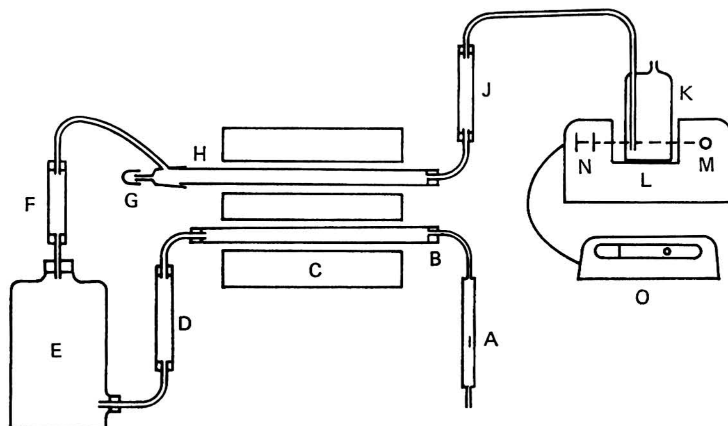


Fig. 1. Absorption cell (all measurements are in millimetres)



A = Flow meter
B = Purification tube
C = Resistance furnace
D = Soda asbestos tube
E = 10-Litre reservoir
F = Soda asbestos and magnesium perchlorate tube
G = Atmosphere trap
H = Combustion tube
J = Manganese dioxide and magnesium perchlorate tube
K = Absorption cell
L = EEL Quantitrator
M = Lamp
N = Filter and photocell
O = Spot galvanometer

Fig. 2. Schematic diagram of apparatus

TITRATION PROCEDURE—

With the photometric end-point detector switched on (lamp, galvanometer and stirrer), the absorbing solution was titrated to an arbitrary deflection (within the blue region) by passing a current of 20 mA. Acid or carbon dioxide was then introduced into the cell as described later and, after complete absorption, the current was switched on simultaneously with starting a stop-watch. The milliammeter reading was immediately adjusted to 20 mA and the titration was continued until the galvanometer spot returned to the starting point. Current and stop-watch were stopped exactly at this point.

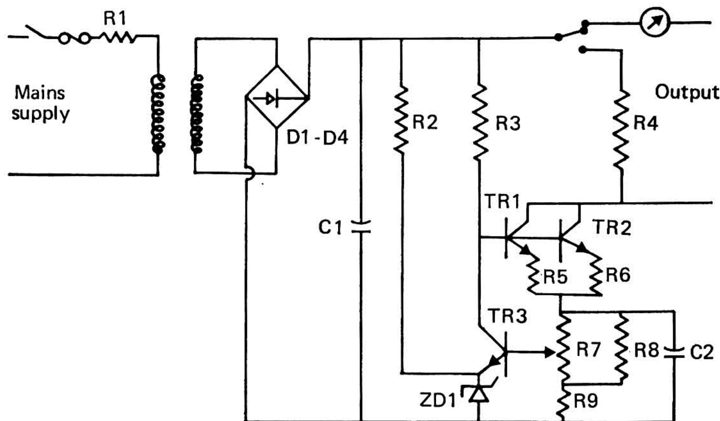


Fig. 3. Circuit diagram of current source (for values of components see Appendix)

ABSORBENT AND ELECTROLYTE—

In preliminary tests the solvent mixture and indicator described by Jones *et al.*[3] were used. The composition of the mixture was as follows: dimethylformamide, 150 ml; monoethanolamine, 5 ml; and thymolphthalein (0·1 per cent. solution) 2 ml (for this work the thymolphthalein was dissolved in dimethylformamide instead of methanol).

Various salts were chosen as possible electrolytes and were dissolved in the solution. The current efficiency, $\frac{\text{calculated titration time}}{\text{observed titration time}} \times 100$, of each electrolyte was measured by titration of known amounts of pure benzoic acid. The most suitable electrolyte was shown by these experiments to be potassium iodide (Table I). More reproducible efficiencies were obtained by replacing the graphite anode by a silver anode, which prevented diffusion of iodine from the anode compartment into the bulk of the solution.

TABLE I

CURRENT EFFICIENCIES OF VARIOUS SALTS DISSOLVED IN A DIMETHYLFORMAMIDE - MONOETHANOLAMINE MIXTURE

Electrolyte	Weight in 75 ml of solvent/g	Efficiency, per cent.	Remarks
LiCl	0·4	—	Deposit formed on cathode
$NaClO_4$	2·0	88 to 94	Range for about twenty results
NaI	2	95 and 97	—
KI	3	94 to 100·5	Range for about twenty results
RbI	0·5	81	—
CsI	0·5	83	—
$MgClO_4$	0·5	—	Apparently no base generated
$CaCl_2$	1	—	Very slow colour change
$BaClO_4$	1	82	Insoluble product formed on cathode
$(C_2H_5)_4NBr$	0·5	80	—
$(C_2H_5)_4NI$	0·5	68	—
NH_4Br..	1	—	Apparently no base generated

It was found that the non-aqueous solution of potassium iodide in dimethylformamide - monoethanolamine mixture formed an insoluble product on titration of carbon dioxide, which restricted the useful life of the absorbent. It was thought that the addition of water would reduce the insolubility and about 4 per cent. of water was added (this amount gave the greatest increase in the solubility of the product compatible with the least loss of end-point definition). The composition of the absorbing solution was therefore changed to the following: dimethylformamide, 78 ml; 0·1 per cent. solution of thymolphthalein in dimethylformamide, 2 ml; water, 3 ml; potassium iodide, 3 g; and monoethanolamine, 3 ml.

TITRATION EFFICIENCY—

A 0·4 per cent. w/v solution of benzoic acid in dimethylformamide (2·0 ml) was added to the titration cell (fitted with a silver anode) by using a burette with a PTFE stopcock. Titration was carried out at currents of 10 and 20 mA, the flow-rate of oxygen being 200 ml minute^{-1}. A blank was performed with 2·0 ml of dimethylformamide. Weighed fused pieces of benzoic acid were also dissolved and titrated. The results obtained are shown in Table II.

TABLE II

TITRATION OF BENZOIC ACID

Benzoic acid taken	Current efficiency, per cent.	
	10 mA	20 mA
8 mg in solution	101·0, 100·3 101·5	101·2, 100·3 99·5, 100·6
20 mg as fused solid.. ..	—	100·8, 100·9

Measured volumes of carbon dioxide were passed into the absorbing solution by using a gas pipette, oxygen being the carrier gas. The solutions were titrated and results given in Table III show the recovery obtained.

TABLE III

TITRATION OF CARBON DIOXIDE

Volume of carbon dioxide/ml	Recovery, per cent.	
	10 mA	20 mA
1·756	99·5, 100·1	99·7
	101·0, 100·3	101·4, 98·9

Later, confirmation of efficient absorption was obtained: two cells were connected in series and the carbon dioxide collected in the second cell was titrated. Carbon dioxide was provided by the ignition of steel samples, by using the combustion train described in the following section.

TABLE IV

ABSORPTION EFFICIENCY

Steel sample	Weight/g	Titration times/s		Percentage of carbon dioxide not absorbed by 1st cell
		1st cell	2nd cell	
		Current 20 mA	Times for 1-mA current divided by 20 for comparison with 1st cell	
B.C.S. 218/3	0·37	254	0·07	0·03
B.C.S. 218/3	0·75	514	0·21	0·04
B.C.S. 220/1	0·075	278	0·00	0·00
B.C.S. 220/1	0·156	576	0·19	0·03
B.C.S. 220/1	0·301	1110	1·18	0·11
B.C.S. 220/1	0·592	2190	3·07	0·14

Results in Table IV indicate no significant loss of carbon dioxide from the first absorbent.

APPLICATION TO STEEL SAMPLES—

Equipment for application of the technique to steel analysis was set up, which consisted of a conventional combustion train with resistance heating (1200° C) coupled to the coulometric cell. The oxygen supply was purified by passage through a heated refractory tube and soda asbestos. Sulphur gases were removed from the stream after combustion by using a manganese dioxide column.

Selected British Chemical Standard samples were analysed by the procedure (see Method). Tin was added to all samples as a combustion initiator and blank determinations with boats containing tin alone were carried out for periods of time similar to those for the samples.

The effect of oxygen flow-rates of 100 to 700 ml minute^{-1} was examined. Results given in Table V show that the optimum rate is 200 to 400 ml minute^{-1}.

TABLE V

INFLUENCE OF FLOW-RATE OF OXYGEN ON THE DETERMINATION OF CARBON IN B.C.S. 218/3 (CERTIFICATE VALUE 0·17 PER CENT. OF CARBON)

Oxygen flow-rate/ml minute^{-1}	100	200	300	400	500	600	700
Carbon, per cent. . .	0·1648	0·1713	0·1702	0·1717	0·1694	0·1705	0·1693
		0·1714	0·1716	0·1701	0·1705	0·1699	0·1691

A total determination time of 8 to 9 minutes was considered acceptable, which was made up of 2 minutes for purging, 2 minutes for burn-off and 4 to 5 minutes for the titration. Sample weights were adjusted according to the carbon content to enable titration to be completed in the time.

The capacity of the absorbent (before precipitation occurred) was found to be about 5 mg of carbon. Fresh electrolyte solution was required after this amount had been absorbed.

A titration current of 20 mA was normally used but 10 mA was more convenient for low carbon levels.

METHOD

REAGENTS—

Indicator solution—Dissolve 0·1 g of thymolphthalein in 100 ml of dimethylformamide.

Absorption solution—To 780 ml of dimethylformamide in a 1-litre bottle add 20 ml of the indicator solution, a solution of 30 g of analytical-reagent grade potassium iodide in 30 ml of water and finally 30 ml of ethanolamine. Mix the solution and transfer 80 to 90 ml to the absorption cell.

APPARATUS—

Oxygen cylinder.

Flow meter—Capable of measuring flow-rates of oxygen up to 800 ml minute^{-1}.

Tube furnace—Capable of operating at 1200° C (or 1300° C if alloy steels are to be analysed). It is convenient to use a two-tube furnace so that the purification tube (used to oxidise carbon monoxide and other carbon compounds) and the combustion tube can be heated in one furnace. These tubes are made of aluminous porcelain and are 76 cm long and 2·2 cm i.d.

An atmosphere trap (through which the samples are introduced) is fitted at the end of the combustion tube, which consists of a modified 29/32 cone and socket. The cone is joined to the combustion tube with rubber tubing. The tubing attached to the socket is drawn out into a narrow tube that can be closed with a rubber cap. Sealed to the side of the socket is a side-arm through which oxygen is passed. By using this trap the sample can be pushed into the furnace while an out-flow of oxygen excludes the atmosphere.

Absorption tower filled with soda asbestos.

A 10-litre aspirator bottle—This acts as a reservoir of gas to prevent suck-back of the absorption solution.

Absorption tower filled with a layer of soda asbestos and a layer of magnesium perchlorate.

Absorption tube—This contains a layer of manganese dioxide on asbestos and a layer of magnesium perchlorate. The manganese dioxide is prepared as follows: shake thoroughly 25 g of ignited asbestos fibre with 400 ml of a saturated solution of potassium permanganate. Add 400 ml of a saturated solution of manganese(II) sulphate and shake the mixture well. Filter on a Buchner funnel under pressure, wash the fibre twice with hot water and dry the cake at 105° C. Break up the solid mass into small granules.

Absorption cell (*see Fig.* 1)—This contains 85 ml of absorption solution.

End-point detection apparatus—This consists of an Evans Electroselenium Limited Quantitrator and galvanometer.

Current source—Capable of producing 20·00 ± 0·01 mA.

Ammeter—Capable of giving a full-scale deflection of 20 mA (*e.g.*, Sangamo - Weston "Sub-Standard" instrument).

Stop-watch—Capable of being read to 0·1 s.

Alpolain No. 3 *refractory combustion boats.*

PROCEDURE—

Weigh a suitable amount (Note 1) of the steel sample into a pre-ignited combustion boat (Note 2). Cover the sample with 0·2 g of de-greased tin foil or granules and then place the boat in the mouth of the combustion tube. Allow the oxygen to flow for 2 minutes to free the train from contamination. Switch on the titration current and allow it to flow until the galvanometer light spot reaches the end-point marked on the scale (Note 3), then switch off the current. Remove the cap from the atmosphere trap at the end of the combustion tube and push the combustion boat into the hot zone of the furnace with a stainless-steel rod. Replace the cap. Adjust the oxygen flow-rate to 400 ml minute^{-1}. As the sample burns (shown by cessation of gas bubbles in the absorption cell) increase the gas flow temporarily to prevent suck-back of the solution (when a gas reservoir is being used, this is necessary only for sample weights greater than 0·5 g). Two minutes after setting the end-point, switch on the currrent, start the stop-watch simultaneously and check that the current is exactly 20·00 mA (or 10·00 mA if required). Allow the current to flow until the galvanometer spot returns to the marked end-point. Stop the stop-watch and switch off the current at the same time. Read the stop-watch and record the titration time. Remove the combustion boat from the furnace tube.

Carry out a blank determination with a combustion boat and tin flux. Allow the same interval of time to elapse between the initial end-point adjustment and the end of the titration as for the sample. At low blank levels use a current of 1 or 2 mA for titration. After nine 4-minute titrations at 20 mA it is necessary to change the absorption solution.

CALCULATION—

$$\text{Percentage of carbon} = \frac{12{\cdot}011}{96487} \times \frac{i \times t}{W \times 10}$$

$$= \frac{0{\cdot}12448 \times i \times t}{W \times 10^4}$$

where i is the current, mA, t is the time, s, and W is the sample weight, g.

NOTES—

1. It is convenient to take a sample weight that will give a titration time of 4 minutes (*i.e.*, a total time of 6 minutes between the initial setting of the end-point and the end of the titration). If necessary a rough titration can be carried out to determine the sample weight required.

However, the following is a guide to sample weights required.

Carbon, per cent.	Sample weight/g
0·08 and below	1·0
0·08 to 0·10	0·75
0·10 to 0·20	0·50
0·20 to 0·40	0·25

If a titration time of much less than 4 minutes is taken, the current should be switched on a little later to maintain a total time of 6 minutes.

2. Combustion boats should be pre-ignited in a muffle furnace at 1200° C. They should then be transferred to a desiccator to cool.

3. In a preliminary titration determine the reading of the galvanometer at which the most rapid change in optical density occurs. Mark this point on a scale and use it as a subsequent end-point. If the galvanometer light spot appears to move irregularly when the current is off, either the position of the gas inlet must be changed or the stirrer speed must be reduced. When the current is on, a stirrer speed that is too low will cause irregular movement.

BLANKS

Contributions to the blank values were reduced as much as possible as follows.

The oxygen stream was purified by passage through a combustion tube heated at 1200° C and then through soda asbestos. Combustion boats were pre-ignited in a muffle furnace at 1200° C. Commercially available low-carbon tin granules wese used. The use of rubber tubing was kept to a minimum by the substitution of glass tubing whenever possible. Ingress of air to the titration cell was prevented by providing a restricted outlet for the oxygen gas. Diffusion of anolyte into the main cell compartment was reduced by fitting a No. 4 porosity sintered-glass disc to the anode arm.

Blanks arising from electrical causes were not significant; fatigue of the photocell was checked and found to be negligible, and no leakage occurred from the current source, which was particularly stable.

Typical blank values over 6 minutes' titration time were 5 μg of carbon, most of which arose from the boat and tin initiator.

Because of the risk of missing the end-point when using a 20-mA current, it was necessary to titrate these low blank tests at a current of 2 mA. This was conveniently achieved by replacing the fixed current source with a dry cell (1·5 V) and an appropriate variable resistor in series.

LIMIT OF DETECTION—

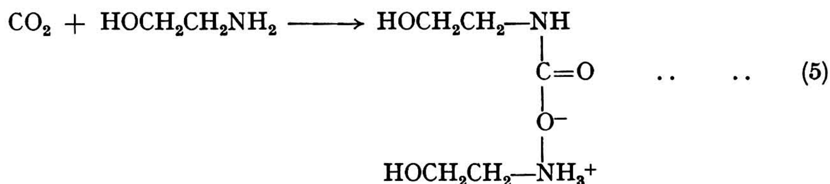
Twenty blank determinations were carried out and a standard deviation was calculated to indicate the limit of detection of the method.

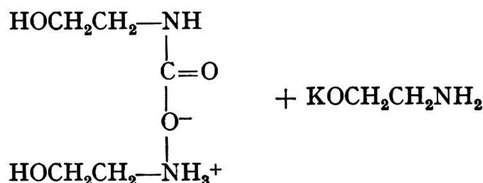
Mean of twenty blank determinations (current of 1 mA) ..	34·1 s
Standard deviation, s	7·7 s (= 1 μg of carbon)
Limit of detection (3 s = 3 μg of carbon) (0·983 probability)	0·0003 per cent. of carbon (1-g sample)

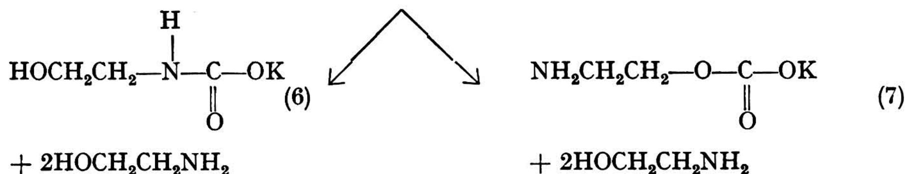
Discussion and results

Reactions—

The following reactions are thought to occur—







In reaction (4) the potassium liberated at the cathode reacts with monoethanolamine to form a base. This is supported by the observation that when the monoethanolamine is omitted from the solution, the current efficiency drops from 100 to 64 per cent., showing that a different reaction (probably with dimethylformamide) is taking place. The sodium derivative of monoethanolamine has been used successfully as a base in non-aqueous titration by other workers.[17] Reaction (5) has been established by Braid *et al.*,[9] who also suggest reactions similar to (6) and (7), but reaction (6) is believed to be the more probable.

As the electrolysis takes place both in the presence and absence of water it has been assumed that water does not take part in the reactions mentioned above.

Apparatus and titration conditions—

The apparatus used in this investigation was based on previous experience with aqueous coulometry (with the exception of the end-point detector). The chief advantages are simplicity and low cost, *e.g.*, the use of a constant current and measurement of time with a stop-watch. The EEL Quantitrator could easily be replaced by a lamp - photocell - galvanometer arrangement constructed in the laboratory.

It should be possible to provide greater flexibility by using a variable titration current, the amount of electricity being measured by an integrator. This could be designed to give high initial titration rates with end-point anticipation, and the photocell output would provide the necessary signals. However, expensive high-quality instrumentation would be needed to avoid loss in precision. In this way an automated rapid control method of high precision could be produced.

ANALYTICAL PERFORMANCE—

Table VI gives the results of triplicate determinations of carbon in a variety of British Chemical Standard steels. Table VII gives the results of replicate determinations of carbon in further samples of B.C.S. steels to provide a measure of the reproducibility of the method. The 95 per cent. confidence limits of the method within this laboratory range from ±0·0007 per cent. of carbon at 0·048 per cent. of carbon to ±0·008 per cent. of carbon at the 0·9 per cent. of carbon level. Certificate values are given for comparison. The limit of detection calculated from the standard deviation of the blank (Wilson's method[18]) is 0·0003 per cent. on a 1-g sample weight.

The performance of the method compares well with that of the low-pressure volumetric method, which it is designed to replace in this laboratory.

TABLE VI

ANALYSIS OF SOME BRITISH CHEMICAL STANDARD STEELS

Sample B.C.S. No.	Weight/g	Certificate values for carbon, mean and (range), per cent.	Carbon found by coulometry, per cent.
431 Mild steel	1	0·019 (0·017 to 0·020)	0·0185, 0·0184, 0·0181
317 3 per cent. Silicon steel ..	1	0·028 (0·026 to 0·029)	0·0272, 0·0274, 0·0271
432 Mild steel	1	0·093 (0·091 to 0·096)	0·0917, 0·0913, 0·0915
239/3 Mild steel	0·35	0·30 (0·29 to 0·30$_5$)	0·292, 0·291, 0·291
434 Mild steel	0·20	0·37 (0·36 to 0·38)	0·366, 0·366, 0·365
264/1 Mild steel	0·20	0·49$_5$ (0·49$_0$ to 0·50$_0$)	0·489, 0·492, 0·489
221/1 Mild steel	0·18	0·60 (0·59 to 0·62)	0·594, 0·593, 0·592

TABLE VII

REPRODUCIBILITY OF ANALYSES OF SOME BRITISH CHEMICAL STANDARD STEELS

B.C.S. sample and certificate values for carbon, per cent.	Mean carbon found by coulometry, per cent.	No. of determinations	Standard deviation, per cent.	Coefficient of variation, per cent.
265/2 Mild, 0·048	0·047 95	20	0·000 35	0·73
333 Austenitic stainless, 0·066	0·065 2	20	0·000 42	0·65
237/1 Mild, 0·10$_5$	0·103 4	18	0·000 91	0·88
218/3 Mild, 0·17	0·170 8	18	0·000 68	0·40
240/2 Mild, 0·41	0·406 4	20	0·002 4	0·59
220/1 7 per cent. Tungsten, 0·93	0·919 9	20	0·004 1	0·45

CONCLUSIONS

A method based on coulometric titration in a partially aqueous medium has been developed for the precise determination of carbon in steel. It is intended for use in reference analysis and involves the use of inexpensive apparatus and a simple technique. However, the application of appropriate instrumentation could provide a method of high precision for routine control purposes.

The reproducibility index of the method (2σ) of 0·0007 per cent. at the 0·05 per cent. of carbon level is comparable with that of the low-pressure volumetric method. As it is based on the fundamental laws of Faraday, no standardisation against analysed samples or pure materials is required. The use of high-vacuum techniques, expensive instrumentation and highly inflammable titrants, characteristic of other methods, is avoided.

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APPENDIX

LIST OF COMPONENTS OF CONSTANT-CURRENT SOURCE (Fig. 3)

Resistors—

R_1	= 750 Ω
R_2	= 33 kΩ
R_3	= 240 kΩ
R_4	= 1 kΩ
R_5, R_6	= 22 Ω
R_7	= 500 Ω (variable)
R_8	= 240 Ω
R_9	= 270 Ω

Transformer—

250/125 V

Capacitors—

C_1	= 100 μF, 350 V
C_2	= 25 μF, 25 V

Semi-conductors—

D_1, D_2, D_3, D_4	= 1B05J400
ZD_1	= ZB6.8
TR_1	= BF259
TR_2	= BF259
TR_3	= 2N698

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The Determination of Yttrium, Europium, Terbium, Dysprosium, Holmium, Erbium, Thulium, Ytterbium and Lutetium in Minerals by Atomic-absorption Spectrophotometry

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A procedure is described for determining yttrium, europium, terbium, dysprosium, holmium, erbium, thulium, ytterbium and lutetium in zirconium and calcium rare earth silicates by atomic-absorption spectroscopy, which involves the use of a lanthanum suppressor and has potential applications to other minerals containing these interferences. Although many of the associated ingredients are found to interfere, the addition of lanthanum overcomes these problems in many instances. Sensitivities and detection limits found with the proposed procedure are given for each element.

EXISTING methods for determining individual rare earths in complex materials are cumbersome and, in general, difficult to carry out. Wet-chemical, emission-spectrographic and spectrophotometric methods[1] have been widely used, and recently X-ray fluorescence[2] and neutron-activation methods[3,4,5,6] have been applied to these determinations. Spectrographic, spectrophotometric and X-ray fluorescence methods require a complex system of standardisation for accurate results. Wet-chemical methods involve complex schemes to separate the rare earths as a group, before isolating each of the individual rare earth elements.

Atomic-absorption methods are generally simpler and are often relatively free from spectral interferences. Several workers have studied the atomic-absorption behaviour of the rare earths,[7 to 15] but, to the authors' knowledge, no method for their determination exists which is accompanied by supporting results.

In the most extensive investigation, Kinnunen and Linsjö[14,15] studied the atomic-absorption behaviour of all of the rare earths, with the exception of thulium, lutetium and cerium. Hollow-cathode lamps for thulium and lutetium were not available at the time of their study, and cerium determinations were unsuccessful. The same authors recommended the use of a calibration graph prepared from a rare earth matrix solution for accurate work, but no results of determinations were given. Recently, Thomas[13] and Fernandez and Manning[9] outlined the conditions for the application of atomic-absorption spectroscopy to the determination of cerium, thulium and lutetium.

In the present work a method is proposed for the atomic-absorption determination of the more sensitive rare earths, including europium. Results are given, which are substantiated by X-ray fluorescence determinations on mineral material.

EXPERIMENTAL

INTERFERENCES—

Table I (see p. 48) summarises a study of the interference effect on the absorbance of a typical rare earth (60 p.p.m. of ytterbium), caused by the commonly associated ingredients of rare earth minerals and rocks. (Other rare earths such as 60 p.p.m. of dysprosium and 100 p.p.m. of holmium gave similar values.) The flame conditions were adjusted to give maximum absorption in each case (with a slightly reducing flame).

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TABLE I

INTERFERENCE STUDY

Interference	Concentration	Absorbance change, per cent.	Corrective step required
Fe	500 p.p.m.	+20	Add 1 per cent. of lanthanum
K	500 p.p.m.	+78	
Mg	5 p.p.m.	0	
	50 p.p.m.	+ 4	
	500 p.p.m.	+20	
Na	5 p.p.m.	+ 7	
	50 p.p.m.	+22	
	500 p.p.m.	+90	
Mn	500 p.p.m.	+20	
Ca	500 p.p.m.	+50	
Ti	500 p.p.m.	−35	
Other rare earths	200 p.p.m.	+13 to +21	
Al	10 p.p.m.	− 7	Add 1 per cent. of lanthanum and use a burner-to-beam height of about 2 cm
	100 p.p.m.	−22	
	500 p.p.m.	−60	
Zr	1000 p.p.m.	−60	As for aluminium and also adjust flame to less reducing conditions until equal absorbances are obtained between zirconium-bearing and zirconium-free solutions
HNO_3	0·1N	0	Standards and samples must have roughly comparable acidities
	1·0N	0	
	3·0N	−15	
HCl	0·1N	0	
	1·0N	−2	
	3·0N	−10	

Under the conditions given in Table I the interference arising from a 2-fold excess of silica and an 8-fold excess of all of the other cations is decreased to a negligible level. One per cent. of lanthanum is the smallest amount that successfully overcomes the interferences; larger amounts have no beneficial effect. No other element, not even strontium, was as effective as lanthanum as a suppressor. The additional adjustments required for eliminating zirconium and aluminium interferences result in 15 to 25 per cent., and at least 50 per cent., decreases in the absorbances, respectively, when compared with conditions giving maximum values.

METHOD

APPARATUS—

A Perkin-Elmer Model 303 atomic-absorption spectrophotometer, with a 5-cm nitrous oxide burner, was used. Table II gives the wavelength, current and slit widths. Westinghouse hollow-cathode lamps were used for dysprosium, holmium, erbium and ytterbium, and Perkin-Elmer Intenstron lamps for yttrium, europium, thulium and lutetium.

TABLE II

INSTRUMENT PARAMETERS

Element	Eu	Tb	Dy	Ho	Er	Th	Yb	Lu	Y
Wavelength/nm	459·4	432·6	421·2	410·4	400·8	409·4	398·8	331·2	410·2
Current/mA	35	30	15	30	25	30	20	30	30
Slit width/mm	0·3	0·3	0·3	0·3	0·3	0·3	0·3	0·1	0·3

REAGENTS—

Rare earth stock solutions, 1000 *p.p.m.*—Dissolve accurately weighed, spectroscopically pure, rare earth oxides (obtained from A. D. McKay Co. Ltd.) in 25 ml of 1 + 1 nitric acid, by gently heating. Wash the solutions into 1-litre calibrated flasks and dilute to the mark with water. Prepare dilute working solutions to cover a wide concentration range, by diluting the required aliquot with sufficient 10 per cent. lanthanum solution to make the final concentration of lanthanum 1 per cent., and sufficient nitric acid to correspond with the acid content of the sample.

Prepare solutions for the interference study from analytical-reagent grade chemicals.

Lanthanum nitrate solution, 10 per cent.—Dissolve a known weight of purified lanthanum oxide, La_2O_3, in 1 + 1 nitric acid and dilute with water to give a nitric acid concentration of 1 per cent. and lanthanum content of 10 per cent. Test this solution to ensure that the rare earths are absent.

PROCEDURE—

The rare earth content of minerals and rocks is usually small and therefore it is often necessary to concentrate the dissolved material in a small volume (5 to 10 ml). The method by which this is done will depend on the composition of the sample, which may give a sample solution containing any of the elements listed above, the interferences from which are overcome with lanthanum.

Zirconium rare earth silicates—The sample (0·5 g) is treated with hydrofluoric acid to effect decomposition and to precipitate the insoluble-fluoride group. The soluble zirconium fluoride is then removed by filtration. After ignition to give the oxides, aided by the addition of a few drops of sulphuric acid, the insoluble material is fused with potassium pyrosulphate and dissolved in dilute acid. The hydroxides are precipitated with ammonia solution and treated as indicated below.

A direct determination of the rare earths without removal of zirconium can be carried out: dissolution of the sample with hydrofluoric acid is followed by heating it to fumes with sulphuric acid to remove fluoride. The resulting precipitate is dissolved in dilute acid and after adding lanthanum the solution is measured by atomic-absorption spectroscopy under the conditions given in Table I. A loss of at least 50 per cent. in the absorbance makes this alternative less attractive when sensitivity is important.

Calcium rare earth silicates—The sample (0·5 g) is decomposed by fusion with carbonate, followed by precipitation of the hydroxide in the dilute acid solution of the fusion mixture. The hydroxide precipitate is treated as indicated below.

Other rare earth silicates—A suitable accurately weighed amount (1 to 10 g) of sample is dissolved by an established procedure.[2,16,17,18,19,20] One precipitation, or a combination of fluoride, hydroxide and oxalate precipitations, depending on the nature of the sample, is used to remove the rare earth group from the high salt content of the dissolution mixture. In the final step a precipitate of hydroxide is obtained and treated as indicated below.

Determination of rare earths in the hydroxide precipitate—Dissolve the precipitate in hot dilute nitric acid in a 50-ml beaker. Wash it thoroughly with hot 1 + 50 nitric acid. Add sufficient lanthanum to give a final concentration of 1 per cent., and evaporate the solution to about 2 ml. Transfer the mixture with washing into a small calibrated flask and dilute to volume with water. Run the solution against standards for each element containing a similar amount of nitric acid and 1 per cent. of lanthanum. Although these steps appear to make the procedure unnecessarily cumbersome, in practice they take a relatively short time and greatly increase the reliability.

TABLE III

RARE EARTH DETERMINATIONS

Element (as oxide)	Zirconium silicate				Calcium rare earth silicate	
	by X-ray	Estimated standard deviation	by atomic absorption*	Standard deviation	by X-ray†	by atomic absorption†
TbO_2	0·04	0·004	0·05	0·007	0·38	0·29
Dy_2O_3	0·31	0·006	0·28	0·012	2·14	2·40
Ho_2O_3	0·09	0·004	0·07	0·002	0·41	0·39
Er_2O_3	0·35	0·007	0·32	0·017	1·01	0·97
Tm_2O_3	0·06	0·006	0·05	0·006	0·13	0·12
Yb_2O_3	0·44	0·008	0·41	0·002	0·66	0·68
Lu_2O_3	0·06	0·006	0·06	0·006	—	0·10
Eu_2O_3	0·01	0·001	0·01	0·001	0·01	0·01
Y_2O_3	3·50	0·07	3·60	0·03	10·3	10·0

* Mean of three determinations.
† Enough sample for only one determination.

RESULTS AND DISCUSSION

A zirconium silicate and a calcium rare earth silicate were subjected to the proposed procedure, and the results obtained are given in Table III. The results are compared with those obtained by X-ray fluorescence on similar materials.

The complete atomic-absorption method for the elements determined takes 3 to 4 hours, compared with about 16 hours by X-ray fluorescence. The precision of the two techniques is about the same, although the sensitivity of the X-ray fluorescence method is greater for lutetium, terbium and dysprosium, and is comparable for the others. Serious rare earth interelemental interference occurs in the europium, holmium, ytterbium and lutetium determinations by X-ray fluorescence.

SENSITIVITY AND DETECTION LIMIT—

Table IV gives the sensitivities and detection limits obtained by the proposed procedure, which are compared with those recorded by Slavin.[21]

TABLE IV

DETECTION LIMITS AND SENSITIVITIES

Element	Sensitivity, p.p.m. per 1 per cent. absorption		Detection limit, p.p.m.	
	Present method	Slavin	Present method	Slavin
Terbium	10	7·5	2	2
Dysprosium	1·0	0·7	0·5	0·4
Holmium	2·0	1·4	0·3	0·3
Erbium	0·8	0·85	0·4	0·1
Thulium	1·0	1·0	0·3	0·15
Ytterbium	0·3	0·17	0·1	0·04
Lutetium	30	15	10	3
Europium	0·7	0·75	0·3	0·2
Yttrium	1·8	1·1	—	—

Determination of lutetium is extremely difficult because it occurs in very small amounts in natural materials, and because it is relatively insensitive to atomic-absorption spectroscopy.

Work is continuing on the atomic-absorption and atomic-fluorescence determination of the lighter, less sensitive, rare earths.

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The Determination of Fluorine in Rocks and Minerals by a Pyrohydrolytic Method

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A rapid pyrohydrolytic method, with simple apparatus, is described for the determination of fluorine in rocks and minerals. The sample is heated with a three-component flux in a stream of moist air, and the liberated hydrogen fluoride is absorbed into an alkaline solution. The recovered fluorine is determined either colorimetrically or by means of a fluoride-specific electrode. The method is suitable for determining fluorine at concentrations down to about 50 p.p.m.

THE distillation method of Evans and Sergeant[1] for the determination of fluorine in rocks and minerals has been shown to be reliable in routine work at this laboratory over a period of years. It is, however, a comparatively long procedure, and the possibilities of a rapid pyrohydrolytic method have been under consideration for some time. In particular, among recent methods of this type, that described by Newman[2] for aluminosilicate analysis has now been examined in detail and, with modifications, forms the basis of our proposed method.

EXPERIMENTAL

Certain alterations were made to the apparatus as used by Newman, the most important of which concerned the outlet condenser and the method of introducing water into the reaction tube. The condenser was found not to be necessary (or desirable) with the reduced air flow of our method, as condensation was apt to prevent complete fluorine recovery. In place of the steam generator required by Newman's system, water is contained in a nickel boat placed in the reaction tube and heated by a burner. This simple arrangement ensures that sufficient water vapour is present during each determination.

Pure vanadium pentoxide, as used by Newman, was found to be an effective flux for most of the rock samples tested, with a few exceptions. In particular, a specimen of micaceous material (muscovite - tremolite schist) failed to yield the expected recovery of 0·40 per cent. of fluorine, and variation of the sample-to-flux ratio did not lead to significantly improved recoveries. Next, mixed fluxes were considered, and a combination of one part of bismuth trioxide with two parts of vanadium pentoxide gave improved recoveries of fluorine, but these appeared to be critically dependent on the degree of grinding of flux with the sample. The addition of sodium tungstate as a third component gave a still more active flux so that fluorine was recovered quantitatively from the micaceous material. In order to avoid spitting of the flux during pyrohydrolysis, it was necessary to prepare pre-fused flux from a mixture of the components. The dense ground flux powder was also an excellent material for further grinding with sample powder. In order to standardise the sample-to-flux ratio, further determinations of the fluorine content of the micaceous schist were made on 200-mg portions mechanically ground with various weights (100, 200, 400 and 800 mg) of the pre-fused flux. Complete recoveries of fluorine were obtained with all but the smallest weight of flux.

To examine the effect on the pyrohydrolysis of varying the amount of water used, the operation was performed successively with 2, 4, 6 and 8 ml of water in the nickel boat. A mechanically ground mixture of the sample of micaceous schist with two parts by weight of three-component flux was used in these tests, which showed complete recoveries of fluorine except when the smallest amount of water was used. The time taken, from the time of lighting the burners, for the water to evaporate completely was 10 to 25 minutes for 2 to 8 ml, respectively.

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The reaction temperature in the ignition tube under working conditions was measured with a thermocouple positioned in place of the sample, and was found to be 700 to 750 °C.

For the purpose of this investigation, the fluoride contents of the absorbent solutions were measured by the fluoride-specific electrode and by the lanthanum - alizarin fluorine blue colorimetric method as described by Greenhalgh and Riley.[3] Good agreement was observed between the two methods over a wide range of fluoride contents.

METHOD

APPARATUS—

The transparent fused silica tube* A (Fig. 1) is similar to that used by Newman and is 450 mm long with an internal diameter of 20 mm and a wall thickness of 2 mm. It is heated by three Bunsen burners, B, C and D, the first two of which are fitted with flame-spreader attachments. The silica boat F (50 × 15 mm) contains the mixture of sample and flux. The nickel boat G (100 × 10 mm, capacity approximately 8 ml) is filled with water for each determination and placed near the inlet end of the tube. Compressed air, after being filtered through a cotton-wool plug, passes into the reaction tube by way of the flow meter E and the emergent air stream bubbles through the alkaline absorbent solution contained in a polythene bottle of approximately 50-ml capacity.

Electrical measuring equipment—This comprised an "Ionalyzer" fluoride-activity electrode, Model 94–09, together with the associated specific-ion meter.

Spectrophotometer—A Unicam SP600 instrument was used.

REAGENTS—

All reagents should be of analytical-reagent grade when possible.

Preparation of flux—In a nickel crucible mix 5 g of bismuth trioxide, 5 g of sodium tungstate and 10 g of vanadium pentoxide. Heat the mixture to fusion over a burner, and pour the melt on to a silica plate, then break up the cooled melt and grind it to powder.

Sodium hydroxide solution—A 0·2 M aqueous solution.

Neutralising buffer solution—Dissolve 15 g of sodium acetate trihydrate and 5 ml of glacial acetic acid in water, and dilute the solution to 100 ml.

Lanthanum - alizarin fluorine blue reagent—This is prepared as described for solution A in the method of Marshall and Wood.[4]

Standard fluoride solution—Dissolve 0·1106 g of dry sodium fluoride in water, and dilute the solution to 500 ml. This solution contains 100 μg ml^{-1} of fluorine. From it prepare, by dilution with water, a dilute standard solution containing 4 μg ml^{-1} of fluorine.

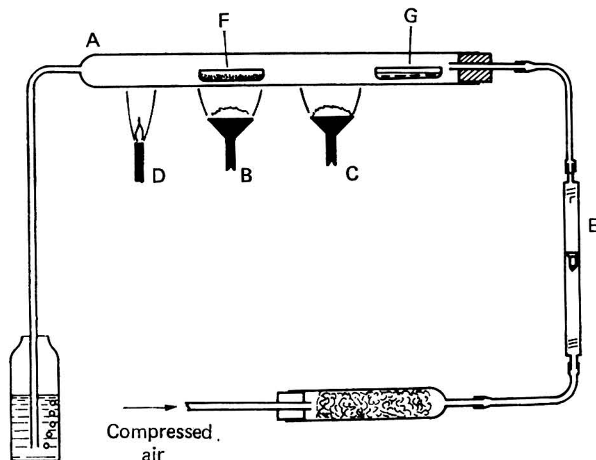

Fig. 1. Details of apparatus

* A suitable tube was made to order by Jencons Ltd., Hemel Hempstead, Herts.

PROCEDURE—

Mix 0·500 g of sample powder with 1·00 g of flux in a mechanical mortar (preferably of agate), then grind the mixture for 30 minutes. Transfer the ground mixture, which is sufficient for duplicate determinations, to a stoppered specimen tube. Transfer 0·600 g of the mixture to the silica boat and place this in position in the reaction tube over burner B (Fig. 1). Fill the nickel boat to the brim with water, and place it in the reaction tube on the inlet side of burner C, as shown in the diagram. The position should be such that about 25 minutes are required for total evaporation of the water after lighting the burners. Prepare the absorbent mixture in the polythene bottle by introducing 5 ml of sodium hydroxide solution and 20 to 25 ml of water, and place the bottle so that the exit end of the reaction tube is just below the surface of the liquid. After connecting the air supply and adjusting the flow-rate to 10 litres per hour, light the two outer burners D and C, then, 2 minutes later, the central burner B. At this point the air flow-rate may need re-adjustment.

TABLE I

DETERMINATION OF FLUORINE IN ROCKS

		Fluorine found, per cent.			
		Pyrohydrolytic method			
Sample	Deviation from standard method	Colorimetric finish	Electrode finish	Distillation method	Other figures
Felsite	None	0·011	0·011	0·012	—
	B	0·011	0·011		
	G	0·013	0·011		
Diabase W–1	None	0·023	0·022	0·023	0·025*
	A	0·023	0·022		
	G	0·025	0·023		
Tonalite, T–1	None	0·047	0·048	0·046	0·045†
	E	0·046	0·047		0·08
	G	0·045	0·046		0·05
Granite, G–1	None	0·065	0·065	0·063	0·07*
	A	0·065	0·065		
	G	0·063	0·064		
Biotitic green schist	None	0·073	0·072	0·074	—
	None	0·073	0·074		
	B	0·073	0·074		
	A	0·073	0·074		
	G	0·071	0·072		
Porphyritic basalt	None	0·127	0·130	0·120	—
	C	0·127	0·129		
	G	0·126	0·126		
Alkali-granite	None	0·181	0·181	0·180	—
	D	0·181	0·183		
	G	0·178	0·180		
Muscovite - tremolite	None	0·395	0·400	0·400	—
	None	0·395	0·400		
	G	0·395	0·395		
Granite	None	1·16	1·18	1·11	—
	F, B	1·16	1·18		
	G	1·14	1·15		

* From the compilation by M. Fleisher.[5]

† From the publication "Standard Geochemical Sample T–1," 1961, Geological Survey Division, Ministry of Commerce and Industry, Tanganyika.

A: Air flow-rate at 5 l hour^{-1}.

B: Air flow-rate at 20 l hour^{-1}.

C, D, E, F: Volume of water added to nickel boat reduced to 5 ml. Time for total evaporation of water and total period of pyrohydrolysis, in minutes, was 17(C), 10(D), 15(E) and 13(F).

G: Sample and flux mixed and ground by hand in an agate mortar for a total time of 10 minutes.

Thirty minutes after lighting burner B remove the polythene bottle, turn off all the burners, and rinse the tip of the outlet tube with water into the polythene bottle. Allow the absorbent mixture to cool, add 5 ml of neutralising buffer solution, then quantitatively

transfer the liquid to a 50-ml graduated flask and dilute to the mark. Determine the fluorine content of the solution either by using the fluoride-specific electrode, or the colorimetric method, as described below.

Ion-specific electrode finish—Prepare a set of fluoride standards in 50-ml graduated flasks by adding to each, by pipette, 5 ml of sodium hydroxide solution and 5 ml of neutralising buffer solution, together with aliquots of standard fluoride solution equivalent to 0·002, 0·010, 0·100 and 1·00 per cent. of fluorine in the original material. Dilute each standard to 50 ml and store in polythene bottles.

With the ion-specific electrode, compare the test solution with the standards according to the manufacturer's instructions.

Colorimetric finish—Transfer an aliquot of the test solution, containing not more than 20 μg of fluorine, to a 25-ml graduated flask. (A 5-ml aliquot is suitable if the fluorine content of the original material does not exceed 0·1 per cent.) Add, by pipette, 10 ml of lanthanum - alizarin fluorine blue reagent, dilute to 25 ml and mix. After 30 minutes measure the optical density at 625 nm of the solution contained in a 1-cm cell with air as reference. Ascertain the fluorine content by reference to a calibration curve covering the range 0 to 20 μg of fluorine in 25 ml of solution.

Results obtained by either method should be corrected for the small blank, which is determined by completing the whole procedure without sample material.

RESULTS

The proposed pyrohydrolytic method was applied to a number of silicate rocks with a wide range of fluorine contents previously established by the distillation procedure; the results are shown in Table I. Also shown are the results of experiments intended to demonstrate that the method is tolerant to the effects of fairly wide deviations from the standard procedure in a number of respects, and some figures obtained by other workers.

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A Simple Method for the Determination of Solvents Retained in Plastic Films and Laminates

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A simple method is described for the determination of solvents retained in polymer films and laminates as a result of the application of inks, coatings or adhesives. A sample of the film or laminate is heated, together with a known amount of a suitable solvent as an internal standard, in a simple modified Kilner jar. After a period of heating in an oven the atmosphere in the headspace of the jar is analysed by gas chromatography with a flame-ionisation detector.

Results given show the precision of the method and its application to actual samples. Replicate analyses on similar samples show a standard deviation of 5 per cent. of the mean value for each solvent determined. The method is currently used in the authors' laboratories for analysing samples containing retained solvents at levels between 1 and 300 mg m^{-2}.

COATINGS, inks and adhesives are usually applied to polymer films as solutions in or mixtures with organic solvents. After their application the film is passed through a drying oven, but small amounts of solvent may diffuse into the film and be retained by it. It is important to know the amounts retained, as these can relate to the odour of the film when used to package foodstuffs, etc. For the past 10 years, these solvents have been determined in our laboratories by gas chromatography after a carrier distillation.[1] Application of this method requires considerable expertise and recoveries are low when it is used with certain films that have come into common use since its inception. Several alternative methods have been described recently. Martin[2] heated a relatively large area of film in a converted vacuum oven and passed a sample of the vapour through a gas-chromatographic detector. Susukida and Okuma[3] heated a small sample with a carrier liquid in a sealed tube and then analysed the carrier liquid. Allavena and Grassi[4] heated a sample of film under an unmeasured reduced pressure and have described a way of introducing an internal standard. These methods are not sufficiently sensitive or simple for our requirements. Wilks and Gilbert[5] heated a sample of film under a measured reduced pressure and analysed the atmosphere in the headspace of the vessel by gas chromatography.

The method described below can be regarded as a logical development of the method of Wilks and Gilbert. The apparatus, operations and calculations have been changed and simplified, and the use of an internal standard has been introduced to improve the precision. An experiment described demonstrates that it is possible to show whether the recovery of each solvent from each film or laminate is complete.

EXPERIMENTAL

APPARATUS AND REAGENTS—

Kilner jars (1 *lb*)—These jars have metal lids. A brass ¼-inch pipe union is sealed with Araldite into a hole drilled through each lid; this firmly holds a rubber septum. Jars and lids can be used many times; after use they can be cleaned by being placed separately in an oven at 100° C for half an hour.

Gas chromatograph with a flame-ionisation detector—This can be fitted with any column that will satisfactorily separate the various solvents from each other and from the internal

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standard. We have found that a suitable liquid phase for our purpose is silicone oil modified by the addition of a small amount of UCON HB2000 (polyalkylene glycol). The proportions used were 9 parts of silicone oil, 2 parts of UCON HB2000 and 100 parts of Chromosorb W.

Internal standard—This can be any solvent that is known to be absent from the sample material. We found butyl acetate or butyl propionate to be suitable for most of our analyses.

PROCEDURE—

About 250 cm^2 of material are crumpled loosely, placed in a clean Kilner jar and the jar is closed. Internal standard (3·00 μl) is injected into the jar through the rubber septum by using a 10-μl syringe. The jar is then placed in an oven at 100° C for the minimum specified period (see below), at the end of which it is removed from the oven and about 2 ml of the atmosphere within are immediately extracted with an all-glass unlubricated syringe and injected into the gas chromatograph. The ratios of the peak heights of the solvents (a, b, c, *etc.*) to that of the internal standard are calculated. The same amount of internal standard as used with the samples, *plus* 2·00 μl of each of the solvents a, b, c, *etc.*, is introduced, for calibration purposes, into each of two more Kilner jars. These jars are heated together with those containing samples.

The content of retained solvent, *e.g.*, a, is given in milligrams per square metre by the expression—

$$\frac{\dfrac{\text{Peak height of solvent a in sample jar}}{\text{Peak height of internal standard in sample jar}}}{\dfrac{\text{Peak height of solvent a in calibration jar}}{\text{Peak height of internal standard in calibration jar}}} \times \frac{20{,}000 \times \text{specific gravity of solvent a}}{\text{Area of sample in square centimetres}}$$

The minimum heating period for each material is determined as follows. The film or laminate sample and internal standard are placed in a Kilner jar as described above and the jar is placed in an oven at 100° C; it is removed from the oven at 10 or 15-minute intervals and 2-ml samples are withdrawn and chromatographed. The jar is replaced in the oven immediately after each 2-ml sample has been taken. The ratios of the peak heights of solvents a, b, c, etc., to that of the internal standard are calculated. The minimum heating time is that after which continued heating produces no further change in the ratios.

The method as described above is applicable for retained solvent levels between 1 and 300 mg m^{-2}. The linearity of the amplified response of the flame-ionisation detector must be shown to be adequate throughout the range of attenuation settings used.

RESULTS

The following results show the precision of the method and illustrate its application to actual samples.

PRECISION IN THE PREPARATION OF CALIBRATION STANDARDS—

Seven jars were prepared, each containing 2·00 μl of toluene and 3·00 μl of butyl acetate (our usual internal standard). Each jar was heated in an oven for a minimum of 45 minutes.

Peak height ratio, toluene/internal standard	2.12, 2.12, 2·08, 2·06, 2·09, 2·10, 2·11
	Mean 2·10 Standard deviation 0·02

DETERMINATION OF MINIMUM HEATING TIME—

This experiment was carried out to ascertain the minimum heating time for the determination of toluene in a laminate of polythene and polypropylene double-coated with saran [poly(vinylidene chloride)].

Time in oven/minutes	15	25	35	45	55	80	90	160	185	210
Peak height ratio, $\frac{\text{toluene}}{\text{internal standard}}$	4·2	6·1	7·0	8·1	8·3	8·7	8·8	8·9	8·8	8·8

From these figures it is deduced that a suitable minimum heating time would be 90 minutes.

REPEATED DETERMINATIONS ON ACTUAL SAMPLES—

The following results were obtained for the determination of toluene and ethyl acetate on adjacent pieces of laminate (polythene adhesive-laminated to polypropylene double-coated with saran). The solvents originate from the adhesive.

									Mean	s.d.
Toluene/mg m^{-2} ..	127	135	119	127	140	122	130	119	127	7
Ethyl acetate/mg m^{-2}..	136	145	124	141	136	133	138	128	135	6

Results as follows were obtained for the determination of toluene on adjacent pieces of printed film (polypropylene with a single saran coating). The toluene originated from the printing ink.

Toluene/ mg m^{-2} .. .. 11·9, 11·4, 12·3, 11·9, 12·5, 11·9, 12·3

Mean 12·0 mg m^{-2} Standard deviation 0·34 mg m^{-2}

RECOVERY EXPERIMENTS—

It is not possible to carry out direct recovery experiments because films or laminates cannot at present be prepared with known amounts of solvent evenly distributed throughout the material. However, one can say that after the minimum heating period the solvents and internal standard are partitioned between the polymer and vapour phases in proportions that remain unchanged during further heating; this indicates that one can conduct the following experiment with each solvent and each film to estimate the percentage recovery obtained in an actual determination. Two sets of calibration standards in Kilner jars are prepared, one normal and the other containing a sample of film; both sets of jars are then heated for the minimum heating time for that solvent and film. The peak height ratios of solvent to standard are measured. Any differences in ratio between the two sets of jars are caused by a preferential absorption of one compound by the film. A comparison of these ratios is a measure of the recovery of that solvent during an analysis of that film. It might be suggested that calibration jars should always contain a sample of the film under analysis, but it is seldom that such a sample known to be solvent free is available.

In the example given below two sets of calibration jars were prepared, of which only one set contained in each jar 250 cm² of polythene laminated to polypropylene double-coated with saran—

	Peak height ratio, toluene/internal standard		
	Calibration standards without laminate		Calibration standards with laminate
	1·82		1·65
	1·82		1·66
	1·77		1·60
	1·86		1·73
Mean ..	1·82	Mean ..	1·66

These results show that for the determination of toluene in this particular laminate the recovery is greater than 90 per cent.

Two solvents that have different physical properties from those of toluene are butanol and 2-ethoxyethanol. Experiments have shown that 100 per cent. of butanol is recovered from polythene and 95 per cent. from polypropylene double-coated with saran and that 85 per cent. of 2-ethoxyethanol is recovered from polypropylene double-coated with saran.

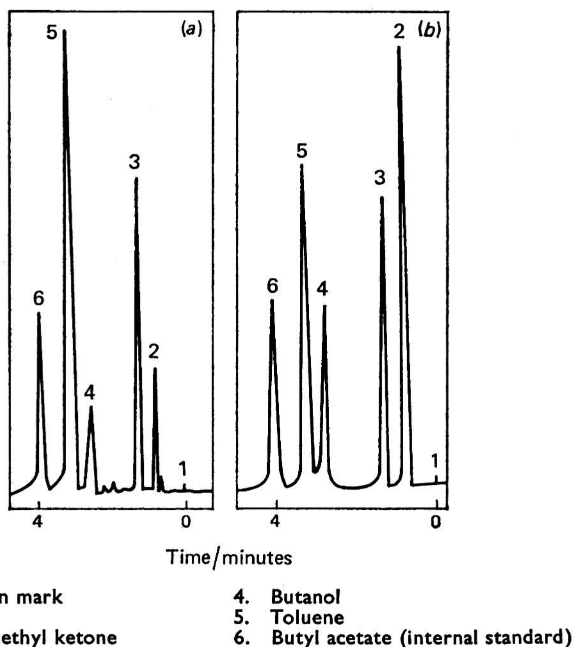
In recovery experiments of this sort it is theoretically possible for recoveries to be over 100 per cent., which could occur when more of the internal standard than of the solvent under test is absorbed into the film. Such recovery figures show by exactly how much one is over-estimating this solvent during an actual analysis.

DISCUSSION

The method described has been devised to determine the amount of solvent retained in a film or laminate. It is currently used in the range 1 to 300 mg m^{-2}, but the range could easily be extended at either end. Although it is still impossible to carry out true recovery experiments, an estimate of recovery can be made by the described procedure. The apparatus has been deliberately kept simple, and 100°C was chosen as a suitable temperature to which

the adapted Kilner jars could be repeatedly heated. We have shown that at 100°C the minimum heating time is reasonably short, being not more than 2 hours and often much less. This method has been used to determine many different solvents in several different substrates. The solvents include ethanol, ethyl acetate, ethyl methyl ketone, 2-ethoxyethanol, propan-1-ol and toluene. The substrates include polythene, polypropylene and cellophane, which occur individually, coated with saran or combined in laminates.

Fig. 1 (*a* and *b*) shows typical chromatograms obtained from a film and a calibration jar.



1. Injection mark
2. Ethanol
3. Ethyl methyl ketone
4. Butanol
5. Toluene
6. Butyl acetate (internal standard)

Fig. 1. (*a*) Chromatogram of solvents from a sample of printed polythene - polypropylene laminate. Chromatograph, Pye 104 with flame-ionisation detector; column, glass, 5 feet × ¼ inch, packed with 9 per cent. silicone oil and 3 per cent. UCON HB2000 on Chromosorb W; oven, 80° C; attenuation × 20,000; and chart speed, 1 cm minute^{-1}. (*b*) Calibration jar prepared for sample shown in (*a*)

Although it is possible, by using a large number of Kilner jars, to keep the gas chromatograph running continuously and thus analyse, say, between six and twenty samples per hour, there is still a delay between receipt of a sample and the completion of the analysis. Most of this delay is caused by the heating time required. It may well be possible, by using a vessel more robust than a Kilner jar, to heat the sample to a temperature just short of polymer decomposition and so reduce the heating period substantially.

In two of the examples given reference was made to polythene laminated to polypropylene double-coated with saran. This laminate was chosen for these examples because the authors have previously found it to be difficult to analyse.

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The Microgasometric Determination of Some Inorganic and Organic Nitrates by Reduction with Iodide Ion and Elemental Iodine

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Simple micro methods are described for the determination of nitrate based on its reduction with iodide or iodine in the presence of halogen acids. Nitric oxide gas is liberated and iodine(I) is the oxidation product from both iodide and iodine.

METHODS for the determination of the nitrate group have been reviewed in a previous work describing the microgasometric determination of nitrate by reduction with iron(II), titanium(III), mercury and hydroquinone.[1] However, reduction with iron(II) and mercury[1] is not specific for nitrate salts as nitramine and nitrate esters are also reduced. The mercury method has the further limitation that any aromatic compounds present are preferentially nitrated, thus giving low results for nitrate ion. Although reduction of nitrates with titanium(III)[1] is specific, the preparation and storage of the reagent are tedious.

The present work shows that iodide and iodine can be used instead of these reductants. The nitramine group and nitrate esters are not reduced under these conditions and aromatic substances do not interfere.

EXPERIMENTAL

APPARATUS—

As previously described.[1,2]

PROCEDURE—

Introduce 3 to 5 mg of the nitrate sample into the reaction vessel, and add 20 to 30 mg of potassium iodide. Displace the air in the apparatus with a stream of carbon dioxide gas at the rate of 100 bubbles minute^{-1} for 5 minutes, or until no air bubbles are collected in the nitrometer. Add 3 to 5 ml of 40 per cent. hydrobromic acid or 35 per cent. hydrochloric acid by using the funnel. Gently heat the reaction mixture for 5 minutes until no further gas bubbles are evolved. Sweep the gaseous products with carbon dioxide through the trap, containing 20 per cent. tin(II) chloride solution in concentrated hydrochloric acid, and collect the nitric oxide over freshly prepared 50 per cent. potassium hydroxide solution in the nitrometer. Carry out a blank experiment.

Reduction with iodine is carried out in the same way, but in this event only hydrobromic acid can be used as reaction medium.

CALCULATION—

$$\text{Nitrate-nitrogen, per cent.} = \frac{(V - v)\,(P - p) \times 273 \times 14{\cdot}01}{(273 + t) \times W \times 224 \times 760}$$

where V is the volume of nitric oxide, v is the blank, both in ml, P is the atmospheric pressure, mm Hg, p is the water vapour pressure, mm Hg, at temperature t, which is the average room temperature and also that of the potassium hydroxide solution contained in the nitrometer, and W is the weight of sample, mg.

As the reaction time is short, no correction for the solubility of nitric oxide is required when fresh potassium hydroxide solution is used in the nitrometer.

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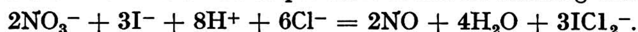
RESULTS AND DISCUSSION

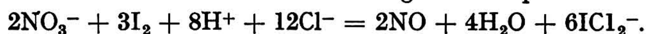
NATURE OF THE REACTION—

Various mole ratios of potassium nitrate and potassium iodide were tried, and the degree of reduction was calculated from the volume of nitric oxide gas produced. Quantitative reduction occurred with 1·5 mole of iodide per mole of nitrate showing that the reaction is—



$$2NO_3^- + 3I^- + 8H^+ + 6Cl^- = 2NO + 4H_2O + 3ICl_2^-.$$

This was confirmed by carrying out reduction of potassium nitrate with various mole ratios of iodine in hydrochloric acid. Three moles of iodine were the minimum amount required for reduction of 2 mole of nitrate according to the equation—



$$2NO_3^- + 3I_2 + 8H^+ + 12Cl^- = 2NO + 4H_2O + 6ICl_2^-.$$

The nitric oxide was identified by the formation of brown fumes when it was exposed to air and by its complete absorption in acidic iron(II) sulphate solution. The aqueous solution of reduction products decolorises indigo carmine and litmus solutions, thus confirming the presence of iodine monochloride.[3]

The mean recovery of the nitric oxide on simple sweeping and collection in the nitrometer was 91·0 per cent. These low results are attributed to volatilisation of the iodine with dissolution of the vapour in the potassium hydroxide to form hypoiodite which, in turn, reacts with the nitric oxide. Attempts were made to absorb the iodine in traps containing alkaline sodium hydrogen sulphite solution, carbon tetrachloride, starch solution and tin(II) chloride solution. The results obtained (Table I) show that 20 per cent. tin(II) chloride solution is the most suitable.

TABLE I

MICROGASOMETRIC DETERMINATION OF POTASSIUM NITRATE (CONTAINING 13·85 PER CENT. OF NITROGEN) BY REDUCTION WITH POTASSIUM IODIDE AND HYDROCHLORIC ACID AFTER IODINE ABSORPTION

Iodine absorption solution	Average nitrate-nitrogen found, per cent.	Average recovery, per cent.
—	12·6	91·0
Sodium hydrogen sulphite, 35 per cent. ..	13·0	94·1
Starch solution, 20 per cent.	13·4	97·0
Carbon tetrachloride..	13·6	98·2
Tin(II) chloride, 20 per cent.	13·8	99·9

DETERMINATION OF NITRATE—

Several nitrate samples were analysed by reduction with potassium iodide in the presence of hydrochloric and hydrobromic acids (Table II). In either medium, quantitative liberation of nitric oxide gas occurred with acid concentrations in the range 6 to 11 N. The maximum deviation was $\pm 0{\cdot}1$ per cent. absolute. With iodine as reductant (Table III) similar results were obtained in hydrobromic acid, but unsatisfactory results were obtained in hydrochloric acid medium.

TABLE II

MICROGASOMETRIC DETERMINATION OF SOME NITRATE SAMPLES BY REDUCTION WITH POTASSIUM IODIDE IN HYDROCHLORIC AND HYDROBROMIC ACID MEDIA

Sample	Nitrate-nitrogen calculated, per cent.	Reduction in hydrochloric acid. Nitrate-nitrogen found, per cent.	Reduction in hydrobromic acid. Nitrate-nitrogen found, per cent.
Potassium nitrate	13·85	13·9	13·8
		13·8	13·8
Barium nitrate	10·71	10·7	10·8
		10·6	10·7
Urea nitrate	11·38	11·4	11·4
		11·3	11·3
Guanidine nitrate	11·47	11·4	11·6
		11·4	11·4
Nitron nitrate	3·73	3·6	3·8
		3·7	3·7

TABLE III

MICROGASOMETRIC DETERMINATION OF SOME NITRATE SAMPLES BY REDUCTION WITH IODINE IN HYDROCHLORIC AND HYDROBROMIC ACID MEDIA

Sample	Nitrate-nitrogen calculated, per cent.	Reduction in hydrochloric acid. Nitrate-nitrogen found, per cent.	Reduction in hydrobromic acid. Nitrate-nitrogen found, per cent.
Potassium nitrate	13·85	12·8	13·8
		12·6	13·7
Barium nitrate	10·71	9·8	10·7
		9·9	10·7
Urea nitrate	11·38	10·7	11·2
		10·5	11·3
Guanidine nitrate	11·47	10·3	11·5
		10·4	11·4
Nitron nitrate	3·73	3·0	3·7
		3·2	3·8

In general, the nitrate was reduced more easily if hydrobromic acid was used instead of hydrochloric acid. Sulphuric acid could not be used because of its reduction to hydrogen sulphide and sulphur.

Attempts to use potassium bromide in presence of hydrochloric or hydrobromic acid proved to be unsuitable for quantitative reduction of nitrate as only 70 per cent. of the theoretical nitrate-nitrogen was recovered as nitric oxide gas.

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A Field Method for the Determination of Iron Oxide Fume in Air

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A field method is described for the determination of iron oxide fume in industrial atmospheres at concentrations up to 20 mg m^{-3}. After collection on a filter-paper the iron oxide is dissolved in a hot solution of hydroxylammonium chloride in hydrochloric acid. The iron solution is quantitatively transferred to a calibrated flask and the red complex of iron(II) - bathophenanthrolinedisulphonate is formed, the intensity of which is determined either spectrophotometrically or by visual comparison with a set of permanent colour standards. The procedure is simple to carry out and the time required for a complete analysis is about 25 minutes.

IRON oxide fume occurs in many industrial processes that involve the melting of iron or steel, *e.g.*, steel manufacture and the welding of iron or steel products. Although generally considered to be only a nuisance,[1] as it reduces visibility and causes damage by soiling, iron oxide occurs widely in industry and has been assigned[2] a threshold limit value of 10 mg m^{-3}. Consequently, and also to make possible the identification of an unknown fume as iron oxide, it appeared that there was a requirement for a simple, rapid field test for the determination of this material in air at its threshold limit value. A review of the literature revealed that no such test had previously been developed. Reynolds and Monkman[3] have described a method for the determination of iron in dustfall samples, but their procedure involves wet ashing and solvent-extraction steps that would greatly limit its use as a field test.

EXPERIMENTAL

COLORIMETRIC DETERMINATION OF IRON—

It was envisaged that any field method devised would involve the collection of the iron-containing fume from the test atmosphere on a suitable filter, dissolution of the iron from the filter and subsequent colorimetric determination with an optional visual or spectrophotometric finish. For use in the colorimetric procedure the widely used reagent for iron, 4,7-diphenyl-1,10-phenanthroline[4] (bathophenanthroline), was chosen. Its main advantages are its specificity for iron(II), adequate sensitivity, rapid formation of the coloured iron bathophenanthroline complex and the excellent visual colour change from colourless to red.

By using standard iron solutions, an initial study was made of a bathophenanthroline procedure described previously,[3] which was satisfactory when used by a skilled operator, but a disadvantage in its use as a field test was the need to extract the iron complex with chloroform. Consequently, attention was turned to the water-soluble disodium salt of bathophenanthrolinedisulphonic acid,[5] the use of which obviated the need for the extraction of the coloured complex with an organic solvent. It had been shown[5] that this reagent has a similar sensitivity to bathophenanthroline for the determination of iron(II) and was not more subject to interference from other chemical species. We therefore decided to determine the various optimum reaction parameters required for the use of this reagent in the proposed field test.

Concentration of disodium bathophenanthrolinedisulphonate reagent—The combining ratio of the iron(II) - bathophenanthrolinedisulphonate ion had previously been found experimentally[5] to be 1 : 3·17. On this basis, the presence of 2 ml of a 0·1 per cent. w/v aqueous solution of the reagent in a final reaction solution volume of 25 ml should have been adequate for determining up to 66 μg of iron(II). In practice, the maximum amount of iron(II) capable of being determined by this system was only 60 μg, equivalent to 86 μg of iron oxide (Fe_2O_3), but this was considered adequate for the purposes of the proposed field test.

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Optimum pH conditions and colour stability—Although it had previously been shown[5] that the red complex of iron(II) and the bathophenanthrolinedisulphonate ion can be formed over a pH range from 2·6 to 9, it was found in the present work that full and rapid colour development (within 5 minutes) commenced at pH 3·7, which was attained by the addition of sodium acetate solution to the acidic solution of the iron oxide fume sample (see Removal of fume samples from filter-papers). Tests showed that the presence of between 6 and 10 ml of a 16·7 per cent. w/v aqueous solution of sodium acetate trihydrate in the final reaction solution of 25 ml stabilised the pH at between 3·7 and 4·2 and yielded constant absorbances for a known amount of iron. To obviate the effect of possible accidental addition in the field of excess of the acid used for dissolution, it was decided to use 10 ml of the buffering acetate solution. The colours produced by this sytem were found to be stable for at least 1 hour. To reduce the number of solutions required it was found possible, as suggested previously,[6] to combine the aqueous hydroxylammonium chloride solution (about 10 per cent. w/v) necessary to maintain the iron as iron(II) in the reaction solution with the sodium acetate solution.

Reagent blank—The iron contamination in several commercial, analytical-grade hydrochloric acids was found to be minimal. Although the quoted maximum limits of iron impurity in the various analytical-reagent grades of hydroxylammonium chloride and sodium acetate trihydrate would have produced reagent blanks above those allowable in the proposed method, tests showed that all of these reagents were free from iron contamination. The maximum reagent blank for the complete procedure was always less than 1 μg of iron which, on the basis of a proposed 2·5-litre sample (see Sample size and visual colour standards), was equivalent to less than 0·6 mg m^{-3} of iron oxide in air, a value sufficiently low to be of no consequence in the proposed field test. In practice, a reagent blank of up to the equivalent of 1 mg m^{-3} of iron oxide in air would be allowable in a visual determination of iron oxide, and would entail a 20 per cent. over-estimation of the iron oxide fume in an atmosphere of half the present threshold limit value.

Sensitivity of the method—By using the parameters established above and a standard iron solution, a calibration graph, which was linear, was constructed over the range 0 to 50 μg of iron (0 to 70 μg of iron oxide). The optical densities of the various solutions were read in a 20-mm cell against a reagent blank at 538 nm, the readings for 5 and 70 μg of iron oxide being 0·11 and 1·53, respectively.

Sample size and visual colour standards—It was necessary to assess the most convenient sample size to be taken, particularly when using visual colour standards. A set of standards representing an acceptable reagent blank and 0·5, 1 and 2 times the present threshold limit value for iron oxide in air (5, 10 and 20 mg m^{-3}) was required. A suitable maximum standard was considered to be 50 μg of iron oxide. Consequently, as a sampling rate of at least 500 ml $minute^{-1}$ was considered necessary, this would involve sampling 2·5 litres of an atmosphere. By using a standard iron solution a set of standards was prepared to satisfy the above requirements, the respective colours of the solutions being easily differentiated visually when viewed through about 10 mm of liquid, and this was selected for use. However, to avoid the use of liquid colour standards in the field, a set of standard discs was prepared, with the co-operation of Tintometer Ltd., representing the intensity of colours produced by collecting

TABLE I

MAXIMUM LEVELS TESTED OF VARIOUS CHEMICAL SPECIES SHOWING NO INTERFERENCE WITH THE DETERMINATION OF 10 μg OF IRON (ABOUT THE EQUIVALENT OF AN ATMOSPHERE CONTAINING HALF THE THRESHOLD LIMIT VALUE OF IRON OXIDE SAMPLED BY THE PROPOSED METHOD)

Chemical species	Weight of interfering species/μg
Fluoride (F^-)	1000
Manganese (Mn^{2+})	1000
Phosphate (PO_4^{3-})	1000
Vanadate (VO_3^-)	1000
Zinc (Zn^{2+})	1000
Tin (Sn^{2+})	200
Nickel (Ni^{2+})	50
Tungstate (WO_4^{2-})	50
Chromium (Cr^{3+})	20
Titanium (Ti^{4+})	20

2·5-litre samples of 1 (reagent blank), 5, 10 and 20 mg m^{-3} atmospheres of iron oxide fume when viewed through a solution thickness of 13·5 mm (the internal diameter of glass tubes used for colour comparisons). With these standards it was possible to determine the iron oxide fume content of an atmosphere to the nearest $\pm$2·5 mg m^{-3} between 0 and 10 mg m^{-3}, and at least to the nearest 5 mg m^{-3} between 10 and 20 mg m^{-3}.

Interferences—The effects on the proposed colorimetric method of several chemical species that might occur together with iron oxide in industrial atmospheres were examined by adding known amounts of these species to a solution containing 10 μg of iron (approximately equivalent to a 2·5-litre sample of an atmosphere containing half the present threshold limit value of iron oxide) prior to determining the latter. Under the test conditions the species listed in Table I did not interfere, at least up to the level indicated. These results concurred with a previous study,[5] which included, among others, several of the species examined in the present work. The ratios (w/w) of the interfering species to iron in Table I were already well in excess of those at which the interfering species, on the basis of their respective threshold limit values,[2] became more of a hazard than the iron oxide.

SAMPLING AND COLLECTION OF IRON OXIDE FUME—

Choice of filter-paper—On the basis of published information,[7] filters such as the Millipore, Type AA, were selected as the most suitable for the collection of iron oxide particulates; they had also previously been shown[8] to have virtually a 100 per cent. collection efficiency for all particulates down to a size of 8 nm when sampling is carried out at a face velocity of less than 0·4 m s^{-1}. In the proposed test with an effective collecting area of 255 mm^2 and a sampling rate of 500 ml $minute^{-1}$, the face velocity across the filter is only 0·033 m s^{-1}.

Removal of fume samples from filter-papers—Iron oxide fume atmospheres were prepared by atomising aqueous iron(III) citrate solutions in the apparatus to be described elsewhere.[9] The use of a 10 per cent. w/v iron(III) citrate solution gave an atmosphere containing about 3·0 mg m^{-3} of iron oxide fume but, as was also found[9] in the preparation of a zinc oxide fume, this concentration was neither reproducible from one run to another nor constant during any one run. A series of iron oxide fume samples (sources) was collected on Millipore filters and the response of each with non-destructive X-ray fluorescence spectrometry was noted. These sources were then used in investigations to find a suitable method of dissolving the collected fume in the field, the amounts of iron remaining on the filter-papers after trials with various methods being also assessed by X-ray fluorescence spectrometry.

Several dissolution agents were tried as follows: 50 per cent. v/v hydrochloric acid alone and with each of tin(II) chloride, iodine, hydrogen peroxide, manganese dioxide and hydroxylammonium chloride. The last, consisting of 2 ml of a 1 + 1 v/v mixture of 50 per cent. v/v hydrochloric acid and 20 per cent. w/v aqueous hydroxylammonium chloride solution, showed promise as over 90 per cent. of the iron oxide on a source was dissolved after a 15-minute extraction on a hot-plate. As it was difficult to reproduce the exact temperature with a hot-plate, a steam-bath was adopted as a standard source of heating; by using this extraction of thirteen iron oxide fume samples (ranging from 5 to 45 μg) with the above dissolution agent gave an average recovery of 96·3 $\pm$ 2·9 per cent., which appeared to be independent of extraction times between 5 and 15 minutes; a 10-minute extraction period

TABLE II

SOLUBILITY IN SUCCESSIVE EXTRACTIONS WITH HYDROCHLORIC ACID - HYDROXYLAMMONIUM CHLORIDE SOLUTION* OF IRON OXIDE FUME SAMPLES TAKEN IN THE VICINITY OF INDUSTRIAL WELDING

Sample	Iron found/μg			Total iron in 1st extraction, per cent.
	1st extraction	2nd extraction	3rd extraction	
1	25·0	1·0	0·0	96·2
2	30·0	0·8	0·0	97·4
3	35·0	0·7	<0·1	97·8
4	63·8	1·7	0·0	97·4
5	180·5	4·3	0·0	97·7

* One millilitre of each of 50 per cent. v/v hydrochloric acid and 20 per cent. w/v aqueous solution of hydroxylammonium chloride.

was chosen. The best extraction results were obtained when the two components of the dissolution agent were combined immediately before use. It was noted with this procedure that the number of micrograms of iron recovered from the various samples of collected fume plotted against the respective X-ray fluorescence responses, in counts s^{-1}, gave a straight line.

The above method of dissolution proved satisfactory for iron oxide fume formed in the burner of the generator,[9] but it was found that iron oxide fused at 1000° C for 8 hours was more difficult to dissolve as only about 75 per cent. of it was soluble under the proposed conditions. A heating time of 40 minutes was required for 95 per cent. dissolution. However, it was considered that this would be an extreme case and that iron oxide fume formed in industry, in either welding or casting operations, would not be so highly fused. To check this view, fume samples were collected in the vicinity of industrial welding operations and the extent of their dissolution was examined in the laboratory; each sample was successively treated three times by the proposed dissolution method and the iron extracted in each step was determined separately. Table II lists the results obtained, which show that simply one extraction would be adequate to dissolve iron oxide fume samples collected in the field.

FIELD METHOD FOR THE DETERMINATION OF IRON OXIDE FUME IN AIR

APPARATUS—

Filter-paper holder—To hold filter-papers 25 mm in diameter.

Filter-papers—Millipore, Type AA, 25 mm in diameter.

Sampling pump—A pump capable of drawing air through the filter-paper in the holder at a constant rate of 0·5 l $minute^{-1}$. (This flow-rate can be achieved by using a suitable critical orifice in conjunction with a pump capable of producing an orifice pressure differential of at least 200 mm of mercury. Alternatively, sampling can be carried out with a pump, by monitoring and controlling the flow-rate with a flow-meter and control valve, respectively.)

Spectrophotometer or colorimeter—An instrument capable of measuring the optical densities of solutions at 538 nm.

Colour standards—A comparator disc, containing coloured glass standards for this test, for use with the Lovibond "1000" comparator is obtainable from Tintometer Limited, Salisbury. Four standards are provided representing 1, 5, 10 and 20 mg m^{-3} of iron oxide in air.

Glass tubes for colour comparison—These were of 13·5 mm i.d. and 10-ml capacity (Tintometer Limited supply pairs of tubes suitable for use in conjunction with the Lovibond "1000" comparator).

NOTE—

All glassware should be rendered free from iron by washing it with concentrated hydrochloric acid.

REAGENTS—

All reagents should be of analytical-reagent grade when possible and all solutions prepared with distilled or de-ionised water. All reagents should be stored in iron-free glass or polythene bottles and kept stoppered except when in use.

Hydrochloric acid (1 + 1 *v/v*)—Dilute concentrated hydrochloric acid with an equal volume of water.

Hydroxylammonium chloride solution, 20 per cent. w/v, aqueous.

Buffer solution—Dissolve 33·3 g of hydroxylammonium chloride and 60 g of sodium acetate trihydrate in 360 ml of water.

Disodium 4,7-*diphenyl*-1,10-*phenanthrolinedisulphonate solution*—Dissolve 100 mg of the reagent in 100 ml of water.

Standard iron solution—Dissolve 0·28 g of precipitated iron powder (or electrolytic iron wire) in 20 ml of hydrochloric acid (1 + 1 v/v) and dilute to 1 litre with water (solution A). To 50 ml of solution A, add 20 ml of hydrochloric acid (1 + 1 v/v) and dilute to 1 litre with water (solution B). To 100 ml of solution B, add 4 ml of hydrochloric acid (1 + 1 v/v) and dilute to 200 ml with water (solution C). Solution C contains 7·0 μg ml^{-1} of iron, *i.e.*, the equivalent of 10·0 μg ml^{-1} of iron oxide (Fe_2O_3).

NOTE—

When using visual colour standards for determining the iron oxide content it is important that the reagent blank should be low. If it has a coloration more intense than that of the 1 mg m^{-3} standard, discard the solutions and prepare again with fresh reagents.

PROCEDURE—

Place a filter-paper in the filter holder, attach the assembly to the pump and draw a sample of air through the paper at a rate of 0·5 l minute^{-1} for 5 minutes. Disconnect the holder from the pump, remove the filter-paper and place it in a small beaker (25-ml) of diameter not less than 25 mm. Add 1 ml of hydrochloric acid (1 + 1 v/v) and 1 ml of the hydroxylammonium chloride solution, cover the beaker with a watch-glass and place it on a steam-bath. After 10 minutes remove the beaker from the bath, transfer the acidic solution to a 25-ml calibrated flask and wash the filter-paper thoroughly with 10 ml of water. Add 2 ml of disodium 4,7-diphenyl-1,10-phenanthrolinedisulphonate solution followed by 10 ml of the buffer solution. Dilute to 25 ml with water, mix well, allow to stand for 5 minutes for maximum colour development and determine the iron as iron oxide either visually or spectrophotometrically as described below.

VISUAL DETERMINATION OF IRON OXIDE—

Fill one colour comparison tube with the reaction solution and another with water. Insert both tubes into the comparator and, by using the comparator disc, ascertain the nearest colour match between sample and standards while viewing through the sample.

SPECTROPHOTOMETRIC DETERMINATION OF IRON OXIDE—

Measure the optical density of the reaction solution in a 20-mm glass cell at 538 nm against a reference solution prepared from all of the reagents used. Determine the amount of iron oxide in the solution by reference to the calibration graph. Calculate the concentration of iron oxide in the sample of air, in milligrams per cubic metre, by dividing the total iron oxide in micrograms by 2·5.

Preparation of calibration graph—To a series of 25-ml calibrated flasks add 0, 1, 2, 3, 5 and 6 ml of the standard iron solution C. To each flask add 1 ml of hydrochloric acid (1 + 1 v/v), 1 ml of the hydroxylammonium chloride solution, 2 ml of disodium 4,7-diphenyl-1,10-phenanthrolinedisulphonate solution and 10 ml of buffer solution. Dilute each to 25 ml with water, mix well, allow to stand for 5 minutes and then measure the optical densities of the solutions in 20-mm cells at 538 nm, with the solution to which no iron was added as reference. Construct a graph of micrograms of iron oxide (Fe_2O_3) against optical density.

APPLICATION AND SCOPE OF THE METHOD—

Although specifically designed for the determination of iron oxide fume in the field, the above method is versatile and can be adapted, with minor modifications, to the field or laboratory determination of other iron-containing species, including metallic iron, in the form of fume or dust, or both. Laboratory tests showed that the dusts of particle size less than 45 μm (respirable range) of both iron oxides (Fe_2O_3 and Fe_3O_4) can be determined.

The method can also be used to determine the concentration of water-soluble iron salts (as iron) in air, a threshold limit value (1 mg m^{-3}) for these having been first established[2] in 1969. Provided a sample of exactly 17·5 litres is taken at a rate between 0·5 and 2·5 l minute^{-1}, then the same set of visual colour standards, *i.e.*, the comparator disc, in this case representing 0·1, 0·5, 1·0 and 2·0 mg m^{-3} of soluble iron salts (as iron), respectively, can be used.

This work was carried out on behalf of the Department of Employment and Productivity Committee on Tests for Toxic Substances in Air. We thank the Government Chemist for permission to publish this paper, and H.M. Factory Inspectorate for arranging the field tests.

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The Determination of Acrylamide in Polyelectrolytes by Extraction and Gas-chromatographic Analysis*

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A method is described for the quantitative extraction of acrylamide from water-treatment grade acrylamide polymers and copolymers. The extraction is performed by shaking the polymer with methanol - water (80 + 20) for 24 hours. An improved gas-chromatographic method has been developed for analysing these extracts and can be conveniently used at levels down to 0·0004 per cent. of acrylamide in polymer; 0·05 per cent. of acrylamide in polymer can be determined with a relative standard deviation of ± 4 per cent.

Only two of the polymers tested contained materials that interfered in the gas-chromatographic determination. A thin-layer chromatographic clean-up technique is described, which enables these polymers to be analysed by gas chromatography with no interference.

THE use of polyelectrolytes in water clarification can improve efficiency and reduce costs. It is important for health that the amount of acrylamide in these chemicals is carefully controlled.

The high chronic toxicity of acrylamide[1] necessitates that its concentration in food and drink be limited to very low levels. At present no methods have been published for determining residues resulting from the use of acrylamide polymers and copolymers. It has, therefore, been necessary to specify the acrylamide content of the polymeric materials and the amounts that can be used.[2] To meet these specifications, methods are required that can be used to determine down to 0·01 per cent. of acrylamide in the polymer.

Analytical techniques, such as bromination,[3] chlorobromination,[4] thiol addition,[5] morpholine addition,[6,7] polarography,[8] ultraviolet spectrophotometry[9] and gas chromatography, have been used to determine sub-microgram amounts of acrylamide. Because even 1 per cent. solutions of high molecular weight polyacrylamides are extremely viscous, methods involving polymer solutions present handling and mixing difficulties below 0·1 per cent. of monomer. Methods for extracting acrylamide from the polymer powder or gel include shaking it with aqueous methanol[8] or Soxhlet extraction with methanol.

Initial attempts in these laboratories to determine residual acrylamide in polyacrylamide powders indicated that large differences existed in the recoveries given by the two extraction methods. Accordingly, an investigation was undertaken to develop a procedure that would be capable of extracting 100 per cent. of the acrylamide from a wide range of polymers. Shaking procedures and Soxhlet extraction were investigated. Gas chromatography was used to determine acrylamide, and an improved column packing was developed.

EXPERIMENTAL

POLYMERS STUDIED—

Twenty polymers from seven manufacturers were studied; these, identified by letters A to T, were all based on polyacrylamide or copolymers of acrylamide and acrylic acid. With the exception of polymer N, the molecular weight of the polymers was greater than one million. Polymers A and B were non-ionic, C to S were anionic and T was cationic.

* This paper is based on WRA Technical Report TP.70.

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GAS CHROMATOGRAPHY—

Several columns and conditions of operation were investigated to find the best system for the determination of solutions of acrylamide in methanol and methanol - water mixtures. A glass column, 1 m × 3 mm i.d., packed with 60 to 80-mesh acid-washed dichlorodimethylsilane-treated Chromosorb W supporting 20 per cent. w/w of Carbowax 20M, was found to be the most suitable, although prolonged conditioning was sometimes necessary to achieve satisfactory results. Of those tested, this column gave the least tailing of solvent and acrylamide. When operated at 170° C with a nitrogen flow-rate of 32 ml minute^{-1} the retention time of acrylamide was 4 minutes 20 seconds. On-column injection gave better results than injection into an unpacked-glass hot zone. Peak broadening was noticed after a few weeks' use of on-column injection for the analysis of extracts; this was caused by the build-up of non-volatile components in the injection zone. The column performance was restored by re-packing the first 10 cm of the column with freshly coated support.

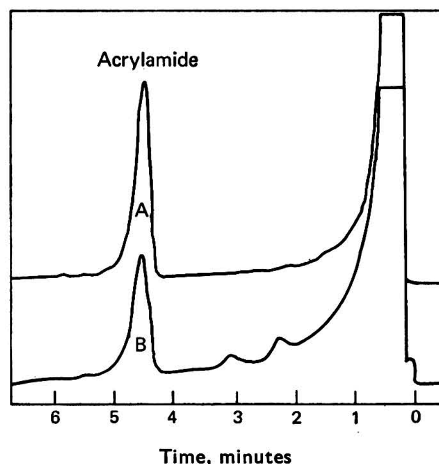

Fig. 1. Gas chromatography of acrylamide: A, 1 μg of acrylamide in 5 μl of methanol + water (80 + 20); and B, 5-μl portion of an extract of polymer containing 0·032 per cent. of acrylamide

A chromatogram of 1 μg of acrylamide in 5 μl of methanol - water (80 + 20) is shown in Fig. 1 (A). Polymer extracts were chromatographed with no difficulty; a typical trace, of a 5-μl portion of an extract of a polymer containing 0·032 per cent. of acrylamide, is shown in Fig. 1 (B). At the lowest levels of acrylamide determined (less than 0·02 per cent. in polymer) some small peaks were apparent; these sometimes interfered in the determination of the acrylamide peak areas.

Quantitative calibrations of the chromatograph were made by measuring peak areas by several different methods. Peak heights were also measured. The amount of acrylamide was then plotted against peak area on log - log paper, for each method of area determination. The amount of acrylamide was also plotted against peak height. A straight-line calibration graph was obtained (Fig. 2) if the peak area was estimated by multiplying peak height by peak width at half height. The relationship between the estimated peak area and the amount of acrylamide injected was not linear on the instrument used. However, a good straight-line calibration was achieved by plotting the results on log - log paper. Methods of measuring peak areas, other than that described above, may be more suitable for use on other instruments; these instruments may give linear calibrations.

The instrument was re-calibrated by using aqueous standards, when aqueous polymer solutions were analysed. The calibration graph differed slightly from that obtained with

methanol - water (80 + 20). No "ghost" peaks were experienced when aqueous solutions were being analysed, provided that the instrument was conditioned between samples by repeated injections of water until no spurious responses were given.

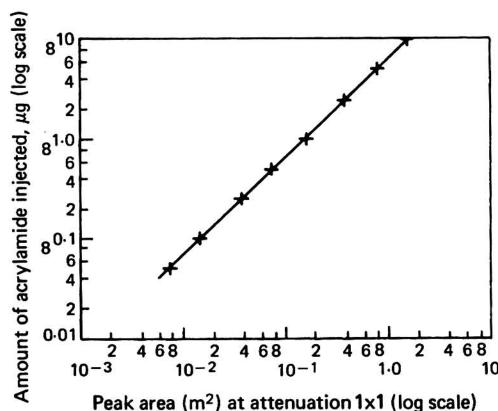

Fig. 2. Calibration of acrylamide *versus* peak area

COLD EXTRACTION OF ACRYLAMIDE FROM POLYMERS—

Polymer powders were shaken with the extracting solvent in a suitable vessel. After settling, 5-μl portions of the supernatant solution were taken for gas-chromatographic analysis.

Graded polymer powders (100 to 120 mesh), as recommended by MacWilliams, Kaufman and Waling,[8] were not investigated as it was considered that sieving of the coarse powders could give rise to non-representative sampling and that the heat generated by grinding the powders could cause losses of acrylamide by evaporation or polymerisation. MacWilliams *et al.*[8] used a mixed-solvent system designed to cause the polymer to swell without dissolving. Manufacturers' literature indicated that other single solvents would also cause the polymers to swell. These solvents were, therefore, compared with the methanol - water (80 + 20) solvent used by MacWilliams *et al.*[8] Preliminary experiments showed that extraction of acrylamide from polymer C reached a maximum after 3 hours when a 2-g sample of polymer was shaken

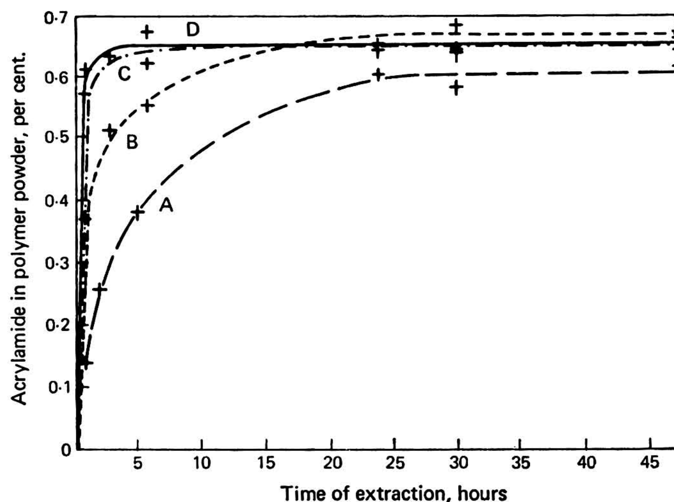

Fig. 3. Cold extraction of polymer I in methanol - water: A, 85 + 15; B, 80 + 20; C, 75 + 25; and D, 70 + 30

with 10 ml of methanol - water (80 + 20). This experiment was repeated with methanol, chloroform and dimethylformamide as the extracting solvents; the acrylamide recoveries after 3 hours, expressed as percentages of the methanol - water (80 + 20) result, were 18, 6 and 2 per cent., respectively. From these results it was concluded that methanol - water was the best extracting solvent tested.

To ascertain the proportions of water and methanol that would give the most satisfactory extraction of acrylamide, 2-g samples of three polymers were shaken with 70 + 30, 75 + 25, 80 + 20 and 85 + 15 methanol - water mixtures until extraction was at a maximum. Portions were taken for determination approximately every hour at the beginning of the experiments, and then less frequently. Initial experiments had indicated that some polymers were more difficult to extract than others. The three used for these experiments were polymers C, I and F: C was easy to extract and had a relatively high acrylamide content, I was difficult to extract and had a relatively high acrylamide content, and F was difficult to extract and had a relatively low acrylamide content.

Maximum recovery of acrylamide from polymer C was 0·62 per cent. of acrylamide in polymer powder, and was constant after shaking it for 1 hour with methanol - water mixtures in the proportions (70 + 30), (75 + 25) and (80 + 20). Acrylamide recovery was only 0·53 per cent. of acrylamide in polymer powder with methanol - water (85 + 15). This level was constant after 4 hours' shaking. The duration of the experiment was 24 hours. The results for polymer I are shown in Fig. 3. Higher proportions of water to methanol gave

TABLE I

EFFECT OF THE AMOUNT OF ACRYLAMIDE EXTRACTED BY 10 ml OF SOLVENT

Polymer	Weight of polymer extracted, g	Mean acrylamide found in polymer powder, per cent.	Standard deviation	Relative standard deviation, per cent.
C	2·0	0·61	±0·01	±2
	0·2	0·60	±0·02	±3
D	2·0	0·093	±0·004	±4
	0·2	0·091	±0·004	±4
I	2·0	0·65	±0·01	±2
	0·2	0·65	±0·02	±3
F	2·0	0·024	±0·001	±4
	0·2	0·023	±0·002	±9

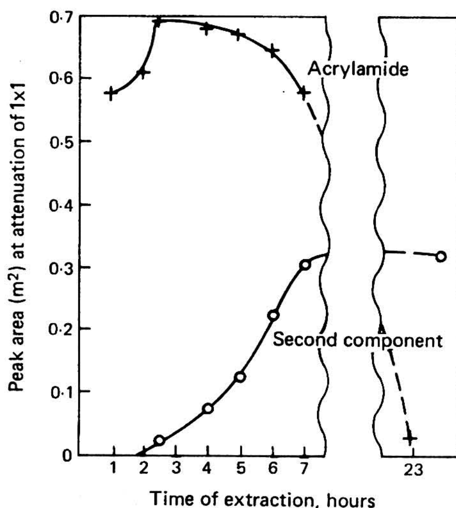

Fig. 4. Soxhlet extraction of polymer C with methanol

faster extraction, and methanol - water (85 + 15) again gave low recoveries. The rate of extraction from polymer F was similar to that from polymer I; however, methanol - water (85 + 15) gave the same acrylamide recoveries as methanol - water mixtures (80 + 20) and (75 + 25) after 24 hours' shaking. No result was obtained for methanol - water (70 + 30) as this caused the polymer to gel. Because methanol - water (75 + 25) caused polymer N to gel, methanol - water (80 + 20) was considered to be the most suitable extracting solvent provided that an extraction time of at least 24 hours was used.

To determine whether the amount of polymer extracted by 10 ml of solvent affected the recovery of acrylamide, 24-hour extracts were prepared with 2·0 and 0·2 g of polymer. Four polymers were investigated, C, D, I and F. C and D were easy to extract and had high and low acrylamide contents, respectively; I and F were difficult to extract and had high and low acrylamide contents, respectively. Eight replicate experiments were performed with each set of conditions. The results of these experiments are shown in Table I. Statistical analysis showed that there was no significant difference at the 10 per cent. level in the percentage of acrylamide found in the polymer, whether 2·0 or 0·2 g of polymer was extracted. During these experiments adequate agitation was found to be extremely important. Initially the polymers were tumbled with the solvent in stoppered test-tubes. This gave consistent results with some polymers, but others tended to aggregate and gave low recoveries when 2·0 g of polymer were extracted. Different shapes of vessel and types of agitation were investigated to overcome these difficulties. The most satisfactory method of agitation was by shaking with a laboratory flask-shaker. A horizontally clamped 1-oz McCartney bottle was a convenient vessel for these experiments, although other types of vessel, such as round-bottomed or conical flasks, were equal in performance.

The maximum weight of polymer N that could be extracted was 1 g, because of its excessive swelling with the solvent; 1 g was therefore the largest amount of polymer that could be extracted with 10 ml of methanol - water (80 + 20) if the extraction method was to be applicable to all of the polymers tested.

To ascertain that 24 hours was a sufficiently long extraction time to be generally applicable, 1-g samples of fourteen polymers (A, E, G, H, J, K, L, M, N, P, Q, R, S and T) were extracted with 10 ml of methanol - water (80 + 20), in addition to the polymers already tested. Portions of the extracts were taken for analysis at similar time intervals to those used in previous experiments. The maximum recovery of acrylamide from all of the polymers tested was reached within 24 hours. The levels of monomer found in the polymers ranged from 0·017 to 0·19 per cent.

Extracts stored on the bench top were stable for about 2 days, then some extracts showed a loss of acrylamide or the appearance of materials that interfered in the gas - liquid chromatographic determinations, or both. Samples stored in a refrigerator were stable for at least 1 week. Longer periods of storage were not investigated.

SOXHLET EXTRACTION—

Polymer powder (10 g) was weighed into a 90 × 19-mm Soxhlet thimble and the top was plugged with cotton-wool. The thimble was then placed in the extraction apparatus, the flask of which contained 40 ml of extracting solvent, and the sample was moistened with the solvent before commencing extraction. After extraction the solution in the flask was cooled and made up to 50 ml with the extracting solvent. Portions of the solution were then taken for analysis. Initial experiments indicated that Soxhlet extraction for 24 hours with methanol gave lower recoveries of acrylamide than extraction with cold aqueous methanol, and that the gas chromatograms of extracts of most of the polymers showed a second component. The amount of this material, which had a retention time of 1·7 relative to acrylamide, increased with extraction time.

Polymer C was extracted with methanol for 23 hours. Portions of the extract were analysed every hour for 7 hours and then after 23 hours; the results are shown in Fig. 4. The maximum acrylamide recovery occurred after 2½ hours, then it decreased. The second component appeared after 2½ hours and then continued to increase throughout the period of extraction. The maximum recovery of acrylamide represented 0·48 per cent. of acrylamide in the polymer powder. The percentage of acrylamide found in polymer C by cold extraction with methanol - water (80 + 20) was 0·61 per cent. This experiment was repeated with polymer I. The maximum recovery of acrylamide occurred at 22 hours, and represented

an acrylamide content of 0·015 per cent. in the polymer powder. The amount of acrylamide found in this polymer by cold extraction with methanol - water (80 + 20) was 0·65 per cent.

The addition of 2 per cent. of glacial acetic acid to hot methanolic extracts of a polymer has been used to inhibit the decomposition of acrylamide. Polymer I was extracted with methanol - glacial acetic acid (98 + 2). Recovery of acrylamide was not complete after 23 hours, and at that time corresponded to about 10 per cent. of the recovery obtained by cold extraction with methanol - water (80 + 20).

As methanol - water (80 + 20) was such an excellent solvent for cold extraction by shaking, it was decided to use it as a solvent in Soxhlet extraction. Maximum acrylamide recovery from polymer I was obtained after 1¾ hours and represented an acrylamide content of 0·05 per cent. compared with 0·65 per cent. by cold shaking. The second component appeared after 1¼ hours and increased throughout the extraction in a similar manner to the results shown in Fig. 4. A mixture of methanol - water - glacial acetic acid (80 + 18 + 2) was then investigated to see whether the presence of the acid would prevent loss of acrylamide. Polymers I, C and N were extracted. Polymer N was tested as it was thought that, because of its low molecular weight, excessive gelation might be experienced when a repeated extraction with an aqueous solvent was performed. This did not occur. Maximum recoveries of acrylamide were identical with recoveries obtained by shaking with methanol - water (80 + 20), and occurred after 3, 2½ and 5 hours for polymers I, C and N, respectively. The extractions were continued for 24 hours, the acrylamide recovered remaining constant for polymer N, but dropping slightly for polymers I and C. No second component appeared in the polymer I or N extracts, and only a very small amount in the polymer C extracts. Although in two instances the acrylamide concentration reached a peak and then dropped, the loss of acrylamide was much less than when glacial acetic acid was not present. The solvent system used with Soxhlet extraction appears to be suitable for the determination of acrylamide in polymers, provided that care is taken to follow the extraction to optimum recovery. It was not as convenient as the cold extraction method.

The second component was not formed when acrylamide was refluxed with anhydrous or aqueous methanol for several days, nor was any loss of acrylamide noted. After refluxing with 0·2 per cent. w/v potassium hydroxide in anhydrous methanol, however, the second component was produced within 15 minutes. It appears, therefore, that the degradation of acrylamide in Soxhlet extracts is catalysed by base extracted from the polymer; if so, it explains the suppression of the reaction by acetic acid. The structure of the second component, which was not acrylic acid, has not been elucidated. It may be formed by the base-catalysed addition of methoxide across the acrylamide double bond, or may be an adduct of acrylamide with itself or acrylic acid.

ANALYSIS OF POLYMER SOLUTIONS—

To check the possibility that aqueous methanol extraction did not remove all of the acrylamide from the polymer powders, some acrylamide determinations were made by gas-chromatographic analysis of aqueous polymer solutions. This was possible only with polymers C and I, which had acrylamide contents greater than 0·5 per cent. The most concentrated aqueous polymer solution that could be injected into the gas chromatograph was 0·1 per cent., as more concentrated solutions are too viscous to give satisfactory injection, so that the determination of acrylamide residues of less than 0·5 per cent. was too close to the limits of sensitivity of the gas chromatograph to give satisfactory results. The investigation was centred on polymer I as it was more difficult to extract by the aqueous methanol method. Three portions of each of four solutions of polymer I were determined. The mean percentage of acrylamide found was 0·65 per cent., and its standard deviation was ±0·02 per cent. These results were not significantly different from the values obtained by aqueous methanol extraction. Three portions of a solution of polymer C were analysed, the mean percentage of acrylamide found being 0·61 per cent. This result was in agreement with those obtained by aqueous methanol extraction.

THIN-LAYER CHROMATOGRAPHY AND INFRARED SPECTROSCOPY—

Gas chromatography does not give positive identification of separated components, as several compounds may behave identically on any one column. It was decided, therefore,

to gain additional information about the identity of the "acrylamide" extracted from polymers. Thin-layer chromatography and infrared spectroscopy were used in the investigation.

Acrylamide was successfully determined chromatographically on silica gel plates with acetone or chloroform - methanol (80 + 20) as the developing solvent. The R_F values in an "S-tank" were 0·75 and 0·42, respectively. The former solvent gave more consistent results. The spots were made visible with the fluorescein - bromine reagent,[10] or by spraying with 0·01 per cent. potassium permanganate in acetone. The latter reagent was more sensitive (down to 0·25 μg) and consistent, the acrylamide showing as yellow spots on a pink background that became yellow after a few hours. The chromatograms could be made permanent for at least 4 days by carefully overspraying with 0·01 per cent. bromophenol blue in acetone - water (20 + 1) before the background had changed. The dye was bleached by the permanganate background but not by the spots, which showed as blue on a buff background. The life of the permanganate spray reagent was, at the most, 30 minutes, and it was prepared, as required, by dilution of a more concentrated aqueous solution with acetone. The bromophenol blue solution could be used for 1 week after preparation.

Portions of extracts of polymers B, C, D, E, M and O were determined by thin-layer chromatography with acrylamide standards. Semi-quantitative evaluation of the sprayed chromatograms showed levels of acrylamide in polymer powder that were not significantly different from the results obtained by gas chromatography. With polymers E and M the acrylamide portions of unsprayed chromatograms, samples and standards were scraped off the plates and the acrylamide was eluted from the adsorbent with methanol - water (80 + 20). The eluates were then analysed by gas chromatography. The recovery of acrylamide standards was 90 per cent. Allowing for this recovery, the percentage of acrylamide found in the polymer powders from the samples that had undergone thin-layer separation (0·083 and 0·10 per cent.) was not significantly different from the percentage found by gas chromatography of the extracts before thin-layer separation (0·085 and 0·10 per cent.). It was, therefore, confirmed that the material in the sample extracts, which was identified as acrylamide by gas chromatography, behaves as acrylamide in thin-layer chromatography.

Because it showed a relatively high acrylamide content, a 20-g extract of polymer C was prepared for infrared spectroscopy. A portion of this extract, equivalent to about 2 mg of acrylamide, was then evaporated to dryness on to potassium bromide, and a disc was pressed. The infrared spectrum of this sample did not correspond to acrylamide, but was so intense that other material was obviously present in a far greater amount than acrylamide. A portion of this extract was determined by thin-layer chromatography and the portion of the chromatogram corresponding to acrylamide was scraped off. The material was then eluted with methanol and the methanol solution was evaporated to dryness on potassium bromide. The spectrum of the disc pressed from this material was identical with that of acrylamide standards treated in the same manner. An estimation of the amount of acrylamide in the polymer powder made from the intensity of the infrared spectrum was between 0·5 and 1·0 per cent., which agreed well with the values of 0·60 to 0·61 per cent. found by gas - liquid chromatography.

Thus by infrared spectroscopic measurements it has been confirmed that the thin-layer chromatographic spot corresponding to acrylamide in the polymer extracts is due to acrylamide. As the gas - liquid chromatographic acrylamide peak behaves as acrylamide on thin-layer chromatography it is confirmed that the gas - liquid chromatographic acrylamide peak is due to acrylamide. This last statement is strictly true only for polymer C, and the above experiments should be repeated for every polymer tested. As all of these polymers are prepared by similar processes however, it is reasonable to assume that this should apply to all of the products.

THIN-LAYER CLEAN-UP—

Gas chromatograms of extracts of polymers B and O showed that materials with similar retention times to that of acrylamide were present, which made determination of acrylamide impossible without further purification of the extracts. Attempts to purify the extracts with ion-exchange resins,[8] silica gel in a similar manner to ion-exchange resins, and column chromatography with silica gel, alumina or Florisil were either only partly successful or totally unsuccessful. Excellent purification was obtained by thin-layer chromatography of a 40-μl

portion of the extract and elution of the acrylamide from the relevant portion of the chromatogram as described previously, but by using re-distilled acetone. Care was taken to prevent loss of acrylamide at the evaporation step, and it was essential to stop the air stream immediately the extract became dry. The most consistent results were obtained when there was no delay between successive steps of the analysis.

Method

Apparatus—

Gas chromatograph—Perkin-Elmer, Model F11, equipped with a glass column, a flame-ionisation detector and an analyser unit.

Recorder—Hitachi Perkin-Elmer 159, 0 to 2·5 mV full-scale deflection.

Column—This was 1 m × 3 mm i.d., glass-packed, with 60 to 80-mesh acid-washed dichlorodimethylsilane-treated Chromosorb W, supporting 20 per cent. w/w of Carbowax 20M. The syringe needle penetrated to a depth of 2 cm into the packing for injection. The temperatures of the column, the injection point and the detector were 180 °C. The nitrogen flow-rate was 32 ml minute^{-1}.

Microsyringes—These were 10-µl Hamilton with 125-mm needle and 50-µl Hamilton with standard needle.

Flask-shaker, Microid.

McCartney bottles, 1 *oz.*

Pipettes—Grade B, 50, 25 and 10 ml.

Calibrated flasks—Eight flasks of 100-ml capacity.

Thin-layer spreader bed, spreader box and plates (200 *mm*^{2})—Quickfit and Quartz.

Thin-layer sample applicator and capillary pipettes—As described by Curtis.[11]

"S"-tank—As described by Bancroft.[12]

Thin-layer chromatographic spray gun—Shandon Scientific Co.

Supply of dry air or inert gas.

Conical flask, 150-*ml capacity.*

Graduated centrifuge tubes, 10-*ml capacity.*

Elution columns—Glass tubing, 30 × 3 mm i.d., drawn to a fine tip and plugged with a small piece of cotton-wool. The columns were washed with acetone before use.

Reagents—

Acetone—Laboratory-reagent grade.

Acrylamide—Laboratory-reagent grade, obtainable from B.D.H. Ltd. (better than 99 per cent. pure).

Methanol—Analytical-reagent grade.

Potassium hydroxide, Analar.

Silica gel G, Merck.

Thin-layer chromatographic spray reagents—Potassium permanganate, 0·01 per cent., in acetone; and bromophenol blue, 0·01 per cent., in acetone - water (20 + 1).

Preparation of standards—

Dissolve 200 mg of acrylamide in methanol - water (80 + 20) in a 100-ml calibrated flask. Make up to the mark with methanol - water (80 + 20). This gives a standard of 2 mg ml^{-1} of acrylamide, or 10 µg of acrylamide per 5 µl of solvent. Dilute to give standards of 5·0, 2·5, 1·0, 0·5, 0·25, 0·1 and 0·05 µg of acrylamide per 5 µl of solvent.

Calibration of gas chromatograph—

Inject 5-µl portions of each of the above standards into the chromatograph with the 10-µl syringe and use amplifier attenuations such that the peak heights are less than 60 per cent. of the full-scale deflection. Measure the area of the acrylamide responses as the product of the peak height and the peak width at half height. Plot peak area against amount of acrylamide injected on log - log graph paper. With the extraction method given below, the calibration graph covers acrylamide in polymer contents of 0·01 to 2 per cent.

Extraction of polymer—

Weigh 1 g of polymer powder into a 1-oz McCartney bottle. Add, by pipette, 10 ml of methanol - water (80 + 20), screw the cap on firmly and shake the bottle to disperse the

polymer. Clamp the bottle horizontally in a Microid flask-shaker and shake it vigorously for 24 hours. Vigorous shaking is essential as insufficient agitation leads to poor extraction. After shaking the bottle remove it from the shaker, stand it upright and allow to settle for 15 minutes.

GAS - LIQUID CHROMATOGRAPHIC ANALYSIS—

Inject a 5-μl portion of sample extract into the gas chromatograph. Measure the area of the acrylamide peak and read off the equivalent amount of acrylamide from the calibration graph. The percentage of acrylamide in the polymer can be calculated as $0{\cdot}2X$, where X is the amount of acrylamide, μg, in the 5-μl portion of extract.

PREPARATION OF THIN-LAYER PLATES—

Shake 30 g of silica gel with 58 ml of distilled water in the 150-ml conical flask for 1 minute. Spread the slurry, 0·25 mm thick, across five 20-cm^2 plates with the spreading apparatus. Allow the plates to dry in air until the surface moisture has evaporated, then activate them at 120° C for 1 hour. Cool and store them in a dry box until required.

THIN-LAYER CHROMATOGRAPHY—

Scrape a band 1 cm wide from the top and two edges of the thin-layer chromatographic plate. With the spotting apparatus apply 40 μl of sample extract to the plate. On either side of the sample apply 40 μl of the 0·25 μg per 5 μl standard. The capillary pipettes were filled by using the 50-μl syringe, and quantitative transfer was achieved by washing the pipettes twice with 5 μl of methanol - water (80 + 20), and applying the solvent also to the plates. Restrict the spot diameters to a maximum of 5 mm. Dry the spots for 5 minutes with a gentle current of dry air, then transfer to the "S"-tank and develop to 100 mm from the spots with acetone as the developing solvent. Dry in air for 1 minute to remove the acetone, cover the sample chromatogram and spray the standard chromatograms lightly, first with the potassium permanganate reagent, then with the bromophenol blue reagent. The acrylamide will be visible as blue spots on a buff background, R_F 0·75. The amount of reagent sprayed affects the spot intensity, and practice is necessary to achieve optimum results.

ELUTION OF ACRYLAMIDE—

Locate the acrylamide in the sample chromatogram by comparison with the visible standards running beside it. Scrape the relevant portion of adsorbent from the plate and pack it into an elution column. Elute the acrylamide into a centrifuge tube with acetone. Collect 0·5 ml of eluate and evaporate this just to dryness at room temperature with a gentle current of dry air. Dissolve the residue in 40 μl of methanol - water (80 + 20). Care must be taken to remove the air stream as soon as the last trace of solvent has evaporated, as failure to do so results in serious loss of acrylamide. Use 5-μl portions of the solution for gas-chromatographic analysis.

RESULTS

GAS CHROMATOGRAPHY—

Ten replicate injections were made of amounts of acrylamide representing the top, middle and bottom sections of the calibration graph. The results and their standard deviations are shown in Table II.

TABLE II

STANDARD DEVIATIONS OF REPLICATE INJECTIONS OF ACRYLAMIDE

Amount of acrylamide injected, μg	Mean peak area (m^2) at attenuation of 1 × 1	Standard deviation, m^2	Relative standard deviation, per cent.
10	1·59	±0·01	±0·6
1·0	0·152	±0·003	±2·0
0·05	$58{\cdot}5 \times 10^{-4}$	$\pm 0{\cdot}2 \times 10^{-4}$	±3·6

The over-all base-line instability at an attenuation of 1 × 1 was 8 mm. The smallest amount of acrylamide that can be detected can be defined as the amount giving a peak of double that height.[13] A 16-mm peak at an attenuation of 1 × 1 would be given by 0·002 μg

of acrylamide. Thus if 1 g of polymer is extracted with 10 ml of solvent and 5 μl of the extract is chromatographed it should be possible to detect 0·0004 per cent. of acrylamide in polymer powder.

EXTRACTION AND ANALYSIS—

The results obtained by the analysis of eight replicate samples of four different polymers under two sets of conditions for each polymer are shown in Table I.

The over-all relative standard deviation of the shaking-extraction gas-chromatographic analysis method of determining acrylamide in polymer powder varied between $\pm$2·0 per cent. when 2 g of polymer with an acrylamide content of 0·61 per cent. was extracted with 10 ml of solvent, and $\pm$9 per cent. when 0·2 g of a polymer with an acrylamide content of 0·021 per cent. was extracted with 10 ml of solvent. From these values (Table I) the relative standard deviations to be expected when extracting 1 g of polymer with 10 ml of solvent have been calculated. These would be $\pm$2 per cent. relative standard deviation at an acrylamide content of greater than 0·2 per cent. in polymer powder, rising to $\pm$4 per cent. between 0·1 and 0·02 per cent. of acrylamide in polymer powder, and about $\pm$6 per cent. at 0·01 per cent. of acrylamide in polymer powder. Comparison of the figures in Tables I and II shows that the errors in the extraction and gas-chromatographic steps are similar at all levels of acrylamide in polymer.

THIN-LAYER CLEAN-UP METHOD—

Eight samples of 0·05 μg of acrylamide, equivalent to an extract of a polymer containing 0·01 per cent. of acrylamide, were taken through the procedure. The mean recovery was 91 per cent. and the standard deviations of individual analyses lay within the limits $\pm$2 per cent. Samples of polymers E and M, which did not contain interfering materials, were also taken through the procedure. Allowing for the above recovery, the percentage of acrylamide found in the polymer powders from the samples that had undergone thin-layer separation (0·083 and 0·10 per cent.) was not significantly different from the percentage found by gas chromatography of the extracts before thin-layer separation (0·085 and 0·10 per cent.).

DISCUSSION

It has been shown that gas chromatography permits small amounts of acrylamide to be determined accurately, and that the component of polymer extracts identified by gas chromatography as acrylamide is in fact acrylamide. Only two of the polymer extracts contained materials that seriously interfere in the gas-chromatographic determination of acrylamide; these extracts were successfully purified by thin-layer chromatography. The method has proved to be quick, reliable and easy to use in these laboratories.

A comparison of acrylamide determinations performed on aqueous solutions of polymers and aqueous methanol extracts has shown that the latter system is capable of extracting all of the acrylamide from a polymer powder. The extraction can be performed by vigorously shaking 1 g of the powder with 10 ml of methanol - water (80 + 20) for 24 hours or by Soxhlet extraction. Other cold extraction conditions give complete extraction of individual polymers, sometimes in a shorter time, but only the above conditions were applicable to all of the polymers tested. Soxhlet extraction leads to breakdown of acrylamide in the boiling solution. However, by choosing a suitable solvent system [*e.g.*, methanol - water - glacial acetic acid (80 + 18 + 2)] all of the acrylamide could be extracted from a polymer powder in a shorter time than with the shaking method. The extract must be analysed at regular intervals during the extraction, as even with the above solvent system the recovery of acrylamide appears to drop after reaching the maximum. Because of the greater attention required with the Soxhlet extraction procedure, the shaking method in the cold is the preferred technique in these laboratories.

CONCLUSIONS

A quantitative extraction of acrylamide from all the acrylamide polymers and copolymers tested was obtained after shaking them with methanol - water (80 + 20) for 24 hours, without previous sample preparation. This was not so with other methods. Flame-ionisation gas chromatography with Carbowax columns has proved an excellent method for the analysis of most of the polymer extracts without further sample preparation. This method is more

sensitive (down to 0·0004 per cent. of acrylamide in polymer) than titrimetric techniques, which are difficult below 0·1 per cent. of monomer in polymer, and does not suffer from interferences arising from acrylic acid, which interferes in the ultraviolet, the polarographic and some titrimetric methods.

A thin-layer clean-up technique has been developed for the two polymers that showed interferences with the gas-chromatographic analysis.

I thank the Director of the Water Research Association for permission to publish this paper, and Mr. M. W. Hart for carrying out much of the practical work. Acknowledgements are also given for the work of the Allied Colloids Co. on thiol addition methods of analysis, and to I.C.I. Ltd. (Dyestuffs Division) for the original work on Soxhlet extraction, extract stabilisation and gas chromatography of acrylamide; the co-operation of these companies is greatly appreciated.

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The Determination of 3,5-Dinitrosalicyl-(5-nitrofurfurylidene)hydrazide (Nifursol) in Animal Feeding Stuffs by Electron-capture Gas Chromatography

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A method is described for the determination of 3,5-dinitrosalicyl-(5-nitrofurfurylidene)hydrazide (nifursol) in animal feeding stuffs. The additive is extracted from the feed with acetonitrile, and after further clean-up with carbon disulphide the nifursol is reacted with boron trifluoride - methanol complex. The product, methyl 3,5-dinitrosalicylate, is determined by gas chromatography with an electron-capture detector.

3,5-Dinitrosalicyl-(5-nitrofurfurylidene)hydrazide (nifursol) is incorporated in poultry feeds at a level of 75 p.p.m. as a histomonacide and growth-promoting compound. It can be determined polarographically[1] and colorimetrically,[2] but neither the polarographic procedure, based on the reduction of the nitro group, nor the colorimetric reaction, involving phenylhydrazone formation, is specific in these determinations. The gas-chromatographic technique described below, which involves electron-capture detection of methyl 3,5-dinitrosalicylate, is sensitive and virtually specific for nifursol, as this derivative cannot be obtained from any other additive in common use. Methyl 3,5-dinitrosalicylate is prepared by cleavage of the nifursol molecule and esterification of the 3,5-dinitrosalicylic acid thus produced; these two stages can be carried out in one operation by the use of boron trifluoride - methanol complex. The resulting methyl 3,5-dinitrosalicylate is separated from interfering substances by solvent partition.

Experimental

Apparatus—

An isothermal gas chromatograph equipped with an electron-capture detector (*e.g.*, Varian Aerograph 1400 series) was used.

Column—Stainless steel, 150 cm in length and 2 mm i.d.

Column packing—Silanised Chromosorb W, 80 to 100 mesh, coated with 5 per cent. neopentyl glycol succinate and 1·75 per cent. orthophosphoric acid (obtained by diluting orthophosphoric acid of sp.gr. 1·75), the support being coated by evaporating a methanol solution of orthophosphoric acid, then adding a chloroform solution of neopentyl glycol succinate and removing the chloroform. The column was conditioned at 230° C for 48 hours.

Operating conditions—Column temperature, 210° C; detector temperature, 210° C; and nitrogen flow-rate, 150 ml minute^{-1}.

Reagents—

Acetonitrile.

Boron trifluoride - methanol complex, containing approximately 14 *per cent. of* BF_3.

Carbon disulphide.

Dimethyl sulphoxide.

Hexane—General-purpose reagent grade.

Hydrochloric acid, approximately 5·5 N—Mix one volume of concentrated hydrochloric acid with one volume of water.

Methyl red—A 0·01 per cent. solution in 50 per cent. ethanol.

Nifursol standard solutions—Dissolve appropriate amounts of nifursol in dimethyl sulphoxide to give solutions containing 100, 200, 300, 400 and 500 μg ml^{-1}.

Sodium sulphate, granular, anhydrous.

Toluene.

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PROCEDURE—

Grind the feed sample to pass through a B.S. 16-mesh sieve and mix thoroughly. Weigh 10·0 g of the prepared feed (for nifursol contents up to 100 p.p.m.) and transfer to a stoppered 250-ml flask. Add 100 ml of acetonitrile and shake the mixture vigorously for 1 hour on a mechanical shaker. (CAUTION—Safety pipettes must be used to measure acetonitrile and solutions containing it.) Centrifuge the suspension and carry out the following operations in a fume cupboard because of the toxic nature of acetonitrile. Transfer 25 ml of the clear supernatant liquor to a 50-ml separator and add 25 ml of carbon disulphide, shake the mixture vigorously for 30 s and allow it to separate. Discard the lower layer and transfer the upper acetonitrile layer to a stoppered vessel.

To a pear-shaped flask of 10-ml capacity that has a neck with a standard ground-glass joint, add 0·1 ml of dimethyl sulphoxide and then, by pipette, 5 ml of the extract. Add anti-bumping granules and evaporate the solution to a small volume on a steam-bath by using a micro-Snyder column.[3] Remove the final traces of acetonitrile with a gentle stream of air. Add 5 ml of boron trifluoride - methanol complex and additional anti-bumping beads, and boil under reflux for 15 minutes. Transfer the contents of the flask to a 50-ml separator by using a Pasteur pipette, wash the flask with 25 ml of 5·5 N hydrochloric acid, and add the washings to the separator. Add 10 ml of hexane and 2 drops of methyl red indicator and shake the mixture for 30 s. (The methyl red enables the two layers to be distinguished.) Transfer the lower aqueous layer to a second separator and shake it with 10 ml of toluene for 30 s. Pass the toluene layer down a column plugged with glass-wool and containing 5 g of anhydrous sodium sulphate; collect the eluate in a stoppered test-tube.

Prepare a series of nifursol standards by adding 0·1 ml of the stock standard solutions to pear-shaped flasks and proceed as described above commencing: "Add 5 ml of boron trifluoride - methanol complex and additional anti-bumping beads, and boil under reflux for 15 minutes. . . ." Inject 5-μl aliquots of samples and standards on to the column by using a microlitre syringe. Measure the peak height of the chromatographic peaks corresponding to methyl 3,5-dinitrosalicylate and compare the sample response with a graph prepared from the standard series. Calculate the nifursol content of the sample from the relationship—

$$A = B \times 116/5$$

where A is the nifursol content of the sample, B is the weight of nifursol indicated by the standard curve and 116/5 is the correction factor accounting for the increased volume of the acetonitrile phase after partitioning with carbon disulphide (*i.e.*, 100 ml increases in volume to 116 ml).

RESULTS

Under the described chromatographic conditions, the retention time of methyl 3,5-dinitrosalicylate was 4·3 minutes. The detection limit for this compound was about 0·25 ng and the peak height - concentration curves were linear over the range 1 to 10 ng.

TABLE I

RECOVERY OF NIFURSOL ADDED TO FEEDS

	Added, p.p.m.	Found, p.p.m.	Recovery, per cent.
Battery layers mash	70·0	69·6, 68·5, 68·5	99·4, 97·9, 97·9
	69·8	68·5, 69·0, 69·5	98·1, 98·8, 99·6
	100·0	99·7, 96·5, 100·3	99·7, 96·5, 100·3
	40·0	44·0, 41·0, 41·8	110·0, 102·5, 104·5
Baby chick crumbs (stated to contain 100 p.p.m. of nitrofurazone and added vitamins)	70·0	67·5, 68·5, 67·5	96·4, 97·9, 96·4
	72·0	67·8, 69·5, 70·0	94·1, 96·5, 97·2
Broiler starter crumbs	70·0	68·7, 70·0, 68·2	98·3, 100·0, 97·5
	65·8	65·0, 66·0, 65·8	98·8, 100·3, 100·0
	100·0	102·0, 103·2, 99·2	102·0, 103·2, 99·2
	40·0	39·5, 41·2, 39·4	98·5, 103·0, 98·5
Coarse grain balancer	70·0	68·5, 67·2, 66·0	97·8, 96·0, 94·2
	69·8	67·3, 71·8, 65·0	96·4, 102·9, 93·1

Nifursol was added as a 1 per cent. solid suspension in talc B.P.

All feeds were commercial samples and no information as to additive content was available except for the baby chick crumbs.

Recoveries of nifursol from medicated samples determined by the procedure described are shown in Table I. Nifursol was added to the feed either as a freshly prepared solution in acetone (the solvent was subsequently removed in a gentle stream of air) or by the addition of a known weight of talc mixture containing 1 per cent. of nifursol.

Discussion

The choice of extractants for nifursol was subject to the dual requirements of affinity for nifursol together with low affinity for other feed components. Of the solvents examined, *viz.*, acetone, methanol, ethyl acetate and acetonitrile, the last gave the lowest level of co-extraction. The subsequent partition between acetonitrile and carbon disulphide removes residual material that interferes with the formation of methyl 3,5-dinitrosalicylate during the reaction with the boron trifluoride - methanol complex. Other solvents that are immiscible with acetonitrile, for example, hexane, cyclohexane and isooctane, were found not to remove such interferences so that low yields resulted. The insolubility of methyl 3,5-dinitrosalicylate in hexane enabled a further clean-up to be introduced before the final extraction with toluene, so that the extract injected on to the gas-chromatographic column is low in co-extractives and gives minimal column contamination.

The reaction of nifursol with boron trifluoride - methanol complex to give methyl 3,5-dinitrosalicylate proceeds rapidly, with a high yield. Typical yields derived from 10 μg of nifursol, when following the hexane and toluene partitioning stages described above, were 75 to 85 per cent. of the theoretical. Some etching of glassware was encountered with the reagent, but this occurred only after prolonged use and is not a drawback.

Selection of a gas-chromatographic column for methyl 3,5-dinitrosalicylate was influenced by the acidic character of the compound arising from its free phenolic group. No previous references to the gas chromatography of this compound could be found, and only by the use of supports modified with orthophosphoric acid, such as those used for dinitrophenols,[4] could non-tailing peaks be obtained. Decomposition of the compound was found to occur when using "flash" injection, and "on-column" injection was used throughout this study. Methyl 3,5-dinitrosalicylate is not known to be commercially available for use as a reference in gas chromatography but it can be readily prepared from 3,5-dinitrosalicylic acid by the Fischer-Speier method of esterification.[5] No interfering gas-chromatographic peaks arising from feed extractives were encountered in this work, and the method is not subject to interferences from other currently used prophylactic additives containing a nitrofurfuraldehyde moiety, *e.g.*, nitrofurazone and furazolidone.

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The Determination of Nicotine in Human Blood by Gas - Liquid Chromatography

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A method has been developed for the extraction and determination of sub-microgram amounts of nicotine in blood. It involves steam distillation of the nicotine followed by solvent partition and column-chromatographic clean-up. The final solution of nicotine in ethanol is injected directly on to the column of a gas - liquid chromatograph fitted with a flame-ionisation detector. The method can be used to determine down to 1 ng of nicotine, and has been applied to the measurement of nicotine levels found in the blood of smokers while smoking cigarettes.

THE metabolism of nicotine is now well understood in several species of animals, including man.[1,2,3] The processes of absorption[4,5,6] and distribution[3,7] of nicotine in humans while smoking have not been so fully investigated, particularly with reference to the pharmacological[8] and physiological[8,9,10] activities. In order to correlate these phenomena it is important to determine quantitatively the amount of nicotine circulating in the body fluids of smokers.[10,11] Recently gas-chromatographic measurements have been made to detect and determine the nicotine present in body fluids.[12,13,14] However, these methods have, in general, been used to determine amounts of nicotine in the urine of smokers,[12,13] in which it is present in larger amounts than those found in venous blood.[14] Adapting these methods for use with blood - nicotine analyses proved difficult, because of the more stringent clean-up procedure required for blood, and the smaller samples normally obtained. The present method has been developed to study nicotine levels in the nanogram range expected[11,14] in the blood of smokers while smoking.

METHOD

APPARATUS—

Steam-distillation apparatus, all glass.

Rotary film evaporator (*Quickfit and Quartz*)—This was used at 40° C and the prevailing pressure was provided by a water pump.

Perkin-Elmer F-11 *gas - liquid chromatograph*—This was fitted with a flame-ionisation detector.

REAGENTS—

All reagents were of analytical-reagent grade, except when stated.

Sodium hydroxide, 10 N, *in glass-distilled water.*

Sulphuric acid, 10 N, *in glass-distilled water.*

Phosphoric acid, 88·0 *per cent.*

Sodium sulphate, anhydrous solid.

Dichloromethane.

Ethanol.

Alumina—A540, supplied by Fisher Scientific Co., and screened to pass 120 to 200 mesh.

Silicone MS antifoam A.

l-Nicotine—Obtained from B.D.H. Ltd.

Quinoline—Obtained from B.D.H. Ltd.

All solvents were further purified before use, by distillation.

PROCEDURE—

Blood samples were obtained from the subjects by venepuncture in the arm.

Dilute 10 ml of the heparinised whole-blood samples with 25 ml of glass-distilled water, make the solution alkaline with 10 ml of 10 N sodium hydroxide solution and add 0·1 g of

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antifoam A. Steam-distil the mixture and collect the first 50 ml of distillate in a flask containing 3 ml of 10 N sulphuric acid. Extract the acidified distillate with 50 ml of dichloromethane and discard the organic phase. Make the aqueous phase alkaline (pH 8 to 9) by adding 10 N sodium hydroxide and extract twice with 50 ml of dichloromethane. Combine the organic layers and dry the mixture over sodium sulphate for 10 to 15 minutes. Filter it through a Whatman No. 1 filter-paper and evaporate the solvent to about 3 ml. Transfer the residue quantitatively to a 10-ml test-tube and add a few drops of phosphoric acid. Evaporate the solvent to 1 ml under a gentle stream of nitrogen. Add 2 to 3 ml of glass-distilled water and shake the test-tube vigorously for 2 to 3 minutes, allow the layers to separate and discard the dichloromethane. Make the aqueous phase alkaline (pH 8 to 9) by adding 10 N sodium hydroxide and extract twice with 2 ml of dichloromethane. Dry the combined dichloromethane layers over sodium sulphate, filter the solution through a Whatman No. 1 filter-paper and evaporate it to dryness in a gentle stream of nitrogen. Transfer the residue quantitatively with 50 per cent. v/v dichloromethane in ethanol to an alumina column (40 × 4 mm) pre-wetted with 50 per cent. v/v dichloromethane in ethanol. Elute with this solvent and collect the first 5 ml of eluate. Evaporate the solvent to dryness under a gentle stream of nitrogen and dissolve the residue in 50 μl of ethanolic quinoline (10 μg ml^{-1}) as an internal standard. For maximum sensitivity, dissolve the residue in 20 μl of ethanolic quinoline (10 μg ml^{-1}). In either instance inject between 1 and 10 μl of this solution directly on to the gas - liquid chromatographic column by using a Hamilton syringe fitted with a 100-mm needle.

GAS CHROMATOGRAPHY—

The gas chromatograph was fitted with a glass column (2 m × 4 mm i.d.), packed with 8 per cent. w/w Carbowax 20M and 2 per cent. w/w potassium hydroxide on acid-washed Chromosorb W, 80 to 100 mesh, treated with hexamethyldisilazane.

The optimum chromatographic conditions for nicotine were: column temperature, 150° C; nitrogen flow-rate, 60 ml minute^{-1}; and gas pressures, hydrogen $1 \cdot 22 \times 10^5$ N m^{-2}, air $2 \cdot 14 \times 10^5$ N m^{-2} and nitrogen $3 \cdot 57 \times 10^5$ N m^{-2}.

The retention time for nicotine was about 13 minutes and about 15 minutes for quinoline.

With quinoline as an internal standard, authentic samples of nicotine and quinoline were injected on to the column, at various levels. The ratio of the peak heights for each level was calculated and was found to be constant over the range of concentrations used (Table I).

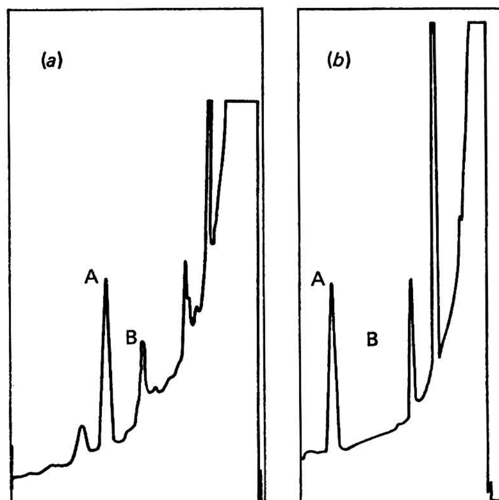

Fig. 1. Chromatograms of (*a*), smoker's blood; and (*b*), non-smoker's blood: A, quinoline; and B, nicotine

TABLE I

STANDARD GRAPHS FOR NICOTINE AND QUINOLINE

Amount injected/ng	Attenuation	Nicotine peak height/mm	Quinoline peak height/mm	Ratio of nicotine peak height to quinoline peak height
100	10 × 1	170	172	0·990
75	10 × 1	123	125	0·985
50	5 × 1	169	168·5	0·999
25	5 × 1	83·5	87·5	0·955
10	2 × 1	79	83·5	0·945
0	1 × 1	0	0	—

RESULTS

With the above procedure, the level of nicotine in the blood of smokers while smoking cigarettes was measured. Control samples of blood were taken from the subjects before smoking the cigarettes. Blood from non-smokers was also used as a control. It can be seen from the chromatograms (Fig. 1) that nicotine was not detected in the blood of non-smokers. Nicotine levels found in the blood of several subjects, smokers and non-smokers, are seen in Table II.

TABLE II

BLOOD LEVELS OF NICOTINE IN HUMAN SMOKERS*

No.	Sex	Number of cigarettes smoked daily	Nicotine per ml of blood/ng A	B†
1	F	<10	23·5	—
2	F	<10	16·5	—
3	F	<10	9·3	—
4	M	10 to 19	37·0	22·5
5	M	10 to 19	22·5	—
6	M	10 to 19	23·0	20·5
7	M	10 to 19	26·7	—
8	F	10 to 19	15·0	5·5
9	F	>20	33·0	—

* Blood from twelve non-smokers of both sexes showed no peaks on the gas chromatograms (see Fig. 1).

† Sample taken on a different occasion.

RECOVERY EXPERIMENTS—

Recovery experiments were conducted by adding known amounts of nicotine to 10-ml samples of human whole blood and taking these through the analytical procedure. The results of these experiments are given in Table III.

TABLE III

RECOVERY GRAPH

Mean of four determinations at each level

Nicotine added to 10-ml blood sample/ng	Ratio of peak heights (nicotine to quinoline)	Mean recovery, per cent.	Recovery range, per cent.
500	0·587	58·7	±4·0
450	0·539	59·9	±4·1
400	0·487	59·9	±3·3
350	0·408	56·3	±3·4
250	0·275	55·0	±4·0
200	0·228	57·0	±5·0
150	0·166	55·3	±5·0
100	0·108	54·0	±2·4
75	0·090	56·2	±3·1
50	0·053	57·8	±5·4
25	0·035	55·9	±5·6

The recovery results were checked by adding carbon-14 labelled nicotine (*l*-[*methyl*-^{14}C]nicotine hydrogen D-tartrate, specific activity 122 µCi mg^{-1} (free base), supplied by the Radiochemical Centre, Amersham) to human blood. After each stage of the method an aliquot was assayed by using liquid scintillation counting. The amounts of nicotine recovered at each stage are given in Table IV.

TABLE IV

RECOVERY OF *l*-[*methyl*-^{14}C]NICOTINE TAKEN THROUGH THE ANALYTICAL PROCEDURE

The experiment was carried out in duplicate at each of two levels of activity: 50·3 µCi (412 ng) and 25·15 µCi (206 ng)

	Loss in individual stage, per cent.		Over-all recovery, per cent.	
Stage *Level*—	412 ng	206 ng	412 ng	206 ng
Steam distillation	14·90	14·30	85·10	85·70
1st acidic extract	0·08	0·06	85·02	85·64
1st basic extract	0·24	0·16	84·58	85·48
2nd acidic extract	1·55	1·30	83·03	84·18
2nd basic extract	4·04	9·10	78·99	75·08
Alumina column	17·00	17·00	61·99	58·08
Final evaporation and dissolution in ethanolic quinoline	7·90	12·00	54·09	46·08

SPECIFICITY—

The specificity of the method was checked by taking a 150-ng sample of *l*-[*methyl*-^{14}C]-nicotine through the analytical procedure described above. Results were obtained by gas - liquid chromatography, on an aliquot of the final solution, giving a result of 84 ng of nicotine (56 per cent. recovery). A second aliquot was applied to a silica gel thin-layer chromatographic plate (Antec kieselgel SL254, silanised) (100 × 200 × 0·1 mm) and run in acetone - water (4 + 1) solvent. The developed plate was scanned on a Berthold scanner equipped with a 2π proportional gas-flow counter. A standard solution of *l*-[*methyl*-^{14}C]-nicotine was also applied to the plate, and the R_F value found to be 0·80 (in the literature[15] the value is 0·82). Integration of the radioactive peak corresponding to nicotine gave a result of 260 counts, corresponding to 73·5 ng of nicotine (49 per cent. recovery). Samples of cotinine, the major metabolite of nicotine, when determined chromatographically under the conditions established for the method had much longer retention times than nicotine (about 180 minutes).

SENSITIVITY—

At a signal-to-noise ratio of 3 : 1 the minimum amount of nicotine that could be detected was 0·2 ng. This represents a practical detection limit for nicotine of 1 ng. In a 10-ml sample of human blood this represents a limit of 0·0001 p.p.m. when using the above method.

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Amperometric Method for the Determination of Propham

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An amperometric diazotisation titration for the determination of active material in technical propham has been investigated. Advantages in comparison with the official CIPAC method are the smaller amount of sample required, the reduced time of hydrolysis and analysis, automation of titration, more accurate evaluation and better reproducibility because no extraction is necessary. The standard deviation was 0·52 compared with 1·04 obtained with the CIPAC method. In addition, the method has been tested successfully with other carbamates and their formulations.

THE Collaborative Pesticides Analytical Committee (CIPAC) has recommended a method for the determination of propham (the common name for isopropyl *N*-phenylcarbamate) in technical material, which is based on hydrolysis and titration of the liberated aniline with sodium nitrite solution.[1]

In the proposed method a mixture of sulphuric and glacial acetic acids is used for hydrolysis instead of phosphoric acid, because of the shorter hydrolysis time. The conditions have been modified, and the end-point of the titration is determined by amperometric (dead-stop) measurement.

EXPERIMENTAL

REAGENTS AND APPARATUS—

Glacial acetic acid.
Sulphuric acid, 50 *per cent.*
Hydrochloric acid, concentrated.
Potassium bromide.
Sodium nitrite solution, 0·1 N—Standardise with anthranilic acid.
All reagents were of analytical-reagent grade.

A magnetic stirrer, an automatic titrimeter, suitable for amperometric measurements (*e.g.*, Potentiograph E436, Metrohm AG, Switzerland), and two platinum-sheet electrodes (surface 1·3 cm^2) were used.

PROCEDURE—

Weigh the sample containing about 200 mg of propham, to the nearest 0·1 mg, into a conical flask with a standard ground-glass joint. Add 10 ml of glacial acetic acid and 20 ml of 50 per cent. sulphuric acid and reflux the mixture for about 10 minutes under a water condenser.

Allow the mixture to cool, add 10 ml of concentrated hydrochloric acid and a solution containing 2 g of potassium bromide in 70 ml of water. Titrate the ice-cooled mixture with 0·1 N sodium nitrite solution while stirring rapidly with a magnetic stirrer. Reduce the rate of titration near the expected end-point, which is indicated by a sharp drop in current (Fig. 1).

Amperometric conditions were as follows: polarisation voltage, 100 mV; measuring range, 1 V per 250 mm; and sensitivity, 50 μA per 50 mV.

The following formula was used for the calculation.

$$\text{Propham, per cent.} = \frac{\text{Volume of 0·1 N sodium nitrite, ml} \times 17·92 \times 100}{\text{Sample weight, mg}}.$$

Poorer quality batches contain measurable amounts of *NN′*-diphenylurea. With these samples results must be corrected by using the CIPAC method,[1] because this impurity is also hydrolysed.

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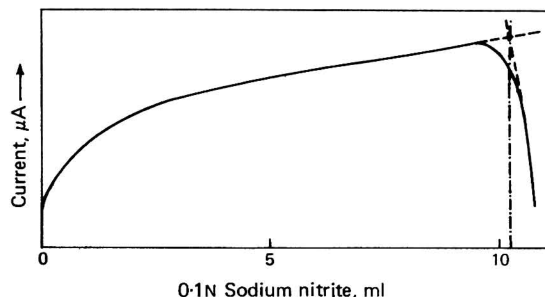

Fig. 1. Amperometric titration curve for propham

Free aniline can be determined by carrying out a blank determination. Weigh the sample containing about 1 g of propham to the nearest 1 mg, add 10 ml of concentrated hydrochloric acid and a solution containing 2 g of potassium bromide in 70 ml of water and immediately begin titrating as described above.

Results and discussion

The results obtained by the method described are presented in Table I. The tabulated values give standard deviations of 0·52 for the amperometric and 1·04 for the CIPAC method. From these results it is apparent that reproducibility of values is more satisfactory when the amperometric method is used.

TABLE I

PERCENTAGE RECOVERY OF PROPHAM

Amperometric method				CIPAC method			
Sample number	Sample weight, mg	Consumption of 0·1 N $NaNO_2$, ml	Propham found, per cent.	Sample number	Sample weight, g	Consumption of 0·5 N $NaNO_2$, ml	Propham found, per cent.
				First operator—			
1	213·1	11·79	99·1	1	2·500 2	28·04	100·5
2	199·2	11·08	99·7	2	2·554 0	28·02	98·3
3	199·2	11·05	99·1	3	2·505 0	28·07	100·4
4	202·5	11·29	99·9	4	2·507 5	28·23	100·9
5	198·7	11·15	100·5	5	2·520 5	27·88	99·1
6	198·9	10·98	98·9	6	2·492 0	27·67	99·5
				Second operator—			
7	199·7	11·10	99·6	1	2·569 0	28·15	98·2
8	200·7	11·25	100·4	2	2·511 0	28·15	100·5
9	200·6	11·15	99·6	3	2·521 0	27·69	98·4
10	200·0	11·15	99·9	4	2·506 5	27·50	98·3
11	199·6	11·12	99·8	5	2·516 0	28·14	100·2
12	200·5	11·08	99·0	6	2·516 5	27·69	98·6

In addition, it must be mentioned that the CIPAC method is difficult to apply, 1 day being needed by an operator to become familiar with the procedure. Also, spot testing is tedious, especially if the approximate content of the sample is unknown.

The described procedure has the following advantages over the CIPAC method:

(*i*) The sample weight required is only about 200 mg instead of 2·5 to 3 g.

(*ii*) Less than half the time is needed for analysis (less than 1 hour instead of 2½ hours), with the time of hydrolysis reduced from 90 to 10 minutes by changing the hydrolysis acids.

(*iii*) After hydrolysis no extraction of aniline is necessary.

(*iv*) Accuracy of evaluation, because titration curves are almost linear. The equivalence point is identical with the intersection point of the titration lines.

(*v*) No colour indicators are necessary, therefore the content of propham in coloured formulations can also be determined.

(*vi*) Better reproducibility of results.

(*vii*) The procedure can be applied to production control when carried out with automatic titrators. However, the proposed method is practicable even in less well equipped laboratories if a potentiometer with a suitable power supply and two platinum-sheet electrodes are available.

The procedure has been successfully tested with other carbamates such as chlorpropham (isopropyl *N*-(3-chlorophenyl)carbamate) and formetanate hydrochloride (3-(dimethylaminomethyleneimino)phenyl *N*-methylcarbamate hydrochloride). In addition, formetanate hydrochloride formulations have been investigated.[2] As only aromatic amine compounds interfere, the mixtures can be coloured and contain non-diazotisable emulsifiers, solvents and other adjuvants.

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An Enzymic Method for the Determination of Skimmed Milk Powder in Raw Sausages

BY R. K. BAHL

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An enzymic method for the determination of skimmed milk powder in raw sausages is described. The method is based upon the estimation of free lactose by its hydrolysis with β-galactosidase to galactose and glucose, the latter being determined by the hexokinase method. The determination is free from interference by reducing sugars and other substances present in sausage ingredients. The method is more rapid, accurate and reliable than other methods currently in use.

EXISTING methods used for the determination of skimmed milk powder in sausages rely upon the chemical determination of calcium[1] or lactose.[2]

The determination of calcium can give rise to unreliable results because the amounts of calcium in the meat and other ingredients used in the manufacture of sausages can vary. It has been observed in this laboratory that the calcium content can vary between 0·019 and 0·042 per cent. in sausages made without the addition of skimmed milk powder. This large variation in the calcium content prevents such a method being used to determine the skimmed milk content of a sample, particularly when one considers that only 0·013 per cent. of calcium represents 1 per cent. of skimmed milk powder.

On the other hand, direct determination based on the lactose content is subject to interference from other reducing sugars, whose concentrations may vary, that are found in sausage meat. Moreover, a marked increase in these reducing sugars during storage makes the situation even worse. Selective fermentation with yeast,[3,4,5] which eliminates these reducing sugars, is a tedious process and has not proved practical for routine laboratory use.

The present paper describes an enzymic method for the determination of skimmed milk powder in raw sausages. The method is relatively simple and is not subject to the disadvantages outlined above.

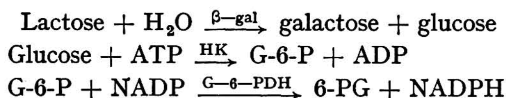
EXPERIMENTAL

PRINCIPLE OF THE METHOD—

The method is based upon the enzymic determination of free lactose.

After preparation of an aqueous extract of the sausage meat, the lactose present is hydrolysed with β-galactosidase (β-gal) to glucose, which is then phosphorylated in the presence of hexokinase (HK) and adenosine triphosphate (ATP) to glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). A sufficiently pure β-galactosidase must be used in order not to hydrolyse maltose or other oligosaccharides that may be present.

The phosphate ester is then oxidised to 6-phosphogluconate (6-PG) with G-6-P dehydrogenase (G-6-PDH) in the presence of nicotinamide-adenine dinucleotide phosphate (NADP), which is accordingly converted to its reduced form (NADPH). The reactions can be represented as follows:



$$\text{Lactose} + H_2O \xrightarrow{\beta\text{-gal}} \text{galactose} + \text{glucose}$$
$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$
$$\text{G-6-P} + \text{NADP} \xrightarrow{\text{G-6-PDH}} \text{6-PG} + \text{NADPH}$$

The NADPH produced in the last reaction is directly proportional to the amount of lactose present, and is used to determine the original concentration of lactose by its light absorption at a wavelength of 340 nm. A blank determination must, however, be carried out, because any glucose originally present in the sausages will form NADPH.

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APPARATUS—

All readings at 340 nm were carried out on a Pye-Unicam SP800 spectrophotometer with 1-cm light path silica cells.

Marburg pipettes of 100-μl and 20-μl capacity were used for transferring small volumes of solution.

REAGENTS—

Glucose test combination kit (hexokinase method)—Obtainable from The Boehringer Corporation (London) Ltd., Catalogue No. 15931 TGAB. According to the supplier's instructions, prepare the following three solutions, and use suspension (iv) undiluted: solution (i), 0·3 M with respect to triethanolamine buffer (pH 7·5) and 0·004 M with respect to magnesium sulphate; solution (ii), 0·012 M NADP; solution (iii), 0·016 M ATP; and suspension (iv), 1 mg ml^{-1} of HK and 2 mg ml^{-1} of G-6-PDH.

β-Galactosidase, pure, specific activity about 30 *U/mg.**

Dialysed iron sol, about 5 *per cent. of iron(III) oxide, laboratory-reagent grade.*

Sodium sulphate solution—Dissolve 200 g of laboratory-reagent grade sodium sulphate decahydrate in distilled water and make up to 1 litre.

Standard solution of lactose—Weigh accurately 0·7 to 1·6 g of analytical-reagent grade lactose monohydrate, and dissolve it in 100 ml of distilled water. Dilute 1 ml of this stock solution to 100 ml with glass-distilled water.

Fehling's solutions Nos. 1 *and* 2—Prepare and standardise before use.

Sucrose, analytical-reagent grade.

Methylene blue solution—Prepare a 1 per cent. w/v solution in distilled water.

PREPARATION OF AN AQUEOUS EXTRACT OF SKIMMED MILK POWDER—

Weigh accurately 0·4 to 1·0 g of skimmed milk powder and macerate it at high speed with 210 ml of distilled water and 20 ml each of 5 per cent. dialysed iron sol and sodium sulphate solution. Filter the mixture through a 15-cm Whatman No. 4 filter-paper. Dilute 10 ml of the filtrate to 100 ml with glass-distilled water.

PREPARATION OF AN AQUEOUS EXTRACT OF SAUSAGE MEAT—

Weigh accurately 25 g of the well minced and mixed sausage meat. Macerate it at high speed with 20 ml each of 5 per cent. dialysed iron sol and sodium sulphate solution and sufficient water (allowing for the previously determined moisture present in the sample) to make the total volume of water 250 ml. Filter the mixture through a 15-cm Whatman No. 4 filter-paper. Dilute 20 ml of the filtrate to 100 ml with glass-distilled water.

LACTOSE DETERMINATION—

In sequence, transfer by pipette 1 ml of test solution, 2 ml of solution (i), 0·1 ml of solution (ii), 0·1 ml of solution (iii) and 0·02 ml of suspension (iv) into each of two silica cells. Mix the contents of each cell with a small plastic paddle or a glass rod with a flattened end.

Add 0·02 ml of distilled water to one cell (blank cell) and 0·02 ml of β-galactosidase suspension to the other cell (test cell).

After incubation at 20 to 25 °C for 1 hour, measure the increase in optical density of the test cell (against the blank cell) at a wavelength of 340 nm. Repeat this measurement after a further 10 minutes to ensure that the reaction is complete.

The concentration of lactose in the test solution (μg ml^{-1}) is calculated from the formula

$$C = \frac{E \times V \times \text{MW}}{\epsilon \times v \times d}$$

where E is the measured increase in optical density change at 340 nm, V is the total volume of the solution in the cell, ϵ is the extinction coefficient of NADPH at 340 nm (6·22 cm^2 μmole^{-1}), MW is the molecular weight of lactose, d is the light path of the cell and v is the volume of the test solution taken for the determination.

For routine determinations it is convenient to prepare daily a fresh mixture of solutions (i), (ii) and (iii) and of suspension (iv) in the ratio of 2·0 : 0·1 : 0·1 : 0·02, and to add 2 ml of the mixture directly to each cell. This mixture is stable for 12 hours at 25 °C and 4 days

* Obtainable from The Boehringer Corporation (London) Ltd., Catalogue No. 15079 EGAY.

at 4 °C. To avoid the use of a large number of expensive cells, the reactions can be carried out in batches with small test-tubes (75 × 12 mm), while the extinction can be measured in a single pair of matched silica cells that can be washed and dried for immediate re-use.

Results

The accuracy of the proposed method for the determination of lactose was established by carrying out assays of lactose monohydrate (analytical-reagent grade) by this method and comparing the results with the titrimetric method of Lane and Eynon[6] in the modification of Tritton.[7] Six separate determinations were made for each method, the results of which are shown in Table I.

Table I

Assay of lactose

	Lactose, per cent. w/w*	Average, per cent. w/w	Standard deviation
Enzymic method	98·40 98·80 98·37 98·70 98·51 98·57	98·56	±0·16
Lane and Eynon's titrimetric method[6] as modified by Tritton[7]	98·61 98·77 98·78 98·64 98·71 98·35	98·64	±0·15

* Here, and in subsequent determinations, lactose was calculated as the monohydrate.

The results obtained by the two methods did not differ significantly.

The proposed method was used to determine the lactose content of different types of sausage mixes specially prepared without skimmed milk powder. The results obtained are shown in Table II.

Table II

Lactose content of various sausage mixes

Sample	Lactose found
Pork sausage mix	Negligible
Beef sausage mix	Negligible
Pork and beef sausage mix	Negligible
Beef, pork and liver sausage mix	Negligible

The results clearly indicate that the amount of free lactose present in the sausage ingredients, other than that in skimmed milk powder, was negligible.

Six separate determinations were also carried out on a single sample of skimmed milk powder to establish the accuracy of the proposed method. The average lactose content was observed to be 47·42 per cent. (with a standard deviation of ±0·23 per cent.).

Table III

Recovery of skimmed milk powder from laboratory-prepared samples of sausage mix

Milk powder added, per cent. w/w	Milk powder found, per cent. w/w	Standard deviation	Recovery, per cent.
0	0	0	0
0·50	0·50	±0·04	100·0
1·00	0·99	±0·01	99·0
1·50	1·48	±0·02	98·7
1·96	1·95	±0·01	99·5

In addition, recovery experiments were carried out on samples of sausage meat with known concentrations of skimmed milk powder. The results, as an average of six determinations at each concentration, are presented in Tables III and IV. In Table III, the samples were prepared under laboratory conditions, whereas the results presented in Table IV refer to samples prepared under factory conditions.

TABLE IV

RECOVERY OF SKIMMED MILK POWDER FROM FACTORY-PREPARED SAMPLES OF SAUSAGE MIX

Milk powder added, per cent. w/w	Milk powder found, per cent. w/w	Standard deviation	Recovery, per cent.
0	0	0	0
0·51	0·50	±0·03	98·0
1·02	1·01	±0·02	99·0
1·44	1·43	±0·01	99·3
1·96	1·96	±0·02	100·0

The results show that the proposed method is applicable to the determination of lactose in skimmed milk powder with a good degree of precision. Satisfactory recoveries were obtained over a wide range of concentration of skimmed milk powder in sausages (0 to 2·0 per cent. w/w).

To investigate the effect of storage on lactose concentration in raw sausages, samples were analysed initially and after storage in a refrigerator at 2 to 4 °C for 24 and 96 hours. The results obtained are shown in Table V.

TABLE V

LACTOSE FOUND IN SAUSAGE MIX AFTER 0, 24 AND 96 HOURS' STORAGE

Sample	Lactose content, per cent. w/w		
	0 hours	24 hours	96 hours
A	1·30	1·30	1·29
B	1·44	1·43	1·41
C	2·01	1·98	1·96
D	3·00	2·95	—

It is therefore evident that raw sausages, if kept at a low temperature, lose only a small proportion of their lactose content.

It was observed at an early stage in the investigation that the various supplies of skimmed milk powder contained different concentrations of lactose. Thus, for the same concentrations of different skimmed milk powders the lactose content could vary. To determine the extent of this variation the lactose contents of various skimmed milk powders available for commercial use in the United Kingdom were determined. The results are shown in Table VI.

TABLE VI

LACTOSE CONCENTRATION IN COMMERCIAL SKIMMED MILK POWDERS

Sample	Country of origin	Process of manufacture	Lactose, per cent. w/w
1	U.K.	Spray dried	51·5
2	U.K.	Roller dried	52·2
3	U.K.	Spray dried	50·0
4	U.K.	Roller dried	52·1
5	New Zealand	Spray dried (low heat)	51·3
6	New Zealand	Spray dried (medium heat)	52·2
7	New Zealand	Spray dried (high heat)	51·4
8	New Zealand	Roller dried	51·1
9	New Zealand	Roller dried	46·8
10	New Zealand	Spray dried	49·6
11	Ireland	Roller dried	48·5
12	Ireland	Spray dried	54·8
13	Holland	Spray dried	55·2
14	Australia	Spray dried	51·9
Average (fourteen different suppliers)			51·3

Standard deviation ±2·1 per cent.

Thus, the difference between the amount of skimmed milk powder added and the amount determined on the basis of an average lactose content of 51·3 per cent. would not be greater than 10 per cent. By using this average lactose figure of 51·3 per cent., the skimmed milk powder content of sausages sold by retailers within the London area was determined. Table VII lists the results obtained for five brands that were sampled weekly over a period of 6 weeks.

TABLE VII

SKIMMED MILK POWDER IN RETAIL SAUSAGES

Brand	Type of sausage	Skimmed milk powder in six weekly samples, per cent. w/w					
		1	2	3	4	5	6
1	Beef	1·4	1·4	1·1	1·2	1·1	1·2
2	Beef and pork	1·4	1·2	1·2	1·4	1·3	1·3
3	Pork	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
4	Pork	1·4	0·9	1·1	0·4	1·1	1·0
5	Beef and pork	1·4	1·3	1·2	1·1	1·5	1·4

CONCLUSIONS

Statistical study of the proposed enzymic method in respect of analytical accuracy, recovery efficiency, and variation of lactose in skimmed milk powder shows that, for the present level of skimmed milk powder in sausages, the results are accurate to within $\pm 0{\cdot}1$ per cent.

The determination is free from interference by reducing sugars and other substances present in sausage ingredients, the error due to these substances being eliminated by use of a sample blank.

The method is rapid and simple and the whole determination takes only 1½ hours, of which the actual working time is less than 15 minutes.

Raw sausage samples can be kept in a refrigerator for up to 4 days before carrying out the analysis, without serious reduction in accuracy. Thus, as a routine laboratory method, the enzymic method offers superior speed, accuracy and reliability compared with the other methods currently in use.

I thank the Directors of J. Sainsbury Limited for their permission to publish this work, Mr. E. F. Williams for his advice throughout the investigation and Mr. M. J. Follett for his help in the preparation of this paper.

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Book Reviews

ION EXCHANGE IN ANALYTICAL CHEMISTRY. By WILLIAM RIEMAN and HAROLD F. WALTON. Pp. xiv + 294. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1970. Price 130s.; $17.50.

This book attempts "to provide analytical chemists with a broad survey of the rôle that ion exchange can and should play in chemical analysis." While it can be argued that the skilled analytical chemist should know his tools, this book, nevertheless, provides an opportunity for fruitful reflective reading. For its intended readership there is the pre-requisite of a careful perusal of the types of ion exchanger and of their preparation, structure and properties as well as the equilibria and kinetics of ion exchange. The authors have succeeded in their aim, although not everyone will be happy with the many mathematical relationships that are used in the text. However, the qualitative explanations are well done and the mathematically disinclined need have no qualms about turning to this book. If criticism is to be made, it is that the mathematical treatment could be fuller and more explicit.

The various applications of ion exchange, both chromatographic and non-chromatographic, are elegantly presented, but without much in the way of experimental detail for specific applications, although this is well compensated for by a wide (but not exhaustive) reference list and name index.

In the treatment on the theory of ion-exchange chromatography, the plate equilibrium theory is emphasised over the mass transfer theory as being "more helpful to the analyst who wishes to calculate from the data of a few elutions the concentration and pH of eluent that will give the most efficient separation of a given mixture." Here again the reviewer feels the treatment might have been fuller, for example, the Cornish thesis (F. W. Cornish, *Analyst*, 1958, **83**, 634) for deducing adequate operating conditions might have been included. However, suggestions for modification at the expense of size must be cautiously made for the book is already too expensive to appear on many individual book-shelves.

The penultimate chapter (65 pages) is devoted to less common exchangers. Although the authors might have brought out their crystal ball at this juncture, they were probably wiser to temper valour with discretion and leave the future to its own devices! The final chapter on the study of complex ions earns its keep in a book that recognises the important rôle of ion-exchange chromatography (over a third of the text is devoted to the subject), which is itself so dependent on complexation for its successes.

The book is well produced with an invitingly pleasant jacket and is likely to be useful to analytical chemists.

J. D. R. THOMAS

CHEMICAL METHODS OF ROCK ANALYSIS. International Series of Monographs in Analytical Chemistry. Volume 36. By P. G. JEFFERY. Pp. xiv + 509. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1970. Price 140s.; $18.50.

The study of chemical methods of rock analysis is a complicated topic with a rapidly growing and voluminous literature. Although books on this subject have been published recently it is sufficiently wide to allow for a variety of treatments. Dr. Jeffery's book, although containing much general information, deals in far greater detail than others on this subject with minor and trace elements. This, in itself, apart from its undoubted general merit, is sufficient justification for publication.

The first six chapters deal with general topics, including the composition of rock material, the availability of geochemical standards, sample preparation and chemical methods of decomposition. Classical schemes of analysis and the more recent rapid methods are reviewed and given in working detail. Chapter six contains a useful introduction to the use of statistical methods in rock analysis.

The remainder, forming the bulk of the book, deals with the determination of about sixty individual elements in major, minor and trace amounts. The major elements are dealt with fully in working detail, as are the more usually determined minor elements, with perhaps the exception of mercury, for which chemical methods are not available for the concentrations normally to be expected in rocks. Each chapter has a useful introductory section dealing with the occurrence and geochemistry of the element. The author was perhaps unwise to attempt the almost impossible task of tackling the complex topic of silver, gold and the platinum metals in just over 5 pages,

4 of which are necessarily devoted to the occurrence of these elements. The remaining page consists half each of a brief account of available analytical methods and a list of references.

Errors and misprints appear to be rare, although the "wetting" of the appetite of the geologist on p. 54 by Shapiro and Brannock's scheme of rapid analysis, is an amusing and notable exception.

An accurate detailed rock analysis now requires wide and up-to-date knowledge and experience on the part of the analyst and experts in this field will be well advised to add this book to their collection of working guides.

W. H. BENNETT

CHEMICAL APPLICATIONS OF FAR INFRARED SPECTROSCOPY. By ARTHUR FINCH, P. N. GATES, K. RADCLIFFE, F. N. DICKSON and F. F. BENTLEY. Pp. viii + 270. London and New York: Academic Press. 1970. Price 100s.; $14.50.

Developments in instrumentation over the past decade have rendered the far infrared region more accessible to analytical study. In particular, the introduction of the laser source into Raman work and the development of small relatively inexpensive computers for the processing of data from interferometers have brought these techniques to the point at which many users of standard infrared instruments will be considering whether the far infrared and Raman have anything additional to offer them. This, then, is an extremely timely book, and has been produced by five authors, well known in the field, who have co-operated successfully in collecting together information on various aspects of the application of far infrared spectra.

Two excellent introductory chapters on instrumentation and techniques are followed by chapters on specific applications. Of particular interest to inorganic analytical spectroscopists will be the chapters on metal - ligand vibrations, *viz.*, chapter 6 on complex inorganic systems and chapter 7 on metal - organic and organic compounds. There are certain omissions in the coverage, as the book was written in the summer of 1968 and considerable developments have taken place since then, most notably in the spectra of liquids, particularly organic, in the 100 to 10 cm^{-1} region. One point worth mentioning is the description of the book in the publisher's blurb as suitable for undergraduates. The present allocation of time in most undergraduate courses to the study of far infrared varies between none and very little, and while the book undoubtedly represents useful reading material for the keen or bright student, it is to my mind aimed more at research workers who either own, or are considering the purchase of, suitable instrumentation. This is not intended as a criticism of the book, which is an excellent treatise on the present state of knowledge in this field.

J. M. OTTAWAY

ANALYTICAL CHEMISTRY OF ZIRCONIUM AND HAFNIUM. By ANIL K. MUKHERJI. Pp. xiv + 280. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1970. Price 90s.; $12.00.

Any detailed publication on the analytical chemistry of zirconium is almost invariably linked with that of hafnium, because the chemical and physico-chemical properties of these two metals, and their corresponding compounds, are so similar. This book deals with the reactions of these two ionic species, under such individual headings as: Gravimetric, Titrimetric, Electrometric, and Absorptiometric Methods.

In these four chapters, and elsewhere in the book, mention is made of the fact that hafnium reacts almost exactly like zirconium, but this should be emphasised more in a publication that deals exclusively with the chemistry of these two metals.

These early chapters cover familiar, although up-to-date ground, and later sections aim to highlight the important features of literature references up to 1967, on aspects involving, *e.g.*, ion-exchange, solvent-extraction and neutron-activation procedures.

The determination of small amounts of either zirconium in hafnium, or hafnium in zirconium, presents problems for the analyst, and the chapter covering these separations, and their separate evaluations, is allocated the largest number of pages (36), and has 146 supporting references, but much of the text is more relevant to the chemistry of large scale industrial manufacturing processes.

The two chapters, Spectrographic and X-ray Analyses, deal largely, as do most of the other sections, with published work, although it is disappointing that here, and elsewhere in the book, the humble efforts of certain workers in the U.K. rank neither for praise nor criticism; not even a mention of their published work.

This is a book for those who are new to the subject, analysts and manufacturers alike, but "older hands" are unlikely to find anything significantly new within its pages.

W. T. ELWELL

PROGRESS IN STEREOCHEMISTRY 4. Edited by B. J. AYLETT, M.A., Ph.D., and MARGARET M. HARRIS, D.Sc., Ph.D. Pp. viii + 389. London: Butterworth & Co. (Publishers) Ltd. 1970. Price 150s.

This volume of "Progress in Stereochemistry" maintains the high standards that we have come to expect in this series. The topics dealt with are well chosen to illustrate the breadth of modern structural chemistry and the strengths and weaknesses of the weapons in the stereochemical armoury. By its very nature a book of this sort must deal with completed researches whose veracity has, in the main, been established, nevertheless the contributors have taken pains to include a number of "awful warnings" to restrain the incautious from an unthinking application of the principles presented.

There is much in this book that will interest the specialist, but in addition it is a mine of information and good sense into which all chemists with any interest in structural problems may dip with profit.

Dr. Hall discusses the stereochemistry of the 2,2′-bridged biphenyls with great clarity and insight, and her contribution admirably supplements the discussion of the biphenyls in Volume 2. In this chapter, however, and to a greater degree than in any of the others, the clumsiness of the use of Roman numerals becomes very apparent. Surely this stilted device could be dropped in favour of bold face Arabic numerals with their greater comprehensibility.

It is to be hoped that the intending authors of the new wave of textbooks of organic chemistry will consult the excellent chapters on the configurational analysis of the carbohydrates and stereochemical correlations. The time has come for the classic methods of Fischer to be presented alongside some of the new and powerful methods now available for the study of the fine stereochemical details of molecules. This should not only point up the brilliance of Fischer's work but illustrate the truism that unambiguous synthesis is still the core of stereochemistry.

Mills and Speakman have contributed a thoughtful chapter on crystal structure analysis, which sets out very clearly the problems to be faced by the crystallographer and the solutions available to him. The practical aspects of these precepts follows immediately in Watson's presentation of the structure of myoglobin.

Both of these discussions are enriched by the use of stereoscopic drawings. Most of these have been produced by computer techniques but Mills and Speakman indicate how hand-drawn diagrams can be prepared, thus providing this reviewer with a great deal of enjoyment. It is good to see stereoscopy brought before a wider audience than the readers of *Acta Crystallographica*. Perhaps more use will be made of these in textbooks in future to help the student master the bugbear of the third dimension.

Dr. Walton has contributed a most useful chapter, which reviews the use and abuse of molecular models. This chapter is well illustrated and is invaluable to teachers of chemistry at all levels as a source of information about commercially available models, which are dealt with in considerable detail and, perhaps equally important, listed with their prices and suppliers. There is also plenty for the inveterate, or impecunious, do-it-yourself addict.

This is a thoroughly enjoyable book.

D. H. MAASS

FLUORINE CHEMISTRY REVIEWS. VOLUME 3. Edited by PAUL TARRANT. Pp. vi + 154. New York and London: Marcel Dekker Inc. 1969. Price $12.50.

This publication, like the two preceding it in this series, is a compilation of four review articles by authors who have specialised in specific facets of fluorine chemistry.

The first paper, entitled "Fluorine Compounds in Anesthesiology" by E. R. Larmen, is an interesting account of the use of fluorinated compounds, including polyhalogenated substances, as anaesthetics. The author describes the physiology of anaesthesia in some detail and relates this to the compounds under review. Data are given about an astonishingly large number of halogenated compounds evaluated as anaesthetic agents. As is to be expected a good deal of space is devoted to the compound 2-bromo-2-chloro-1,1,1-trifluoroethane, commonly known as halothane, and probably the most widely used fluorinated anaesthetic. The author cites 109 references; it is a very useful article. Another paper, by M. Fild and O. Glemser, deals with the pentafluorophenyl compounds of antimony, arsenic and phosphorus. This is a relatively new field of fluorine chemistry, which the authors have reviewed comprehensively, having included some of the most recent work.

A group of Russian scientists has contributed the longest paper, "Reactions of Fluoro-olefins with Electrophilic Reagents" by B. L. Dyatkin, E. P. Mochalina and I. L. Knunyants, from which it is evident that one of the authors (I.L.K.) has himself done much work on this subject.

W. R. Cullen, of the University of British Columbia, has contributed a paper on the fluoro-alicyclic derivatives of metals and metalloids. Here again the author has presented a vast amount of detail as well as dealing in a fairly conventional way with the chemical properties of a wide range of the compounds in his review.

This book will be of interest to those who have made these subjects their especial study, but is very expensive.

R. J. HALL

THE CHEMISTRY OF FATS AND OTHER LIPIDS. VOLUME 9. POLYUNSATURATED ACIDS. Part IV. Edited by RALPH T. HOLMAN. Pp. iv + 453–585. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1970. Price 50s.; $6.75.

Part 4 of the ninth volume of this series of authoritative books on lipid chemistry deals with the biosynthesis of unsaturated fatty acids in higher plants, the inter-relationship of unsaturated fatty acids with anti-oxidants *in vivo*, and the rôle of these acids in human nutrition and metabolism. Although none of the subjects is treated in a predominantly or even remotely analytical manner, they are all of great importance and human interest. That the body requires polyunsaturated fatty acids in its diet is now well established, and we know much about the effect of adding them where there have been deficiencies. Many of the studies have been made with infants, and dramatic effects have resulted. Photographs in this book show the return to normal of childrens' faces from a state indicating no hope of recovery, in 2 months in the case of an eczematous dermatitis with a caloric addition to the diet of only 2 per cent. of trilinolein, and in only 5 days in a case of acro-dermatitis enteropathica treated with human milk and diodoquin. It is clear that such corrective diets cannot work as quickly for adults, but their value is accepted. Polyunsaturated acids are hypocholesteremic, partly by increasing excretion of cholesterol and partly by conferring optimum availability for synthesis of the phospholipids and cholesterol esters required for making the lipoprotein complexes needed to accompany any surge of fats or cholesterol in the diet; without the added polyunsaturated acids the complexes must be obtained from the tissue stores in the body. This is why both saturated fats and cholesterol accelerate deficiencies of essential fatty acids, and why the amount so used up should be restored in the diet.

Deficiencies of polyunsaturated acids are apparent in abetalipoproteinemia, a genetic syndrome of malabsorption and serum lipid abnormality, and in rare diseases such as cholesterol acyl transfer. Speculation continues about other effects. The acids may be related to the nutritional disease of kwashiorkor through protein deficiency of the lipoproteins; and pro-oxidant conditions in the tissues, which lead to oxidation of polyunsaturated acids at abnormally high rates, may provide ideal conditions for leprosy and tuberculosis to thrive. Deficiency of $\omega 3$ and $\omega 6$ acids during maturation of the brain may be responsible for multiple sclerosis, which is known to have a lower incidence in regions where seed and fruit fats rich in 18:3 acids are consumed in quantity.

If these findings and speculations do no more, they point to the vastly increased contributions made to the study of lipid chemistry by modern methods of analysis that have made the separation and identification of individual fatty acids possible. Thirty years ago our analytical methods were relatively crude and although we could separate the fatty acids of a fat into classes often we could not recognise minor constituents or even some of the major ones. Now we can separate even positional isomers; and the results of the study of their effects are well shown in this book.

K. A. WILLIAMS

Errata

SEPTEMBER (1970) ISSUE, p. 801, 7th line. *For* "mg" *read* "ng"

IBID., p. 802, legend to Fig. 7, 3rd line, *For* "mg" *read* "ng"

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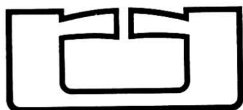

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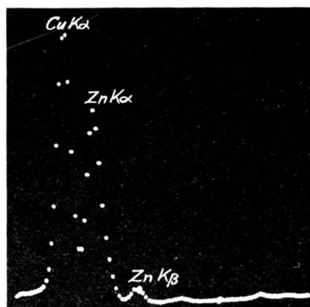

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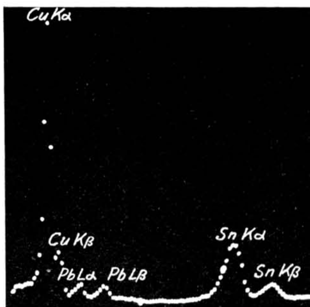

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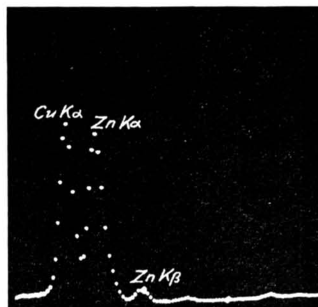

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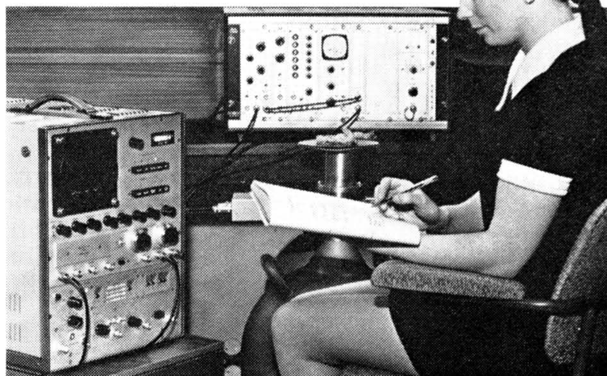

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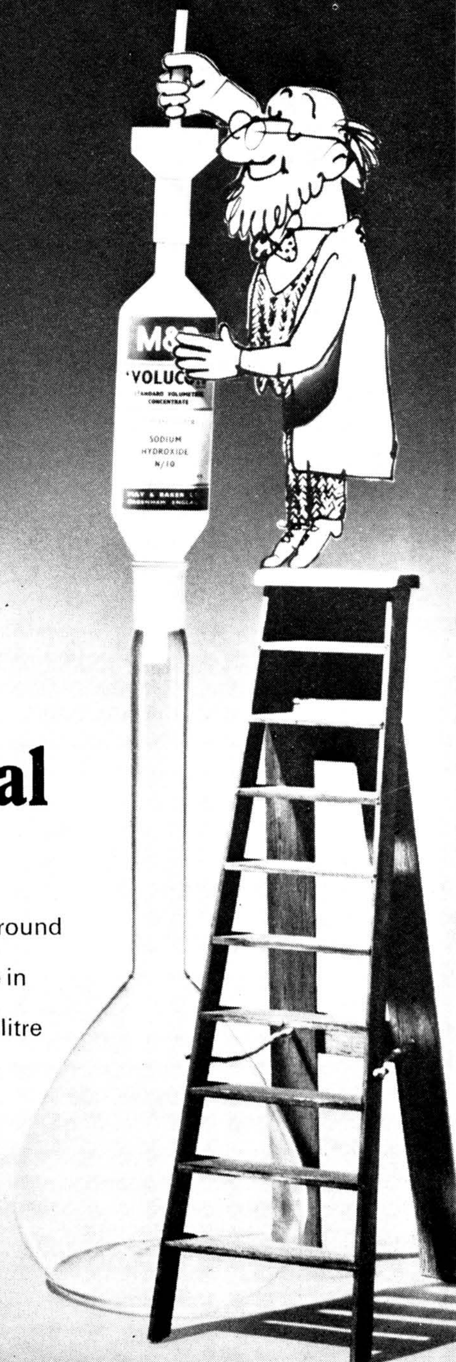

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A Simple Method for the Determination of Solvents Retained in Plastic Films and Laminates

A simple method is described for the determination of solvents retained in polymer films and laminates as a result of the application of inks, coatings or adhesives. A sample of the film or laminate is heated, together with a known amount of a suitable solvent as an internal standard, in a simple modified Kilner jar. After a period of heating in an oven the atmosphere in the headspace of the jar is analysed by gas chromatography with a flame-ionisation detector.

Results given show the precision of the method and its application to actual samples. Replicate analyses on similar samples show a standard deviation of 5 per cent. of the mean value for each solvent determined. The method is currently used in the authors' laboratories for analysing samples containing retained solvents at levels between 1 and 300 mg m^{-2}.

J. T. DAVIES and J. R. BISHOP
The Metal Box Company Limited, Research and Development Department, Kendal Avenue, Westfields Road, Acton, London, W.3.

Analyst, 1971, **96**, 55–58.

The Microgasometric Determination of Some Inorganic and Organic Nitrates by Reduction with Iodide Ion and Elemental Iodine

Simple micro methods are described for the determination of nitrate based on its reduction with iodide or iodine in the presence of halogen acids. Nitric oxide gas is liberated and iodine(I) is the oxidation product from both iodide and iodine.

S. S. M. HASSAN
Research Microanalytical Laboratories, Chemistry Department, Faculty of Science, Ain Shams University, Cairo, U.A.R.

Analyst, 1971, **96**, 59–61.

A Field Method for the Determination of Iron Oxide Fume in Air

A field method is described for the determination of iron oxide fume in industrial atmospheres at concentrations up to 20 mg m^{-3}. After collection on a filter-paper the iron oxide is dissolved in a hot solution of hydroxylammonium chloride in hydrochloric acid. The iron solution is quantitatively transferred to a calibrated flask and the red complex of iron(II) - bathophenanthrolinedisulphonate is formed, the intensity of which is determined either spectrophotometrically or by visual comparison with a set of permanent colour standards. The procedure is simple to carry out and the time required for a complete analysis is about 25 minutes.

D. W. MEDDLE and R. WOOD
Department of Trade and Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1.

Analyst, 1971, **96**, 62–66.

The Determination of Acrylamide in Polyelectrolytes by Extraction and Gas-chromatographic Analysis

A method is described for the quantitative extraction of acrylamide from water-treatment grade acrylamide polymers and copolymers. The extraction is performed by shaking the polymer with methanol - water(80 + 20) for 24 hours. An improved gas-chromatographic method has been developed for analysing these extracts and can be conveniently used at levels down to 0·0004 per cent. of acrylamide in polymer; 0·05 per cent. of acrylamide in polymer can be determined with a relative standard deviation of $\pm$ 4 per cent.

Only two of the polymers tested contained materials that interfered in the gas-chromatographic determination. A thin-layer chromatographic clean-up technique is described, which enables these polymers to be analysed by gas chromatography with no interference.

B. T. CROLL
The Water Research Association, Medmenham, Marlow, Buckinghamshire.

Analyst, 1971, **96**, 67–77.

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The Determination of 3,5-Dinitrosalicyl-(5-nitrofurfurylidene)-hydrazide (Nifursol) in Animal Feeding Stuffs by Electron-capture Gas Chromatography

A method is described for the determination of 3,5-dinitrosalicyl-(5-nitrofurfurylidene)hydrazide (nifursol) in animal feeding stuffs. The additive is extracted from the feed with acetonitrile, and after further clean-up with carbon disulphide the nifursol is reacted with boron trifluoride - methanol complex. The product, methyl 3,5-dinitrosalicylate, is determined by gas chromatography with an electron-capture detector.

B. B. WHEALS and R. E. WESTON

Department of Trade and Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1.

Analyst, 1971, **96**, 78–80.

The Determination of Nicotine in Human Blood by Gas - Liquid Chromatography

A method has been developed for the extraction and determination of sub-microgram amounts of nicotine in blood. It involves steam distillation of the nicotine followed by solvent partition and column-chromatographic clean-up. The final solution of nicotine in ethanol is injected directly on to the column of a gas - liquid chromatograph fitted with a flame-ionisation detector. The method can be used to determine down to 1 ng of nicotine, and has been applied to the measurement of nicotine levels found in the blood of smokers while smoking cigarettes.

I. E. BURROWS, P. J. CORP, G. C. JACKSON and B. F. J. PAGE

Department of Chemistry, Huntingdon Research Centre, Huntingdon.

Analyst, 1971, **96**, 81–84.

Amperometric Method for the Determination of Propham

An amperometric diazotisation titration for the determination of active material in technical propham has been investigated. Advantages in comparison with the official CIPAC method are the smaller amount of sample required, the reduced time of hydrolysis and analysis, automation of titration, more accurate evaluation and better reproducibility because no extraction is necessary. The standard deviation was 0·52 compared with 1·04 obtained with the CIPAC method. In addition, the method has been tested successfully with other carbamates and their formulations.

GENO KYNAST and HANS HAHN

Schering AG, Zentrale Analytik, Werk Wolfenbüttel, 334 Wolfenbüttel, Halchtersche Strasse 33, Germany.

Analyst, 1971, **96**, 85–87.

An Enzymic Method for the Determination of Skimmed Milk Powder in Raw Sausages

An enzymic method for the determination of skimmed milk powder in raw sausages is described. The method is based upon the estimation of free lactose by its hydrolysis with β-galactosidase to galactose and glucose, the latter being determined by the hexokinase method. The determination is free from interference by reducing sugars and other substances present in sausage ingredients. The method is more rapid, accurate and reliable than other methods currently in use.

R. K. BAHL

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Analyst, 1971, **96**, 88–92.

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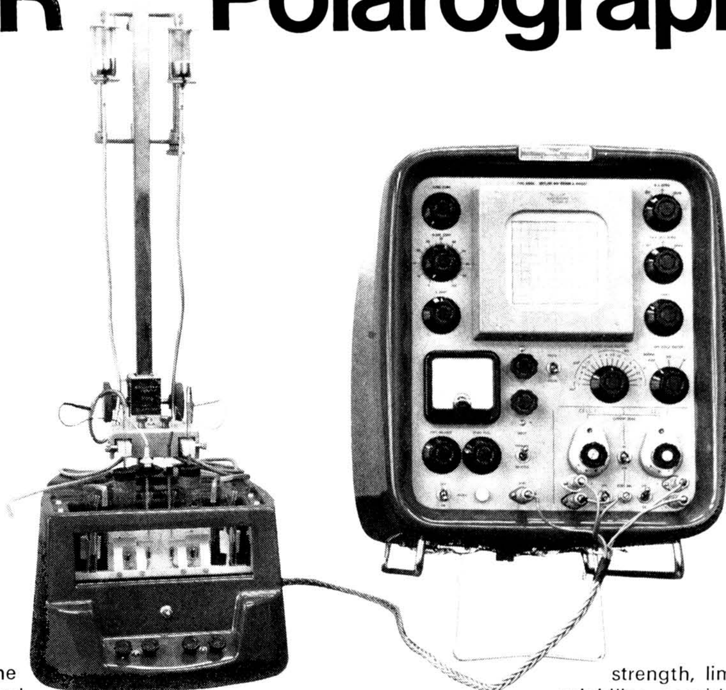

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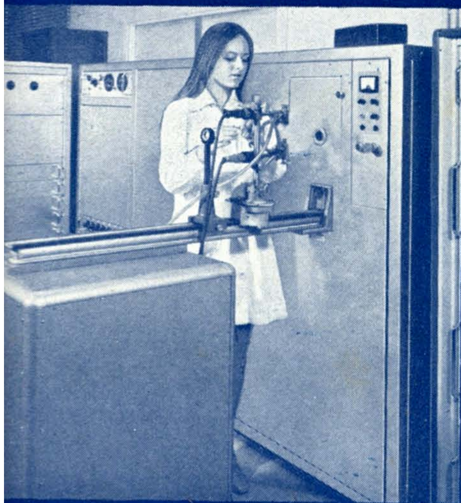

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