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Volume 103 No 1226 May 1978

Summaries of Papers in this Issue

Simultaneous Techniques in Thermal Analysis

A Review

Summary of Contents

Keywords: *Review*; *thermal analysis*; *derivative and differential methods*; *simultaneous methods*

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Analyst, 1978, **103**, 417–437.

Solid-state Mercury(I) Chloride Electrode for Determining 0.1–1.0 μg ml^{-1} Levels of Chloride in Boiler Water and Other High-purity Waters

A solid-state electrode based on mercury(I) chloride and mercury(II) sulphide has been developed for determining chloride concentrations of 0.01–1.0 μg l^{-1} in boiler water. The greater sensitivity of the electrode compared with that of silver - silver chloride electrodes enables concentrations as low as 0.01 μg ml^{-1} to be determined by a simple manual technique. The total standard deviations at chloride concentrations of 0.5, 0.1 and 0.01 μg ml^{-1} were 0.025, 0.005 and 0.015 μg ml^{-1}, respectively. The electrode can be prepared easily in the laboratory from commercially available materials and a Růžička Selectrode. The only significant interference is from iron(III) ions and this interference can be eliminated by adding fluoride ions to the sample.

Keywords: *Chloride determination*; *water analysis*; *potentiometry*; *chloride-selective electrode*; *mercury(I) chloride electrode*

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Central Electricity Research Laboratories, Kelvin Avenue, Leatherhead, Surrey, KT22 7SE.

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The Analyst

Simultaneous Techniques in Thermal Analysis*

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Summary of Contents

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Introduction

The development of simultaneous thermoanalytical methods has taken place during the past two decades and this development has many aspects. However, within the scope of this review we wish first to analyse the causes, motives, aims and trends that led finally to the development of simultaneous methods.

In the 1950s thermal analysis entered a new phase of development. The accuracy obtainable with classical methods had not met more stringent requirements and could not be increased by improving the measuring devices.

In those days absolute temperature values could be measured by use of thermocouples with an accuracy higher by orders of magnitude than, for example, that with which decomposition temperatures could be determined by means of differential thermal analysis (DTA). For instance, two literature values for the decomposition temperature of calcium carbonate obtained by DTA are 800 and 900 °C. These values were determined with an error of only ± 1 °C, but in spite of this, and on the basis of these data, we cannot state with a greater certainty than ± 50 °C that the decomposition temperature of calcium carbonate is 850 °C.

Also, mass change could be determined with an accuracy higher by orders of magnitude than that with which the amounts of two components could be determined if the decomposition processes of the components overlapped. Thus, if the sample mass became constant between the two decomposition processes, the amount of each component could easily be determined with an error of only $\pm 0.2\%$. However, if the two processes overlapped, then, as shown in Fig. 1, the error could increase to $\pm 20\%$ or more.

Accordingly, it became evident that a further development in thermal analysis could only be expected if new principles of examination were introduced and new measuring techniques developed. As a consequence, intensive research activity began. One of the most significant results of this activity was the birth and rapid development of simultaneous methods.

* Plenary Lecture presented at the First European Symposium on Thermal Analysis, Salford University, September, 1976.

Development of Simultaneous Methods

The aims that were characteristic of this development were, in addition to multiplying the information obtainable, to increase resolution, to standardise the experimental conditions and to increase selectivity. Experimental conditions were manipulated in order to realise these aims.

The idea of coupling single classical thermoanalytical methods is straightforward, as it is well known that extension of an examination by use of an additional method increases both the certainty of interpretation and the accuracy of evaluation of the thermoanalytical curves not in a proportional, but in a multiple, way. This idea is demonstrated by the following example. Knowledge of the mineral composition of bauxites is important from the point of view of alumina production. However, as Fig. 2 shows, for this extreme example (selected for the purposes of demonstration), the composition of a bauxite sample cannot be determined at all if a thermobalance alone is used. The possible components are as follows (with decomposition temperatures in parentheses): boehmite, α-$Al_2O_3.H_2O$ (520 °C); diaspore, γ-$Al_2O_3.H_2O$ (540 °C); kaolinite, $Al_2O_3.2SiO_2.2H_2O$ (570 °C); alunite, $K_2SO_4.3Al_2O_3.3SO_3.6H_2O$ (570 °C) and $K_2SO_4.3Al_2O_3.3SO_3$ (790 °C); and calcite, $CaO.CO_2$ (800 °C). The thermogravimetry (TG) curve alone cannot indicate which components are present and the decomposition of which components was responsible for the two-step mass-loss process.

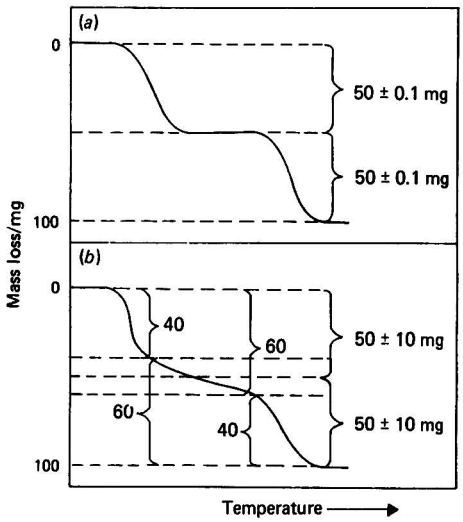


Fig. 1. Accuracy of mass measurement by means of thermogravimetry: (*a*), when the decomposition processes of the components of a sample do not overlap; and (*b*), when overlap occurs.

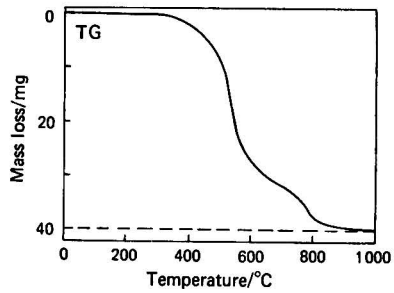


Fig. 2. TG investigation of a bauxite.

As Fig. 3 shows, the composition of the sample cannot be stated even if the derivative thermogravimetry (DTG) curve is also recorded, though the situation undoubtedly becomes clearer. The DTG peaks indicate the single temperature values characteristic of the different mineral components more accurately than does the TG curve. However, the presence of four bauxite minerals or, more precisely, three combinations of these four, is still possible. These combinations are: boehmite and alunite; boehmite, alunite and kaolinite; and boehmite, kaolinite and calcite.

The DTA curve offers further important information. Although it is not sufficient for the complete solution of the problem, it reduces the number of possible combinations to two, as can be seen from Fig. 4. A small exothermic DTA peak can be seen at 960 °C, which is characteristic of kaolinite only and clearly proves the presence of this mineral. The two possible combinations are, therefore: boehmite, alunite and kaolinite; and boehmite, kaolinite

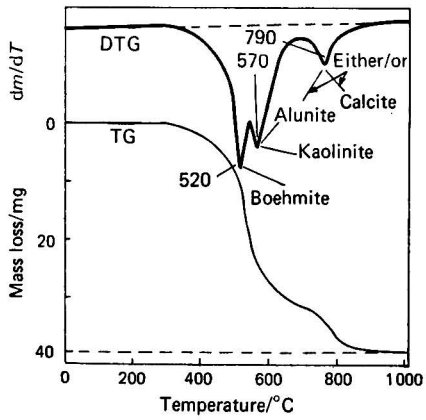


Fig. 3. Simultaneous TG and DTG investigation of the same bauxite as in Fig. 2.

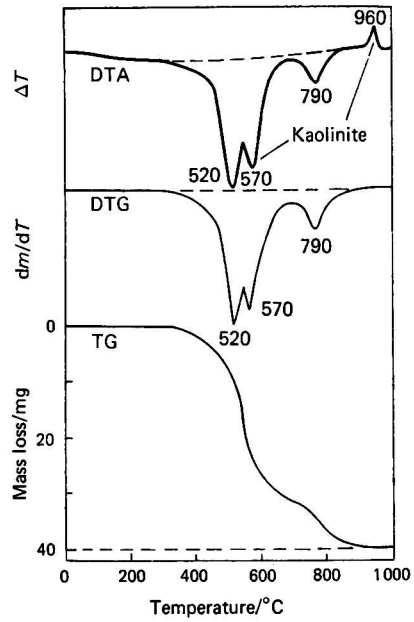


Fig. 4. Simultaneous TG, DTG and DTA investigation of the same bauxite as in Figs. 2 and 3.

and calcite. Accordingly, in this instance the simultaneous TG, DTG and DTA examinations must be completed by use of one of the evolved gas analytical (EGA) methods in order to determine whether sulphur(VI) oxide or carbon dioxide is liberated, *i.e.*, whether the sample contains alunite or calcite, respectively, and if possible to determine the amount of the gaseous decomposition product.

As Fig. 5 shows, by using EGA in addition to the other methods, not only can the qualitative composition of the sample be identified with certainty, but also the amount of each identified component can be determined with satisfactory accuracy.

Difficulties of Interpretation

Despite the fact that the combination of different methods is connected with the abovementioned advantages, up to the 1950s, apparently for no reason, thermoanalysts used only single thermoanalytical methods for their investigations. The question of the reason for this choice of method arises.

One of the causes undoubtedly lies in the difficulty of the common interpretation and evaluation of curves recorded by means of different thermoanalytical devices. This difficulty is shown for a TG and a DTA curve in Fig. 6. The curves demonstrate the thermal decomposition of the same dolomite sample, investigated by using a thermobalance to obtain the TG curve, and a classical steel block type DTA apparatus. On comparing the courses of the two curves, immediately two contradictions can be seen. Firstly, the TG curve represents the thermal decomposition as a one-step process while the DTA curve shows it as a two-step process. The explanation of this phenomenon lies in the different character of the two curves and the poor resolution of the TG curve. As will be shown below, this problem could be solved successfully by the derivation of the TG curve. At least as serious is the other contradiction. According to the two curves, the thermal decomposition of the dolomite did not take place in the same temperature range. According to the TG curve, the sample had totally decomposed below 900 °C, while according to the DTA curve, only the first part of the decomposition had taken place below this temperature and the decomposition was complete only at a temperature 100 °C higher. This phenomenon

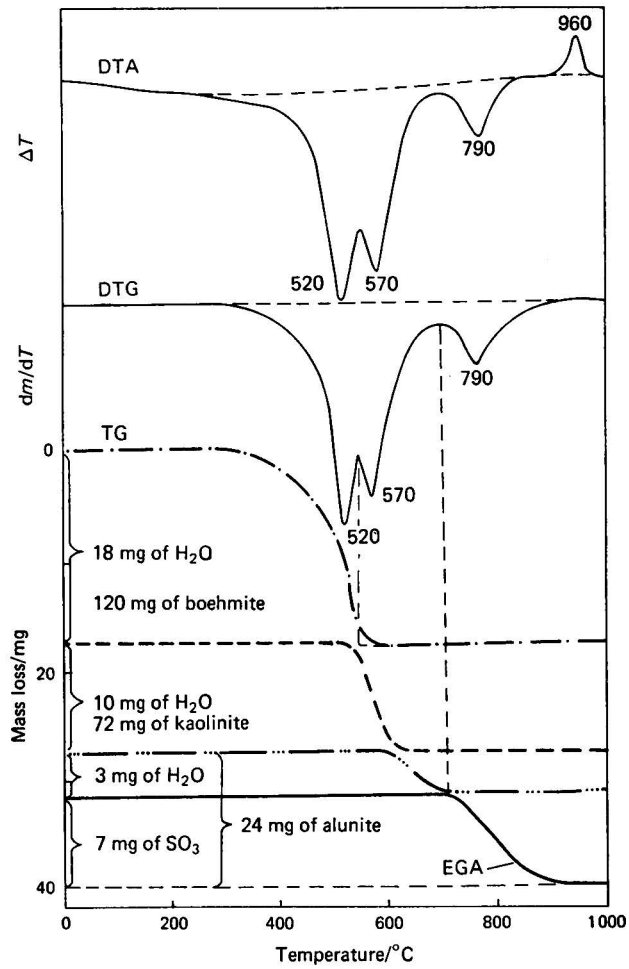


Fig. 5. Simultaneous TG, DTG, DTA and EGA investigation of the same bauxite as in Figs. 2, 3 and 4.

can be explained by the fact that different experimental conditions lead inevitably to different results. In order to eliminate this difficulty, thermoanalysts made efforts to standardise the experimental conditions.

According to the thermoanalytical method applied the thermal changes examined are described in the form of either integral or differential functions. The two different kinds of curve illustrate the transformations in different ways, because of the difference in their resolution.

We can detect a difference between two thermal effects described by two curves of different character, even if these curves demonstrate thermal processes, such as mass and enthalpy changes, that occur simultaneously. For instance, even the most experienced thermoanalyst could not decide with certainty at first glance whether the TG and DTA curves in Fig. 7 illustrate the decomposition of the same bauxite sample or that of two different bauxites of similar but not identical composition.

Derivative Methods

With the development of derivative methods this problem was fully eliminated. This statement is supported by Fig. 8, in which, in addition to the simultaneously recorded DTA

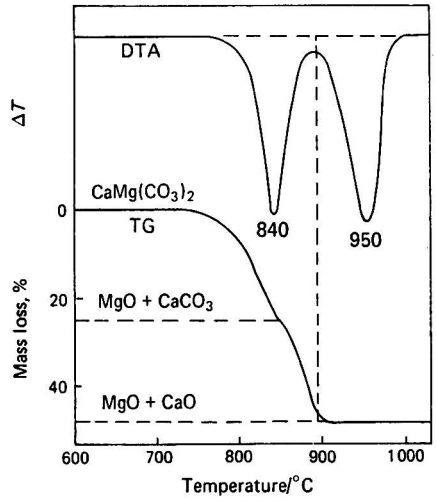


Fig. 6. Parallel TG and DTA examinations of dolomite.

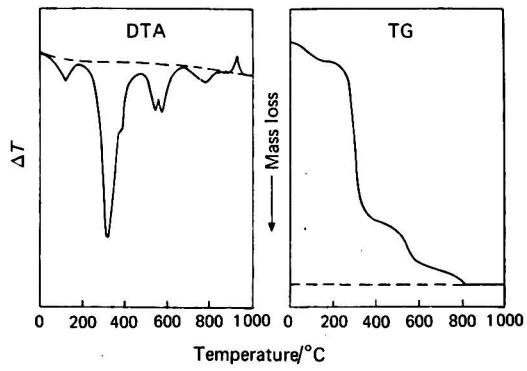


Fig. 7. Parallel DTA and TG examinations of bauxite.

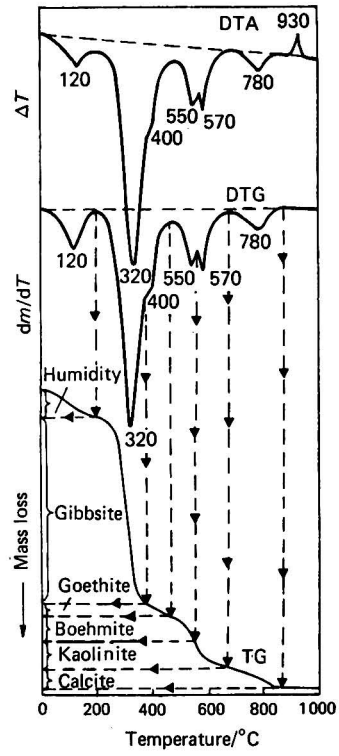


Fig. 8. Simultaneous TG, DTG and DTA investigations of the same bauxite as in Fig. 7.

and TG curves of the above-mentioned bauxite sample, the DTG curve is shown. It can be seen that the courses of the DTG and DTA curves are similar, which occurs because owing to their mathematical relationship their characters are identical. Thus the DTG curve creates a basis for the comparison of TG and DTA curves.

However, the recording of the DTG curve has further, even more significant, advantages than that just mentioned. The DTG curve, owing to its high resolution, is of significant help in selecting the characteristic temperatures as well as in describing the whole course of the transformation, and it therefore makes possible the reliable determination of the qualitative composition of multi-component samples. Thus, in Fig. 8, while reactions that follow one another overlap in the TG curve, these reactions can clearly be distinguished in the DTG curve. So, from knowledge of the TG curve alone, we could not decide whether the step on the TG curve in the temperature interval between 500 and 700 °C is due to the decomposition of boehmite, diaspore or kaolinite, or to all of them together, but the DTG curve shows without any doubt that the sample contains boehmite and kaolinite. Further, by projecting the 560 °C minimum of the DTG curve on to the TG curve, even the amount of these two minerals can be determined with a limited accuracy, which is an important additional advantage of recording the DTG curve.

These advantages were suspected by all those thermoanalysts who were making efforts to develop the various differential and derivative methods in thermal analysis. Table I is a list of the pioneers in the field. Dejean first suggested the derivation of thermoanalytical functions in 1905. His measuring technique was theoretically correct but owing to practical difficulties his method was not used. The method of de Keyser, published in 1953, also has more theoretical than practical significance. The first derivative method that found practical application was worked out in 1954. The technique, based on the principle of induction, is suitable for recording a DTG curve.

These initiatives were soon followed up and in the course of a single decade numerous measuring techniques were worked out for the derivation of thermoanalytical curves (DTA, TG), thermal analysis (TA), thermodilatometry (TD), evolved gas detection (EGD), evolved gas analysis (EGA), electrical conductivity analysis (EC) and thermomagnetic analysis (TM). Although the thermoanalytical curves can easily be derived by use of computers, for various reasons conventional methods are used in preference even now. As these computer techniques can technically be classed among computer rather than thermoanalytical methods, they are not listed in Table I.

In Table I differential and derivative methods are distinguished. On theoretical considerations derivative methods are favoured as differential methods, owing to the principle

TABLE I

DERIVATIVE AND DIFFERENTIAL METHODS

Derivative	Differential	Date	Workers	Reference
TA		1905	Dejean	1
	TG	1953	de Keyser	2
TG		1954	Paulik and co-workers	3, 4
TG		1955	Paulik and co-workers	5, 6
	TG	1955	Lambert	7
TG		1956	Waters	8
TG, DTA, EGD		1959	Campbell *et al.*	9
	DTA	1959	Freeman and Edelman	10
TD		1961	Paulik *et al.*	11, 6
TD		1961	Paulik *et al.*	12, 6
TD		1963	Wilburn and Hesford	13
	DTA	1963	Knöfel	14
TG		1965	Garn	15
TA		1965	Rupert	16
EGA		1966	Paulik and co-workers	17, 18
EGD		1966	Pannétier	19
TG		1969	Byrd	20
	TG	1972	Price	21
EC		1974	Forster, *et al.*	22
TM		1975	Moskalewicz	23

of difference formation, are liable to smaller or greater errors, while derivation can be regarded as the ideal limiting case in difference formation.

Standardisation of Experimental Conditions

The 1950s saw the introduction of another innovation, the basic idea of which is as follows. If examinations are carried out in such a way that different thermal variables are measured in a single sample, then for each variable the experimental conditions will be identical and there will not be any shift in the course of the curves obtained. This idea is the basic principle of simultaneous methods.

Fig. 9 demonstrates the thermal decomposition of dolomite. The curves in Fig. 9(*a*) were obtained by using a DTA apparatus and a deriving thermobalance separately, while the curves in Fig. 9(*b*) were recorded by applying the simultaneous TG, DTG and DTA technique. It can be seen that in the latter instance the DTA and DTG curves are congruent. Curves recorded by using two different devices differ in phase and in form. By observing the two pairs of curves we can form an idea of the difficulties that thermoanalysts had in the common interpretation and evaluation of thermal curves recorded by means of separate devices. Also, we can understand why the literature contains data for transformation temperatures of the same material that often differ by 50–100 °C.

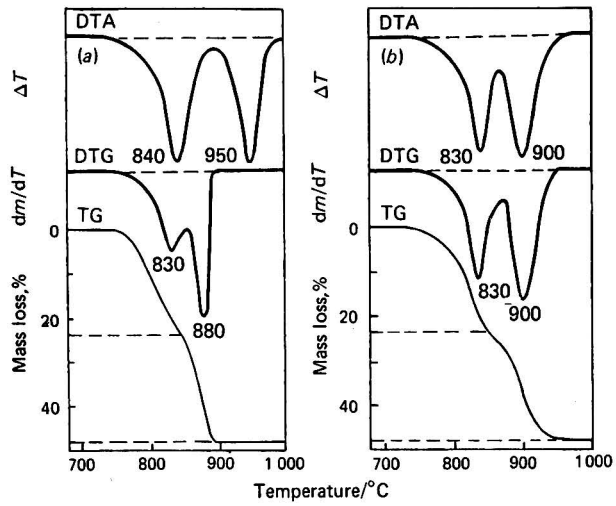


Fig. 9. (*a*), Parallel and (*b*), simultaneous TG, DTG and DTA examinations of the same dolomite as in Fig. 6.

The explanation for this phenomenon is as follows. Most of the decomposition reactions of inorganic compounds are processes that lead to an equilibrium. In a closed system at equilibrium, decomposition takes place according to the correlation existing between decomposition pressure and temperature. For example, the decomposition pressure of calcium carbonate as a function of temperature is shown in curve 1 in Fig. 10. However, in general, in thermoanalytical investigations the sample is not examined in a closed space but in an open sample holder at atmospheric pressure and in the presence of air or of an inert gas. Therefore, the composition of the gas in contact with the solid phase is changing continuously in an uncontrollable way. The TG curve in Fig. 10 describes this situation for a given practical example. The single values of the partial pressure of carbon dioxide correspond to the points given on the TG curve. These values were obtained by extrapolation, according to the principle shown, from the curve of decomposition pressure *versus* temperature. However, it should be noted that the curve was constructed by making certain suppositions and neglecting certain factors, so that the picture obtained is only qualitative. In spite of this the figure characterises the exceedingly complex process taking place in the sample. It is well known that under conditions of thermoanalytical investiga-

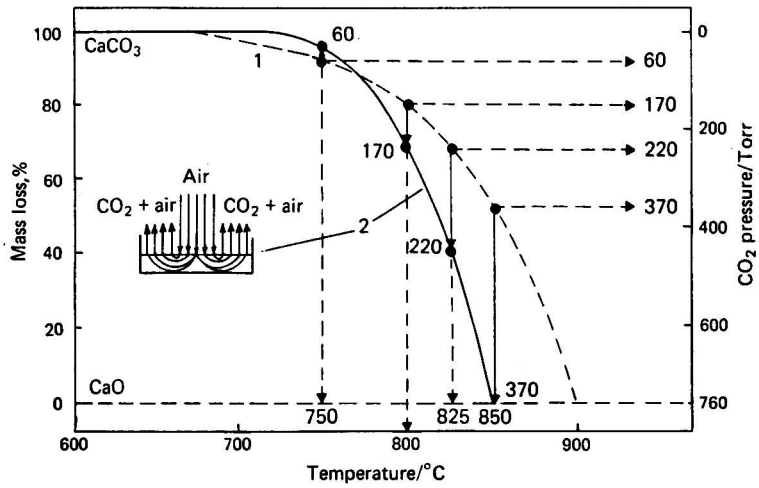


Fig. 10. 1, Decomposition pressure of $CaCO_3$ *versus* temperature; and 2, TG curve of $CaCO_3$ obtained by using a low-walled crucible.

tions reactions leading to an equilibrium are composed of many chemical and physical partial processes, some of which impede the transformation while others promote it. The path of the transformation is determined by the infinite succession of alternations taking place continuously in the formation and resolution of micro-equilibrium states.

For example, when the dissociation of calcium carbonate begins, carbon dioxide will appear within the sample, in the space between the grains of the substance. If the partial pressure of this carbon dioxide approaches the value of the decomposition pressure that corresponds to the actual temperature, then the decomposition will slow down. It will even stop if the values of the theoretical and actual pressures become equal. The fact that the reaction leads to an equilibrium produces an impeding effect on the progress of the decomposition, *i.e.*, it sets an upper limit to the decomposition rate. However, the momentary quasi-equilibrium will always overbalance and the decomposition will start again because, owing to the increased temperature, only a higher concentration of carbon dioxide will cause a new quasi-equilibrium to be established.

In addition, another factor hastens the decomposition. The mixture composed of carbon dioxide and air diffuses continuously outwards from the sample, while air diffuses towards the centre of it. The carbon dioxide released is then replaced by amounts of newly formed carbon dioxide. However, the increase in sample temperature that actually promotes the

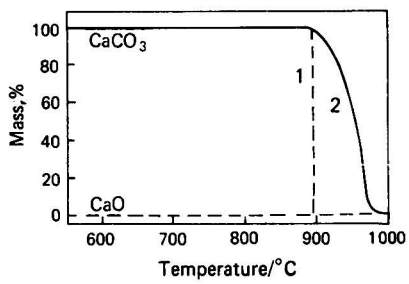


Fig. 11. Effect of heat transfer on the course of decomposition of $CaCO_3$: 1, theoretical curve for isothermal conditions and an atmosphere of CO_2; and 2, TG curve obtained with a dynamic heating programme and in an atmosphere of CO_2.

process occurs without hindrance only until the sample has taken up an amount of heat that corresponds to its heat capacity. In general, the thermal conductivity of the substances investigated is poor. Therefore, the sample is not able instantaneously to absorb from its surroundings the amount of heat necessary for the transformation to take place as this amount is greater by orders of magnitude than its heat capacity. For example, for calcium carbonate the molar heat capacity, C_P, is 26 cal mol^{-1} °C^{-1} at 890 °C and $\Delta H^0_{diss.}$ is 42 600 cal mol^{-1}. This is the reason why transformations generally take place slowly, as is demonstrated by curves 1 and 2 in Fig. 11. Curve 1 illustrates the ideal course of decomposition of calcium carbonate. In constructing this theoretical curve isothermal conditions and the presence of a carbon dioxide atmosphere were assumed. Curve 2 was recorded with a 10 °C min^{-1} dynamic heating programme and in an atmosphere of carbon dioxide. Owing to the atmosphere of carbon dioxide the decomposition process was made independent of the gas transport, as we have supposed in constructing curve 1. Accordingly, the difference between the courses of the two curves can be attributed solely to the effect of the slow heat transfer (see also Fig. 18).

Thus the progress of the decomposition is governed by the rate of temperature increase and of gas diffusion, which will be such that the momentary equilibrium of the system always corresponds to the correlation between decomposition pressure and temperature.

Fig. 12 demonstrates the different gas-diffusion conditions obtaining in two sample holders of different shape. Comparison of the partial pressure values of carbon dioxide, indicated at the corresponding points of the TG curve, shows that the concentration of carbon dioxide within the sample changed in the two experiments in different ways. For example, when, in the low-walled sample holder, the decomposition of the sample was complete, the temperature was at 850 °C, while the partial pressure of carbon dioxide was at 370 Torr. In contrast, at the same temperature and pressure values, only 40% of the sample was decomposed in the high-walled sample holder and the decomposition was complete only at 890 °C. However, the partial pressure of carbon dioxide in the sample had reached 680 Torr at this temperature.

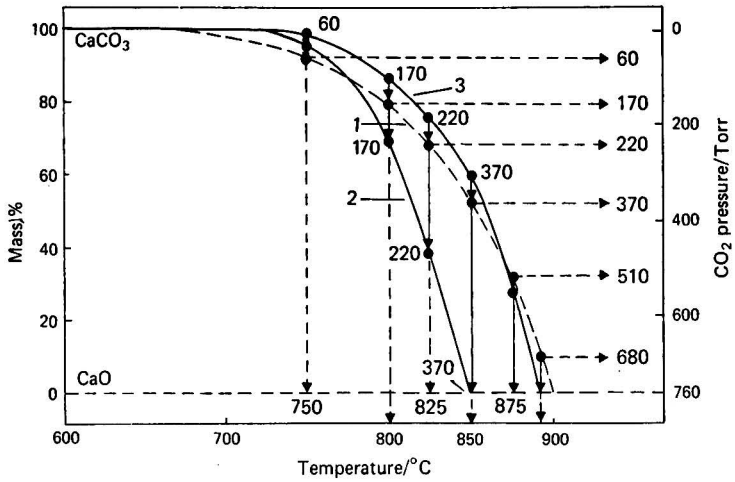


Fig. 12. 1, Decomposition pressure of $CaCO_3$ *versus* temperature; 2, TG curve obtained with a low-walled crucible; and 3, TG curve obtained with a high-walled crucible.

It follows from the above that all of the experimental conditions that may influence the rate of gas diffusion and also the rate of heat transfer exert a significant influence upon the course of thermal decomposition. Such conditions are, for example, the amount of sample, its layer thickness and compactness, size of the grains, heating rate, composition and pressure of the gaseous atmosphere and shape and size of the sample holder.

However, for the sake of completeness it should be noted that in some instances, owing to the occurrence of even slower partial processes than those mentioned above (nucleus forma-

tion, nucleus growth, gas diffusion through the compact new phase, etc.) the transformation process will be even more complicated.

On the other hand, for endothermic reactions not leading to an equilibrium, the situation is simpler, as the course of such transformations is independent of the concentration of the gaseous decomposition products and in most instances it is disadvantageously influenced only by the slow heat transfer.

In the 1950s, recognition of these factors led to research activity to find appropriate means for the standardisation of experimental conditions, but a real breakthrough in this field could successfully be achieved only by the introduction of simultaneous techniques. The solution to the problem, which was to use a single sample, was exceedingly simple and the results obtained were ideal.

Despite the rapid and wide application of simultaneous techniques, the parallel application of individual methods remained current and they are still employed nowadays. Great efforts were made to standardise the experimental conditions in the course of the development of these latter methods too. At the present stage of development both types of measurement have advantages and disadvantages. In the construction of equipment for simultaneous techniques, just in the interest of coupling different methods we are often compelled to select less precise techniques of measurement, while methods applied in parallel compensate for the lack of standard results with greater accuracy.

Although the principle of examination precludes the possibility of using identical experimental conditions when applying complementary methods, these methods do not play a subordinate role among combined techniques. Let us remember, for example, the importance of complementary X-ray spectroscopy, infrared spectroscopy and electron microscopy.

In spite of the importance of all the combined methods mentioned, in what follows we shall deal only with simultaneous measuring techniques.

TG - DTA

Among thermoanalytical methods TG and DTA are the two that yield the greatest and the most valuable information about the substance investigated. Consequently, it is understandable that the coupling of these two methods was the first to be attempted. Researchers who made efforts in this direction are listed in Table II. It can be seen that the first equipment of this type was constructed in 1955, and was suitable for the simultaneous recording of TG, DTG and DTA curves. This initiative was soon followed by several others.

TABLE II

SIMULTANEOUS DTA, TG AND DTG

Method	Date	Workers	Reference
DTA - TG - DTG	1955	Paulik *et al.*	24, 25
DTA - TG	1957	Powell	26
DTA - TG	1959	Papailhau	27
DTA - TG	1960	Blazek and Cisar	28
DTA - TG	1960	Reismann	29
DTA - TG	1961	Piece	30
DTA - TG	1962	Torkar *et al.*	31
DTA - TG	1962	Formanek and Dykast	32
DTA - TG	1962	Kissinger and Newman	33
DTA - TG	1963	McAdie	34
DTA - TG	1963	Krüger and Bryden	35
DTA - TG - DTG	1964	Wiedemann	36
DTA - TG	1964	Khristianov and Korovyatnikov	37
DTA - TG - DTG	1964	Saito *et al.*	38
DTA - TG	1965	Charsley and Redfern	39
DTA - TG	1968	Patai *et al.*	40

EGA and EGD

Apart from TG and DTA methods, the examination of the gaseous decomposition products that evolve on heating may perhaps furnish the greatest and the most useful information

regarding both the composition of the substance investigated and the nature of the reactions taking place in the material. For this purpose a number of different methods have been developed, which are usually divided into two groups, EGD and EGA. According to the measurement technique, the character of the information obtained and the field of application each of these groups can be further divided into two sub-groups.

A number of the EGD methods (Table III) are based on the detection of gas evolution by means of a thermal conductivity cell or a gas density detector (Group I). The method of examination is simple and the equipment is cheap, but the information obtained is modest. The equipment clearly indicates the evolution of gas and conclusions can be reached based upon the amount of gas evolved. However, these methods are not adequate for the examination of the quality of the evolved gas. In spite of this the combination of these methods with other thermoanalytical methods, for example with DTA or TD, or with mass spectrometry (MS), may be very useful, as they can contribute to the better interpretation of the results obtained and enable physical transformations and decomposition reactions to be clearly distinguished.

TABLE III

EGD METHODS

Method	Method combined with	Date	Workers	Reference
Group I—				
Thermal conductivity and densitometry	DTA	1960	Lodding and Hammel	41
	DTA	1961	Ayres and Bens	42
	DTA	1961	Garn and Kessler	43
	DTA	1962	Wendlandt	44
Group II—				
Volumetric	DTA	1953	Teitelbaum and Berg	45
	DTA	1957	Gordon and Campbell	46
Barometric	DTA	1964	Charles	47
	TG	1965	Bancroft and Gesser	48
	DTA	1967	Guenot *et al.*	49
	TG	1968	Maicock and Pai Verneker	50
	DTA	1969	Bousquet *et al.*	51

An example of the use of these techniques of examination is given in Fig. 13. The DTA curve of barium perchlorate hydrate is shown, and the interpretation of this curve alone would be difficult, but if the EGD curve is recorded simultaneously, as shown, then the common interpretation of the two curves permits us without risk of major error to state the following. The first three maxima on the EGD curve indicate the release of water, while the fourth is due to the escape of oxygen. The first maximum on the DTA curve can probably be explained by the melting of the hydrate, the fifth and sixth maxima by changes in crystal modification, and the seventh may be due to the melting of the anhydride.

With the help of gas volumetric and gas barometric examinations (Table III, Group II) the amount of gas evolved can also be determined, but obtaining these curves is only an indirect means for the determination of the quality of the evolved gases. In addition to recording the curves of the gaseous decomposition products, it is also necessary to record the TG curve. Owing to the difference in the relative molecular masses of gases, in a favourable instance we shall be able to select from possible gaseous decomposition products the most probable one.

The principle of this examination is demonstrated for a simple example in Fig. 14. Here the EGD and TG curves of a calcium oxalate sample can be seen. The former indicates that in the three periods of the decomposition the volume of evolved gases was equal, but owing to the difference in the relative molecular masses of water, carbon monoxide and carbon dioxide, the sizes of the three steps in the TG curve are different. In more complicated instances this numerical difference forms the basis of calculations.

The essential difference between the methods of EGD and EGA lies in the fact that with EGA both the quality and the amount of the gaseous decomposition products can be determined in a selective way. However, in addition to problems of standardisation, the

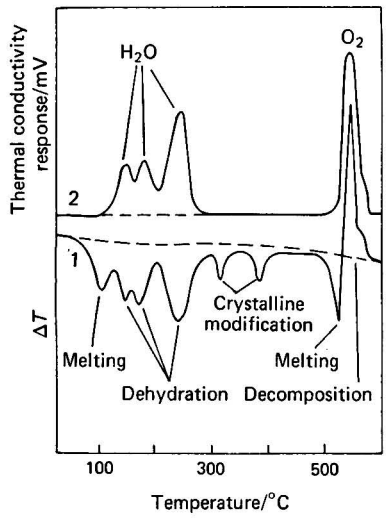


Fig. 13. Simultaneous DTA (1) and EGD (2) examinations of $Ba(ClO_4)_2.xH_2O$.

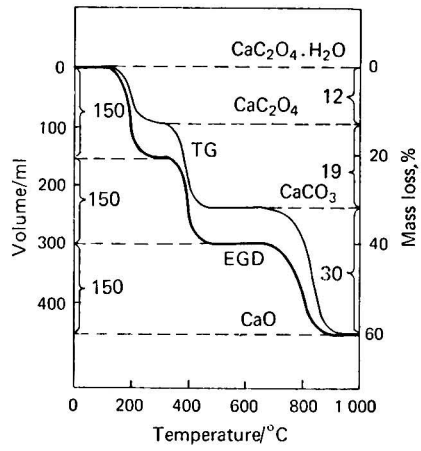


Fig. 14. Simultaneous TG and EGD examinations of $CaC_2O_4.xH_2O$.

separation of overlapping transformations, or in other words increasing the selectivity of the examination, causes the greatest trouble to thermoanalysts.

We have already shown, for the determination of boehmite, alunite and kaolinite, the usefulness of EGA methods in separating overlapping reactions. A similarly convincing example is shown in Fig. 15. During the preparation of aluminium sulphate, if the optimum

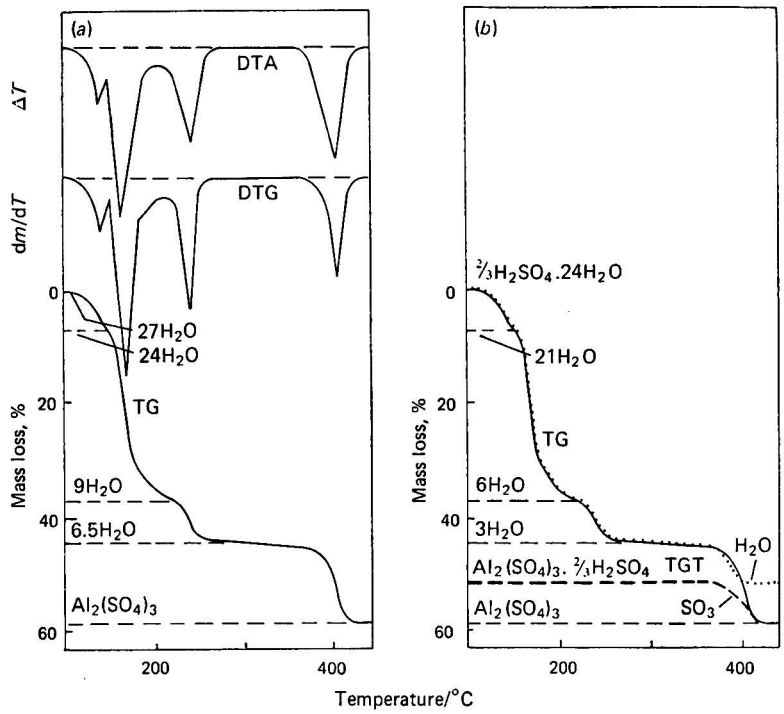


Fig. 15. Simultaneous DTA, TG, DTG and EGA examinations of (*a*), $Al_2(SO_4)_3.xH_2O$ of unknown composition and (*b*), $Al_2(SO_4)_3.\frac{2}{3}H_2SO_4.24H_2O$.

conditions are not observed, then instead of $Al_2(SO_4)_3.18H_2O$ the acid salt is precipitated. When this salt is dehydrated sulphur(VI) oxide is also evolved. In fact, the course of the TG, DTG and DTA curves would not call our attention to this possibility. Further, even an EGD curve would not indicate the water and sulphur(VI) oxide separately. In contrast, the curve denoted SO_3 in Fig. 15 clearly shows that with the departure of the last two water molecules at 450 °C two thirds of a molecule of sulphur(VI) oxide is also released. Without performing the simultaneous EGA examination, we were not able even to notice the evolution of sulphur(VI) oxide. At the same time, having obtained the curve for the release of sulphur(VI) oxide we not only can determine the amount of sulphur(VI) oxide but may also be prevented from drawing incorrect conclusions regarding the composition of the sample and the kinetics of the process.

The sulphur(VI) oxide curve was obtained by applying the technique of thermal gas titrimetry (TGT) (Table IV). With the help of this technique, based on the principle of titrimetric analysis, the amounts of the gaseous decomposition products of inorganic compounds can be determined in the presence of one another.

TABLE IV

EGA METHODS

Method	Method combined with	Date	Workers	Reference
Group I—				
TGT	DTGT, DTA, TG, DTG	1966	Paulik *et al.*	52, 17
Gas infrared spectroscopy ..	TG	1967	Hegedüs and Kiss	53
Absorption liquid conductimetry	TG, DTA	1964	Keattch	54
Absorption liquid thermometry ..	DTA	1963	Chamberlain and Green	55
	DTA	1963	Notz and Jaffe	56
Group II—				
GC	TG	1963	Cano	57
	DTA	1966	Bandi *et al.*	58
	TG	1968	Chiu	59
	DTA	1969	Bollin	60
	DTA	1975	Yamada *et al.*	61
	DTA	1975	Mercier	62
MS	EGD	1965	Wendlandt and Southern	63
	EGD, DTA	1965	Langer *et al.*	64
	EGD, DTA	1966	Wendlandt *et al.*	65
	EGD, TG	1968	Zitomer	66
	TG	1969	Wilson and Hamaker	67
	EGD, TG, DTA	1969	Smith and Johnson	68
	EGD, DTA, TG	1969	Wiedemann	69
	DTA	1969	Gaulin *et al.*	70
	EGD, DTA, TG, DTG	1969	Redfern *et al.*	71
	TG	1969	Brown *et al.*	72
	DTA	1969	Langer and Bradly	73
	TG, GC	1971	Chang and Mead	74
	DTA, TG	1973	Gibson	75
	GC	1974	Merritt *et al.*	76
Thermoparticulate analysis ..	DTA, TG	1960	Doyle	77
Thin-layer chromatography ..	DTA	1967	Rogers	78

A similar possibility for the selective determination of gaseous decomposition products is offered by infrared spectroscopy and by conductimetric or thermometric measurements on a liquid in which the gases have been absorbed (Table IV, Group I).

Examination of Decomposition of Organic Compounds

It is known that the decomposition of organic compounds is in most instances a very complex process. Solid, liquid and gaseous decomposition products are formed in the course of the countless reactions taking place in parallel or consecutively. For the selective investigation of these products and processes the methods discussed so far are not suitable. However, these processes can be examined if, for example, gas-chromatographic (GC), mass

spectrometric (MS), thin-layer chromatographic or thermoparticulate analyses are combined with DTA and TG measurements. The earliest of these methods are listed in Table IV, Group II. Particularly good selectivity can be attained when GC and MS are applied together. In this instance the gaseous decomposition products are separated by means of a gas chromatograph into different fractions, which are then further examined by means of a mass spectrometer. As these examinations can be performed only stepwise, generally the EGD curve is simultaneously recorded in order to determine the gas-evolution process. The equipment required for these analyses is expensive and requires skill in handling; however, it is indispensable when the kinetics and mechanism of the thermal decomposition of organic compounds are to be studied.

TD and ETA

We think TD has been neglected up to now, yet it provides useful information about changes occurring in the crystal structure of inorganic compounds, which is an area in which other thermoanalytical methods are not very useful. Two examples of the use of TD are shown in Fig. 16. It is known that kaolinite loses its water of constitution between 400 and 800 °C. During the course of a solid-state reaction it transforms first into metakaolinite and then into mullite at about 950 °C. The other example, barium chloride dihydrate, first loses its water of crystallisation and in the temperature interval 350–850 °C a significant recrystallisation process takes place in the anhydrous material. In the vicinity of 900 °C, just before the substance melts, a modification from the α to β crystal form occurs.

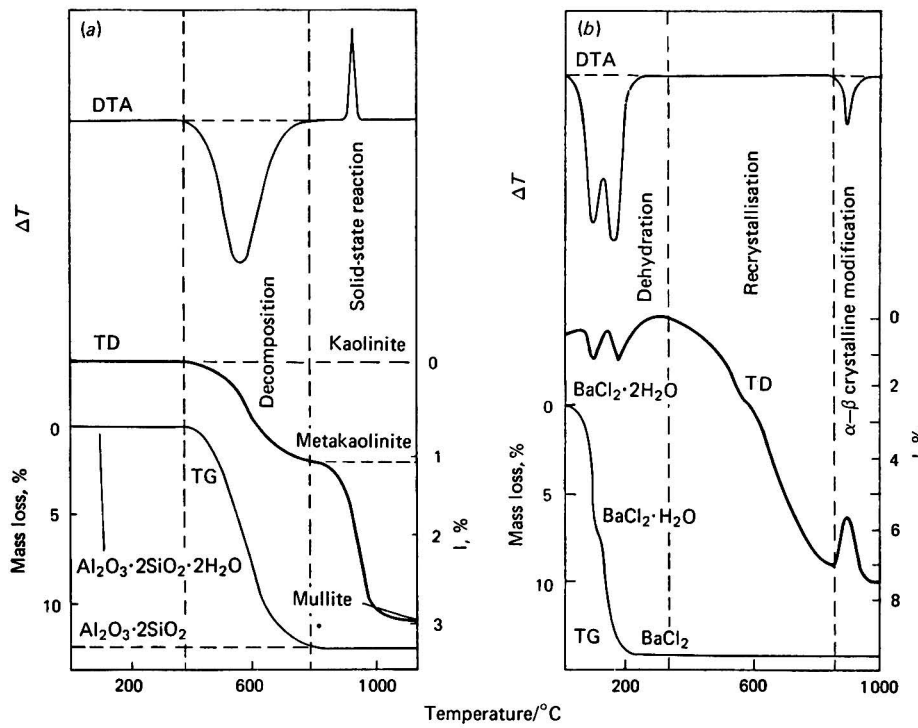


Fig. 16. Simultaneous DTA, TG and TD examinations of (*a*), $Al_2O_3.2SiO_2.2H_2O$ and (*b*), $BaCl_2.2H_2O$.

As the shape of the TD curves in the figures proves, the course of all the processes, the solid-state reaction and also the thermal decomposition, the recrystallisation and crystal modification, can be followed by this method. However, so far only in the field of coal, ceramic and silicate chemistry has TD found wide application, and even here mostly as a single method. The reason for the neglect of this technique probably lies in the difficult interpretation of the TD curve, as in general it undoubtedly gives a more complicated picture

than the TG or DTA curves. For example, in thermal decomposition the sample decomposes and an amorphous or microcrystalline phase is formed. The recrystallisation that generally follows is in most instances protracted and overlaps the previous process. While the DTA and TG curves indicate only the first process, the TD curve depicts both processes, which causes difficulties in its interpretation. Therefore, in addition to the derivation of this curve, the simultaneous recording of other thermoanalytical curves, *e.g.*, DTA or DTG (Table V), can greatly contribute to the easier and more reliable evaluation of the TD curve.

TABLE V

TD METHODS

Method combined with	Date	Workers	Reference
DTA	1956	Lehman and Gatzke	79
DTA	1958	Koehler	80
DTA	1959	Pearce and Mardon	81
DTD, DTA	1961	Paulik *et al.*	11
DTD, DTA, TG, DTG ..	1961	Paulik *et al.*	82, 11
DTD, DTA, TG, DTG, TGT, DTGT	1971	Paulik and Paulik	83

Emanation thermal analysis (ETA) is a method cognate with the previous one (Table VI). With the help of this method all those chemical and physical processes can be followed which actually cause the transitional migration of the lattice elements in crystals. However, the results of the two kinds of examination differ from and therefore supplement one another.

TABLE VI

ETA METHODS

Method combined with	Date	Workers	Reference
TG	1961	Bussiere *et al.*	84
DTA, TD	1965	Zaborenko *et al.*	85
DTA, TD	1969	Balek	86
DTA, EGD	1972	Habersberger and Balek	87
DTA, TG	1973	Emmerich and Balek	88

EC Methods

An example of the applicability of EC methods coupled with other thermoanalytical methods (Table VII) can be seen in Fig. 17. This figure shows the results obtained with a thallium stearate sample. According to the DTA curve the substance melts immediately after its second polymorphous transition, but it forms an isotropic liquid only after the mesomorph - liquid transition. Now, as the course of the EC curve proves, these last two

TABLE VII

EC METHODS

Method combined with	Date	Workers	Reference
DTA	1959	Satava	89
DTA	1960	Berg and Burmistrova	90
DTA	1960	Budnikov *et al.*	91
DTA	1963	Pannetier *et al.*	92
X-ray	1965	Bessonov and Ustyantsev	93
TG, DTG, DTA	1967	Chiu	94
DSC	1969	Carrol and Mangravite	95
DTA	1970	David	96
TD	1970	Judd and Pope	97
DTA, TD, ETA	1970	Balek	98
DTA	1973	Halmos and Wendlandt	99
DTA, EGD	1975	Berg and Shlyapkina	100
TG	1975	Juranic *et al.*	101

processes can also be followed by measurement of electrical conductivity. This example is a simple one, but the information-multiplying effect of the method has its real significance in more complicated instances.

Also, remarkable results were obtained recently by thermoanalysts who coupled the methods of thermal X-ray analysis, photometric thermal analysis, hot-stage microscopy, thermomagnetic analysis, dynamic reflectance spectroscopy and thermoacoustic analysis with the methods of TG, DTA, EC, MS, EGD and TD (Table VIII).

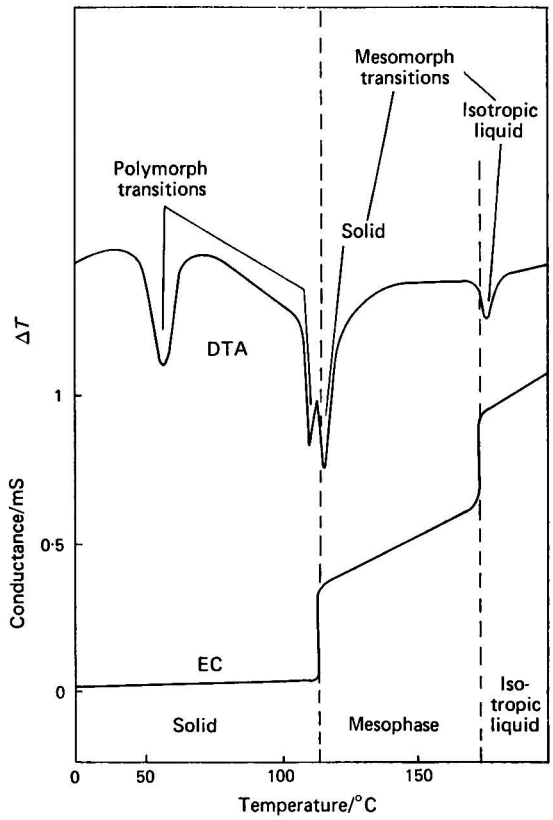


Fig. 17. Simultaneous DTA and EC examinations of thallium stearate.[102]

Manipulation of Experimental Conditions

The picture we intend to give would not be complete without consideration of the manipulation of experimental conditions. For example, the beneficial effect exerted upon the resolution or the selectivity if a vacuum or high pressure are applied is well known. Further, semi-micro techniques can be used to improve results, and many other similar attempts have been made to achieve the same purpose by the selection of experimental conditions. However, we shall mention only two subjects here.

An example was shown above (Fig. 10) to demonstrate that in the course of decomposition, the concentration of the gaseous decomposition products within the sample is continuously changing in an uncontrollable way. This condition greatly influences the whole course of the decomposition. By altering the experimental conditions the concentration of the gaseous decomposition products, and therefore the course of the decomposition, changes too (Fig. 12). Some thermoanalysts wished to eliminate this effect by ensuring a "self-generated" atmosphere. These researchers wanted to solve this problem by use of sample holders whose shape, as shown in Table IX, makes it possible for the gaseous decomposition products

TABLE VIII

THERMAL X-RAY ANALYSIS

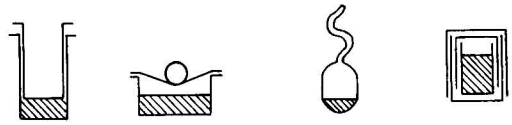
Method	Method combined with	Date	Workers	Reference
Thermal X-ray analysis	DTA	1965	Wefers	103
	DTA	1967	Ravich	104
	DTA, EC	1967	Bessonov *et al.*	105
	DTA	1968	Barret *et al.*	106
	EGD, MS	1973	Wiedemann	107
Photometric thermal analysis	DTA	1972	David	108
	TG	1972	Loehr and Levy	109
	DTA	1972	Barrall and Johnson	110
	TD	1974	Chrony	111
Hot-stage microscopy	DTA	1966	Miller and Sommer	112
	DTA	1967	Dichtl and Jeglitsch	113
	DTA	1968	Van Tets and Wiedemann	114
Thermomagnetic analysis	TG	1966	Simmons and Wendlandt	115
Dynamic reflectance spectroscopy	EGD	1970	Wendlandt and Bradley	116
Thermoacoustic analysis	DTA	1975	Chatterjee	117

to be released easily, while the diffusion of air from the opposite direction is possibly hindered. They postulated that a pure self-generated atmosphere would immediately be created in the reaction space at the beginning of the decomposition. Thus, the stability of the partial pressure of the gaseous decomposition products would make the course of the decomposition unambiguous.

However, as it turned out later, this solved the problem only to a certain extent. Owing to the poor thermal conductivity of the sample and the rapid increase in temperature, a temperature drop occurs within the sample. Accordingly, the slow heat transfer is also responsible for the delayed transformation, which takes place in a broad temperature interval (Fig. 11).

TABLE IX

SAMPLE HOLDERS FOR A SELF-GENERATED ATMOSPHERE



Type*	Date	Workers	Reference
P	1960	Garn and Kessler	118
BV	1960	Forkel	119
C	1962	Amiel and Paulmier	120
P	1962	Claudel	121
BV	1962	Pannetier *et al.*	122
BV	1963	Dormieux	123
C	1964	Lagier *et al.*	124
L	1971	Paulik and Paulik	125, 126

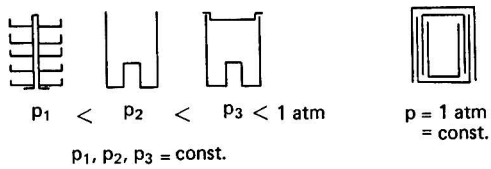
* P, piston; BV, ball-valve; C, capillary; L, labyrinth.

Researchers wanted to overcome this problem by working out a "quasi-static" heating programme (Table X). The idea was that if the temperature increase in the sample was controlled in such a way that the decomposition reaction took place at a very low and constant rate, then the error caused by the slow heat transfer may be totally eliminated.

Two kinds of heating control systems were developed. One, by limiting the rate of mass change, dm/dT, produced quasi-isothermal heating conditions for TG and simultaneous EGA investigations. The other, thermoanalysis with a constant rate of decomposition

TABLE X

METHODS WITH QUASI-STATIC HEATING



Conditions	Method	Pressure	Heating control	Date	Workers	Reference
Quasi-static heating	TG	—	dm/dT	1962	Erdey *et al.*	127
	TCRD	Vacuum	dp/dT	1969	Rouquerol	128
	TG	p_1, p_2, p_3	dm/dT	1971	Paulik and Paulik	129
Self-generated atmosphere +	TG	1 atm	dm/dT	1972	Paulik and Paulik	130
quasi-static heating	TG, EGA	1 atm	dm/dT	1973	Paulik and Paulik	131

(TCRD), produced a heating control system in vacuum, under which the pressure of the gaseous products became constant, and the correlation between the constant gas flow being evolved and the temperature was recorded.

However, the final solution to the problem has been brought about by the simultaneous application of a quasi-static heating programme and a sample holder ensuring a self-generated atmosphere. The gradual progress of this technique and the beneficial effect of its application are shown by the TG curves in Fig. 18. The curves demonstrate the decomposition of calcium carbonate under different experimental conditions. Curves 1 and 2 were recorded at a heating rate of 10 °C min^{-1}. For curve 1 a conventional open crucible

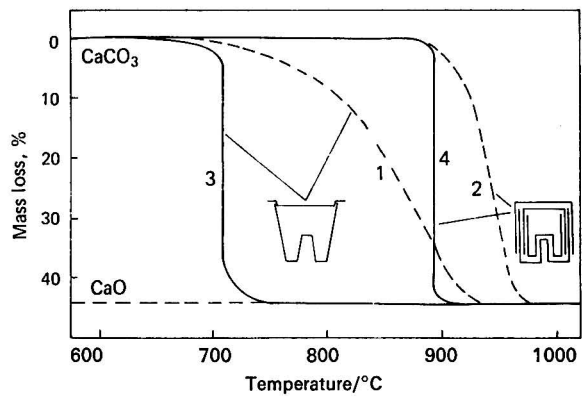


Fig. 18. Decomposition of $CaCO_3$ investigated under (1 and 2) a dynamic and (3 and 4) a quasi-static heating programme. Sample holder: 1 and 3, conventional; and 2 and 4, labyrinth.

was used, while for curve 2 a labyrinth sample holder was used in order to ensure a self-generated atmosphere. Curves 3 and 4 were similarly recorded by using the two different kinds of sample holder, but with a quasi-static heating programme, and the calcium carbonate decomposed under quasi-isothermal conditions, *i.e.*, the temperature of the sample did not change during the decomposition process. It is evident that quasi-isothermal conditions greatly increase the selectivity and resolution of the examination, as well as the accuracy of the qualitative and quantitative determinations. However, there is a difference even between curves 3 and 4. According to curve 4 the decomposition of calcium carbonate took place at 895 °C, *i.e.*, at its "normal" decomposition temperature, known from physical chemistry. This situation is of significance from the point of view of standardisation.

Conclusion

In the presentation of the tables our primary intention was to summarise the simultaneous methods that have been reported in a manner that can be easily read. It must be appreciated that a complete survey of the literature is not given here, and the material presented has necessarily been selective.

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Solid-state Mercury(I) Chloride Electrode for Determining 0.1–1.0 μg ml^{-1} Levels of Chloride in Boiler Water and Other High-purity Waters

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A solid-state electrode based on mercury(I) chloride and mercury(II) sulphide has been developed for determining chloride concentrations of 0.01–1.0 μg l^{-1} in boiler water. The greater sensitivity of the electrode compared with that of silver - silver chloride electrodes enables concentrations as low as 0.01 μg ml^{-1} to be determined by a simple manual technique. The total standard deviations at chloride concentrations of 0.5, 0.1 and 0.01 μg ml^{-1} were 0.025, 0.005 and 0.015 μg ml^{-1}, respectively. The electrode can be prepared easily in the laboratory from commercially available materials and a Růžička Selectrode. The only significant interference is from iron(III) ions and this interference can be eliminated by adding fluoride ions to the sample.

Keywords: *Chloride determination*; *water analysis*; *potentiometry*; *chloride-selective electrode*; *mercury(I) chloride electrode*

In order to avoid acid attack in boilers, the concentration of chloride in boiler waters must be kept at very low levels [in Central Electricity Generating Board (CEGB) practice a maximum of 0.2–2 μg ml^{-1}, depending on the type of boiler]. The established CEGB manual potentiometric method[1] and absorptiometric methods based on mercury(II) thiocyanate are insufficiently sensitive for chloride concentrations much below 0.1 μg ml^{-1}, such as occur in many boilers. The range of the potentiometric method has been extended by using a flow cell whose temperature is precisely controlled[2] and that of the absorptiometric method by first concentrating the chloride by a co-precipitation technique.[3] In both methods the analytical procedure is fairly complicated.

The existing potentiometric method uses electrodes based on silver chloride, but electrodes made from mercury(I) chloride should be more suitable for determining low concentrations of chloride because of the lower solubility of the mercury(I) salt. The difficulty of handling mercury - mercury(I) chloride electrodes has largely restricted them to use in reference electrodes, but an experimental solid-state ion-selective electrode with a membrane composed of a compressed mixture of mercury(II) sulphide and mercury(I) chloride[4] showed promise of being easy to use. We have combined the same membrane materials with a Růžička Selectrode[5] to produce a solid-state chloride-selective electrode that can be made easily from commercially available components and is suitable for determining chloride at concentrations as low as 0.01 μg ml^{-1} by a simple manual technique.

Theoretical

As with calomel electrodes, the presence of chloride in a solution disturbs the solubility product equilibrium of mercury(I) chloride [equation (1)], but there is also a simultaneous solubility equilibrium for mercury(II) sulphide [equation (2)]. The two solubility equilibria are linked by the disproportionation equilibrium of mercury(I) ions [equation (3)]:

$$Hg_2Cl_{2(s)} \rightleftharpoons Hg_2^{2+}{}_{(aq)} + 2Cl^-{}_{(aq)} \quad .. \quad .. \quad .. \quad (1)$$
$$K_{Hg_2Cl_2} = \{Hg_2^{2+}\}\{Cl^-\}^2$$

$$HgS_{(s)} \rightleftharpoons Hg^{2+}{}_{(aq)} + S^{2-}{}_{(aq)} \quad .. \quad .. \quad .. \quad (2)$$
$$K_{HgS} = \{Hg^{2+}\}\{S^{2-}\}$$

$$Hg_2^{2+}{}_{(aq)} \rightleftharpoons Hg^{\circ}{}_{(l)} + Hg^{2+}{}_{(aq)} \quad .. \quad .. \quad .. \quad (3)$$
$$K_d = \{Hg^{2+}\}/\{Hg_2^{2+}\}$$

The e.m.f. of an electrode containing mercury(I) chloride and mercury(II) sulphide both in equilibrium with an aqueous solution of chloride ions should be given by a form of the Nernst equation, which is expressed in terms of either a chloride or sulphide response:

$$E = E^{\circ}_{Hg_2Cl_2} - k \log\{Cl^-\} \quad .. \quad .. \quad .. \quad .. \quad (4)$$

$$= E^{\circ}_{HgS} - \frac{k}{2} \log\{S^2\} \quad .. \quad .. \quad .. \quad .. \quad (4a)$$

where $k = RT\ln 10/F$ is the slope factor and $\{S^{2-}\}$ is the activity of the sulphide ion dissolved from the electrode. From equations (1)–(3)

$$\{S^{2-}\} = K_{HgS}\{Cl^-\}^2/K_d K_{Hg_2Cl_2} \quad .. \quad .. \quad .. \quad (5)$$

and hence from equations (4) and (4a)

$$E^{\circ}_{Hg_2Cl_2} = E^{\circ}_{HgS} - \frac{k}{2} \log(K_{HgS}/K_d K_{Hg_2Cl_2}) \quad .. \quad .. \quad .. \quad (6)$$

Experimental

Apparatus

Potentials were measured with an Orion 801 digital pH meter reading to 0.1 mV and displayed on a Servoscribe 2s chart recorder. When two electrodes were being used simultaneously they were switched in turn through the pH meter by an Orion 855 automatic electrode switch.

The reference electrodes were of the mercury - mercury(I) sulphate type with a ground-glass sleeve liquid junction and 1 mol l^{-1} sodium sulphate filling solution (Electronic Instruments Ltd., Type 1380–230).

Preparation of chloride electrodes

A sensitising mixture was prepared by mixing red mercury(II) sulphide (BDH, Optran grade) and mercury(I) chloride in equimolar amounts and grinding them in an agate mortar until the mixture was uniformly pink. The mercury(II) sulphide had been washed for 15 min with each of two portions of carbon disulphide, washed with acetone and air dried before use. Because of their toxicity these materials should be handled with care.

Radiometer F3012 Universal Selectrodes were impregnated with the above mixture by rubbing the mixture into the exposed graphite surface with a glass rod.[5]

Reagents

Water. Town mains water was distilled in a stainless-steel still (Manesty Machines Ltd., Liverpool), and the distillate passed through a twin-column mixed-bed de-ionisation unit (Elga Products Ltd., Model B106/2). The conductivity of this water was less than 0.1 μS cm^{-1} at 20 °C as it left the unit and was found by a modification of the method of Rodabaugh and Upperman[3] to have a chloride content of about 0.7 μg l^{-1}. This water was used in the preparation of all standard or reagent solutions.

Standard chloride solutions. A stock solution (1 000 μg ml^{-1} of chloride) was prepared by dissolving 1.649 g of sodium chloride in water and making up to 1 l in a calibrated flask. Further standard solutions were prepared by successive dilution of this solution.

Nitric acid, 0.1 mol l^{-1}. A solution was prepared by dilution of 6.25 ml of concentrated nitric acid (BDH, Aristar grade) to 1 l.

Mercury(II) sulphide. Commercially available mercury(II) sulphide (BDH, Optran grade) was used for most experiments, but a batch of material was also prepared in the laboratory as follows.

Sodium sulphide nonahydrate (AnalaR grade) (12 g) was dissolved in about 70 ml of water and the solution filtered through a 0.45-μm Millipore filter. The filtrate was added to a solution of 13.7 g of mercury(II) nitrate (AnalaR grade) in about 100 ml of water. The precipitate was filtered off, washed with de-ionised water and dried.

The material prepared by precipitation was black β-cinnabar but the commercial product was the red α-cinnabar form.

Analytical Procedure

E.m.f. measurements were made with the electrodes immersed in stirred 50-ml portions of standard or sample solution to which 5 ml of 0.1 mol l^{-1} nitric acid had been added. Between each measurement the electrodes were immersed in a stirred rinsing solution of 0.01 mol l^{-1} nitric acid for about 2 min until the e.m.f. was less negative than that recorded with any of the solutions being analysed (about -30 mV for solutions below 0.1 μg ml^{-1} of chloride). The analytical measurement therefore was always obtained from an electrode responding to an increase in concentration, which procedure gave the best over-all response time, and, in addition, contamination of one sample or standard solution by a more concentrated predecessor was prevented. Concentrations of chloride were obtained from a calibration graph prepared by making measurements, as above, with standard solutions.

Before the start of each batch of analyses the ground-glass sleeve of the reference electrode was flushed by allowing a few drops of the internal filling solution to flow out. If this precaution was neglected, the e.m.f. tended to drift, as also occurred after a time when reference electrodes with ceramic frit junctions were used.

Results

Electrode Characteristics

Nature of the membrane material

Electrodes were sensitised with equimolar mixtures of mercury(I) chloride and either the red or black form of mercury(II) sulphide. The electrodes with the red form had Nernstian responses (57–59 mV per decade) at concentrations above 1 μg ml^{-1}, while those made with the black form had calibration slopes 1–2 mV per decade lower and were also much slower to respond.

When the red mercury(II) sulphide was washed with carbon disulphide, the sensitivity and response time of the electrodes were further improved. Electrodes prepared with two batches of red mercury(II) sulphide had almost identical characteristics. Because of its poorer performance, no further tests were made using the black mercury(II) sulphide.

Electrodes prepared with mercury(I) chloride only were still sensitive to chloride, but the e.m.f. difference was only 45 mV between 0.1 and 1 μg ml^{-1} solutions (*cf.*, Table II) and about 30 min were required for a steady potential to be established; for these reasons no detailed work was done with electrodes of this type.

Variations between electrodes

The amount of sensitising mixture in the membrane and the pressure exerted in forming it on the end of the electrode did not affect the performance of the electrode. The four Radiometer F3012 electrodes used during the tests gave similar results; the potentials of electrodes in the same solution rarely differed by more than 2 mV, regardless of the concentration of the solution. Removal of half of the membrane and exposure of the graphite substrate had no deleterious effect on the electrode and did not change the standard potential or the calibration slope.

Conditioning of electrodes

Freshly impregnated electrodes showed a sluggish response and a lower sensitivity compared with the same electrode after use for 1 d. Immersion overnight in a 10 μg ml^{-1} chloride solution was effective in conditioning the electrodes.

Optimum pH for chloride determinations

At pH values of 3 or above, the formation of hydroxo complexes of mercury reduces the sensitivity of the electrode at the lower end of the concentration range (<10 μg ml^{-1}). The addition of nitric acid to the sample reduced this interference (Table I) and it was found that there was little practical difference in the sensitivity over the range 10^{-3}–10^{-2} mol l^{-1} of nitric acid. Once enough acid has been added to overcome the hydroxide interference, the shift in the potential with increasing acid concentration at constant chloride concentration is a more likely source of error than small variations in sensitivity; a concentration of 10^{-2} mol l^{-1} of nitric acid was, therefore, adopted for the analytical procedure, as the greater buffer capacity was considered advantageous.

TABLE I

EFFECT OF ACIDITY ON ELECTRODE RESPONSE (ELECTRODE E.M.F. IN MILLIVOLTS)

Nitric acid concentration/ mol l^{-1}	Chloride concentration/μg ml^{-1}				
	0*	0.1	1.0	10	100
0	−67	—	−94	−141	−204
10^{-3}	−13	−34.5	−83.5	—	—
10^{-2}	−24	−47.5	−96	−155	−213

* De-ionised water, no added chloride.

Effect of stirring

Without stirring, the electrode took 20–30 min to reach an equilibrium e.m.f., compared with 5 min in a stirred solution. The rate of stirring had little effect (<2 mV) on the equilibrium e.m.f.

Effect of light

The potential decreased (indicating an apparently higher chloride concentration) when the electrode was exposed to more intense light and increased when it was shaded. The effect was reversible in all instances. Switching off the laboratory fluorescent lights on a dull day caused a 2-mV change in potential within 1 min and similar changes could be produced by variations in the intensity of sunlight. For the main body of the tests the electrodes were fixed in an opaque holder which fitted on top of black-painted 100-ml beakers, thus excluding virtually all light from the membrane of the electrode.

Resistance

The resistance of the cell formed by the chloride electrode and the reference electrode immersed in 50 ml of 0.1 μg ml^{-1} chloride solution to which 5 ml of 0.1 mol l^{-1} nitric acid had been added was 30 kΩ, as measured with an Avometer. This resistance is small enough to allow the cell potentials to be measured with many types of digital voltmeter; potentials measured with a Solartron LM 1420.2 digital voltmeter were within 0.1 mV of those measured on a pH meter with a high input impedance.

Observed and predicted e.m.f.s

The e.m.f. observed when the electrode is immersed in a 1 μg ml^{-1} chloride solution at 25 °C and pH 2.5 (obtained by addition of 5 ml of nitric acid per 50 ml of solution) can be compared with the values predicted by equations (4) and (4a). The standard potentials[6] are $E^{\circ}_{HgS} = -750$ mV and $E^{\circ}_{Hg_2Cl_2} = 268$ mV and the chloride ion activity is 2.32×10^{-5} mol l^{-1} (allowing for dilution and using activity coefficients calculated from the Davies equation[7]); hence

$$E = E^{\circ}_{Hg_2Cl_2} - 59.16 \log\{Cl^-\} = 542 \text{ mV}$$

From equation (5) and the equilibrium constants[6] for equations (1)–(3),

$$\{S^{2-}\} = 1.2 \times 10^{-43} \text{ mol l}^{-1}$$

and hence

$$E = E^{\circ}_{HgS} - 29.58 \log\{S^{2-}\} = 520 \text{ mV}$$

The observed values, after correction for the potential of the reference electrode, were in the range 530–537 mV. The agreement between the observed and predicted e.m.f.s is acceptable, particularly in view of the uncertainty in the value of $K_{Hg_2^{2+}}$.

Performance Tests

The electrodes used for the following tests were made with red mercury(II) sulphide that had been washed with carbon disulphide and were immersed in stirred chloride solutions containing 0.1 mol l^{-1} nitric acid (5 ml per 50 ml) in darkened glass beakers. Electrodes were conditioned overnight in 10 μg ml^{-1} chloride solution before being used for the first time. Unless otherwise stated, the solutions were brought to a temperature of 25 °C in a water-bath before being analysed.

Concentration range

The response of the electrode is Nernstian from at least 1 000 down to about 0.35 μg ml^{-1}. Below that level the calibration graph becomes increasingly curved. Fig. 1 shows the curved portion of a typical calibration graph. The slope of the linear portion (1–10 μg ml^{-1}) was −57.8 mV per decade increase in concentration, with a standard deviation of 0.25 mV per decade increase in concentration (estimated from the least-squares fit of the points to a straight line). It is possible to use the non-linear part of the calibration graph to measure chloride concentrations down to 0.01 μg ml^{-1} and the sensitivity can be increased by working at reduced temperatures.

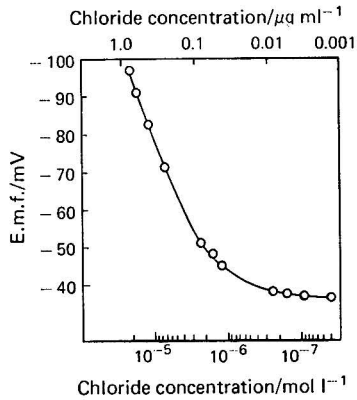

Fig. 1. Calibration of chloride ion-selective electrode.

Precision

Over a period of 3 d, five batches of five standard solutions each were analysed in duplicate. The e.m.f. values were normalised with respect to the mean e.m.f. reading for the 1 μg ml^{-1} standard in each batch and the within-batch, between-batch and total standard deviations were calculated. The results for one electrode are shown in Table II. A second electrode immersed in the same solutions at the same time gave almost identical results, in which neither the mean values of the normalised e.m.f.s nor the standard deviations were significantly different, at the 5% level, from those in Table II (t- and F-tests, respectively).

Table III shows the recorded e.m.f. values for the two electrodes in batches of 1.0 μg ml^{-1} chloride solution. The correlation coefficient between the two sets of e.m.f.s was 0.97, showing the high degree of co-variance between electrodes immersed in the same solution at the same time. It is inferred that the changes in e.m.f. are caused less by the variability of the chloride electrodes than by factors that could affect both simultaneously, *e.g.*, small changes in temperature, pH or liquid junction potential.

TABLE II

PRECISION OF MEASUREMENTS OF CHLORIDE CONCENTRATIONS

Chloride concentration/ μg ml^{-1}	Δ e.m.f./ mV*	Standard deviation†		
		Within-batch	Between-batch	Total
1.0	0.0	0.63 (0.03)	—	—
0.5	17.6	0.93 (0.025)	0 (0)	0.93 (0.025)
0.1	51.1	0.83 (0.004)	NS‡	0.91 (0.004)
0.05	59.3	0.74 (0.005)	NS‡	0.75 (0.005)
0.01	64.6	2.04 (0.015)	0 (0)	2.04 (0.015)

* E.m.f. normalised with respect to 1 μg ml^{-1} solution, *e.g.*, $\Delta_{0.1} = E_{0.1} - E_{1.0}$.
† Figures in parentheses are standard deviations in concentration units (μg ml^{-1}).
‡ NS = non-significant at the 5% level.

TABLE III

VARIABILITY OF E.M.F.S OF TWO ELECTRODES IN 1.0 μg ml^{-1} CHLORIDE SOLUTION

Electrode number	E.m.f./mV									
	1		2		3		4		5	
	A*	B*	A	B	A	B	A	B	A	B
23	−106.0	−106.3	−103.3	−103.8	−103.2	−105.2	−102.3	−101.4	−100.5	−100.9
24	−102.2	−103.4	−101.4	−101.8	−101.0	−102.3	−99.9	−99.3	−98.3	−98.9

* A and B are the first and second 1 μg ml^{-1} solutions, respectively, in each batch.

Accuracy

The accuracy of the analyses using the electrode was tested both by analysing samples of boiler water from five power stations and comparing the results with those obtained by the mercury(II) thiocyanate absorptiometric method,[8] and by spiking the samples with 0.1 μg ml^{-1} of chloride and measuring the recovery. The results are shown in Table IV.

TABLE IV

ANALYSIS OF BOILER WATERS

Station	Location	Chloride content/μg ml^{-1}		
		Absorptiometry: sample concentration	Potentiometry	
			Sample concentration	Recovery of 0.1 μg ml^{-1} spike
A	Boiler 1(A)	0.17	0.17	0.11
	Boiler 1(B)	0.18	0.17_5	0.11
B	Unit 4(A)	0.08	0.10	0.11
	Unit 4(B)	0.09	0.10	0.10
C	Unit 1	0.11	0.08	0.10
	Unit 2	0.07	0.06	0.11
D	Unit 1	0.01	0.01	0.10
	Unit 3	0.01	0.01	0.10
E	Boiler 1	0.01	0.01	0.10
	Boiler 2	0.03	0.01	0.10

Response time

The response for a change from de-ionised water to 0.1 μg ml^{-1} chloride solution or from 0.1 to 1.0 μg ml^{-1} chloride solution was complete in 5 min, but changes in the reverse direction took longer (15–20 min). A better over-all time (approximately 5 min) for the analytical procedure was obtained by immersing the electrode in stirred 0.01 mol l^{-1} nitric acid solution for about 2 min before the next solution was analysed.

Interferences

Substances that could occur in power station waters were tested for their interference effects by observing the change in e.m.f. of the electrode when 100-μl portions of concentrated standard solutions of the interferents were injected into a mixture of 50 ml of 0.1 μg ml^{-1} chloride solution and 5 ml of 0.1 mol l^{-1} nitric acid. Dilution of the chloride solution on addition of the interferent solution was calculated to cause a change of less than 0.05 mV, which would not have been detectable on the pH meter. The concentrations of interferents tested were generally much higher than those expected in power station waters. The following (separately) caused an interference no greater than 0.001 μg ml^{-1} in the determination of 0.1 μg ml^{-1} of chloride: 2 μg ml^{-1} of SO_4^{2-}, 200 μg ml^{-1} of carbon dioxide, 0.43 μg ml^{-1} of silicon dioxide, 120 μg ml^{-1} of CH_3COO^-, 20 μg ml^{-1} of PO_4^{3-}, 12.6 μg ml^{-1} of Cu^{2+}, 20 μg ml^{-1} of Ni^{2+}, 5 μg ml^{-1} of Ca^{2+} + 5 μg ml^{-1} of Mg^{2+}, 2 μg ml^{-1} of Zn^{2+}, 10 μg ml^{-1} of Cr^{3+}, 0.1 μg ml^{-1} of Fe^{2+}, 0.02 μg ml^{-1} of Fe^{3+}, 10 μg ml^{-1} of ammonia, 1 μg ml^{-1} of hydrazine, 2 μg ml^{-1} of cyclohexylamine or 100 μg ml^{-1} of morpholine.

The addition of 20 μg ml^{-1} of SO_4^{2-} caused a bias of +0.03 μg ml^{-1} in the determination of 0.1 μg ml^{-1} of chloride and 0.1 μg ml^{-1} of Fe^{3+} caused a bias of −0.034 μg ml^{-1}, which was eliminated by the addition of fluoride.

Anions that form insoluble mercury(I) salts will interfere if present in such concentrations that they can displace chloride from the mercury(I) chloride in the membrane. The most strongly interfering ions of this type are sulphide, cyanide, iodide and bromide, but these are not normally present in power station waters. The interference effects of some ions can be eliminated, as in the proposed analytical procedure, by acidifying the solution, *e.g.*, hydroxide, carbonate and hydrogen phosphate. Sulphate interfered at much lower concentrations than predicted by this mechanism, probably because the solubility of mercury(I) chloride was enhanced by the formation of a soluble $Hg_2SO_4^0$ complex.

Substances that form strong complexes with mercury(II) ion will promote the disproportionation of mercury(I) ion, causing chloride to be released from the membrane and the electrode to indicate a higher chloride concentration than was present in the sample solution. Under the conditions of the analytical procedure, 16 μg ml^{-1} of sulphite caused an interference equivalent to 3 μg ml^{-1} of chloride because of the reaction

$$Hg_2Cl_2 + 2SO_3^{2-} \rightleftharpoons Hg(SO_3)_2^{2-} + Hg^0 + 2Cl^-$$

Cations that form strong chloro complexes will reduce the concentration of free chloride, but this interference can be overcome by adding a substance that will compete with chloride for the interferent but not for mercury(I) ions. The only important interferent of this kind likely to be present in boiler water is iron(III), the effect of which is eliminated by adding fluoride ions, *e.g.*, 0.1 μg ml^{-1} of Fe^{3+} reduced the reading obtained with 0.1 μg ml^{-1} of chloride by 0.034 μg ml^{-1}, but with 20 μg ml^{-1} of fluoride present the interference effect was less than 0.01 μg ml^{-1} of chloride. Iron(II) and other divalent metal ions had no detectable interference effects at the concentrations tested, which are much higher than those likely to be found in boiler water.

Effect of temperature

The Nernstian sensitivity of the electrode increases with temperature, according to the factor $RT\ln 10/F$, but at reduced temperatures, because the solubility of mercury(I) chloride is suppressed, the linear calibration range is extended to lower concentrations. In addition, there is a shift in the standard potential of the cell formed by the chloride and reference electrode pair to more negative e.m.f. values as the temperature decreases. The combined effect of these factors was tested by measurements made at three temperatures (Table V).

For the analysis of solutions containing less than 1.0 μg ml^{-1} of chloride, the shift in the standard potential is a greater source of error than the change in sensitivity. For measurements below about 0.05 μg ml^{-1} of chloride, it may be worth working at reduced temperatures to obtain greater sensitivity, in spite of the longer response times, *e.g.*, about four times longer at 7–10 °C than at 25 °C.

TABLE V

EFFECT OF TEMPERATURE ON E.M.F.

Concentration of chloride/μg ml^{-1}	E.m.f./mV		
	25 °C	15 °C	7 °C
1.0	−87	−108.5	−115
0.1	−39	−59.5	−67.5
0.01	−25	−32	−35
0*	−22.5	−24	−29

* De-ionised water, no added chloride.

Lifetime of the electrode

Each application of the sensitising mixture gave a membrane with a lifetime of at least 2 months. When the response of the electrode became sluggish the membrane was shaved off the end of the electrode with the tool provided and the exposed tip re-impregnated with more of the sensitising mixture. The Růžička Selectrode should last for years, its lifetime being limited only by the loss of material each time an old membrane is shaved off. The sensitising mixture can be stored for at least 6 months without ill-effect. Table VI shows the potentials recorded during the life of a typical electrode. As the measurements were made at ambient temperature, the potentials could not be expected to be constant over the period of trial, but no trend in the calibration slope (exemplified by the e.m.f. difference in Table VI) or the standard potential could be discerned.

TABLE VI

ELECTRODE RESPONSE AS A FUNCTION OF TIME

The electrode was prepared on May 25th, 1976.

Date of test	E.m.f./mV		E.m.f. difference/mV
	Chloride content 1 μg ml^{-1}	Chloride content 0.1 μg ml^{-1}	
26.5.76	−147.2	−96.5	50.7
10.6.76*	−104.2	−53.9	50.3
14.6.76	−103.4	−53.2	50.2
21.6.76	−101.9	−50.8	51.1
20.7.76	−107.7	−57.5	50.2
23.8.76	−108.3	−55.7	52.6
1.9.76	−105.1	−54.4	50.7

* Reference electrode changed.

Discussion

Lechner and Sekerka[4] made electrodes with pelleted membranes of the same materials as we have used and obtained generally similar results, although their response times were shorter. The precision of analyses with their electrode[9] was poorer than that reported here, *e.g.*, at 0.1 μg ml^{-1} they obtained a relative standard deviation of 14% compared with 4% in this work. The performance of the pelleted membranes depended on the temperature and duration of compression and at best 3 h at 150 °C were required for the preparation of a successful electrode, whereas variations in preparing the impregnated graphite electrode had no appreciable effect on the performance.

The solid-state chloride-selective electrode described in this paper is suitable for the analysis of power station waters containing 0.01–1.0 μg ml^{-1} of chloride, using a simple

manual technique. Higher concentrations can also be determined, if required. At 0.1 μg ml^{-1} of chloride, better precision can be obtained with this electrode than with the silver - silver chloride type[1] (0.004 μg ml^{-1} compared with 0.04 μg ml^{-1} for the total standard deviation), although at 1 μg ml^{-1} the two electrodes are equally precise. At concentrations below 1 μg ml^{-1} the mercury(I) chloride electrode is much more sensitive than the silver chloride types, *e.g.*, the difference between the e.m.f.s observed in 1.0 and 0.1 μg ml^{-1} chloride solutions is 51 mV for the mercury(I) chloride electrode and 19 mV for silver chloride electrodes and between 0.1 and 0.01 μg ml^{-1} the corresponding differences are 13.5 and 2 mV, respectively. The mercury(I) chloride electrode is slower to reach equilibrium than the silver chloride type, taking 5 min to reach a steady e.m.f. where the latter requires less than 1 min. The mercury(I) chloride electrode is similar in precision at 1 μg ml^{-1} of chloride to the mercury(II) thiocyanate absorptiometric method,[10] but its precision is better at lower concentrations. Power station waters are unlikely to contain sufficient concentrations of interfering substances to bias the results of analyses and generally good agreement was obtained when samples of boiler water from several power stations were analysed using the electrode and the mercury(II) thiocyanate absorptiometric method. Good recoveries of spikes were obtained from the same samples and there was no evidence of an effect from iron(III) ions, the most likely source of interference.

The sensitivity of the electrode could make it suitable for monitoring very low concentrations of chloride (<0.01 μg ml^{-1}), especially if used in a flow-cell at a reduced and carefully controlled temperature, as has been done with silver chloride electrodes.[2] Further work is in hand to assess the performance of the electrode in continuously flowing solutions at low temperatures.

This work was carried out at the Central Electricity Research Laboratories and is published by permission of the Central Electricity Generating Board.

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Influence of Ascorbic Acid on the Matrix Interferences Observed During the Carbon Furnace Atomic-absorption Spectrophotometric Determination of Lead in Some Drinking Waters

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Nine samples of drinking water taken from a range of locations in England and Scotland have been analysed for lead by using carbon furnace atomic-absorption spectrophotometry. Spiking experiments have been carried out in order to determine the severity of the matrix interference. The suppression of the lead signals ranged from 22 to 84%. No relationship was found to exist between the hardness of a water sample and its suppression effect.

Further spiking experiments carried out in the presence of 1% *m/V* of ascorbic acid showed that the suppression effect of eight of the water samples was reduced to a level of less than 5%. The remaining water sample gave a suppression of 18%. This water was not the hardest examined, nor did it give the highest suppression in the previous experiment.

The natural lead contents of the nine waters were determined both by carbon furnace atomic-absorption spectrophotometry in the presence of ascorbic acid and by a method that involves solvent extraction - flame atomic absorption. Statistical analysis, using a *t*-test, indicated that there was no significant difference (at the 95% confidence level) in the results obtained by using the two techniques.

Keywords: *Lead determination*; *drinking water*; *carbon furnace atomic-absorption spectrophotometry*; *matrix interferences*; *ascorbic acid*

The analysis of drinking waters for lead by carbon furnace atomic-absorption spectrophotometry is made difficult by severe suppression of the lead signal caused by sample matrix constituents. The use of the method of standard additions appears to be an obvious way of surmounting the difficulty but this procedure is time consuming and, with signal suppressions as high as 84%, may still not yield a result.

Various attempts to overcome these matrix effects were made, but without success. The addition of sulphuric acid or phosphoric acid partially overcame the effects due to magnesium but increased those due to calcium. Campbell and Ottaway[1] suggested that analysis in the presence of 10% *V/V* nitric acid overcomes the suppression of lead by calcium. It was found, however, that while this suppression was reduced to the 10–15% level, a severe loss of sensitivity, attack on atomiser components and spreading on drying, with resultant loss of precision, also occurred.

Thompson *et al.*[2] have proposed the use of lanthanum-treated tubes for the analysis of water for lead and cadmium and reported a reduction of suppression effects to the ± 7% level.

Our earlier paper[3] on the use of ascorbic acid showed that its addition at the 1% *m/V* level overcomes numerous single-element interferences on the lead signal. Some further work has been carried out on the use of ascorbic acid in the determination of lead in some drinking waters in order to study its effectiveness in overcoming multiple interferences arising from a natural, rather than a synthetic, matrix.

Experimental

Apparatus

A Perkin-Elmer HGA-72 heated graphite tube atomiser installed in a Perkin-Elmer 403 atomic-absorption spectrophotometer fitted with the optical modification was used together

with a Telsec chart recorder of 10 mV f.s.d. and a response time of 0.3 s for full-scale deflection.

Reagents

Lead nitrate. Specpure (Johnson Matthey Chemicals Ltd.).
Nitric acid (sp. gr. 1.42). Aristar (BDH).
Ascorbic acid. Standard Laboratory Reagent (Fisons).

Operating Conditions

All lead peak-height determinations were made on 50-μl volumes and the mean (coefficient of variation better than 5%) of at least four replicate injections was taken for each solution. Measurements were made using the lead absorbing line at 283.3 nm and a spectral band width of 0.7 nm (slit 4). The thermal programme used was: drying at 100 °C for 45 s; thermal destruction at 450 °C for 30 s; atomisation at 2 080 °C for 7.5 s; and maximum temperature burn-out for 5 s; all temperatures as shown on the instrument indicator. Automatic background correction was used throughout. The gas-stop facility was not used.

All samples were made 0.015 M with respect to nitric acid on collection.

Effect of Sample Matrix on the Lead Signal

In order to determine the effect of the sample matrix on the lead signal a spiking experiment was carried out.

Three millilitres of distilled, de-ionised water were added to an acid-washed, dry, 50-ml calibrated flask and sufficient sample was added to make up to the mark. The spiked sample was prepared by adding 2 ml of distilled, de-ionised water and 1 ml of a 2.5 μg ml^{-1} solution of lead (as nitrate) to the 50-ml flask prior to the addition of sample. Pasteur pipettes were used to make accurate adjustments of the final volume. The above procedure produced a solution containing 0.050 μg ml^{-1} of added lead.

The solutions obtained by this procedure were measured for apparent lead concentration by comparison with lead standard solutions in 0.015 M nitric acid.

Some of the matrix constituents of the samples were determined to ascertain whether or not a simple relationship between these and suppression of the lead signal existed.

Effect of Addition of Ascorbic Acid

To ascertain the effectiveness, if any, of ascorbic acid in overcoming matrix interferences, the experiment was repeated in the presence of 1% m/V of ascorbic acid in samples, spiked solutions and standard solutions. The ascorbic acid concentration of 1% m/V was achieved by replacing 2 ml of the water added to the calibrated flasks with 2 ml of 25% m/V ascorbic acid solution.

The resulting solutions were measured for apparent lead concentration by comparison with lead standard solutions in 1% m/V ascorbic acid and 0.015 M nitric acid.

No reagent blanks for lead were found during this study. However, other batches of ascorbic acid, even from the same manufacturer, have been found to contain lead.

Determination of Lead by Solvent Extraction - Flame Atomic-absorption Spectrophotometry

The lead content of the samples was also determined by a method, based on that proposed by the Department of the Environment and the National Water Council Standing Committee of Analysts, currently used in this Laboratory. The method consists in extraction of the lead as its ammonium tetramethylenedithiocarbamate complex into 4-methylpentan-2-one, followed by measurement by means of flame atomic-absorption spectrophotometry.

Results and Discussion

Effect of Sample Matrix on the Lead Signal

The results of the spiking experiment without the addition of ascorbic acid, given in Table I, exhibit a range of signal suppressions varying from 22 to 84%. Table II gives

some data concerning the matrix constituents of the waters examined, together with the conductivity of each sample.

It can be seen that no simple correlation exists between the matrix constituents determined and the degree of observed signal suppression. For example, sample D, with calcium, magnesium, chloride and sulphate concentrations of 98, 29, 180 and 170 μg ml^{-1}, respectively, caused a suppression of 44%, whereas sample E, with calcium, magnesium, chloride and sulphate concentrations of only 54, 14, 53 and 76 μg ml^{-1}, respectively, gave the highest suppression (84%) found in this study.

TABLE I

SUPPRESSION OF LEAD PEAK HEIGHT BY DRINKING-WATER SAMPLES

	Apparent concentration of lead			
Sample	Sample/μg ml^{-1}	Sample containing 0.050 μg ml^{-1} of added lead/μg ml^{-1}	Apparent amount of lead added/ μg ml^{-1}	Suppression, %
A	Not detected	0.027	0.027	46
B	0.044	0.081	0.037	26
C	0.067	0.106	0.039	22
D	Not detected	0.028	0.028	44
E	Not detected	0.008	0.008	84
F	Not detected	0.038	0.038	24
G	Not detected	0.014	0.014	72
H	0.0028	0.0205	0.0177	65
I	0.0064	0.0280	0.0216	57

Further, although samples B and F gave similar suppressions of 26 and 24%, respectively, they possessed widely different matrices, which could indicate that the effect of a single interferent is modified by other species present in the matrix.

TABLE II

SOME MATRIX CONSTITUENTS (μg ml^{-1}) AND THE CONDUCTIVITY VALUES OF THE DRINKING-WATER SAMPLES EXAMINED

Sample	NH_3 as N	Albuminoid nitrogen	Nitrite as N	Nitrate as N	Oxygen absorbed from $KMnO_4$*	Chloride as Cl	Alkalinity as $CaCO_3$	Hardness as $CaCO_3$	Iron	Zinc	Copper
A	0.03	0.05	0.001	1.0	1.5	7	25	40	0.04	0.1	0.3
B	0.01	0.03	0.001	1.0	1.4	4	15	20	0.04	0.1	0.17
C	0.01	0.01	0.001	5.8	0.2	11	230	260	0.04	3.5	0.12
D	0.05	0.27	0.001	7.0	1.5	180	130	360	0.04	0.05	0.01
E	0.03	0.07	0.001	3.4	0.5	53	120	180	0.04	0.1	0.005
F	0.01	0.01	0.002	1.0	0.2	22	130	140	0.3	0.6	0.005
G	0.01	0.03	0.002	8.8	0.1	25	250	320	0.005	6.5	0.01
H	0.01	0.01	0.001	4.0	0.1	47	150	240	0.04	0.05	0.039
I	0.02	0.03	0.002	4.6	0.1	27	60	140	0.10	3.2	0.005

Sample	Total residual chlorine	Free residual chlorine	Cadmium	Fluoride	Calcium	Magnesium	Sulphate	Sodium	Potassium	Conductivity/ μS cm^{-1}
A	0.05	0.05	0.001	0.1	10	1.6	15	4.4	0.5	120
B	0.05	0.05	0.001	0.1	3.4	1.0	9	12.4	0.7	80
C	0.05	0.05	0.001	0.1	98	2.1	60	18.0	1.8	480
D	3.2	2.0	0.002	0.1	98	29	170	14.9	4.4	790
E	0.05	0.05	0.001	0.60	54	14	76	39.5	3.4	540
F	0.05	0.05	0.001	0.1	52	2.6	34	11.9	2.7	320
G	0.05	0.05	0.001	0.80	112	2.8	24	13.8	2.1	600
H	0.05	0.05	0.001	0.135	48	26	80	47.8	3.6	530
I	0.05	0.05	0.001	0.4	45	2.5	6	12.8	2.2	310

* Oxygen absorbed in 4 h at 26.7 °C from N/80 $KMnO_4$ solution (an empirical measurement of organic materials).

Effect of Addition of Ascorbic Acid

The results of the spiking experiment in the presence of 1% *m/V* of ascorbic acid, given in Table III, indicate the effectiveness of ascorbic acid in overcoming the observed suppression effects of drinking-water matrices on the lead signal. It can be seen that with the exception of sample H the suppression effects have been reduced to a level of 5% or less.

The complexity of the matrix interference system is again indicated by the fact that the interference associated with sample D, which exhibited the highest suppression of 84%, was completely eliminated, whereas that associated with sample H, which had a smaller suppression of 65%, could only be reduced to the 18% level by the addition of ascorbic acid.

TABLE III

SUPPRESSION OF LEAD PEAK HEIGHT BY DRINKING-WATER SAMPLES AFTER ADDITION OF ASCORBIC ACID

	Apparent concentration of lead			
Sample	Sample/μg ml^{-1}	Sample containing 0.050 μg ml^{-1} of added lead/μg ml^{-1}	Apparent amount of lead added/ μg ml^{-1}	Suppression, %
A	Not detected	0.049	0.049	2
B	0.061*	0.111	0.050	0
C	0.081*	0.129	0.048	4
D	Not detected	0.048	0.048	4
E	Not detected	0.051	0.051	−2
F	Not detected	0.050	0.050	0
G	0.0202*	0.0702	0.050	0
H	0.0087*	0.0495	0.0408	18
I	0.0126*	0.0600	0.0474	5

* These results should be multiplied by the dilution factor of 50/47 to give natural lead levels based on the original water sample.

Accuracy

A measure of the accuracy of the results obtained for lead by means of the carbon furnace atomic-absorption spectrophotometric analysis of samples treated with ascorbic acid can be gained from the results of the spiking experiment shown in Table III. A further measure of the accuracy of these results was gained by comparison with results obtained by using the method involving solvent extraction followed by measurement by means of flame atomic-absorption spectrophotometry.

It can be seen from Table IV that the difference in the values obtained by means of the two techniques was less than 10% relative. Statistical analysis of the data, using a *t*-test, indicated that there was no significant difference (at the 95% confidence level) in the results obtained by using the two different methods.

TABLE IV

COMPARISON OF RESULTS FOR LEAD IN DRINKING WATER OBTAINED BY THE ASCORBIC ACID METHOD AND THE SOLVENT EXTRACTION - FLAME ATOMIC-ABSORPTION SPECTROPHOTOMETRIC METHOD

	Concentration of lead $\pm$ 2σ*/μg ml^{-1}	
Sample	Ascorbic acid method	Solvent extraction - AAS method
A	Not detected	Not detected
B	0.063 $\pm$ 0.003	0.058 $\pm$ 0.003
C	0.086 $\pm$ 0.003	0.092 $\pm$ 0.002
D	Not detected	Not detected
E	Not detected	Not detected
F	Not detected	Not detected
G	0.0215 $\pm$ 0.0002	0.0218 $\pm$ 0.0006
H	0.0096 $\pm$ 0.0007	0.0098 $\pm$ 0.0006
I	0.0133 $\pm$ 0.0005	0.0147 $\pm$ 0.0038

* 2σ values are from a limited number (3–5) of independent determinations carried out at different times and using fresh sample aliquots and standards.

Limit of Determination

The limit of determination for lead in drinking water using the addition of ascorbic acid is 0.002 μg ml^{-1} with a 50-μl sample volume and measurement at the 283.3-nm line. This limit is of the same order as that obtained with the solvent extraction - flame atomic-

absorption spectrophotometric procedure. Further improvement could be expected on using a larger sample volume and the lead absorbing line at 217 nm. The use of the gas-stop facility may also prove beneficial.

Conclusions

The suppressions of the lead signal exhibited by the nine waters examined, which were gathered from a wide range of geographical locations, bore no simple relationship to the hardness of the water or to the concentration of any of the individual matrix constituents studied. The complexity of this situation demonstrates that suppression effects on lead measured in artificial, single-interferent systems cannot be extrapolated to real, multi-interferent matrices. Also, the effectiveness of ascorbic acid in combating the suppression of the lead signal was not found to be related to the composition of the matrix or the actual degree of suppression caused by the matrix.

Although the use of ascorbic acid was not completely successful in overcoming interference in the determination of lead, the suppression effect was greatly diminished, to less than 5% for eight of the water samples and to 18% for the remaining sample.

Where suppression by the matrix reduces the lead signal to below the detection limit, the addition of ascorbic acid may be beneficial owing to (1) the reduction of the suppressive interference and (2) the enhancement of the lead signal, as previously reported.[3]

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Qualification of Estimates for Total Trace Elements in Foodstuffs Using Measurement by Atomic-absorption Spectrophotometry

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The qualification of results for small concentrations of elements in foodstuffs implies a knowledge of the accuracy of a method when applied to foodstuffs and an assessment of the variation in results that exists in the application of that method.

An attempt is made to describe the problems inherent in obtaining such qualifications, and to suggest a standard procedure for accomplishing these aims. From data obtained for a particular method, a statistical appreciation will give confidence limits and detection limits that can be applied to subsequent results obtained, depending upon the nature of the exercise involved.

Keywords: *Accuracy and variation of results*; *limit of detection*; *statistical appreciation*; *foodstuffs analysis*; *atomic-absorption spectrophotometry*

Little work has been published on the statistical appreciation of analytical methods. General accounts do exist[1,2–5] and between-laboratory testing has been described in detail.[6] Exceptionally, certain types of instrumental measurement, such as radiochemical counting techniques[7] and emission spectrography,[8] have been considered at length and some aspects developed, notably by Kaiser and Menzies,[9] for analytical procedures in general.

An approach used for instrumental measurement, however, is frequently not practical when applied to a complete analytical method. The outcome is often a method that is not well qualified, or that is arbitrarily qualified, *e.g.*, in the determination of elements using atomic-absorption spectrophotometric measurement it is usual to quote a limit of detection of twice the instrument background variation. This may be satisfactory as an instrument qualification if the analyst is measuring levels 100-fold greater than this limit, but, if the determination is in the region of the true limit of detection of the method, the quotation of an instrument background variation is of little consequence. Without qualification it is not possible to ascertain the usefulness of a method, compare the results from two different methods or give a practical appreciation of an individual method when results are used for a more profound but tenuous purpose than monitoring. This is particularly so in the determination of trace-element contaminants in foodstuffs and other biological matrices, where they are usually present at levels just within or beyond the capacity of existing methodology.

This paper illustrates some of the difficulties in obtaining valid laboratory qualifications, the means of obtaining estimates for them and the reliance that can be placed upon a result for a total element level in foodstuffs.

The Problem

An analytical method for the measurement of any determinand should be qualified to an extent that enables any subsquent result obtained by an analyst on defined matrices to have some meaning. It is generally accepted that the *accuracy* of results, *i.e.*, the agreement between a mean of estimates and the correct value, must be tested by defining any systematic bias from the true value throughout the range of determinand concentrations measured. The *variation* of results obtained must be known, *i.e.*, the standard deviation at the extremity of the concentration determined must be calculated. The level below which the variation creates intolerable uncertainty in determinand values must be deduced, giving a limit of detection for results.

Accuracy

An analytical method for the determination of trace elements in foodstuffs, using measurement by flame atomic-absorption spectrophotometry, involves prior destruction of the organic matrix by, *e.g.*, wet acid digestion or dry ashing, followed possibly by a separation and/or a concentration stage. Such a method may suffer from losses of an element, particularly during the chemically violent destruction stage, and hence a check on the accuracy of the method is essential. It is also necessary to ascertain that the laboratory conditions are such that contamination does not cause too high results for small amounts of these elements.

Foodstuffs vary widely in organic composition and the digests obtained after destruction of the organic matrix may similarly vary in inorganic composition. The latter variation may introduce errors into the measurement stage because of interference effects. This could apply to the digests obtained from many foodstuffs and for those obtained from foodstuffs that contain an unusually high inorganic composition could cause major interference; in such instances the method would be invalidated.

Another aspect that must not be overlooked is the possibility that an element may be present in more than one chemical form. Although this may not apply to all elements, it has been suggested that in samples of marine origin, arsenic may exist as As^{3+}, As^{5+}, as specific methylated arsenic compounds or as complex high relative molecular mass organic compounds of unknown composition.[10,11] Similarly, mercury in samples of marine origin occurs mainly as methylmercury compounds, but these occur infrequently in other foodstuffs.

The accuracy of a method should therefore be established not only for the method itself, but also in its application to a range of different foodstuffs and ideally to several examples of a particular type of foodstuff. The measure of this accuracy will be the bias obtained from true values for results on homogeneous food samples, and this bias will be systematic or random. In the ensuing account, systematic bias is considered to invalidate results and random bias to increase the variation inherent in the results.

Variation

Suppose that there exists a foodstuff A, which is an example of foodstuffs A_1, A_2, A_3, . . . If foodstuff A is sampled, there will be a sample B that is representative of A but similar samples B_1, B_2, B_3, . . . could be drawn. If an element C is measured in sample B, that element may exist in the sample in forms C_1, C_2, C_3, . . . If element C in sample B of foodstuff A is now determined by analyst D, that analyst is representative of analysts D_1, D_2, D_3, . . . Finally, if analyst D measures C in sample B of foodstuff A, there will be an uncertainty E associated with the measurement.

The final result will be an estimate subject to variation, and this variation will depend in part on measurement, analyst, the elemental form in the foodstuff, sampling and the foodstuff itself.

The analytical uncertainty E is inevitable; it is the variation inherent in a method (including pre-treatment) reflected by replicate measurement when all other factors are fixed. The main aim of monitoring is not measurement, however, but results, in particular a result starting from a sample B of a foodstuff A and proceeding through a fixed method, and it is confidence associated with that one result that is of interest. It follows that the analytical uncertainty of a result must include also the variation from sampling, and this total uncertainty must be determined by replicate analysis. It will also include variation during pre-treatment of a series of samples and the variation in instrument performance during measurement of such a series. The variance of this analytical uncertainty is the experimental error variance, s_0^2, from which the standard deviation, the repeatability s_0, of an analyst on a matrix may be obtained.

No two analysts perform in an identical manner. In any laboratory it is not usually possible for one analyst to specialise in one type of analysis and hence a laboratory qualification is necessary, embracing a cross-section of analysts; any new analyst must work within this laboratory qualification. (It should be noted that in collaborative exercises this variation assumes the variation between laboratories.) While it may be inferred what other laboratories could achieve, an analyst's main preoccupation should be with the laboratory of which he is a member. Let the variance contribution from analysts be s_a^2.

We have so far considered only foodstuff A, but there are unlimited kinds of foodstuffs.

This statement as it stands means little, but if, because foodstuffs differ, variation occurs in the application of a method, or part of it, it can be said that variation occurs because of the nature of the foodstuff. Hence, if some food matrices are such that they take longer to digest than others, this may be a cause of variation in results for the total element determined in foodstuffs generally. Also, the chemical state of the element to be determined may differ wholly or partly from one foodstuff to another, or even in examples of the same foodstuff. This may introduce variation into the breakdown of molecular species, or hinder release from bindings with high molecular weight organic molecules to the inorganic species measured. There is therefore a measure of uncertainty attached to the application of a method to foodstuffs, which also includes the nature of the element in the food matrix. (It is assumed, within the context of bias, that a method is generally applicable.) Let the variance caused by matrices be denoted by s_m^2.

Depending upon the experimental design, the variation caused by pre-treatment and measurement between series of blanks, standards and one sample, and also the variation inherent in the blank for each series, will be contained within either the variance s_a^2 or s_m^2.

The variation inherent in the determination of an element can therefore be said to depend on the experimental error, the analyst and the nature of the matrix, and the standard deviation, s, of an estimate on a single sample by any analyst on any food matrix will be $\sqrt{s_o^2 + s_a^2 + s_m^2}$. This expression is similar to that described by Youden and Steiner[6] for the reproducibility of inter-laboratory exercises. But, as with accuracy, a range of foodstuffs and a sufficient number of analysts are difficult to encompass.

Limit of Detection

Two quantities are quoted for the use of atomic-absorption spectrophotometers: the limit of detection is taken to be the concentration equivalent to twice the background noise variation at the wavelength of measurement, and the sensitivity is the concentration corresponding to 1% absorption. Neither is of importance except as a guide to instrumental behaviour and/or the condition of the element emission sources.

Many workers use the variation in the signal or response of the total reagent blank of the method as a basis for calculation. This is hardly tenable if the method involves treatment of the blank in a different manner to the samples, *e.g.*, reduction of the volume of acid used in digestion by boiling is not comparable to the violent oxidation reaction involved in the destruction of organic matter. Also, the varying ionic composition of sample digests may be the cause of random bias, defined as variation, in the remainder of the method, which will not be reproduced in the sample blank.

A few workers have advocated detection limits based upon the variation of the response (or signal) from a determinand at a level similar to the limit of detection (let the standard deviation be s_d). In such instances, the variation in response (or signal) of the reagent blank of the method (standard deviation s_b) is taken into account and the detection limit is then based upon a multiple of the standard deviation $\sqrt{s_d^2 + s_b^2}$ (refs. 7, 12 and 13). However, again it is results that are of primary interest and, for this discussion, results for an element in different foodstuffs with different elemental forms and obtained by different analysts.

It is reasonable to assume that the limit of detection must be based in some way on the variation in results, or the variation in recovery results, obtained for very small amounts of element and hence must be contiguous with the assessment of variation in estimating results.

A Design

Before proceeding to a design for obtaining satisfactory qualification, it is necessary to accept the concept of a complete analytical procedure, which has been described in detail elsewhere.[2,9] The most important aspects that need to be defined are the food matrices to which the method is applicable and the particular analytical method used.

This may appear to be straightforward, but it is impossible to design a method that can be applied to all food matrices and that will remain an efficient analytical procedure. In the first instance, the technique for initial destruction of organic matter might not be generally satisfactory, *e.g.*, in samples of marine origin a normal wet oxidation may not break down complex compounds of organically bound arsenic that are present to an inorganic species.

Alternative methods of destruction have to be used, such as wet oxidation with vanadium(V) oxide[14] or dry combustion.[15] Normal wet oxidation will be satisfactory, however, for the determination of arsenic in other foods, as far as is known at present. Similarly, digests obtained from foodstuffs vary widely in inorganic composition, *e.g.*, it is possible for liver or tomato products to contain levels of copper that interfere in the determination of nickel by its extractive chelation into an organic solvent and measurement by flame atomic-absorption spectrophotometry,[16] or excessive iron levels may interfere in lead and cadmium determinations by a similar method. These examples do not mean that a method should be discarded on account of the few occasions on which it is not applicable, but rather that those occasions should be defined, by a knowledge of the composition of the samples under examination and by careful consideration of the interference effects that apply to the method.

The application of an analytical method to foodstuffs should also include a knowledge of the homogeneity of samples, but this cannot be assessed until results from a selection of foodstuffs are obtained. It is not unreasonable to assume that foodstuff samples are homogeneous until proved otherwise.

The details of the analytical method must be clearly defined so as to include, for instance, the type of atomic-absorption spectrophotometer, as the performance of an instrument depends upon its design.

It has been mentioned that trace contaminant levels are often outside or just within the limits of existing methodology. A calibration graph should define the lower and upper range of measurement and should be linear. Few analysts would be prepared to project a response graph, even if linear, beyond the upper calibration point, yet it is not uncommon to find results derived from readings obtained below the lowest calibration point. It follows that once a limit of detection has been established, a calibration graph must be prepared that contains a calibration point equivalent to or near to this limit of detection. If difficulty is experienced in measuring such a standard, then clearly the limit of detection is false at that time.

In addition, it must be known whether the experimental pre-treatment and, in particular, instrument measurement are randomised to include standards and samples in any order, or whether the experimental factors involve the determination of a level in a single sample prepared and measured discretely from other samples and standards. Measurements by atomic-absorption spectrophotometry in some modes suffer from memory effects, which, even if eradicated, may occasionally re-occur either directly or indirectly via interference effects. A standard deviation obtained from a number of single determinations may not reflect that obtained in routine series use.

It has been recommended that replicate total analyses of a sample should be carried out, for instance, on different days. However, this will introduce variation attributed to analysts or matrices (through varying instrument performance or varying pre-treatment) into the experimental error variance and distort the ideal of a standard deviation that reflects the performance of an analyst for a sample. In any proving exercises, replicate total analyses should therefore be carried out within a series but not in consecutive positions within the series.

Accuracy

Having designed the method to take account of such tenets, and having defined circumstances where adaptation or alternative methods are desirable, the most important aspect to consider is the accuracy of the results obtained for trace elements in foodstuffs.

Only the methodology can be checked for accuracy by measuring the recovery of an added element, in a discrete form, from foodstuffs, and this should always be a preliminary step in establishing an analytical method. The accuracy of results, however, can be confirmed only by obtaining agreement with certified levels in standard reference materials. At present, there are three such materials: NBS bovine liver, NBS orchard leaves and Bowen's kale, for which much information exists.[17] It is necessary for these reference materials to be dehydrated in order to maintain a long shelf-life and the mass of sample taken should reflect the wet mass of similar material if material in the latter state is normally examined. Many trace-element levels within the above reference materials do not reflect those normally present in foodstuffs and therefore it is impossible to encompass the range estimated for

these trace elements. It is hoped that eventually other reference materials will become available that will enable the range of an element normally present to be qualified adequately for various food matrices.

Results should be obtained at least in duplicate, by a selection of analysts, on undried material, as it has been reported that some elements in certain food matrices occur as volatile organo-elemental forms that are lost at temperatures below 100 °C.[18–20] Therefore, the moisture content should be measured on a separate sample of the reference material and element levels re-calculated to reflect the application of the total analytical method.

Variation

The accuracy of the analytical method will have been tested initially by measuring the recovery of an element, in discrete form, added to a selection of foodstuffs. The advantages that accrue from this exercise, however, will be wider than a check on the accuracy of the method. From the results it should be possible to estimate the contribution to the total variation from analytical uncertainty, analysts and matrices, to test for significance for these variance sources, to calculate relevant standard deviations and to establish confidence limits.

If ten food matrices, varying widely in composition, are taken as representative of food matrices generally, the recovery should be tested at a minimum of three levels. If two of these are at the lower end of the range for measurement, such a system will give information regarding the limit of detection. Ideally, this should be done in duplicate by a selection of analysts (not less than 10) at each of the levels on each food matrix. Few laboratories will have available this statistically desirable number of analysts and will be fortunate to have three analysts competent to deal with new methodology; even so, each analyst would produce 20 values at each recovery level. This is too many and the criteria described earlier, with one exception, will be satisfied by each of three analysts obtaining recovery values, each on three or four different food matrices at each level. The exception is when contributions to variation from different chemical states of an element occur in different foodstuffs and the above exercise would understate relevant standard deviations. There could be a further penalty, which, however, is less evident when dealing with metallic contaminants, as it is possible to find examples of most foodstuffs in which the level of these is very small. This will not be so with the group of minor nutrient elements, which will invariably be present at levels above trace amounts. Consequently, the naturally occurring levels will distort the recovery figures and any statistical information calculated, because the total recovery will be higher than the fixed amount added. In this instance relevant standard deviations will be overstated.

As a check on the above exercise and the possible distortions, it will be necessary to obtain similar statistical information from results obtained on a number of individual foodstuffs, again using the same selection of analysts and carrying out duplicate determinations. The standard deviations obtained will not contain contributions from matrix variation, but may reflect the possible chemical states of an element if sufficient foodstuffs are investigated. Provided that the exercise covers the range of an element normally determined, any gross anomalies in comparison with the first exercise should become apparent and will include, through the repeatability, an assessment of the homogeneity of individual foodstuffs. For this exercise, standard reference materials, as mentioned above, or laboratory internal reference materials should be used. Each laboratory should have a series of such materials in order to monitor analyst performance, the method to include instrument performance at the measuring stage, and also to search for possible aerial contamination. Indeed, it is often recommended that any series of determinations should always include duplicate determinations on one of these internal reference materials for this purpose.

These single foodstuffs will include some that contain low levels of an element, enabling the limit of detection to be deduced from the variation found and giving a comparison with values obtained from the recovery experiments.

Statistical Considerations

Before proceeding to examples of such a design, it is necessary to consider the basic statistical formulae and tests to be used. The three factors in analytical methodology that

require consideration are the mean of results, the standard deviation of results and individual results obtained after the development stage of a method.

The true mean of an infinitely large number of defined results normally distributed, or to be more practical a very large number of results, $x_1, x_2, \ldots, x_i, \ldots, x_N$ of size N is denoted by μ, the standard deviation is denoted by σ and the variance is related to these by the expression

$$V = \sigma^2 = \frac{\sum_{i=1}^{N} (x_i - \mu)^2}{N}$$

However, the mean of a small sample of the above results, normally distributed, $x_1, x_2, \ldots, x_i, \ldots, x_n$, size n, is an estimate of μ and is denoted by $\bar{x}$, the standard deviation is denoted by s such that $N\sigma^2 \approx (n-1)s^2$ and the variance is

$$V = s^2 = \frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}$$

In analytical chemistry, only s is calculated and this is an estimate of σ, to which uncertainty attaches. A derived term of use for assessing trends is the coefficient of variation, which is the standard deviation of a series of results divided by their mean multiplied by 100%. It is an assumption that a series of defined laboratory results are normally distributed,* but provided that this assumption is accepted distributions derived from the normal distribution can also be applied to analytical results. These are the t-distribution for small series of results and the χ^2 (chi-squared) distribution for standard deviations.

If σ were known, for a probability $p = \alpha$, there is a $100(1-2\alpha)\%$ confidence that any result x_i will lie between $\mu \pm \mu_\alpha\sigma$, where μ_α is a factor obtainable from the normalised distribution table, *e.g.*, 95% confidence limits† will be $\mu \pm 1.96\sigma$.

As in the design outlined and in general in analytical chemistry when only series of small numbers of results are possible, only s is known. The corresponding $100(1-2\alpha)\%$ confidence limits for a result x_i in this instance will be $x_i \pm t_\alpha s$, where t_α is a factor obtained from t-distribution tables. If $n = 10$ then 95 C.I. for x_i will be $\pm 2.26s$; if $n > 30$ then the t-distribution approximates to the normal distribution.

The 95 C.L. of a mean $\bar{x}_i$ of n_i results of a parent population of size n, standard deviation s, is given by $\bar{x}_i \pm t_\alpha s/\sqrt{n_1}$, where t_α is a factor obtained from distribution tables for n results. Irrespective of whether the n_1 results form part of a population of n results that are not normally distributed, each mean from n_1 results will form a distribution of means that will tend to be normally distributed, as the size of each sub-group increases.

Even if the parent population of results is normal or assumed to be normal, the distribution of the variance, and hence the standard deviation, s, from n results, will not be normally distributed and the confidence limits for a calculated s are deduced from an asymmetric distribution, the χ^2 distribution. Thus, for 95 C.L., the true standard deviation, σ, will lie in the interval between $s[(n-1)/\chi^2_{0.025}]^{\frac{1}{2}}$ and $s[(n-1)/\chi^2_{0.975}]^{\frac{1}{2}}$. If $n = 10$, this means that the true standard deviation lies between 0.69 and $1.83s$. This is of fundamental importance, as will be seen in the calculation of limits of detection.

To compare the values of two variances, ${\sigma_1}^2$ and ${\sigma_2}^2$, from estimates ${s_1}^2$ and ${s_2}^2$ for ϕ_1 and ϕ_2 degrees of freedom, a null hypothesis is set up that ${\sigma_1}^2$ and ${\sigma_2}^2$ are the same (the F-test). The ratio ${s_1}^2/{s_2}^2$ for ϕ_1 and ϕ_2 degrees of freedom will be the measure of the probability, α, from F-tables, that it is not so. If the alternative hypothesis is that ${\sigma_1}^2 \neq {\sigma_2}^2$, this is a double-sided test; if the alternative hypothesis is that ${\sigma_1}^2 > {\sigma_2}^2$, this is a single-sided test.

* Kaiser[21] has brought attention to the danger of acceptance of normal distributions to laboratory results and this must be repeated. Tschebyscheff's inequality states that

$$[\text{probability } (x_i - \bar{x}) > k\sigma] < 1/k^2$$

When σ is known, this gives for $k = 2$ a probability (2α) of less than 0.25 that a result will be outside the range $\pm 2\sigma$ for any distribution, but for a normal distribution the probability (2α) that a result is outside the range $\pm 2\sigma$ is only 0.046.

† 95% confidence limits are abbreviated to 95 C.L. hereafter, and 95% confidence interval to 95 C.I.

The F-test is of importance in the analysis of variance, where mean squares (variance) from two sources can be tested, whether the results population is normally distributed or not. The denominator variance is always fixed in this instance and the test is therefore always single-sided.

Analysis of Variance

The technique of analysis of variance is based on the assumption that all results of a large population N are normally distributed and there should be no significance in the difference of variances from groups of results of size n. If there is, it is caused by a difference in the means of the results from groups of size n.

An analysis of variance of data will permit the following:

1. significance of variances from different sources to be tested;
2. the standard deviations to be calculated singly, or in total from component parts;
3. estimates of the confidence limits of standard deviations to be deduced;
4. the significance of differences in mean values for each source of variation to be tested.

Two common types of this system are the hierarchical (nested) and the cross-classification. An example of the first is the system of a bulk sample from which sub-samples are drawn and complete analyses carried out for a determinand on each sub-sample. The result of each analysis is dependent upon an intermediate factor, *viz.*, the sub-sample of the bulk. An example of the second system is a material analysed by three methods in five different laboratories. Each result is classified independently with regard to all sources of variation. In cross-classification systems, only the interaction between two variables can be calculated; in the hierarchical system, with which the design exercises are concerned, the variation of sub-groups within groups is calculated because of dependence. It is also considered that examples of analysis of variance emanating from these exercises are random in nature, *i.e.*, only some foodstuffs are considered from a large number of foodstuffs and, similarly, analysts are considered as representative of a large population of analysts. For fixed or random systems, estimated mean squares will differ.[22]

It is not proposed to consider the calculations for this system and reference should be made to suitable texts.[22–24] Two examples will be described that are typical for data accumulated in exercises detailed in design.

Example 1

This example concerns a foodstuff examined for an element by four analysts. Each analyst makes duplicate determinations, within a series of different samples, starting for each determination from 10 g of the foodstuff and proceeding through a digestion with final measurement upon 100 ml of acidified digest. The results stated as milligrams per kilogram (Table I) are corrected for blank contributions from reagents and working conditions by subtraction of the mean of the blanks for each analyst series. The expression of

TABLE I

EXPERIMENTAL RESULTS FOR AN ELEMENT IN A FOODSTUFF

	Analyst			
	1	2	3	4
Results/mg kg^{-1} ..	12.5, 13.1	12.9, 13.2	13.0, 13.1	12.6, 12.2

results to three digits is sufficient for the calculation of relevant factors in trace element analysis. The mean of the results is 12.87 mg kg^{-1} and the range is 12.2–13.2 mg kg^{-1}. These data may be treated as a simple one-factor hierarchical classification, which is shown in Table II.

The information to be gathered from Table II can be summarised as follows.

1. The mean square obtained between analysts can be tested for significance against that obtained from experimental error. The ratio is 4.45 for 3 and 4 degrees of freedom for the numerator and denominator. From F-tables, the 0.05 probability ratio is 6.59.

TABLE II

ANALYSIS OF VARIANCE OF TRANSFORMED RESULTS

Source of variance	Sum of squares	Degrees of freedom	Mean square	Estimate of
Between analysts	0.584	3	$M_a = 0.194\,7$	$\sigma_0^2 + 2\sigma_a^2$
Experimental error — variance ..	0.175	4	$M_o = 0.043\,8$	σ_0^2
Total	0.759	7	(0.108 4)	—

More than one chance in twenty therefore exists that variation caused by analysts could come from experimental error, and this is not significant at the 5% level. (If M_a was significant then this exercise would be discredited.)

2. The standard deviation from analytical uncertainty (repeatability) can be obtained from the mean square, M_0, an estimate of σ_0^2. In this account, for a small number of degrees of freedom, the symbol s will always be used in order to avoid confusion.

$$s_0 = \sqrt{0.043\,8} = 0.209 \text{ mg kg}^{-1}$$

As $M_a > 0$ but is not significant, s_a^2 can be obtained from

$$s_a^2 = \frac{M_a - M_0}{2} = 0.075\,5$$

The standard deviation defined by reproducibility, s, is obtained from

$$s = \sqrt{s_0^2 + s_a^2} = \sqrt{0.043\,8 + 0.075\,5} = 0.345 \text{ mg kg}^{-1}$$

3. The 95 C.L. for a standard deviation can be quoted. Because of the small number of degrees of freedom, for s_0 these will have a wide range, from 0.60×0.209 to $2.87 \times 0.209 =$ 0.125–0.600 mg kg^{-1}. It may be noted that even with 10 degrees of freedom the 95 C.L. would be 0.146–0.366 mg kg^{-1}. Similar confidence limits for s_a can be calculated with the caution that s_0 should have 10 degrees of freedom and, even so, such limits will be approximate in nature.[23]

4. To ascertain whether each analyst mean falls significantly outside that expected, 95 C.L. can be calculated from $12.87 \pm t_\alpha s_0/\sqrt{2}$, where $t_\alpha = 2.78$ for 4 degrees of freedom. The confidence limits for the means are 12.45 and 13.27 and analyst 4 is on the lower limit.

5. The relevant 95 C.I. for a single result can be calculated for the repeatability and reproducibility from $t_\alpha s_0$ for 4 degrees of freedom and $t_\alpha s$ for an assumed 7 degrees of freedom to give values of ± 0.58 and ± 0.82 mg kg^{-1}.

The total of these derived factors can be summarised as in Table III, the standard deviations being quoted to two digits only.

TABLE III

REPLICATE ANALYSIS OF A FOODSTUFF FOR AN ELEMENT

Parameter	Value	Parameter	Value
Foodstuff	X	Range/mg kg^{-1}	12.2–13.2
No. of results	8	Significance (analysts) ..	Not significant
No. of analysts	4	Repeatability %	1.6
Mass of foodstuff/g	10	Repeatability μg	2.1
Level of element/mg kg^{-1} ..	12.9	Repeatability mg kg^{-1} ..	0.21
Amount of element/μg ..	129	Reproducibility % ..	2.8
		Reproducibility μg ..	3.7
		Reproducibility mg kg^{-1} ..	0.37
		95% confidence interval/mg kg^{-1} s_0 ..	± 0.58
		95% confidence interval/mg kg^{-1} s ..	± 0.82

Example 2

This example is concerned with the recovery of 50 μg of the same element, added in inorganic form, to 10-g samples of eight different foodstuffs. As before, the procedure involves a digestion with final measurement on 100 ml of acidified digest. To simulate the design previously described, three analysts are used and this is an example of an unbalanced two-way hierarchical system, which is random in pattern. It is assumed that the element in question is not present in excessive amounts in the foodstuff, but these levels are determined in duplicate in the same series of determinations as the duplicated recovery experiments for each foodstuff, no two foodstuffs being included in any one experimental series. Each level is corrected by the mean of series blanks. The net recoveries, after subtraction of the mean base levels, expressed as a percentage are displayed in Table IV and the analysis of variance in Table V. The mean of the results in Table IV is 100.3% and the range is 92–109%.

TABLE IV

RECOVERY LEVELS FOR AN ELEMENT ADDED TO FOODSTUFFS

	Analyst							
	A			B			C	
Foodstuff	1	2	3	4	5	6	7	8
Recovery, %	102, 95	103, 104	92, 93	98, 100	107, 106	99, 97	109, 100	101, 98
Mean, %	98.5	103.5	92.5	99	106.5	98	104.5	99.5

TABLE V

ANALYSIS OF VARIANCE OF TRANSFORMED RESULTS

Source of variance	Sum of squares	Degrees of freedom	Mean square	Estimate of
Between analysts	43.33 } 276	2 } 7	$M_a = 21.66$ } $M_{m1} = 39.4$	$\sigma_0^2 + 2\sigma_m^2 + 2\bar{m}\sigma_a^2$
Between matrices, within analysts	232.66	5	$M_m = 46.53$	$\sigma_0^2 + 2\sigma_m^2$
Experimental error variance	75.00	8	$M_0 = 9.36$	σ_0^2
Total	351	15	(23.4)	—

The mean square M_a in Table V is an estimate of the term shown, where $\bar{m}$ represents the number of matrices per analyst calculated from the equation[23]

$$\bar{m} = \frac{M^2 - \sum_{i=1}^{3} m_i^2}{(k-1)M} = \frac{64 - 22}{2 \times 8} = 2.63$$

The information obtainable from Table V can now be summarised as in example 1.

1. Because this is a random pattern, the significance from mean squares between matrices, M_m, is tested against M_0, but the mean square between analysts, M_a, must be tested against M_m. Before proceeding to the calculation, it is necessary to accept certain statistical tenets. If M_m is significantly greater than M_0 and if M_m or M_a is greater, but not significantly so, than M_0 and M_m, respectively, then s_a^2 and s_m^2 can be calculated according to normal practice. If M_a is smaller than M_m, the best estimate for s_a^2 is zero and similarly for s_m^2 if $M_m < M_0$. In both instances, significance must be further tested by obtaining better estimates for M_m or correspondingly M_a if the ratio M_m/M_0 is less than unity.

In this example, the latter ratio is 4.97, which is significant for a 0.05 probability level and the ratio of M_a/M_m is less than unity. The best estimate for s_a^2 is therefore zero and hence a better estimate, M_{m1}, of 39.4 is obtained by re-calculation. The ratio M_{m1}/M_0 remains significant for a 0.05 probability level.

2. The relevant standard deviations can be calculated as follows (the use of significant mean squares will be discussed later):

$$s_o = \sqrt{9.36} = 3.06\%$$

$$s_m = \sqrt{\frac{M_{m1} - M_o}{2}} = \sqrt{15.02} = 3.88\%$$

$$s_a = 0$$

$$s = \sqrt{15.02 + 9.36} = 4.94\%$$

3. The 95 C.L. for s_o will be 0.68×3.06 to 1.92×3.06 or 2.08–5.88%.

4. The 95 C.L. for matrix means will be $100.3 \pm t_\alpha s/\sqrt{2}$, where t_α is 2.13 for 15 degrees of freedom. These limits are 89.8 and 110.8%.

5. The relevant 95 C.I. for the repeatability and reproducibility for a single result can be calculated from $t_\alpha s_o$, where t_α for 8 degrees of freedom will be 2.31, and the C.I. ±7.1%, and from $t_\alpha s$, where t_α for an assumed 15 degrees of freedom will be 2.13, and the C.I. ±10.5%.

The total of these derived factors can be summarised as in Table VI.

TABLE VI

RECOVERY OF ELEMENT ADDED TO FOODSTUFFS

Parameter	Value
No. of foods examined ..	8
No. of results	16
No. of analysts	3
Amount of element added/μg	50
Mean recovery, %	100
Range of recovery, % ..	92–107

Parameter		Value
Significance	Matrix, % ..	5
	Analysts, %	Not significant
Repeatability	%	3.1
	μg	1.5
	mg kg^{-1} ..	0.15
Reproducibility	% ..	4.9
	μg ..	2.5
	mg kg^{-1} ..	0.25
95% confidence interval/mg kg^{-1}	s_o ..	±0.36
	s ..	±0.53

Discussion

It is assumed for the purposes of this discussion that series of laboratory results around a mean are normally distributed. Firstly, the standard deviation will be considered. Secondly, the accuracy of a result and how the standard deviation, indirectly through an analysis of variance, may be an aid in assessing accuracy will be considered. Lastly, the limit of detection, the magnitude of which is decided by the standard deviation of results when low levels are determined, will be considered. Having assessed each of these in the evaluation of a method, the influence of the standard deviation and the limit of detection on the reporting of a result (or a mean of results) obtained subsequently by the method will be considered.

Standard Deviation

Of the factors that define an analytical method, the standard deviation is that for which most information is normally gathered. In the examination of foodstuffs for trace elements in general, the standard deviation for the variation between results by an analyst on a foodstuff can be defined as repeatability, signified by s_o in examples 1 and 2. Clearly, a measure of this factor cannot be obtained for each foodstuff by each analyst over the range normally determined. Hence, values must be obtained for separate points in the range, at best, for analysts concerned with foodstuffs constituting a total exercise or by known analysts

on representative foodstuffs. It is within this context that the performance of new analysts must be tested to the standards originally set. Values for this factor will include any random bias associated with the design and with the experimental technique, and will be derived from the recovery of an element in discrete form added to a cross-section of foodstuffs and for an element in natural form in a number of foodstuffs, both covering the range of levels of the element normally present.

The standard deviation, defined as reproducibility, s, for different points in the range normally determined, will be the projection of the variation in results for competent analysts in general on foodstuffs in general to which the method is applicable. That is, it will include random bias within the context of an exercise involving different foodstuffs and different analysts (and including between-series variation and blank variation), but not systematic bias that invalidates the method in application. If the variance component from food matrices is significant, statisticians could formally discredit the standard deviation, s, but in the very definition of the problem random bias might be expected to happen, as in example 2. Accordingly, s must be calculated so as to take account of matrix variance whether significant or not within limits that will be discussed later. The same argument does not apply to significance from analysts.

There are, therefore, for any method in general application, two sets of standard deviations which encompass the range of element normally determined.* In practice, which set should define the variation of results in subsequent exercises will depend upon the nature of the exercise undertaken. If the design in this paper is followed, the variation will always be based upon s, *i.e.*, for a single result on a foodstuff obtained by a known analyst or for a foodstuff not normally examined. If comparison is sought between foodstuffs or between discrete samples of the same foodstuff, and these are examined in separate series of determinations and even though with known analysts, the variation must also be based upon s.

In examples 1 and 2, it was made clear that any calculated standard deviation is in itself an estimate and the 95 C.L. for this estimate will be decided by the asymmetric distribution, the χ^2 distribution. The smaller the number of degrees of freedom, the wider the 95 C.L. becomes. Thus, in the two examples, for 4 and 8 degrees of freedom these limits for the repeatability are 0.60 to $2.87s_0$ and 0.68 to $1.92s_0$, respectively. In a set of calculated standard deviations in the range normally determined, any difference in the particular standard deviation for a point in that range should not be considered abnormal or observations made without consideration of these limits. This will apply especially when comparing standard deviations obtained at similar levels, by recovery exercises and replicate analyses of individual foodstuffs, with the object of seeking distortion, from matrix effects, differing elemental forms and inhomogeneity. (It should be noted that attempts to achieve homogeneity in foodstuffs, when it is possible to improve the texture, carry with them considerable risks of contamination at the low levels of element determined in foodstuffs when a wide range of different elements are being monitored.)

In Tables III and VI, each standard deviation has been expressed in three ways. Often, in qualification of a method, samples of varying masses, particularly reference materials, are used in order to simulate the mass of wet foodstuff or to enable an acceptable amount of element to be determined. Clearly, the assessment of trends by comparison of standard deviations at different levels requires an expression in terms of the actual amount, the coefficient of variation being also helpful, and this applies also to subsequent application of the method. Comparison of the standard deviation of different techniques for an element must be made, however, in terms of concentration.

The last comparison is of interest in monitoring foodstuffs for lead. Levels of lead in foodstuffs seldom exceed 0.40 mg kg^{-1}, which coincidentally is a level seldom exceeded in

* It should be noted these standard deviations will be for levels in foodstuffs that approach the limit of detection of existing methods. For concentrations two orders of magnitude greater than the detection limit, a graph of the coefficient of variation against concentration becomes asymptotic, *i.e.*, the coefficient of variation tends to a limiting value. For concentrations below this magnitude, the graph becomes a sharply rising curve.[25] A general linear equation relating any standard deviation s to concentration C, such as $s = aC + b$,[26] where a and b are constants, is not necessarily tenable for low element concentrations and s cannot be calculated from estimates s_1 and s_2 for points intervening between concentrations C_1 and C_2. It must also be accepted there could be a limiting value for the absolute standard deviation of a result as the concentration decreases, and hence the range normally encountered will decide the number of points at which the standard deviations are calculated.

human whole-blood samples (≡40 μg per 100 ml of blood). Several methods, all of which involve measurement by atomic-absorption spectrophotometry, have been evolved that include stages varying from having no pre-treatment to prior destruction of organic matter. Measurements can be made on a solvent extract after chelation,[16,27] by basically the Delves cup technique,[28,29] or a carbon rod atomiser,[30] a heated graphite furnace[31–33] or hydride generation can be used[34]; the references cited here are a selection only. With the exception of the last method, which is novel and for which insufficient data are available, together with the direct application to foodstuffs of measurement with the heated graphite furnace, the standard deviation has been well documented for blood samples and a number of foodstuffs. Invariably, the determination of approximately 0.40 mg kg^{-1} of lead in such samples is attained to a standard deviation within the range 0.015–0.037 mg kg^{-1} for 5–12 replicates, irrespective of the method used. In the absence of experimental information to the contrary, this is taken to define the reproducibility, and for 12 replicates is the confidence range for a standard deviation of 0.020 mg kg^{-1} (≡2 μg per 100 ml of blood). What does this imply for levels obtained in routine use in similar exercises subsequently?

The 95 C.I. for a single result will be $2.2 \times 0.020 = \pm 0.044$ mg kg^{-1}. On the basis of quoted figures, it seems reasonable to assume that when a single level of 0.40 mg kg^{-1} of lead in foodstuffs or in whole blood samples is determined, a result will lie in the range 0.356–0.444 mg kg^{-1} (36–44 μg per 100 ml of blood). How that single result is reported now becomes a matter of interest. The 95 C.I. decrees that the digit in the first decimal place cannot be guaranteed, still less the second decimal place. If it is to exist as a single figure, it must be reported as 0.4 mg kg^{-1} by taking a reporting interval of similar magnitude to the confidence interval, suitably rounded. (If the 95 C.I. had been ±0.022 mg kg^{-1}, then the reporting interval would be to the nearest 0.05 mg kg^{-1}.) If replicate measurements are made, the standard deviation for a result will be only marginally affected.[6] If replicate total analyses are carried out, in triplicate, the standard error of the mean obtained in the above example will be $0.020/\sqrt{3} = 0.012$ mg kg^{-1}, and the 95 C.I. of this mean will be ±0.025 mg kg^{-1}. (The standard deviation and t_α factor will remain based upon that obtained originally for 12 replicates.) The 95 C.L. for such a mean will therefore be 0.37_5 and 0.42_5 (37.5–42.5 μg per 100 ml of blood), which is not a great improvement.

When results are used for other purposes, such as in a survey of the levels of an element in samples of a particular foodstuff, it could be the mean that is of interest. If only the mean is quoted, this could be obtained from individual results calculated to three digits. If the standard deviation was independent of concentration, a mean of approximately 0.4 mg kg^{-1} of lead for 25 samples of a foodstuff, determined independently, could be quoted to an analytical 95 C.I. of $\pm 0.044/\sqrt{25} = \pm 0.009$ or as 0.40, 0.42 mg kg^{-1}, etc. A problem arises when individual levels are required together with the mean, in order to display, for instance, the distribution of results. If the number of results is small, the quoting of individual results within the ideal of confidence limits could cause truncation of the distribution, giving a poor estimate of the mean. In such circumstances, provided that it is clearly understood that each single result is an estimate, reporting intervals other than $\pm t_\alpha s$ could be adopted. Thus, in the example of lead in foodstuffs, reporting intervals could be halved to 0.05 mg kg^{-1}, but this problem is of greater importance in the region of the limit of detection.

Accuracy

It will have become increasingly evident from the above that total qualification of trace-element levels for a range of foodstuffs is difficult to accomplish or unattainable in practice. The most important of these qualifications, and the hardest to ensure, is the accuracy of a defined method in application to foodstuffs generally.

In example 2, the mean recovery of 50 μg of the element added in ionic form is, by chance, 100%. The recovery of the method is assured for a cross-section (a small range) of foodstuffs at this level. Similar assurance would be required for other amounts at the extremities of the levels of this element likely to be encountered. However, this would not imply that the element is recoverable to this extent from all food matrices or that the element in unknown form in any food matrix is recoverable. The only certain method of ensuring the

accuracy is by agreement with standard reference materials with an authenticated content of the element. At present, this is not possible because of their small number.

If the results detailed in example 1 were from a reference material with a certified level of 13.00 mg kg^{-1} of element, the data could be abridged, as detailed elsewhere,[5] to show the bias *i.e.* 12.87 −13.0 = −0.13 mg kg^{-1}, with 95 C.I. for the mean based upon 8 readings from $t_{\alpha}s/\sqrt{8}$, which would be ±0.29 mg kg^{-1}. In such an instance, the agreement would be satisfactory, but the accuracy of the method in application to foodstuffs generally would remain unproved on the basis of one or of a small number of such agreements. If the certified value had been 13.50 mg kg^{-1}, the bias would be −0.63 mg kg^{-1} and the mean obtained would be significantly different from the expected value. Even if agreement was attained with certified values from other authenticated reference materials, until the cause had been ascertained the application of the existing methodology would lead to uncertain results.

Indirectly, data obtained for interference effects will be an aid in defining the application when the ionic composition of the foodstuffs is known; the technique of assessing interference in analytical methodology has been described.[5,35] However, for these uncertain circumstances, recourse to any additional information on the accuracy of results is welcomed. For this the analysis of variance is important and, in particular, the interpretation of significance from different sources of variance that could enable one to decide whether there is systematic or random bias within the context of this design. For instance, if experiments on the recovery of an element in discrete form using a defined method consistently display no analyst significance, whereas replicate analyses of individual foods conversely display significance, then the defined method is faulty in application to foodstuffs. As different analysts are not equivalent, an element that exists in different forms could be revealed by analyst significance.

This would be an example of systematic bias invalidating the method. Random bias would emanate from many different sources that are experimental in origin or tenable within the design outlined. The objective of trace-element analysis of foodstuffs is to obtain a method that is as generally applicable as possible. It will not be surprising, therefore, if random bias introduces significance to variance when applying a method to different foodstuffs, but it would not be expected to happen for results at low levels in the region of minimum detection as the repeatability, s_0, expressed as the coefficient of variation, will be high. The experimental error variance, s_0^2, could therefore be high in relation to the variance from matrices. As levels increase to 100-fold of this region of minimum detection, the repeatability expressed as the coefficient of variation would be low and random bias in relation to the lowered repeatability could become distinguishable by significance testing (this cannot apply to analyst significance). In example 2, the matrix significance must not be considered abnormal but, in order to define any unusual source of the over-all matrix significance, the means of each duplicate should be tested as described using the reproducibility, s, as the relevant standard deviation (in example 1, s_0 would be the relevant standard deviation). In this example, no foodstuff is significantly outside the expected range, but this exercise could have been repeated on two different levels of element giving 24 duplicate means, and from random statistical considerations for this number at least one and perhaps two would be expected to exceed relevant statistical ranges for means. Some care is therefore required in the interpretation of significance testing.

Another aspect for judgement, which would be an aid in considering accuracy, is the assessment of trends in the standard deviations with concentration in recovery exercises and in replicate analyses of a series of different foodstuffs for an element. Accepting that each standard deviation is an estimate subject to an asymmetric range of values, any serious discrepancy in a decreasing gradation, expressed as the coefficient of variation, with increasing amount determined in these factors (taking account of possible heterogeneous behaviour for s_0) should merit further consideration.

It will therefore be seen that the initial ideal of proving accuracy by comparison with standard reference materials, in the present circumstances, has to be elaborated so as to use whatever information is available. The mathematical probabilities will give rise to deductions that will involve logic or opinion, and will not be based upon readily recognised factors.

Limit of Detection

A definition of the limit of detection is "the smallest amount of a component to be determined, which is still large enough to be detected,"[1] and is generally expressed as amount or concentration. In the ensuing account it is accepted that instrumental signals are readily converted into concentration, or preferably amounts, by a linear calibration function.

Kaiser and Menzies[9] while basing this limit upon the variation of blank measures, extended the limit of detection to a more general case of the variation of the measure of small but discrete amounts in the region of the limit of detection, $\bar{x}$, and, taking into account the mean value of the method blank also, gave a general equation for this limit of detection:

$$\bar{x} = \bar{x}_{b_1} + 3\sigma^* \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

If one assumes that by measures, responses or signals are implied, then using a high probability (0.0014 to give a one-sided confidence limit of 99.86%) a factor of 3 will eliminate errors that originate from random variation of noise being mistaken for an analytical signal.

Roos[13] pointed out that for a concentration above a certain critical level, the criterion of detection, in addition to a risk of concluding that a determinand is present when it is not, there will be a risk of concluding that a determinand is absent when in fact it is not. As such, the true detection limit is double that shown in equation (1) and, excluding the mean blank level, is similar to the limit of guarantee of Kaiser and Menzies, which is $\bar{x}_{b_1} + 6\sigma$.*

Two terms, the critical level L_C and the detection limit L_D, have also been defined,[7] which are essentially equivalent to Kaiser and Menzies' two definitions, but are based only upon the variation of a signal from small amounts of determinand and do not take in account the magnitude of the signal from the blank. This is reasonable as the signal from the method blank is automatically subtracted from a gross signal to give the sample signal, but implies acceptance of the variation being measured in the region of the limit of detection that is of interest. This acceptance is important as the standard deviation may vary in absolute terms with the amount of element measured. There is interest only in a limit of detection being exceeded and hence any confidence limit is one-sided. For a probability of 0.05 corresponding to a 95 C.L. for an infinite population, this limit is defined by 1.64σ, not 1.96σ. In the examples quoted,[7] for such a probability for a signal response, $S + B$, containing some of the substance being determined, measured with a paired blank response B, or a sample containing none of the substance being estimated, L_C and L_D are described as follows:

$$L_C = 1.64\sqrt{\sigma^2_{S+B} + \sigma^2_B} \text{ and } L_D = 3.29\sqrt{\sigma^2_{S+B} + \sigma^2_B} \quad .. \quad .. \quad (2)$$

If both $S + B$ and B are in the region of the limit of detection, these expressions become $1.64\sqrt{2}\sigma_B$ and $3.29\sqrt{2}\sigma_B$. The application of equation (2), however, assumes that σ is known and in general only s can be estimated in analytical chemistry.

The systems described are based generally upon the variation of measurement, related to the determinand by a calibration factor, but the limit of detection of a method essentially defined by the critical level L_C must be based upon the variation inherent in the complete analytical method in application and not on the measurement stage of that method alone. If it depends upon a standard deviation, it has been shown in this text that more than one standard deviation defines a method, *i.e.*, repeatability or reproducibility, and so the limit of detection is no longer invariate. As there may be different confidence limits for a single result, depending upon whether it is obtained on representative foodstuffs with known analysts or on unknown foodstuffs with any analyst, there may be different limits of detection for the two sets of circumstances. For the design described in this paper, the reproducibility should be used for calculation, unless both s_0 and s are the same or similar, in which event statistical practice would decree which standard deviation should be used. This factor may be further influenced, depending on whether replicate measurements or replicate total analysis are involved, in a manner similar to their influence upon the standard deviation.

Good reason has already been given why this factor should not be based upon the variation of a total reagent blank. This does not mean that a limit, so calculated, might not be a valid estimate, but it makes the assumption that random bias from sources other than

measurement will not contribute to variation and this is an untenable assumption to make until proved, when it would be too late, if true, to accept. (It should be noted that any calculation based upon the variation of the reagent blank must take account of variation of this blank measured at random in a series of blanks, standards and samples.) The limit of detection must therefore be based upon the variation of results obtained by a method in application to foodstuffs containing low levels of the element. In dealing with results, the variation of these results will include automatically also the variation inherent in blanks paired with each result. As it must be clear that ranges of foodstuffs, levels and analysts are required for qualification of trace-element levels in foodstuffs, the number of results will necessarily be lower than desired. The *t*-factor must therefore be applied at some level of probability to an equation for the detection limit involving the standard deviation, *i.e.*, $t_\alpha s$. There is one proviso, namely that replication of samples with a mean below the detection limit will give a skewed distribution of results as zero is the least figure that can be reported and such samples will understate the standard deviation calculated from that sample.

To exemplify the following argument for an acceptable probability level, suppose that s_0 has been estimated for a level of element at the limit of detection for 8 degrees of freedom (the calculation of confidence limits for s is more complicated and reference should be made to advanced statistical texts). Suppose a probability level of 0.025, corresponding to a one-sided confidence limit of 97.5%, was selected. For 8 degrees of freedom the *t*-factor is 2.31 and hence the detection limit would be $t_{0.025}\, s_0 = 2.31 s_0$. However, s_0 is an estimate, in itself subject to variation, as is the total standard deviation, s. Kaiser and Menzies[9] brought attention to this point, which is of fundamental importance. Thus, for this example the 95 C.L. for s_0 itself are 0.68 and $1.92 s_0$. If, as could happen once in 20 times, the true value σ_0 was greater than $1.92 s_0$, then the limit of detection calculated on $2.31 s_0$ would be equivalent to $(2.31/1.92)\sigma_0 = 1.21\sigma_0$, and instead of the detection limit being spuriously exceeded once in 40 results through random chance when the element was not present, it would be exceeded once in every 9 results if the series of results was large. If one accepts an equal probability of an element at a level the same as the detection limit being declared absent when it is in fact present, seven out of nine results only could be guaranteed to be greater than zero. When detection limits are calculated from small numbers of results, it is preferable to give a higher probability, 0.005, corresponding to a one-sided confidence limit of 99.5%, which is readily referred to in *t*-tables. The detection limit for this example would then become $3.36 s_0$ and would be equivalent to $(3.36/1.92)\sigma_0 = 1.75\sigma_0$ and so, at worst, the detection limit would not be exceeded for a large number of results by chance more than once in 25 times and 11 out of every 12 results would be guaranteed as being greater than zero.

In the determination of lead in both foodstuffs and blood samples, for levels of approximately 0.4 mg kg^{-1}, a standard deviation of 0.02 mg kg^{-1} was indicated, in general, representing the reproducibility based upon 12 replicates. If for similar circumstances the same standard deviation had been obtained at 0.1 mg kg^{-1}, a calculated limit of detection would be $3.11 \times 0.02 = 0.062$ mg kg^{-1}. There would be a high degree of confidence that such a result implies it would be greater than zero *and that is all.* The nature of the exercise and the relevant confidence limits will decide how such a level will be reported. The 95 C.L. for this level would be 0.018 and 0.104 mg kg^{-1} and a single result of 0.062 mg kg^{-1} would therefore be reported as 0.1 mg kg^{-1} and a result of <0.062 mg kg^{-1} would be reported as <0.1 mg kg^{-1}. If a survey of values for lead included many results in this region (0–0.1 mg kg^{-1}), the mean could be quoted as for higher values from individual results calculated to three digits but, if individual results are required together with a mean, great care would be required in order to avoid truncation of data. If, as detailed under Standard Deviation, reporting intervals could be halved to 0.05 mg kg^{-1}, the intervals 0–0.025 $\rightarrow$ 0, 0.026–0.074 $\rightarrow$ 0.05 and 0.075–0.124 $\rightarrow$ 0.10 would have to be adopted, which discredits the ideal of a limit of detection for a single result. Clearly, a limit of detection must be calculated in the evaluation of a method and its use must be in defining whether levels subsequently obtained have a meaning within the context of the exercise undertaken.

The factors obtained for $t_{0.005}$ for 8 and 16 degrees of freedom are 3.36 and 2.92, respectively. This range is very similar to the empirical factor used by Kaiser and Menzies, but it refers to variation in results and not to variation of a signal response, whether it be caused by discrete element or reagent blank. It will be possible to assess the effectiveness of such

calculations for estimates of the limit of detection, together with other qualifications, in succeeding publications that describe the determination of cadmium, lead and nickel by a method involving chelation from acidic solution and extraction into organic solvent with measurement by flame atomic-absorption spectrophotometry[16]; and for arsenic, antimony and tin by measurement with an electrothermal atomisation technique after prior evolution from digests as the respective hydrides. The observations contained in this account are based upon results obtained with these methods and their application subsequently in obtaining many thousands of results. The order in which this account and its applications are presented should not be taken as being deficient of much practice. It is equally certain that circumstances will exist that invalidate the system, so it should not be considered definitive.

Conclusions

With the design suggested, provided that a strictly defined analytical procedure is used, the accuracy of a method for the determination of trace elements in foodstuffs can be assessed. This should be achieved at the extremities of the range normally determined and should include as many types of foodstuff as possible and a selection of competent analysts.

On the basis of information obtained, bias can be treated as systematic or random. If the former, the method is invalidated for part of its application. If the latter, and significance testing of variance components through means indicates only random bias, this becomes part of the variation of a method. Such a system will involve the calculation of two standard deviations for several points in the range normally estimated. The first, emanating from an analyst (or known analysts) upon a matrix (or representative matrices), will be the repeatability; the second standard deviation will be the variation likely from any analyst upon any matrix to which the method is applicable and will be the reproducibility. From these, confidence limits for a single result can be calculated that will define how that single result is reported, and will depend upon the exercise involved. From these two standard deviations obtained for low levels of an element, the limits of detection for the element in foodstuffs generally can be deduced, completing the qualifications by the analytical procedure for any result and establishing the validity of subsequent results.

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Analysis of Metals Using a Glow-discharge Source with a Fluorescent Atomic Vapour as Spectral-line Isolator

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A compact and rugged spectral-line isolator based on atomic fluorescence from atoms generated by a low-pressure gas discharge has been constructed. The device is bolted to a standard glow-discharge source. A pulsed electrical current generates the atoms and the fluorescence is measured by means of a gated integrator. Several types of metals have been analysed, *e.g.*, steel, cast iron, aluminium and gold. Good precisions and accuracies have been obtained.

Keywords: *Metal analysis*; *atomic-fluorescence spectrometry*

The value of a low-pressure gas-discharge sputtering cell as a means of isolating resonance emission lines has been adequately demonstrated by Walsh and co-workers,[1–3] who used it for the measurement of the primary source intensity in atomic-absorption analyses. In their measurements, the primary source radiation was focused on to an atomic cloud of the analytical element generated by the discharge. The energy absorbed and re-emitted as fluorescent radiation is highly selective if the cathode of the glow discharge is pure. It is also directly proportional to the intensity of the resonance analytical line(s) emitted by the primary source. Gough *et al.*[4] used such a sputtering cell as both atom reservoir and resonance-line isolating device in conjunction with high-intensity lamps as sources of irradiation in atomic-fluorescence analyses.

Butler *et al.*[5] showed that a sputtering cell can be used for the isolation and detection of relatively weak emission from a Grimm-type glow-discharge emission source. Less than 100 p.p.m. of copper could be determined in aluminium using a lock-in amplifier to discriminate between the d.c. emission from the sputtering cell and the analytical signal induced by the modulated radiation from the glow-discharge lamp. In a later refinement of this work,[6] an important advance was made in that the current of the sputtering cell was pulsed while being irradiated continuously by the glow-discharge source. A gated integrator was used to measure the fluorescence signal several milliseconds after the sputtering cell current had been switched off. At this stage, the emission from the cell had subsided to zero while there were still sufficient numbers of free neutral atoms in the irradiated region above the cathode to cause absorption of the analytical resonance line and subsequent fluorescence. In this way, the measuring system could not "see" the high emission intensity from the cell and so the shot noise caused by this high signal level was eliminated from the analytical signal and detection limits could be lowered significantly.

In the last two experiments mentioned above, the sputtering cell was a separate loose Pyrex sealed-off unit, which presented problems concerning optical alignment and de-gassing of the spectral-line isolating lamp. In fact, although the principle of operation and potential of the system were well illustrated, it was not robust or sturdy enough to be used in a factory or at a smelter works site.

This paper describes the further development of the spectral-line isolator (SLI) as an instrument suitable for industrial use. The apparatus has been applied to several problems of industrial interest, *e.g.*, the determination of copper and magnesium in nodular cast iron (S.G. iron), silver in gold and copper in aluminium.

Experimental

The glow-discharge emission source (RSV Präzisionsmessegerate, Hechendorf, West Germany) is of the type described by Grimm.[7] A schematic diagram of the SLI is shown in Fig. 1. It consists of a brass cylinder (110 × 50 mm) containing a shallow hollow cathode of the analytical element, a lens ($f = 50$ mm, $d = 40$ mm) for focusing the radiation from the

glow-discharge lamp directly above the hollow cathode, a gas inlet and a pumping port. A side-arm attached at right-angles to the brass cylinder in line with the hollow cathode allows observation of the fluorescent radiation. In order to improve the light-collection efficiency, another lens of focal length 50 mm ($d = 40$ mm) focuses the active part of the atom cloud on to a diaphragm in front of the photomultiplier tube. The SLI is fixed to the glow-discharge source by three screws so that no alignment problems arise.

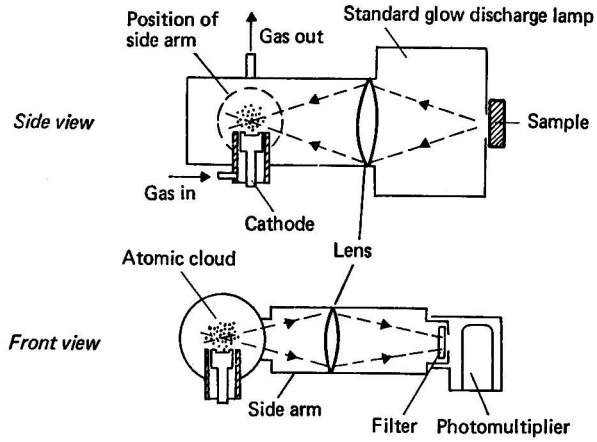

Fig. 1. Schematic diagram of apparatus.

The SLI is continuously pumped, sharing one of the two pumps of the standard glow-discharge emission source. In order to ensure optimum speed and stability under factory conditions, a separate pump is advisable. A needle valve controls the argon pressure in the SLI and a separate supply of argon is used to ensure that the pressure remains constant. The pressure is adjusted so that the ratio of the fluorescence to emission signal is a maximum (about 200 Pa). The flow-rate of gas through the lamp is restricted by an orifice of diameter 1 mm to approximately 10 cm^3 min^{-1}. As a result of electrical time constants in the measuring circuits, a small emission signal from the atomic cloud persists for some time after the termination of the current pulse applied to the hollow cathode.

The shallow hollow cathode of the SLI has an external diameter of 10 mm, a recess 7 mm in diameter and a depth of 2 mm. The hollow cathode gives results similar to a cathode with a flat surface but has the advantage that its burning voltage is lower for the same current. Both the anode and the cathode are electrically insulated from the body of the lamp. The cathode is enclosed with electrically insulating material to prevent the discharge from burning to its sides. Gas flows in between cathode and sheath upwards through the region where the light from the emission source is focused (15 mm above the top of the cathode), to the pumping port.

The heart of the electrical measuring system is a dual-gated integrator (Molectron Corp., Sunnyvale, Calif., USA), which is operated as follows. At the beginning of the measuring cycle, the discharge to the detector cathode is switched on for 4 ms. The first gate of the integrator measures between 6 and 8 ms while the second gate is set to measure for the same length of time (2 ms) just before the end of the cycle (the cycle period is either 40 or 60 ms). The difference in signal between the first and second gates represents the fluorescent intensity. At the time when the second gate is measuring, all of the atoms generated by the discharge pulse have diffused away and there is no more fluorescence. This measurement is used to correct the signal for stray light of the (d.c.) emission source reaching the photomultiplier. The current applied to the SLI cathode is adjusted so that approximately 40% absorption takes place during the first measuring period. The currents necessary for cathodes of copper and silver are 15 and 10 mA, respectively. These relatively low currents result in the SLI cathode having a very long life. If the absorption exceeds 50%, the fluorescence signal decreases as a result of excessive re-absorption of fluorescent light. The absorption and

therefore the concentration of ground-state atoms in the light path gradually decays to zero in 30–40 ms. The rate of decay determines the period of operation. For copper and silver a period of 60 ms was used, but for magnesium a shorter period of 40 ms could be used. It was found that operation of the gated integrator synchronous with the mains frequency (in multiples of the mains period of 20 ms) resulted in a large reduction in electrical noise.

A pre-amplifier with a gain of 30 was used before the gated integrator. The high emission signal emitted by the SLI while the current pulse is applied is short-circuited to earth by an FET switch in order to avoid saturation of the pre-amplifier.

Results

Magnesium in Steel

In the determination of magnesium in steel, a problem encountered was that with a pure magnesium cathode in the SLI, no steady discharge could be obtained. The discharge tended to burn to small spots on the cathode surface, which became very hot, and was probably caused by an oxide layer on the surface of the pure magnesium. This problem was overcome by using an alloy of approximately 20% magnesium - 80% zinc for the cathode, which gave a steady discharge. The current (4-ms pulse) necessary for 35% absorption of the primary beam was 40 mA, but even this relatively higher current caused negligible electrode erosion.

A solar-blind photomultiplier (Hamamatsu R166) was used as the light detector. Radiation emitted by the glow-discharge lamp and reflected from the end window and walls of the SLI caused a high background signal and consequent noise on the analytical signal. This could be avoided to a great extent by using an interference filter (peak at 285.2 nm for magnesium) in front of the photomultiplier. As the stray light is mainly gas and matrix-element (iron) emission lines for this application, this radiation is then prevented from reaching the photomultiplier. However, the low transmission of the filter (10–20%) rendered the signal so low that a poor signal to noise ratio was again obtained. The only alternative was to omit the filter and to reduce the stray light as much as possible by using baffles in the incident beam and lining the walls and end window of the SLI with black absorbing material.

A set of analysed samples containing between 0.022 and 0.10% of magnesium was available for testing the performance of the SLI in the application where magnesium in nodular (S.G.) cast iron had to be determined. Fig. 2 shows the analytical graph obtained. The points are average intensities of three measured values. Except for the point marked No. 39, the individually measured values deviated from the calibration graph by not more than 3%. The signs of these deviations differed, showing them to be random measurement errors, and the value of 3% can be considered as a measure of the accuracy of determination for a single measurement. Sample No. 39 showed a systematic deviation and the true magnesium concentration was later found to be 6% higher than that given.

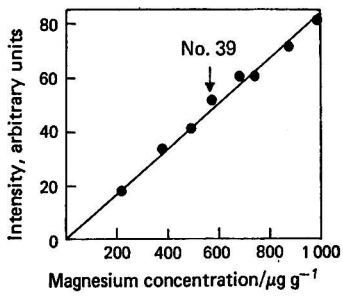

Fig. 2. Calibration graph for magnesium in S.G. iron.

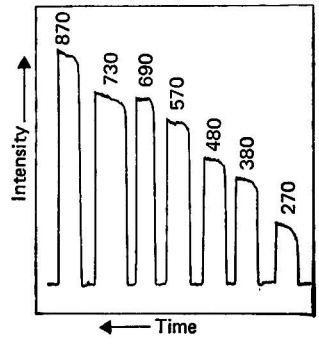

Fig. 3. Signals recorded for different samples, showing different burn-off characteristics of samples (magnesium in S.G. iron). Numbers on peaks denote magnesium concentrations (μg g⁻¹).

The accuracy of determination could probably be improved to better than 2% if the burn-off characteristics of the glow-discharge lamp (emission source) could be improved. Fig. 3 shows the direct recordings of the signals from the detector. It is clear that different samples show different burn-off characteristics, which makes assessment of the signal level uncertain. This characteristic of the glow-discharge source is well known and can be minimised by using a more energetic burn-in discharge condition.

Reproducibility of measurement was determined by measuring 22 values of the fluorescence signal given by a sample containing 0.063% of magnesium. A new burn spot was selected for each measurement, and as only four spots could be accommodated on the 35-mm diameter sample, the sample had to be re-surfaced after each set of four burns. A relative standard deviation of 1.4% was obtained. The sample burning time was 2 min. The average value of the signal during the last minute was measured. Fig. 4 shows five consecutive values of the signal as registered on the chart recorder for this sample. No long-term drift was noticeable over the 1½-h measuring period.

For all of these measurements the glow-discharge lamp current was 100 mA. The source was current stabilised and the burning voltage varied between 1100 and 1200 V for individual measurements.

It has been mentioned that a solar-blind photomultiplier was used as the detector. The risk therefore existed that if zinc were present in the sample, it could cause fluorescence at 213.9 nm in the vapour of the magnesium - zinc cathode. To avoid this, a nickel sulphate solution filter[8] that transmitted about 90% at 285.2 nm but absorbed totally at 213.9 nm was used in front of the photomultiplier. Commercially available glass filters, such as the Schott UG 11 filter (0.5 mm thick), would achieve the same purpose. Other resonance zinc lines did not interfere. This was tested by measuring absorbance with a zinc hollow-cathode lamp as primary source instead of the glow-discharge source. No absorption of the zinc 307.6-nm line could be measured.

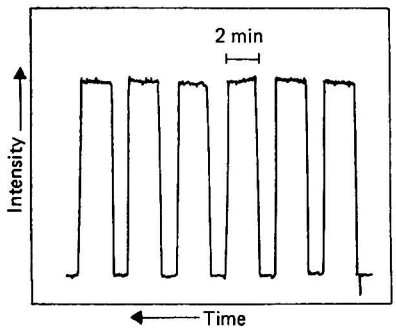

Fig. 4. Consecutive recordings of signal illustrating reproducibility of measurement.

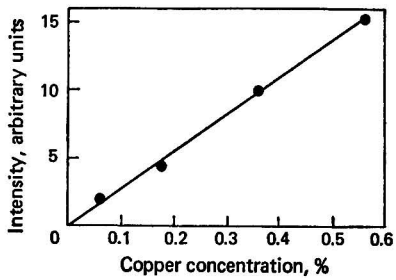

Fig. 5. Calibration graph for copper in steel.

Copper in Steel and Aluminium

Fig. 5 shows the analytical graph obtained for copper in steel. The graph is linear up to 0.56% of copper, the highest standard available. A pure copper cathode was used in the SLI. A current of 15 mA (4-ms pulse) proved sufficient to cause 40–50% absorption in the vapour cloud so generated. A 1P28 photomultiplier with a 324.7-nm narrow-band interference filter was used in order to eliminate stray light.

Fig. 6 shows the calibration graph obtained for copper in aluminium. Again, the graph is linear up to the highest standard available, *viz.*, 4.4% of copper. A matrix effect well known in glow-discharge emission analysis is noticeable, *viz.*, the deviation of the reading from the graph for samples that have high magnesium contents.

Copper and Silver in Gold

For the fluorescence detection of silver in gold, a pure silver cathode was used. The optimum current in this instance was only 10 mA. A 1P28 photomultiplier with a 338.0-nm interference filter was used. Fig. 7 shows the calibration graph obtained for silver concentrations of up to 0.2%. Copper in gold exhibited a similar linear graph for concentrations of up to 0.2%. The measuring period for both copper and silver was extended to 60 ms, as these atoms seem to take longer to diffuse out of the region where the light from the emission source is focused.

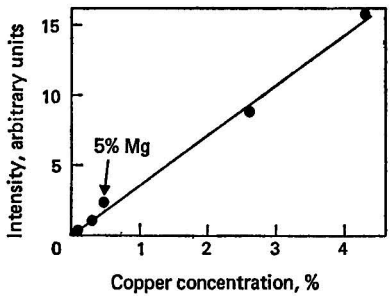

Fig. 6. Calibration graph for copper in aluminium.

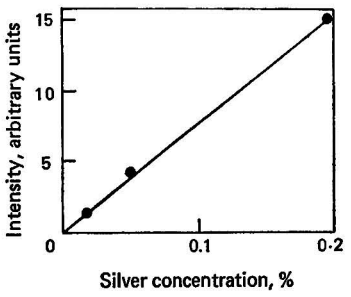

Fig. 7. Calibration graph for silver in gold.

Samples containing up to 5% of copper and silver showed that the calibration graphs in these regions are curved, the curvature being evident at the 1% concentration level. This effect is due to self-absorption and reversal in the glow-discharge emission source, which would be expected in view of the high sputtering rate of the gold metal and the fact that the resonance line intensities are measured. It can, of course, be reduced by decreasing the energy of the glow-discharge source.

Detection Limits

The detection limits obtained were estimated from the noise and signal levels of low-concentration samples and blanks when they were available. Defining the detection limit as that concentration which gives a signal equal to twice the peak-to-peak noise level as registered on a chart recorder, the values obtained are as given in Table I.

TABLE I

DETECTION LIMITS OBTAINED

Element	Matrix	Detection limit, p.p.m.
Magnesium	Steel	20
Copper	Steel	10
Copper	Aluminium	25
Copper	Gold	2
Silver	Gold	3

Conclusion

The fluorescent spectral line isolator proved to be a sensitive yet robust, non-dispersive spectrometer for the isolation of resonance lines emitted by the glow-discharge emission source. Its resolution is very high, being determined by the absorption line width in the cell, which is mainly determined by Doppler broadening. It is mechanically stable and is insensitive to temperature and humidity changes and can therefore be used in almost any environment.

The SLI is at present used for single-element determinations only. Preliminary experiments, however, showed that very little signal is lost by pumping the lamp from ports not situated directly opposite the cathode. It is therefore possible to position a second cathode

directly opposite the first. Pulsing the two cathodes alternately and using a two-channel electronic measuring system should enable dual-element measurements to be made. If a single photomultiplier tube is used for measurement of the fluorescent radiation intensity, the use of separate filters for each element will not be possible and a solar-blind photomultiplier will have to be used. Such an instrument is under construction and will be evaluated soon.

A fact that should be borne in mind is that this type of detector is limited to the measurement of resonance-line intensities, and analyses at high concentrations of analyte will not be possible, *e.g.*, by selecting a non-resonant emission line as is done in ordinary emission spectrometry. As the absorption line profile in the SLI is very narrow (gas temperature probably lower than 500 K and negligible pressure broadening), the peak of the emission line is essentially measured and the slightest amount of self-absorption in the emission source will be detected. This problem can be avoided to a great extent, however, by using low-power conditions in the glow-discharge emission source.

The very short optical path and the fact that both the sample and SLI are in low-pressure argon atmospheres suggest the use of this instrument for the determination of carbon and other elements that have their resonance lines in the vacuum ultraviolet region. This aspect is being investigated and will be reported in the near future.

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Radiochemical Neutron-activation Analysis of Sulphide Ores Using Zinc Diethyldithiocarbamate as Extraction Reagent*

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A procedure for the analysis of lead sulphide and mixed sulphide ores for silver, arsenic, gold, cadmium, copper, manganese, antimony and zinc was developed with emphasis on the determination of the low gold to silver and arsenic to antimony ratios. Radiochemical neutron-activation analysis was necessary and a solvent-extraction technique has been developed. In the first separation step arsenic(III) chloride was extracted from the ore solution with benzene. The results are compared with the values obtained after separation of arsenic by distillation.

Gold(III), silver(I), copper(II), cadmium(II) and several other trace elements were extracted with zinc diethyldithiocarbamate in chloroform, whereas antimony(V) remained in the aqueous phase. The activities of the samples were counted on a germanium(lithium) well-type detector and compared with those of known volumes of standard solutions. Chemical yields were determined by re-activation.

The combination of conventional arsenic separation and this newly developed diethyldithiocarbamate extraction technique proved to be a very efficient and reliable method for the analysis of sulphide ores.

Keywords: *Sulphide ore analysis*; *neutron-activation analysis*; *radiochemical separation*; *zinc diethyldithiocarbamate*; *gamma-ray spectrometry*

In the course of studies concerned with the chemical composition and origin of ancient Greek silver,[1,2] we have analysed argentiferous lead sulphide ores and mixed sulphide ores from Laurion near Athens and some islands of the Aegean Sea with the aim of determining the provenance of the ancient silver used for coins. This paper deals with the radiochemical procedure for sulphide ore analyses.

The ore samples and deposits, mainly from the site of Laurion, Attica, Greece, were characterised by their mineralogical and elemental compositions. The main aim of this study was to establish whether argentiferous ores and metallic silver can be linked by distinct trace-element concentrations or elemental ratios, which are considered typical for a sulphide ore deposit. For this reason and considering the trace elements detected in the silver coins,[3] the emphasis of this work was on the gold to silver ratios of ores compared with those of coins, because the ratio of these elements is probably least affected by the smelting and refining processes. We have also studied whether any other trace-element contents, such as arsenic, antimony or copper, or the ratios of those elements might be used to determine the provenance of ancient Greek silver.

To test the validity of this assumption, we started with the well documented example of archaic Athenian silver, which originated from the famous mines of Laurion. Most of the analysed Athenian silver coins that belong to the Asyut hoard (time of burial about 475 BC) contain only a few hundred parts per million of gold.[2,3] The highest silver content of the lead sulphide ores (galena) from Laurion studied in this work is about 0.5%. As the occurrence of gold is not geochemically associated with galena, we expected rather low concentrations of gold in those ores.

Neutron-activation analysis, with its high sensitivity for the elements of interest and especially for gold, is a suitable method for solving this problem. However, after irradiation

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of the ore samples with thermal neutrons, the main activity is due to copper-64 and antimony-122. The latter isotope interferes, especially with the 559-keV photopeak of arsenic-76, and increases the Compton background substantially so that gold-198 (γ-radiation, 412 keV) cannot be determined instrumentally with a germanium(lithium) detector. Therefore, a radiochemical procedure has to be applied for the separation of the interfering elements.

Principle of the Method

The diethyldithiocarbamate anion (DDC) is widely used in analytical chemistry as a chelating agent for extractions both in supra- and in sub-stoicheiometric amounts.[4,5] Successive sub-stoicheiometric extractions from a multi-element system result in a certain extraction order, which has been established by several workers[6–9] at various pH values, for example by Wyttenbach and Bajo[10] for 0.1 N sulphuric acid: gold(III), mercury(II), thallium(III), silver(I), copper(II), bismuth(III), antimony(III), tellurium(IV), molybdenum(VI), selenium(IV), indium(III), arsenic(III), lead(II), cadmium(II) and zinc(II). Arsenic(V) and antimony(V) are not extracted by DDC.

Wyttenbach and Bajo[10] showed that this extraction order also represents the inverse order of replacement. Therefore, the extraction with supra-stoicheiometric amounts of MDDC as reagent gives a fraction with a high yield of the elements to the left of M in the above order in the organic phase (where M = metal cation). By applying this method with different MDDC compounds, even samples of complex composition can be analysed rapidly with good separation efficiency and with a minimum of chemical operations. A detailed outline of the theory of this method was given by Wyttenbach and Bajo.[10] Applications to water,[11] biological material,[12] silicate rocks[13] and silver coins[14] have been reported. We have used $Zn(DDC)_2$ to separate gold, silver, copper and cadmium from antimony. Copper-64 has a short half-life of 12.70 h; therefore, it does not interfere seriously with the gamma-ray spectrometric measurements after a few days' cooling time. Arsenic, however, under these conditions remains together with antimony in the aqueous phase and has to be separated in a specific procedure.

Two methods were considered for this separation. Firstly, the classical separation of arsenic by distillation from an acidic solution of high hydrochloric acid content at 110 °C; with the exception of germanium, no other elements are distilled under these conditions. Secondly, arsenic in the trivalent state can be extracted quantitatively into benzene from an aqueous phase that is more than 9 M in hydrochloric acid.[15] Only germanium(IV) is also extracted into the organic phase, while antimony(III) and tin(IV) remain in the acidic phase.[16] The solvent-extraction method has proved to be less time consuming, and the separation of arsenic from antimony was as good as with the distillation technique.

Experimental

Preparation of Zinc Diethyldithiocarbamate Reagent

Ten grams of zinc sulphate ($ZnSO_4.7H_2O$) were dissolved in 150 ml of water. A solution of 14 g of sodium diethyldithiocarbamate ($NaDDC.3H_2O$) in 100 ml of ethanol was slowly added and the mixture stirred for 30 min. The precipitate was filtered off, washed with 500 ml of water and dried at 70 °C overnight. The crystalline product was dissolved in 400–500 ml of chloroform and filtered. The filtrate was mixed with 150 ml of ethanol and covered with a watch-glass. After evaporation of about half of the solvent, the crystals were filtered off, washed twice with absolute ethanol and dried in air. A 0.03 M solution of $Zn(DDC)_2$ in chloroform was prepared.

Sample Preparation and Neutron Irradiation

Approximately 30-g amounts of lead sulphide ore and mixed sulphide ore were selected from samples and each was ground in an agate ball mill, sieved through a 60-μm sieve and mixed thoroughly. Amounts of about 100 mg of each prepared sample and two standards were packed in highly pure polyethylene containers and irradiated in the carrousel position of the Heidelberg TRIGA reactor at a neutron flux of about 2×10^{12} neutrons cm^{-2} s^{-1} for 2 h. The standards were prepared by pipetting chemical standard solutions on to filter-

paper. Gamma-ray self-shielding is not important under these conditions (less than 2% at 511 keV and about 1% at 1116 keV). After a cooling period of 1 d, the samples were measured instrumentally on top of a germanium(lithium) well counter of resolution $\leqslant$2.7 keV in order to determine copper, manganese, antimony, zinc and in some instances silver. The photopeaks were accumulated and stored and the data were processed on-line with a Digital Equipment Corp. (DEC) system consisting of a PDP 11/40 computer, a cartridge disk, a magnetic tape unit and a fast printer.[3] Immediately after this measurement, the radiochemical separation was started (Fig. 1). One run with two ore samples and two standards lasted about 4 h. For re-activation, 50-μl amounts of the $Zn(DDC)_2$ extracts were pipetted on to filter-paper situated in polyethylene containers for the determination of chemical yields. Together with pipetted carrier solutions (100% yield), they were irradiated for 4 h at a flux of about 2×10^{12} neutrons cm^{-2} s^{-1} in the Heidelberg TRIGA reactor.

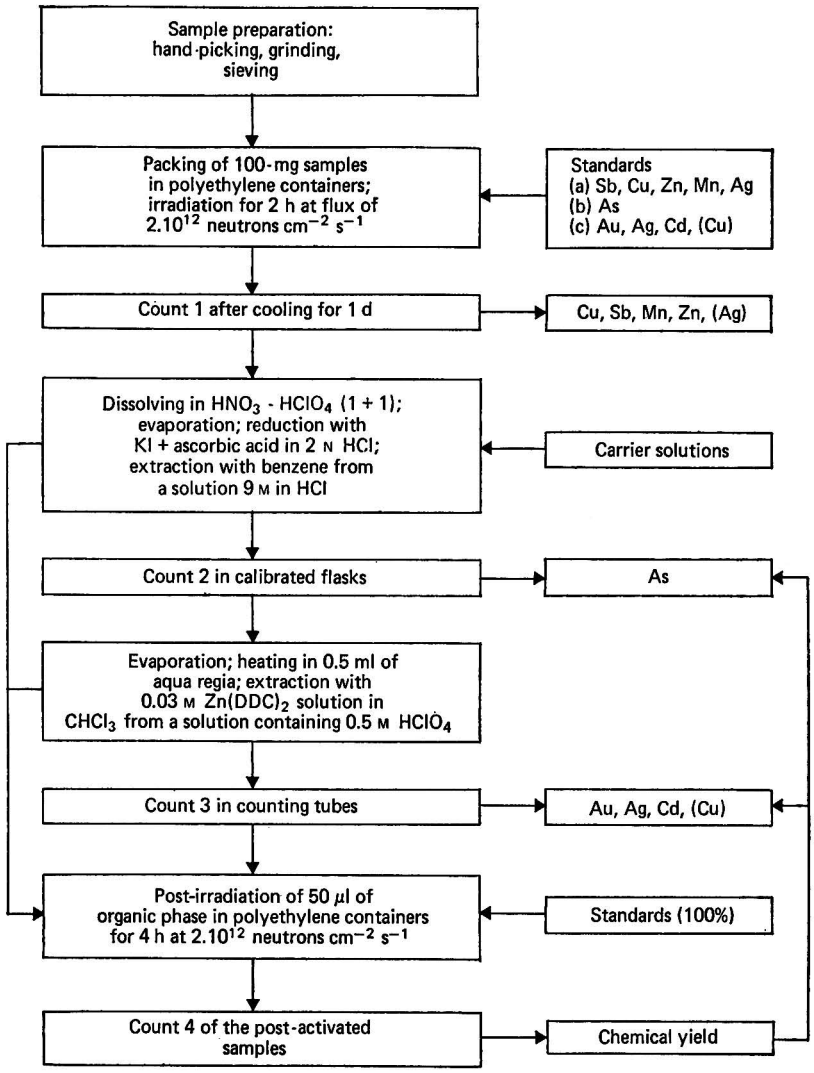

Fig. 1. Scheme of analysis for lead sulphide ores and mixed sulphide ores by neutron activation.

Distillation of Arsenic

The amounts of each carrier element added varied from 30 μg to 2 mg; they were adjusted so as to yield reasonably strong activities of all of the elements after re-activation. Carrier solutions, 1 ml of concentrated perchloric acid and 1 ml of concentrated nitric acid were pipetted into a distillation flask. The ore sample was added and the flask was immediately connected with a distillation apparatus consisting of a dropping funnel, a thermometer, a Liebig condenser and a gas inlet. A conical flask cooled with ice and containing 5 ml of water served as a receiver. On gentle heating, the reaction started and the heating was continued until white fumes appeared. Concentrated hydrochloric acid (5 ml) was added to the distillation flask, the contents of which were evaporated to half of the volume, and 5 ml of concentrated hydrobromic acid were added. Distillation was continued at 110 °C until the temperature began to increase. A continuous flow of nitrogen was passed through the apparatus during the entire procedure. The solution in the receiver was transferred into a 25-ml calibrated flask, diluted to 25 ml with water and the flask was put on the germanium(lithium) detector in fixed geometry and counted.

Extraction of Arsenic

Amounts of carrier solutions as described above, 0.5 ml of concentrated perchloric acid and 0.5 ml of concentrated nitric acid were pipetted into a 50-ml extraction vessel of length about 15 cm, equipped with a hollow plug, and the ore sample was added. The size of the plug was about 60 × 15 mm, and the ground-glass portion was connected to the extraction vessel after sample decomposition.[14] On gentle heating of the glass vessel, the sulphides were easily dissolved and a white precipitate of lead sulphate was formed. The heating was continued until all of the nitric acid was fumed off. In order to reduce arsenic(V) to arsenic(III), 100 mg of potassium iodide and 25 mg of ascorbic acid were dissolved in a few millilitres of 2 M hydrochloric acid and added to the sample solution, which was kept on a boiling water bath for 15 min. After cooling, 25 ml of benzene were pipetted into the extraction vessel and enough ice-cold concentrated hydrochloric acid was added to make the acid solution at least 9 M in hydrochloric acid. The mixture was shaken vigorously for about 10 min to extract arsenic(III) into the benzene and subsequently centrifuged. At this stage, the organic phase showed the purple colour of dissolved iodine. An aliquot of 20 ml of the organic phase was pipetted into a 25-ml calibrated flask, diluted to the mark with benzene and counted. The arsenic standard was dissolved in 1 M nitric acid in a calibrated flask of the same size and counted in the same geometry on the germanium(lithium) detector (Fig. 1).

Extraction with Zinc Diethyldithiocarbamate

After addition of 1 ml of concentrated nitric acid, the aqueous phase from the extraction vessel was evaporated almost to dryness by use of an oil-bath and cooled. To ensure that all of the gold had dissolved, the residue was treated with 0.5 ml of aqua regia, 0.25 ml of concentrated perchloric acid was added and the nitric acid carefully fumed off. The solution was diluted with 10 ml of 0.5 M perchloric acid and gold(III), copper(II), silver(I) and cadmium(II) were extracted with 5 ml of 0.03 M $Zn(DDC)_2$ in chloroform, when most of the antimony(V) remained in the aqueous phase. The mixture was shaken for 10 min and subsequently centrifuged. A 4-ml volume of the organic phase was pipetted into a polyethylene counting tube and measured in the well of the germanium(lithium) detector. The errors caused by antimony-122 and antimony-124 might be reduced by further treatment of the organic portion with an antimony hold-back carrier solution (see Fig. 3).

Results and Discussion

The efficiency of the separation of arsenic from antimony with benzene was tested over a range of arsenic to antimony ratios varying by a factor of 10^3. Almost 100% of arsenic but less than 0.4% of antimony are extracted into the benzene phase under the given conditions (Fig. 2). If there was any need, the amount of antimony could be reduced by another factor of 100 by washing the benzene solution with concentrated hydrochloric acid.

In Fig. 3 the gamma-ray spectra A and B of sulphide ore C3 (W 110) from Laurion containing 95% of lead sulphide and 0.48% of silver, in addition to other constituents, are shown

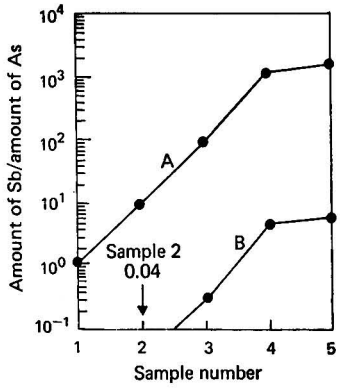

Fig. 2. Trial separation of As from Sb with benzene for five samples of various Sb/As ratios. As and Sb standard solutions were pipetted, dried and irradiated, and the activities of ^{76}As and ^{122}Sb counted on a Ge(Li) detector after benzene extraction. Line B shows the amounts calculated. Line A shows the Sb/As abundance ratio before extraction.

before and after radiochemical processing. The spectra show that a good separation with $Zn(DDC)_2$ has been achieved, and the photopeaks of silver-110m, gold-198 and cadmium-115 show up in spectrum B of the extracted organic phase. The chemical yields for the extracted elements were gold >95, silver >80, copper >80 and cadmium >70%. The pH of the aqueous phase that has been chosen is too low for a quantitative extraction of cadmium, as cadmium has been shown[17] to be extracted quantitatively from perchloric acid only up to 0.2 M. Gold(III), silver(I) and copper(II) are known to be extracted with satisfactory efficiency from solutions with the acidity used in this work.[10,14] It may also be possible

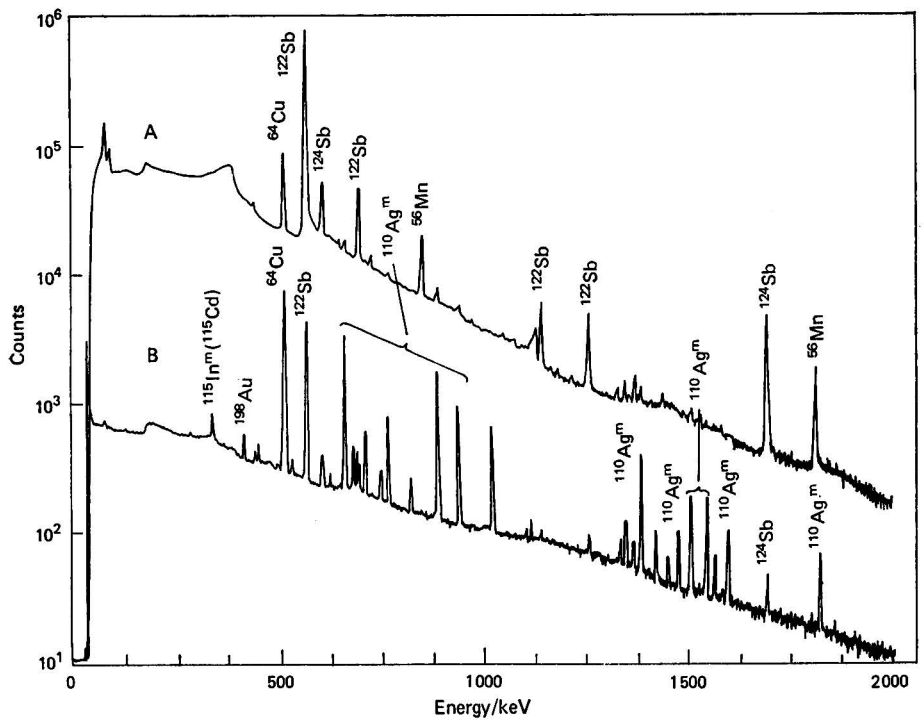

Fig. 3. γ-Ray spectrum of neutron-activated ore sample C3 (W 110) from the site of Laurion. Sample mass 100 mg. A, Before radiochemical separation; B, after extraction with $Zn(DDC)_2$. Sum peaks are not marked. The strong peak of 1022 keV in spectrum B, which has been measured in the well of the Ge(Li) detector, is due to the sum of annihilation radiation.

that the MDDC complexes are slowly decomposed in strongly acidic solutions, which would lower the chemical yields. It has been shown recently that an extraction time of 2 min would be sufficient.

We also determined the arsenic contents in three Laurion sulphide ores by benzene extraction and by distillation, and found good agreement between the two methods (Table I).

TABLE I

DETERMINATION OF ARSENIC BY EXTRACTION WITH BENZENE AND BY DISTILLATION

	Arsenic content, p.p.m.	
Sulphide ore sample from Laurion	Arsenic extracted with benzene as arsenic(III) chloride	Arsenic distilled as arsenic(III) chloride
C3 (E7)	4.9 ± 0.4	5.1 ± 1.7
A4	0.9 ± 0.1	0.6 ± 0.1
A5	16.8 ± 1.5	16.8 ± 1.3

In Table II the results of replicate analyses for two lead sulphide ore samples from the site of Laurion are shown. On average, the precision was equal to or less than 5% for copper, antimony, manganese and zinc, equal to or less than 10% for silver, arsenic and cadmium, and 25% for gold. The precision for gold and silver could possibly be improved by replacing $Zn(DDC)_2$ with $Ni(DDC)_2$ in which instance only mercury(II), silver(I) and gold(III) would be extracted. However, it was more important for this study to characterise the ores with as many minor and trace elements as possible in one run. We have been able to show that the gold to silver ratio can be a valuable indicator for the determination of the provenance of ancient silver, while no conclusions can be drawn from the arsenic to antimony ratio at present.

TABLE II

REPLICATE ANALYSES OF LEAD SULPHIDE ORE SAMPLES FROM THE LAURION SITE

Sample	Silver, p.p.m.	Gold, p.p.m.	Copper, p.p.m.	Arsenic, p.p.m.	Antimony, %	Manganese, p.p.m.	Zinc, %	Cadmium, p.p.m.
C8 (W110)	4 800 ± 360	0.071 ± 0.004	870 ± 60	45.3 ± 1.2	1.07 ± 0.01	870 ± 30	0.37 ± 0.01	60 ± 4
C8 (E7)	4 400 ± 400	0.047 ± 0.009	1 460 ± 70	5.0 ± 0.1	0.92 ± 0.05	186 ± 6	<0.02	26 ± 9

The great interest of Professor W. Gentner in this work is appreciated. We are grateful to Ms. Karin Trauner for many chemical experiments and to Mr. Klaus Oberfrank for assistance in gamma-ray spectrometry and data handling. We thank Priv.-Doz. Dr. A. Wyttenbach and Dipl.-Chem. S. Bajo, Eidgenössisches Institut für Reaktorforschung, Würenlingen, Switzerland, for valuable discussion and advice, and the staff members Mr. Jünger and Mr. Anasensl of the TRIGA reactor, Deutsches Krebsforschungszentrum, Heidelberg, for the neutron irradiations. Financial support by the Stiftung Volkswagenwerk is gratefully acknowledged.

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Pyrolysis - Mass Spectrometry of Textile Fibres

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A procedure for pyrolysis - mass spectrometry is described and the spectra (mass pyrograms) of various textile fibres are presented. The method is compared with infrared spectroscopy for the forensic characterisation of synthetic fibres. Samples of less than 5 μg can be analysed.

Keywords: *Textile fibre characterisation*; *pyrolysis - mass spectrometry*; *infrared spectroscopy*

The forensic analysis of synthetic fibres usually involves the characterisation and comparison of two or more samples and in this laboratory techniques such as microscopy, infrared spectroscopy and thin-layer chromatography combined with ultraviolet spectroscopy of extracted dyes are the methods in current use. In practice the amount of material available may be as little as a single fibre and in such circumstances the infrared method in particular is unable to provide the same amount of information as might be obtainable from a larger sample. Pyrolysis - mass spectrometry (Py - MS)[1,2] is an analytical technique capable of providing useful data on very small polymeric samples and this paper reports on its use for fibre characterisation and compares the method with infrared spectroscopy.

Experimental

Fibre Pyrolysis

Single fibres 0.5–10 mm in length were heated in a helium stream using a Curie-point (Pye) or filament pyrolyser (Chemical Data Systems Pyroprobe, Model 190). On the Pyroprobe samples were generally pyrolysed isothermally for 20 s at a selected temperature (normally 600 or 800 °C), but some of the fibres were sequentially pyrolysed at increasing temperatures in the range 400–900 °C. For the Curie-point method a 15-s pyrolysis time was used. Fibres were mounted for analysis by crimping into a flattened wire for the Curie-point method or by insertion into a quartz tube mounted in the filament coil of the Pyroprobe.

Either pyrolyser was connected to an empty glass column, 45 cm × 6.3 mm o.d. × 2 mm i.d., heated at 200 °C in the oven of a Varian 2700 gas chromatograph. The pyrolyser was mounted outside the oven and was flushed with helium at 15 ml min^{-1} in order to sweep the pyrolysate into the empty column. The empty column provided a simple means of broadening the pyrolysate band before it entered the mass spectrometer.[3]

Mass Spectrometry

A VG Micromass 12F mass spectrometer was used under standard electron-impact conditions: electron energy 70 eV, emission current 100 μA, accelerating voltage 4 kV and source temperature 240 °C. The pyrolysate emerging from the empty glass column passed into the mass spectrometer via a length of glass-lined stainless-steel tubing and a glass jet separator.

A mass range of 25–250 a.m.u. was scanned at 1 s per decade with a magnet re-set time of 1 s. After pyrolysis 35 scans were collected, with data acquisition and storage achieved with a VG 2040 data system. The 25 most intense spectra of each series were integrated using standard software and a specially written Fortran IV program to produce a composite mass spectrum (*i.e.*, a mass pyrogram). The ions of *m/e* 28, 32, 40 and 44 were not included in the integration procedure as they are associated with air in the system and only those ions with intensities greater than 0.1% of that of the base peak were included in the processed data.

An FIT program was written in Fortran IV to allow the data-processing equipment to compare two sets of data. The FIT equation was defined as

$$\mathrm{FIT} = 1\,000 \left[1 - \frac{\sum_{m=25}^{m=200} (a-b)^2}{\sum_{m=25}^{m=200} (a^2+b^2)} \right]$$

where a and b are the intensities of the same ion of mass m in the spectra of samples A and B. The individual ion intensities were expressed as a fraction of the total ion current (*i.e.*, they were normalised) in order to compensate for variations in sample size and efficiency of pyrolysis. The FIT factor ranges between 1 000 (for perfectly matched mass pyrograms) and 0 (for completely dissimilar pyrograms). A comparison of the pyrolysers was made by the replicate analysis of ten samples of the same nylon 6 fibre and FIT factors were determined for the resulting 45 pairs of pyrograms for each series.

Infrared Spectroscopy

Single fibres 1 cm long (mass 3–5 μg) were sealed into glass capillary tubes 2.5 cm × 1 mm i.d., together with 2–3 μl of solvent, and were heated in an oven at 80–100 °C to effect solution. The solvent selected was dependent upon the type of fibre, *e.g.*, dimethylformamide (acrylics), *m*-cresol (nylons and polyesters), acetone (cellulose acetate) and chloroform (cellulose triacetates). The contents of the tubes were poured on to a silicone-coated Petri dish to form a film, which was washed with ethanol, dried and pressed in a 1-mm lead microdisc between layers of potassium bromide. The microdisc was then analysed by using a suitable infrared spectrophotometer with beam condensing facilities.

Results and Discussion

Fibres examined under the experimental conditions described gave results that were sufficiently reproducible to be used for characterisation. This finding is in agreement with previous studies using the same instrumentation.[2,3] With the exception of Nomex [Fig. 1(*g*)] and Kevlar [Fig. 1(*h*)], which did not pyrolyse efficiently at 600 °C and were pyrolysed at 800 °C, all of the samples were pyrolysed at 600 °C. This temperature was found experimentally to provide the highest yields of characteristic pyrolysis products with both pyrolysers. The mass pyrograms shown in Figs. 1–9 are reproduced directly from the computer output. They were all obtained by using the Pyroprobe 190, which was operated at 600 °C unless another temperature is stated. In discussing the data an attempt has been made to assign probable identities to significant ions, but it must be noted that these have not been established experimentally but were deduced from the chemical composition of the fibre and experience gained in the pyrolysis - gas chromatography of such materials.

Comparison of Pyrolysers

The average FIT factors calculated for the 45 comparisons between the ten samples of nylon 6 were as follows: Curie-point pyrolyser, 975.9 (coefficient of variation 1.6%); and filament pyrolyser (Pyroprobe), 994.9 (coefficient of variation 0.3%).

The reproducibility of the Pyroprobe was found to be slightly better than that of the Curie-point pyrolyser and fibre handling was easier. Although both pyrolysers produced characteristic mass pyrograms when operated at optimum temperatures, the form of the pyrogram was influenced by the pyrolyser and for comparative analysis it is essential to use the same equipment.

Sensitivity

The sensitivity of the method was limited principally by the handling difficulties associated with very small samples (*i.e.*, less than 1 mm in length). No instrumental difficulties were experienced in the analysis of 2-mm lengths of single fibres (mass 0.5–1 μg).

Comparison of Polymers

Nylons

Typical mass pyrograms for different types of nylons are shown in Fig. 1 and the major features that permit classification are shown in Table I.

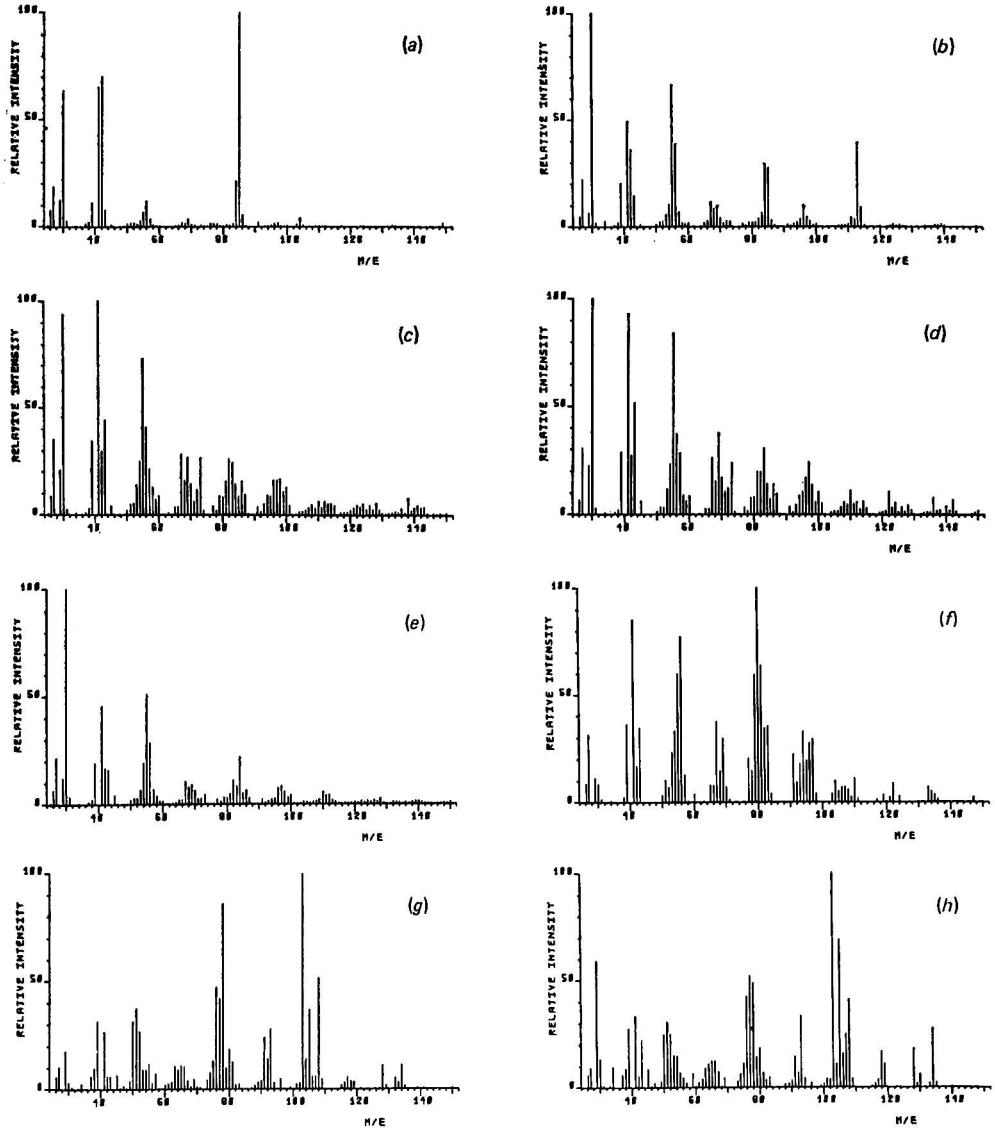
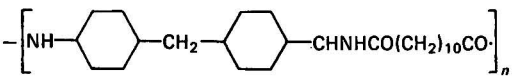
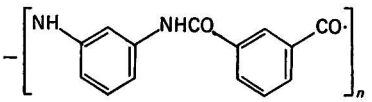
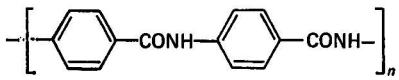


Fig. 1. Mass pyrograms of nylon fibres pyrolysed at 600 °C. (*a*), Nylon 4, Tajmir, Alrac Co.; (*b*), nylon 6, Firestone Synthetic Fiber Co.; (*c*), nylon 6.10, Grayni, Slack Brothers; (*d*), nylon 11, Rilsan, Rhodiaceta (Lyon); (*e*), nylon 6.6, Columbian Rope Co.; (*f*), nylon, Qiana, Du Pont UK Ltd.; (*g*), nylon, Nomex, Du Pont Co. (Burlington Industries), pyrolysed at 800 °C; and (*h*), nylon, Kevlar, sample provided by the Shirley Institute, pyrolysed at 800 °C.

The mass pyrograms of nylon 4 [Fig. 1(*a*)] and nylon 6 [Fig. 1(*b*)] are consistent with these materials pyrolysing to produce large amounts of their respective monomers, *viz.*, butyrolactam and caprolactam.

TABLE I

FEATURES PERMITTING THE CLASSIFICATION OF NYLONS BY PYROLYSIS - MASS SPECTROMETRY

Nylon type	Structural unit	*m/e* Base peak	*m/e* Other prominent peaks
4 ..	$-[NH(CH_2)_3CO-]_n$	85	30, 41, 42, 84
6 ..	$-[NH(CH_2)_5CO-]_n$	30	41, 55, 84, 85, 113
6.10 ..	$-[NH(CH_2)_6NHCO(CH_2)_8CO-]_n$	30 or 41	Discriminated by minor peaks
11 ..	$-[NH(CH_2)_{10}CO-]_n$	30 or 41	Discriminated by minor peaks
6.6 ..	$-[NH(CH_2)_6NHCO(CH_2)_4CO-]_n$	30	41, 55, 84
Qiana		80	41, 55
Nomex		103	Discriminated by pyrolysis at 600 °C
Kevlar		103	

Nylon 6.10 [Fig. 1(*c*)] and nylon 11 [Fig. 1(*d*)] gave results that varied in having either *m/e* 30 or 41 as the base peak but with *m/e* 55 as the third most abundant ion. Despite the variability of the base peak, other spectral features in the region of *m/e* 60–150 are sufficiently characteristic to allow unambiguous classification.

Nylon 6.6 [Fig. 1(*e*)] produced pyrograms similar to that of nylon 6, but the weakness or absence of *m/e* 113 in the spectra from the former material was a characteristic feature. The prominent ion *m/e* 84 in the nylon 6.6 pyrogram was consistent with the presence of cyclopentanone.[4]

Qiana gave very characteristic pyrograms but it has not been possible to assign identities to the major ions [Fig. 1(*f*)].

Nomex and Kevlar are chemically very similar and it was not found possible to discriminate between these two fibres by Py - MS [Fig. 1(*g*) and (*h*)]. A base peak of *m/e* 103 attributable to $C_6H_5CN^+$ or $C_6H_5NC^+$ was the dominant feature of both pyrograms. The close similarity of the pyrolysates of these materials is not immediately obvious from the data illustrated in Fig. 1, which reflects the quantitative variability of pyrolysates from these materials. The reason for this variability is not understood but it is far greater than that displayed by other polyamides and may be related to the thermal stability of these fibres or to pyrolysate interactions before entering the mass spectrometer. During experiments with the sample of Nomex fibre in our collection it was noticed that heating of this fibre at 400–600 °C using the Pyroprobe gave rise to a product with a mass spectrum matching that of *NN*-dimethylacetamide. It was assumed that this compound was introduced during the fibre processing and although it is not necessarily distinctive to Nomex such information could be of value in a forensic context.

Cellulose acetate and triacetate

Fibres of this type differ in the extent to which the fully acetylated product has been hydrolysed and the pyrograms of both [Fig. 2(*a*) and (*b*)] show major peaks at *m/e* 43, 45 and 60, probably indicative of the acetic acid formed on pyrolysis. Variations in the intensities of major and minor peaks were not sufficiently reproducible to allow the discrimination of acetates from triacetates, although the pyrograms were unlike those of any other fibre class investigated.

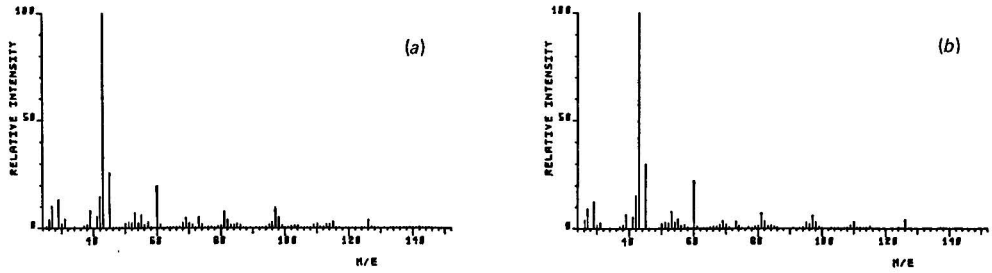


Fig. 2. Mass pyrograms of cellulose acetate and triacetate fibres pyrolysed at 600 °C. (*a*), Cellulose acetate, Silene, SNIA Viscosa; and (*b*), cellulose triacetate, Tricel, British Celanese Ltd.

Polyesters

Four types of polyester were studied. The sample of A-Tell was readily distinguishable from other polyesters in giving a pyrogram [Fig. 3(*a*)] with a base peak of *m/e* 94 ($C_6H_4O^+$). The other three materials gave similar mass pyrograms [Fig. 3(*b*)–(*d*)] with a base peak of *m/e* 105 ($C_6H_5CO^+$) and major peaks at *m/e* 77 ($C_6H_5^+$), 122 ($C_6H_5COOH^+$) and 149 ($OCC_6H_4COOH^+$). The relative intensity of the last ion showed great variability between replicate runs of all three fibres, but was always significantly lower in Kodel. This, together with a higher ratio of *m/e* 43 to 51, appears to allow Kodel to be discriminated from Terylene or Dacron. The method was unable to distinguish between the latter fibres, which differ chemically only in the end grouping of the polymer chains.

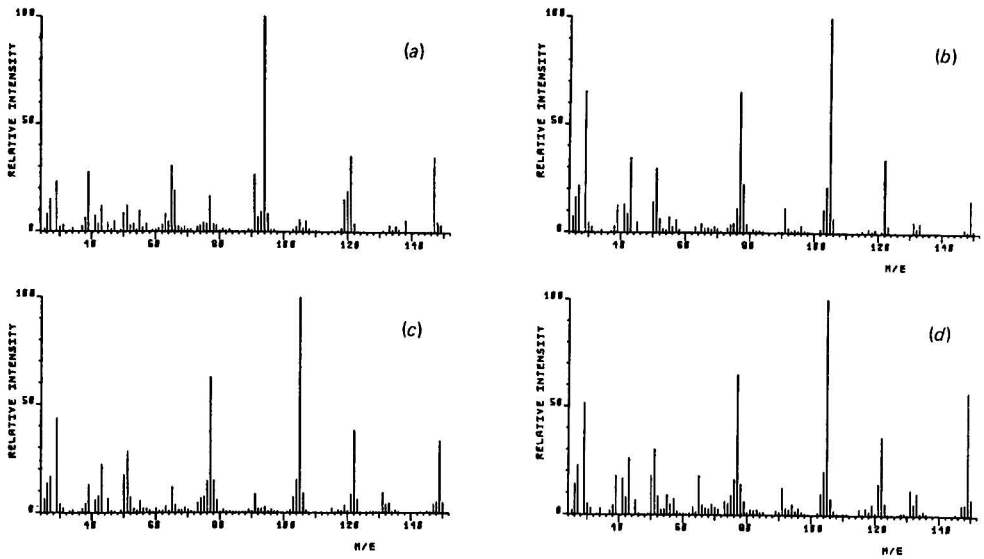


Fig. 3. Mass pyrograms of polyester fibres pyrolysed at 600 °C. (*a*), Polyester [poly(ethylene oxybenzoate)], A-Tell, Nippon Rayon Co.; (*b*), polyester (cyclohexanedimethanol - dimethyl terephthalate copolymer), Kodel IV, Cane Mills; (*c*), polyester [poly(ethylene terephthalate)], Dacron, Columbian Rope Co.; and (*d*), polyester [poly(ethylene terephthalate)], Trevira (g-1), Farbwerke Hoechst AG.

Acrylic fibres

This class of fibres is defined as having at least 85% of acrylonitrile in the final polymer and as would be expected the incorporation of a comonomer makes only a minor difference to the resulting mass pyrogram. A 100% acrylic fibre, Crylor, produced a characteristic pyrogram [Fig. 4(*a*)] with a base peak of *m/e* 66 ($NCCH_2CH{=}CH^+$) and major ions at *m/e*

105 and 119 ($C_7H_5N^+$). Zefran (an acrylonitrile - vinylpyrrolidone copolymer) gave an identical pyrogram. Acrilan (an acrylonitrile - vinyl acetate copolymer) was found to give a slightly different result [Fig. 4(*b*)] with an enhanced peak at *m/e* 43 (CH_3CO^+). The other common acrylic fibre, Courtelle (a terpolymer containing acrylonitrile and methyl acrylate), also gave a modified pyrogram with significantly enhanced peaks at *m/e* 41 and 54 [Fig. 4(*c*)].

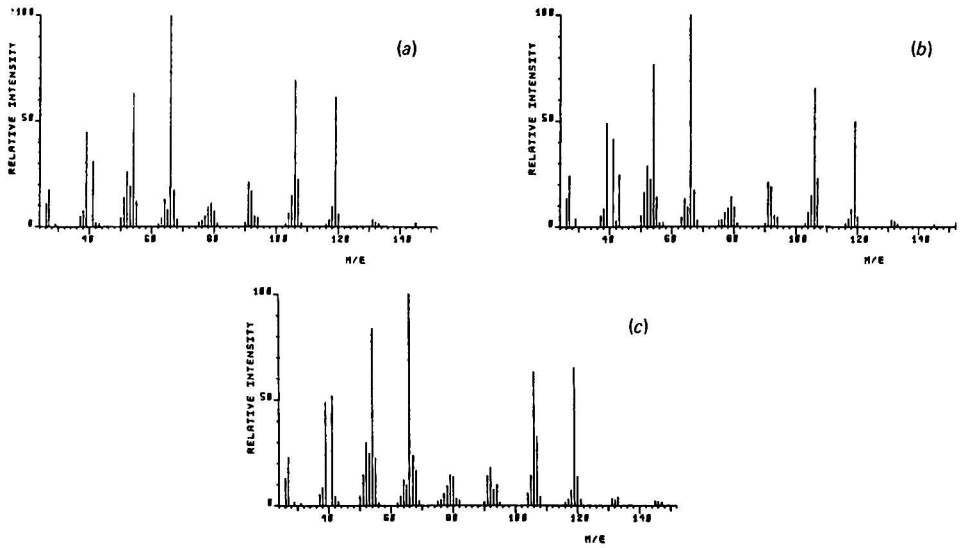


Fig. 4. Mass pyrograms of acrylic fibres pyrolysed at 600 °C. (*a*), Polyacrylonitrile, Crylor, Rhodiaceta (Lyon); (*b*), polyacrylonitrile - poly(vinyl acetate) copolymer, Acrilan, Israel Chemical Fibres Ltd.; and (*c*), terpolymer containing acrylonitrile and methyl acrylate, Courtelle, Courtaulds UK Ltd.

Modified acrylics (modacrylics)

Modacrylics, *i.e.*, materials containing between 35 and 85% of acrylonitrile, surprisingly gave rise to pyrograms [Fig. 5(*a*)–(*e*)] that showed little resemblance to those of acrylic fibres. In each instance a base peak of *m/e* 41 and intense peaks at *m/e* 39 and 27 were the principal features of the spectra. The reproducibility of replicate runs with modacrylics was poorer than for any other class of fibre. This irreproducibility complicates the task of fibre discrimination. The variation in the relative intensity of the minor peaks may provide a basis for discrimination.

Polyolefins

Both polyethylene and polypropylene fibres break down to give distinctive mass pyrograms [Fig. 6(*a*) and (*b*)]. The major ions are attributable to aliphatic hydrocarbons, *e.g.*, *m/e* 43 ($C_3H_7^+$), 55 ($C_4H_7^+$), 57 ($C_4H_7^+$), 69 ($C_5H_9^+$) and 83 ($C_6H_{11}^+$). The mass pyrograms can be distinguished from those of the other fibre classes analysed.

Poly(vinylidene chloride) and poly(vinyl chloride)

The mass pyrogram of Saran [poly(vinylidene chloride)] [Fig. 7(*a*)] is very similar to that obtained in other studies.[5] The major ions are *m/e* 36, 38 (both associated with HCl^+), with smaller contributions from *m/e* 61, 63 ($CH_2{=}C^+{-}Cl$), 96, 98 ($CH_2{=}C^+{-}Cl_2$), 146 and 148 ($C_6H_4Cl_2^+$).

In contrast, poly(vinyl chloride) fibres gave a pyrogram notable for the absence of ions due to HCl^+ [Fig. 7(*b*)]. This difference can possibly be explained by the adsorption of acid on active sites before entry into the mass spectrometer and could be reconciled with the detection of HCl^+ in the instance of Saran due to a much higher yield of acid. Whatever the explanation, the mass pyrogram of poly(vinyl chloride) contains many features attributable

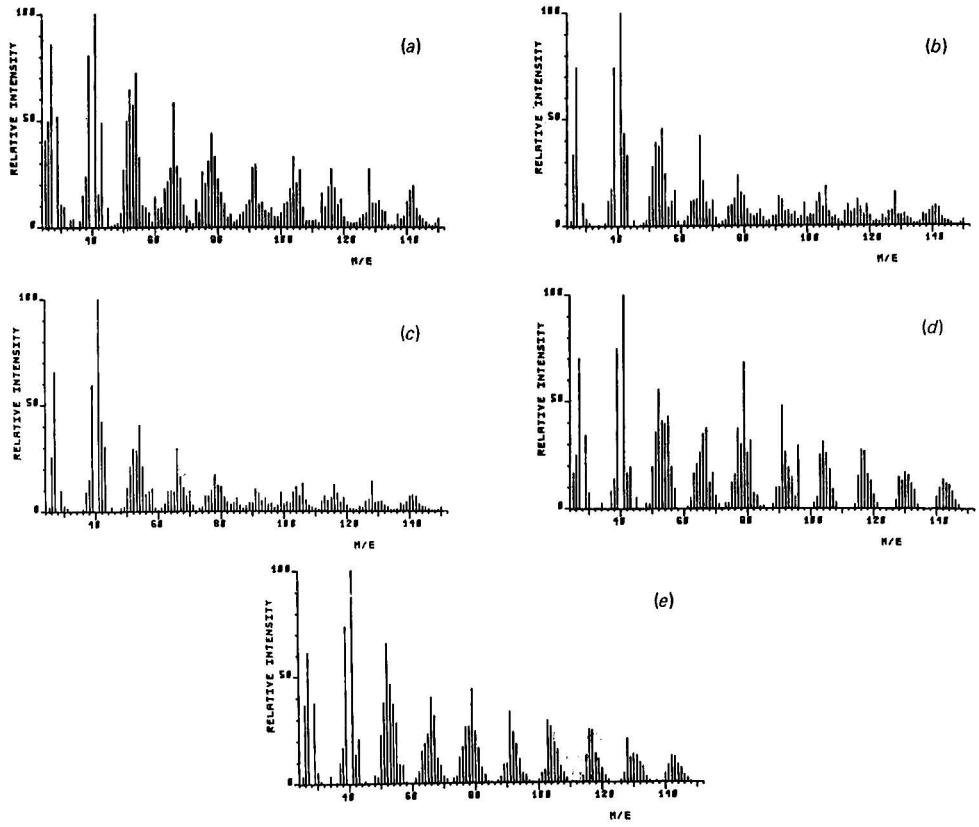


Fig. 5. Mass pyrograms of modacrylic fibres pyrolysed at 600 °C. (*a*), Teklan, Courtaulds UK Ltd.; (*b*), Verel, Type HB, Eastman Chemical International; (*c*), Verel, Type F, Eastman Chemical International; (*d*), Dynel, Type 180, Union Carbide Corporation; and (*e*), Kanekalon (high bulk), Kanegafushi Chemical Industrial Co.

to aromatic hydrocarbons, presumably formed by free-radical recombination after pyrolysis. Thus the major ions are *m/e* 78 ($C_6H_6^+$), 91 ($C_7H_7^+$), 104 ($C_8H_8^+$), 115 ($C_9H_7^+$) and 128 ($C_{10}H_8^+$).

Two modified PVC fibres, Vinyon (a copolymer with vinyl acetate) and Bristrand (a copolymer with styrene), gave significantly different pyrograms to that of PVC [Fig. 7(*c*) and (*d*)]. With Vinyon, ions at *m/e* 43, 45 and 60 are indicative of the presence of acetic acid in the pyrolysate. The Bristrand fibre produced a pyrogram displaying features that

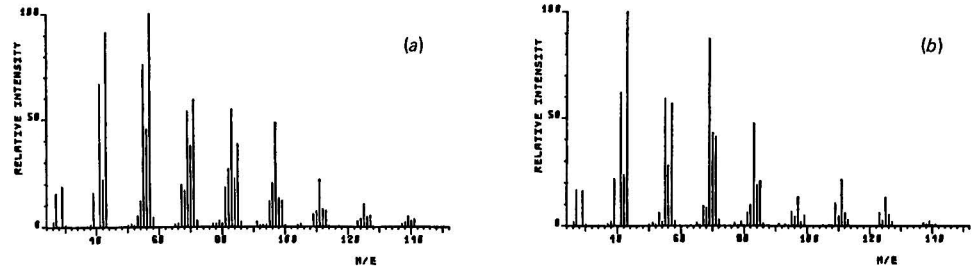


Fig. 6. Mass pyrograms of polyolefins. (*a*), Polyethylene, Suddeutsche CFAG, pyrolysed at 800 °C; and (*b*), polypropylene, Magyar Viscosa, pyrolysed at 600 °C.

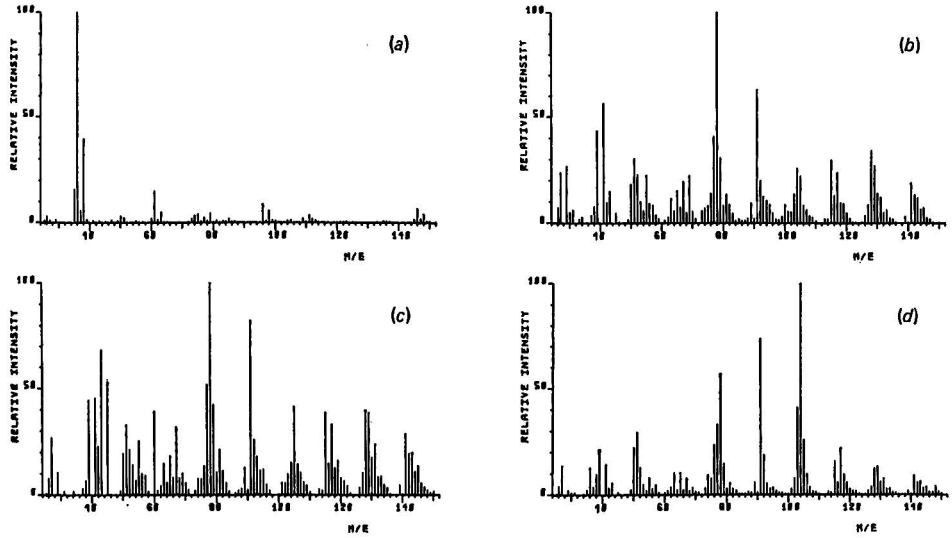


Fig. 7. Mass pyrograms of poly(vinylidene chloride) and poly(vinyl chloride) fibres pyrolysed at 600 °C. (*a*), Poly(vinylidene chloride), Saran, Thiokol Fibres Canada Ltd.; (*b*), poly(vinyl chloride), Rhovyl, Rhovyl SA, France; (*c*), poly(vinyl chloride) - poly(vinyl acetate) copolymer, Vinyon, F.M.C. Corporation (American Viscose Division); and (*d*), poly(vinyl chloride) - polystyrene copolymer, Bristrand, Polymers Incorporated.

closely resembled those of polystyrene (Fig. 8), but it could be differentiated from the latter by the intensity of the *m/e* 91 peak.

Polystyrene

The mass pyrogram of a polystyrene fibre, Kilmarn (Fig. 8), is distinctive and closely resembles the published mass spectra of styrene. The principal ions are *m/e* 104 ($C_8H_8^+$), 103 ($C_8H_7^+$) and 78 ($C_6H_6^+$). The intensity of the *m/e* 91 ion is higher in the mass pyrogram than in the normal electron-impact mass spectrum of styrene and this difference can be attributed to the formation of toluene during pyrolysis.

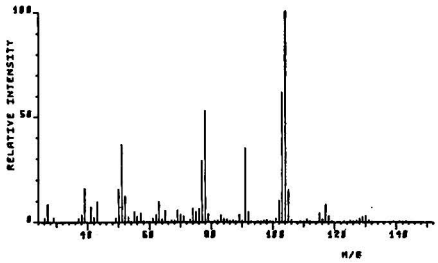


Fig. 8. Mass pyrogram of polystyrene fibre pyrolysed at 600 °C, Kilmarn, Polymers Incorporated.

Natural fibres

A selection of natural fibres together with viscose rayon gave characteristic mass pyrograms [Fig. 9(*a*)–(*e*)], but the complexity of these materials makes it difficult to interpret the data although their value as "fingerprints" is obvious. Interestingly, the pyrogram of viscose [Fig. 9(*e*)] is different to that of natural cellulose [Fig. 9(*d*)] with respect to variation in the ratio of *m/e* 29 to 43.

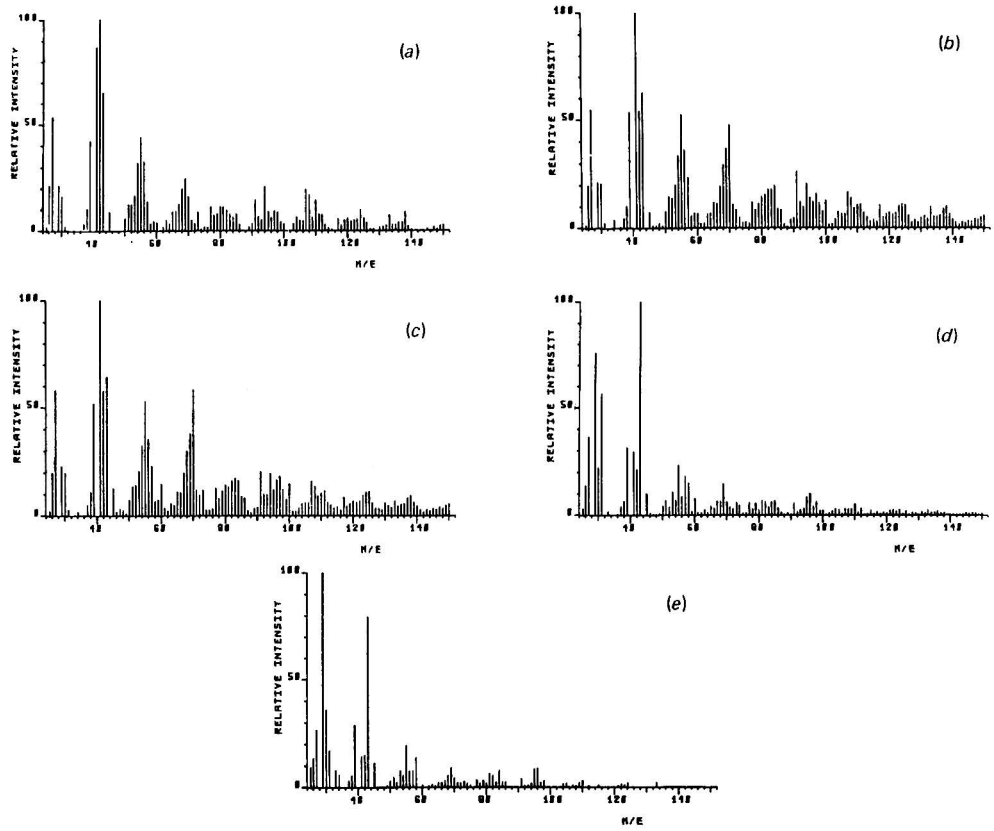


Fig. 9. Mass pyrograms of natural fibres and viscose rayon pyrolysed at 600 °C. (*a*), Japanese raw silk (*Bombyx mori*); (*b*), natural sheep wool; (*c*), Caucasian head hair, dark brown; (*d*), cotton (*Gossypium* sp.), Brazil; and (*e*), viscose (bright), Courtaulds (Canada) Ltd.

Comparison of Pyrolysis - Mass Spectrometry with Infrared Spectroscopy

All of the samples analysed in this study had been previously examined by infrared spectroscopy. When a sufficient amount of material is available the latter technique is superior to Py - MS for discriminating fibres, particularly with those samples in which copolymerisation significantly modifies the infrared spectrum of the parent polymer, *e.g.*, the modacrylics. Nevertheless, in a blind trial conducted with 12 fibres (each 1 cm in length, mainly nylons) Py - MS was used to identify every fibre correctly, whereas infrared spectroscopy led to only two correct assignments. Certainly for the nylons, and probably also for most other fibres, Py - MS has the following advantages over infrared spectroscopy:

(1) much smaller samples can be examined;
(2) fibres containing appreciable amounts of filler can be assigned with greater confidence than with infrared spectroscopy;
(3) the analysis time is shorter, typically 20–30 min, depending on the computer handling facilities and peripherals available. However, it should be pointed out that batch handling of fibres for infrared analysis does significantly reduce this time advantage.

Limitations of Pyrolysis - Mass Spectrometry

The major factor limiting our approach to Py - MS at present is the poor reproducibility for certain types of polymer. It is not clear how this irreproducibility arises but if it could be overcome then it is likely that far greater discrimination could be achieved by attention

to the fine structure of mass pyrograms. Obviously we are constrained by the need to work with equipment normally utilised for gas chromatography - mass spectrometry,[3] rather than a custom-built instrument[1] and reaction in the empty glass column used to spread out the pyrolysate band may be contributing to the variability of some of the results.

A further disadvantage of the Py - MS procedure described, in comparison with infrared spectroscopy, becomes apparent when the pyrogram is used for interpretation rather than as a fingerprint. The ions contributing to the mass pyrogram result from two vigorous decomposition processes, *i.e.*, a thermal and an electron-impact fragmentation, and not surprisingly, it is often difficult to relate the final ions to the starting polymer. The use of chemical ionisation to reduce fragmentation within the mass spectrometer could yield results more amenable to qualitative interpretation,[6] and pyrolysis - gas chromatography followed by mass spectrometry is an obvious method of providing information on the significance of particular ions in a mass pyrogram.

Conclusions

This study indicates that Py - MS provides a rapid and sensitive method for the characterisation of synthetic fibres, which could have a wider range of application than infrared spectroscopy for forensic fibre examination. The equipment required for the technique is expensive but those laboratories currently using a mass spectrometer and data system could adapt their instruments at low cost. Provided that the reproducibility of Py - MS can be improved it should become a powerful method for the microanalysis of a wide range of natural and synthetic polymers.

The infrared spectroscopic method described in this paper has been in use for some years in this laboratory. The development of this procedure was the work of R. Cook and co-workers at the Metropolitan Police Forensic Science Laboratory.

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Determination of Probenecid in Serum by High-performance Liquid Chromatography

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The determination of probenecid in serum samples by using high-performance liquid chromatography is described. The method gives satisfactory results over the normal therapeutic range, namely up to 150 μg ml^{-1} of probenecid in serum, and is not affected by metabolites of the drug. The method does not require derivatisation of the drug, as in gas - liquid chromatographic procedures, and is less subject to interferences than spectrophotometric procedures. It has been used in analysis of several hundred serum samples and has given a satisfactory performance in respect of precision and accuracy.

Keywords: *Probenecid determination*; *serum*; *high-performance liquid chromatography*

Probenecid [4-(dipropylamino)sulphonylbenzoic acid] is a uricosuric agent that has recently been used in conjunction with penicillin derivatives in the treatment of various diseases. Probenecid reduces the excretion of penicillins by the kidneys and thus enables therapeutic levels of the penicillins to be achieved with a lower initial dose. The normal dose of probenecid used to obtain this effect is 1 g daily.

Several methods have been described for the determination of probenecid in body fluids,[1–5] mainly employing spectrophotometric or gas - liquid chromatographic methods for the final quantification step. The disadvantages of these methods are that the spectrophotometric methods are not specific and the gas - liquid chromatographic methods require derivatisation of the sample before the injection on to the column.

High-performance liquid chromatography was investigated as an alternative method. The extraction of probenecid from serum, followed by injection of the non-derivatised extract into the chromatographic column, should give advantages of specificity and time compared with the other methods.

Experimental

Reagents

All reagents were of analytical-reagent grade unless otherwise stated.

Potassium chloride.

Citric acid.

Disodium hydrogen orthophosphate.

Sodium hydroxide.

Potassium dihydrogen orthophosphate.

Diethyl ether.

Acetonitrile. Reagent grade.

Buffer, pH 4.0. Dissolve 35.5 g of disodium hydrogen orthophosphate in 250 ml of water, adjust the pH to 4.0 with approximately 40 g of citric acid, then dilute to 500 ml with water.

Buffer, pH 6.0. Dissolve 27.2 g of potassium dihydrogen orthophosphate in 500 ml of water, adjust the pH to 6.0 with sodium hydroxide solution and dilute to 1 l with water.

Mobile phase. Dilute 50 ml of pH 6.0 buffer to 700 ml with water, add 300 ml of acetonitrile, mix and de-gas by applying a vacuum immediately before use.

Apparatus

The high-performance liquid chromatograph was assembled using the following components: a constant-volume, syringe type, high-pressure pump (Metering Pumps Ltd., London; 3000 lb in^{-2}), a high-pressure septum injector head (Perkin-Elmer, Part No. 0087-3015) and a suitable Bourdon-type pressure gauge.

The column used, 25 cm $\times$ 4 mm i.d., was made of stainless steel, and was fitted with a stainless-steel sinter at the outlet and a 2 mm thick PTFE sinter at the inlet. Injections

were made into this sinter. The packing material was Partisil, particle diameter 10 μm, modified by treatment with octadecyltrichlorosilane to give a reversed-phase type of chromatography.

The detector used was a variable-wavelength monitor (Model 212, Cecil Instruments Ltd., Cambridge) equipped with an 8-μl flow-through cell. The detector was operated at 252.5 nm and the signal was recorded on a flat-bed recorder (W & W, Model 1100). Injections were made using a 10-μl high-pressure syringe (SGE, Type 10BLR).

The operating conditions for this system were as follows: flow-rate, 1 ml min^{-1} (about 50 bar); detector range, 0.05 A full-scale deflection (f.s.d.); and recorder range, 10 mV f.s.d.

The apparatus used in the extraction procedure was normal laboratory glassware, together with a centrifuge and a vortex mixer.

Procedure

Preparation of standard

Weigh accurately about 30 mg of probenecid, dissolve it in methanol and make the volume up to 100.0 ml. This is the stock solution. Dilute a 5.0-ml aliquot of stock solution to 50.0 ml with methanol to give a 30 μg ml^{-1} working standard solution.

Pipette an appropriate volume (usually 2.0 ml) of working standard solution into a 12-ml stoppered centrifuge tube and remove the methanol by blowing with air. Add 1.0 ml of distilled water to the residue and swirl in a vortex mixer to dissolve the residue. Process this standard solution as for the serum samples. The concentration of this solution will be 60 μg ml^{-1} when 2.0 ml of working standard solution are used in its preparation.

Preparation of samples

Pipette a 1.0-ml aliquot of serum sample into a 12-ml stoppered centrifuge tube and to it add 1.0 ml of pH 4.0 buffer and approximately 1 g of potassium chloride. Stopper the tube and vortex mix for about 15 s, then add 8.0 ml of diethyl ether from a dispensing pipette. Stopper the tube, vortex mix thoroughly for 1 min and centrifuge at 4000 rev min^{-1} for 5 min.

Transfer a 5.0-ml portion of the ether layer into a second tube, add an anti-bumping granule and remove the ether by evaporation on a water-bath at 40–50 °C. Final traces of ether should be removed by gentle purging of the tube with a stream of air or nitrogen. Add 1.0 ml of chromatographic mobile phase to the residue and mix briefly on a vortex mixer.

Chromatographic procedure

Establish a stable solvent flow and base-line conditions. Inject 8-μl portions of the standard solution extract until reproducible peak heights are obtained, then inject 8-μl portions of sample extract. Inject a further portion of the standard extract after every six sample injections.

Rinse the syringe at least five times with each solution prior to injection of that sample. This ensures a negligible carry-over of solutions between injections.

Calculation

Measure the probenecid peak heights of the standard extract and of the sample extract. Take a mean value of the peak heights for the two standard injections on either side of a sample. Then,

$$\text{Probenecid } (\mu g\ ml^{-1}) = \frac{\text{sample peak height} \times \text{standard concentration}}{\text{standard peak height}}$$

A typical chromatogram is shown in Fig. 1.

Investigation of Experimental Variables

Extraction from serum

When extracting probenecid from serum it was found that coagulated blood proteins formed a semi-solid "plug" at the aqueous surface. The solvent used in the extraction should

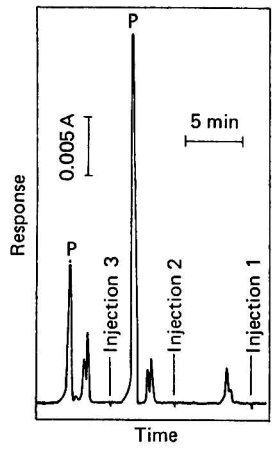


Fig. 1. Chromatograms of serum containing probenecid. (1), Serum blank; (2), a 4-h serum sample containing 75 μg ml^{-1} of probenecid; and (3), a 12-h serum sample containing 28 μg ml^{-1} of probenecid. The probenecid peaks are marked P.

therefore, for convenience of recovery, have a density lower than that of the aqueous phase, be readily removable by evaporation and be immiscible with water. Solvents evaluated included ethyl acetate, butyl acetate and a 2:1 mixture of 1-chlorobutane with chloroform. It was found that some of these solvents did not extract probenecid completely and others were difficult to evaporate or were too miscible with the aqueous phase. Diethyl ether was finally chosen, but it was found that the reduction in volume of the solvent due to loss into the aqueous phase (diethyl ether has a solubility in water of 6.9% at 20 °C) resulted in a bias leading to high recoveries of 103–107% when assayed against external standards. The adoption of a standard that was passed through the extraction procedure obviated the errors arising from this effect.

Linearity of response

Linearity of the response was established by injection of standard solutions containing from 10 to 100 μg ml^{-1} of probenecid. The mean peak heights of three injections of each of these solutions were plotted against the concentration of the solution injected. The linear response graph was found to pass through the origin, with a slope of 2.5 mm per μg ml^{-1}. The level of 100 μg ml^{-1} here corresponded to 160 μg ml^{-1} in serum because only a 5-ml volume of the 8 ml of diethyl ether extract was removed. The sensitivity of the procedure, taken as being that of a peak of height equal to twice the base-line noise, was generally 1 μg ml^{-1}, depending on the instrumental conditions. In order to ensure a constant absorptivity for probenecid at the detector, it was necessary to buffer the mobile phase to pH 6, thus ensuring that the same ionic form of probenecid was measured in each determination.

Possible interferences

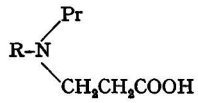
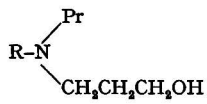
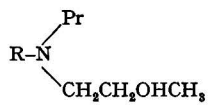
Probenecid metabolites. The chromatographic behaviour of probenecid metabolites was investigated, and the results are shown in Table I. The probenecid peak was well separated from all of the metabolite peaks and hence no interference could occur.

Co-administered epicillin. The co-administration of probenecid and penicillins is now relatively common so that the method should ideally be unaffected by large doses (about

TABLE I

RETENTION DATA FOR PROBENECID METABOLITES

Column and mobile phase as in text. Flow-rate, 1 ml min^{-1}.

Metabolite*	Retention time/min
	1.67
$R–NH_2$	2.00
	2.20
	2.25
R–NH–Pr	2.30
$R–NPr_2$ (probenecid)	3.50

* R = $–SO_2–Ph–COOH$; Ph = C_6H_5; Pr = n-C_3H_7.

4 g) of penicillins. This was tested *in vivo* by obtaining serum samples from a subject who had taken 4 g of epicillin (α-amino-3,6-dihydrobenzylpenicillin). Samples were taken at 0, 0.5, 1, 2, 3, 4, 5, 6, 9, 12, 24 and 30 h after the epicillin had been administered, extracted by using the above method and the chromatograms were examined for possible interferences. No peaks other than those due to the solvent were found in samples taken up to 2 h after administration. In the 3–30-h samples a small peak was eluted at 3.2 min (probenecid was eluted at 3.4 min). The maximum height of this peak was 2.5 mm, which occurred at the 4-h sample, this height being approximately the same as that of a peak representing 1 μg ml^{-1} of probenecid. This small peak was of short duration and in all instances was completely eluted by the start of the probenecid peak. No positive interference could thus occur. This small peak does not correspond to epicillin, which is not extracted from serum by this method, but may be due to a metabolite of epicillin.

Other drugs. Some other drugs that might be found with probenecid in serum were examined by using the chromatographic system described. The retention times for these drugs are given in Table II.

TABLE II

RETENTION TIMES FOR SOME SELECTED DRUGS

Column and mobile phase as in text. Flow-rate, 1.2 ml min^{-1}.

Compound	Retention time/min
Aspirin	1.6
Ampicillin	1.95
Epicillin	2.00
Potassium penicillin G	2.05
Probenecid	2.9
Phenazone	4.0
Niflumic acid	5.8–8 (tailing)
Flufenamic acid	10.5–15 (tailing)

Results and Discussion

This assay method was originally developed in order to give comparative data in clinical

trial studies in which probenecid was administered in different dosage forms, alone and in conjunction with epicillin. Recovery experiments were therefore carried out at probenecid concentrations that approximated to the levels expected over the initial, clinically significant, part of the trial, *i.e.*, from 0 to 12 h. The recovery and precision results are given in Table III.

TABLE III

RECOVERY AND PRECISION RESULTS FOR PROBENECID IN WATER AND IN SERUM

Matrix	Probenecid added/ $\mu g\ ml^{-1}$	Mean recovery, %	Coefficient of variation, %	Number of determinations
Water	30	97.8, 98.7	*	2
	60	96.8, 98.2	*	2
	90	99.3, 101.1	*	2
Serum	5	96.2	3.6	12
	30	95.3	1.0	8
	60	97.7	1.1	6
	100	100.4	0.9	10

* Both values determined are given.

The coefficients of variation of probenecid peak heights from standard injections over 3–6-h periods were 1.8% (6 values), 2.4% (6 values), 2.0% (5 values) and 2.4% (11 values) on four separate days. These results indicate that the chromatographic conditions are relatively stable, as these values represent the cumulative error due to contributions from syringe inaccuracies and to variation in injection technique, eluent flow-rate, detector lamp stability and peak measurement.

Between 35 and 50 samples can be assayed in an 8-h period. Over 400 samples, from several subjects in a clinical trial, have been assayed for probenecid.

Samples of probenecid metabolites were kindly donated by Dr. W. D. Conway of the State University of New York at Buffalo, N.Y., USA.

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Polarographic Method for the Identification of 1,4-Benzodiazepines

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The polarographic behaviour of 12 therapeutically important 1,4-benzodiazepines in Britton - Robinson universal buffers, pH 4.0 and pH 12.0, has been investigated. Differences in the polarographic peak potentials of these compounds in these media are explained. The rates of hydrolysis of certain benzodiazepines in acidic solution were investigated. Bromazepam and flunitrazepam, both of which possess a strongly electron-withdrawing substituent on the 5-*o*-phenyl group, were found to undergo rapid acid hydrolysis. On the basis of these findings, and taking into account the extraction profile of some of the compounds over a pH range, a scheme is devised for the identification of any one or more of 12 1,4-benzodiazepines. It is suggested that this procedure would be applicable to the analysis of unknown formulations or body fluids in forensic cases where the parent compound exists in relatively high concentrations compared with its metabolites.

Keywords: *1,4-Benzodiazepine identification*; *polarography*

The 1,4-benzodiazepines are one of the most frequently prescribed group of drugs in the UK and, with the ever increasing number of compounds in this series that are used for therapeutic purposes, there is a need for the development of rapid methods of identification, particularly for use in forensic situations, *e.g.*, identification of a particular 1,4-benzodiazepine in unknown formulations or body fluids. The most commonly employed method of identification is thin-layer chromatography of the intact compounds or of their acid hydrolysis products, *i.e.*, benzophenones.[1] In the examination of body fluids, a separation step such as adsorption-column chromatography[2] or solvent extraction[3,4] is usually employed prior to thin-layer chromatography. Identification is usually effected by comparison of R_F values, the colours produced by chromogenic spraying and spot patterns produced by the drug and its metabolites.[2,3,5] Gas - liquid chromatography using the nickel-63 electron-capture detector appears to be the method of choice for the determination of the 1,4-benzodiazepines.[6–9]

Smyth and co-workers[10–16] have investigated the acid - base properties and polarographic behaviour of many of the members of this group of drugs. Groves[17] has made a detailed study of the mechanism of hydrolysis of 5-fluorophenyl-1,4-benzodiazepines[17] and compared the rates of hydrolysis of other non-fluorinated 1,4-benzodiazepines.[18] This paper shows how a knowledge of these physico-chemical properties can be used to devise a scheme for the identification of 12 1,4-benzodiazepines: medazepam (**I**), chlordiazepoxide (**II**), potassium chlorazepate (**III**), bromazepam (**IV**), diazepam (**V**, X = Cl, Y = CH_3, Z = H, W = H), oxazepam (**V**, X = Cl, Y = H, Z = OH, W = H), prazepam (**V**, X = Cl, Y = cyclopropyl, Z = H, W = H), lorazepam (**V**, X = Cl, Y = H, Z = OH, W = Cl), nitrazepam (**V**, X = NO_2, Y = H, Z = H, W = H), clonazepam (**V**, X = NO_2, Y = H, Z = H, W = Cl), flunitrazepam (**V**, X = NO_2, Y = CH_3, Z = H, W = F) and flurazepam (**V**, X = Cl, Y = $(CH_2)_2$ NEt_2, Z = H, W = F). This scheme involves the combination of simple solvent-extraction procedures and polarographic examination of these extracts, in some instances following acid hydrolysis. It is not intended to be an absolute method of identification but it is suggested that it can be used as a rapid method for obtaining information complementary to that obtained by other identification techniques. The study has been confined to parent compounds so that it would be directly applicable to the identification of unknown formulations and in overdose cases where a relatively high concentration of the free parent drug still remained in the particular body fluid.

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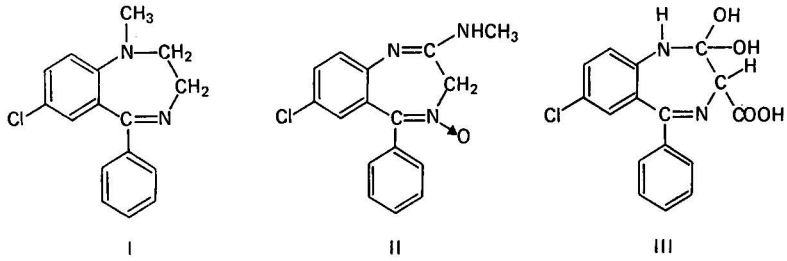
† Present address: Beecham Products, Block F2-F, Brentford, Middlesex.



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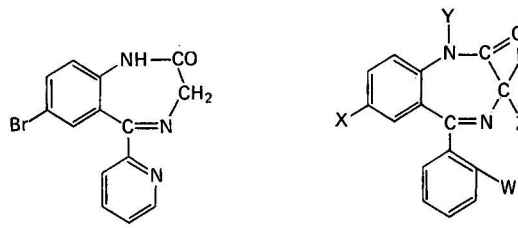


IV V

Experimental

Apparatus

Instrumentation

A PAR Model 174A Polarographic Analyser produced by Princeton Applied Research Corporation, N.J., USA, was used in conjunction with a Servoscribe 15 recorder throughout this study. A three-electrode operation was employed using a platinum counter electrode. The dropping-mercury electrode (D.M.E.) used had an outflow velocity of 2.571 mg s^{-1} and a drop time of 3.46 s at the potential of the calomel electrode and at a mercury pressure of 55 cm in 1 M potassium chloride solution.

Polarographic cells

For investigations on the polarographic behaviour in different buffer solutions, the cell shown in Fig. 1 was used. This was based on a 25-ml Quickfit flask and was suitable for volumes of solution from 2 to 20 ml, the optimum volume being about 5 ml. The platinum-wire counter electrode was sealed into the glass of the vessel, as were the inlet tubes for passing gas into and over the sample solution. A Radiometer-type saturated calomel electrode (S.C.E.) was used as the reference electrode.

As it was intended to carry out and monitor the hydrolysis in the polarographic cell, a

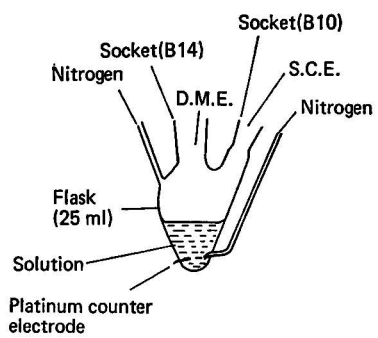


Fig. 1. Polarographic cell.

vessel was required that could be maintained at constant temperature. The cell shown in Fig. 2 was designed and constructed specifically for this purpose. It is based on a 25-ml three-necked, pear-shaped flask that is enclosed in an outer glass jacket. Water at the required temperature could be pumped between the inner and outer vessels. A tap provided at the bottom of the cell allowed for emptying and cleaning purposes. Narrow-bore glass tubes were sealed into the apparatus to pass nitrogen through or over the solution. The cell functioned satisfactorily with solution volumes from 2 to 15 ml, but in practice 5–10 ml was ideal. The reference electrode was again a Radiometer-type saturated calomel electrode. A platinum wire sealed into a glass tube served as the counter electrode. Water was circulated through the jacketed cell from a thermostatically controlled water-bath. A 10-ml volume of aqueous solution in the cell could be brought from room temperature to any temperature in the range 25–45 °C in 10 min and maintained at that temperature within ±0.05 °C. Throughout this study, the hydrolyses were performed at 25 °C. The passage of nitrogen saturated with water vapour either through or over the solution did not alter the temperature.

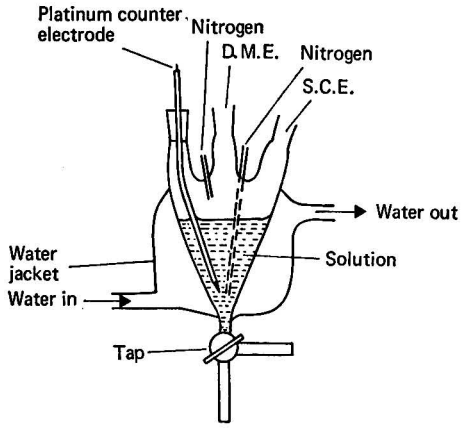


Fig. 2. Polarographic cell with water jacket used for hydrolysis studies.

Reagents

Supporting electrolyte

A modified Britton - Robinson universal buffer was used to provide buffer solutions of pH 4.0 and 12.0. The stock solution of this buffer (pH 1.8) contained 0.04 M acetic acid, orthophosphoric acid and boric acid. The stock solution was adjusted to the required pH by adding 0.2 M sodium hydroxide solution until the desired pH had been reached, as monitored by using a pH meter. All reagents and solvents used were of analytical-reagent grade.

Stock solutions of the benzodiazepines

Stock solutions of concentration 1×10^{-3} M were freshly prepared in AnalaR methanol. The addition of 0.1 ml of solution to 9.9 ml of the buffer solution would produce a working concentration of 1×10^{-5} M.

Experimental Techniques

Polarographic behaviour in pH 4.0 *and* 12.0 *Britton - Robinson buffers*

For each run, 0.1 ml of the stock solution of the benzodiazepine was pipetted into a 10-ml calibrated flask and then diluted to the mark with the appropriate buffer solution. After thorough mixing, the solution was placed in the polarographic cell shown in Fig. 1, with the S.C.E. and the D.M.E. positioned in the cell, and nitrogen was passed through the solution for 10 min in order to remove oxygen from the solution. After de-gassing, nitrogen was

passed over the solution and the polarogram was recorded using the following polarographic conditions: mode, differential-pulse polarography (DPP); initial potential, 0.0 V *versus* S.C.E.; scan rate, 5 mV s^{-1}; chart speed, 3 cm min^{-1}; modulation amplitude, 100 mV; low pass filter, 0.3 s; potential scan range, 3.0 V; scan direction, negative; current range, 2 or 5 μA; drop time, 1 s.

Hydrolysis

The addition of 0.1 ml of the stock solution to 9.9 ml of acid in the thermostatically controlled cell would produce a working concentration of 1×10^{-5} M. The concentration of methanol in the solution was therefore only 1%. The hydrolysis of each compound included in this study was carried out in 0.1 M hydrochloric acid, which was prepared from a Volucon ampoule. The latter solution exhibited an acceptable polarographic background at the current ranges employed.

A 9.9-ml volume of 0.1 M hydrochloric acid was pipetted into the thermostatically controlled cell and the oxygen removed by passing nitrogen through the solution for 10 min. During this time, the contents of the cell attained the required temperature. After de-gassing, nitrogen was passed over the solution and the S.C.E., platinum counter electrode and D.M.E. positioned in the cell. The polarogram of the acid alone was recorded using the polarographic conditions described above for the buffer solution. Subsequently, 0.1 ml of the methanolic stock solution of the benzodiazepine was introduced into the cell by means of an automatic zero pipette. This addition was achieved by partially removing the platinum counter electrode and inserting the pipette through the neck into the cell. Timing was begun when approximately half of the 0.1-ml aliquot had been added. The solutions were thoroughly mixed by passing a stream of nitrogen through the cell for about 1 min. The polarogram of the solution was recorded after a convenient time.

Preliminary experiments indicated that the method of recording the entire polarogram would be suitable for the present investigation. The use of a concentration of electroactive species of 1×10^{-5} M precluded the use of d.c. polarography as the polarograms at this concentration exhibit a certain degree of slope that makes measurement of the limiting current difficult. Also, the half-wave reduction potentials of some of the reactants and products are close (with a difference of less than 100 mV) and so would not be resolved as individual waves when using d.c. polarography. The cathode-ray polarograph could have been employed but again, accurate measurement of the limiting current of two waves close to each other would be difficult. As a result of these considerations, differential-pulse polarography (DPP) was chosen as the means by which to record the polarograms. This technique has the required sensitivity and would allow a compromise between speed of recording and resolution of the polarographic waves. The shape of the peaks, without serrations, also allows for a more convenient way of measuring concentration.

Results and Discussion

Polarographic Behaviour

The peak reduction potentials, E_p, for each of the compounds were measured from consideration of the potential scan rate and the chart speed. Table I shows the E_p values (volts *versus* S.C.E.) obtained in the buffer solutions of pH 4.0 and 12.0.

In pH 4.0 buffer, chlordiazepoxide shows three peaks, at −0.37, −0.73 and −1.17 V, corresponding to the reduction of the =N→O, C=N and N=C moieties, respectively.[15] In pH 2.0 buffer it gives one peak at −1.24V. In pH 4.0 buffer, bromazepam shows two peaks, at −0.40 and −1.12 V (see Fig. 3). The electronegative influence of the 5-pyridyl moiety shifts the reduction of the azomethine C=N group from −0.7–0.80 V, as observed in other benzodiazepines, to −0.40 V. The wave at −1.12 V for bromazepam corresponds to a reduction process involving the 5-pyridyl group.[11] In pH 12.0 buffer a well defined peak was observed at −0.99 V. At low concentrations, the waves at −1.17 and −1.12 V for chlordiazepoxide and bromazepam, respectively, (in pH 4.0 buffer) can be masked by the reduction of the supporting electrolyte or other impurities co-extracted from a body fluid. When in a mixture bromazepam and chlordiazepoxide are best differentiated polaro-

TABLE I

E_p VALUES OF SOME BENZODIAZEPINES IN pH 4.0 AND pH 12.0 BRITTON - ROBINSON BUFFERS

1,4-Benzodiazepine	E_p/V *versus* S.C.E.	
	pH 4.0 buffer	pH 12.0 buffer
Chlordiazepoxide	−0.37, −0.73, −1.17	−1.24
Bromazepam	−0.40, −1.12	−0.99
Nitrazepam	−0.16, −0.78	−0.61, −1.23
Clonazepam	−0.15, −0.73	−0.60, −1.22
Flunitrazepam	−0.16, −0.73	−0.61, −1.20
Oxazepam	−0.76	−1.50
Lorazepam	−0.74	−1.45
Diazepam	−0.74	−1.15
Prazepam	−0.73	−1.22
Flurazepam	−0.72	−1.10
Potassium chlorazepate ..	−0.73	−1.15
Medazepam	−0.82	−1.23

graphically by the peaks observed in pH 12.0 buffer (Fig. 4). The peak at −0.73 V at pH 4.0 for chlordiazepoxide is not used as it is also observed in other benzodiazepines, *e.g.*, see Fig. 5, which shows the differential-pulse polarograms of a mixture of bromazepam, chlordiazepoxide and flunitrazepam in pH 4.0 and 12.0 buffers. Flunitrazepam is included in Figs. 3–5 as an example of a 1,4-benzodiazepine giving a typical C=N reduction in pH 4.0 and 12.0 buffers.

Nitrazepam, clonazepam and flunitrazepam can be differentiated from all other 1,4-benzodiazepines included in this study by the peak observed at −0.16 V in pH 4.0 buffer, corresponding to the reduction of their NO_2 group. At pH 12.0 this reduction is observed at −0.60 to −0.61 V and this peak could also be used for identification.

Figs. 6 and 7 show the differential-pulse polarograms of some other 1,4-benzodiazepines which have only one reducible group, *i.e.*, the 4,5-azomethine group. Oxazepam, lorazepam, diazepam, prazepam and medazepam give well defined peaks in pH 4.0 buffer, but the peak potentials do not differ significantly except for medazepam, which is reduced at a potential of the order of 75 ± 15 mV more negative than the remainder. The peak heights for the first two are approximately twice the size of the other peaks as they are reduced

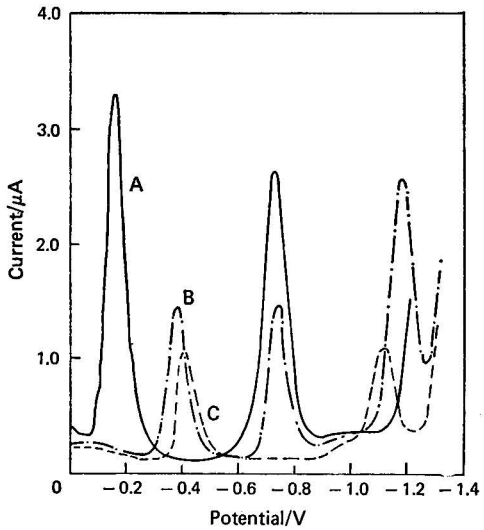


Fig. 3. Differential-pulse polarograms of: A, flunitrazepam; B, chlordiazepoxide; and C, bromazepam, as 1 × 10^{-5} M solutions in pH 4.0 buffer solution.

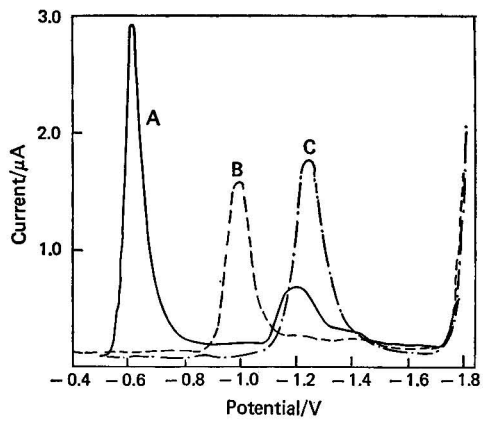


Fig. 4. Differential-pulse polarograms of: A, flunitrazepam; B, bromazepam; and C, chlordiazepoxide, as 1 × 10^{-5} M solutions in pH 12.0 buffer solution.

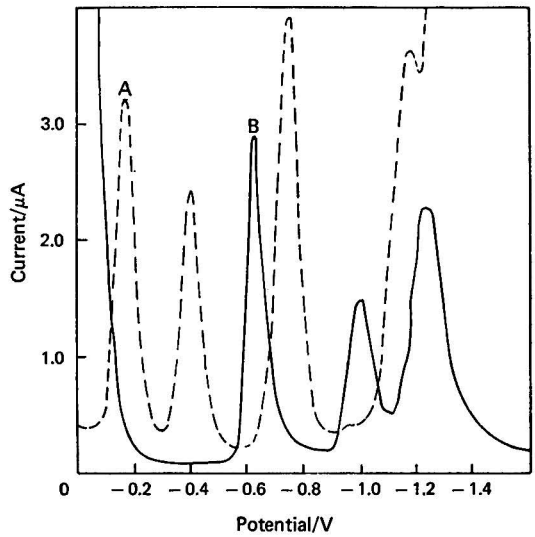
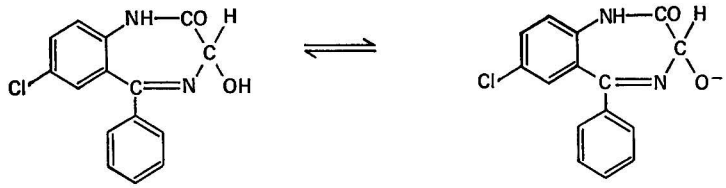


Fig. 5. Differential-pulse polarograms of a mixture of bromazepam, chlordiazepoxide and flunitrazepam as 1×10^{-5} M solutions in: A, pH 4.0 buffer solution; and B, pH 12.0 buffer solution.

in four-electron processes that involve reductive dehydroxylation of the C-3 position.[14] Oxazepam and lorazepam show different polarographic behaviour in pH 12.0 buffer in that they give rise to relatively small broad peaks at substantially more negative potentials than diazepam, prazepam and medazepam. This effect is a polarographic manifestation of the acid - base equilibrium in which species **VI** is reduced at a more negative potential than the neutral molecule, and is also due to repulsion of the anion (**VI**) from the negatively



VI

charged mercury surface. This behaviour can be used to differentiate oxazepam and lorazepam from diazepam, prazepam and medazepam and also to a certain extent from each other. Diazepam can be differentiated from prazepam and medazepam in this buffer as their E_p values differ by 70 mV. Flurazepam and potassium chlorazepate give reduction potentials of −0.72 and −0.73 V in pH 4.0 buffer and −1.10 and −1.15 V in pH 12.0 buffer, but these can be separated prior to polarographic analysis by using their unique acid - base properties, as discussed below under Analytical Applications.

Hydrolysis Studies

The aim of these investigations was to differentiate between members of groups of 1,4-benzodiazepines which it was not possible to resolve by polarography and/or solvent extraction. The three nitro-containing substances are an example of such a group.

In 0.1 M hydrochloric acid at 25 °C, flunitrazepam was found to hydrolyse much faster than either clonazepam or nitrazepam, and has a half-life of about 50 min. Fig. 8(*a*) shows the differential-pulse polarograms obtained for flunitrazepam at 1×10^{-5} M in 0.1 M hydrochloric acid at 25 °C. The times indicated are those that elapsed between the start of the

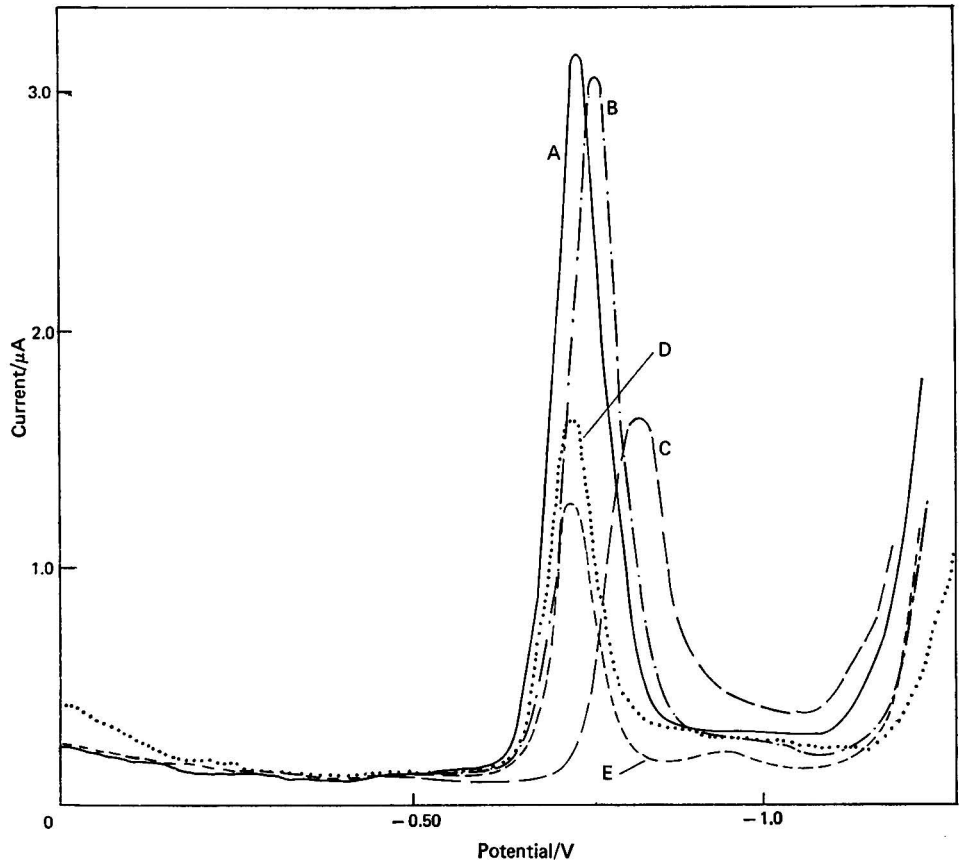
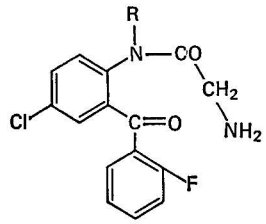


Fig. 6. Differential-pulse polarograms of: A, lorazepam; B, oxazepam; C, medazepam; D, diazepam; and E, prazepam, as 1×10^{-5} M solutions in pH 4.0 buffer solution.

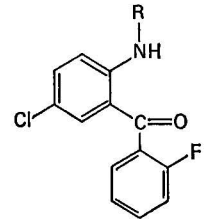
reaction and the start of the recording. Fig. 8(*b*) and (*c*) shows the polarograms obtained for the acid hydrolyses of clonazepam and nitrazepam, respectively. The decrease in the polarographic peak currents caused by the disappearance of the reactants in 0.1 M hydrochloric acid, and the corresponding increase in the reaction products of these three compounds are shown in Fig. 9.

These results indicate that flunitrazepam can be differentiated from the other two nitro-containing benzodiazepines on the basis of its rate of hydrolysis in 0.1 M hydrochloric acid. Clonazepam hydrolysed at a slightly faster rate than nitrazepam (Fig. 9) but this could not be used to differentiate between these two compounds. Provided that no other benzodiazepines were present in the unknown sample, they could be differentiated by their peak potentials in pH 4.0 buffer (Table I).

Groves[18] has studied the mechanism of hydrolysis of 1,4-benzodiazepines in dilute acidic solutions (pH 0–2) and found that such reactions occur relatively rapidly when there is an electron-attracting group in the 5-phenyl ring, *e.g.*, 5-*o*-fluorophenyl-1,4-benzodiazepines. In addition, these reactions are most rapid in the region of the pK_a value corresponding to the azomethine group and fall off at pH $< pK_a$ and pH $> pK_a$. This suggests that the mechanism involves acid catalysis of the non-protonated benzodiazepine and benzophenones of type **VII** are generally formed. In strong acids (pH < 0), benzophenones of type **VIII** are formed and it is these species that are used in identification procedures involving thin-layer chromatography.



VII



VIII

The half-lives ($t_{\frac{1}{2}}$) of these reactions and the corresponding changes in potential are given in Table II for flunitrazepam, clonazepam and nitrazepam. The slower rate observed for clonazepam presumably occurs as a result of the interference of the large chlorine atom in the hydrolytic reactions that involve the azomethine group. This interference could come

TABLE II

HALF-LIVES OF HYDROLYSIS REACTIONS AND CORRESPONDING CHANGES IN POTENTIAL OF FLUNITRAZEPAM, CLONAZEPAM AND NITRAZEPAM

E_p values in volts *versus* S.C.E.

	Medium						
	1.0 M hydrochloric acid			0.1 M hydrochloric acid			
Compound	$t_{\frac{1}{2}}$/s	E_p (product)	E_p (reactant)	$t_{\frac{1}{2}}$/s	E_p (product)	E_p (reactant)	$E_p(NO_2)$
Flunitrazepam ..	237	−0.547	−0.675	25	−0.584	−0.723	−0.069
Clonazepam	1069	−0.520	−0.654	285	−0.562	−0.701	−0.069
Nitrazepam	672	−0.552	−0.665	652	−0.589	−0.755	−0.069

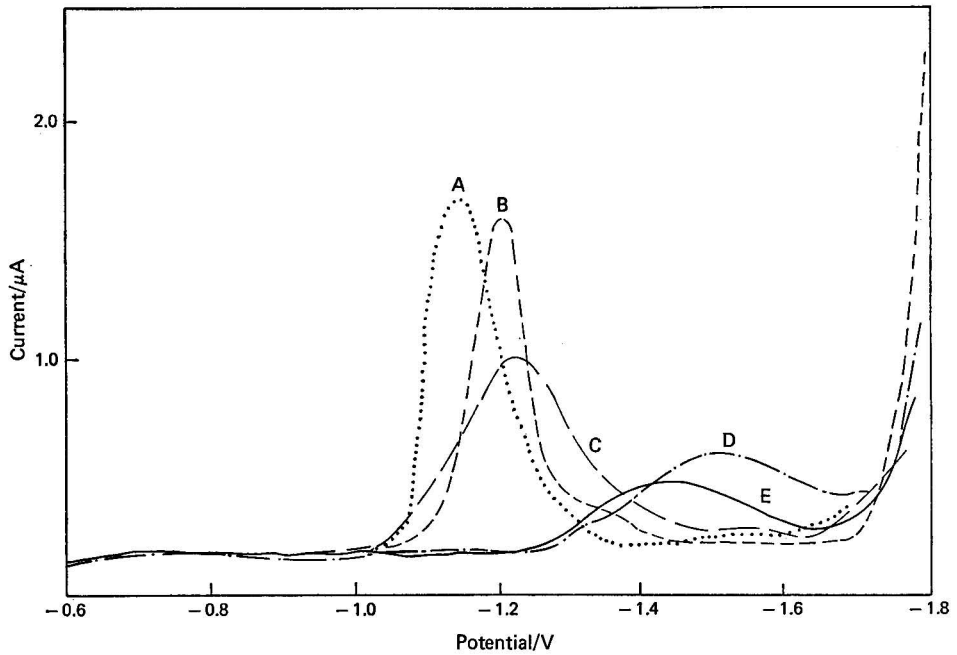


Fig. 7. Differential-pulse polarograms of: A, diazepam; B, prazepam; C, medazepam; D, oxazepam; and E, lorezapam as 1×10^{-5} M solutions in pH 12.0 buffer solution.

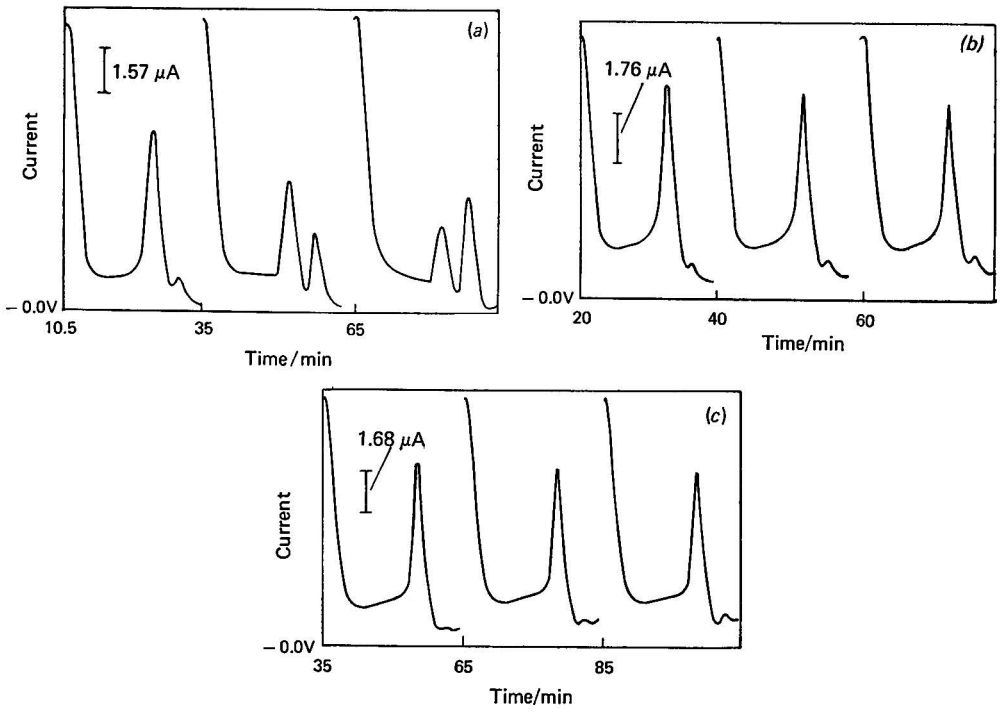


Fig. 8. Differential-pulse polarograms of: (*a*), flunitrazepam; (*b*), clonazepam; and (*c*), nitrazepam, as 1×10^{-5} M solutions in 0.1 M hydrochloric acid at 25 °C.

about by direct shielding of the group by chlorine and/or twisting the phenyl ring at position 5 out of plane with the azomethine group, causing a reduction in conjugative interaction.

These results seem to indicate that the presence of a relatively positively charged nitrogen atom in the azomethine group results in a relatively rapid hydrolysis in dilute acid solutions. Bromazepam, which contains a 5-pyridyl substituent, was found to react about ten times faster than flunitrazepam in 0.1 M hydrochloric acid with a $t_{\frac{1}{2}}$ of 5 min. Its rate of reaction increases with decreasing pH at pH values less than the pK_a value of the azomethine group and Smyth *et al.* have proposed an alternative hydrolytic mechanism in these dilute acid solutions.[11]

Analytical Applications

The results of the polarographic studies on the 1,4-benzodiazepines show that it is possible to identify bromazepam and chlordiazepoxide in an unknown sample if a peak is found in pH 4.0 buffer in the potential range −0.37 to −0.40 V. These two compounds can be differentiated by polarography in pH 12.0 buffer, determination of the rate of acid hydrolysis in 0.1 M hydrochloric acid in which chlordiazepoxide is stable or formation of the copper - bromazepam complex, as described elsewhere. If a peak occurs at approximately −0.16 V, a nitro-containing 1,4-benzodiazepine is suspected. Rapid acid hydrolysis in 0.1 M hydrochloric acid will confirm the presence of flunitrazepam ("rapid" being defined as a marked decrease in the peak current corresponding to the azomethine group occurring within 15 min). Nitrazepam and clonazepam can be differentiated by the peak potentials corresponding to their azomethine reductions (−0.78 and −0.73 V, respectively) in pH 4.0 buffer provided that no other benzodiazepines are present. In such a sample, the azomethine reduction peak (plus the hydroxylammonium reduction peak) will appear abnormally low in relation to the nitro reduction peak (in theory, they should be identical in pH 4.0 buffer, but in practice, the differential-pulse peak corresponding to the nitro reduction is higher than the

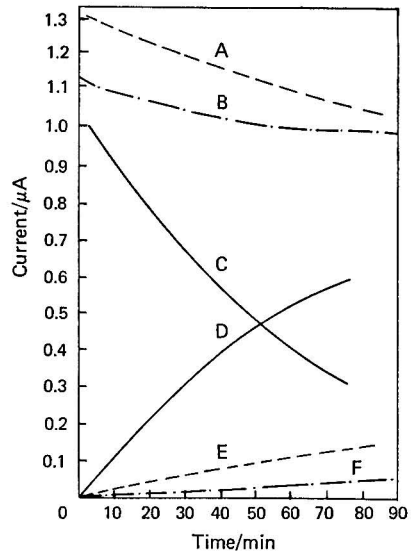
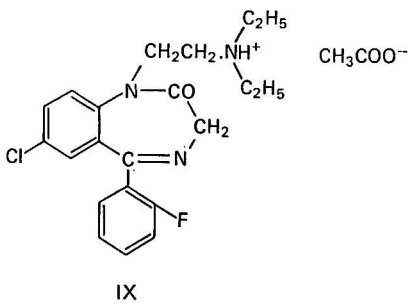


Fig. 9. Hydrolysis of clonazepam, nitrazepam and flunitrazepam as 1×10^{-5} M solutions in 0.1 M hydrochloric acid at 25 °C. A, Clonazepam; B, nitrazepam; C, flunitrazepam; D, reaction product of flunitrazepam; E, reaction product of clonazepam; and F, reaction product of nitrazepam.

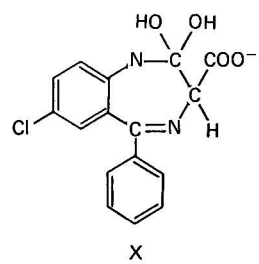
sum of the other two reduction peaks). This effect occurs because differential-pulse polarography is not particularly responsive to the reduction of aromatic hydroxylamines. If only one peak is obtained and it occurs between −0.70 and −0.76 V, the presence of a benzodiazepine with only the electroreducible azomethine group present is suggested. Oxazepam and lorazepam can be differentiated from diazepam, medazepam and prazepam by polarography in pH 12.0 buffer. The same buffer can be used to differentiate oxazepam and lorazepam as their peak potentials differ by 50 mV but, because of the broad nature of these peaks, this difference is of limited value.

Diazepam can be differentiated from medazepam and prazepam in pH 12.0 buffer as it is reduced 70 mV earlier. Medazepam is reduced 90 mV more negatively than prazepam in pH 4.0 buffer.

The acid - base equilibria that exist in aqueous solutions of flurazepam[13,18] and potassium chlorazepate[12] have been the subject of previous publications. Briefly, flurazepam is extractable at neutral and alkaline pH but inextractable at acid pH (of the order of 3.0) owing to the formation of an extractable ion pair (**IX**) in the former pH region. The other



IX



X

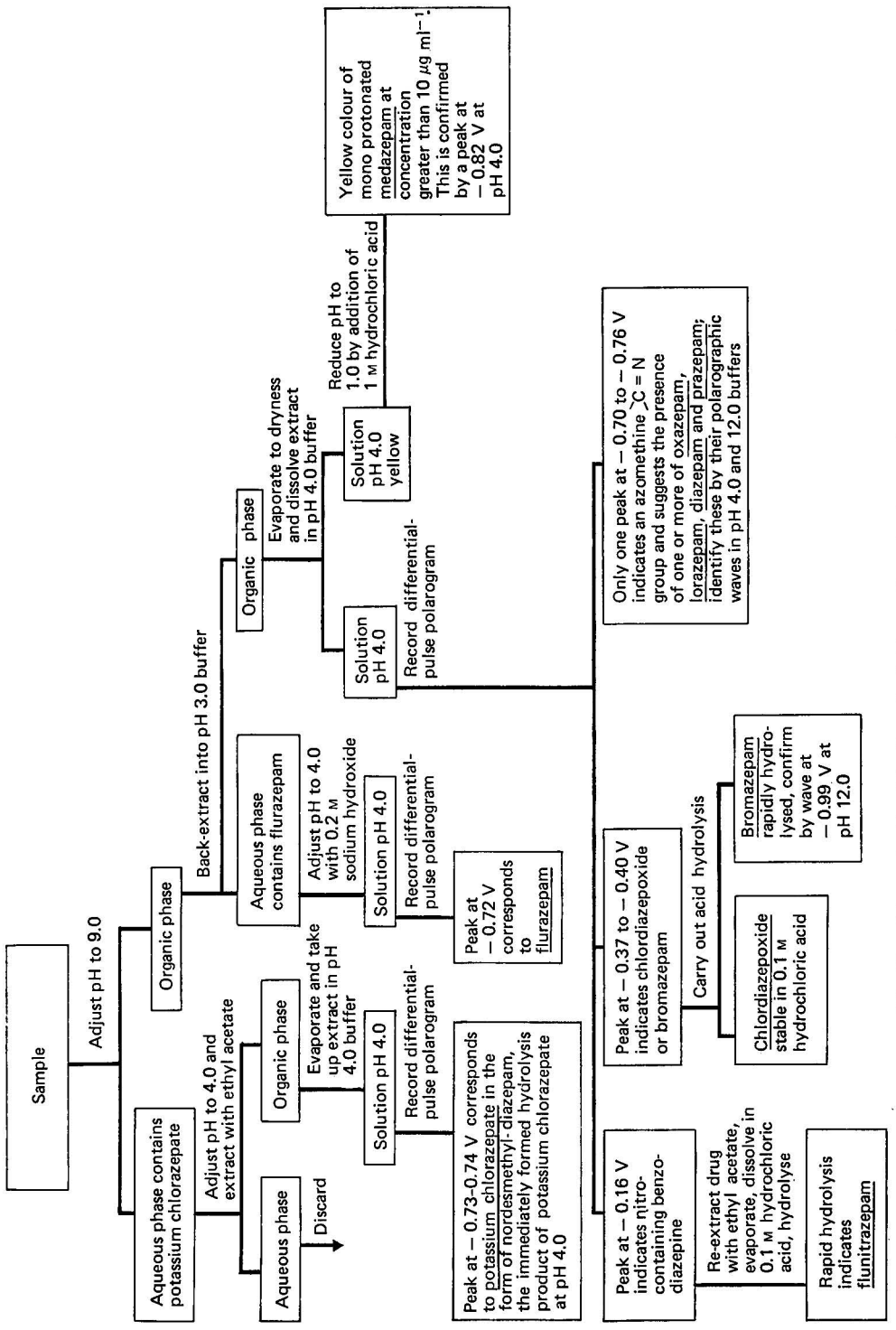


Fig. 10. Scheme for identification of a 1,4-benzodiazepine in an unknown sample.

11 1,4-benzodiazepines are extracted at pH 3.0 with a high efficiency. Potassium chlorazepate is the only 1,4-benzodiazepine of the 12 under study that possesses a carboxylic acid group. Because of the formation of an inextractable species (**X**) in solutions of neutral and alkaline pH this is the only 1,4-benzodiazepine of this group that is not extractable at pH 9.

The scheme shown in Fig. 10 is therefore suggested for the identification of any one of the above-mentioned 1,4-benzodiazepines in an unknown formulation or in body fluids in forensic cases where the parent compound exists in relatively high concentrations compared with its metabolites. It should be possible to identify the drugs at concentrations of 10^{-5}–10^{-7} M. Reproducibility of results can be achieved only by strict control of the polarographic conditions given in the section on Experimental Techniques.

It would be expected that polarograms of all 12 benzodiazepines included here would be recorded under appropriate conditions before an identification is attempted. This procedure would allow for the inevitable differences in peak potentials that occur from one laboratory to the next. However, the relative positions of these peaks would not be affected.

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Analytical Methods Committee

REPORT PREPARED BY THE MEDICINAL ADDITIVES IN ANIMAL FEEDS SUB-COMMITTEE "A"

Spectrophotometric Determination of Ronidazole in Animal Feeds

Keywords: *Ronidazole determination*; *animal feeds*; *spectrophotometry*

The Analytical Methods Committee has received and approved for publication the following Report from its Medicinal Additives in Animal Feeds Sub-Committee "A."

Report

The constitution of the Sub-Committee responsible for the preparation of this Report was: Mr. J. Markland (Chairman), Mr. R. J. Anderson, Mr. A. G. Croft, Mr. C. E. Dodd, Mr. R. Fawcett, Dr. K. Field, Mr. R. S. Hatfull, Mr. G. E. Kitson, Mr. D. H. Mitchell and Mr. J. A. Stubbles, with Mr. P. W. Shallis as Secretary.

Introduction

Ronidazole [(1-methyl-5-nitroimidazol-2-yl)methyl carbamate] is used in poultry feeds for the control and treatment of blackhead; it is also used in pig feeds for the treatment and control of dysentery and as a growth-promoting agent. The normal level of inclusion of the drug in a feed is between 60 and 120 mg kg^{-1}.

Experimental and Results

The most promising method available when the Sub-Committee began its work was that proposed by Szalkowski and Kanora.[1] In this method, the nitro group of the drug is split off by alkaline hydrolysis, which is followed by diazotisation of 4-aminobenzoic acid by the liberated nitrite ion and then coupling with *N*-2-aminoethyl-1-naphthylamine to form a coloured complex. The absorption of the coloured complex is measured at 550 nm in a 10-mm cell.

Preliminary work on the method by some members of the Sub-Committee indicated that it was not specific for ronidazole as other drugs that contain a nitro group, such as dimetridazole and nitrofurazone, interfered. There was also some evidence to suggest that interference was encountered when grass meal or fish meal was present in the sample.

It was realised that a gas - liquid chromatographic method might prove to be the best for the determination of ronidazole. However, in the absence of any suitable method at that time, the Sub-Committee decided to standardise the conditions of the spectrophotometric method. Subsequently, a gas - liquid chromatographic method based on the formation of a volatile silyl derivative of ronidazole in which no interference from the presence of grass meal, fish meal or other drugs in the feed was observed has been proposed by Harris *et al.*[2]

The conditions of the method proposed by Szalkowski and Kanora[1] were examined in detail by members of the Sub-Committee. It was found that in common with most other methods of this type, the batch and grade of aluminium oxide used appeared to have a profound effect on the recovery of the drug and in this instance the type and grade of floridin earth used also had an effect. Acceptable batches and types of both materials are available and it is suggested that the suitability for the purpose of the aluminium oxide and the floridin earth should first be checked by taking a standard through the procedure; a recovery of at least 90% should be obtained.

In their preliminary work, some members had found that recoveries of ronidazole added to animal feeds were good, despite variable and sometimes large feed blank values. The method makes use of liberated nitrite ion to diazotise 4-aminobenzoic acid and therefore any adventitious contamination with nitrite after cleavage of the molecule will give rise to an apparent ronidazole content. As a result of the exploratory work carried out by

members, a few modifications were incorporated into the published method. A collaborative test was then carried out by applying the modified method to the determination of ronidazole added within each laboratory to a circulated sample of feedingstuff at a level of 60 mg kg^{-1}; the results are shown in Table I.

TABLE I

DETERMINATION OF RONIDAZOLE IN AN ANIMAL FEED BY THE RECOMMENDED METHOD

Laboratory	Ronidazole added/ mg kg^{-1}	Ronidazole found/ mg kg^{-1}	Recovery, %	Feed blank/ mg kg^{-1}	Corrected recovery, %
A	60	63.6, 64.2	106.0, 107.0		
	60	52.8, 54.0	88.0, 90.0		
	60	58.8, 57.6	98.0, 96.0		
B	60	56.3	93.8	7.9	80.7
	60	55.1	91.8	7.9	78.7
C	60	51.5	85.8	5.2	77.2
	60	51.6	86.0	2.5	81.8
	60	51.0	85.0	2.5	80.8
D	60	60.5	100.8	4.4	93.5
	60	55.8	93.0	6.4	82.3
	60	57.6	96.0	3.6	90.0
E	60	63.0	105.0		
	60	55.0	91.7		
F	60	69.0	115.0	4.5	107.5
	60	69.0	115.0	4.5	107.5
G	64.8	67.3	103.9	7.0	93.1
	64.8	75.7	116.8	7.0	106.0
H	60	61.0	101.7		
	60	68.0	113.3		
	60	60.0	100.0		
	60	59.0	98.3		
	60	57.0	95.0		

Recommendation

The Sub-Committee recommends that the method given in the Appendix should be used for the determination of ronidazole in animal feeds known to be free from grass meal, fish meal and interfering nitro compounds.

APPENDIX

Recommended Method for the Determination of Ronidazole in Feeds

Scope and Field of Application

The method is for the determination of ronidazole in animal feeds in the absence of grass meal, fish meal and other drugs containing a nitro group that will interfere.

Principle

Ronidazole is extracted from the feed with hot methanol and the extract is cleaned up on a column of aluminium oxide and floridin earth. The extracted drug is hydrolysed with alkali in the presence of copper and the nitrite formed is used to diazotise 4-aminobenzoic acid, which is then coupled with *N*-2-aminoethyl-1-naphthylamine to form a purple complex. The absorbance of the complex is measured at 550 nm.

Reagents

Methanol.

Butan-1-ol.

Sodium chloride.

Aluminium oxide. For chromatography (see Note 1).

Floridin earth. See Note 1.

Copper(II) sulphate solution. Dissolve 2.5 g of anhydrous copper(II) sulphate in sufficient water to produce 100 ml of solution.

Alkaline copper solution. Add 1.0 ml of the copper(II) sulphate solution to 100 ml of a 5% *m/V* aqueous solution of sodium hydroxide and mix thoroughly. Prepare this solution freshly before use.

4-Aminobenzoic acid solution. Dissolve 400 mg of 4-aminobenzoic acid in 250 ml of water, add 100 ml of hydrochloric acid (sp. gr. 1.18) and mix. Dilute to 500 ml with water and mix thoroughly. Cool the solution in an ice - water bath before use.

N-2-aminoethyl-1-naphthylamine reagent solution. Dissolve 50 mg of *N*-2-aminoethyl-1-naphthylamine hydrochloride in 50 ml of water. Prepare this solution freshly each day.

Ronidazole stock solutions. (A). Dissolve 30.0 mg of pure ronidazole in sufficient methanol to produce 100 ml of solution. This solution is stable for 1 month if stored in a tightly stoppered container and protected from light.

$$1 \text{ ml of solution} \equiv 300 \ \mu g \text{ of ronidazole.}$$

(B). Transfer 4.00 ml of solution A into a 100-ml calibrated flask, dilute to the mark with water and mix thoroughly. This solution is stable for 1 week if stored in a tightly stoppered container and protected from light.

$$1 \text{ ml of solution} \equiv 12 \ \mu g \text{ of ronidazole.}$$

Ronidazole working standard solution. Transfer 10.0 ml of solution B into a 100-ml calibrated flask, dilute to the mark with water and mix thoroughly. Prepare this solution freshly each day.

Preparation of Chromatographic Column

Into the bottom of a glass tube about 400 mm long and 11 mm i.d., constricted at its bottom end to a diameter of 4–5 mm, place a small plug of Pyrex glass-wool. Place 3.0 g of aluminium oxide into the column and assist settling by gently tamping it with a glass rod. On top of the aluminium oxide place 2.0 g of the floridin earth and also tamp this lightly with a glass rod.

Procedure

Weigh accurately sufficient of the finely divided sample to contain about 600 μg of ronidazole and transfer it into a 250-ml flat-bottomed flask. Add 100.0 ml of methanol, place a magnetic stirrer bar in the flask and attach the flask to a reflux condenser. Heat, with stirring, on a magnetic-stirrer hot-plate to maintain a gentle reflux for 30 min and then immerse the flask with the condenser still attached in cold water until it reaches room temperature. Transfer the mixture to centrifuge tubes and centrifuge for about 5 min.

Transfer 5.0 ml of the clear extract to the top of the chromatographic column and allow it to drain through under gravity. Collect the eluate in a 50-ml centrifuge tube. Wash the column with three 5-ml portions of methanol and collect the washings in the same tube. Add 0.1 ml of glacial acetic acid, place the tube in a water-bath at 50 °C and evaporate to dryness by passing over the surface of the liquid a stream of compressed air carefully controlled to avoid splashing (see Note 2).

Dissolve the residue in 10.0 ml of chloroform, add 25.0 ml of water, stopper the tube, shake it for 5 min on a mechanical shaker and then centrifuge for about 5 min. Transfer two 10.0-ml portions of the aqueous (top) layer into separate 50-ml centrifuge tubes (A_1 and A_2). To further separate 50-ml centrifuge tubes transfer two 10.0-ml portions of ronidazole working standard solution (B_1 and B_2) and two 10.0-ml portions of water (C_1 and C_2).

To the tubes A_2, B_2 and C_2 add 5.0 ml of water and mix. To the tubes A_1, B_1 and C_1

add 5.0 ml of alkaline copper solution and mix thoroughly. Place all tubes (without stoppers) into a stirred water bath at 80 °C, allow 1–2 min for the tubes and their contents to become warm and then insert the stoppers tightly. After 1 h, remove the tubes from the water-bath, momentarily loosen and replace the stoppers and then immerse the tubes in an ice - water bath until the temperature has been reduced to 3–5 °C (about 5 min). Remove the tubes from the ice - water bath, add 5.0 ml of cold 4-aminobenzoic acid solution to the contents of each tube and set them aside for 2 min. Then add 1.0 ml of *N*-2-amino-ethyl-1-naphthylamine reagent solution to the contents of each tube, mix and set them aside at room temperature for 20 min. Finally, add 5.0 g of sodium chloride and 5.0 ml of butan-1-ol to the contents of each tube, insert the stoppers and shake each tube vigorously for 2–3 min. Allow the layers to separate and with a pipette carefully transfer the butanol layer to a small (about 15-ml) centrifuge tube. Spin the tubes in a centrifuge at about 2 000 rev min^{-1} for 3 min. Measure the absorbances of the clarified butanol solutions from each of the tubes in 10-mm cells against butan-1-ol.

Calculation of Results

Calculate the concentration of ronidazole in the feed sample from the expression

$$\text{Ronidazole/mg kg}^{-1} = \frac{Z \times 100 \times X}{2 \times W \times Y}$$

where

X = corrected absorbance of sample, *i.e.*, $(A_1 - A_2) - (C_1 - C_2)$.
Y = corrected absorbance of standard, *i.e.*, $(B_1 - B_2) - (C_1 - C_2)$.
Z = mass of ronidazole (micrograms) in the standard (usually 12 μg).
W = mass of feed (grams) taken for analysis.

NOTES—

1. Various makes and grades of aluminium oxide, both neutral and basic, and of floridin earth have been found to be satisfactory. Before use a standard should be put through the column, which can be considered to be satisfactory for use if a recovery of at least 90% is obtained.
2. It is essential to remove impurities from the compressed air supply used at the evaporation stage. For this purpose, pass the air through a train of three gas-washing bottles, the first containing a saturated solution of potassium hydroxide, the second concentrated sulphuric acid saturated with chromium(III) oxide and the third Pyrex glass-wool.

References

1. Szalkowski, C. R., and Kanora, J., *J. Ass. Off. Analyt. Chem.*, 1969, **52**, 101.
2. Harris, J. R., Baker, P. G., and Alliston, G., *Analyst*, 1977, **102**, 580.

Analyst, May, 1978, Vol. 103, pp. 513–520

Analytical Methods Committee

REPORT PREPARED BY THE MEDICINAL ADDITIVES IN ANIMAL FEEDS SUB-COMMITTEE "B"

Identification of Prophylactic and Growth-promoting Drugs in Animal Feedingstuffs

Keywords: *Prophylactic drugs*; *growth-promoting drugs*; *feedingstuffs analysis*; *thin-layer chromatography*

The Analytical Methods Committee has received and approved for publication the following Report from its Medicinal Additives in Animal Feeds Sub-Committee "B."

Report

The constitution of the Sub-Committee responsible for the preparation of this Report was: Dr. D. R. Williams (Chairman), Mr. A. G. Croft, Mr. G. Drewery, Mr. R. Fawcett, Dr. K. Field, Mr. R. S. Hatfull, Dr. R. McEwan and Mr. G. H. Smith, with Mr. P. W. Shallis as Secretary.

Introduction

As part of its programme of work, the Sub-Committee is required to recommend methods that can be used to confirm the presence of a declared prophylactic or growth-promoting drug in an animal feedingstuff. Although individual chemical tests for many of the permitted drugs are known, they are not necessarily specific for any one drug. The Sub-Committee decided that, although the primary object of the work was to provide individual tests that could be used to confirm the presence of a declared drug in a feedingstuff, it would be advantageous if all the individual tests could be integrated into a composite scheme. Such a scheme had already been proposed by Hammond and Weston[1] and it was decided to attempt to apply this published scheme to the Sub-Committee's requirements. Not all of the drugs of interest to the Sub-Committee were covered in the published scheme and some preliminary work was carried out to extend the scheme to cover these drugs.

As a result of the collaborative work carried out by the Sub-Committee, it is recommended that the individual tests given in the Appendix should be used, as appropriate, to confirm the presence of a declared drug in a medicated feedingstuff. A procedure for the identification of ethopabate is included but, owing to the very low level at which this drug is incorporated in animal feeds, the procedure can seldom be successfully applied. It is given here only for guidance and will be replaced when a more successful procedure has been developed. In each individual test an indication of the chromatographic fraction in which the drug will be found is given. The named fraction is the one in which it is most likely to appear but experience has shown that on occasions it will appear in fractions other than the expected one.

APPENDIX

Identification of Prophylactic and Growth-promoting Drugs in Animal Feedingstuffs

Scope and Field of Application

The method is for confirming the presence of declared prophylactic and growth-promoting drugs in animal feedingstuffs.

Principle

The feed samples are extracted with methanol or with a mixture of acetonitrile and chloroform. The extract is passed through a column of aluminium oxide and the eluate is collected. Further elutions from the column with different solvents are carried out and the eluted fractions are concentrated and subjected to thin-layer chromatography on silica-gel plates. The drugs are identified from their positions on the plate, their reactions to different spray reagents and by examination of the plate in daylight and in ultraviolet light. A standard is included on every plate for comparison.

Reagents

Aluminium oxide. For chromatography, neutral, 100–250 mesh, Brockman activity 1.

Silica gel G. For thin-layer chromatography.

Chloroform.

Methanol.

Acetonitrile - chloroform (4 + 1). Mix 80 ml of acetonitrile with sufficient chloroform to give 100 ml.

Acetonitrile - chloroform (1 + 1). Mix 20 ml of acetonitrile with 20 ml of chloroform.

Methanol - ammonia solution. Mix 80 ml of methanol with 20 ml of ammonia solution (density 0.88 g ml^{-1}).

Development solvent A. Mix 90 ml of chloroform with 10 ml of methanol.

Development solvent B. Mix 50 ml of ethanol with 50 ml of 1 M hydrochloric acid.

Development solvent C. Mix 80 ml of ethanol with 20 ml of ammonia solution (density 0.88 g ml^{-1}).

Development solvent D. Mix 10 ml of dimethylformamide with 90 ml of chloroform.

1,2-Diaminoethane, anhydrous.

Dragendorff's reagent.

(*i*) *Stock solution.* Add 2.6 g of bismuth triiodide and 7.0 g of sodium iodide to 25 ml of glacial acetic acid and boil for a few minutes. Remove the precipitated sodium acetate on a sintered-glass funnel, add 8 ml of ethyl acetate to 20 ml of the filtrate and store in a dark glass bottle.

(*ii*) *Spray solution.* Mix 10 ml of the stock solution with 25 ml of glacial acetic acid and 60 ml of ethyl acetate. (The detection sensitivity of this reagent is increased by spraying the plate finally with 0.1 N sulphuric acid.)

Ehrlich's reagent. Dissolve 1 g of 4-dimethylaminobenzaldehyde in 30 ml of hydrochloric acid (density 1.18 g ml^{-1}) and add 180 ml of butan-1-ol.

Picryl chloride reagent. Dissolve 1 g of picryl chloride in 100 ml of ethanol.

Phenylhydrazine reagent solution. Dissolve 0.25 g of phenylhydrazine in 25 ml of water and add 25 ml of hydrochloric acid (density 1.18 g ml^{-1}).

Silver nitrate spray reagent. Add 50 mg of silver nitrate to 45 ml of acetone and then add distilled water dropwise until the solid dissolves.

Apparatus

Chromatographic column. Length 300 mm, internal diameter 10 mm, fitted with a poly-tetrafluoroethylene stopcock.

Centrifuge.

Mechanical shaker (or magnetic stirrer).

Thin-layer chromatographic equipment.

Ultraviolet lamp. With 254- and 350-nm tubes.

Procedure

Preparation of column

Insert a small plug of cotton-wool in the bottom of the column and then slowly add 5 g of aluminium oxide, tapping the column gently during the addition. Immediately before use, wash the aluminium oxide with 50 ml of water and allow it to drain. Then wash it with 50 ml of methanol and allow it to drain. Apply gentle suction to the lower end of the column for about 20 min to ensure the complete removal of methanol.

Preparation of plates

Shake 30 g of silica gel G with 60 ml of water for 1–2 min. Use this slurry to coat six 200 × 200 mm plates with a layer 0.25-mm thick; commercially available pre-coated plates can also be used. Activate all plates before use by heating at 100 °C for 1 h.

Preparation of chromatographic tank

Line the tank with filter-paper and equilibrate before use with 100 ml of the appropriate development solvent (A–D).

Extraction and column chromatography

Two different procedures are given below; the one to be used for any given drug will be specified appropriately.

Method A. To 10.0 g of the prepared feedingstuff add 40 ml of acetonitrile - chloroform (4 + 1) and shake or stir for 1 h. Pour the slurry as completely as possible into a 50-ml centrifuge tube and centrifuge at about 2 500 rev min^{-1} for 3 min. Decant the supernatant liquid on to the aluminium oxide column and collect the eluate (fraction 1) in a 50-ml beaker at a rate of about 2 drops per second. Then pass the following through the column successively, for each collecting the eluate in a separate beaker: 5 ml of chloroform (fraction 2), 40 ml of chloroform (fraction 3) and finally 10 ml of methanol (fraction 4). Evaporate fraction 1 to dryness on a steam-bath under a stream of air, avoiding excessive heating, and dissolve the residue in 1 ml of acetonitrile - chloroform (1 + 1). By using a stream of air, reduce the volumes of both fractions 3 and 4 to 1 ml (examination of fraction 2 is unnecessary).

Method B. To 10.0 g of the prepared feedingstuff add 40 ml of methanol, stir or shake for 15 min and then centrifuge at about 3 500 rev min^{-1} for 3 min. Decant the supernatant liquid on to the aluminium oxide column and elute with 25 ml of methanol. Collect the eluate in a 100-ml beaker and evaporate it to a volume of about 1 ml on a steam-bath under a stream of air.

Thin-layer chromatography

Spot on to a prepared thin-layer plate (*i*) 20 µl of the appropriate sample extract, (*ii*) 10 µl of the sample extract, overspotted with 10 µl of standard drug solution, and (*iii*) 20 µl of the standard drug solution. (Note that these volumes are reduced when the presence of nifursol is being investigated.) Develop the plate and render the spots visible as described for the particular drug under investigation.

Interpretation of results

The chromatogram of the sample extract (spot *i*) will, if the drug under investigation is present, have a spot at the same R_F value as, and be similar in colour to, the spot obtained for the standard solution (spot *iii*). The spot obtained for the sample extract overspotted with the standard (spot *ii*) must show no evidence of separation into two components.

Identification of Acinitrazole

Standard solution

Prepare a solution of acinitrazole in methanol containing 1.5 mg ml^{-1}, or of similar concentration to that expected in the sample

Extraction and column chromatography

Carry out by method A. The acinitrazole is expected to be present in fraction 4.

Thin-layer chromatography

Develop the plate with solvent A until the solvent front has moved 150 mm. Remove the plate from the tank, allow to dry in air, examine it in ultraviolet light (254 nm) and note the response. Spray the plate with 1,2-diaminoethane and note the response. Interpret the results as described above.

Identification of Amprolium

Standard solution

Prepare a solution of amprolium in methanol containing 0.6 mg ml^{-1}, or of similar concentration to that expected from the sample.

Extraction and column chromatography

Carry out by method B.

Thin-layer chromatography

Develop the plate with solvent B until the solvent front has moved 150 mm. Remove the plate from the tank, allow it to dry in air, examine it in ultraviolet light (254 nm) and note the response. Spray the plate with Dragendorff's reagent and note the response. Interpret the results as described on p. 515.

Identification of Buquinolate

Standard solution

Prepare a solution of buquinolate in chloroform containing 1.0 mg ml^{-1}, or of similar concentration to that expected from the sample.

Extraction and column chromatography

Carry out by method A. The buquinolate is expected to be in fraction 3.

Thin-layer chromatography

Develop the plate with solvent A until the solvent front has moved 150 mm. Remove the plate from the tank, allow it to dry in air, examine it in ultraviolet light (254 nm) and note the response. Spray the plate with Dragendorff's reagent followed by 0.1 N sulphuric acid and note the response. Interpret the results as described on p. 515.

Identification of Clopidol

Standard solution

Prepare a solution of clopidol in methanol containing 1.25 mg ml^{-1}, or of similar concentration to that expected from the sample.

Extraction and column chromatography

Carry out by method A. The clopidol is expected to be in fraction 4.

Thin-layer chromatography

Develop the plate with solvent A until the solvent front has moved 150 mm. Remove the plate from the tank, allow it to dry in air, examine it in ultraviolet light (254 nm) and note the response. Spray the plate with picryl chloride, expose the plate to ammonia vapour and note the response. The red spot formed, which fades after 2–3 min, indicates the presence of clopidol. Interpret the results as described on p. 515.

Identification of Dinitolmide

Standard solution

Prepare a solution of dinitolmide in acetonitrile - chloroform (1 + 1) containing 1.0 mg ml^{-1}, or of similar concentration to that expected from the sample.

Extraction and column chromatography

Carry out by method A. The dinitolmide is expected to be in fraction 1.

Thin-layer chromatography

Develop the plate with solvent A until the solvent front has moved 150 mm. Remove the plate from the tank, allow it to dry in air, examine it in ultraviolet light (254 nm) and note the response. Spray the plate with 1,2-diaminoethane and note the response. Interpret the results as described on p. 515.

Identification of Ethopabate

Standard solution

Prepare a solution of ethopabate in methanol containing 0.04 mg ml^{-1}, or of similar concentration to that expected from the sample.

Extraction and column chromatography

Carry out by method A. The ethopabate is expected to be in fraction 4.

Thin-layer chromatography

Develop the plate with solvent A until the solvent front has moved 150 mm. Remove the plate from the tank, allow it to dry in air, examine it in ultraviolet light (254 nm) and note the response. Spray the plate with Ehrlich's reagent, heat the plate to 100 °C for 5 min and note the response. Interpret the results as described on p. 515.

Identification of Furazolidone

Standard solution

Prepare a solution of furazolidone in methanol containing 0.6 mg ml^{-1}, or of similar concentration to that expected from the sample.

Extraction and column chromatography

Carry out by method A. The furazolidone is expected to be in fraction 1.

Thin-layer chromatography

Develop the plate with solvent A until the solvent front has moved 150 mm. Remove the plate from the tank, allow it to dry in air, examine it in ultraviolet light (254 nm) and note the response. Spray the plate with 1,2-diaminoethane or with Dragendorff's reagent and note the response. Interpret the results as described on p. 515. (Note that occasionally interference from co-extracted materials hampers the positive identification of furazolidone. If this occurs, it is necessary to repeat the test, but using solvent C to develop the plate.)

Identification of Halquinol

Standard solution

Prepare a solution of halquinol in chloroform at a similar concentration to that expected in the sample.

Extraction and column chromatography

Carry out by method A and collect all four fractions. Then elute the halquinol from the column with 15 ml of 1 M sulphuric acid, collecting the eluate in a separating funnel. Add 15 ml of chloroform, shake the funnel, allow the phases to separate and run off the lower (chloroform) layer. Reduce the volume of the chloroform extract to about 1 ml under a stream of air. Spot this solution on to the prepared thin-layer chromatographic plate.

Thin-layer chromatography

Develop the plate with solvent A until the solvent front has moved 150 mm. Remove the plate from the tank, allow it to dry in air, examine it in ultraviolet light (350 nm) and note the response. Spray the plate with silver nitrate spray reagent and note the response. Interpret the results as described on p. 515.

Identification of Nicarbazin

Nicarbazin is an equimolecular mixture of 1,3-bis(4-nitrophenyl)urea (component 1) and 2-hydroxy-4,6-dimethylpyrimidine (component 2).

Standard solution

Prepare a standard solution of nicarbazin in methanol containing a similar concentration to that expected in the sample.

Extraction and column chromatography

Carry out by method B.

Thin-layer chromatography

Develop the plate with solvent A until the solvent front has moved 150 mm. Remove the plate from the tank, allow it to dry in air, examine it in ultraviolet light (254 nm) and note the response. Component 1 will give a positive reaction with both 1,2-diaminoethane and Dragendorff's reagent. Component 2 will give a positive reaction with Dragendorff's reagent, the colour developing only after several hours provided that the plate is not sprayed with 0.1 N sulphuric acid, and with Ehrlich's reagent, for which a colour will develop on heating the plate at 100–105 °C for 10–15 min. Interpret the results as described on p. 515.

Identification of Nifursol

Standard solution

Prepare a solution of nifursol in acetone containing a similar concentration to that expected in the sample.

Extraction and column chromatography

Carry out by method A and collect all four fractions. Then elute the nifursol from the column with 25 ml of methanol - ammonia solution, collecting the eluate in a 50-ml beaker. Evaporate the eluate just to dryness on a steam-bath and dissolve the residue in 1 ml of acetone.

Thin-layer chromatography

Spot on to the prepared thin-layer chromatographic plate (*i*) 10 μl of the sample extract, (*ii*) 5 μl of the sample extract, overspotted with 5 μl of standard nifursol solution, and (*iii*) 10 μl of standard nifursol solution. Develop the plate with solvent D until the solvent front has moved 150 mm. Remove the plate from the tank, allow it to dry in air, examine it in ultraviolet light (254 nm) and note the response. Spray the plate with phenylhydrazine reagent solution, heat the plate at 100 °C for 5 min and note the response. Interpret the results as described on p. 515. (In daylight, nifursol gives a yellow spot that appears only just above the base line.)

Identification of Nitrofurazone

Standard solution

Prepare a solution of nitrofurazone in methanol containing 0.6 mg ml^{-1}, or of similar concentration to that expected from the sample.

Extraction and column chromatography

Carry out by method A. The nitrofurazone is expected to be in fraction 4.

Thin-layer chromatography

Develop the plate with solvent A until the solvent front has moved 150 mm. Remove the plate from the tank, allow it to dry in air, examine it in ultraviolet light (254 nm) and note the response. Spray the plate with 1,2-diaminoethane and note the response. Interpret the results as described on p. 515.

Identification of Nitrovin

Standard solution

Prepare a solution of nitrovin in methanol containing 0.1 mg ml^{-1}, or of similar concentration to that expected from the sample.

Extraction and column chromatography

Carry out by method A. The nitrovin is expected to be in fraction 4.

Thin-layer chromatography

Develop the plate with solvent A until the solvent front has moved 150 mm. Remove the plate from the tank, allow it to dry in air, examine it in ultraviolet light (254 nm) and note the response. Spray the plate with 1,2-diaminoethane and note the response. Interpret the results as described on p. 515.

Identification of Sulphaquinoxaline

Standard solution

Prepare a solution of sulphaquinoxaline in methanol containing 1.0 mg ml^{-1}, or of similar concentration to that expected from the sample.

Extraction and column chromatography

Carry out by method A. The sulphaquinoxaline is expected to be in fraction 1.

Thin-layer chromatography

Develop the plate with solvent A until the solvent front has moved 150 mm. Remove the plate from the tank, allow it to dry in air, examine it in ultraviolet light (254 nm) and note the response. Spray the plate with Ehrlich's reagent and note the response. Interpret the results as described on p. 515.

Identification of Decoquinate

Standard solution

Prepare a solution of decoquinate in chloroform containing 0.4 mg ml^{-1}, or of similar concentration to that expected from the sample.

Extraction and column chromatography

Carry out by method A. The decoquinate is expected to be in fraction 3.

Thin-layer chromatography

Develop the plate with solvent A until the solvent front has moved 150 mm. Remove the plate from the tank, allow it to dry in air, examine it in ultraviolet light (254 nm) and note the response. Spray the plate with Dragendorff's reagent followed by 0.1 N sulphuric acid and note the response. Interpret the results as described on p. 515.

Identification of Dimetridazole

Standard solution

Prepare a solution of dimetridazole in chloroform containing 1.0 mg ml^{-1}, or of similar concentration to that expected from the sample.

Extraction and column chromatography

Carry out by method A. The dimetridazole is expected to be in fraction 1.

Thin-layer chromatography

Develop the plate with solvent A until the solvent front has moved 150 mm. Remove the plate from the tank, allow it to dry in air, examine it in ultraviolet light (254 nm) and note the response. Spray the plate with either Ehrlich's reagent or Dragendorff's reagent and note the response. Interpret the results as described on p. 515.

Identification of Methyl Benzoquate

Standard solution

Prepare a solution of methyl benzoquate in acetonitrile - chloroform (1 + 1) containing 0.1 mg ml^{-1}, or of similar concentration to that expected from the sample.

Extraction and column chromatography

Carry out by method A. The methyl benzoquate is expected to be in fraction 1.

Thin-layer chromatography

Develop the plate with solvent A until the solvent front has moved 150 mm. Remove the plate from the tank, allow it to dry in air, examine it in ultraviolet light (254 nm) and note the response. Spray the plate with Dragendorff's reagent followed by 0.1 N sulphuric acid and note the response. Interpret the results as described on p. 515.

Identification of Pyrimethamine

Standard solution

Prepare a solution of pyrimethamine in chloroform containing 0.05 mg ml^{-1}, or of similar concentration to that expected from the sample.

Extraction and column chromatography

Carry out by method A. The pyrimethamine is expected to be in fraction 3.

Thin-layer chromatography

Develop the plate with solvent A until the solvent front has moved 150 mm. Remove the plate from the tank, allow it to dry in air, examine it in ultraviolet light (254 nm) and note the response. Spray the plate with Dragendorff's reagent followed by 0.1 N sulphuric acid, and note the response. Interpret the results as described on p. 515.

Identification of Robenidine

Standard solution

Prepare a solution of robenidine in acetonitrile - chloroform (1 + 1) containing 0.3 mg ml^{-1}, or of similar concentration to that expected from the sample.

Extraction and column chromatography

Carry out by method A. The robenidine is expected to be in fraction 1.

Thin-layer chromatography

Develop the plate with solvent A until the solvent front has moved 150 mm. Remove the plate from the tank, allow it to dry in air, examine it in ultraviolet light (254 nm) and note the response. Spray the plate with 1,2-diaminoethane and note the response. Interpret the results as described on p. 515.

Reference

1. Hammond, P. W., and Weston, R. E., *Analyst*, 1969, **94**, 921.

Analyst, May, 1978, Vol. 103, pp. 521–524

Analytical Methods Committee

REPORT PREPARED BY THE IRON SUB-COMMITTEE

General Method for the Determination of Iron with 4,7-Diphenyl-1,10-phenanthroline (Bathophenanthroline)

Keywords: *Iron determination*; *4,7-diphenyl-1,10-phenanthroline*; *bathophenanthroline*; *spectrophotometry*

The Analytical Methods Committee has received and approved for publication the following Report from its Iron Sub-Committee.

Report

The constitution of the Sub-Committee responsible for the preparation of this Report was: Mr. A. G. Hill (Chairman), Professor E. Bishop, Dr. L. E. Coles, Dr. E. J. McLauchlan, Mr. D. W. Meddle, Mr. M. J. Pater, Mr. C. A. Watson and Mr. C. Whalley, with Mr. P. W. Shallis as Secretary.

Introduction

The Iron Sub-Committee of the Analytical Methods Committee has earlier recommended a general method for the determination of iron with 1,10-phenanthroline.[1] The work was carried out at the request of the British Standards Institution, acting on behalf of the International Standards Organisation (ISO), who needed a general method that could be used in all specifications that require the determination of iron content. After considering the available reagents and methods, the Sub-Committee concluded that the use of 1,10-phenanthroline, a reagent already widely used in ISO specifications for the determination of iron, offered adequate sensitivity for the purpose. A standardised version of this method was evaluated and was recommended to ISO for use as a general method.

The Sub-Committee then proceeded to the second phase of its programme, which was to carry out investigations that would lead to the recommendation of the best general method for determining iron. Several reagents are available that will form coloured complexes with iron that can be extracted into an organic solvent, and of these perhaps the best known and most widely used is 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline). This reagent was recommended for the determination of iron by Smith *et al.*[2] and, since its introduction, has been proposed by many different workers for determining iron in a variety of sample materials.

The bright red complex of bathophenanthroline with iron(II), which is formed in the pH range 2–9, has a molar absorptivity of about 22 000, which is approximately twice that of the 1,10-phenanthroline - iron(II) complex in aqueous solution. Moreover, as the iron(II) - bathophenanthroline complex can be extracted into an organic solvent and concentrated, it offers considerable advantages in terms of sensitivity over the use of 1,10-phenanthroline. Interferences in the method are few and the major problems that can be encountered have been summarised previously.[3]

The Sub-Committee therefore decided to investigate the bathophenanthroline method as it was so widely used and recommended. It was realised that other reagents, such as the disodium salt of 4,7-diphenyl-1,10-phenanthrolinedisulphonic acid, FerroZine and 1,3,5-triazine, had been recommended for the determination of iron, but that these were, at best, unlikely to be appreciably better than bathophenanthroline and were in any event much less widely available.

Atomic-absorption spectrophotometry is another technique that it was considered could possibly provide a general method for determining iron, and some work done by the Sub-Committee is the subject of a separate report.[4]

Experimental

The bathophenanthroline method as recommended by Cluley and Newman[3] was selected by the Sub-Committee for investigation, except that ascorbic acid was to be used as the reducing agent as it can be obtained with a lower iron content that can hydroxylammonium chloride. It was suggested to the Sub-Committee that propylene carbonate was a satisfactory alternative solvent to chloroform for the iron(II) - bathophenanthroline complex, and it was decided to investigate this at the same time.

A collaborative test was arranged in which the nine solutions already distributed to the collaborators for use in the work on the 1,10-phenanthroline method[1] were to be used. Each laboratory was asked to carry out at least two determinations of the iron contents of the nine solutions by the method given in Appendix I and also by virtually the same method but with propylene carbonate as the extraction solvent instead of chloroform. As the use of chloroform is now restricted in some laboratories, two of the collaborators also carried out some work by the method given in Appendix I but with 1,1,1-trichloroethane as the extraction solvent.

Results and Discussion

The results of the determination of iron at three different levels in each of the three sample materials are shown in Table I and indicate that extraction of the iron(II) - bathophenanthroline complex into chloroform provides a good general method for the determination of iron. Only one set of results is given in Table I for the work in which the iron(II) - bathophenanthroline complex was to have been extracted into propylene carbonate, as nearly all members found this to be unsatisfactory under the conditions employed. Of the two laboratories that carried out some work in which the coloured complex was extracted into 1,1,1-trichloroethane, one prepared only a calibration graph covering the range 5–60 μg of iron, which was satisfactory and from which it was concluded that the sensitivity when using 1,1,1-trichloroethane was very similar to that when using chloroform. The other

TABLE I

DETERMINATIONS OF IRON IN SOLUTIONS OF TECHNICAL MATERIALS BY THE RECOMMENDED METHOD USING BATHOPHENANTHROLINE AND EXTRACTION INTO CHLOROFORM

		Sample									
		Aluminium sulphate			Ammonium sulphate			Sodium tetraborate			Instru-
Laboratory	Value	A	B	C	A	B	C	A	B	C	ment
1	Mean*/μg ml^{-1}	2.185	3.802	6.708	1.505	3.984	5.418	1.301	2.742	5.678	H700
	R.S.D.,† %	1.80	1.80	0.35	2.50	0.89	0.57	0.48	2.33	1.20	
2	Mean/μg ml^{-1}	2.247	4.008	7.036	1.500	4.102	5.595	1.172	2.925	5.914	SP 1800
	R.S.D., %	1.56	1.50	0.68	0.94	0.40	0.99	0.54	0.43	0.92	
3	Mean/μg ml^{-1}	2.121	3.854	7.004	1.487	3.992	5.456	1.148	2.583	5.712	SP 500
	R.S.D., %	5.17	3.16	4.25	3.57	3.29	4.60	3.70	6.80	3.40	
4	Mean/μg ml^{-1}	2.168	3.790	6.667	1.496	3.907	5.356	1.112	2.745	5.686	SP 1750
	R.S.D., %	0.73	0.33	0.45	2.27	0.09	0.33	1.70	0.65	0.16	
5	Mean/μg ml^{-1}	2.104	3.825	6.712	1.553	3.956	5.502	1.169	2.806	5.698	SP 500
	R.S.D., %	0.41	0.81	0.30	0.74	0.24	0.73	1.50	0.26	0.65	
6	Mean/μg ml^{-1}	2.045	3.834	6.834	1.547	3.939	5.602	1.258	2.769	5.623	DRGT
	R.S.D., %	0.177	2.83	0.53	1.90	0.92	1.12	0.0	0.26	0.65	
7	Mean/μg ml^{-1}	2.258 8	4.052 5	7.183 5	1.554 1	4.138 7	5.590 5	1.197 3	2.788 2	5.907 7	Cary 16
	R.S.D., %	0.127	0.216	0.373	0.237	0.062	0.177	0.188	0.082	0.064	
7‡	Mean/μg ml^{-1}	2.247 4	4.069 2	7.205 0	1.572 4	4.174 7	5.665 9	1.220 0	2.835 3	5.996 3	Cary 16
	R.S.D., %	0.251	0.094	0.132	0.081	0.527	0.113	0.131	0.111	0.296	
Intra-laboratory	Mean R.S.D.,§ %	1.48	1.34	0.88	1.53	0.80	1.08	1.03	1.39	0.87	
Inter-laboratory‖	Mean/μg ml^{-1}	2.172	3.904	6.919	1.524	4.024	5.511	1.197	2.774	5.777	
	R.S.D., %	3.58	3.01	3.14	2.07	2.49	1.69	5.10	3.50	2.41	

* Mean of three separate complete experiments.

† Relative standard deviation expressed as a percentage = 100 × standard deviation/mean.

‡ Extraction into propylene carbonate carried out in parallel with the chloroform method and at the same time. The high photometric accuracy, wide scale, logarithmic amplification and close thermostatting (0.02 °C) of the Cary 16 instrument warrant the additional significant figure for laboratory 7.

§ The average of the relative standard deviations within individual laboratories.

‖ The unweighted mean of all 24 results for each sample, with the over-all R.S.D. as defined in the second footnote. No results have been rejected.

laboratory applied the method with extraction of the coloured complex into 1,1,1-trichloroethane to the determination of iron in the nine circulated solutions; the results are given in Table II.

TABLE II

DETERMINATION OF IRON IN SOLUTIONS OF TECHNICAL MATERIALS BY THE RECOMMENDED METHOD WITH EXTRACTION INTO 1,1,1-TRICHLOROETHANE

The figures in parentheses are the means from Table I.

Iron found/μg ml^{-1}								
Aluminium sulphate			Ammonium sulphate			Sodium tetraborate		
A	B	C	A	B	C	A	B	C
2.29	3.90	7.01	1.66	3.98	5.71	1.16	2.84	5.82
2.12								
2.25								
(2.17)	(3.90)	(6.92)	(1.52)	(4.02)	(5.51)	(1.20)	(2.77)	(5.78)

Recommendation

The Sub-Committee recommends that the method given in Appendix I should be used for the determination of iron when the highest sensitivity is required. It is also recommended that if necessary chloroform can be replaced with confidence by 1,1,1-trichloroethane as the extraction solvent.

APPENDIX

Recommended General Method for the Determination of Iron with Bathophenanthroline

Object

A general spectrophotometric method for determining iron involving the use of 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline) is described.

Principle

Iron(II) forms a bright red complex with bathophenanthroline within the pH range 2–9. Any iron(III) present is reduced to iron(II) with ascorbic acid. The coloured complex is extracted into chloroform (or 1,1,1-trichloroethane) and its absorbance is measured at the maximum at about 533 nm.

Reagents

Ascorbic acid solution, 100 g l^{-1}. Prepare freshly each week.

Acetate buffer solution, 1 M *in acetate* (*see Note* 1). Dissolve 68 g of sodium acetate trihydrate and 28.6 ml of glacial acetic acid in 800 ml of water, adjust the pH to 4.6, if necessary, with sodium hydroxide solution or acetic acid, add 1 ml of ascorbic acid solution and dilute to 1 l with water. Transfer the solution into a separating funnel, add 10 ml of bathophenanthroline solution, mix and set aside for 15 min. Extract by shaking vigorously with 10-ml portions of chloroform successively until the solution is colourless. Discard the extracts, extract with two further 10-ml portions of chloroform and also discard the extracts. Store the solution in an iron-free glass or polyethylene container.

Bathophenanthroline solution, approximately 0.001 M. Dissolve 0.332 g of 4,7-diphenyl-1,10-phenanthroline in 1 l of absolute industrial methylated spirit. Store the solution in an iron-free glass bottle.

Ethanol. Industrial methylated spirit, 74 o.p.

Chloroform (*or* 1,1,1-*trichloroethane*). Analytical-reagent grade.

Perchloric acid, 60% *m/m*.

Ammonia solution, 5 M.

Standard iron solution, 1 mg ml^{-1}. Dissolve 8.65 g of ammonium iron(III) sulphate in 50 ml of concentrated nitric acid and dilute to 1 l with water.

Dilute standard iron solution, 10 μg ml^{-1}. Dilute 10.0 ml of the standard iron solution to 1 l with water; this solution must be freshly prepared.

Procedure

Transfer 10 ml of the sample solution containing between 0.25 and 100 μg of iron (see Note 2) into a 100-ml Squibb's-type separating funnel, add 1 ml of 60% *m/m* perchloric acid and 5 ml of ascorbic acid solution, mix and add 10 ml of bathophenanthroline solution. Set aside for 5 min, then add 2 ml of 5 M ammonia solution and 10 ml of buffer solution, mix and set aside for a further 10 min. Add 10 ml of chloroform (see Note 3), shake the funnel vigorously for 30 s, allow the layers to separate and run the chloroform layer into a 25-ml calibrated flask containing 1 ml of ethanol. Extract similarly with two further 5-ml portions of chloroform, run the extracts into the same 25-ml calibrated flask and dilute to the mark with ethanol. Measure the absorbance of this solution in a 10-mm cell at 533 nm against the reagent blank.

Preparation of Calibration Graph

Transfer appropriate portions of the dilute standard iron solution covering the range 0–100 μg of iron into separate Squibb's-type separating funnels. Dilute the contents of each funnel to 10 ml with water and proceed with each as described for the sample, beginning at ". . . add 1 ml of 60% *m/m* perchloric acid . . ." Construct a graph of iron content, in micrograms, against absorbance.

NOTES—

1. When necessary, the acetate buffer solution can be replaced by a citrate or tartrate buffer solution without further modification to the method.
2. By altering the final volume and also the path length of the spectrophotometer cell used, the method can be applied to other ranges of iron contents, as indicated in Table III. In each instance the figure in line (a) is the amount of iron, in micrograms, that will give an absorbance reading of about 0.004 and that in line (b) is the amount of iron, in micrograms, that will give an absorbance reading of about 1.6.

TABLE III

APPLICATION OF METHOD TO DIFFERENT RANGES OF IRON CONTENTS

		Final volume/ml				
		5	10	25	50	100
Cell path length/mm						
5	(a)	0.1	0.2	0.5	1.0	2.0
	(b)	40	80	200	400	800
10	(a)	0.05	0.1	0.25	0.5	1.0
	(b)	20	40	100	200	400
20	(a)	0.025	0.05	0.13	0.25	0.5
	(b)	10	20	50	100	200
40	(a)	0.013	0.025	0.06	0.13	0.25
	(b)	5	10	25	50	100

3. In all instances where chloroform is referred to in the method, it can be replaced if desired by 1,1,1-trichloroethane.

References

1. Analytical Methods Committee, *Analyst*, 1978, **103**, 391.
2. Smith, G. F., McCurdy, W. H., jun., and Diehl, H., *Analyst*, 1952, **77**, 418.
3. Cluley, H. J., and Newman, E. J., *Analyst*, 1963, **88**, 3.
4. Analytical Methods Committee, *Analyst*, 1978, **103**, in the press.

Book Reviews

LIQUID CHROMATOGRAPHY DETECTORS. By R. P. W. SCOTT. *Journal of Chromatography Library, Volume* 11. Pp. x + 248. Amsterdam, Oxford and New York: Elsevier. Distributed by Elsevier North-Holland in USA and Canada. 1977. Price $34.50; Dfl84.

The text of this volume is divided into four parts, covering the general characteristics of liquid-chromatographic detectors, descriptions of bulk and solute property detectors, and their selection and use. The means of detection covered include those of ultraviolet, fluorimetric, polarographic, heat of adsorption, spray impact, radioactivity, electron-capture, transport, dielectric constant, refractive index, electrical and thermal conductivity, density, interferometer, vapour pressure and gas density bridge. In addition, a complete chapter is devoted to spectroscopic detectors and includes descriptions of liquid chromatography - ultraviolet and liquid chromatography - mass spectrometry systems.

The author discusses the properties of the detectors, their effect on the quality of the chromatographic separations and the precision of the analytical results obtained, descriptions being given for the measurement of these properties. One of the chapters deals solely with practical hints on their operation, while another covers the special techniques of the differential and integral modes of detection and the technique of "vacancy chromatography."

Over the last decade, there have been numerous publications on liquid chromatography in its various forms and at present there seems little lessening of this output. This particular volume has been written by an acknowledged expert in the field and, while some of the content has been covered adequately elsewhere, the book has something extra to offer. For example, there is an interesting description of the spray electrification effect employed as a detector, mainly for use with reversed-phase liquid chromatography, and the use of a rotating gauze disc carrier in a transport system.

The text is clear and easy to follow and understand. The total number of references given is about 120; the index is short but the contents pages are very helpful. Very few specific practical applications are included but the book nevertheless is likely to find its way into many industrial and college libraries.

D. SIMPSON

APPLICATIONS OF ION-SELECTIVE MEMBRANE ELECTRODES IN ORGANIC ANALYSIS. By GEORGE E. BAILESCU and VASILE V. COSOFRET. Pp. xii + 235. Chichester: Ellis Horwood. Distributed by John Wiley in Australia, New Zealand, South-East Asia, Canada, Europe and Africa and by Halsted Press in North and South America and the rest of the world. 1977. Price £16: $30.40.

This is a readable and well organised monograph, consisting of a first part devoted to the main classes and principles of ion-selective electrodes as a base for the main part, over three-quarters of the text being devoted to the complete process of analysis of organic materials, from pre-treatment designed to render the sought species in a detectable form to the actual determination by ion-selective electrodes.

It is devoted, as its title implies, to organic analysis *per se* and is not in any way concerned with determining inorganic ions such as calcium and potassium in body fluids. It is very much concerned with determinations of substances such as glucose, urea, choline and its esters. Ways and means of rendering these in a form detectable by ion-selective electrodes are therefore an important part of the discussion. Thus, taking glucose as an example, attention is given to the enzyme-liberation process of hydrogen peroxide, which can then be determined by the depletion of iodide following its reaction with the peroxide, which is catalysed by molybdate ions. Alongside is discussed the alternative determination of the enzyme-liberated hydrogen peroxide by direct amperometry with a catalytic platinum electrode.

The determination of bound halogens is accompanied by discussions of oxygen flask and combustion train techniques as a means of conversion into ionic forms. More routine determinations are frequently accompanied by discussions of automated methods employing ion-selective electrodes. There is a genuine attempt by the authors to be critical in approach but, of course, in this they are frequently bedevilled by the scantiness of detail of some literature presentations, especially with less common determinations such as that of tetraphenylarsonium ions.

There are some deficiencies in the first part, for example, the range of electrodes depicted on

p. 4 is deceivingly restricted, but otherwise this serves as a good introduction to the newcomer to ion-selective electrode methods. The second part is easily accessible according to different readers' interests. The main chapter headings themselves provide adequate guide-lines but further subdivisions such as heterocyclic aromatic compounds into penicillins, uric acid and vitamins, and hydroxy compounds into alcohols, vicinal glycols and epoxy resins, make for real ease of reference to any part of the text.

All in all, the book invites browsing and its good index and copious references (664 in all) make for further delving, but must the price be so high? J. D. R. THOMAS

ENVIRONMENTAL *N*-NITROSO COMPOUNDS—ANALYSIS AND FORMATION. PROCEEDINGS OF THE FOURTH WORKING CONFERENCE HELD IN TALLINN, ESTONIAN SSR, 1–3 OCTOBER 1975. Edited by E. A. WALKER, P. BOGOVSKI and L. GRICIUTE. *IARC Scientific Publications, Number* 14. Pp. xviii + 512. Lyon: International Agency for Research on Cancer. Distributed by the World Health Organization. Available in the UK through HM Stationery Office. 1976. Price Swfr110; $45.

The International Agency for Research on Cancer conducts a programme of research concentrating particularly on the epidemiology of cancer and the study of potential carcinogens in the human environment. This publication is a record of the papers presented at the fourth biennial conference in 1975, organised by IARC, on the analysis and formation of *N*-nitroso compounds. It describes the advances that had been made at that time in their identification and determination and reports on their formation and occurrence. Studies of this type demand reliable analytical techniques and several papers discuss the use of gas chromatography coupled with chemical ionisation - mass spectroscopy to identify and determine the very low levels of nitrosamines that may be significant. Non-volatile compounds pose another problem and various detection methods coupled with high-performance liquid chromatography have been investigated. Of particular interest to those concerned with the safety of food is an investigation into methods suitable for monitoring food supplies. This demands the use of techniques available in the ordinary routine laboratory. It was concluded that a gas-chromatographic technique using a nitrogen oxide selective detector warranted further investigation, particularly into the catalytic cleavage of nitrosamines.

Much detailed work is reported on the occurrence and formation *in vivo* and *in vitro* of nitrosamines. Of particular interest are investigations undertaken in areas where an abnormally high incidence of oesophageal cancer is recorded. Detailed investigations of the nitrosamine contents of the food intake of typical families in the area were compared with controls. At the time of reporting, no significant differences had been discovered.

This book is a fascinating account of the state of knowledge and the advances that had been made at the time of the meeting at which the papers were presented. Although it records many successes, the list of recommendations prepared by three sub-committees set up at the conference indicates the vast amount of work that is still required in this field in order to establish the formation and the role of these compounds in the study of cancer.

This collection of papers is to be recommended not only to the reader intimately concerned with the subject, but also to those who are more generally concerned with the presence of minor impurities in foodstuffs. P. S. HALL

BIOMEDICAL APPLICATIONS OF IMMOBILIZED ENZYMES AND PROTEINS. Volume 2. Edited by THOMAS MING SWI CHANG. Pp. xx + 359. New York and London: Plenum. 1977. $47.40.

This volume contains, as its first 14 chapters, the applications of immobilised enzymes in the biomedical fields of diagnostics and public health. While some are now well established (*e.g.*, their use in automated and semi-automated analysis and in enzyme linked immunosorbent assay), their application in the diagnosis of bacterial infections, parasitic diseases and in the field of virology are still fascinating and exciting. The future world of the enzyme-based sensor is foretold with chapters on enzyme electrodes (now commercially available) and the thermal enzyme probe. Using column antibody immobilisation technology, a strategy to exploit this to simplify and automate radioimmunoassay is outlined that eliminates pipetting and centrifuging. This first part of the book is completed by suggestions of possible applications of the volatile enzyme product method.

The last eight chapters, under the heading of "Perspectives," are spectacularly novel and exciting with viewpoints from the physiologist, physicist, biochemist and chemist of future applications of immobilized enzyme technology. I found intriguing the concept of mechanosensitive and sound-sensitive enzyme systems as chemical amplifiers of weak signals. The development of multi-step enzyme systems and recycling coenzyme-dependent immobilised enzyme systems is already feasible. Artificial organs and cells are realistic targets of the technology. Already the first nylon-tube enzyme supports reviewed in one of the chapters are on sale under the tradename CATALINKS and can last for 4 000 analyses with negligible activity loss while giving a useful life of 7 000 analyses. The potential of soluble cross-linked enzyme polymers for enzyme therapy mentioned in the book has yet to be fulfilled. However, this book should be read by clinical chemists, biochemists and physiologists and serves notice to instrument manufacturers that immobilised enzymes are no longer research toys but are, and will be, integral parts of modern instruments. Congratulations on an excellent book and one I highly recommend.

S. A. BARKER

NUCLEAR MICROANALYSIS. By VLADO VALKOVIĆ. *Garland Reference Library of Science and Technology, Volume* 10. Pp. xii + 415. New York and London: Garland Publishing. 1977. Price $27.

This book consists of five chapters, as follows: Fundamentals of Radioactivity (107 pp.); Nuclear Reactions (43 pp.); Charged Particle Activation Analysis (70 pp.); Neutron-activation Analysis (50 pp.); and Charged Particle Induced X-ray Emission Spectrometry (100 pp.). Theoretical aspects of the above topics are well described in a readable style. Practical matters are less evenly covered, except in the last chapter. Some applications are included, notably the determination of the elemental composition of fossil fuels. Chemical methods are scarcely mentioned, even in the section on neutron activation.

In summary, this book will be useful for teachers wishing to up-date their knowledge of applied nuclear physics, and for research students interested in non-destructive analytical techniques. Chapter 5 on charged particle induced X-ray emission contains material not readily available elsewhere.

H. J. M. BOWEN

NIOSH MANUAL OF ANALYTICAL METHODS. Second Edition. Volume 1. Part I. NIOSH MONITORING METHODS. Volumes 2 and 3. Part II. STANDARDS COMPLETION PROGRAM VALIDATED METHODS. By DAVID G. TAYLOR. Volume 1, pp. xii + Methods P & CAM 102–262. Volume 2, pp. vi + Methods S1–134. Volume 3, pp. vi + Methods S135–391. Cincinatti, Ohio: U.S. Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health. 1977. Price $8.75, $9.75, $9 ($11, $12, $11.25 outside USA), respectively.

DOCUMENTATION OF THE NIOSH VALIDATION TESTS. By DAVID G. TAYLOR, RICHARD E. KUPEL and JOHN M. BRYANT. Pp. xxx + Methods S1-385. Cincinatti, Ohio: U.S. Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Division of Physical Sciences and Engineering. 1977. Price $15.50 ($19.50 outside USA).

In 1971, when occupational safety and health standards for some 400 chemical substances were adopted by the US Department of Labor, there were few analytical procedures for accurately monitoring the exposure of workers to such substances. Three years later the National Institute for Occupational Safety and Health (NIOSH) published a manual containing 39 analytical methods covering 130 of the toxic substances, and a 3-year programme was undertaken jointly by NIOSH and OSHA to produce properly evaluated methods for the remainder. The result is the current Second Edition of the manual, which now contains full details of 337 sampling and analytical methods for use in industrial hygiene environmental monitoring, covering various elements from aluminium to zirconium, inorganic compounds from arsine to zinc oxide, and organic compounds from acetic anhydride to xylidine. Some of the procedures are useful for measuring toxic constituents in blood, urine or body tissues, but most are intended for use in air exposure monitoring.

Part I (Volume 1) contains 110 methods developed by NIOSH or its contractors, each classified according to its reliability on a five-point scale (recommended, accepted, tentative, operational,

proposed). A box form at the beginning of each gives the basic data in a standard manner, *e.g.*, analyte, matrix, range, precision, classification, date issued, date revised. The method format is also standardised and perhaps best illustrated simply by listing the main headings as they are used: 1, Principle of the Method; 2, Range and Sensitivity; 3, Interferences; 4, Precision and Accuracy; 5, Advantages and Disadvantages of the Method; 6, Apparatus; 7, Reagents; 8, Procedure; 9, Calibration and Standards; 10, Calculations; 11, References.

A new section has been included in the Second Edition; Part II (Volumes 2 and 3) includes 227 methods that were evaluated and validated for accuracy and precision in the joint NIOSH/OSHA Standards Completion Programme during 1974–76. These are class B (accepted methods), but have not been field tested. They follow the same standardised format as those in Part I.

Last but by no means least is a fourth and even more massive tome entitled "Documentation of the NIOSH Validation Tests." This presents an introduction to the programme and the statistical protocol used but, more important for the practising analyst, the data reports. These form the main bulk of the volume and give full detailed technical information to support the validation of 216 of the sampling and analytical methods.

Taken as a whole, the prospect of dealing with 7 kg of printed paper seems daunting, but good indexing and a sensible format make it easy to find one's way through the mass of detailed information. It is certain that no analyst with a serious interest in the determination of contaminants in the working atmosphere would want to be without this compilation. The total price of about £25 is far outweighed by the benefits of having not only 337 assessed methods but also the back-up data on which the assessments of many of them were made. My one very minor criticism is that the volumes are in paperback, probably to keep the costs within bounds, and the quality of the binding does not augur well for the survival intact of what are likely to be well thumbed books.

G. E. PENKETH

THE DETERMINATION OF VINYL CHLORIDE. A PLANT MANUAL. 3rd Edition. Edited by W. THAIN. Pp. vi + 156. London: Chemical Industries Association Limited. 1977. Price £20 (CIA members, £15).

This book presents the considered views of a specialist committee of the Chemical Industries Association on the best analytical methods for the control of vinyl chloride concentrations in and around vinyl chloride monomer and poly(vinyl chloride) manufacturing plants. The methods cover the analysis of spot samples, area monitoring and personal monitoring and a brief account is given of the philosophy of monitoring adopted by UK manufacturers. Each method is complete in itself and contains information that will enable the user to choose the most appropriate method for his application. A loose-leaf format has been used to facilitate updating, but this will only be of value if information is given as to the procedure for notifying and issuing of new or revised methods.

In addition to the methods, the book contains a series of analytical notes containing information on various instruments that have been used in vinyl chloride measurement, useful calibration and sampling techniques and a list of vinyl chloride monomer monitoring services available in the UK.

A considerable amount of effort was expended on method development for monitoring vinyl chloride and the Chemical Industries Association is to be congratulated on bringing together all this information in such a digestable form. The co-operation between various companies to solve common problems is a worthwhile exercise and it is to be hoped that it will continue in the production of manuals for other toxic compounds.

J. CHARLTON

Errata

JANUARY (1978) ISSUE, p. 105. Table I: heading of first column should read "Arsenic, parts per 10^9." Reference 5: the authors should be Van Loon, J. C., Knechtel, J. R., and Pitts, A. E.

Corrected
V. Ph.
19th October 1978

Influence of Ascorbic Acid on the Matrix Interferences Observed During the Carbon Furnace Atomic-absorption Spectrophotometric Determination of Lead in Some Drinking Waters

Nine samples of drinking water taken from a range of locations in England and Scotland have been analysed for lead by using carbon furnace atomic-absorption spectrophotometry. Spiking experiments have been carried out in order to determine the severity of the matrix interference. The suppression of the lead signals ranged from 22 to 84%. No relationship was found to exist between the hardness of a water sample and its suppression effect.

Further spiking experiments carried out in the presence of 1% *m/V* of ascorbic acid showed that the suppression effect of eight of the water samples was reduced to a level of less than 5%. The remaining water sample gave a suppression of 18%. This water was not the hardest examined, nor did it give the highest suppression in the previous experiment.

The natural lead contents of the nine waters were determined both by carbon furnace atomic-absorption spectrophotometry in the presence of ascorbic acid and by a method that involves solvent extraction - flame atomic absorption. Statistical analysis, using a *t*-test, indicated that there was no significant difference (at the 95% confidence level) in the results obtained by using the two techniques.

Keywords: *Lead determination*; *drinking water*; *carbon furnace atomic-absorption spectrophotometry*; *matrix interferences*; *ascorbic acid*

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Analyst, 1978, **103**, 447–451.

Qualification of Estimates for Total Trace Elements in Foodstuffs Using Measurement by Atomic-absorption Spectrophotometry

The qualification of results for small concentrations of elements in foodstuffs implies a knowledge of the accuracy of a method when applied to foodstuffs and an assessment of the variation in results that exists in the application of that method.

An attempt is made to describe the problems inherent in obtaining such qualifications, and to suggest a standard procedure for accomplishing these aims. From data obtained for a particular method, a statistical appreciation will give confidence limits and detection limits that can be applied to subsequent results obtained, depending upon the nature of the exercise involved.

Keywords: *Accuracy and variation of results*; *limit of detection*; *statistical appreciation*; *foodstuffs analysis*; *atomic-absorption spectrophotometry*

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Analyst, 1978, **103**, 452–468.

Analysis of Metals Using a Glow-discharge Source with a Fluorescent Atomic Vapour as Spectral-line Isolator

A compact and rugged spectral-line isolator based on atomic fluorescence from atoms generated by a low-pressure gas discharge has been constructed. The device is bolted to a standard glow-discharge source. A pulsed electrical current generates the atoms and the fluorescence is measured by means of a gated integrator. Several types of metals have been analysed, *e.g.*, steel, cast iron, aluminium and gold. Good precisions and accuracies have been obtained.

Keywords: *Metal analysis*; *atomic-fluorescence spectrometry*

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Analyst, 1978, **103**, 469–474.

Radiochemical Neutron-activation Analysis of Sulphide Ores Using Zinc Diethyldithiocarbamate as Extraction Reagent

A procedure for the analysis of lead sulphide and mixed sulphide ores for silver, arsenic, gold, cadmium, copper, manganese, antimony and zinc was developed with emphasis on the determination of the low gold to silver and arsenic to antimony ratios. Radiochemical neutron-activation analysis was necessary and a solvent-extraction technique has been developed. In the first separation step arsenic(III) chloride was extracted from the ore solution with benzene. The results are compared with the values obtained after separation of arsenic by distillation.

Gold(III), silver(I), copper(II), cadmium(II) and several other trace elements were extracted with zinc diethyldithiocarbamate in chloroform, whereas antimony(V) remained in the aqueous phase. The activities of the samples were counted on a germanium(lithium) well-type detector and compared with those of known volumes of standard solutions. Chemical yields were determined by re-activation.

The combination of conventional arsenic separation and this newly developed diethyldithiocarbamate extraction technique proved to be a very efficient and reliable method for the analysis of sulphide ores.

Keywords: *Sulphide ore analysis*; *neutron-activation analysis*; *radiochemical separation*; *zinc diethyldithiocarbamate*; *gamma-ray spectrometry*

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Analyst, 1978, **103**, 475–481.

Pyrolysis - Mass Spectrometry of Textile Fibres

A procedure for pyrolysis - mass spectrometry is described and the spectra (mass pyrograms) of various textile fibres are presented. The method is compared with infrared spectroscopy for the forensic characterisation of synthetic fibres. Samples of less than 5 μg can be analysed.

Keywords: *Textile fibre characterisation*; *pyrolysis - mass spectrometry*; *infrared spectroscopy*

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Determination of Probenecid in Serum by High-performance Liquid Chromatography

The determination of probenecid in serum samples by using high-performance liquid chromatography is described. The method gives satisfactory results over the normal therapeutic range, namely up to 150 μg ml^{-1} of probenecid in serum, and is not affected by metabolites of the drug. The method does not require derivatisation of the drug, as in gas - liquid chromatographic procedures, and is less subject to interferences than spectrophotometric procedures. It has been used in analysis of several hundred serum samples and has given a satisfactory performance in respect of precision and accuracy.

Keywords: *Probenecid determination*; *serum*; *high-performance liquid chromatography*

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Polarographic Method for the Identification of 1, 4-Benzodiazepines

The polarographic behaviour of 12 therapeutically important 1,4-benzodiazepines in Britton - Robinson universal buffers, pH 4.0 and pH 12.0, has been investigated. Differences in the polarographic peak potentials of these compounds in these media are explained. The rates of hydrolysis of certain benzodiazepines in acidic solution were investigated. Bromazepam and flumitrazepam, both of which possess a strongly electron-withdrawing substituent on the 5-*o*-phenyl group, were found to undergo rapid acid hydrolysis. On the basis of these findings, and taking into account the extraction profile of some of the compounds over a pH range, a scheme is devised for the identification of any one or more of 12 1,4-benzodiazepines. It is suggested that this procedure would be applicable to the analysis of unknown formulations or body fluids in forensic cases where the parent compound exists in relatively high concentrations compared with its metabolites.

Keywords: *1,4-Benzodiazepine identification*; *polarography*

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Spectrophotometric Determination of Ronidazole in Animal Feeds

Report prepared by the Medicinal Additives in Animal Feeds Sub-Committee "A."

Keywords: *Ronidazole determination*; *animal feeds*; *spectrophotometry*

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Identification of Prophylactic and Growth-promoting Drugs in Animal Feedingstuffs

Report prepared by the Medicinal Additives in Animal Feeds Sub-Committee "B."

Keywords: *Prophylactic drugs*; *growth-promoting drugs*; *feedingstuffs analysis*; *thin-layer chromatography*

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General Method for the Determination of Iron with 4,7-Diphenyl-1,10-phenanthroline (Bathophenanthroline)

Report prepared by the Iron Sub-Committee.

Keywords: *Iron determination*; *4,7-diphenyl-1,10-phenathroline*; *bathophenanthroline*; *spectrophotometry*

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