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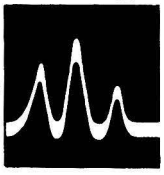

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Simultaneous Determinations in Flow Injection Analysis A Review

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Summary of Contents

Keywords: *Review; simultaneous determinations; flow injection analysis*

Introduction

The concept of simultaneous determinations used in this paper is related to the determination of two or more species in the same sample with a single flow injection analysis (FIA) system.

The need to measure several parameters rapidly in the same sample in areas such as clinical chemistry, environmental pollution and industrial control has urged the development of automated methods of analysis which in both the continuous and discrete modes offer the possibility of carrying out simultaneous determinations. In the discrete mode, the same sample must be present in as many locations as there are parameters that are going to be determined, there being a single detection system. In the continuous mode or segmented flow analysis, the sample is split up into as many channels as there are parameters that are going to be determined. Reaction and detection units exist for each of them.

Although research on FIA has undergone great development in recent years, commercialisation of the method is still limited.[1] One of the reasons for this gap can be attributed to the small number of simultaneous determinations that have been described. We are convinced that a broader development of this area, relatively unexplored in FIA, will stimulate the development of FIA instruments, especially for application in clinical analysis, where the need to know several parameters is very frequent, principally in samples of blood and urine.

In this paper, a critical review of FIA methods that permit the simultaneous determination of several species is presented. The most elementary classification of these methods can be made on the basis of taking the correlation between the detection unit and the species to be determined. Thus, there is one group of methods in which a detector for each species to be analysed exists. Another group consists of methods in which the number of detectors is lower than the number of species to be determined. Figs. 1 and 2 show the most important manifolds used in both types of methods. However, this review has been constructed following the classification of these methods according to whether the conventional methodology is used or whether they are based on differential kinetic analysis, such as that which appears in Table 1, where the main simultaneous determinations developed by FIA are summarised. Table 1 also gives the detection and injection systems and the principle of each of them.

Conventional FIA Methodology

Simultaneous Determinations with Several Detectors

The relative location of the detectors in simultaneous FIA systems permits a sub-classification according to whether their configuration is in series or in parallel. Their characteristics differ considerably.

In series

The most common examples are related to the use of the potentiometric technique [Fig. 1(*a*)]. The use of ion-selective

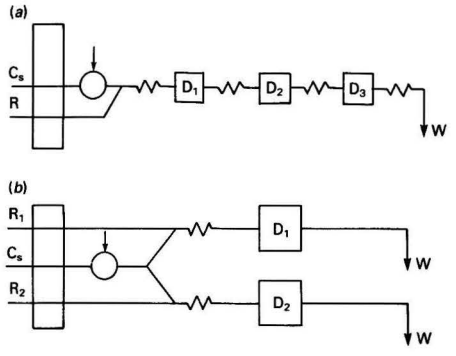

Fig. 1. Schematic diagrams of FIA manifolds for simultaneous determinations with several detectors (D). (*a*) In series configuration; and (*b*) parallel configuration with splitting up of the sample channel. C_s, Sample; R_1 and R_2, reagents; and W, waste

electrodes placed sequentially in an FIA system permits the determination of several species simultaneously. A typical case of this type of determination is that developed by Virtanen[2] for the determination of Na(I), K(I), Ca(II) and Cl^- in serum by the use of ion-selective electrodes in which the flow impinges laterally on both the sensors and the reference, which is located behind them and in the same position. Regression equations that correct for interferences from some species in the determination of others have been established.

The over-all determination of Ca(II) and pH in serum described by Hansen *et al.*,[3] in a paper in which they proposed several FIA methods for the determination of calcium in serum and water, is carried out with the same scheme as in the above-mentioned example [Fig. 1(*a*)], with the difference that the additional channel merges with the main channel in the measurement cell. The system consists of a flow-through capillary electrode, suitable for the measurement of pH in blood, and a calcium-selective electrode, which the flow finally meets after passing through the tubular electrode. The reference electrode, located at the bottom of the calcium cell, receives both the sample flow after impact with the calcium sensor and an additional channel of buffer whose purpose is to dilute the sample so that the composition of the solution that surrounds the reference electrode remains almost constant. The level, which is also constant in the measurement cell, is established with a differential pump. For other simultaneous determinations (nitrate and potassium or nitrate and sodium) with ion-selective electrodes, the same workers utilised a configuration in which the sensors are not in series, but with an angle between them of slightly under 180°.

A special manifold for simultaneous measurements that cannot be carried out in series, but which can be included under this heading, has been applied to the determination of glucose and urea using enzymatic electrodes for both species.[4] The positions of the electrodes in the flow cell are diametrically opposed. Two recorders permit the signal corresponding to each sensor to be obtained. A peculiarity of the system is the absence of injection. The sample is aspirated for 1 s by a pump located behind the detection cell. A subsequent 2-s washing step permits the return to the base line.

Also with differential characteristics, but suitable for inclusion here, is the simultaneous determination of pH, calcium, potassium, chloride, etc., by ion-sensitive field effect transistors (ISFETs), which facilitate the *in vivo* measurements of these species when several ion-selective membranes are located on the different gates of the ISFET.[5] These sensors have still not been sufficiently developed or applied to specific problems.

In parallel

It is possible to make a division based on the manner in which the sample reaches each detector. The most usual division is that where splitting up of the sample bolus occurs in a regular and reproducible manner, each part passing to each detector, or the use of several valves working simultaneously. Less usual, but interesting, is the case in which the two parts of a zone sampling are used: a portion extracted from the original bolus is sent to one detector and the remainder of the same bolus is directed to the other detector. Each of these types is commented on below.

Single injection, with splitting up of the sample. These determinations (with slight modifications) correspond to the scheme in Fig. 1(*b*). A system with photometric and turbidimetric detection has been developed for the determination of Cl^- and SO_4^{2-} by the displacement of the $Hg(SCN)_4^{2-}$ complex in the presence of Fe(III) and the formation of barium sulphate,[6] respectively. It consists of a sampler that works in an 18-s cycle, so that it has a capacity of 200 samples per hour. The almost immediate splitting up of the sample bolus is replaced by the formation of two sub-systems, a channel of the appropriate reagent merging with each of them. The length of the reactor of each sub-system is a function of the intrinsic characteristics of each reaction. To compensate for the decrease in pressure in these sub-systems, tubing of suitable diameter and length is placed after each detector. In this way, a homogeneous division of the sample is obtained.

For the simultaneous determination of up to four species (Na, K, Mg and Ca) by the use of two optical detectors with different characteristics (emission and absorption), Basson and Van Standen[7] designed an FIA system and applied it to surface, ground and domestic waters, with an automatic sampling frequency of 128 h^{-1}. The injection is carried out in an aqueous carrier, with which the reagent [Li(I), La(III), Cs(I), Cl^- and NO_3^- solution] immediately merges. This solution is split up after the mixture coil reaches the corresponding detectors. One of the sub-boluses enters an atomic-absorption spectrophotometer (dual-channel analyser), where Ca and Mg are determined, while the other sub-bolus enters a flame photometer for the measurement of Na and K. Although these workers did not specify that the emission system is a double-channel one, their claim for 500 analyses per hour permits us to conclude that the last detector holds this characteristic, in common with the first one. The lack of carry-over between samples and the advantageous comparison with the automated segmented flow techniques confirm the usefulness of the proposed technique.

A modification of the scheme in Fig. 1(*b*) consists of an additional channel that merges with each of the auxiliaries R_1 and R_2 before their confluence with the main ones, and was used for the simultaneous determination of nitrite and nitrate[8] by the traditional modified Griess reaction (Shinn method). It consists of two almost identical FIA sub-systems, the difference being the inclusion of a reducing column and the aspiration of this waste to mitigate the effect of the compactness of the column on the flow. In the higher sub-system, the nitrite determination is carried out. In the lower one, the existence of the reducing column [cadmium granules coated batch-wise with copper(II) sulphate solution] causes nitrate reduction with an efficiency of 80–90% and permits, after the confluence with the reagent channels, the analysis of the total mixture, the nitrate concentration being obtained by difference.

Simultaneous multi-injection. Simultaneous multi-injection has been utilised by Slanina *et al.*[9] for the determination of three species by the use of a multi-channel spectrophotometer. The determination of chloride, ammonium and nitrate is carried out by utilising a three-injection valve system, the loops of which are simultaneously filled by a single

sample stream. Each valve injects the corresponding sample in an aqueous stream that merges with a reagent suitable for the determination of the species to be analysed in that sub-system. The reactions that serve as a basis for the identification (and counting) of each species are methylene blue formation, displacement of the $Hg(SCN)_4^{2-}$ complex and non-chemical reaction for NH_4^+, Cl^- and NO_3^-, respectively.

Zone sampling. In their first paper on this technique, Reis *et al.*[10] outlined the possibility of its application to the simultaneous determination of species that require different degrees of sample dispersion, and later[11] applied it to the determination of aluminium and iron in plant digests. The spectrophotometric determination of aluminium with Eriochrome Cyanine R by FIA requires a high degree of sample dispersion. In contrast, the determination of iron in acidic samples (nitric acid - perchloric acid) of plant digests is usually performed without sample dilution when an air - acetylene flame is employed. The most appropriate FIA system for this simultaneous determination with minimum consumption of reagents consists of a triple injector - commutator in which the sample is aspirated to fill the corresponding sample loop. When the injector is switched to the injection position the following occur simultaneously: (*a*) the selected volume of sample is inserted into the carrier 1, (*b*) the second sample loop is moved towards the area of movement of the sample and (*c*) the reducing reagent starts to fill the corresponding loop. The injection of the sample gives a sample zone that is sent through the dispersion coil, the second sample loop and the transmission line towards the atomic-absorption spectrophotometer. On switching the injector back to the filling position to start a new cycle, the sample zone passes through the second loop. This simple movement causes the simultaneous introduction of both the sample portion inside it and the selected volume of the reducing reagent into the corresponding carrier stream. The two zones flow together with the Eriochrome Cyanine R.

One of the greatest possibilities for the future of simultaneous determinations by FIA is the use of a single multi-detector, such as an inductively coupled plasma (ICP). The capacity to utilise a spectrometer of this type in conjunction with FIA has been shown by Greenfield.[12]

Simultaneous Determinations with a Single Detector

With sequential injection

The Brazilian research group directed by Bergamin has published a technique for the determination of several species by the use of the merging zones principle and a triple injector - commutator, which they termed "simultaneous determinations," but which correspond more exactly to the characteristics of "sequential determinations," as they do not utilise a single injection or several simultaneous injections to determine different species, but by ingenious modifications of the injector - commutator they succeed in making each injected sample provide the form for measurement of a simple species. Representative examples are commented on below.

For the determination of nitrite and nitrate,[13] the injector is modified in such a manner that one of the loops is used for the reagent injection [sulphanilamide + *N*-(1-naphthyl)-ethylenediammonium chloride]; of the other two injectors, one is normal and the other has a reducing column incorporated, and they are used alternatively. The confluence of the reagent bolus with that of the sample from the normal loop permits the nitrite determination. The subsequent combination of the reagent with the sample bolus from the loop with the reducing column incorporated makes the determination of both species possible. The use of a reducing pre-valve through which the sample passes or not also provides a similar means of determining these species.[14]

The same group utilised a more complicated triple injector - commutator for the sequential determination of nitrogen and phosphorus in plant material.[15] The methods of determination are based on the Berthelot and molybdophosphate reactions, for which the measurement wavelength is 630 nm in both instances. The injector works in two positions. In one of them the sample and reagents for phosphorus determination are placed in the analytical system while the sample and the reagent involved in the nitrogen determination start to fill their corresponding loops. First the sample merges with the molybdate solution and the resulting bolus merges subsequently with the ascorbic acid solution. When the injector is switched on again, a new portion of sample now merges with the alkaline phenol reagent, and this sample plus reagent mixture merges later with the hypochlorite solution. The volumes of sample injected are different for each species to be determined and the volumes of the reagents are also different, depending on the optimum characteristics of each determination.

The non-simultaneous nature of the determination is once again clearly established in the sense that the term "simultaneous" is used in analytical terms meaning measurements of several species in the same sample or in different portions of the same samples but with measurements carried out at the same time. For this reason, although they are included in this section as simultaneous determinations with a single detector, they can be considered as a particular variant of the other types included.

Splitting up of the stream with two cells aligned in the same optical path. This type of simultaneous determination does not fit any of the proposed schemes and corresponds to an ingenious manifold designed by Stewart and Růžička[16] in the early days of the technique for the determination of nitrogen and phosphorus using a single spectrophotometer. It consists of a main channel through which an acidic stream circulates. The sample is injected into this and split into two channels, and the appropriate reagent merges with each of them. The two flow-through cuvettes are identical and aligned in the optical path of the sample beam. A coil inserted immediately after the injection point has the object of avoiding the effect of variation of the injection speed on sample splitting. This occurs at a ratio 4 : 1 (P : N) as the split point of the sample proceeds through a short transmission line (5 cm) into the phosphate branch and through the log (470 cm) phasing coil into the nitrogen line. The role of a coil of 470 cm in the nitrogen sub-system placed immediately after the splitting of the sample is to delay the passage of the sample plug in the nitrogen line until the sample zone in the phosphate line passes the flow cell and clears the optical path below the 1% absorbance level. Therefore, in the common record, the peaks corresponding to the two species do not overlap.

With a pH gradient. This restricted, but interesting, mode of simultaneous determination by conventional FIA, involving a single detector and single injection, is based on the use of a pH carrier that is different to the sample. If the volume of the latter is sufficiently large, when it reaches the detector two zones of different characteristics exist that are close to the interphases with pH values different from that of the central zone of the plug. The characteristics of these regions can be used to determine several species in the same sample. An example of this type is the simultaneous determination of Pb(II) and V(V)[17,18] with 4-(2-pyridylazo)resorcinol (PAR). The complexes of PAR with these cations exist in the following pH ranges: at pH below 3 only the V(V) complex exists, in the pH range 3–9 the V(V) and Pb(II) chelates co-exist, and above pH 9 only the Pb(II) complex is detected. When a PAR solution in NH_4^+ - NH_3 buffer (pH 9.9) is used in an FIA system such as that shown in Fig. 2(*a*), but without an additional confluence channel, and a sample of a mixture of those cations at a sufficiently acidic pH is injected, at the moment at which the sample plug reaches the detector a pH gradient is obtained that allows the existence of plug zones in which only one of the complexes is formed.

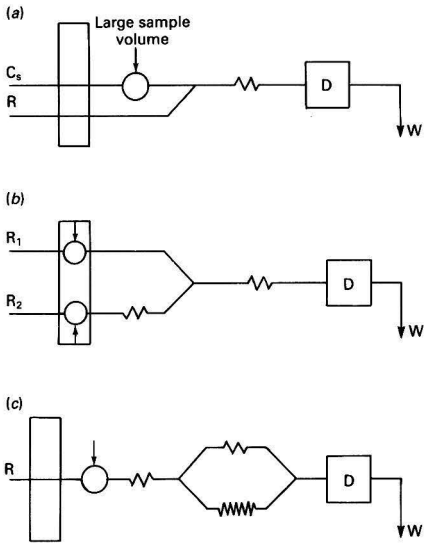

Fig. 2. Schematic diagrams of FIA manifolds for simultaneous determinations with a single detector. Several peaks are obtained for each volume injected. (*a*) Based on the establishment of a concentration gradient; (*b*) with simultaneous double injection and an asymmetric merging configuration; and (*c*) based on the splitting up of the sample channel into two reactors, which merge in front of the detector

A pH gradient can also be obtained by the injection of an acidic or basic solution in a basic or acidic carrier, respectively. The existence of these gradients together with the kinetic dissociation of the PAR - M(II) complexes has been exploited by Betteridge and Fields[19] for the simultaneous determination of Co(II) - Mn(II) and Ni(II) - Cu(II) mixtures in the presence of a similar amount of Co(II). Determinations of Bi, Th and Cu have been carried out with this FIA mode.[20]

With ion-exchange. FIA and ion-exchange association provide the possibility of carrying out simultaneous determinations such as that of zinc and cadmium based on their inhibitory effect on the luminol - hydrogen peroxide chemiluminescence system catalysed by Co(II).[21] The FIA configuration used is that shown in Fig. 1(*a*) with the previous confluence of an auxiliary channel with each of those shown. The four primary channels merge in the following manner: luminol and hydrogen peroxide on the one hand and the catalyst and the sample on the other. The subsequent confluence of the two resulting channels gives rise to the catalysis or inhibition of the monitored reaction. The carrier of the sample is formed by an acidic solution (0.1 M HCl + 0.5 M NaCl) in which the sample and eluent solutions are injected immediately before the anion-exchange column and in a sequential manner in time to prevent overlapping of the eluates. The method is sensitive, as it permits the determination of zinc and cadmium in the ranges 10–100 and 20–200 ng ml^{-1}, respectively. A time of 3 min per sample is required.

FIA Methods Based on Differential Kinetics

One of the advantages of kinetic analysis methods over equilibrium methods is the possibility of carrying out simultaneous determinations based on the different rates of their reactions with a common reagent. In spite of this being a promising aspect, it has several important limitations that restrict its applicability. Firstly, it should be emphasised that it is not easy to find chemical systems in which significantly important differences can be established in the experimental conditions between two or more reaction rates. On the other hand, the differential kinetic methods described do not have a very high level of accuracy and/or reproducibility, because slight disturbances produced by the diversity of samples or by slight changes in the working conditions lead to a low precision in comparison with other manual or kinetic techniques.

FIA is an important alternative in the development of differential kinetic methods, because it imparts technical advantages over manual procedures and also can be applied to chemical systems of reduced half-life (1–10 s). For extra-fast systems (half-life of the order 10^{-3}–10^{-1} s) FIA is not suitable and it is necessary to apply stopped-flow technology, which is very complex and is expensive owing to the high precision required in carrying the sample towards the detector and in the data processing systems.

Few differential kinetic methods have been developed for FIA. For a description of the most significant contributions the systematisation in Table 1 has been adopted, according to the number of detectors that the FIA system contains.

FIA Systems with Two Detectors

This consists of a differential kinetic mode with a simple principle. The signal produced by the reactant bolus is measured at two different times, t_1 and t_2, in each detector. A scheme of two determinations of this type proposed by Jensen and co-workers[22,23] is shown in Fig. 1(*a*). Both are based on the displacement reactions of the complexes (ligand interchange).

The first determination of this type is based on the different dissociation rates of the Mg(II) and Sr(II) complexes with CDTA (*trans*-1,2-diaminocyclohexane *N,N,N′,N′*-tetraacetate):

$$M\text{–}CDTA^{2-} + H^+ \underset{k_M^{CDTA}}{\overset{k_H^{M\text{-}CDTA}}{\rightleftharpoons}} HCDTA^{3-} + M^{2} \qquad (1)$$

the dissociation of the Sr(II) complex being 100 times faster than that of the Mg(II) complex. If Cu(II) exists in the solution, it acts as a scavenger, according to the fast reaction

$$HDCTA^{3-} + Cu^{2+} \xrightarrow{k_{Cu}^{CDTA}} Cu\text{–}CDTA^{2-} + H^+$$

If the concentration of Cu(II) is high enough, the following occurs:

$$k_{Cu}^{CDTA}\,[Cu^{2+}] \gg k_M^{CDTA}\,[M^{2+}]$$

Reaction (1) determines the over-all process rate, which is monitored spectrophotometrically by the absorption ($\lambda_{max.}$ = 320 nm) of the Cu–CDTA complex. The sample, a mixture of Mg(II) and Sr(II) complexes with CDTA in excess, merges with a Cu(II) solution. A short coil takes the reaction mixture to detector 1. By this time, t_1, the dissociation of the complex formed initially has not started, so that the signal is due to the Cu–CDTA complex formed with the free ligand. The total concentration (Mg + Sr) is deduced by difference. The sample is carried by a relatively long reactor and after a time $t = t_2 - t_1$ the signal is measured in detector 2. At this moment, the Mg–CDTA complex is not dissociated, while the strontium complex is 50% dissociated. The Cu–CDTA complex formed is related directly to the Sr(II) concentration and the Mg(II) concentration is obtained by difference. The assembly is similar for the Mg(II) + Ca(II) mixture, using a [2.2.1] cryptand[24] as a ligand and sodium ion as a scavenger.

Table 1. Simultaneous determinations by FIA

	Detection system		Injection system	Principle	References
Conventional FIA methods	With several detectors	In series	Single injection	Several ion-selective electrodes with a single reference electrode	2, 3, 4, 5
		In parallel	Single injection	With splitting up of the flow after the injection	6, 7, 8
			Multi-injection	With a valve for each parameter and multi-channel detector	9
		Single multi-detector	Zone sampling	Collection of part of the injected bolus that is directed to other detector	10, 11
				ICP	12
	With a single detector		Sequential injection	Use of different reagents for different samples according to the parameter to be determined	13, 14, 15
			Single injection	Splitting up of the flow with two flow cells aligned in the same optical path	16
				pH gradient	17, 18, 19, 20
				Ion exchange	21
FIA methods based on differential kinetics	With several detectors	In series	Single injection	Measurements at different times in each detector	22, 23, 25
				Combination conventional FIA and stopped flow	26
	With a single detector		Single injection	Splitting up of the sample with a double path cell	27
				Splitting up the flow in two different reactors and subsequent confluence	28
				Different measurement times in the two bolus - reagent inter-phases	29
			Multi-injection	Double injection of two aliquots of sample that pass through different reactors and merge before the detector	30

FIA Systems with Multi-detection

Hooley and Dessy[25] recently proposed a significant FIA system. In addition to developing a feedback system for the control and regularisation of flow that will certainly solve this critical aspect of FIA, they proposed interesting schemes relating to photometric systems of detection based on LEDs (light emitting diode sources). The paper is orientated towards kinetic determinations based on multiple measurements. The multiple detection system consists of a quartz reactor tube with a series of independent detection units with an LED and a photodetector. Its signal is monitored by an electronic data-processing system. A plug of reactant sample passes successively through each measurement point at different times in such a manner that it is possible to process as many types of kinetic data as there are detectors. The relative locations of these detectors depends on the flow and rate reactions considered. It is an ideal system for kinetic determinations, and very useful for the simple determination of rate constants. These workers proposed the simultaneous determination of Mg(II) and Zn(II) by differential kinetics, using the logarithmic extrapolation method.

FIA Systems with a Single Detector

This is the simplest mode and includes those systems which are classified as "differential kinetics" but which do not truly meet all the requirements for such methods. Basically, a detector provides two different signals, or a signal increase, or both, at two times (or at a time increase) that coincide(s) with different reaction times. In general the method of "proportional equations" can be used for the calculation of the concentration of a species in the mixture starting from the signals obtained and with a knowledge of the corresponding rate constants. When a signal and an increase are used [the FIA-gram peak and stopped-flow interval (delay time)] the method for the calculation is different.

Several different FIA configurations for carrying out these determinations will be considered, making a distinction between systems with a double and single injection.

With single injection

Combination of conventional FIA with stopped flow. Recently, Kagenow and Jensen,[26] studying the application of FIA to the development of differential kinetic methods, carried out a simultaneous determination of Ca(II) and Mg(II) by the stopped-flow technique, based on the same reactions of displacement and formation of coloured complexes as described above. In this instance the Mg - L complex undergoes rapid dissociation whereas the Ca - L complex dissociates more slowly when K^+ is used as a scavenger. The FIA scheme is as shown in Fig. 2(*a*), automated in this instance. The sample contains Ca(II), Mg(II), the same buffer as the carrier and an excess of ligand, [2.2.2] cryptand.[24] In the other channel is a solution of the same buffer that contains the scavenger ion and *o*-cresolphthalein complexone. Both channels merge at a confluence point in a micromixing chamber (*ca.* 3 μl) and, after passing through a short reactor, enter a photometric detector where the magnesium and calcium chelates with the complexone are detected. The former is completely formed when it arrives at the flow cell; the latter is developed during the delay time.

With splitting up of the flow and double path cell. Betteridge and Fields[27] recently proposed a two-point simultaneous kinetic determination of cobalt and nickel based on the different rates of ligand substitution of the citrate complexes of both metals using PAR as a scavenger. The stream containing the citrate complexes merges with another stream containing the scavenger. The stream is then split up into two channels and EDTA, which halts the reaction, is added to the shortest channel. In the other channel a coil measuring about 3 m is heated at 45 °C to complete the reaction. EDTA is also added after heating. Each stream passes through a double-

path cell consisting of a Perspex block with two holes drilled in the light path of a single spectrophotometer. Two peaks are obtained. The application of conventional proportional methods allows the determination of cobalt and nickel in a mixture, with slightly high relative errors. It is interesting that of the interferences from several ions, such as copper, are eliminated by masking with the EDTA after the formation of the Ni - PAR and Co - PAR complexes, which are not dissociated by EDTA.

With splitting up of the flow in two different reactors and their subsequent confluence. A very simple alternative for carrying out differential kinetic methods by FIA is based on the splitting up of the flow after the sample injection, passing the two sub-boluses through two reaction tubes with different geometrical characteristics and subsequent confluence before reaching the detector [Fig. 2(*c*)]. The different geometrical and hydrodynamic properties of the two channels provide different residence times for each of them, and an FIA-gram with two peaks is obtained. The overlap of these peaks depends on the relative lengths of the channels. A manifold has been utilised for the analysis of mixtures of Co(II) and Ni(II) ions, which have different rates of reaction with 2-hydroxybenzaldehyde thiosemicarbazone.[28] The different contributions of the two complexes to the final absorbance in each peak permits the establishment of two equations for the determination of the individual ions.

Different measurement times in the two bolus–reagent interphases. A new possibility for carrying out simultaneous determinations based on differential kinetics by FIA consists in the injection of an unusually large sample volume so that the mixture of the sample plug and reagent carrier is only produced in the two inter-phases. If there is a sufficient time interval between them before reaching the detector [depending on the V_i/V_R ratio (V_i = injection volume and V_R = reactor volume)], two signals or FIA peaks are obtained. This method has been applied to the determination of Co(II) and Ni(II) in mixtures based on the above-mentioned complexation reaction.[29]

FIA configuration with double injection

In 1980, Kagenow and Jensen[30] described an FIA system with a synchronised double injection valve and a single detector for the determination of Mg(II) and Sr(II) ions. The principle of the method means that two boluses of different composition are simultaneously injected and run through tubing of different lengths so that they reach the detector sequentially and give two peaks in the FIA-gram.

The chemical principle of the determination is to use the traditional reactions of ligand displacement, often utilised in differential kinetics. The complexes between [2.2.1] cryptand[24] and alkaline earth metals have different stabilities. In this instance the dissociation constants, K_H^{ML}, of the Mg(II) and Sr(II) complexes are very different, $K_H^{MgL} \gg K_H^{SrL}$. To obtain a sufficiently high reaction rate, a scavenger ion such as K^+ is used, which picks up the ligand at high speed, the reverse reaction being insignificant when an excess of KNO_3 is present in the medium. To monitor the presence of alkaline earth metal ions in the system it is essential to use an additional ligand that forms colour complexes with both in a fast reaction. The FIA scheme with which this study has been carried out is shown in Fig. 2(*b*).

Conclusions

Few simultaneous FIA determinations have so far been described, such methods accounting for only about 8% of papers published on FIA. This is surprising, considering that FIA can easily be adapted to carrying out the determination of several species in a single sample. Undoubtedly when simultaneous analyses of samples of great practical interest (in clinical analysis, contamination, environmental fields, etc.) are carried out, commercial FIA instrumentation will be developed, especially automated apparatus. Such simultaneous determinations are also of value in small control laboratories, where automation is not essential.

In view of the general characteristics of FIA, in our opinion it is not difficult to design devices for simultaneous determinations, in the same way as when the development of segmented continuous flow analysers began. When these systems are developed, FIA will have clear advantages over other automated analytical methods owing to its intrinsic characteristics of rapidity, simplicity, versatility and low cost. Until these objectives have been attained, other automated methods surpass FIA in this respect.

Among all the systems described, it should be emphasised that the methods based on differential kinetics suffer from the difficulties inherent in this methodology and, therefore, their application in routine analyses must be approached with caution.

Similar considerations apply to the methods based on pH gradient and zone sampling, the results of which are hardly influenced by the working conditions, which can lead to problems of reproducibility. In our view, the development of simultaneous routine determinations must be based on the use of several detection units or multi-detectors and by multiple injection, or on simple splitting up of the injected bolus. Other developments have a markedly academic character, which is why their adaptation to the routine analysis of real samples is very difficult.

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Determination of Free Cyanide in Mineral Leachates

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Cyanide leaching is a common process by which metals, *e.g.*, silver and gold, can be extracted from ores and related materials. This paper describes an automatic flow injection system for the determination of free cyanide in samples from leaching processes.

The sample is injected into a carrier stream, which is merged with a flowing reagent stream. The reagent stream contains sodium hydroxide together with a trace of cyanide and these optimise the solution composition for detection by a cyanide ion-selective electrode. A working range of 10–1 000 p.p.m. is covered with a sample rate of 120 h^{-1}. Sample recoveries compared excellently with a titrimetric procedure and a short-term repeatability study on a sample gave a mean value of 306.5 ± 7.4 p.p.m. at the 95% confidence level. The method selectivity, with particular reference to metal - cyanide complexes, is also discussed. The technique is applicable to process control as well as process development.

Keywords: *Free cyanide determination; flow injection analysis; ion-selective electrode; leachate; minerals*

Leaching is a hydrometallurgical process in which some of the components of an ore are selectively dissolved. The suitability of such processes for the treatment of different ores is dependent on the relationship between the extent of dissolution and the reagent consumption.

Cyanide ion is a common leaching agent for the dissolution of silver and gold from ores and related materials and we were faced with a situation in which it would be necessary to analyse a large number of leachates, containing from less than 10 to 1 000 p.p.m. of free cyanide, in order to study the cyanidation process, initially on a laboratory scale and later on a production scale. The main requirements of any analytical method are precision, specificity and speed and we decided that flow injection analysis (FIA), a simple, flexible and rapid technique originally described by Růžička and Hansen[1] and reviewed by Betteridge,[2] provided the ideal solution.

Our first task was to decide what form of detection we would employ and we consulted the literature for methods that would be suitable for conversion into an FIA system. Vogel[3] described a titrimetric procedure with silver nitrate in the presence of ammonia but we discovered that many of the samples contained metal - cyano complexes with lower stability constants than the silver - cyanide complex and thus a true free cyanide concentration could not be obtained. Bark and Higson[4] gave an excellent review on reported methods, which they categorised under two main headings, namely colorimetric or titrimetric, and since their review several other colorimetric procedures have been described.[5–8] Colorimetric reactions have been extensively used in flow injection systems but a closer examination of the above procedures revealed that they are directed towards the determination of trace and ultra-trace amounts of cyanide and are therefore not applicable to the levels of cyanide present in leachates. Colorimetric methods also involve the formation of a cyano complex and would be subject to the same problem encountered with the titrimetric procedure.[3]

The development of the cyanide ion-selective electrode has resulted in the publication of several procedures that either determine cyanide directly[9–14] or use the electrode to indicate a titrimetric end-point.[10,13,15]

Tóth and Pungor[10] reported that the electrode measures only cyanide ions and does not respond to hydrocyanic acid. They carried out a detailed study of metal - cyanide complexes and reported that those with a stability constant lower than that of the silver - dicyanide complex gave a response.

The electrode was therefore ideally suited to our needs as we required to determine cyanide available to complex silver and this should include cyanide from complexes of lower stability. Ion-selective electrodes have been employed as selective detectors in flow injection systems[16–18] and it appeared probable that the cyanide ion-selective electrode incorporated in such a system would satisfy our requirements.

Experimental

Reagents

Cyanide stock standard solution. Dissolve 4.0 g of sodium hydroxide and 2.500 g of KCN in about 700 ml of de-ionised water and dilute to 1 l. The resulting solution contains 1 000 p.p.m. of cyanide and can be standardised by silver nitrate titration.[3] Standard cyanide solutions in the range 10–1 000 p.p.m. were prepared by dilution of this solution.

Ionic strength adjuster. Dissolve 40 g of sodium hydroxide in about 800 ml of de-ionised water, add 200 µl of cyanide stock solution and dilute to 1 l.

Carrier solution. De-ionised water.

Apparatus

A flow diagram of the system is shown in Fig. 1. The reagents were pumped by a Watson Marlow 501S variable speed pump (10 rev min^{-1} full speed) fitted with Tygon blue pump tubes and operated at 8 rev min^{-1} to give a measured flow stream of 3 ml min^{-1}. Transmission tubing and mixing coils were constructed from 0.8 mm i.d. PTFE tubing using the Altex micro-plumbing system (supplied by Anachem Limited). The detector cell has three components. A cyanide ion-selective electrode (Orion Model 90-06) and a single-junction reference

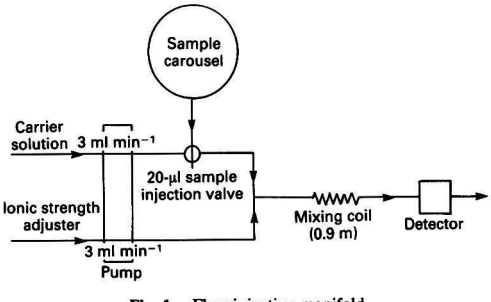

Fig. 1. Flow injection manifold

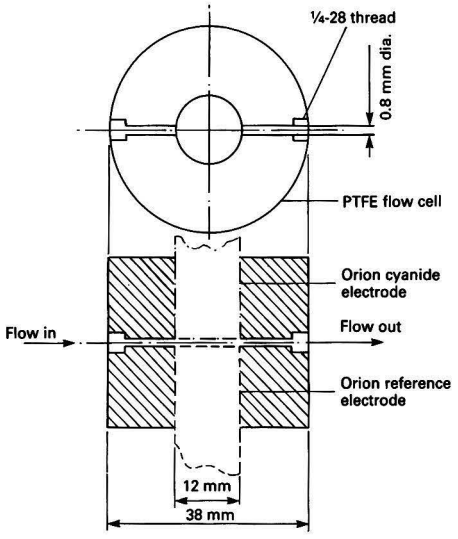

Fig. 2. Detector cell

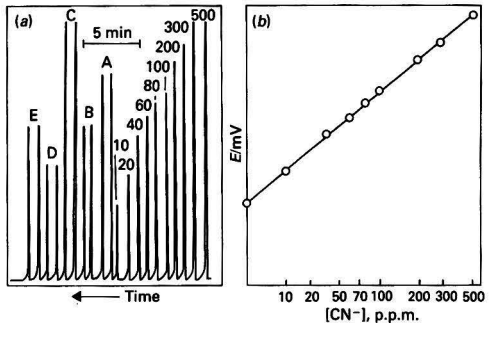

Fig. 3. Typical detector response and corresponding calibration. (*a*) Reading from right to left the first 10 peaks are standard solutions. The cyanide concentration (in p.p.m.) is given by the number above each. (*b*) Calibration graph corresponding to standards described in (*a*)

electrode (Orion Model 90-01) were fitted into our own design of PTFE flow-through holder (Fig. 2). Response of the detector cell was monitored on a chart recorder by way of an Orion Model 801 Ionalyzer millivolt meter. Sample injection was by a coupled autosampler injection valve assembly[19] fitted with a 20-µl sample loop.

Calibration graphs were prepared by processing standard solutions of sodium cyanide. The resulting peak height was plotted *versus* the logarithm of the cyanide concentration and a linear graph was obtained. A working range of 10–1 000 p.p.m. free cyanide was employed using two-decade graph paper and a typical calibration graph is shown in Fig. 3.

The peaks labelled A–E in Fig. 3 represent leachate samples and the computed cyanide concentrations can be seen in Table 1.

Results and Discussion

Table 2 shows results for the determination of free cyanide in leachate solutions by both the flow injection procedure and the titrimetric method of Vogel.[3] The results show excellent

Table 1. Sample results from Fig. 3

Sample	[CN⁻], p.p.m.
A	170
B	48
C	460
D	22
E	50

Table 2. Method comparison

	[CN⁻], % *m/V*	
Solution	Titration	FIA-ISE
1	0.130	0.128
2	0.128	0.128
3	0.105	0.105

Table 3. Comparison of results obtained from two different leaching experiments

	[CN⁻], % *m/V*	
Sample	Exp. A	Exp. B
Feed	0.130	0.129
½h	0.115	0.024
1h	0.120	0.022
2h	0.120	0.015
4h	0.114	0.008
7h	0.116	0.008
24h	0.114	0.008
Wash	0.011	0.004

agreement between the two independent methods. This was possible because, unlike most of the samples to be analysed, the leachates chosen for this comparison did not contain cyanide complexes, which would interfere with the titrimetric procedure.

Table 3 shows results obtained for the analysis of samples from two leaching experiments and it can be seen that in both experiments the leaching process was effectively completed after 4 h. However, the relative amounts of reagent consumed by the experiments differed markedly. The samples listed in Tables 1 and 3 contained metal - cyano complexes and could not be analysed by the titrimetric procedure. The short-term repeatability on a sample solution was as follows: number of determinations (n) = 27; mean cyanide concentration = 306.5 p.p.m.; S_{n-1} (standard deviation) = 3.6 p.p.m.; and repeatability (95% confidence limit) = ±7.4. Precision, speed and specificity were the main features considered to be important for this method and flow injection analysis is ideally suited to such requirements. These systems can employ a wide variety of detectors and this, combined with the facility for separation procedures such as solvent extraction, can lead to a highly specific method. Another outstanding advantage of flow injection analysis is that system constants and timing sequences are maintained within far closer limits than is possible in manual processes, which results in excellent repeatability for a process control procedure. Good recoveries are confirmed by the agreement with the titrimetric procedure (Table 2).

The selectivity of the cyanide ion electrode with respect to its response to cyano species has already been discussed. There are, however, ions other than cyanide that can cause a response from the electrode, and Table 4 lists the maximum allowable concentration of the more common interfering anions. Reference 20 states that sulphide must be absent from the analytical solution and as the majority of materials leached by cyanide are sulphide based, this would seem a major problem. However, during the cyanidation process oxygen is used for the dissolution of precious metals and under these conditions sulphide components are oxidised to thiosulphate, which does not interfere.

Table 4. Common interferents and tolerable levels for the cyanide ion-selective electrode

Interferent	Maximum ratio	Maximum tolerable level in 10^{-5} M CN^- (0.26 p.p.m.)/M
Cl^-	10^6	10
Br^-	5×10^3	5×10^{-2}
I^-	0.1	1×10^{-6}
S^{2-}	Must be absent	Must be absent

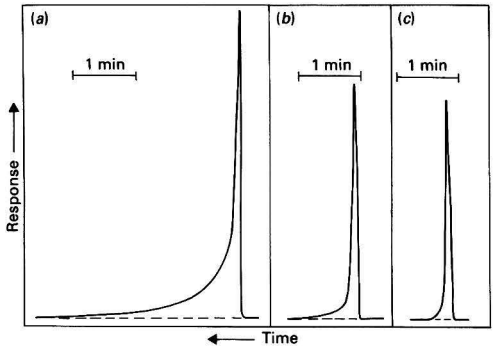

Fig. 4. Effect on sample rate of the addition of trace amounts of cyanide to the reagent stream. (*a*) No cyanide; (*b*) 0.2 p.p.m.; and (*c*) 0.4 p.p.m.

The conventional procedure for the determination of cyanide by an ion-selective electrode involves the immersion of the indicator and reference electrodes in a stirred pH buffered solution containing a known aliquot of sample. The experimental solution contains sodium hydroxide, which maintains the pH above 10.5, ensuring that all uncomplexed cyanide is in the ionic form, and provides a constant background ionic strength and, therefore, a constant cyanide activity coefficient, allowing the e.m.f. to be equated to concentration. These conditions are also necessary in the flow injection system, hence the ionic strength adjuster stream in the manifold assembly (Fig. 1). However, initial experiments using only sodium hydroxide in this reagent revealed that it took 3 min to return to the base line after sample injection, *i.e.*, an effective sample rate of 20 h^{-1}. We suspected that the electrode was exhibiting a memory effect and a simple static test confirmed this. Memory effects occur on transferring from a high to a very low or zero concentration of analyte. We decided that we could reduce this effect by dosing the ionic strength adjuster solution with a trace amount of analyte ion. The results of these additions are illustrated in Fig. 4 and the addition of 0.4 p.p.m. of cyanide to the reagent reduced the base line return time to 30 s, thus raising the sample rate from 20 to 120 h^{-1}.

The flow injection system has several other advantages over conventional procedures. For example, the system is extensively automated, very robust and reliable, and as a result requires minimum operator skill and attention. Also, from the time the sample is removed from the sample cup until it is taken to waste it is transported around a totally enclosed system. This greatly reduces the risk of sample contamination and virtually eliminates the possibility of the accidental release of HCN gas to the atmosphere.

Conclusion

The proposed method is rapid, specific and precise and has been demonstrated to be suitable for the analysis of free cyanide in leachates over a working range of 10–1 000 p.p.m. The system can be applied to the analysis of samples from laboratory or pilot plant evaluations or as a process control method.

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Automatic Methods for the Determination of Total Inorganic Iodine and Free Iodide in Waters

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Methods for the determination of total inorganic iodine and free iodide, based on the catalytic effect of iodide on the destruction of the thiocyanate ion by the nitrite ion, have been developed and automated. These methods use a Technicon AutoAnalyzer system, and a throughput of 20 samples per hour was achieved. Results obtained for a range of United Kingdom drinking waters showed that the total inorganic iodine method had a coefficient of variation of the order of 3%, a detection limit of 0.2 μg l^{-1} of I and a recovery of added iodine of 90–108%. The free iodide method had a coefficient of variation of the order of 10%, a detection limit of 0.4 μg l^{-1} of I and a recovery of added iodine of 89–109%. The effects of possible interfering substances on both methods have been investigated and shown to be negligible for normal drinking waters. The stability of dilute iodine solutions stored in containers made of different materials has also been evaluated.

Keywords: *Automatic methods; total inorganic iodine determination; iodide determination; water analysis*

A project to develop and test methods for the determination of iodide species in fresh and potable waters was undertaken at the Laboratory of the Government Chemist for the Standing Committee of Analysts under a contract with the Department of the Environment.

A survey of the literature revealed two methods with a detection limit of the order of 1 μg l^{-1}. These methods were those of Dubravčič,[1] which utilises the catalytic effect of iodide on the reduction of cerium(IV) sulphate by arsenious acid, and of Sveikina,[2] in which iodide catalyses the destruction of the thiocyanate ion by the nitrite ion. Proskuryakova *et al.*[3] have compared the two methods and concluded that the Sveikina method has marginally better sensitivity and precision.

Experimental

The colorimetric finish of the Sveikina method, in which iodide catalyses the destruction of the orange iron(III) thiocyanate by the nitrite ion has been previously automated in this Laboratory by Moxon and Dixon[4] using a Technicon AutoAnalyzer system. The final flow diagram used for this paper is a modification of this and is shown in Fig. 1. The reaction can be represented by

$$2CNS^- + 3NO_2^- + 3NO_3^- + 2H^+ \rightarrow 2CN^- + 2SO_4^{2-} + 6NO + H_2O$$

Using the range expansion setting of ×4, a suitable calibration graph for the range 0–5 μg l^{-1} of I was obtained. When series of drinking waters were run, the peaks were found to be irregular and erratic. It was found that there was a relationship between the hardness of the waters and the peak irregularity, and this was removed when the alkaline earth metals were precipitated out with potassium carbonate. Subsequently, samples and standards were made up in 0.3% potassium carbonate solution. This addition of alkali prolonged the stability of the standard solutions from 6 h to 8 d, but led to a decrease in the sensitivity of the method. However, extra sensitivity was obtained by doubling the concentration of nitric acid in the ammonium iron(III) sulphate reagent and halving the concentration of potassium thiocyanate reagent. Under these new conditions, regular and precise peaks were obtained for both standard and sample solutions. When the effects of interfering ions were examined, it was found that chloride at a level of 400 mg l^{-1} gave a 20% increase in the response to a 4 μg l^{-1} of I standard solution. To overcome this interference, a large excess of chloride in the form of sodium chloride solution was introduced into the sample stream and it was found that as well as removing the interference effect of chloride, the sensitivity of the method was greatly increased. This enhancement of the catalytic effect of iodide by chloride has also been noted by Dubravčič,[1] who also offered a theory as to its mode of action.

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Determination of Total Inorganic Iodine and Free Iodide

A considerable proportion of the total inorganic iodine present in water can consist of iodate. System A, described in Fig. 1, recovered iodate quantitatively and gave a measure of the total inorganic iodine in solution. In order to determine free iodide only, the oxidation - reduction potential of the reaction mixture was adjusted so that iodate was not reduced to iodine or iodide. This was achieved by (a) reducing the concentration of nitric acid in the ammonium iron(III) sulphate reagent, (b) reducing the concentration of the sodium chloride reagent and (c) reducing the concentration of the potassium thiocyanate reagent. These changes caused a corresponding decrease in sensitivity. The final manifold system shown in Fig. 1 has two different sets of reagents. Set A was used for the determination of total inorganic iodine in water over the range 0.2–5.0 μg l^{-1} of I and set B was used for the determination of free iodide in water over the range 0.4–5.0 μg l^{-1} of I. A comparison of the concentrations of free iodide and total inorganic iodine in a range of drinking waters determined by the proposed methods is shown in Table 1.

Table 1. Amounts of free iodide and total inorganic iodine in a range of United Kingdom drinking waters

Sample source	Total iodine/μg l^{-1}	Free iodide/μg l^{-1}
London (borehole)	28.0	28.0
Harrogate	2.2	0.9
Fife	7.8	7.8
Bristol	4.1	2.1
Nottingham	14	3.5
Amesbury	4.3	0.9
Oxford	4.3	1.3
Gloucester	3.2	3.2
Dunoon	1.1	0.9
Nuneaton	2.8	1.6

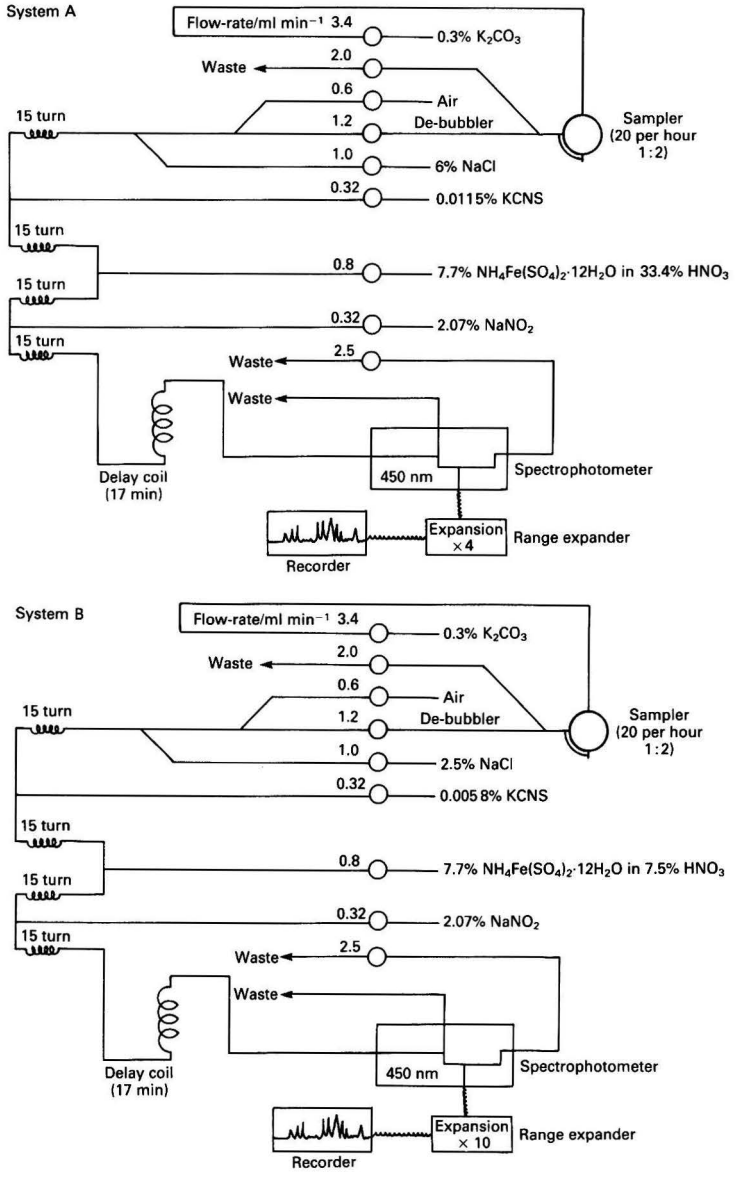

Fig. 1. Flow diagrams for the determination of total inorganic iodine in waters (system A) and free iodide in waters (system B)

Stability of Standard and Sample Solutions

When trials were run on a series of test waters, it was found that the total inorganic iodine values increased over a period of days, especially when drinking water straight from the tap was used. This phenomenon was investigated by leaving standard, blank and drinking water solutions made up in 0.3% potassium carbonate in containers made of different materials and analysing them over different periods of time. All the containers were previously thoroughly washed with concentrated nitric acid and rinsed copiously with distilled water. Drinking water samples were centrifuged for 5 min at 50 Hz after addition of potassium carbonate to remove any precipitate and organic impurities. The results are summarised in Table 2.

The results show a slight increase in the iodine concentration of solutions stored in glass and polythene containers. The cause of this increase is unlikely to be contamination as all containers were washed with concentrated nitric acid and rinsed copiously with distilled water before use. In all solutions, except that made up with laboratory drinking water taken straight from the tap, no significant increase occurred when polystyrene bottles were used for storage. There may be

Table 2. Changes in the iodine concentration of standards, blanks and drinking water solutions made up in 0.3% potassium carbonate in different containers over a period of days

Test solution	Container	Iodine concentration/μg l^{-1} After 1 h	After 1 d	After 3 d	After 8 d
4 μg l^{-1} solution	Glass calibrated flask	3.9	4.0	4.0	4.2
	Polyethylene bottle	3.9	3.9	4.0	4.0
	Polystyrene bottle	3.9	3.9	3.9	3.9
Blank solution	Glass calibrated flask	0.0	0.2	0.4	0.5
	Polyethylene bottle	0.0	0.1	0.2	0.3
	Polystyrene bottle	0.0	0.0	0.0	0.0
Laboratory drinking water straight from tap	Glass calibrated flask	4.7	5.5	5.7	5.8
	Polyethylene bottle	4.5	5.5	5.8	5.6
	Polystyrene bottle	4.5	5.4	5.7	5.7
Cambridge drinking water	Glass calibrated flask	5.4	—	5.8	6.1
	Polystyrene bottle	5.4	—	5.5	5.4
Hertford drinking water	Glass calibrated flask	4.4	—	5.0	5.0
	Polystyrene bottle	4.5	—	4.6	4.5
Oxford drinking water	Glass calibrated flask	5.6	—	6.0	6.0
	Polystyrene bottle	5.7	—	5.9	5.8

surface effects occurring in glass and polyethylene containers but there is no direct evidence for this. The larger increase observed in laboratory tap water was thought to be due to the influence of dissolved gases such as chlorine, carbon dioxide or oxygen. A series of experiments were run to test the effects of removing dissolved gases by boiling or purging with nitrogen and also monitoring pH changes over a period of time, but the results were inconclusive. After 3 d, the iodine level of the laboratory tap water remained constant.

The results show that it is necessary to adopt a standardised sampling procedure. In this laboratory, all water samples were at least 3 d old on receipt, and these were made up in 0.3% potassium carbonate solution in polypropylene calibrated flasks, centrifuged and then stored in polystyrene bottles (30-ml universal containers obtained from Sterilin Ltd., Teddington, Middlesex, were found to be suitable). Fresh tap water was treated in the same way, but allowed to stand for 3 d before analysis.

Methods For the Determination of Total Inorganic Iodine and Free Iodide in Water

All chemicals used should be of analytical-reagent grade and glass-distilled water should be used in preference to de-ionised water.

Reagents for the determination of total inorganic iodine (system A)

Standard iodide solution, 4 g l^{-1} *of I*. Dissolve 0.5232 g of potassium iodide, previously dried in an oven at 105 °C for 2 h, in distilled water and dilute to 100 ml in a calibrated flask.

Standard iodide solution, 40 mg l^{-1} *of I*. Dilute 10 ml of the standard iodide solution (4 g l^{-1} of I) to 1000 ml with distilled water in a calibrated flask (stable for 1 month).

Standard iodide solution, 200 μg l^{-1} *of I*. Dilute 5 ml of the standard iodide solution (40 mg l^{-1} of I) to 1000 ml with distilled water in a calibrated flask. Store in a polythene or polystyrene bottle (stable for 1 month).

Working solutions. Into 200-ml calibrated flasks pipette 5, 4, 3, 2, 1 and 0 ml of standard iodide solution (200 μg l^{-1} of I). Add 2 ml of 30% *m/V* potassium carbonate solution and dilute to 200 ml with distilled water. These are the working standards. Store in polystyrene bottles and prepare freshly every 2 weeks.

Potassium carbonate solution, 30% *m/V*. Dissolve 300 g of potassium carbonate in distilled water and make up to 1 l.

Potassium thiocyanate solution, 0.0115% *m/V*. Dissolve 0.115 g of potassium thiocyanate in distilled water and make up to 1 l.

Sodium nitrite solution. Dissolve 4.14 g of sodium nitrite in distilled water and dilute to 200 ml (stable for 1 d only).

Sodium chloride solution, 6% *m/V*. Dissolve 60 g of sodium chloride in distilled water and dilute to 1 l.

Ammonium iron(III) sulphate reagent. Dissolve 77 g of ammonium iron(III) sulphate $[NH_4Fe(SO_4).12H_2O]$ in approximately 300 ml of distilled water. Add 334 ml of concentrated nitric acid (sp. gr. 1.42) and make up to 1 l. Heat on a hot-plate until all traces of solid dissolve.

Reagents for the determination of free iodide (system B)

Standard iodide solutions. These are exactly the same as those described in system A.

Potassium carbonate solution, 30% *m/V*. Dissolve 300 g of potassium carbonate in water and dilute to 1 l.

Sodium nitrite solution, 2.07% *m/V*. Dissolve 4.14 g of sodium nitrite in distilled water and dilute to 200 ml (stable for 1 d only).

Sodium chloride solution, 2.5% *m/V*. Dissolve 25 g of sodium chloride in distilled water and dilute to 1 l.

Potassium thiocyanate solution, 0.0058% *m/V*. Dissolve 0.058 g of potassium thiocyanate in distilled water and dilute to 1 l.

Ammonium iron(III) sulphate reagent. Dissolve 77 g of ammonium iron(III) sulphate $[NH_4Fe(SO_4).12H_2O]$ in approximately 400 ml of distilled water. Add 75 ml of concentrated nitric acid (sp. gr. 1.42) and make up to 1 l. Warm until all traces of solid dissolve.

Sodium oxalate reagent. Dissolve 5 g of sodium oxalate in 100 ml of 5% *V/V* sulphuric acid (this reagent is toxic).

Apparatus

A centrifuge with a speed of 50 Hz and glass or polypropylene centrifuge tubes of 150-ml capacity were used. Polystyrene bottles of 30–50-ml capacity were employed.

An AutoAnalyzer system for colorimetric analysis was utilised. The results shown in this paper were obtained using a Technicon AutoAnalyzer 1 system with a range expansion facility that was operated and maintained in accordance with the instructions given in the Operator Instruction Manual.[5]

Procedure

Wash all glassware and polystyrene containers with concentrated nitric acid and rinse copiously with distilled water

Table 3. Effect of added ions on the determination of (A) total inorganic iodine concentration and (B) free iodide concentration in a 4 μg l^{-1} standard iodide solution

Element added	Concentration/ mg l^{-1}	Maximum concentration in drinking waters/ mg l^{-1}	(*A*) Total I found/ μg l^{-1}	(*B*) Free I found/ μg l^{-1}
Zn^{2+}	1	0.69	4.0	3.9
Cu^{2+}	1	0.36	4.0	3.8
Li^{2+}	1	0.01	4.1	3.8
Pb^{2+}	0.1	0.046	4.0	3.9
Fe^{3+}	2	1.5	4.0	3.8
Mn^{3+}	1	0.06	3.9	3.9
Ni^{2+}	0.2	0.013	4.0	4.2
Hg^{2+}	0.005	0.001	3.9	4.2
Co^{2+}	0.2	0.011	4.0	4.2
Mg^{2+}	30	23	4.1	4.0
Cl^-	400	245	4.0	4.0
Br^-	1	—	4.0	4.1
F^-	1	—	4.1	4.3
IO_3^-	0.004	—	8.0	4.0
SO_4^{2-}	200	—	4.1	4.5
Humic acid	20	—	3.9	0.0
Iodoform	1	—	5.6	5.6
Methyl iodide	0.1	—	4.2	4.2

Table 4. Variations in peak heights of standard solutions and total inorganic iodine concentrations of samples run on each of five consecutive days

Standard solution peak heights (% full-scale deflection)—

Concentration/ μg l^{-1}	Mean peak height	Standard deviation	Coefficient of variation, %
5	62.9	0.4	0.6
4	52.2	0.6	1.1
3	41.6	0.9	2.2
2	28.1	1.2	4.2
1	15.3	0.6	3.7

Total inorganic iodine concentration of drinking water—

Sample source	Mean total I concentration/ μg l^{-1}	Standard deviation/μg l^{-1}	Coefficient of variation, %
London (borehole)	28.0	0.76	2.7
Harrogate	2.2	0.06	2.7
Fife	7.8	0.54	6.9
Bristol	4.1	0.10	2.4
Nottingham	14.0	0.54	3.9
Amesbury	4.3	0.10	2.3
Oxford	4.3	0.10	2.3
Gloucester	3.2	0.08	2.5
Dunoon	1.1	0.07	6.4
Nuneaton	2.8	0.04	1.4

Table 5. Variations in peak heights of standard solutions and free iodide concentrations of samples run on each of five consecutive days

Standard solution peak heights (% full-scale deflection)—

Concentration/ μg l^{-1}	Mean peak height	Standard deviation	Coefficient of variation, %
5	20.5	0.3	1.5
4	16.7	0.5	3.0
3	12.6	0.5	6.0
2	8.4	0.5	6.0
1	4.5	0.3	6.4

Free iodide concentration of drinking waters—

Sample source	Mean free I concentration/ μg l^{-1}	Standard deviation/μg l^{-1}	Coefficient of variation, %
London (borehole)	28.0	1.1	3.9
Harrogate	0.9	0.17	19.9
Fife	7.8	0.86	11.0
Bristol	2.1	0.05	2.4
Nottingham	3.5	0.49	14.0
Amesbury	0.9	0.17	19.0
Oxford	1.3	0.22	17.0
Gloucester	3.2	0.7	5.3
Dunoon	0.9	0.0	0.0
Nuneaton	1.6	0.14	8.7

before use. Dispense 1.0 ml of 30% *m/V* potassium carbonate solution into a 100-ml calibrated flask. Make up to 100 ml with a water sample and shake well. Centrifuge the resulting solution for 5 min at 50 Hz. Decant about half of the solution into polystyrene bottles. Allow fresh tap water to stabilise for 3 d before analysis.

Set up the manifold system shown in the flow diagram (Fig. 1) and use the appropriate set of reagents for either total inorganic iodine determination (A) or free iodide determination (B). Load the sample tray with a set of working standards followed by 20 samples interspersed with a working standard every fifth sample. Complete the series with another set of working standards and run at a rate of 20 per hour.

If deposits of iron(III) thiocyanate occur, these may be removed by running two sample cups of sodium oxalate reagent through the system at the end of a run.

Calculation

Plot a calibration graph of the mean standard peak heights against their respective iodine concentrations. The iodine concentration of a sample is obtained by comparing its peak height with the calibration graph. Multiply the result by 1.01 to compensate for the addition of potassium carbonate.

Results

Effects of Interferences

The effects of ions commonly occurring in drinking waters that could cause possible interference were tested, and the results, representing a mean of three determinations are shown in Table 3. The maximum values for the concentrations of elements in drinking waters are those reported by Zoetman and Brinkmann.[6]

Table 6. Recovery of added iodide from water samples using the total inorganic iodine method

Sample source	Original iodine concentration/ $\mu g\ l^{-1}$	Iodide added/ $\mu g\ l^{-1}$	Calculated total/$\mu g\ l^{-1}$	Iodine found/ $\mu g\ l^{-1}$	Recovery, %
London (borehole)*	2.8	2.0	4.8	4.8	100
Harrogate	2.2	2.0	4.2	4.0	90
Bristol	2.05	2.0	4.05	4.0	99
Bristol*	2.05	2.0	4.05	4.0	99
Nottingham*	2.8	2.0	4.8	4.6	96
Amesbury*	2.15	2.0	4.15	4.2	101
Oxford*	2.15	2.0	4.15	4.5	108
Gloucester	1.6	2.0	3.6	3.8	106
Nuneaton	1.4	2.0	3.4	3.5	103

* These samples were diluted.

Table 7. Recovery of added iodide from water samples using the free iodide method

Sample source	Original iodide concentration/ $\mu g\ l^{-1}$	Iodide added/ $\mu g\ l^{-1}$	Calculated total/$\mu g\ l^{-1}$	Iodide found/ $\mu g\ l^{-1}$	Recovery, %
Slough	1.3	2.0	3.3	3.6	109
Catterick	0.7	2.0	2.7	2.4	89
Hexham	1.2	2.0	3.2	3.2	100
Royston	0.6	2.0	2.6	2.7	104
Braintree	1.2	2.0	3.2	3.5	109
Benson	1.0	2.0	3.0	3.0	100

Table 8. Comparison of the proposed method for total inorganic iodine with that of Keller *et al.*[7] for a range of waters

	Iodine concentration/$\mu g\ l^{-1}$	
Sample source	Proposed method	Keller's *et al.* method
Slough	11.5	12.7
Oakington	5.5	6.0
Catterick	0.7	0.7
Hexham	2.3	2.9
Royston	4.5	5.2
Harrogate	2.2	1.7
Braintree	13.7	13.9
Benson	5.8	6.5
London (borehole)	28.0	30.0
Fife	7.8	5.8
Nottingham	14.0	15.4
Dunoon	1.1	1.1
London (tap)	5.8	5.9

The results show that interferences will not present any major problems in the analysis of drinking waters for either total inorganic iodine or free iodide. The greater variability of the values for free iodide is due to the lower sensitivity and precision of the method.

Precision of the Methods

To obtain a measure of the precision of the methods, a number of drinking waters from different areas in the United Kingdom were analysed on each of five consecutive days. The results are summarised in Table 4 for total inorganic iodine and Table 5 for free iodide. Variations in the peak heights of working standard solutions and individual iodine results are expressed by the standard deviation from the mean. This shows that the general precision of the total inorganic iodine method is of the order of 3% and that of the free iodide method is of the order of 10%.

Accuracy of the Methods

No certified water sample against which to test the accuracy of the methods could be found, and no suitable referee method was available. An indication of the accuracy of the method was therefore obtained by adding known amounts of iodide to water samples that had been previously analysed for total inorganic iodine and free iodide. The results are summarised in Tables 6 and 7, where the results shown for spiked samples represent a mean of three separate determinations.

As a further check, the results obtained by the total inorganic iodine method were compared with those obtained using the method of Keller *et al.*,[7] which is based on the method of Dubravčić[1] [cerium(IV) sulphate - arsenious acid], modified for an AutoAnalyzer system. The results are sumarised in Table 8, where the values given are the means of three determinations.

The mean recovery of iodide by the total inorganic iodine method and the free iodide method are 100 ± 5.4% and 102 ± 7.5%, respectively. The results show reasonable agreement with those of Keller *et al.*[7] The effects of interferences on the method of Keller *et al.* were not determined and could explain some of the minor differences.

Limit of Detection

The limit of detection was taken to be the concentration at which the signal was three times greater than the noise of the base line. This gave a limit of detection of 0.2 $\mu g\ l^{-1}$ of I for the total inorganic iodine method and 0.4 $\mu g\ l^{-1}$ of I for the free iodide method.

Conclusion

The automatic methods for the determination of total inorganic iodine and free iodide were shown to be applicable to normal drinking waters. The same manifold system was common to both methods and the sensitivity, precision and accuracy were shown to be adequate. The effects of possible interfering ions were shown to be negligible for normal

drinking waters, and once the system has been set up it is simple to operate and has a throughput of 20 samples per hour.

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Accuracy of Determination of the Electrical Conductivity and the pH Value of River Waters: Results of Water Authority Tests Made for the Harmonised Monitoring Scheme of the Department of the Environment

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The Department of the Environment, in collaboration with the Regional Water Authorities, has initiated a Scheme for the Harmonised Monitoring of Inland Fresh Waters in England and Wales. The Scottish Development Department has been closely associated with the development of this Scheme, and has introduced a similar scheme in Scotland in collaboration with the River Purification Boards. To achieve the required comparability of results, each of the 11 participating laboratories (drawn from 10 Regional Water Authorities and 1 River Purification Board) takes part in an analytical quality control (AQC) programme; this work is coordinated by the Water Research Centre. The general approach adopted to AQC has already been described, and this paper presents the results of tests made on the determination of pH value and electrical conductivity in river waters. The accuracy requirements for both determinands (that the total error on a single result should be not larger than 0.2 unit, for pH, and not larger than 20% of sample conductivity or 5 μS cm^{-1}, whichever is the greater, for conductivity) were essentially achieved by all 11 participating laboratories. Inter-laboratory tests on the determination of pH were, however, restricted to the distribution of buffer solutions because river samples examined were not sufficiently stable.

Keywords: *River water analysis; electrical conductivity determination; pH determination; accuracy of results; analytical quality control*

The Scheme for the Harmonised Monitoring of the Quality of Inland Fresh Water has been described in detail.[1] It is intended to provide objective data on river water quality so that accurate assessments can be made of long-term trends in the qualities of rivers and of the amount of materials discharged by them to the sea. The Scheme complements monitoring carried out for regional or local purposes and one of its essential aims is to achieve comparability of the results from all participating laboratories. To that end, special investigations have been made to establish suitable sampling locations and to define the necessary sampling frequency. Sampling procedures have been recommended, and each participating laboratory carries a specially designed programme of tests to ensure that its analytical results are of adequate accuracy for the Scheme.

The Water Research Centre (WRC) is under contract to the Department of the Environment to advise on and to coordinate this analytical quality control (AQC) programme.

The need for, and details of an approach to, a planned AQC system for this and similar monitoring schemes have been discussed elsewhere.[2] In view of the growing interest in achieving comparable results from a number of laboratories, it was thought useful to describe the AQC work for the Harmonised Monitoring Scheme and to present the results for different determinands. This paper considers the determination of pH and electrical conductivity; earlier papers describe the work for chloride,[3] ammoniacal nitrogen,[4] total oxidised nitrogen and nitrite[5] and suspended solids[6] and subsequent papers will deal with other determinands of importance in rivers.

Organisation of the Work

A Committee was formed to plan the collaborative work and has representatives† from the Department of the Environment (DOE), the Scottish Development Department, each Regional Water Authority (RWA), the Scottish River Purification Boards and the WRC. This Committee decided to adopt the approach to AQC described elsewhere,[2] each determinand being studied in two phases.

Phase (i). One laboratory in each of the 10 RWAs and one in Scotland participated, the WRC acting as coordinating laboratory.[2]

Phase (ii). After satisfactory results have been obtained in phase (*i*), those laboratories act as coordinators of tests within RWAs and in Scotland. Certain RWAs are not involved in this stage because all analyses for Harmonised Monitoring are made by one laboratory.

This paper deals only with phase (*i*).

Required Analytical Accuracy

The following requirements for analytical accuracy were agreed for the determination of electrical conductivity: maximum tolerable bias, 10% of the electrical conductivity of the sample or 2.5 μS cm^{-1}, whichever is the greater; maximum tolerable total standard deviation, 5% of the electrical conductivity of the sample or 1.25 μS cm^{-1}, whichever is the greater.

For pH determination, the following requirements were established: maximum possible bias, 0.1 pH unit; maximum possible standard deviation, 0.05 pH unit.

Analytical Quality Control

The approach followed was exactly as presented previously[2]; no attempt is made here, therefore, to explain the reasons underlying the various activities described below. The participating laboratories were as follows: Anglian WA, Regional Standards Laboratory, Cambridge; Northumbrian WA, Howdon Laboratory, Wallsend; North West WA, Rivers Division Laboratory, Warrington; Severn-Trent WA, Regional Laboratory, Finham; Southern WA, Resource Planning Laboratory, Otterbourne; South West WA, Rivers and Marine Laboratory, Exeter; Thames WA, Thames Conservatory Division Laboratory, Reading; Welsh WA, Chester Area Laboratory, Chester; Wessex WA, Bristol Avon Division Laboratory,

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† The names of the representatives at the time the work reported here was carried out are given in the Appendix.

Saltford; Yorkshire WA, Headquarters Laboratory, Leeds; Forth RPB, Headquarters Laboratory, Edinburgh. The sequence of participating laboratories in the above list does not relate to the order of numbering of laboratories in the tables.

Analytical Method

All laboratories employed the methods recommended by the DOE/NWC Standing Committee of Analysts.[7] In one laboratory (laboratory 11), calibration buffers and samples were diluted (10 ml of sample/buffer + 1 ml of water) prior to pH determination as part of a procedure for the prevention of cross-contamination in an automated sample dispensing system. This practice was noted only after the laboratory had completed the tests described here. The results of this laboratory in these studies, involving tests of precision on buffer solutions and river samples and tests of inter-laboratory bias using buffer solutions only, were of adequate accuracy but the laboratory was informed that the dilution of river samples for pH measurement was likely to introduce unacceptable bias to analytical results for such samples.

Effect of Temperature on the Measurement of pH and Conductivity

For both determinands considered in this paper, the temperature of the sample at the time of measurement will affect any analytical result obtained. This effect is one in which the true pH value or conductivity of the sample, rather than merely the error associated with measurement, is dependent on sample temperature. Thus variations in the temperature of measurement will, unless eliminated or corrected for, contribute to between-laboratory bias. Such factors will adversely affect laboratories' comparability, apart from any analytical errors that may be present. The approach to this question of sample temperature was slightly different for the two determinands.

For conductivity measurements, it was not possible, because of the practices in use in the various laboratories, to agree that all samples should be measured at a single temperature. All but two laboratories (which measured at 25 °C) performed measurements at 20 °C. There was also the possibility that, if certain samples proved inadequately stable, on-site measurement at ambient temperature would have to be considered. The likely bias introduced by the correction of measured conductivity to conductivity at some standard temperature was therefore examined. The temperature dependence of conductivity has been investigated by Wagner[8] for various types of potable water and by Smith[9] for samples from lakes and rivers. Values of the temperature correction factor (the fractional change in conductivity per degree) appropriate for correction of conductivity to 20 °C from 25 °C range from 0.019 to 0.026. The use of a correction value from one end of the range (*i.e.*, 0.019) when the true value for the water in question was from the other end of the scale of values (*i.e.*, 0.026) would result in a bias in reported results of approximately 4%. This unlikely event was considered in relation to the target for maximum possible bias of 10% and it was decided that correction is not likely to introduce important between-laboratory bias. It is important to note, however, that should the true correction factor of the water concerned lie outside the range given above or that correction is made over a range greater than 5 °C, the correction of conductivity data for temperature might introduce unacceptable bias.

In pH measurement, the option of correcting results for temperature of measurement is not available because the pH value of a sample does not behave in as predictable a manner as the conductivity. Here one laboratory (laboratory 10) performed determinations at 25 °C and the remaining ten at 20 °C. It was agreed that the temperature of samples for pH determination would be controlled to within ±1 °C. (For conductivity determinations, it appeared that such stringent control of temperature is not essential; however, knowledge of the sample temperature to within ±1 °C is necessary if an unacceptable bias is not to be introduced when temperature correction is applied.) Allowance has been made in the data archive for pH measurements to be made at ambient temperature, should it prove necessary to make on-site determinations.

Within-laboratory Precision Tests

Each laboratory then carried out the same programme of tests to assess the precision of its analytical results. On each of ten days, each laboratory made duplicate determinations, in random order, on appropriate standard solutions and portions of two river waters having, respectively, a determinand value near the lowest and highest routinely reported for the Harmonised Monitoring Scheme.

For conductivity, the standards were two solutions of potassium chloride of 0.1 and 0.9 of the upper concentration range of the laboratory's method. Duplicate conductivity blank determinations were also performed in each batch of analyses. The results for the standards were blank corrected to allow for possible between-batch variation in the conductivity of the water used to prepare the standard. Results for river samples were not blank corrected.

For pH, the standards consisted of three buffer solutions having nominal pH values of 4.0, 7.0 and 9.0. In an attempt to ensure constant composition for the buffer solutions, fresh samples were prepared for each batch of analyses. This eliminated the possibility of batch to batch variability caused by instability in the buffers. Potential differences between freshly prepared portions of the same buffer solution were minimised by their being made up from sachets of buffer powder, selected at random, from the same manufacturer's batch. Sachets of such buffer powder were distributed by the WRC to all laboratories. This procedure gave a preliminary check on between-laboratory bias, provided that portions of the distributed buffer powder were assumed to be identical.

It was recognised that problems of drifting sample pH, caused by interaction with atmospheric carbon dioxide, might be encountered if successive aliquots of a river sample were removed from the same container for analysis in any given batch. To overcome this potential problem a bulk sample of river water was collected and split into 20 separate containers of the type used routinely for such samples.

Each determination of river water pH was then made on a freshly opened container, every effort being made to ensure that the 20 sub-samples were identical. This approach was adopted because it was considered more likely that a sample could be split homogeneously than that a single, large sample would remain unchanged, once opened, during a batch of analyses.

On completion of the tests, each laboratory analysed its results to obtain estimates of within-batch (s_w), between-batch (s_b) and total (s_t) standard deviations,[2] where $s_t = (s_w^2 + s_b^2)^{\frac{1}{2}}$. The values of s_t were compared with the appropriate target value using an *F*-test and were accepted as satisfactory provided s_t was not significantly greater ($p = 0.05$) than the appropriate target.

Such a treatment of the results of tests on river waters could, if the sample were unstable from one batch to another, give a falsely inflated estimate of between-batch variability and hence of total standard deviation. In the conductivity determinations, as none of the estimates of laboratories' standard deviations was greater than the appropriate target value, it can be concluded that such effects, if they occur, are not of importance. For pH determination, where these effects are likely to be of greater magnitude, it was agreed that, for real

Table 1. Determination of electrical conductivity—results of precision tests. Target total standard deviation: 5% or 1.25 µS cm^{-1}, whichever is the greater

Laboratory No.	Standard solution 1: Mean conductivity/µS cm^{-1}	Standard deviation/µS cm^{-1} s_w	s_b	s_t	Relative total standard deviation, %	Standard solution 2: Mean conductivity/µS cm^{-1}	Standard deviation/µS cm^{-1} s_w	s_b	s_t	Relative total standard deviation, %	Low-conductivity river water: Mean conductivity/µS cm^{-1}	Standard deviation/µS cm^{-1} s_w	s_b	s_t	Relative total standard deviation, %	High-conductivity river water: Mean conductivity/µS cm^{-1}	Standard deviation/µS cm^{-1} s_w	s_b	s_t	Relative total standard deviation, %
1	657.2	0.6	2.8	2.9	0.4	6113.8	9.0	23.8	25.4	0.4	513.4	0.7	2.0	2.2	0.4	2132.0	5.0	5.2	7.2	0.3
2	64.1	0.4	0.7	0.8	1.5	620.5	2.2	5.8	6.2	1.0	42.5	0.4	0.9	1.0	2.3	325.6	1.9	6.6	6.8	1.9
3	201.0	0.7	NS*	0.9	0.4	1701.1	3.9	8.3	9.1	0.5	218.8	0.5	1.1	1.3	0.6	1501.5	3.9	4.5	6.0	0.4
4	727.4	8.0	NS	9.1	1.2	6614.0	13.0	14.6	19.6	0.3	750.1	8.0	NS	9.5	1.3	13495.0	31.3	51.6	60.3	0.4
5	200.8	0.4	1.0	1.1	0.5	1801.9	1.1	1.5	1.9	0.1	824.6	0.7	1.4	1.6	0.2	1153.8	0.7	2.7	2.8	0.2
6	176.7	2.1	4.1	4.6	2.6	798.9	2.4	9.5	9.8	1.2	164.5	4.5	6.1	7.6	4.6	341.3	6.7	13.6	15.1	4.4
7	101.5	3.5	4.1	5.4	5.3†	906.2	6.2	11.2	12.8	1.4	665.5	5.0	6.1	7.9	1.2	762.5	6.6	NS	9.2	1.2
8	102.5	3.3	NS	3.6	3.5	881.7	5.2	25.2	25.8	2.9	132.3	1.6	3.8	4.1	3.1	636.2	5.1	13.2	14.1	2.2
9	143.2	1.5	4.7	4.9	3.4	1385.8	14.0	13.5	19.4	1.4	674.1	5.3	11.4	12.6	1.9	1229.9	14.7	59.4	61.2	5.0
10	199.2	0.3	0.8	0.8	0.4	1766.0	3.9	9.6	10.4	0.6	256.0	1.7	NS	2.0	0.8	2154.0	3.2	12.7	13.1	0.6
11	49.1	0.1	NS	0.2	0.4	489.8	0.3	0.7	0.8	0.2	52.0	1.6	NS	1.7	3.3	389.0	2.8	3.2	4.3	1.1

* NS, not significant.

† Not significantly greater than the target value.

Table 2. Determination of pH—results of precision tests. Target total standard deviation, 0.05 pH unit. Where the estimate of total standard deviation calculated on the basis that samples are stable with respect to pH exceeds the precision target a revised estimate, in which the possible effect of sample instability, between batches, is not included, has been calculated. This estimate is shown in parentheses beneath the original estimate

Laboratory No.	Buffer solutions, pH 4: Mean pH	Standard deviation, pH units s_w	s_b	s_t	pH 7: Mean pH	s_w	s_b	s_t	pH 9: Mean pH	s_w	s_b	s_t	River water samples, Low pH: Mean pH	s_w	s_b	s_t	High pH: Mean pH	s_w	s_b	s_t
1	4.016	0.0055	0.0097	0.0110	6.995	0.0097	0.0356	0.0370	9.205	0.0050	0.0067	0.0084	7.465	0.0265	0.0306	0.0405	8.137	0.0184	0.0191	0.0265
2	4.010	0.0039	0.0067	0.0077	6.952	0.0070	0.0115	0.0135	9.215	0.0059	0.0130	0.0142	6.792	0.0844	0.118	0.145*	7.727	0.0641	0.0293	0.0705*
																(0.0851)*				(0.0650)
3	4.015	0.0000	0.0412	0.0412	7.020	0.0224	NS†	0.0253	9.185	0.0158	0.0179	0.0239	8.122	0.0193	0.0625	0.0654	8.490	0.0224	0.0398	0.0456
4	4.114	0.0267	0.0329	0.0424	7.046	0.0201	0.0378	0.0428	9.280	0.0216	0.0414	0.0467	7.216	0.0332	0.0450	0.0560	8.114	0.0617	NS	0.0617
5	3.999	0.0018	0.0089	0.0091	7.015	0.0091	0.0012	0.0091	9.270	0.0018	0.0163	0.0164	7.511	0.0189	0.0244	0.0309	8.174	0.0226	0.0226	0.0317
6	4.029	0.0032	0.0227	0.0226	6.978	0.0039	0.0432	0.0434	9.117	0.0071	0.0222	0.0233	7.870	0.479	NS	0.479*	8.062	0.0241	0.114	0.116*
																(4.480)*				(0.0392)
7	3.984	0.0217	0.0073	0.0229	6.972	0.0109	0.0226	0.0251	9.224	0.0160	0.0195	0.0249	8.102	0.0217	0.0137	0.0256	8.108	0.0190	0.0161	0.0249
8	4.100	0.0230	NS	0.0278	6.930	0.0232	0.0251	0.0342	9.173	0.0292	NS	0.0292	6.933	0.0102	NS	0.0119	7.809	0.0217	NS	0.0255
9	3.983	0.0077	0.0204	0.0218	6.901	0.0102	0.0481	0.0492	9.122	0.0143	0.0222	0.0264	7.600	0.0136	0.128	0.129*	8.018	0.0149	0.145	0.153*
																(0.036)				(0.059)
10	3.882	0.0055	0.0251	0.0257	6.991	0.0050	0.0197	0.0203	9.282	0.0084	0.0153	0.0174	7.487	0.0335	0.0351	0.0485	7.929	0.0340	NS	0.0385
11	4.056	0.050	0.0257	0.0262	6.983	0.0129	0.0208	0.0245	9.153	0.0096	0.0175	0.0199	6.573	0.0446	0.1661	0.172*	8.593	0.0251	0.0546	0.0600
																(0.049)				

* Exceeds target.

† NS, not significant (*F*-test, 0.05 probability level).

samples where estimates of standard deviation that exceeded the target were obtained, another estimate of total standard deviation would be calculated using a value, s_{bp}, for the pooled between-batch standard deviation arrived at from the buffer solutions:

$$s_{bp} = \left[\frac{s_b^2(\text{pH } 4) + s_b^2(\text{pH } 7) + s_b^2(\text{pH } 9)}{3} \right]^{\frac{1}{2}}$$

The revised estimate of total standard deviation was obtained from

$$s_t^2 = s_w^2 \text{ (sample)} + s_{bp}^2$$

If this estimate was not significantly greater than the target (F-test at the $p = 0.05$ level) then the precision was regarded as satisfactory.

The results of the precision tests are summarised in Tables 1 and 2.

The results of the tests on conductivity determination indicate that the precision is satisfactory. Those for pH show two instances of failure to meet the target (laboratories 2 and 6 for the low pH river water). An examination of the analytical profiles of these samples revealed that they were of low alkalinity and conductivity (12 and 15 mg l^{-1} of $CaCO_3$ and 51 and 160 μS cm^{-1}, respectively). Such samples are likely to present problems in the determination of pH, owing to possible changes in true pH during measurement (by interaction with atmospheric carbon dioxide) and difficulties involving the liquid junction potential of the reference electrode. It was decided to proceed with the programme of tests and to return to the problem of pH measurement in water of low ionic strength/poor buffer capacity at a later stage.

Each laboratory then set up a statistical quality control chart[2] based on the analysis of a standard solution in each batch of analyses. These charts are intended to aid the continuing, long-term assessment of accuracy and are not considered further here.

Tests for Between-laboratory Bias

To complete this initial phase of AQC, direct checks of between-laboratory bias were made as follows. For conductivity determination, the WRC distributed portions of one standard solution and two river water samples. For the determination of pH, portions of two buffer solutions were circulated. Single determinations were made on each solution on each of five days, and the results obtained are summarised in Tables 3 and 4, the 90% confidence limits for each mean being calculated from the results of the five determinations for each solution.

All solutions distributed were examined for their stability prior to the test. The power of the stability test was such that a true change in determinand value (over a period of storage of 2 weeks, in darkness, at room temperature) of 2% for conductivity or of 0.025 pH unit for pH would have been detected at the 95% confidence level. These stability tests revealed that the river water samples examined were not adequately stable with respect to pH, hence the use of buffer solutions only for this determinand.

To assess whether or not the bias of a laboratory exceeded the target value, the following procedure was adopted. Let the mean result and its 90% confidence interval of laboratory i be denoted by $x_i + L_i$. The value of the maximum possible bias of laboratory i (95% confidence level) was then calculated as

$$(x_i + L_i - X) \text{ if } x_i > X$$

or

$$(x_i - L_i - X) \text{ if } x_i < X$$

where X is the true value of the distributed standard solution.

The results in Tables 3 and 4 indicate that all laboratories meet the target for maximum possible bias for both determinands (except for a wholly marginal failure by one laboratory for one of the buffer solutions).

Laboratory 4 reported its conductivity results after measurement at 25 °C and its results were corrected to 20 °C using a factor of 0.020 μS cm^{-1} $°C^{-1}$. Its results remain within target if a factor from the other end of the likely scale of values (*i.e.*, 0.026) is used.

Laboratory 10 provided information on the temperature dependence of the buffer solutions by performing determinations at both 20 and 25 °C. These results, which are included in Table 4, indicate no important difference in values obtained.

Table 3. Results for tests of inter-laboratory bias—conductivity. The target for maximum possible bias is 10% of the conductivity of the sample or 2.5 μS cm^{-1} (whichever is the larger)

	River water A				Standard solution				River water B			
Laboratory No.	Mean/ μS cm^{-1}	Standard deviation/ μS cm^{-1}	Difference from mean of all results, %	Maximum possible bias,* %	Mean/ μS cm^{-1}	Standard deviation/ μS cm^{-1}	Difference from WRC value, %	Maximum possible bias,* %	Mean/ μS cm^{-1}	Standard deviation/ μS cm^{-1}	Difference from mean of all results, %	Maximum possible bias,* %
1	650.0	2.55	1.58	1.96	660.0	1.73	2.01	2.26	212.8	3.42	2.26	3.83
2	614.0	5.48	4.05	4.86	626.0	5.48	−3.25	−4.05	201.6	2.07	−3.12	−4.07
3	661.4	2.61	3.36	3.74	669.0	3.00	3.40	3.84	217.0	1.00	4.28	4.73
4	642.6	2.05	0.98	1.29	653.2	1.79	1.51	1.78	208.1	1.10	0.53	1.03
5	643.2	1.48	0.51	0.73	650.4	1.14	0.53	0.69	211.0	0.00	1.39	1.39
6	627.6	1.52	−1.92	−2.15	656.2	2.77	1.42	1.83	194.2	3.27	−6.68	−8.17
7	646.4	6.23	1.01	1.94	664.0	5.48	2.63	3.43	206.6	3.65	−0.68	−2.39
8	634.0	2.00	−0.98	−1.22	634.4	4.83	−0.56	−1.27	207.4	1.95	−0.33	−1.23
9†	638.8	2.28	−0.17	−0.51	655.4	7.27	0.51	1.57	211.6	3.58	1.68	3.32
10‡	635.0	2.24	−0.77	−1.10	641.4	3.65	−0.87	−1.40	208.6	1.34	0.24	0.86
10§	650.3	6.18	1.63	2.55	652.6	6.14	0.09	0.99	212.9	1.82	2.31	3.14
11	645.2	2.49	0.83	1.20	657.2	4.82	1.58	2.29	213.4	1.14	2.55	3.07
Mean of all laboratories		639.9			652.08				208.1			
WRC value					647							

* The values given for maximum possible bias are quoted at a confidence level of 95%.

† Laboratory 9 was unable to participate in this test within the time available owing to the breakdown of its instruments. Its results are reported but have not been used to calculate the mean result of laboratories.

‡ Results of measurements at 20 °C—used in calculation of the mean result of laboratories.

§ Results of measurements at 25 °C, corrected to conductivity at 20 °C using α = 0.02 $°C^{-1}$. Those for laboratory 4 were used in the calculation of the mean result of laboratories, but those for laboratory 10 were not because values obtained at 20 °C were available.

Table 4. Results of tests of inter-laboratory bias—pH. Target maximum possible bias, 0.1 pH unit

	Buffer solution A					Buffer solution B				
Laboratory No.	Mean pH	Standard deviation, pH units	* 90% confidence limits	Difference from nominal value, pH units	Maximum possible bias, pH units	Mean pH	Standard deviation, pH units	* 90% confidence limits	Difference from nominal value, pH units	Maximum possible bias, pH units
1	6.874	0.018	0.017	−0.007	−0.024	7.404	0.018	0.017	−0.025	−0.042
2	6.872	0.008	0.008	−0.009	−0.017	7.424	0.005	0.005	−0.005	−0.010
3	6.960	0.019	0.018	0.079	0.097	7.420	0.020	0.019	−0.009	−0.028
4	6.930	0.019	0.018	0.049	0.067	7.468	0.015	0.014	0.039	0.053
5	6.978	0.004	0.004	0.097	0.101*	7.506	0.005	0.005	0.077	0.082
6	6.830	0.026	0.025	−0.051	−0.076	7.392	0.013	0.012	−0.037	−0.049
7	6.870	0.007	0.007	−0.011	−0.018	7.404	0.005	0.005	−0.025	−0.030
8	6.862	0.004	0.004	−0.019	−0.023	7.400	0.007	0.007	−0.029	−0.036
9	6.846	0.009	0.009	−0.035	−0.044	7.374	0.017	0.016	−0.055	−0.071
10 (a)†	6.840	0.010	0.010	−0.041	−0.051	7.400	0.010	0.010	−0.029	−0.039
(b)‡	6.838	0.015	0.014	−0.043	−0.057	7.408	0.015	0.014	−0.021	−0.035
11	6.908	0.022	0.021	−0.027	0.048	7.430	0.017	0.017	0.001	0.018
Mean of all laboratories	6.888					7.420				
Nominal value	6.881					7.429				

* Exceeds target.
† (a) Results of 20 ± 1 °C; used in calculation of the mean result of laboratories.
‡ (b) Results at 25 ± 1 °C.

Table 5. Results of inter-laboratory follow-up test—conductivity. Values in μS cm^{-1} (corrected to 20 °C using a correction factor of 0.022 °C^{-1}). The results of laboratories 3 and 7 can be rejected as statistical outliers (p = 0.05). This has not been done in the results shown here because such rejection makes no difference to the conclusions drawn regarding the ability of laboratories to meet the Harmonised Monitoring requirements for analytical accuracy for conductivity determination. Target maximum possible bias, 10%

Laboratory No.	Mean	Standard deviation	* 90% confidence limits	Difference from mean of all results, %	Maximum possible bias, %
1	800.00	0.00	0.00	1.32	1.32
2	788.00	4.47	4.26	−0.20	−0.74
3	865.00	0.00	0.00	9.55	9.55
4	798.33	1.26	1.20	1.11	1.26
5	804.00	2.92	2.78	1.82	2.18
6	819.20	0.84	0.80	3.75	3.85
7	646.20	1.30	1.24	−18.16*	−18.32*
8	782.40	1.34	1.28	−0.91	−1.07
9	772.20	4.44	4.23	−2.20	−2.74
10	793.88	0.00	0.00	0.54	0.54
11	816.41	1.28	1.22	3.39	3.55
Mean of all laboratories	789.6				

* Exceeds target.

It was concluded, therefore, that satisfactory freedom from between-laboratory bias had been achieved for conductivity determination on both the standard solution and on two real samples and for pH determination on the buffer solutions.

Routine AQC

To attempt to ensure that the required accuracy of results is maintained, AQC is now an integral part of the routine analysis for the Harmonised Monitoring Scheme. As stated above, primary reliance for this purpose is placed on within-laboratory AQC using statistical quality control charts. However, to obtain direct checks on between-laboratory bias, portions of samples are distributed at intervals to all laboratories. Since the completion of the preliminary tests only one such distribution (of a river sample for conductivity and a buffer solution for pH) has so far taken place. The results for these tests are shown in Tables 5 and 6. Not all laboratories adhered to the agreed procedure of temperature control for the measurement of pH (see note in Table 6). Despite this, results of adequate accuracy were obtained. Such freedom from important temperature dependence of pH value, whilst being observed for a buffer solution, cannot be relied upon where river samples are concerned.

It appears, from these results, that reasonably satisfactory accuracy has been maintained, although the occasional deviations emphasise the need for continuing AQC.

Discussion

Throughout this programme of AQC there were very few instances of failure to meet targets. It is therefore reasonable to assume that the preliminary tests of precision and between-laboratory bias demonstrated that laboratories were capable of achieving adequate accuracy for the determination of electrical conductivity.

For pH, unambiguous tests of inter-laboratory bias by means of the analysis of a distributed river sample proved impossible owing to sample instability. The use of buffer solutions in such tests means that there was no direct

Table 6. Results of inter-laboratory follow-up test—pH. Target maximum possible bias, 0.1 pH unit

Laboratory No.	Mean pH	Standard deviation, pH units	* 90% confidence limits	Difference from mean of all results, * pH units	Maximum possible * bias, pH units
1	6.218	0.011	0.010	−0.024	−0.035
2	6.264	0.009	0.009	0.022	0.030
3	6.330	0.000	0.000	0.088	0.088
4	6.192	0.008	0.008	−0.050	−0.058
5	6.248	0.004	0.004	0.006	0.010
6	6.244	0.025	0.024	0.002	0.025
7	6.228	0.008	0.008	−0.014	−0.022
8	6.200	0.000	0.000	−0.042	−0.042
9	6.266	0.022	0.021	0.024	0.044
10	6.168	0.013	0.012	−0.074	−0.087
11	6.309	0.007	0.007	0.067	0.074
Nominal pH value of buffer solution . .	. . 6.2 (from tables)				
Mean of all laboratories	. . 6.242				
WRC (measured) value . .	. . 6.231				

* Exceeds target.

demonstration that adequate accuracy can be achieved for the determination of pH. However, inter-laboratory tests involving buffer solutions showed that the effects of biased calibration were acceptably small in all laboratories. The participating laboratories demonstrated that, for pH determinations made on most river water samples, the required analytical precision was achieved. There is evidence that the determination of the pH value of poorly buffered waters and/or waters of low ionic strength poses special problems which will require additional investigation.[10,11] It is intended that this subject will be considered further by the Committee.

The sequential approach to AQC followed in this work involves a relatively large amount of work in each laboratory and a relatively long period to complete all tests. However, these very points provide many opportunities for unsuspected errors to be revealed, thereby facilitating recognition and elimination of problems so that a permanently sound basis is established for routine achievement of the required accuracy. Where, as with the determinands discussed here, the accuracy requirements proved to be relatively lax compared with the accuracy that could be readily achieved, the sequential approach to AQC allowed rapid progress to be made. Where the accuracy requirements are stringent relative to what is easily obtainable, this approach provides the only systematic way in which these standards of accuracy may be attained.

In making the tests described here, not all laboratories followed the agreed procedure, involving the measurement of sample pH at a fixed temperature (usually 20 °C) ±1 °C. This did not lead to any difficulty in achieving the required accuracy when buffer solutions were analysed but no evidence is available that allows this conclusion to be extended to real samples. In the absence of further information on this subject, it has been agreed by the Committee that temperature control to ±1 °C is desirable and should be applied routinely to all pH determinations for the Harmonised Monitoring Scheme, apart from any performed *in situ*.

Conclusions

The targets chosen for the accuracy of results for the determination of electrical conductivity appear to be suitable for the Harmonised Monitoring Scheme and capable of achievement for the river waters tested. It proved impossible to check fully compliance with accuracy requirements for the determination of pH, but it was demonstrated that the analytical precision was adequate for most types of sample and that the calibration bias was acceptably small. There is, however, a need for further work on the determination of pH on samples of low ionic strength and/or low buffer capacity.

Continuing care is needed to ensure that adequate accuracy is maintained. Subsequent AQC is, in addition to normal precautions, based on the use of quality control charts and the analysis of solutions distributed at intervals by the WRC.

These studies by the Committee have now demonstrated, for chloride, ammonia, total oxidised nitrogen, nitrite, suspended solids, conductivity and pH, a procedure for ensuring permanent comparability of results from a group of laboratories. It is hoped to report the results of further studies, on different determinands, in subsequent papers.

Although the analytical work reported here was performed by Regional Water Authority and River Purification Board laboratories, the coordination of the work was carried out by the Water Research Centre under contract to the Department of the Environment, whose permission to publish this paper is acknowledged.

Appendix

The following are or have been members of the Analytical Quality Control (Harmonised Monitoring) Committee in the period during which this work was performed: Dr. P. R. Hinchcliffe, Mr. J. G. Flint, Mr. L. R. Pittwell, Mr. R. Donachie, Mrs. C. Brown, Mr. N. Taylor, Dr. R. J. D. Otter and Mr. P. H. Garnett (Department of the Environment); Mr. A. L. Wilson, Mr. D. J. Dewey, Dr. D. T. E. Hunt and Mr. M. J. Gardner (Water Research Centre); Mr. M. J. Beard (Southern Water Authority); Mr. B. E. P. Clement and Mr. R. Lamb (Welsh Water Authority); Mr. N. Croft, Mr. M. G. Firth and Mr. D. Best (Yorkshire Water Authority); Dr. B. T. Croll (Anglian Water Authority); Mr. D. V. Hopkin (Thames Water Authority); Mr. J. G. Jones and Mr. A. Poole (Wessex Water Authority); Mr. B. Milford and Mr. B. Dale (South West Water Authority); Mr. T. Hooton (Scottish Development Department); Mr. J. B. Allcroft and Mr. A. Hollington (North West Water Authority); Mr. W. Wollers (Northumbrian Water Authority); Dr. K. C. Wheatstone and Mr. K. Bamford (Severn-Trent Water Authority); Mr. J. E. Saunders (Welsh Office); Mr. I. R. M. Black (Forth River Purification Board).

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Reference Electrodes for Use in the Potentiometric Determination of Chloride
Part I. Assessment of Mercury - Mercury(I) Sulphate Electrodes

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Mercury - mercury(I) sulphate reference electrodes are used in the potentiometric determination of chloride. There have been various reports of their erratic behaviour, which is especially serious because chloride-selective electrodes are often working at the limits of their sensitivity in highly pure waters, such as condensed steam and boiler water. The loss of precision in e.m.f. in such circumstances has a much larger effect on the precision in concentration terms than when electrodes are working in their usual (Nernstian) sensitivity range. Commercial mercury - mercury(I) sulphate electrodes have been tested over several weeks of continuous operation so that their performance can be assessed when used under conditions similar to those of chloride monitoring, *i.e.*, in pH 4.4 acetate buffer solution with silver chloride electrodes and 0.01 mol l^{-1} nitric acid solution with mercury(I) chloride electrodes. Electrodes were obtained from two manufacturers and were of two types—one with ground-glass sleeve liquid junctions and one with a ceramic-frit junction. The latter was the best of those tested. The rate of change of its potential with respect to a calomel electrode was less than 0.5 mV over 100 h and the standard deviation of its potential over a week's operation was about 0.3–0.5 mV. Deviations of potential previously reported with this type of electrode could not be reproduced in this work. Some electrodes with ground-glass sleeve junctions became erratic after 2–4 d in the nitric acid solution normally used with mercury(I) chloride sensing electrodes. Their performance was better in the acetate buffer medium used with silver chloride sensing electrodes but inferior to that of similar electrodes with ceramic frit junctions.

Keywords: *Chloride determination; potentiometry; mercury - mercury(I) sulphate electrodes; reference electrodes*

The determination of chloride is important for power station operations as chloride is one of the main causes of corrosion in boilers. In high-pressure drum boilers the chloride level should be below 200 μg l^{-1} and should be known so that the boiler-water chemistry can be adjusted appropriately. In once-through boilers, as in modern nuclear power stations, the feed water should contain less than 2 μg l^{-1} of chloride. Chloride levels in condensed steam can indicate condenser leaks. Continuous monitoring of the chloride level is normal for once-through boilers and becoming more common for drum boilers.

Most potentiometric methods of analysis use either silver -silver chloride or calomel reference electrodes, but leakage of concentrated (≥3 mol l^{-1}) potassium chloride solution from these electrodes makes them unsuitable for use with chloride-selective electrodes. Mercury - mercury(I) sulphate reference electrodes are generally used for chloride determinations, because leakage of their sodium or potassium sulphate filling solutions does not affect chloride-selective electrodes. Although the thermodynamics of the mercury - mercury(I) sulphate electrode are well characterised,[1] practical difficulties have been observed with commercially produced forms of reference half-cell, which have not always exhibited the expected constancy of e.m.f.[2–4]

The difficulties experienced with mercury - mercury(I) sulphate electrodes are intermittent and depend on the configuration of the liquid junction and the experimental conditions; thus Torrance and Wilson[2] and Marshall and Midgley[3] found the ground-glass sleeve type of junction preferable to the ceramic-frit type, while Richardson[5] found the opposite. These problems were not investigated systematically, but their origins may lie in the precipitation of mercury(I) chloride at the interface between the sample and reference solutions. Mercury(I) sulphate is relatively soluble ($K_s = 6 \times 10^{-7}$ mol^2 l^{-2}) and the concentrations of mercury(I) ions in 1 mol l^{-1} sodium sulphate reference solution would cause precipitation to occur with μg l^{-1} levels of chloride. On prolonged immersion of a mercury - mercury(I) sulphate reference electrode in a chloride solution, a deposit may sometimes be seen around the liquid junction.

The aim of this work was to assess the performance of commercial mercury - mercury(I) sulphate reference electrodes over several weeks of continuous use in media similar to those used in potentiometric analysis for chloride, *i.e.*, pH 4.4 ammonium acetate - acetic acid buffer for silver chloride electrodes and 0.01 mol l^{-1} nitric acid for mercury(I) chloride electrodes. Analysis for chloride in condensed steam involves the least sensitive part of an ion-selective electrode's calibration, that in which the e.m.f. is directly proportional to concentration[4,6] and precision of the e.m.f. has a larger relative effect on the precision of analysis than at higher concentrations where the e.m.f. is proportional to the logarithm of the concentration. In this respect, the performance of the mercury - mercury(I) sulphate electrode is more critical than that of the reference electrodes used for most potentiometric analyses. The products of the main manufacturers of mercury - mercury(I) sulphate electrodes available in the UK were surveyed and representative electrodes were selected.

Experimental

Apparatus

The electrodes used are listed in Table 1. The glass sleeve of electrode Q fitted badly and was replaced by a silicone-rubber sleeve recommended by the manufacturers.[7] The Pye 305 calomel electrode was taken as the master reference electrode, which completed an electrochemical cell with each of the other electrodes, and the Pye 360 was included as a check on the system, these both being well characterised types of electrodes.[8]

Table 1. Electrodes tested

Manufacturer's identification	Electrode type	Filling solution	Liquid junction	Coding
EIL 33 1370 230	$Hg-Hg_2SO_4$	1 mol l^{-1} Na_2SO_4	Frit	K, L
EIL 33 1380 230	$Hg-Hg_2SO_4$	1 mol l^{-1} Na_2SO_4	GGS*	M, N
Beckman 40455	$Hg-Hg_2SO_4$	K_2SO_4 (saturated)	GGS*†	P, Q
Pye 360	Ag-AgCl	3 mol l^{-1} KCl	Frit	R
Pye 305	$Hg-Hg_2SO_4$	3 mol l^{-1} KCl	Frit	

* GGS, ground-glass sleeve.
† The ground-glass sleeve on electrode Q was changed for one of silicone rubber after two weeks.

Measurements were made as described previously.[8,9] The electrodes were switched in turn to a Corning 110 pH meter by a modified signal multiplexer and the e.m.f.s were recorded by a data logger connected to the recorder output terminals of the pH meter.

The electrodes were placed in a Perspex flow cell housed in a cabinet and the temperatures of both the flow cell and cabinet were controlled (at 25 °C for most of the tests) as described previously.[8]

Reagents

All reagents were of AnalaR grade (obtained from BDH Chemicals).

Nitric acid, 5 mol l^{-1}. Concentrated nitric acid (317 ml) was diluted to 1 l.

Standard chloride solution, 10 000 mg l^{-1}. Sodium chloride (16.49 g) was dissolved in water and made up to 1 l in a calibrated flask.

Standard chloride solution, 1 000 mg l^{-1}. Sodium chloride (1.649 g) was dissolved in water and made up to 1 l in a calibrated flask.

Nitric acid working solutions. These were prepared in 96-l batches by dilution of 192 ml of 5 mol l^{-1} nitric acid. Some batches also contained either 96 ml of 1 000 mg l^{-1} chloride solution or 96 ml of 10 000 mg l^{-1} chloride solution.

Acetate buffer working solution. Concentrated acetic acid (114 ml) was added to about 9 l of de-ionised water, followed by 55 ml of concentrated ammonia solution (sp. gr. 0.88). The solution was allowed to cool and made up to 10 l with de-ionised water. This solution was equivalent to the mixture of sample and buffer streams in the Technicon chloride monitor[10] and had a pH of 4.4.

Procedure

The tests were carried out at 25 °C in the flow cell and cabinet described above. The test solution was pumped from the reservoir to a header above the cabinet. The solution then flowed under gravity into the flow cell at a rate of about 8 ml min^{-1} (controlled by a capillary restriction). The solution in the flow cell was stirred by a magnetic bar.

Results

Interpretation

Because every measurement of e.m.f. involves two electrodes, a strictly unambiguous assessment of the performance of individual electrodes is impossible. A practical determination of performance characteristics can be made by taking as a "master" electrode, one which is known to enable reproducible e.m.f.s to be obtained when it is used in conjunction with a variety of other electrodes. All the e.m.f. measurements in this work were made with respect to a calomel electrode, which is widely accepted as having "good" properties. Over periods of 3–14 weeks, however, even calomel electrodes are subject to variations in e.m.f.[8] and a further check on the results was desirable.

Table 2. Short-term standard deviation in 0.01 mol l^{-1} nitric acid

Electrode	Short-term standard deviation measured at close of week No.*/ mV				Range over 22 weeks	
	1	8	15	22	Min.	Max.
K	0.067	0.067	0.097	0.123	0.042	0.127
L	0.084	0.082	0.140	0.126	0.052	0.311
M	0.110	0.298	0.107	0.227	0.053	1.62
N	0.071	0.141	0.140	0.097	0.042	0.560
P	0.047	0.067	0.149	0.132	0.053	0.149
Q	†	1.77	0.133	0.490	0.047	1.77
R	0.042	0.042	0.117	0.067	0.00	0.117

* Calculated from 10 readings at 1-min intervals.
† Not available—see Table 1.

The check consisted of including in the tests a silver - silver chloride reference electrode of a type previously found satisfactory,[8] so that the performance of mercury - mercury(I) sulphate electrodes could also be judged against another type of well characterised electrode. The results obtained with the silver - silver chloride electrode are not treated in detail below, but the silver - silver chloride calomel pair gave the most constant potential of all the electrode pairs, thus confirming the choice of master electrode.

Tests in 0.01 mol l^{-1} Nitric Acid Medium for Use with Mercury(I) Chloride Electrodes

Test conditions

The recommended ionic medium for use with mercury(I) chloride electrodes is 0.01 mol l^{-1} nitric acid.[3] The test solution contained no chloride ion for weeks 1–3, 1 mg l^{-1} chloride for weeks 4–13 and 10 mg l^{-1} chloride for weeks 14–22. The chloride ion was included at fairly high levels in an attempt to accelerate the appearance of the deviations previously reported.[2–4]

Short-term variations in potential

The short-term variation in the e.m.f.s was determined from ten consecutive readings 1 min apart. The time interval was dictated by the requirements of the data logger, but in terms of continuous monitoring 10 min is a suitably short period. An extract of the results is shown in Table 2.

The standard deviations of electrodes with ceramic-frit junctions (K and L) varied over a comparatively small range and tended to change in parallel with one another, indicating the influence of a factor common to all the electrodes, *e.g.*, temperature, stirring rate or the behaviour of the master calomel reference electrode. Although the largest standard deviations occurred in weeks 15, 17, 20 and 22, there was no consistent change in the standard deviations over the period of test either with time or with chloride content.

The electrodes with sleeve-type junctions (M, N, P and O) varied considerably in performance; their standard deviations could be as small as those of the electrodes with frit junctions,

Table 3. Drift of standard potential relative to calomel electrode in nitric acid solution

Electrode	Drift rate during week No./mV per 100 h						
	1	4	7	11	15	18	22
K	0.3	−0.04*	−0.1	0.2	−0.2	−0.2*	−0.8†
L	0.3	−0.1*	−0.1	−0.1	−0.3	−0.6	−1.0*
M	−5.5	−0.4	3.0	−3.3†	−3.5†	−3.2†	−3.4†
N	0.9	−0.4	0.9	0.1	−1.0	−0.4	−2.8†
P	−1.0*	−0.8	−0.8*	−1.1†	−0.8	−1.1*	−1.2†
Q	‡	−4.0	−7.4	2.6	3.9	0.9	−2.1*
R	1.0†	0.4†	0.007	1.6†	0.5†	0.4†	0.5*

* The linear fit to the results had a correlation coefficient ($|\rho|$) >0.7.
† The linear fit to the results had a correlation coefficient ($|\rho|$) >0.9.
‡ Not available—see Table 1.

Table 4. Standard deviation of e.m.f. during 1 week in nitric acid solution

Electrode	Standard deviation during week No. */mV						
	1	4	8	11	15	18	22
K	0.3	0.2	0.3	0.2	0.2	0.9	0.4
L	0.3	0.2	0.3	0.3	0.2	0.3	0.5
M	4.1	2.5	3.1	1.5	1.9	1.7	1.7
N	2.6	2.0	2.4	1.9	1.3	1.8	1.6
P	0.4	0.4	0.6	0.5	0.6	0.6	0.6
Q	†	2.7	6.9	2.1	3.3	2.2	1.3
R	0.4	0.2	0.2	0.8	0.3	0.2	0.3

* Calculated from approximately 70 2-h readings in each week.
† Not available—see Table 1.

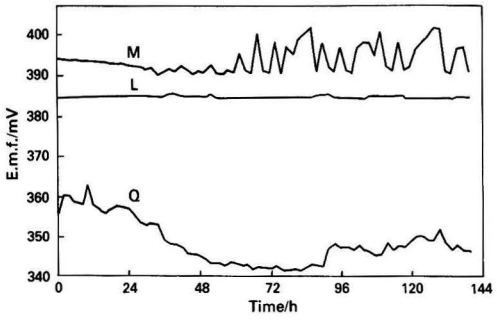

Fig. 1. Change of e.m.f. with time during seventh week of operation in 0.01 mol l^{-1} nitric acid for electrodes with (L) ceramic-frit, (M) ground-glass sleeve and (Q) silicone-rubber sleeve liquid junctions

but at times were much larger. Changes in the standard deviations were not concerted, in contrast to the electrodes with frit junctions. Electrode P, with a ground-glass sleeve, was as good as the frit electrodes, but electrode Q, with a silicone-rubber sleeve, was the worst of those tested.

Drift in standard potential

Drift is defined here as the tendency of the e.m.f. to change persistently in one direction over a given period of time. The rate of drift was determined from the gradient of a linear correlation of the 2-h readings of e.m.f. against time over a period of a week. The gradients obtained were almost always significantly different from zero (*t*-test, 95% confidence limits). An extract of the results is shown in Table 3 and the drift over a typical week's operation is shown in Fig. 1 for three electrodes.

Electrodes with ceramic-frit junctions (K, L) had small rates of drift, rarely exceeding 1 mV per 100 h in any one week. Electrode P with a ground-glass sleeve junction was almost as good, but the other electrodes with sleeve junctions (M, N and Q) had high rates of drift, often changing in direction and magnitude from week to week. Fig. 1 shows that the linear drift rates for electrodes M and Q were only rough indicators of performance, as potentials fluctuated widely within the period of test. Electrodes with low rates of drift did not show these fluctuations (*e.g.*, curve L in Fig. 1).

Fluctuations of electrode potential

Fluctuations such as those for curves M and Q in Fig. 1 can lead to as much analytical error as does drift. As a measure of this source of error, the standard deviations of the 70 or so 2-h readings taken each week were calculated. These results contain contributions from short-term (random) variations, from systematic changes (drift) and from long-term fluctuations, which may not be random.

The results in Table 4 confirm the impression given by the results for drift; the ground-glass sleeve electrodes M and N and electrode Q with the silicone-rubber sleeve showed much the largest variations. Electrode P with the ground-glass sleeve junction was very consistent, while the smallest variations were shown by frit electrodes K and L.

Effect of temperature

The temperature in the flow cell was raised or lowered in 5 °C steps over the range 20–30 °C, two cycles being completed. The steady e.m.f. of each electrode at each temperature was noted. The temperature coefficient of each type of electrode with respect to the 3 mol l^{-1} calomel electrode is shown in Table 5. These results may be converted to the standard hydrogen electrode scale by adding −0.4 mV K^{-1}. Fig. 2 shows that the e.m.f. of the mercury - mercury(I) sulphate electrode follows the temperature change very closely.

Table 5. Temperature coefficients at 25 °C

Electrode	Electrode filling solution	Temperature coefficient*/ mV K^{-1}	Standard deviation†/ mV K^{-1}
K–N	1 mol l^{-1} Na_2SO_4	−0.39	0.02
P	Saturated K_2SO_4	−0.37	0.03

* Mean of all results for each type of electrode.
† Standard deviation of the mean temperature coefficients.

Table 6. Short-term deviations in acetate buffer solution

	Short-term standard deviation at close of week No. */mV		Range over 10 weeks	
Electrode	1	10	Min.	Max.
K	0.082	0.084	0.047	0.110
L	0.063	0.042	0.042	0.162
M	0.071	0.094	0.052	0.106
N	0.108	0.063	0.048	0.157
R	0.067	0.092	0.067	0.216
M_S	0.092	0.105†	0.032	0.105†

* Calculated from 10 readings at 1-min intervals.
† Tests terminated after 8 weeks.

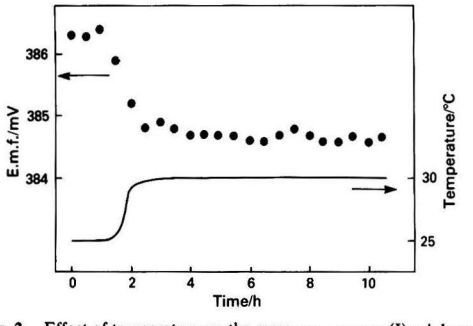

Fig. 2. Effect of temperature on the mercury - mercury(I) sulphate electrode in nitric acid solution

Tests in Acetate Buffer Solution for Use with Silver Chloride Electrodes

The ionic medium generally used in the Central Electricity Generating Board (CEGB) for measurements with silver chloride electrodes is an ammonium acetate - acetic acid buffer solution at pH 4.0–5.0.[2,6,10] The test solution was 0.2 mol l^{-1} acetic acid half neutralised with ammonia solution.

Only electrodes K–N were tested. The earlier performance of electrode Q was too poor to warrant further work and electrode P gave similar results in 0.01 mol l^{-1} nitric acid solution to the ceramic-frit electrodes. In a separate series of tests, the performance of electrode M was assessed with the breather hole closed, so as to simulate a sealed reference electrode. For brevity, the results with this electrode, designated M_S, have been included in the following sections because the test conditions were the same.

Short-term variations in potential

The short-term variance in the e.m.f.s. was determined from ten consecutive readings 1 min apart. Table 6 shows an extract of the results, which did not differ greatly in magnitude from those obtained in 0.01 mol l^{-1} nitric acid, although the spread of results was smaller. The standard deviations obtained from electrodes with ground-glass sleeves (M, N) were no worse than those from similar electrodes with ceramic frits (K, L), in contrast to the results in the nitric acid medium.

Table 7. Drift of standard potential relative to calomel electrode in acetate buffer solution

	Drift rate during week No. /mV per 100 h			
Electrode	1	2	6	10
K	−3.1*	−0.2	0.4†	−0.6†
L	−3.5*	0.01	0.6†	−0.5*
M	−4.1*	−0.6*	0.6	−0.1
N	−3.8*	−0.2	4.4*	−0.8†
R	−3.9*	−0.1	1.3*	−0.01
M_S	7.7†	−2.4	−2.1	—

* Linear fit gave correlation coefficient ($|\rho|$) >0.9.
† Linear fit gave correlation coefficient ($|\rho|$) >0.7.

Table 8. Standard deviation of e.m.f. during 1 week in acetate buffer solution

	Standard deviation during week No. */mV			
Electrode	1	2	6	10
K	1.7	0.1	0.1	0.3
L	1.9	0.1	0.2	0.2
M	2.2	0.3	0.6	0.8
N	2.1	0.2	1.2	0.4
R	2.1	0.1	0.4	0.1
M_S	4.0	2.4	1.7	—

* Calculated from approximately 70 2-h readings in each week.

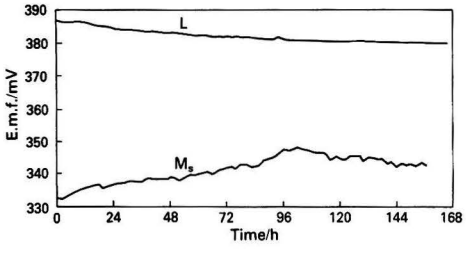

Fig. 3. Change of e.m.f. during the first week of the test for (M_S) sealed and (L) ceramic-frit electrodes

Drift of standard potential

In general, the same considerations apply as in nitric acid solutions. Table 7 gives an extract of the results obtained from a linear correlation of e.m.f. against time. The superior performance of the ceramic-frit junction over the ground-glass sleeve junction is less pronounced than in nitric acid solutions (Table 3). Electrode M_S, however, showed larger rates of drift than before it was sealed. Fig. 3 shows the change of e.m.f. with time for electrode M_S compared with that for electrode L.

Fluctuations of electrode potential

As for nitric acid medium, the standard deviation of the 70 or so 2-h readings collected each week was taken as an indication of instability, including drift, random noise and long-term fluctuations. An extract of the results (Table 8) shows that all the mercury - mercury(I) sulphate electrodes and particularly those in ground-glass sleeves showed less variability than in nitric acid solutions. Comparison of Tables 3 and 7 suggests that changes in the rate of drift do not account for the reduction in variability (except for electrode M). Fluctuations such as those seen in graph M of Fig. 1 were not observed in the acetate medium and hence the variability was lower. The results for the sealed electrode M_S were much higher than for the others, showing the effect of the slow fluctuations visible in Fig. 3.

Effect of temperature

The mercury - mercury(I) sulphate electrodes behaved similarly in the acetate and nitric acid media and the temperature coefficient in the acetate medium (−0.45 mV K^{-1}) was close to that obtained in nitric acid (Table 5).

Discussion

Electrode Performance

In all these tests, electrodes K and L with ceramic-frit junctions performed very well; the rate of change of the standard potential with respect to a calomel electrode was less than 0.5 mV over 100 h and the standard deviation of the e.m.f. over a week's operation was about 0.3–0.5 mV. These results are almost as good as obtained previously for the best calomel and silver - silver chloride reference electrodes[8] and as good as those for the silver - silver chloride electrode included in these tests for comparison. The electrodes required no attention beyond topping up the internal filling solution every 6–8 weeks, but there is a potential problem with the tendency of sodium sulphate decahydrate to precipitate from the solution. The concentration of 1 mol l^{-1} sodium sulphate used in the EIL electrodes is close to saturation at 25 °C and over a period of weeks enough water evaporates through the breather hole for crystals to appear inside the electrode. At temperatures below about 18 °C, sodium sulphate tends to precipitate from 1 mol l^{-1} solution. It is possible for these crystals to form a plug that will impede the flow of solution through the frit, leading to less stable e.m.f.s. (see below). If the plug is formed between the mercury - mercury(I) sulphate element and the frit, it is possible for the electrical resistance to rise so much that the system is effectively open-circuit, although this did not occur during these tests. The use of 0.5 mol l^{-1} sodium sulphate solution[4] would avoid these problems.

Electrodes M and N with ground-glass sleeve junctions did not perform so well, especially in the nitric acid medium. These electrodes tended to give erratic e.m.f.s after 2–4 d of continuous operation. This may be because the internal filling solution emptied relatively quickly (needing to be replenished every week), so that towards the end of the week there was insufficient pressure to maintain a flow of solution through the junction. When the solution was prevented from flowing freely by closing the electrode's breather hole, the performance was much worse than before and previous experience[4] showed that good results were obtained in continuous operation if the electrode had a 50-cm head of filling solution so as to maintain the flow.

The ground-glass sleeve on one of the electrodes (Q) fitted very poorly, so that the electrode drained dry in about 2 d and went open-circuit. Replacement of the ground-glass sleeve with a silicone-rubber sleeve according to the manufacturer's instructions gave an electrode of markedly inferior performance to all the others tested. This electrode had a low rate of loss of filling solution and behaved similarly to the EIL ground-glass sleeve electrode when it had its breather hole sealed (M_S). Electrode P with a properly fitting ground-glass sleeve was better than the other ground-glass sleeve electrodes, although not as good as the electrodes with ceramic frits. At first, it needed to be topped up weekly with filling solution, but in the sixth week the level scarcely decreased. Crystals of potassium sulphate had formed round the spacer holding the mercury - mercury(I) sulphate element in place, and so sealed the electrode. No change in performance was apparent once the electrode had become sealed, but this condition was not allowed to persist and the electrode was unblocked. The saturated filling solution used in electrode P is more likely to give trouble of this sort than the unsaturated solutions of electrodes K–M. Another cause of inconvenience with electrode P is that the sleeve can drop off. This did not occur when the electrode was in place in the flow cell, but it needed to be handled more carefully than the other designs.

The general performance of mercury - mercury(I) sulphate electrodes with ceramic-frit junctions contradicts previous experience in this laboratory, where the ground-glass sleeve junction had been preferred.[2,3] Experience with plant operation, however, has favoured the ceramic-frit type. It is inferred that there are considerable variations between batches of commercially produced electrodes and also between individuals of the same batch (*e.g.*, electrodes P and Q).

Results with electrodes having a low rate of outflow of reference solution indicate that sealed reference electrodes would probably not be suitable for continuous analysis; similar conclusions were drawn[8] for the calomel electrode commonly used in other potentiometric analyses. For continuous industrial analysis, it would be convenient if mercury - mercury(I) sulphate reference electrodes could be fitted with side-arms for connection to a reservoir of sulphate solution.[4] Apart from reducing the frequency with which the electrode has to be refilled, such a reservoir would help to maintain the flow of solution through the liquid junction, which the present work shows to be important for optimising the performance of these reference electrodes.

Badly manufactured ceramic frits or ground-glass sleeves can result in unstable liquid junctions and hence variable potentials.[8] The potentials will vary more, the larger is the difference between the mobility of anions and cations in solution, as is apparent from a consideration of liquid-junction potentials. As the ratio of the mobilities of hydrogen and nitrate ions is about 5 : 1, while for ammonium and acetate ions it is less than 2 : 1, greater variability would be expected in nitric acid solution than in ammonium acetate solution and this was observed. The variation of the liquid-junction potential with pH did not agree very well with that calculated by the Henderson equation,[11] *e.g.*, in nitric acid medium the e.m.f. of an electrode with a ground-glass sleeve junction (M) changed almost linearly by −15 mV per pH unit in the pH range 1.8–2.2, compared with the predicted −6 mV per pH unit. Over ranges larger than about 0.4 pH unit, the non-linearity of the relationship became obvious. The addition of inert electrolytes such as potassium nitrate or potassium chloride to 0.01 mol l^{-1} nitric acid solution caused increases of about 0.5 mV per 10 mmol l^{-1} of salt, which agreed with Henderson equation calculations.

Consequences for Chloride Analysis

The results show that reference electrodes are commercially available having acceptably constant e.m.f.s. for use in determining chloride. Of the electrodes tested, those with ceramic-frit junctions were more suitable for use in continuous monitoring as those with ground-glass sleeve junctions needed frequent attention if errors were to be avoided. The erratic behaviour reported[3] with both ceramic-frit and ground-glass sleeve electrodes in nitric acid solution was not reproduced in this work, although the fluctuating e.m.f.s of some ground-glass sleeve electrodes in nitric acid solution seemed similar except for the lower frequency.

Acceptable performance could be obtained in the nitric acid medium used with mercury(I) chloride sensing electrodes[3,4] and the acetate buffer which in CEGB practice[6] is used with silver chloride sensing electrodes. Because the reference electrodes generally gave more constant e.m.f.s in the acetate buffer, this medium should be preferred for use with silver chloride electrodes. It is not, however, suitable for use with the mercury(I) chloride electrodes, which are generally used at the lowest levels of chloride.

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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Reference Electrodes for Use in the Potentiometric Determination of Chloride
Part II.* Quinhydrone Electrodes

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Reference electrodes consisting of platinum or gold electrodes immersed in solutions of constant pH saturated with quinhydrone have been tested for use in potentiometric analysis for chloride. The solution inside the reference electrode is matched in composition with the main components of the treated sample solution (*i.e.*, after addition of reagents to control pH and ionic strength but excluding the determinand). In this way the effect of the liquid-junction potential is minimised. The electrodes have a sealed construction and need no maintenance.

The potentials of newly made electrodes change at a greater rate than those of the mercury - mercury(I) sulphate reference electrodes normally used in chloride analysis, but after 3–4 weeks the rate of change of potential is similar to that obtained from the best commercial mercury - mercury(I) sulphate electrodes. Older electrodes develop anomalous temperature responses.

The quinhydrone reference electrode is comparable to the best mercury - mercury(I) sulphate electrodes in media used with mercury(I) chloride sensing electrodes (typically 0.01 mol l^{-1} nitric acid). In the media of higher pH used with silver chloride sensing electrodes (typically pH 4.3–5.0 acetic acid - ammonium acetate buffer solution), the performance of the mercury - mercury(I) sulphate electrode is improved, while that of the quinhydrone electrode deteriorates; the former would, therefore, generally be preferred.

This work has provided the basis for similar but improved electrodes, using more chemically stable constituents, which will be described in Part III.

Keywords: *Chloride determination; potentiometry; quinhydrone electrodes; reference electrodes*

The reference electrode is a necessary part of any electrochemical cell used in potentiometry and its properties often have a very significant influence on the characteristics of a potentiometric method. Thus, it has been demonstrated for mercury - mercury(I) sulphate electrodes[1] and calomel and silver - silver chloride electrodes[2,3] that the configuration of the reference electrode affects both the short-term precision and long-term stability of e.m.f. measurements. In addition, the reference electrode is generally as significant as the sensing electrode in contributing to the temperature coefficient of the cell.

Conventional reference electrodes consist of metal - metal salt electrodes immersed in concentrated solutions of the corresponding sodium or potassium salts separated from the test solution by a physical barrier that permits minimum mass transport of solution out of the electrode and enables electrical contact to be maintained by electrolytic diffusion. This latter part of the electrode, the liquid junction, is the most common source of unreliable e.m.f.s; badly constructed junctions may allow excessive mass transport of solution across the junction or be of excessively high electrical resistance. In either instance the performance of the potentiometric cell will not be optimal and may be unacceptable. Even good junctions may deteriorate with use, mainly because of chemical deposition in the pores; this has been demonstrated for silver - silver chloride[3] and mercury - mercury(I) sulphate electrodes.[4,5]

Comparable problems rarely arise from the inner metal - metal salt electrode unless it is allowed to dry out. This, however, means that the maintenance requirements of the reference electrode are generally greater than those of the sensing electrode, at least for glass and solid-state electrodes. Sealed, maintenance-free, reference electrodes have been made, but their performance has hitherto been markedly inferior to that of conventional designs, which permit a small outflow of solution from the electrode.[1–3]

The aim of this work was to devise reference electrodes that would avoid the problems associated with conventional reference electrodes without sacrificing their good points. The following properties were desirable.

(*i*) The inner electrode should give a potential that can be established reproducibly whenever and wherever the electrode is made.

(*ii*) The potential of this electrode should be stable in a solution of constant composition for several weeks if not months.

(*iii*) The electrode, once assembled as a whole, should require no maintenance.

(*iv*) The electrode as a whole should have a relatively low resistance, *i.e.*, <50 kΩ.

(*v*) The temperature dependence of the electrode potential should be similar to that for the ion-selective electrode being used (typically 0.2–0.5 mV K^{-1}) and should not suffer from serious hysteresis effects.

(*vi*) The liquid-junction potential between the inner reference solution and the outer test solution should be constant, and preferably zero.

(*vii*) The inner reference solution should not contain substances that might interfere with the sensing electrode or react with substances in the test solution such that the e.m.f. would be influenced by that reaction.

(*viii*) It should be possible to use either the same electrode with a very wide range of test solutions, or it should be possible to adjust the electrode precisely to suit a specific type of test solution. The balance of advantage depends on the application. Versatility is often very important for the measurement of widely ranging parameters such as pH and redox potential and in potentiometric titrations, because the sample often changes considerably during the course of the titration. For direct potentiometry with ion-selective electrodes, however, it may be advantageous to have a reference electrode specifically designed for each type of analysis.

* For Part I of this series see p. 439.

In this work, emphasis was placed on devising reference electrodes for use with chloride-selective electrodes. In analytical methods using ion-selective electrodes, it is desirable for solutions to have a constant ionic strength and a reasonably high specific conductivity (typically >100 μS cm^{-1}). If the sample solutions do not meet these requirements, it is normal to add an inert electrolyte in order to achieve the desired conditions. In many instances it is also necessary to control the pH of the solution in order to prevent direct interference by hydrogen or hydroxide ions or indirect interference caused by hydrolysis of metal ions or protonation of anions. In most instances the pH is also the most important factor in determining the liquid-junction potential. The systems proposed in this report rely on the control of the sample pH. Although this work was confined to reference electrodes for use in chloride determinations, the principles involved should be applicable to almost any potentiometric system in which the pH is controlled. Chloride electrodes are used widely, and problems with reference electrodes in these systems have been reported.[4,5] Because these electrodes are generally working in their limiting response ranges,[6] the precision of the measurements is particularly important.

The first system chosen was based on quinhydrone (benzoquinhydrone), which has been extensively used for pH measurement.[7] It was expected to meet requirements (*i*), (*iii*), (*iv*), (*vi*), (*vii*), (*viii*) and the temperature coefficient part of (*v*), but the long-term stability (*ii*) was unknown, as was the temperature hysteresis part of (*v*). This paper describes the results of tests with the new reference electrodes in conditions similar to those used in potentiometric analysis for chloride, *i.e.*, in pH 4.5 ammonium acetate - acetic acid buffer solution for silver chloride electrodes and 0.01 mol l^{-1} nitric acid for mercury(I) chloride electrodes.

Theory

Quinhydrone is a 1:1 molecular complex of *p*-benzoquinone (quinone) and its hydroquinone (quinol). When dissolved in water, these two components form an electrochemically reversible oxidation - reduction system in which hydrogen ions also participate:

$$C_6H_4O_2 + 2H^+ + 2e^- \rightleftharpoons C_6H_4(OH)_2$$

If a platinum or gold electrode is immersed in the solution, its potential is given by equation (1).

$$E = E^\circ + \frac{k}{2}\log\frac{a_Q(a_H)^2}{a_{QH_2}} = E^\circ + \frac{k}{2}\log\frac{c_Q}{c_{QH_2}} + \frac{k}{2}\log\frac{f_Q}{f_{QH_2}} - k.\text{pH} \quad \ldots (1)$$

where the subscripts Q and QH_2 refer to quinone and hydroquinone, respectively, and *a*, *c* and *f* are activities, concentrations and activity coefficients, respectively. As the two components are added as the complex quinhydrone, $c_Q = c_{QH_2}$ and, as they are non-electrolytes, $f_Q = f_{QH_2} = 1$ so the second and third terms on the right-hand side of equation (1) are equal to zero. It can now be seen that the quinhydrone electrode acts as a pH electrode.

A platinum electrode immersed in a solution of constant pH saturated with quinhydrone should, therefore, constitute an electrode of invariant potential. Over long periods of time, however, the potential may change. The two factors most likely to cause such changes are inter-diffusion of the sample and reference solutions, which would change the pH itself, and reaction of either quinone or hydroquinone with substances dissolved in the reference solution, so changing the ratio c_Q/c_{QH2} in equation (1).

The first of these factors may be eliminated almost completely by matching the solution inside the reference electrode to that outside. The composition of the solution in which the electrodes are immersed is dominated by reagents added to control the pH and ionic strength of this solution. The solution inside the reference electrode should contain the same reagents in the same concentrations as in the external solution, but with the addition of an excess of quinhydrone.

Experimental

Apparatus

Experimental electrodes

The electrodes used are listed in Table 1. In each instance a commercially produced electrode was the structural basis of the experimental electrode. EIL RJ23/1 electrodes (A and B) were modified by removing the mercury and mercury(I) chloride from the element and then cutting away the empty glass sheath from around the platinum contact. The other electrodes were modified Russell series SR electrodes, the usual calomel element being replaced by a 1.25 mm diameter platinum or gold wire. In both types of electrode the breather hole in the seating of the element was sealed with silicone rubber and the liquid junction was formed at a ceramic frit.

Commercial reference electrodes. The Pye 305 calomel electrode (3 mol l^{-1} potassium chloride filling) was taken as the master reference electrode, which completed an electrochemical cell with each of the other electrodes and the Pye 360 silver - silver chloride electrode (3 mol l^{-1} potassium chloride filling) was included as a check on the system, these being well characterised types of electrodes with ceramic-frit junctions.[2] Commercial mercury - mercury(I) sulphate reference electrodes with both ceramic-frit and ground-glass sleeve junctions were tested simultaneously, as described elsewhere,[1] so that direct comparisons of the two types of electrode could be made.

Instrumentation. Measurements were made as described previously.[2,8] The electrodes were switched in turn to a Corning 110 pH meter by a modified signal multiplexer and the e.m.f.s were recorded by a data logger connected to the recorder output terminals of the pH meter.

The electrodes were placed in a Perspex flow cell housed in a cabinet and the temperatures of both the flow cell and cabinet were controlled (at 25 °C for most of the tests).

Reagents

All reagents were of AnalaR grade (obtained from BDH Chemicals).

Nitric acid, 5 mol l^{-1}. Concentrated nitric acid (317 ml) was diluted to 1 l.

Standard chloride solution, 10000 mg l^{-1}. Sodium chloride (16.49 g) was dissolved in water and made up to 1 l in a calibrated flask.

Standard chloride solution, 1000 mg l^{-1}. Sodium chloride (1.649 g) was dissolved in water and made up to 1 l in a calibrated flask.

Table 1. Experimental reference electrodes

Filling solution*	Electrode material	Coding
0.01 mol l^{-1} nitric acid	Pt	A, B, V, X, Y
0.01 mol l^{-1} nitric acid	Au	U
0.1 mol l^{-1} acetic acid + 0.1 mol l^{-1} sodium acetate	Pt	C, D
0.1 mol l^{-1} acetic acid + 0.1 mol l^{-1} ammonium acetate	Pt	E, F, G
0.1 mol l^{-1} acetic acid + 0.1 mol l^{-1} ammonium acetate	Au	W
0.15 mol l^{-1} potassium hydrogen phthalate adjusted to pH 4.4 with potassium hydroxide	Pt	H, J

* The solutions were saturated with quinhydrone.

Nitric acid working solutions. These were prepared in 96-l batches from 192 ml of 5 mol l^{-1} nitric acid made up with de-ionised water. Some solutions also contained 96 ml of 1 000 mg l^{-1} or 96 ml of 10 000 mg l^{-1} chloride solution.

Acetate buffer working solution. Concentrated acetic acid (114 ml) was added to about 9 l of de-ionised water, followed by 55 ml of concentrated ammonia solution (sp.gr. 0.88). The solution was allowed to cool and made up to 10 l with de-ionised water. This solution is equivalent to the mixture of sample and buffer streams in the Technicon chloride monitor.[9]

Electrode filling solutions

Nitric acid filling solution. An excess of quinhydrone was added to a portion of the chloride-free nitric acid working solution.

Ammonium acetate filling solution. An excess of quinhydrone was added to a portion of the acetate buffer working solution.

Sodium acetate filling solution. Sodium acetate trihydrate (6.80 g) was dissolved in about 400 ml of de-ionised water. Glacial acetic acid (2.85 ml) was added and the whole was made up to 500 ml. A glass electrode was immersed in the solution and more glacial acetic acid was added dropwise until the pH was the same (4.3) as in the acetate buffer working solution. An excess of quinhydrone was then added.

Phthalate filling solution. Potassium hydrogen phthalate (7.66 g) was dissolved in about 220 ml of de-ionised water. Solid potassium hydroxide was added until the pH of the solution was the same as that of the acetate buffer working solution. The solution was made up to 250 ml and an excess of quinhydrone was added. The concentration of potassium hydrogen phthalate was chosen so as to give a solution of approximately the same osmotic pressure as the acetate buffer working solution.

Procedure

Most of the tests were carried out in the flow cell and cabinet described above and any exceptions were noted. The test solution was pumped from the reservoir to a header above the cabinet. The solution then flowed under gravity into the flow cell at a rate of about 8 ml min^{-1} (controlled by a capillary restriction). This arrangement avoided the electrical noise associated with pumping. The solution in the flow cell was stirred by a magnetic bar.

Results

Interpretation

Because every measurement of e.m.f. involves two electrodes, a strictly unambiguous assessment of individual electrodes is impossible. A practical determination of performance characteristics was obtained by measuring the e.m.f. with respect to a calomel electrode that was known from previous studies[1,2] to have good properties (as judged by several different criteria). A further check consisted of including in the tests a silver - silver chloride reference electrode previously found satisfactory,[2] so that the performance of the quinhydrone electrodes could be judged against that of a well characterised electrode. The results obtained with the silver - silver chloride electrode[1] confirmed the choice of master electrode.

Electrodes for Use With Mercury(I) Chloride Sensing Electrodes

The recommended ionic medium for use with mercury(I) chloride electrodes is 0.01 mol l^{-1} nitric acid.[4,5] The quinhydrone electrodes tested were A, B, U, V, X and Y from Table 1.

Effect of pH

The effects of variation in pH were tested by injecting portions of 1 mol l^{-1} nitric acid solution into 50-ml portions of de-ionised water in the cell containing electrode V and the calomel electrode. Fig. 1 shows that the e.m.f. of the quinhydrone electrode varied in accordance with the liquid-junction potential calculated from Henderson's equation.[7]

Effect of ionic strength

The effect of varying the ionic strength was tested by injecting portions of a solution containing 3 mol l^{-1} potassium chloride and 0.01 mol l^{-1} nitric acid into 50 ml of 0.01 mol l^{-1} nitric acid. The e.m.f. of electrode V was measured with respect to the calomel electrode. Changes in e.m.f. were not discernible (*i.e.*, <0.1 mV) until more than 2.5×10^{-3} mol l^{-1} of chloride had been added. The over-all change for the addition of 0.22 mol l^{-1} chloride was +0.6 mV compared with −0.4 mV calculated from the Henderson equation.[7]

Short-term variance

The short-term variation in e.m.f. for electrodes A and B was determined from ten consecutive readings 1 min apart. The time interval was dictated by the requirements of the data logger, but in terms of continuous monitoring 10 min is a suitably short period. An extract of the 22 weeks' results is shown in Table 2. The standard deviations varied over a comparatively small range and tended to change in parallel with one another, indicating some common sensitivity to external factors, perhaps temperature, stirring rate or the master calomel reference electrode. Although the largest standard deviations occurred in weeks 15, 17, 20 and 22, there was no consistent change in the standard deviations over the period of test. These standard deviations were as small as those obtained[1] with the silver - silver chloride and the best of the mercury - mercury(I) sulphate reference electrodes.

Drift of standard potential

Drift is defined here as the tendency of the e.m.f. to change persistently in one direction over a given period of time. Comparing successive readings (2 h apart) might give one measure of drift, but in no instance was the drift calculated in this way significantly different from zero (*t*-test, 95% con-

Table 2. Short-term standard deviations in 0.01 mol l^{-1} nitric acid

	Short-term standard deviation during week No. */mV					
					1–22	
Electrode	1	8	15	22	Min.	Max.
A	0.067	0.067	0.106	0.170	0.048	0.170
B	0.053	0.074	0.129	0.088	0.042	0.129

* Calculated from 10 readings at 1-min intervals.

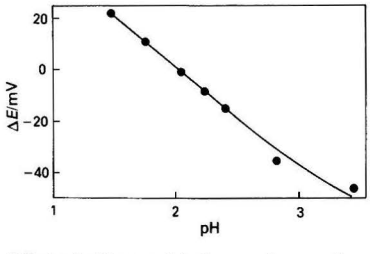

Fig. 1. Effect of pH on quinhydrone reference electrode with 0.01 mol l^{-1} nitric acid filling solution. The solid line shows the calculated change in liquid junction potential

fidence limits), *i.e.*, these differences reflect short-term noise rather than a long-term effect such as the drift. Even when readings 24 h apart were compared, drifts significantly different from zero could not always be discerned, and it was concluded that the interpretation of these 24-h results was still being obscured by random errors.

When the e.m.f.s were linearly correlated against time over a week, the gradient obtained was almost always significantly different from zero (*t*-test, 95% confidence limits). This gradient was, therefore, taken as the best indication of the drift and an extract of the results is shown in Table 3. The potentials of the quinhydrone electrodes drifted at higher rates than those of the best mercury - mercury(I) sulphate electrodes[1] for the first 5 weeks of the trial, but thereafter the rates were similar. The worst mercury - mercury(I) sulphate electrodes had much higher and less consistent drift rates than the quinhydrone electrodes.

Tests with quinhydrone reference electrodes incorporating organic acid solutions (below) showed much higher initial rates of drift than were observed in these tests with quinhydrone - nitric acid electrodes. The quinhydrone - nitric acid electrodes were prepared about 6 weeks before the long-term tests started and so a period of rapidly changing standard potentials might have been missed. New quinhydrone - nitric acid reference electrodes U and V were prepared and immediately tested. The initial rate of drift of standard potential (which persisted steadily for 4 weeks) was −3.6 mV per 100 h for both electrodes, which was much smaller than was observed with organic acid filling solutions (Tables 7 and 9).

The use of a gold inner electrode in electrode U made no difference to the rate of drift. This was tried because platinum has been reported[7] as catalysing the oxidation of hydroquinone, which might have accounted for at least some of the drift in potential. In a further trial with electrodes in which the filling solutions had (X) or had not (Y) been purged with nitrogen before use, the drift rates were again identical.

Fluctuation of electrode potentials

When mercury - mercury(I) sulphate reference electrodes were tested,[1] the standard deviation of the 70 or so 2-h readings was taken as a measure of the constancy of the electrode potential. This was done because those electrodes often showed not a consistent drift, but one combined with fairly large and long-lasting fluctuations in potential. Because the potentials of the quinhydrone electrodes drifted consistently in one direction, without large fluctuations, this measure is not necessary to describe the electrode performance. It is, however, included here for comparison with the mercury - mercury(I) sulphate electrodes. For electrodes A and B after week 1 of the results shown in Table 3, the standard deviation of the 2-h readings was in the range 0.2–0.5 mV throughout the test, which is as good as that observed with the best mercury - mercury(I) sulphate electrodes and much better than with the worst.

Effect of temperature

The temperature in the flow cell was varied in steps over the range 15–35 °C, two cycles being completed. The steady e.m.f. at each temperature was noted and the temperature coefficients with respect to the 3 mol l^{-1} calomel electrode are shown in Table 4. The results may be converted to the standard hydrogen scale by subtracting 0.4 mV K^{-1}.

The potentials of electrodes U and V followed the temperature closely but the coefficient was larger than that calculated from literature values.[7] Electrodes A and B, however, exhibited anomalous behaviour. Although their potentials first changed rapidly in the expected direction, once the temperature had steadied the change in e.m.f. reversed direction, so that the starting potential was passed again and the final change was opposite to the initial change. Fig. 2 shows the course of such a change for electrode B. The temperature coefficient calculated from the maximum change in the initial direction is reported as the "peak" coefficient in Table 4 and that calculated from the final potential is reported as the "steady" coefficient.

The reason for the reversal in potential is not known. Temperature-dependent chemical equilibria involving quinone, hydroquinone or hydrogen ions could be the cause, but would not be expected in the quinhydrone-saturated nitric acid solution unless some unknown impurities were present. Any reactions that did occur were apparently reversible, as the e.m.f. returned to its original value when the initial temperature was regained. Slow recovery after a change in temperature was observed for sealed calomel reference electrodes containing saturated potassium chloride solutions.[2] In these electrodes the concentration of dissolved chloride determines the potential, so that a slowly attained solubility equilibrium in the interior of the electrode can explain the long recovery times. Because the potential of the quinhydrone electrode is determined by the ratio of quinone and hydroquinone concentrations, it should not be affected by the solubility of quinhydrone in a way comparable to the effect of potassium chloride on the calomel electrode.

Table 3. Drift of standard potential relative to the calomel electrode in nitric acid solution

	Drift rate during week No. /mV per 100 h						
Electrode	1	4	8	11	15	18	22
A	−1.6*	−0.6†	−0.8†	−0.05	−0.4†	−0.7*	−0.4†
B	−1.6*	−0.6	−0.8†	−0.3†	−0.4	−0.7*	−0.4†

* |Correlation coefficient| > 0.9.
† |Correlation coefficient| >0.7.

Table 4. Temperature coefficients (*versus* 3.0 mol l^{-1} calomel electrode) in 0.01 mol l^{-1} nitric acid

Electrode	Temperature coefficient/ mV K^{-1}	Standard deviation*/ mV K^{-1}
U, V	−0.63	0.05
A, B		
Peak†	−0.53	0.03
Steady†	+0.26	0.03
Calculated	−0.38	—

* Standard deviation of the mean of 2 determinations for each electrode.
† See text.

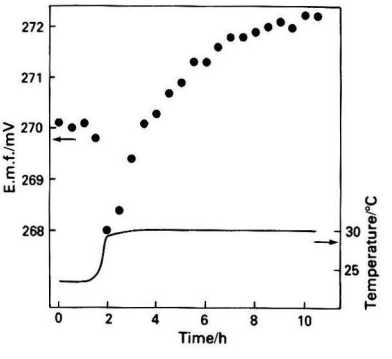

Fig. 2. Anomalous temperature response of aged electrode (B) in 0.01 mol l^{-1} nitric acid medium

Electrodes U and V, which behaved as expected, were 6–8 weeks old when the temperature coefficients were determined. Electrodes A and B, however, were over 6 months old. When electrode A was disassembled, the inner platinum electrode had a tarry coating. A possible explanation is that the quinone and hydroquinone formed electroactive polymers, the existence of which might account for the slow reversal in potential, either because of changes in solubility (*cf.*, the saturated calomel electrode above) or because the electron-transfer reactions at the electrode are themselves slow. The accumulation of such products would have been much greater in the older electrodes A and B than in U and V.

The error produced by making measurements at a temperature different from that at which calibration took place can only be assessed if the sensing and reference electrodes are considered simultaneously. With a mercury(I) chloride sensing electrode, the errors with a new quinhydrone electrode such as V should be smaller than with a mercury - mercury(I) sulphate reference electrode. Compared with aged quinhydrone electrodes, however, the mercury - mercury(I) sulphate electrode would be much better, because of the time it takes the aged quinhydrone electrode to reach equilibrium after a change in temperature; during this time the precision of analysis of spot samples would be reduced and a spurious drift could be indicated in continuous analysis.

With a silver chloride sensing electrode the errors caused by temperature changes would be less with a mercury - mercury(I) sulphate reference electrode, because its temperature coefficient is closer to that of the sensing electrode.

Precision in manual analysis

The precision of measuring electrode potentials in flowing streams of constant composition has been discussed above. In manual analysis there is an additional source of variability arising from the need to establish a new liquid junction every time the electrode is removed from one solution, rinsed and placed in another solution.

The within-batch standard deviations of measurements with a mercury(I) chloride sensing electrode (Ionel SL-01) used in conjunction with both a quinhydrone electrode (B) and a mercury - mercury(I) sulphate electrode (M from the preceding paper[1]) were determined at three chloride concentrations (0.1, 0.5 and 1 mg l^{-1}). Measurements were made with five portions of each solution, in random order. The procedure was that of Marshall and Midgley.[4]

The results in Table 5 show that the precision of measurements made with the quinhydrone reference electrode is at least as good as that obtained with mercury - mercury(I) sulphate electrodes.

Table 5. Precision in manual analysis

Electrode	Within-batch standard deviation/mV: 0.1 mg l^{-1} Cl^-	0.5 mg l^{-1} Cl^-	1 mg l^{-1} Cl^-
Quinhydrone*	0.54	0.60	0.16
Mercury - mercury(I) sulphate*	1.04	0.38	0.30
Mercury - mercury(I) sulphate†	0.63	0.93	0.83

* Measured with respect to Ionel SL-01 chloride electrode, 4 degrees of freedom (this work).

† Measured with respect to Růžička Selectrode, 5 degrees of freedom.[4]

Table 6. Short-term standard deviations in acetate buffer solution

Electrode	Short-term standard deviation during week No. */mV: 1	10	1–10: Min.	Max.
C	0.053	0.053	0.032	0.085
D	0.052	0.095	0.052	0.095
E	0.097	0.097	0.032	0.097
F	0.057	0.070	0.032	0.125

* Calculated from 10 1-min readings.

Table 7. Drift of standard potential relative to calomel electrode in acetate buffer solution

Electrode	Drift rate during week No./mV per 100 h: 1	2	6	10
C	−20*	−4.3*	−0.3†	−0.4†
D	−20*	−4.7*	−0.6†	−0.6†
E	−21*	−4.6*	−0.8*	−1.6*
F	−20*	−4.4*	−0.2	−0.6*
W	−21*	−6.6*	−0.7	

* |Correlation coefficient| >0.9.

† |Correlation coefficient| >0.7.

Electrodes for Use with Silver Chloride Sensing Electrodes

The ionic medium generally used[9,10] with silver chloride electrodes is an ammonium acetate - acetic acid buffer at pH 4.3–5.0. The electrodes tested were C–F and W in Table 1. The test solution was 0.2 mol l^{-1} acetic acid half neutralised with ammonia solution. The same solution, saturated with quinhydrone, was, therefore, used as the internal solution of electrodes E, F and W. Electrodes C and D, which had sodium acetate instead of ammonium acetate, were included in order to show whether any part of the e.m.f. changes for electrodes D and F could be attributed to the reaction of ammonia with quinhydrone.[7]

Because the working solution was buffered and had a high ionic strength, tests on the effect of variations in pH and ionic strength were considered to be unnecessary, in contrast to the tests in nitric acid solutions.

Because the acetate - quinhydrone electrodes had such a high initial rate of drift of e.m.f., a further series of tests was carried out using an acetate - quinhydrone electrode retained from the earlier tests (F), a freshly prepared acetate - quinhydrone electrode (G) and phthalate - quinhydrone electrodes (H and J). The last type of electrode was chosen for comparison with the acetate - quinhydrone type because of the success reported by Cooper and Hand[11] with a 0.05 mol l^{-1} potassium hydrogen phthalate - quinhydrone electrode.

Short-term variance

The short-term variance in the e.m.f.s was determined from ten consecutive readings 1 min apart. Table 6 shows an extract of the 10 weeks' results. The standard deviations did not differ much from those obtained in 0.01 mol l^{-1} nitric acid, although the spread of results was smaller. The results did not indicate any advantage for either the sodium acetate filling solution (C and D) or the ammonium acetate solution (E and F).

Drift of standard potential

In general, the same considerations apply as in nitric acid solutions, but the rates of drift were much larger. Table 7 gives an extract of the results obtained from a linear correlation of e.m.f. against time. The rates of drift of the quinhydrone electrodes were very high at the start of the test, but they decreased progressively until after 5 weeks they were not consistently larger than those of mercury - mercury(I) sulphate electrodes.[1] Fig. 3 shows typical traces during the first (G) and eleventh (F) weeks of operation. The drift was not linear during the first week, but was so thereafter. The potentials always changed in the same direction, whereas for mercury - mercury(I) sulphate electrodes the drifts changed erratically in direction from week to week.

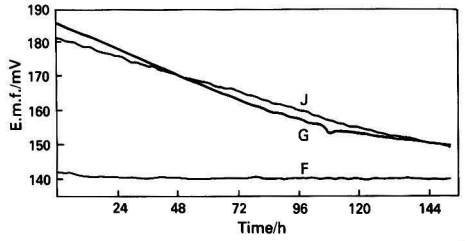

Fig. 3. Changes of e.m.f. with time during first week of test for quinhydrone - acetate (G) and quinhydrone - phthalate (J) electrodes and during eleventh week of test for quinhydrone - acetate electrode (F)

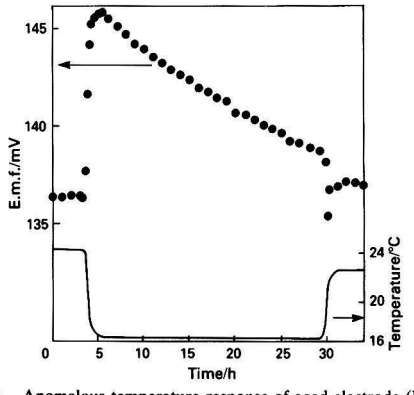

Fig. 4. Anomalous temperature response of aged electrode (D) in acetate buffer

Table 7 also includes results from a later test of an electrode with a gold inner electrode (W). This was tried because platinum has been reported[7] as catalysing the oxidation of hydroquinone, which might account for at least some of the drift in potential. As with nitric acid-filled quinhydrone reference electrodes, however, the nature of the inner electrode did not affect the rate of drift.

Fluctuations of electrode potential

As above, the standard deviation of the 70 or so 2-h readings collected each week was taken as an empirical indication of instability, including drift, random noise and long-term fluctuations, mainly for comparison with mercury - mercury(I) sulphate electrodes.[1]

An extract of the results (Table 8) shows that the variations were initially much larger than those for nitric acid solutions but that after a few weeks they were about the same. It is inferred that these results are dominated by the rates of drift, which were high at first but decreased with time.

Effect of temperature

Qualitatively, the electrodes showed similar features to those observed in nitric acid medium. The potential of the electrode that was about 6 weeks old (W) followed the temperature changes rapidly and reversibly, but the temperature coefficient (-1.10 ± 0.05 mV K^{-1}) was greater in magnitude than predicted (-0.58 mV K^{-1}) from literature values of the standard electrode potential[7] and the characteristics of acetate buffers.[12]

With older electrodes (3 months), the temperature responses were anomalous, as in nitric acid medium, but the effects were exaggerated for a decrease in temperature and reduced for an increase (*cf.*, Figs. 2 and 4); the net result is that the temperature cofficients for the peak (-1.1 mV K^{-1}) and steady potentials (-0.25 mV K^{-1}) have the same sign.

Table 8. Standard deviation of e.m.f. during 1 week in acetate buffer solution

	Standard deviation during week No.*/mV			
Electrode	1	2	6	10
C	11	1.8	0.1	0.2
D	11	1.9	0.2	0.3
E	11	1.9	0.2	0.7
F	10	1.8	0.1	0.3

* Calculated from approximately 70 2-h readings in each week.

Table 9. Drift of standard potential relative to calomel electrode in acetate buffer solution

	Drift rate during week No./mV per 100 h		
Electrode	1	3	7
F	−0.8*	−0.8	−0.1
G	−24†	−3.1†	−0.9†
H	−19†	−1.4	−0.5
J	−21†	−3.9*	−0.8

* |Correlation coefficient| > 0.7.
† |Correlation coefficient| > 0.9.

Table 10. Standard deviation of e.m.f. during 1 week in acetate buffer solution

	Standard deviation during week No.*/mV		
Electrode	1	3	7
F	0.4	0.7	0.2
G	11	1.5	0.4
H	8.6	1.3	2.0
J	9.5	2.2	3.0

* Calculated from approximately 70 readings each week.

Tests with the phthalate - quinhydrone system

Electrodes H and J from Table 1 were used and for comparison both new (G) and old (F) acetate - quinhydrone electrodes were included in the tests.

Short-term variance. The results for all electrodes were in the same range as before (standard deviations of 0.05–0.1 mV), the phthalate - quinhydrone electrodes showing no distinctive properties.

Drift of standard potential. Table 9 shows that the results for electrodes (H and J) with phthalate buffer filling solutions were very similar to those for new electrodes with an acetate filling, both in this test (G of Table 9) and previously (Table 7). The well aged acetate - quinhydrone electrode (F) continued to show the low drift rates found in the later stages of the tests above. Fig. 3 shows the course of the e.m.f. changes during the early part of the test. The similarity of curves G and J suggests that the composition of the buffer (at a given pH) is of little significance for the constancy of the e.m.f.

Fluctuations of electrode potential. As before, the standard deviation of 2-h readings collected each week was taken as an indication of the instability of the electrode potential. An extract of the results (Table 10) shows the same trends as before; the largest variations are associated with high rates of drift at the start of the test. The phthalate - quinhydrone electrodes H and J behaved very similarly to acetate - quinhydrone electrodes of the same age (G and also see Table 8).

Discussion

Quinhydrone Reference Electrodes

Electrodes with the nitric acid filling solution performed almost as well as the best mercury - mercury(I) sulphate electrodes, although their standard potentials drifted more in the first few weeks after preparation (Table 3). Compared with mercury - mercury(I) sulphate electrodes, the quinhydrone reference electrodes were affected more by pH variations in the test solution but less by the ionic strength. The precision of analysis with the two types of electrode was about the same. Over periods of more than a few days the quinhydrone electrodes were preferable to the standard EIL mercury - mercury(I) sulphate electrodes with ground-glass sleeves.

The quinhydrone electrodes needed no maintenance, compared with the frequent refilling required for mercury - mercury(I) sulphate electrodes. At the end of the tests the quinhydrone electrodes were stored for 18 months with their tips immersed in 0.01 mol l^{-1} nitric acid solution before their potentials were checked again. As the e.m.f.s were within 2 mV of the readings 18 months previously, storage presents no problem.

Quinhydrone reference electrodes with organic acid filling solutions showed much higher rates of change of standard potential than either quinhydrone - nitric acid electrodes or mercury - mercury(I) sulphate electrodes, particularly over the first few weeks after assembly. Practical application of the electrode would depend on the acceptability of a long period of conditioning.

The reasons for the high rates of drift are not clear from the known reactions of quinhydrone.[7] Oxygen does not oxidise hydroquinone in acid solution, for kinetic rather than thermodynamic reasons, as milder reagents can effect oxidation. If a catalyst were present oxidation might occur, but replacing the most obvious catalyst, the platinum metal of the inner electrode, by a gold electrode had no effect on the drift, nor had purging the filling solution of oxygen. In alkaline solutions hydroquinone is oxidised by oxygen to form semiquinone radicals, but polymeric products similar to humic acids have also been observed. The reactions depend on both the pH and the buffer system used to maintain that pH and their course in a new system cannot be confidently predicted.

To check the stability of quinhydrone - buffer systems outside the electrode, 0.005 mol l^{-1} solutions of quinhydrone in 0.01 mol l^{-1} nitric acid and 0.15 mol l^{-1} potassium hydrogen phthalate media were examined spectrophotometrically. The absorbance at 440 nm was measured every hour for 16 h. The pale yellow nitric acid solution increased in absorbance only slowly (0.0026 h^{-1}) but the phthalate buffer solution changed more rapidly (0.019 h^{-1}) and became redder. The absorbance of the phthalate - quinhydrone solutions increased across the entire visible spectrum, the original peak at 440 nm becoming a shoulder. The absorbance increased linearly over the 16-h period for both solutions. The rates of increase were the same (within experimental error) in unstoppered and stoppered cuvettes, contrary to the expected result if oxygen were being consumed.

Other types of reaction, such as those reported with ammonium salts and amino compounds, did not appear to be significant, because the electrodes prepared with ammonium acetate filling solutions changed at the same rate as those prepared with sodium acetate (compare electrodes C and D with E and F in Table 7).

Quinhydrone solutions taken from the electrodes V and W after 6 months were analysed by normal-pulse polarography with a PAR Model 264 analyser. The cathodic wave (quinone reduction) was greatly diminished compared with that for a fresh solution, more so for the acetate buffer filling than for the nitric acid filling. The anodic wave (hydroquinone oxidation) changed much less; in nitric acid solution the wave decreased and in the acetate buffer solution the wave increased. These results, summarised in Table 11, are in accord with observations by other techniques but also reveal some anomalies.

Inserting the polarographically determined concentrations in equation (1) enables the e.m.f. to be calculated and the results agree closely with observation. The direction of the change in e.m.f. is typical of a loss of the oxidised species (quinone), and this accords with the lack of effect from oxygen in both potentiometric and spectrophotometric studies, as this would affect the reduced species. The response of the quinhydrone - water system to the loss of quinone is anomalous. In a saturated system, the product (K) of the quinone and hydroquinone concentrations should be a constant, which was calculated to be 3.1×10^{-4} $mol^2\ l^{-2}$ from solubility and dissociation data.[7] When freshly saturated solutions of quinhydrone were analysed polarographically, the value of 3.2×10^{-4} $mol^2\ l^{-2}$ was obtained with both 0.01 mol l^{-1} nitric acid and pH 4.5 acetate buffer solutions, but the aged electrode filling solutions gave much lower values. The excess of solid quinhydrone present inside the electrodes failed, therefore, to re-equilibrate as expected. The simplest explanation for this would have been that lack of mixing inside the electrode prevented saturation being reached, and that possibly the deposition of polymeric products[7] would further inhibit the dissolution of the quinhydrone. Adding an excess of fresh quinhydrone to the supernatant solution from the electrode however, raised the product to $1.3–1.7 \times 10^{-4}$ $mol^2\ l^{-2}$. It is inferred that in aged solutions the quinone and hydroquinone concentrations are no longer governed by equilibrium with solid quinhydrone. The nature of the equilibria involved is unknown and would require a study beyond the scope of this work.

The temperature response of the aged quinhydrone reference electrode remains problematical. The effect is worse (in showing larger changes and slower recoveries) with filling solutions of higher pH (*e.g.*, in acetate buffer compared with 0.01 mol l^{-1} nitric acid). The electrodes with the worst temperature response are also those showing the highest rates of drift. It may be that the products of the reactions discussed above are implicated in the temperature response, but no

Table 11. Composition of electrode filling solution

Electrode filling solution	Quinone/ mol l^{-1}	Hydroquinone/ mol l^{-1}	K/ $mol^2\ l^{-2}$	E.m.f. change from fresh solution/mV	
				Observed	Calculated
Nitric acid (0.01 mol l^{-1})					
Freshly saturated ..	1.8×10^{-2}	1.8×10^{-2}	3.2×10^{-4}	—	—
Aged electrode (V) ..	3.1×10^{-3}	1.1×10^{-2}	3.8×10^{-5}	17	17
Acetate buffer (pH 4.5)					
Freshly saturated ..	1.7×10^{-2}	1.9×10^{-2}	3.2×10^{-4}	—	—
Aged electrode (W) ..	4.7×10^{-4}	3.1×10^{-2}	1.5×10^{-5}	55	54

reports of this have been found in the literature. In continuous analysis the temperature of the electrode system is almost always controlled and the temperature response of the electrodes is then of only minor importance.

Reference Electrodes for Chloride Determinations

Mercury(I) chloride sensing electrodes

For measurements in the nitric acid medium used with mercury(I) chloride electrodes, the results in this work do not indicate a clear preference for a quinhydrone reference electrode over a good mercury - mercury(I) sulphate electrode.[1] The quinhydrone electrode, however, has the advantage of requiring no maintenance and experience of mercury - mercury(I) sulphate electrodes in different laboratories indicates the uncertainty of finding a good electrode of this type.

Silver chloride sensing electrodes

The performance of mercury - mercury(I) sulphate electrodes is better in the acetate buffer solutions commonly used with silver chloride electrodes[9,10] than in nitric acid solutions,[1] but the quinhydrone electrodes with acetate filling solutions show significant drifts in standard potential. For these conditions the mercury - mercury(I) sulphate electrode would normally be preferred.

The convenience of using quinhydrone reference electrodes could best be achieved by changing the ionic medium to nitric acid, as with the mercury(I) chloride electrode. Such a medium has been used successfully with silver chloride electrodes.[13] Another possibility is the sulphuric acid medium,[14] which should give results for a quinhydrone - sulphuric acid reference electrode similar to those obtained in this work with nitric acid.

Further Developments

Although the quinhydrone electrodes offer some advantages over mercury - mercury(I) sulphate electrodes, the need for a conditioning period in order to avoid the initial high rates of drift is inconvenient and the temperature response is not fully explained. It is likely that these problems arise from the formation of products from as yet unidentified reactions. A reference electrode system based on a quinone - hydroquinone system of greater chemical stability was, therefore, investigated.[15]

This work was carried out at the Central Electricity Research Laboratories and is published with the permission of the Central Electricity Generating Board. I thank Mr. C. Gatford for doing the polarographic work.

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NOTE—References 1 and 15 are to Parts I and III of this series, respectively.

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Amperometric Determination of Glycerol and Triglycerides Using an Oxygen Electrode

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A new triglyceride or glycerol measurement system is described based on the indirect electrochemical monitoring of NADH via its reaction with oxygen by horseradish peroxidase. Derivative amperometric signals due to oxygen depletion provide for a one-point kinetic analysis. Lipase hydrolyses triglycerides to glycerol and glycerol dehydrogenase catalyses the reaction of glycerol with NAD to produce NADH. Serum measurements are demonstrated.

Keywords: *Glycerol and triglyceride determination; oxygen electrode; lipase; NADH; horseradish peroxidase*

Serum triglyceride analysis is a crucial test in the clinical diagnosis and classification of hyperlipidaemia.[1,2] Hyperlipidaemia, primarily known as a coronary risk factor,[1,3,4] is also associated, directly and indirectly, with many other disorders.[1,4] Several methods for triglyceride determination have been developed.[5–11]

Recent determinations are primarily enzymatic and employ hydrolysis of triglycerides to glycerol with lipase.[5–8] The generated glycerol is most often quantitatively related to NAD (NADH) by one of two enzymatic pathways, either by equation (1)[5,7,9,10] or by equations (2)–(4),[6,8,11]

$$\text{Glycerol} + \text{NAD}^+ \xrightarrow{\text{GDH}} \text{dihydroxyacetone} + \text{NADH} + \text{H}^+ \quad (1)$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[\text{Mg}^{2+}]{\text{GK}} \text{glycerol phosphate} + \text{ADP} \quad (2)$$

$$\text{Phosphoenolpyruvate} + \text{ADP} \xrightarrow[\text{Mg}^{2+}]{\text{PK}} \text{pyruvate} + \text{ATP} \quad (3)$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{lactate} + \text{NAD}^+ \quad (4)$$

where GDH represents glycerol dehydrogenase; GK, glycerol kinase; PK, pyruvate kinase; and LDH, lactate dehydrogenase. A proportional change in NADH absorbance at 340 nm[5,6,8,11] or fluorescence at 450 nm[9,10] is measured.

NADH has also been monitored electrochemically in clinical methods.[7,12–14] Electrochemical measurements eliminate many of the difficulties encountered in photometric detection, such as the overlap of absorbing species and turbidity. Biamperometric measurements of hexacyanoferrate(II) in a flow system were used to monitor NADH by the following reaction[7,12]:

$$\text{Fe(CN)}_6^{3-} + \text{NADH} + \text{H}^+ \xrightarrow{\text{Diaphorase}} \text{Fe(CN)}_6^{4-} + \text{NAD}^+ \quad (5)$$

NADH may be indirectly monitored amperometrically[15] using a membrane oxygen electrode[16] according to the reaction[14]

$$\text{NADH} + \text{H}^+ + \tfrac{1}{2}\text{O}_2 \xrightarrow[\text{Mn}^{2+}]{\text{HRP}} \text{NAD}^+ + \text{H}_2\text{O} \quad (6)$$

where HRP represents horseradish peroxidase.

In this work, triglycerides were hydrolysed with lipase. The glycerol produced was oxidised with NAD in the presence of glycerol dehydrogenase. The NADH produced was then reoxidised by dissolved oxygen in the presence of horseradish peroxidase. The rate of decrease in the oxygen concentration was monitored amperometrically. Under the appropriate conditions, the maximum rate of change in the oxygen concentration was proportional to the initial triglyceride concentration. Preliminary studies with serum illustrate the feasibility of serum assays.

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Experimental

Reagents

All chemicals were of analytical-reagent grade unless otherwise specified.

Glycine (ICN Pharmaceuticals), 0.1 M, and Tris (Sigma), 0.05 M, were prepared with de-ionised, distilled water and adjusted to a pH between 7 and 9 with 1 M HCl and 1 M KOH solution. All solutions were freshly prepared daily in these buffers. Olive oil (O-1500), tristearin (T-6503), lipase (L-3126, 56 U mg^{-1}), GDH (G-3755, 2.68 U mg^{-1}), NAD (N-7004) and HRP (P-8250, 150 U mg^{-1}) were all obtained from Sigma.

Apparatus

A glucose analyser (Beckman Instruments) was used for all tests in conjunction with a Linear Instruments dual-pen strip-chart recorder. One pen input was connected to the d.c. amplifier potentiometer output for the direct signal, while the derivative signal was taken from the derivative amplifier potentiometer. Helena Laboratories Quickpettes (5–50 and 50–250 µl) and Beckman Lancer micropipettes (5, 10 and 50 µl) were used for make-up and injection of the solutions.

Procedure

For the determination of glycerol, the following solutions were sequentially added to the reaction cell: 750 µl of buffer and 50 µl each of NH_4^+ (2.4 M NH_4Cl), Mn^{2+} (4.8 mM $MnCl_2.4H_2O$), GDH (0.65 U per run) and NAD (0.166 M) solutions. The NAD solution was kept on ice until ready for use.

The standard or sample (50 µl) was then added and allowed to incubate at 33 °C for 300 s with stirring. Glycerol standards were prepared by serial dilution with buffer solutions or serum - buffer (1 + 4) solutions. Then 50 µl of HRP (30 U per run) solution was injected as the trigger and the rate of oxygen consumption was recorded.

In determining triglyceride levels, two separate procedures were followed. A known amount of triglyceride was suspended in buffer or 1 + 4 serum - buffer solution (with heating to facilitate the suspension) immediately before hydrolysis. In the first procedure, 750 µl of standard or sample suspension were added to a test-tube, followed by 250 µl of lipase suspension (500 U per run) and 50 µl of Ca^{2+} solution (1.0 M $CaCl_2.2H_2O$). The mixture was allowed to incubate at 37 °C for 15 min with occasional shaking. Aliquots of this solution (50 µl) were then analysed for glycerol as described above.

In the second procedure, a 500-µl portion of buffer solution was added to the reaction chamber of the analyser along with

Table 1. Simplex optimisation of the GDH - HRP system

Vertex	NH_4^+/ M	Mn^{2+}/ mM	GDH/ U	NAD/ mg	HRP/ U	Incubation time/ min	Response, arbitrary units
1	0.02	0.25	0.50	5.0	14	4.0	240
2	0.17	0.25	0.50	5.0	14	4.0	235
3	0.15	0.36	0.50	5.0	14	4.0	250
4	0.14	0.26	0.80	4.5	13	4.0	205
5	0.14	0.26	0.50	8.3	13	4.0	210
6	0.14	0.26	0.50	5.2	22	4.0	340
7	0.14	0.26	0.50	5.2	14	5.5	270
8	0.15	0.29	0.20	6.9	18	4.5	255
9	0.15	0.29	0.40	2.2	20	4.7	225
10	0.14	0.26	0.50	8.3	13	4.0	210
11	0.11	0.33	0.40	5.1	21	4.9	315
12	0.15	0.35	0.40	5.1	23	5.2	320
13	0.14	0.31	0.50	8.4	19	5.2	285
14	0.14	0.26	0.40	7.5	26	5.8	360
15	0.12	0.31	0.70	5.5	24	5.7	390
16	0.12	0.33	0.40	5.0	29	4.8	345
17	0.13	0.31	0.50	2.6	29	4.9	370
18	0.16	0.29	0.60	5.4	30	5.2	405
19	0.12	0.24	0.65	5.5	30	5.0	415

250 µl of lipase suspension, 50 µl each of NH_4^+ and Mn^{2+} solutions, 5 µl of Ca^{2+} solution, and finally 50 µl of standard or sample. This mixture was allowed to react for 10 min then 50 µl each of the GDH and NAD solutions were added and incubation was continued for another 300 s to produce NADH before the HRP trigger injection.

Results and Discussion

The reaction sequence was as follows:

$$\text{Triglyceride} + 3H_2O \xrightarrow[Ca^{2+}]{\text{Lipase}} \text{glycerol} + 3\,\text{fatty acids} \quad (7)$$

$$\text{Glycerol} + NAD^+ \xrightarrow[NH_4^+]{\text{GDH}} \text{dihydroxyacetone} + NADH + H^+ \quad (8)$$

$$NADH + H^+ + \tfrac{1}{2}O_2 \xrightarrow[Mn^{2+}]{\text{HRP}} NAD^+ + H_2O \quad (9)$$

The particular ions (NH_4^+, Mn^{2+} and Ca^{2+}) associated with each reaction have been found to be the principal activators for the enzymes.[17] Other potentially influential compounds will be discussed later.

Glycerol Determination

Reactions (8) and (9) were performed simultaneously in the determination of glycerol. Simplex optimisation[18] was employed in determining optimum reagent concentrations in the two-step glycerol measurement system. The results are summarised in Table 1.

Glycerol dehydrogenase has been reported to exhibit optimum activity at pH 8–10 and at 10^{-3}–10^{-1} M NH_4^+, as well as being inhibited by Na^+ and solutions of high ionic strength.[19,20] In this work the following optimum reagent concentrations were found for reaction (8) [when combined with reaction (9)]: NH_4^+ = 0.12 M; and GDH = 0.65 U per run (Table 1). High ionic strength and Na^+ concentration were avoided by diluting the serum samples. The variables of GDH activity and NH_4^+ concentration were also optimised individually and independently (Figs. 1 and 2). Differences from the simplex optimisation method were probably due to intervariable dependencies. An optimum pH of 8.0 for the two-step system (Fig. 3) was the same as that previously reported for reaction (9).[14] At pH values above 8.0, Mn^{2+} tends to precipitate, causing irreproducible reaction rates for reaction (9).[14] Of the two buffers tested, Tris resulted in signals 40% larger than those using glycine buffer.

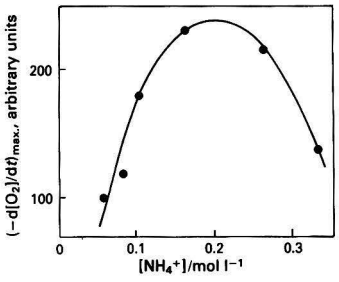

Fig. 1. Effect of NH_4^+ concentration on the GDH - HRP system

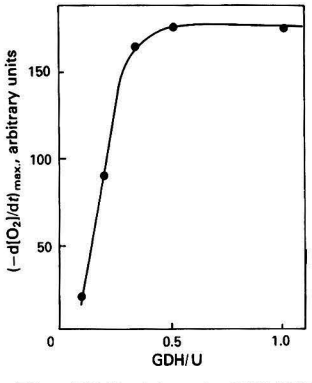

Fig. 2. Effect of GDH activity on the GDH - HRP system

The indicator (trigger) reaction (9) was that developed by Cheng and Christian.[14] Considerable variation in the reaction rate and reproducibility has been found with respect to substrates, reaction conditions and possible mechanisms.[14,21] It was necessary that reaction conditions be slightly alkaline for adequate reproducibility.[14] Similar variation in reagent dependence was seen in this study, with the exception of the lack of an absolute requirement for Mn^{2+} (although the reaction was accelerated in the presence of Mn^{2+}). In coupling the peroxidase reaction to the GDH reaction, optimum

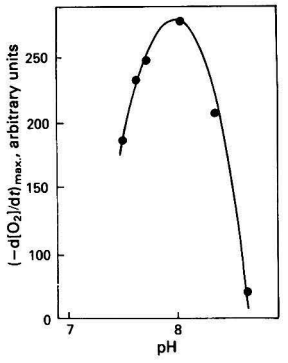

Fig. 3. Effect of pH on the GDH - HRP system

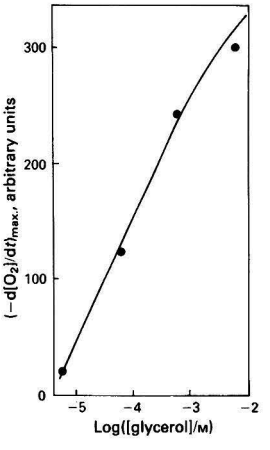

Fig. 4. Semilogarithmic plot of the calibration graph for the determination of glycerol in standard aqueous samples

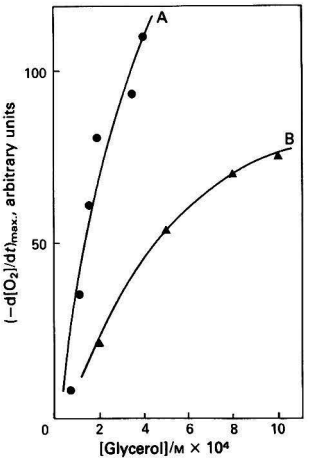

Fig. 5. Calibration graphs for glycerol determined in (A) standard aqueous samples and (B) serum control samples

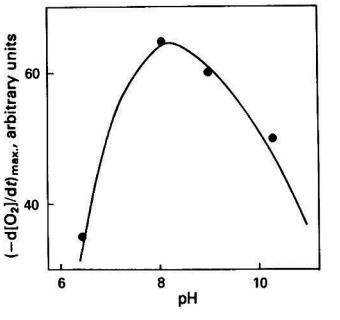

Fig. 6. Effect of pH on the lipase hydrolysis of triglycerides (by the external hydrolysis procedure)

reagent concentrations for the second reaction were Mn^{2+} = 0.24 mM and HRP = 30 U per run (Table 1). These concentrations were higher than previously employed.[14,22]

It was observed that the GDH reaction (8) proceeded much more slowly than reaction (9), which adversely affected the analysis time and the sensitivity. This problem was approached by firstly increasing the NAD concentration (0.01 M NAD or 5.5 mg per run, from simplex optimisation), secondly, maintaining the reaction at 33 °C and thirdly, incubating for 5 min. In incubating, however, feedback inhibition by both NADH (competitively) and dihydroxyacetone (uncompetitively) takes place,[20] which resulted in a non-linear calibration graph (approximately a logarithmic response over wide concentration ranges, as shown in Fig. 4) and reduced sensitivity compared with that of the NADH - oxygen reaction[14] (detection limit 5×10^{-5} M glycerol for aqueous standards and 1×10^{-4} M glycerol for serum samples) (Fig. 5). A within-run precision of 3% was obtained. This could be reduced further by combining reagents in a single stock solution, thus decreasing the number of required pipette measurements. Note in Fig. 5 that the response for glycerol is reduced in serum, necessitating calibration using serum controls.

Triglyceride Determination

Lipase catalyses the hydrolysis of triglycerides to glycerol and fatty acids. The hydrolysis of triglycerides [reaction (7)] was performed either in series with (internal hydrolysis) or separate from (external hydrolysis) the glycerol reaction. Complete hydrolysis is slow and not substrate specific.[19,20] The optimum pH and effect of several activators such as Ca^{2+}, Na^+, bile salts and emulsifiers are reported to vary significantly with different isoenzymes.[23-25]

With the external hydrolysis scheme, optimum activity for the hog pancreas isoenzyme was seen at pH 7.5 (Fig. 6). Ca^{2+} and bile salt activation of the lipase was obtained using 0.05 M Ca^{2+} externally or 0.005 M Ca^{2+} internally and 0.05% *m/V* sodium taurocholate (bile salt). Na^+ and gum arabic (emulsifier) were found to have no significant effect on hydrolysis. α-Chymotrypsin, a non-specific esterase known to increase the rate of hydrolysis,[6] was observed to have no effect in these studies. Of the two standard substrates used, olive oil was found to be more reactive and more convenient than solid tristearin. Olive oil was determinable down to 25 mg dl^{-1} (0.3 mM) (Fig. 7) with a relative standard deviation of 8%. Analysis by internal hydrolysis was faster and more sensitive than by external hydrolysis, by a factor of two in both

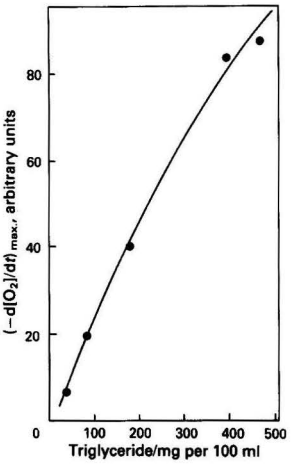

Fig. 7. Calibration graph for the determination of triglycerides in standard aqueous samples (by the external hydrolysis procedure)

instances. Sequential samples could be analysed more rapidly following external hydrolysis, however.

Calibration must be carried out concurrently with sample analyses. The presence of lipase influenced the behaviour of reaction (9), at times giving a significant blank or slightly inhibiting HRP, and at other times substantially activating the catalysis. It was necessary to recharge the electrode and change the membrane on a weekly basis. It appears as though the presence of oils and suspensions has adverse surface effects on the PTFE membrane.

Conclusions

A new reaction sequence is proposed for the determination of glycerol and triglycerides. It utilises selective enzyme catalysis coupled with specific kinetic electrochemical detection. Optimum reagent concentrations and solution conditions were evaluated. Results demonstrate the feasibility of this new approach to the difficult problem of effective triglyceride analysis and its application to serum.

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Use of Infrared Spectroscopy for the Analysis of the Average Properties of Nonylphenol - Ethylene Oxide Condensates

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The use of infrared spectroscopy is proposed for the determination of some average properties of nonylphenol - ethylene oxide condensates such as average relative molecular mass, average degree of condensation, the hydrophobic - lipophilic balance (*HLB*) and percentage of ethylene oxide. The method, based on the existence of a regression law between the logarithm of the surfactant properties and the logarithm of the ratio of the heights of the bands at 960 and 840 cm^{-1}, is precise (the relative standard deviations for a series of eight independent analyses are 0.9% for *HLB*, 1% for percentage of ethylene oxide and 1.6% for relative molecular mass) and accurate; no blank correction is required and there is no constant relative error.

Keywords: *Nonylphenol - ethylene oxide condensates; non-ionic surfactants; infrared spectroscopy*

Methods proposed for the analysis of non-ionic surfactants[1] include gas chromatography, high-performance liquid chromatography, UV - visible spectrophotometry based on complex formation with ammonium cobaltothiocyanate(II) and other instrumental methods such as atomic-absorption spectrometry, X-ray fluorescence spectrometry, potentiometry and polarography.

However, in the analysis of raw materials and reaction products and in the quality control of these compounds, it is interesting to know the composition rather than the concentration. For the characterisation of surfactants, thin-layer chromatography has been employed, relating the R_F values to relative molecular mass.[2]

Danes and Casanovas[3] described the use of mass spectrometry for the analysis of non-ionic surfactants. The mass spectra of unseparated non-ionic surfactants indicate the type of surfactant, aliphatic or aromatic character and the presence or absence of nitrogen in the molecule. The degree of ethoxylation is calculated from the inlet temperature required to produce a good spectrum. Nuclear magnetic resonance spectroscopy has been used for the characterisation of non-ionic surfactants, and it permits the determination of relative molecular mass, degree of condensation and hydrophobic - lipophilic balance (*HLB*) from absolute integrals of different types of hydrogen atoms in the molecule[4] or through the utilisation of empirical equations.[5] In previous work we compared these two strategies for determining the average properties of nonylphenol - ethylene oxide condensates, evaluating their analytical characteristics and concluding that the direct treatment of the NMR data constitutes a precise and accurate method that is generally applicable to compounds of the same chemical nature from different suppliers.

IR spectroscopy has been utilised in the quantitative determination of non-ionic surfactants and as a detection method in chromatographic techniques.[6-9] However, it has not been directly utilised in their characterisation.

The purpose of this work was to apply IR spectroscopy to the determination of the average relative molecular mass (M), average degree of condensation (x) and average *HLB* of nonylphenol - ethylene oxide condensates and to evaluate the analytical features and possibilities of the proposed method.

Experimental

Apparatus

The IR data were obtained using a Pye Unicam Model SP-2000 spectrophotometer employing sodium chloride cells for the direct analysis of viscous samples.

The NMR data were obtained using a Perkin-Elmer Model MS-12 spectrometer equipped with a double resonance accessory. Sample tubes were made by Phedelco. All data were taken at the magnet gap temperature.

To apply the ISO standard method,[10] glassware was constructed according to the specifications in the standard.

Reagents

Nonylphenol - ethylene oxide condensates. Samples of the series Nemol K-34, K-36, K-38, K-39, K-539, K-1030, K1032, K-1033 and K-1035 (Masso and Carol), Renex 647, 697, 688, 690, 682 and 678 (ICI Pharma) and Arkopal 40, 60 and 80 (Hoescht) were used.

Tetramethylsilane (TMS) solution, 3.5% m/m in distilled carbon tetrachloride.

Surfactant solution, 50% m/m in TMS solution.

General Procedure

Standard method

As standard method for evaluating the accuracy, the ISO 2270–1972 method for iodimetric determination of ethylene oxide groups[10] was used.

Between 20 and 100 mg of surfactant are weighed exactly in a small glass cup and then the surfactant and weighing vessel are introduced into a spherical flask; 3 g of potassium iodide are added and a cooling tube is connected to the flask.

After purging the system with a flow of nitrogen for 20 min, it is placed in a glycerine bath at 165 ± 1 °C, and 5 ml of H_3PO_4 (80%) are added. The reaction is continued for 30 min without stopping the gas flow, connecting an absorption tube containing 10 ml of potassium iodide solution to the cooling tube.

The flask is taken out of the bath and allowed to cool to below 80 °C. The gas flow is then stopped and the contents of the absorption tube are poured through the cooling tube into the flask. Both the cooling and absorption tubes and also their joints are washed with potassium iodide solution and distilled water.

The contents of the flask are titrated with 0.1 N sodium thiosulphate solution using starch as indicator.

IR method

A drop of surfactant is placed between two NaCl windows and its IR spectrum is registered.

The ratio of the band heights at 960 and 840 cm^{-1} (h_2/h_1) is calculated, and the value obtained is interpolated in the

variation law that correlates this parameter with relative molecular mass, degree of condensation and *HLB* obtained from previously analysed samples.

NMR method

An amount of 500 ± 1 mg of surfactant is weighed, dissolved in an equivalent amount of TMS in CCl_4 solution and the NMR spectrum recorded between 0 and 10 p.p.m. TMS is used as a zero scale reference.

From the integration values of three types of hydrogen atom, we can calculate the degree of condensation and the alkyl radical composition, as each surfactant molecule contains four hydrogen atoms from the aromatic group, which can be used as an internal reference to determine the number of hydrogen atoms corresponding to the remainder of the peaks. From the degree of condensation and alkyl radical composition, the relative molecular mass, *HLB* and percentage of ethylene oxide of this compound[11] can be calculated.

This method was used to determine these parameters for a series of samples. These parameters were employed as standards for obtaining the variation laws of h_2/h_1 *versus M, x* and *HLB*.

Results and Discussion

IR Spectrum of Nonylphenol - Ethylene Oxide Condensates

Fig. 1 shows the IR spectrum of a nonylphenol - ethylene oxide condensate. From the spectrum of one series of these surfactants, including degrees of condensation from 4 to 17, we have verified that the relative size of the bands appearing at 840 and 960 cm^{-1} and corresponding to a band vibration of aromatic group C–H bonds is modified, the first band is greater than the second for compounds of a smaller degree of condensation, so that this relationship becomes inverted as the surfactant molecule condensation increases.

To determine peak height, the base line used is a tangent at the base of the peak. The ratio of the band heights (h_2/h_1) (band height at about 960 cm^{-1}/band height at about 840 cm^{-1}) was obtained from three independent spectra for each of the compounds studied. Table 1 gives these values and their standard deviations.

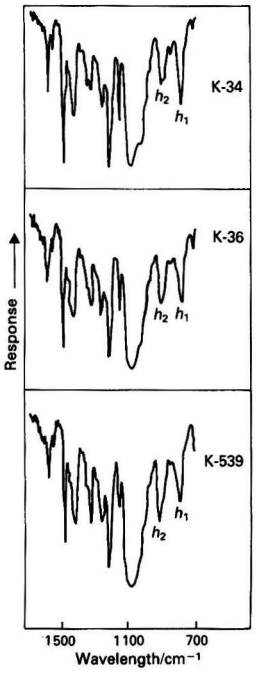

Fig. 1. IR spectra of representative nonylphenol - ethylene oxide condensates

Table 1. Average properties of surfactants obtained by the IR method and the NMR and ISO standard reference methods, with standard deviations (*s*)

Sample	NMR method								IR method								Standard method	
	h_2/h_1	s_h	*M*	s_M	*x*	s_x	*HLB*	s_{HLB}	*M*	s_M	*x*	s_x	*HLB*	s_{HLB}	%EO	s_{EO}	%EO	s_{EO}
Nemol																		
K-34	0.80	0.02	440	30	4.6	0.3	9.9	0.2	380	16	3.7	0.3	9.4	0.2	43	1	42.9	0.3
K-36	1.02	0.01	560	10	7.2	0.1	11.88	0.07	610	12	8.1	0.3	12.3	0.1	58.0	0.7	—	—
K-38	1.10	0.02	690	20	10.0	0.4	13.3	0.1	720	24	10.4	0.6	13.4	0.3	64	1	59.0	0.5
K-39	1.11	0.05	710	20	10.7	0.3	13.68	0.04	730	70	11	2	13.5	0.7	64	4	—	—
K-539(1)*	1.130	0.006	770	40	12.0	0.8	14.2	0.2	760	8	11.4	0.2	13.84	0.09	66.0	0.4	65.0	0.4
K-539(2)*	1.10	0.02	690	40	10.5	0.6	13.93	0.05	720	20	10.5	0.5	13.5	0.2	64	1	65.1	0.3
K-1030	1.22	0.01	800	20	12.4	0.4	14.1	0.1	890	19	14.7	0.5	15.1	0.2	72.8	0.9	—	—
K-1032	1.21	0.01	830	50	13.4	0.4	14.6	0.1	870	21	14.2	0.5	14.9	0.2	72	1	70.4	0.5
K-1033	1.32	0.02	940	50	16	1.0	15.1	0.1	1030	24	18.7	0.7	16.4	0.2	80	1	—	—
K-1035	1.23	0.06	1040	60	17.8	0.8	15.5	0.3	910	86	15	2	15.3	0.8	74	4	74.0	0.5
Renex																		
647	0.87	0.01	460	10	5.1	0.1	10.4	0.2	440	15	4.8	0.3	10.3	0.2	47.4	0.9	—	—
697	0.96	0.03	530	20	6.4	0.2	11.4	0.2	540	2	6.60	0.05	11.48	0.03	53.7	0.2	—	—
688	1.11	0.01	690	10	9.8	0.2	12.96	0.2	740	19	10.9	0.5	13.6	0.2	65	1	—	—
690	1.11	0.04	770	20	11.9	0.1	14.0	0.2	730	52	11	1	13.6	0.5	65	3	—	—
682	1.16	0.03	800	20	12.8	0.4	14.47	0.01	800	40	12	1	14.3	0.4	68	2	—	—
678	1.17	0.01	970	50	16.5	0.8	15.37	0.05	820	14	12.7	0.4	14.4	0.1	68.9	0.7	—	—
Arkopal																		
40	0.830	0.008	440	20	4.3	0.2	9.57	0.05	410	8	4.1	0.1	9.8	0.1	45.0	0.6	—	—
60	0.95	0.01	500	10	6.0	0.2	11.3	0.2	540	13	6.5	0.3	14.4	0.2	53.4	0.8	—	—
80	1.09	0.02	670	40	8.9	0.8	13.5	0.2	710	22	10.1	0.5	13.3	0.2	63	1	—	—

* K-539(1) and (2) correspond to two different consignments of the same product.

Table 2. Empirical equations used in the calculation of the average relative molecular mass, degree of condensation and *HLB* of nonylphenol - ethylene oxide condensates from the IR spectral data

$\text{Log}\, h_2/h_1 = \text{f}(\log M)$	$y = -1.367 + 0.493x$	$r^2 = 0.9$
$\text{Log}\, h_2/h_1 = \text{f}(\log x)$	$y = -0.270 + 0.306x$	$r^2 = 0.93$
$\text{Log}\, h_2/h_1 = \text{f}(\log HLB)$	$y = -0.967 + 0.894x$	$r^2 = 0.93$

Empirical Equations

Three regression models were tested in order to establish the laws that govern the variation of h_2/h_1 with M, x and HLB:

$$h_2/h_1 = \text{f}(P)$$
$$\log (h_2/h_1) = \text{f}(P)$$
$$\log (h_2/h_1) = \text{f}(\log P)$$

Table 2 shows the regression equations between h_2/h_1 values experimentally determined by IR spectroscopy and M, x and HLB obtained by NMR (Table 1), and their regression coefficients (r^2). The double logarithimic model [$\log h_2/h_1 = (\log P)$] gives the best regression for the three parameters considered.

Determination of M, x, HLB and Percentage of Ethylene Oxide (%EO) of Nonylphenol - Ethylene Oxide Condensates by IR Spectroscopy

The experimental value of $\log h_2/h_1$ for each of the compounds studied was interpolated in the empirical equation $\log h_2/h_1 = \text{f}(\log P)$ and the corresponding values of M, x and HLB were obtained. Table 1 gives the results obtained and the standard deviations.

Because the standard used as the reference allows only the determination of the percentage of ethylene oxide, this was determined from the x and M values obtained by IR spectroscopy according to the equation

$$\%\text{EO} = \frac{44.052x}{M} \times 100$$

Table 1 gives the values obtained and the standard deviations.

Proposed Method

A drop of surfactant is placed between two NaCl windows, the heights of the bands at 960 and 840 cm^{-1} are measured and their ratio is calculated.

The values of h_2/h_1 are interpolated in the regression equation $\log h_2/h_1 = \text{f}(\log P)$ obtained from IR spectra of standard samples of this type or of previously analysed compounds.

Precision

The precision of the method for the IR determination of relative molecular mass, degree of condensation, *HLB* and percentage of ethylene oxide, established from eight independent tests on a Nemol K-539 sample, were 1.6, 2.6, 0.9 and 1%, respectively.

Accuracy

The accuracy of the method was evaluated from a comparison between the percentages of ethylene oxide determined following the ISO method[10] and those determined by the proposed IR method. From the regression between all the values obtained for six samples analysed by IR spectroscopy and by the standard method,[12,13] as the corresponding variances for each sample analysed were homogeneous according to the Hartley test,[14] it was deduced by application of two independent statistical tests to the slope and the intercept of the regression curve that the proposed method does not require a blank correction (the intercept is statistically equal to zero) and there is no constant relative error (the slope is statistically equal to unity) at a probability level of 95%.

The accuracy in the determination of the percentage of ethylene oxide is 1–2%; only in one instance was a difference of 8% found.

Conclusions

The analysis of the average properties of nonylphenol - ethylene oxide condensates by IR spectroscopy is a sufficiently precise and accurate method, with the advantages of being rapid, not requiring tedious sample manipulation and involving common instrumentation found in most analytical and control laboratories.

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Spectrophotometric Determination of Vanadium in Fuel Oil and Stack Gas Using 2-[2-(3,5-Dibromopyridyl)azo]-5-dimethylaminobenzoic Acid

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Vanadium(V) reacts with 2-[2-(3,5-dibromopyridyl)azo]-5-dimethylaminobenzoic acid in aqueous acetone solution to give a blue colour with an absorbance peak at 640 nm. The colour in aqueous acetone solution was stable and the system conformed to Beer's law; the optimum range for measurement in 1.0-cm cells was 0.05–0.5 p.p.m. The reagent was very sensitive and reacted with vanadium(V) to form a 1 : 1 complex. The apparent molar absorptivity of the vanadium complex was 5.95×10^4 l mol^{-1} cm^{-1}. Common anions and cations did not interfere. Interferences from copper, iron and nickel can be avoided by using the 8-hydroxyquinaldine extraction method. The proposed method is simple and sensitive and has been applied to the determination of vanadium in fuel oil and stack gas.

Keywords: *Vanadium determination; spectrophotometry; 2-[2-(3,5-dibromopyridyl)azo]-5-dimethylaminobenzoic acid; fuel oil; stack gas*

Vanadium is one of the most important micro-elements in environmental chemistry because of its toxic nature and, therefore, the determination of vanadium in environmental samples, especially fuel oil, stack gas and coal fly ash, is of great importance. Vanadium is usually present at very low concentrations in most samples of interest and its accurate determination can be accomplished only by techniques that are very sensitive and selective.

A study of some azo compounds containing various heterocycles has been made with the object of preparing sensitive organic reagents that have molar absorptivities of the order of 10^5 l mol^{-1} cm^{-1} for different metals; such organic reagents remain rare, except perhaps for some ion-association complexes. In this connection, the synthesis of new azo dyes containing *N*,*N*-dialkylaminobenzoic acid and the determination of cobalt and iron with 2-[2-(3,5-dibromopyridyl)azo]-5-dimethylaminobenzoic acid (3,5-diBr-PAMB) have been reported previously.[1–4]

In this work, a spectrophotometric method has been developed for the determination of vanadium using 3,5-diBr-PAMB, and micro-amounts of vanadium in residual fuel oil and stack gas were determined satisfactorily.

Experimental

Apparatus and Reagents

The apparatus used was as described previously.[3]

3,5-DiBr-PAMB solution in dimethylformamide, 0.05% *m/V*. This solution was stable for several months when stored in an amber-coloured bottle.

Buffer solution, pH 2.8. This solution was prepared by adding 600 ml of re-distilled water to 250 ml of 0.2 M potassium hydrogen phthalate solution and adjusting the pH to 2.8 with 0.2 M hydrochloric acid.

Buffer solution, pH 10.0. A mixture of 0.2 M boric acid and 0.2 M potssium chloride solution was adjusted to pH 10.0 with 0.2 M sodium hydroxide solution.

Other buffer solutions. Used for examining the effect of pH, *e.g.*, 1 M sodium acetate - 1 M hydrochloric acid (pH 1.0–5.0).

8-Hydroxyquinaldine solution, 1% *m/V*. A 1.0-g amount of pure 8-hydroxyquinaldine was dissolved in 2 ml of acetic acid and diluted to 100 ml with re-distilled water.

Vanadium(V) standard solution. A 1.48-g amount of ammonium metavanadate was dissolved in 200 ml of nitric acid (4 + 96) and diluted to 500 ml with re-distilled water in a calibrated flask. This solution contained 1 mg ml^{-1} of vanadium.

Procedure

Transfer 10 ml of the slightly acidic sample solution containing up to 10 μg of vanadium into a 20-ml glass-stoppered test-tube. Add 3 ml of potassium hydrogen phthalate buffer and 0.4 ml of 0.05% *m/V* 3,5-diBr-PAMB solution. Mix well and allow to stand for 3 min. Add 6 ml of acetone and dilute to 20 ml with re-distilled water. Measure the absorbance at 640 nm against a reagent blank.

Results and Discussion

Absorption Spectra and Molar Absorptivity

Vanadium(V) and 3,5-diBr-PAMB form a blue complex that is soluble in various organic solvents. In this work, we chose aqueous acetone solution as the solvent because it gave good reproducibility.

The absorption spectra of the vanadium(V) complex and the reagent itself in aqueous acetone solution are shown in Fig. 1. The vanadium complex in aqueous acetone solution exhibited an absorbance maximum at 640 nm and the apparent molar absorptivity of the vanadium complex was 5.95×10^4 l mol^{-1} cm^{-1} at 640 nm.

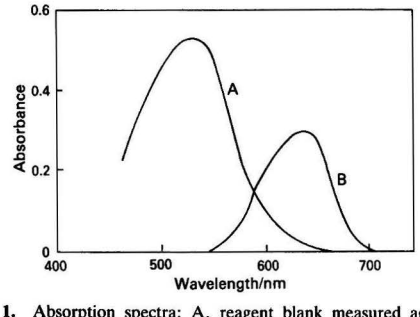

Fig. 1. Absorption spectra: A, reagent blank measured against water; and B, vanadium complex containing 5.0 μg of vanadium(V) measured against reagent blank

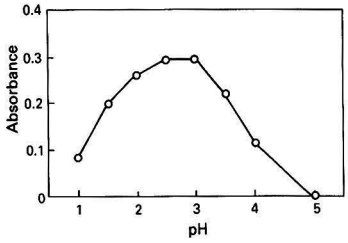

Fig. 2. Effect of pH on the formation of vanadium(V) complex. Absorbance measured at 640 nm against reagent blank

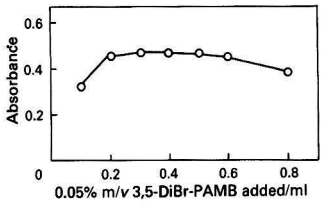

Fig. 3. Effect of reagent concentration on the formation of vanadium(V) complex; concentration of vanadium(V), 0.4 μg ml^{-1}

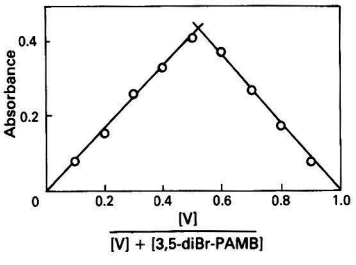

Fig. 4. Composition of vanadium(V) complex by the continuous variations method. Vanadium(V) complex in aqueous acetone solution: pH, 2.8; wavelength, 640 nm; and concentration, 8 × 10^{-5} M

Effect of pH

The absorbance was at a maximum at pH 2.5–3.0, and was constant throughout this range (Fig. 2). Subsequent determinations were therefore carried out at pH 2.8.

Effect of Reagent Concentration

The absorbances of a series of solutions containing 8.0 μg of vanadium and various amounts of 0.05% *m/V* 3,5-diBr-PAMB solution were measured (Fig. 3). It was found that 0.4 ml of 0.05% *m/V* 3,5-diBr-PAMB solution sufficed to complex 8.0 μg of vanadium. Also, it was found that the addition of more than 6 ml of acetone were necessary for maximum and constant colour development.

Nature of the Complex

The empirical formula of the vanadium(V) complex was studied by continuous variations and molar ratio methods. A typical graph showed unequivocally that a 1 : 1 vanadium - reagent complex is formed (Fig. 4). Similar results were obtained from a molar ratio plot. The absorbance at 640 nm was stable for at least 24 h.

Table 1. Tolerance limits for the determination of vanadium. The solution contained 6.0 μg of vanadium(V)

Ion or compound added	Amount tolerated/mg
Cl^-, NO_3^-, ClO_4^-, SO_4^{2-}	100
Potassium iodide, phenol	50
Ag(I), Al(III), As(III), Ba(II), Bi(III), Ca(II), Cd(II), Cr(VI), Hg(II), Li(I), Pb(II), Rb(I), Se(IV), Sb(III), Si(IV), Sr(II)	0.5
Mn(II), Mo(VI), Tl(I), Zn(II)	0.1
Sn(IV)	0.05
Mg(II), Ti(IV)	0.01
W(VI)	0.005
Pd(II)	0.001
Co(II), Cu(II), Fe(III), Ni(II)	0.000 5

Table 2. Determination of vanadium in synthetic mixtures. The solution contained 12.0 μg of vanadium(V)

Composition of mixture/μg	Amount of vanadium found/μg*
Cu(II) 10, Fe(III) 10, Ni(II) 10	12.0
Cu(II) 10, Fe(III) 200, Ni(II) 20	12.0
Al(III) 150, Cu(II) 10, Fe(III) 200, Ni(II) 10, Mn(II) 40, Ti(IV) 40	12.0

*Average values of three determinations.

Calibration Graph

The calibration graph was linear over the range 0–10 μg of vanadium in aqueous acetone solution. The relative standard deviation for 20 results at the 6.0 μg of vanadium level was 1.2%. The Sandell sensitivity was 8.6 × 10^{-4} μg cm^{-2}.

Effect of Diverse Ions

Numerous cations and anions were examined by applying the method to a fixed amount of vanadium in the presence of increasing amounts of the ion being studied. The tolerance limit was taken as the amount that caused an error of ±2% in the absorbance. For the determination of 6.0 μg of vanadium by this method, foreign ions can be tolerated at the levels given in Table 1.

Cobalt(II), copper(II), iron(II), nickel(II) and palladium(II) form intensely coloured complexes with 3,5-diBr-PAMB and therefore interfere in the general procedure for the determination of vanadium. Copper, iron and nickel, which usually accompany vanadium in fuel oil and stack gas, can be easily removed by the 8-hydroxyquinaldine extraction method.[5]

Table 2 shows the results for the determination of vanadium in synthetic mixtures using 8-hyroxyquinaldine. From these results, the following procedure was established.

Procedure for the Analysis of Synthetic Mixtures

Place an aliquot of the synthetic sample solution in a 100-ml beaker. Add 4 ml of 1% *m/V* 8-hydroxyquinaldine solution and adjust the pH to about 10 with 20% *m/V* sodium hydroxide solution. Add 5 ml of boric acid - potassium chloride buffer (pH 10.0). Then, transfer the mixture into a 100-ml separating funnel, add 10 ml of chloroform and shake vigorously for 5 min. Discard the organic layer, transfer the aqueous layer into a beaker and adjust the pH to 2.8 with 1 M hydrochloric acid solution. Evaporate the solution on a hot-plate to about 10–15 ml, then, cool and dilute to 20 ml with re-distilled water in a calibrated flask. Place 10 ml of this solution into a 20-ml glass-stoppered test-tube and continue the determination as described under Procedure.

Table 3. Determination of vanadium in JPI standard samples

Sample	Vanadium content, p.p.m. Certified value	Found Average*	Found Standard deviation
JPI Lot No. 79ML001	8.8 ± 0.5	8.77	0.349
JPI Lot No. 79MH001	79 ± 2	79.2	3.20

* Average values of ten determinations.

Table 4. Determination of vanadium in stack gas

Sample	Vanadium content/mg m^{-3} Proposed method	JIS method*
1	0.83	0.82
2	1.11	1.02
3	1.10	1.05
4	2.85	2.60
5	2.63	2.55

* Atomic-absorption spectrophotometry.

Applications

The 3,5-diBr-PAMB method has been applied satisfactorily to the determination of vanadium in various materials.

Determination of vanadium in residual fuel oil standard sample

Weigh a 2-g sample of residual fuel oil into a 100-ml quartz glass beaker, add 2 ml of sulphuric acid and carbonise by heating. Ash the residue in an electric furnace at 500 °C and dissolve the residue in 5 ml of dilute nitric acid (1 + 1) and 1 ml of sulphuric acid. Evaporate the resulting solution to fumes of sulphuric acid, cool, transfer into a 50-ml calibrated flask and dilute to the mark with re-distilled water.[6] Take suitable aliquots of this solution and carry out the determination according to the procedure for the analysis of synthetic mixtures.

Table 3 shows the results of the determination of vanadium in Japan Petroleum Institute (JPI) standard samples of residual fuel oil. It can be seen that the results obtained by the proposed method are in good agreement with the certified values.

Determination of vanadium in stack gas

Collect a dust in stack gas (0.5 m^3) on a silica glass microfibre thimble according to the method for measuring dust content in flue gas.[7] Ash the silica glass microfibre thimble in an electric furnace at 500 °C and transfer the residue into a platinum dish. Add 10 ml of nitric acid and 15 ml of hydrofluoric acid and evaporate almost to dryness. Add 10 ml of nitric acid, 5 ml of hydrofluoric acid and 5 ml of perchloric acid and heat to dryness. Dissolve the residue in 30 ml of dilute nitric acid (2 + 98) with heating, cool, transfer into a 100-ml calibrated flask and dilute to the mark with re-distilled water.[8] Take suitable aliquots of this solution and carry out the determination according to the procedure for the analysis of synthetic mixtures.

Samples from five boilers using different residual oils were analysed by the proposed method and the results are compared with those obtained by atomic-absorption spectrophotometry in Table 4.

Conclusion

2[2-(3,5-Dibromopyridyl)azo]-5-dimethylaminobenzoic acid reacts with vandium(V) in aqueous acetone solution to form a blue complex. The vanadium(V) complex formed is very stable in aqueous acetone solution and the stoicheiometric ratio is 1 : 1 (V : reagent). The calibration graph is linear over the range 0–10 μg of vanadium in 20 ml of aqueous acetone solution and the apparent molar absorptivity is 5.95 × 10^4 l mol^{-1} cm^{-1} at 640 nm. 3,5-DiBr-PAMB reacts with only a few metal ions. The method is relatively free from interferences because the interferences due to copper, iron and nickel can be removed by the 8-hydroxyquinaldine extraction method. Satisfactory results were obtained when the method was applied to residual fuel oil standard samples and stack gas.

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Thermal Wave Imaging of Multi-layered Films

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Monitoring of the physical structure of a multi-layered composite film presents difficult analytical problems. Thermal wave imaging is used to distinguish between different layers of a photographic film and to provide information on the internal three-dimensional distribution of chromophores.

Keywords: *Multi-layered film monitoring; thermal wave imaging; chromophores; photoacoustics*

Advances in thin-film technology have led to a wide range of new organic film materials with differing properties and applications. Many of these films are composite materials fabricated from individual layers with different physical and chemical properties. The properties of the different layers are tailored to give the completed composite film the desired optical, chemical or physical characteristics. Examples of these kinds of structures include packaging materials, photographic materials and dry reagent clinical test strips.[1] The analytical characterisation of films of this type presents some severe problems, and many new questions arise in the assessment of the physical integrity and quality of the film. Thus, for example, in quality control the distribution of materials in the sample, as well as the total amount present, is of importance. It is frequently necessary to establish whether each layer in a composite is of the correct thickness and to monitor any diffusion of components from one layer into others. The detection of defects or inclusions in the sample layers together with analysis of the topography of each layer is also useful in characterising a sample. Deviation from the product specifications for any of these parameters can produce regions of the composite film with dramatically different properties from those required. Conventional spectroscopy will seldom produce the information on the spatial distribution of absorbing species required for the characterisation of such films. To overcome this problem a combination of conventional UV - visible or IR spectroscopy together with sectioning of the samples and their microscopic examination is normally used. Such methods are time consuming and destroy the sample. They cannot easily be used to monitor variations across the plane of the film sample.

In a previous paper[2] we reported the use of thermal wave imaging using the photoacousitic effect for the characterisation of optically thin films supported on opaque substrates. This previous study was restricted to single-layer polymer films of 6–70 μm thickness. In this paper we describe the extension of this work to the study of multi-layered composite films. The material chosen for this study was a photographic colour reversal film in which the individual layers of the structure are semi-transparent and approximately 5 μm thick.

Experimental

Apparatus

A block diagram of the apparatus employed is shown in Fig. 1. The thermal wave imaging system used was of the same general form as that described previously.[2] The light source was a krypton ion laser (Innova 90-K, Coherent UK Ltd.) operated at 467, 531 or 652 nm with an output power ranging from 70 to 100 mW. The laser beam was amplitude modulated using an acousto-optic modulator (Model 304, Coherent UK Ltd.). After beam expansion and spatial filtering, the beam was scanned using a pair of orthogonal front surface mirrors mounted on limited-rotation galvanometer motors controlled by the computer. The scanned beam was focused on to an

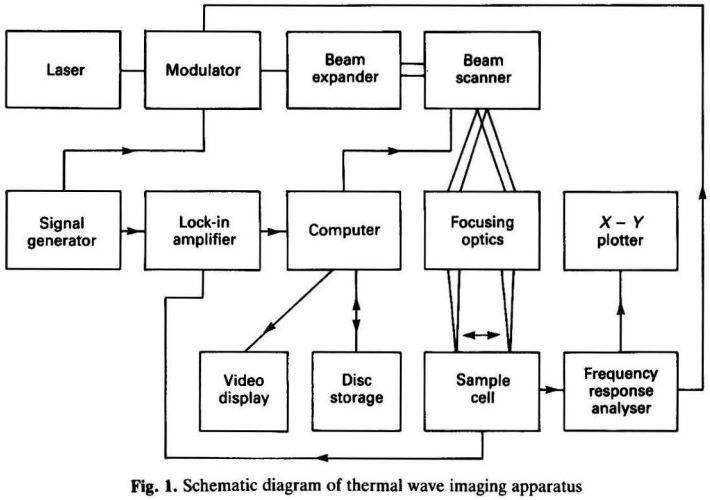

Fig. 1. Schematic diagram of thermal wave imaging apparatus

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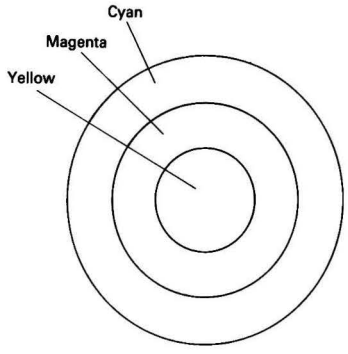

Fig. 2. Multi-coloured original target used to generate film sample

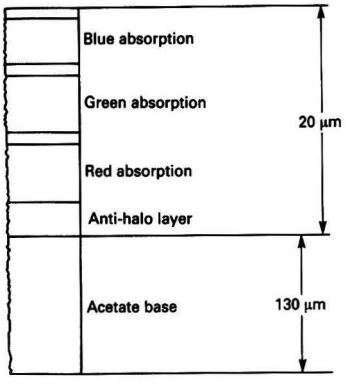

Fig. 3. Cross-section of a typical colour reversal film

image plane using a telecentric biconvex lens. The spot size at the image plane was approximately 10 μm with a positioning precision of 10 μm. The maximum scan area was 10 × 10 mm. The sample was contained in a commercial gas-microphone photoacoustic cell (Model OAS 401, EDT Research Ltd., UK) positioned at the image plane using an X - Y - Z translation stage. The photoacoustic signal was recovered using a two-phase lock-in amplifier (Model 5206, EG&G Brookdeal Ltd., UK). The amplitude modulation waveform was derived from a low frequency function generator (Model 3300A, Hewlett Packard Ltd., UK). The controlling computer was a purpose built image processing system constructed in our laboratory. The system was based on a Z80 microcomputer operated under CP/M with dual floppy disc drives for program and data storage. Additional paged memory was provided to facilitate handling of the large amounts of data used in imaging applications.

Processed images were displayed on a monochrome monitor using a graphics controller card, which provided a resolution of 256 × 256 pixels and 16 grey levels (Model RGB256, Matrox Inc., Canada). A full description of this thermal wave imaging system will be published later.

Frequency response measurements were carried out using a transfer function analyser (Solartron 1250, Solartron Instruments Ltd., UK). For frequency response measurements the modulation signal was provided by the internal oscillator of the Solartron 1250. Phase and amplitude responses were plotted on a digital plotter (Model HP7470A, Hewlett Packard Ltd., UK). No beam scanning was carried out during frequency response measurements; instead the sample was aligned using a translation stage.

Materials

In order to prepare a multi-layered sample of known absorption spectrum and with a known distribution of chromophores, a multi-coloured target was photographed on to modern colour reversal film. The target was 20 cm in diameter and consisted of concentric rings of yellow, magenta and cyan (Fig. 2). This was photographed using Ektachrome 200 daylight colour reversal film (Kodak Type 5076) to produce an image size on the film approximately 3 mm in diameter.

The structure of a colour reversal film is shown in Fig. 3. The film consists of a series of thin coatings of photosensitive or filter components dispersed in gelatine applied to a triacetate polymer base.[3-5] The top photosensitive layer absorbs strongly in the blue, the second in the green and the lower layer in the red region of the visible spectrum. The photosensitive layers are separated by guard and filter layers. Each photosensitive layer is approximately 5 μm thick and the total photographic emulsion thickness is approximately 20 μm. The supporting polymer base is 130–150 μm thick. During processing the three colour-sensitive layers are converted into complementary coloured dye layers; thus the blue sensitive layer becomes yellow, green becomes magenta and red cyan. The amount of dye in each layer is inversely proportional to the amount of light absorbed by the photosensitive material. By controlling the spectrum of light incident on the film it is possible to produce mixtures of dye concentrations in the layers from zero in all layers (white light) to maximum in each layer (black). For these preliminary experiments we used the complementary colours of yellow, magenta and cyan. Each colour zone in the original activates one dye layer in the final image. As the colours were not spectrally matched to the film characteristics, it is likely that the other dye layers were active to a small degree. However, the bulk of light absorption would still be found in the dye layer closest in colour to the corresponding zone of the target, and any residual absorption in other layers would not significantly change the results obtained.

Results and Discussion

Fig. 4 shows the phase and amplitude response of each of the coloured zones in the sample for a frequency range of 20–500 Hz. The measurements were made using the the 531-nm green line of the krypton ion laser. The amplitude response is dominated by the relative absorption coefficients of the different dyes at the laser wavelength employed, the yellow dye showing the weakest signal at all frequencies. The signal amplitude is also modified by the depth at which the light is absorbed. Thermal waves originating in deeper layers will be attenuated relative to those generated near the surface due to the rapid damping of thermal waves as they propagate through a solid.[2] However, because the depth information contained in the attenuation of the thermal waves is convoluted with the optical density information, the amplitude response of the sample cannot easily be used to obtain analytical information. The phase of the photoacoustic signal is a function of the response characteristics of the photoacoustic cell and signal processing electronics, the thermal properties of the sample and the depth at which incident radiation is absorbed, and is insensitive to the optical absorption coefficient of the absorbing layer.[2]

For this experiment the response of the cell, associated electronics and the thermal properities of the sample may be regarded as constant; therefore, the variations in phase response for each colour zone should be due to the different depths of the dye layers beneath the sample surface. The

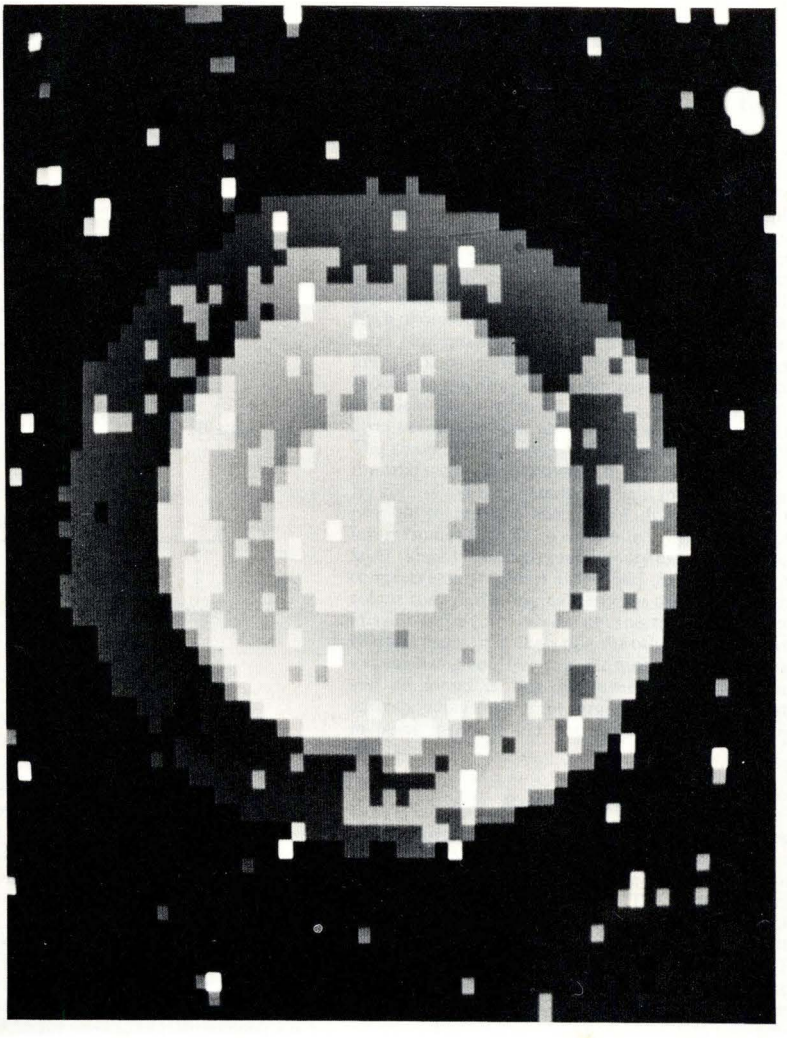

Fig. 5. Thermal wave phase image of multi-layered film

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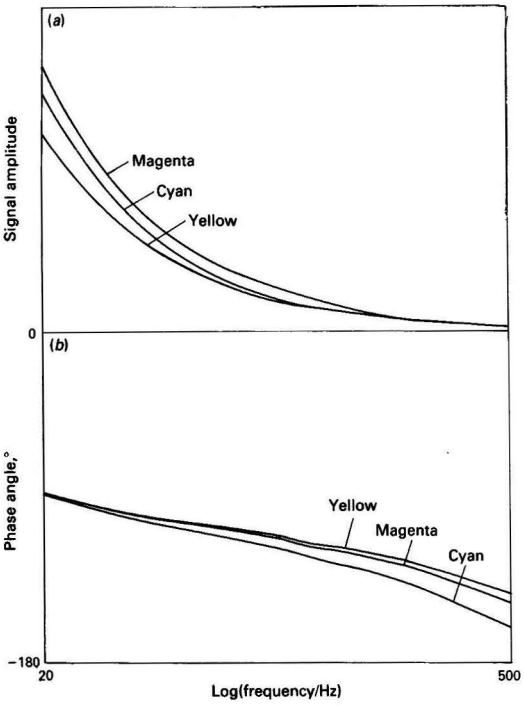

Fig. 4. Frequency response of the photoacoustic signal deriving from each of the sample zones: (*a*) signal amplitude; (*b*) signal phase

phase lag observed for each of the colour zones increases with increasing frequency. This is consistent with a time delay between the absorption of light and detection of the thermal waves at the surface, which is constant for each zone. Throughout the frequency range the yellow zone shows the smallest phase lag, indicating that this absorption zone is closest to the surface. The magenta and cyan zones show a progressively increased phase lag. The phase response of the sample thus indicates that the order of the dye layers from the top is yellow, magenta and cyan in accordance with the known structure of the film.[3,5] The experiment was repeated using the 467-nm line (blue) and the 650-nm line (red) from the laser source. The amplitude responses of the zones were similar at those wavelengths to those observed at 531 nm except that the order of amplitude responses changed according to the absorption coefficient of the different dyes at the respective wavelengths. Thus, predictably, at 467 nm the order was magenta > yellow > cyan and at 650 nm cyan > yellow > magenta. The phase response showed no dependence on the wavelength employed, reinforcing the observation that the phase response depends only on the relative distribution of chromophores and not on their optical absorption characteristics.

The region surrounding the target in the original was white, producing a region in the image with virtually no absorption in the visible. For comparison purposes, the amplitude and phase response of this region were also examined. As expected, the amplitude of the photoacoustic signal for this area of the sample was very low compared with the coloured zones, which have a much higher absorption coefficient. However, this region showed a stable phase lag slightly greater than that observed for the cyan zone. It is possible that the feature being located in this instance corresponds to the interface between the emulsion layers and the acetate substrate of the film.

Fig. 5 shows a phase image obtained for the sample. The 531-nm line was used with an input power to the optical train of 70 mW; the modulation frequency was 270 Hz. The scan covered approximately 4 × 4 mm using 64 points on each axis giving a total of 4096 pixels. The lock-in amplifier time constant was 300 ms and the dwell time for each pixel, 600 ms. Increasing phase lag has been plotted as increasing image density. The darker areas thus correspond to long time delays and the lighter areas to short time delays and shallow features. To make the most use of the limited grey-scale resolution, contrast compression was employed. The different zones in the film sample are clearly resolved in the image showing the excellent depth resolution obtainable using this technique.

To test the model for the observed phase response, the frequency response was remeasured for the three coloured zones with sample inverted and illuminated through the substrate. It was expected that a completely different frequency response would be observed as the heat generated by light absorption under these conditions must propagate through the thick acetate layer before detection. In addition the dye layers had reversed their original order. Initial results were difficult to interpret; at low frequencies some changes were observed, but at higher frequencies the response was almost identical with that observed for front surface illumination. The phase angles of the different absorbing zones were the same and the amplitude was reduced, but only by a factor of two. This behaviour was observed at frequencies up to 1500 Hz. These results contradicted the model developed by ourselves and other workers,[2,6,7] and it was difficult to reconcile the abnormally high signal amplitude and the lack of phase shift with the proposed physical processes. Further consideration led us to conclude that, as the sample was placed loosely in the sample tray of the photoacoustic cell, heat generated in the dye layers was able to propagate to the rear of the sample (the front surface of the film) and generate a photoacoustic signal in the cell. Thus, as the thermal waves had a shorter distance to travel to the rear of the sample in the inverted sample, the signal amplitude at this surface was greater than at the front surface and therefore dominated the observed signal.

This hypothesis was tested by thermal blocking of the rear surface of the sample with a thermally thick buffer layer to prevent any signal generation at the rear surface. An opaque material could not be used, as the strong signal generated by light absorption in an opaque substrate masked all others. Thermally thick samples were therefore prepared by carefully attaching a 250 μm thick clear acetate sheet to the film sample using a thin layer of transparent aerosol spray adhesive (3M Spraymount, 3M United Kingdom Ltd.). Two samples were prepared; in one the acetate sheet was attached to the acetate base of the photographic film, in the other the acetate sheet was attached to the emulsion side of the film. The first sample was illuminated through the emulsion side, and the amplitude and phase response were recorded. The results obtained for this sample were the same as those shown in Fig. 4; the extra thickness of acetate at the rear of the sample did not change the sample response. The second sample was illuminated through the acetate base of the photographic film as before, but now no signal could escape through the rear of the sample from the absorbing layers.

The amplitude of the detected photoacoustic signal was reduced by approximately two orders of magnitude at low frequencies, and at higher frequencies could not readily be detected above the noise level. The phase response exhibited substantial changes at low frequencies compared with the first sample, and at high frequencies the phase could not be reliably determined owing to the high noise levels. The ability to distinguish between the different layers of the emulsion was lost in this configuration. The phase and amplitude response

of a film sample illuminated through the substrate is difficult to explain fully; however, the marked decrease in signal amplitude and the loss of resolution in the depth profile is consistent with current models.[2,6,7]

These observations illustrate the importance of ensuring that the sample is thermally thick over the modulation frequency range to be used when studying optically thin films.

Conclusions

Thermal wave imaging may be used to obtain information about the distribution of chromophores in multi-layered optically thin films, and layers as thin as 5 μm can be readily resolved. This technique may be valuable for experimental studies in the fabrication and subsequent behaviour of composite films, as well as for quality control during manufacture. The technique described cannot be used for resolving overlapping absorbing features, but recent developments in impulse response measurement in thermal wave imaging[8] offer good prospects for the examination of more complex samples.

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Application of Automation and Computerisation to a Soil Testing Laboratory

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The soil testing laboratory operated by the Ministry of Agriculture and Fisheries at Ruakura Soil and Plant Research Station analyses about 45000 soil samples annually. Tests include soil pH and determination of plant available P, K, Mg, Ca and Na. This paper outlines a development programme in which laboratory automation and computers have been combined to increase the analytical capacity of the laboratory and to prepare it to operate a fully computerised fertiliser recommendation scheme. Sample and data handling systems are described for the various procedures.

In addition to increased efficiency, benefits from the developments include greater accuracy and precision, flexibility in laboratory operation and improved staff morale.

Keywords: *Soil analysis; automation; computerisation; fertiliser recommendation scheme*

A soil testing service has been provided by the New Zealand Ministry of Agriculture and Fisheries (MAF) laboratories since 1949. During the late 1970s two important factors had to be considered. The laboratory had to cope with steadily increasing numbers of samples without any increase in staff and space. Secondly it was envisaged that the MAF would introduce a fertiliser recommendation scheme that would require computer facilities for satisfactory implementation. These challenges were met at Ruakura by a development programme that involved automating laboratory techniques and methods of analysis and producing laboratory results on computer magnetic media. The Ruakura soil testing laboratory analyses about 45000 soil samples annually. Soil pH and determinations of available P, K, Mg, Ca and Na are measured for all samples received. This set of data is known as the "quick-test" service.

Prior to automation, a segmented continuous flow analyser with chart recorder output was used for phosphate determination; pH was measured with a digital pH meter and a four-channel emission/atomic-absorption flame spectrometer was used for Ca, K, Na and Mg. The development concept was automatic processing of chemical instrument output and storage of results on computer media (tape or disk).

An important factor was that each chemical instrument should be autonomous. One solution to the concept would have been a mainframe computer simultaneously controlling or servicing each instrument. However a shut-down of the mainframe would necessitate virtually complete shut-down of the laboratory. A second important factor was that the system(s) should be user orientated so that staff would readily use and accept the system and feel that they were being assisted by technology rather than controlled by it. Obviously many other factors such as reliability, ease of operation and flexibility were also considered.

Apple microcomputers were chosen as the laboratory based equipment to be used for processing chemical instruments. Other microcomputers were probably suitable, the final decision being determined by availability. Individual instrument output is on printer and floppy disk with subsequent transfer of data to a minicomputer (PDP 11/34) housed in a main computer room.

The basic design is shown in Fig. 1. Each chemical instrument is serviced by a microcomputer, which provides output on a printer and floppy disk. Each microcomputer is hardwired to the mincomputer for subsequent transfer of data. Collation and final reports, as well as long-term storage, are provided by the minicomputer.

Procedures

Phosphate Determination

Available phosphate is extracted using the Olsen *et al.* hydrogen carbonate method.[1] The phosphate content of the extract is then determined using segmented continuous flow analysis (SCFA), the chemistry being based on the Murphy - Riley molybdenum blue method.[2] The SCFA analyser operates at a sampling rate of 70 per hour; with the inclusion of standards and blanks 60 soil extracts are analysed per hour.

Initially an on-line approach was attempted, a recorder retransmit slide-wire being connected to an analogue to digital converter. This approach did not succeed. The major problem was the time lag between insertion of sample and recorder response, which, depending on the chemistry, could be up to 20 min. Obviously, this involved a similar delay between the occurrence and observation of a malfunction or error.

An on-line system also required the computer to be programmed with an intended chain of events rather than an actual occurrence. This, coupled with the inherent time lag, caused many problems. Therefore an off-line approach was considered.

The proposal was to use a sonic digitiser (SAC 6–20) to process recorder chart-output from the SCFA analyser and this offered distinct advantages: the microcomputer was not dedicated to a specific method and therefore could be utilised to process any method having a chart-recorder output; the operator initialised the system with the events that had actually occurred rather than those which were intended; and development of the system and laboratory analyses could be carried out independently.

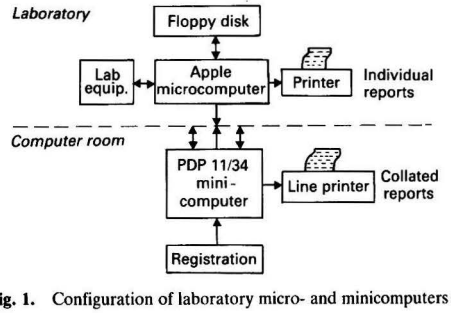

Fig. 1. Configuration of laboratory micro- and minicomputers

The digitiser unit consists of a magnetic base plate with two independent microphones placed on adjacent sides. The microphones detect signals from the unit's sonic pen. Recorder charts are held in position on the base plate using composite magnetic strips. Full details of the electronics, interfacing and microcomputer control were described by Jordan *et al.*[3]

The sonic pen emits an acoustic signal when depressed on to the base plate surface. This signal is then converted into the X - Y ordinates of the point where the pen touches the plate.

In operation the system is first initialised (via the microcomputer keyboard) with the date, number of samples, batch and sample identifications; the technician then "digitises" base lines, standards and samples in a set sequence. Depending on the experience of the technician and complexity of the chart, the complete operation takes 1–2 min. Note that manual processing of a chart (5 standards plus 40 samples) would take 15–20 min. As mentioned above the microcomputer provides results as hardcopy and on floppy disk.

The software includes many useful features to provide safeguards, corrections and cope with abnormalities. For example the microcomputer will alert the operator should he/she attempt to "digitise" out of sequence. Off-scale samples or those swamped by excessive carryover can be deleted (for later processing), or if detected whilst the batch is being analysed repeated at the end of the same batch. The system also provides correction for base line drift and carryover.

Soil pH

Soil pH is determined as a suspension of 12 ml of soil plus 25 ml of distilled water, which is allowed to stand overnight before the actual pH measurement. The automation of this analysis progressed through several stages.

Initially the concept was developed by utilising surplus equipment. A scintillation counter sample chain was adapted for movement of samples. A Fisons dispenser mechanism was used to achieve vertical movement of the electrode into and out of the soil suspension. These components are the basic part of the equipment currently in use.

Control and output proceeded via several stages as new microprocessor equipment came on to the market. In the meantime general plans for complete automation of the laboratory were crystallising. Hence, in the final version, control is effected by an Apple microcomputer. Full details were documented by Jordan *et al.*[3]

Although the pH robot is a completely different unit from the digitiser the principles of operation are essentially the same. The robot is initialised with sample numbers, identification, date, etc., after which operation is automatic. The basic speed of the equipment is 120 samples per hour of which 110 are soil suspensions being analysed.

Soil Cations

In the "quick test" method for soil cations 4.4 ml of <2 mm air-dry soil are shaken with 20 ml of 1 M neutral ammonium acetate solution for 2 min. The filtered extract is then analysed by flame emission spectrometry for K, Ca and Na and by atomic-absorption spectrometry for Mg. The instrument used is a four-channel flame spectrometer built at Ruakura[4] from a surplus Techtron 100 atomic-absorption spectrometer by the addition of three emission detectors around the burner.

Automation of this instrument was relatively simple and again was described by Jordan *et al.*[3] The sampler is a converted ISCO Golden Retriever fraction collector operating at 140 samples per hour, which, allowing for standards, is equivalent to 120 real samples per hour. Recently the system has been modified so that the microcomputer also controls the sampler. The Apple microcomputer multiplexes the four channels and computes sample concentrations by comparison with standards. Output is similar to the digitiser and pH instruments.

Instrument Output

Fig. 2 shows a typical printout from the flame spectrometer; the latter is used as an example, the other two channels being basically the same. ORDER describes the curve-fitting procedure (linear regression) used by the microcomputer. This is invariably linear (1) or quadratic (2) although the program has since been modified to accommodate other functions. FIT is a mnemonic for the correlation coefficient (R^2) for the curve fit. (4) For Ca indicates that $R^2 \geqslant 0.9999$. A minimum acceptable fit is set by the operator and if R^2 is not achieved the computer brings this to the operator's attention. In addition, original standard readings are calculated from the curve-fit as though they were unknown samples. This provides further information on the curve-fit and is particularly useful for diagnosing the reason(s) for a poor curve-fit. For these tests the minimum curve-fit is set at 3 (*i.e.* $R^2 > 0.999$). The batch identification (BEV) and date are merely for the operator's convenience in identifying groups of data, and they are not used subsequently. The sample identification numbers (entered at initialisation) are then printed along with sample results. The final part of the printout indicates the disk (V22) and file (21) where the data for the batch are stored.

Quality Control

The first sample of each group of 20 is always a reference sample of known constitution. If any reference result is outside narrowly defined limits then the whole batch is repeated (after the reason for the error has been identified and corrected). In total five reference samples with a wide range of values are used.

```
*STANDARDS*                                        7/4/83

TEST      CA        K        MG        NA

FIT        4        5.4      7.7       2.8
ORDER      2        2        2         2

           0        0        0         -.5
           5.1      10       15        26.7
           9.9      20       30        48.3
           20       40       60        101.2


*SAMPLES*

BATCH.....BEV                                      7/4/83

TEST      CA        K        MG        NA

90080      5        3        8         8
90081      7        27       29        15
90082      4        11       25        6
90083      4        10       20        5
90084      4        8        27        7
90085      4        16       19        7
90086      6        7        36        7
90087      8        9        36        16
90088      8        11       43        22
90089      8        10       38        19
90090      9        5        20        13
90091      9        10       21        13
90092      9        8        21        13
90093      4        5        10        9
90094      4        5        11        9
90095      3        6        11        8
90096      5        7        15        9
90097      4        3        10        7
90098      6        4        15        9
90099      5        6        11        10

===DATA FILED IN SOILDATA,V22,F17===
```

Fig. 2. Typical microcomputer printout from a four-channel flame spectrometer

As a further quality control check, 20 samples are exchanged each month between the Ruakura soil test laboratory and the MAF laboratory at Invermay Agricultural Research Station. These results are analysed statistically and conclusions regularly monitored.

Data Processing

A two-tier system of processing is used. The manipulation of data from individual channels (pH, digitiser, cations) is carried out at the microcomputer level. Subsequently, data are transferred into the minicomputer for collation and reporting. As indicated earlier the results from the three channels are stored on separate floppy disks. Full editing facilities are available for the data using a file utility program (FUTIL). In addition there are facilities for entering results via the computer keyboard, and this is used for tests, such as phosphate retention,[5] which are not yet automated.

A FUTIL file can have four distinct states. All files are designated E(empty) when the disk is first set-up and also when a file is deleted it is designated E(empty). When a file is first filled with new data it is designated F(full). The technician changes this status to A(accepted) when satisfied that the data are correct. F and A files cannot be re-used for new data. On instruction from the laboratory staff A files are then transferred into the minicomputer and automatically re-designated T(transmitted). The T files can be used for new data along with E files. The advantages of this system are (*i*) full editing facilities at each microcomputer station; and (*ii*) data can only be transferred into the minicomputer on instruction from an operator. This not only acts as a safeguard against the transmission of incorrect data but ensures that the laboratory staff are controlling the system.

On arrival at Ruakura samples go through a registration procedure. Every sample (or group of samples) arrives with a relevant submission form that provides the source, history and other details of the sample(s). The latter are arranged in groups of 20 which is termed a batch, for which a laboratory worksheet is prepared. Information included on the latter is sample identification, extra analyses required (it is assumed that all samples required the basic "quick-test" analyses), submission form numbers. Thus, the worksheet contains the minimum information that allows the identification of each sample and the analysis it requires and the subsequent allocation of results to the correct submission forms.

In all subsequent laboratory work, whether data processing or the physical handling of materials, the samples are retained in their batches of 20. A batch does not lose its identity until transfer of results to the minicomputer.

Collation

The next stage is entry of worksheet details directly into the minicomputer, and this is usually carried out within 1–2 d of registration. At this point the minicomputer contains full details of all registered samples in form files. Analytical results are held in the minicomputer in results files. It is important to note that the transfer of data from the three microcomputer stations and entry of registration details can all be carried out independently.

On instruction from laboratory staff the minicomputer completes an update. This operation matches the laboratory analytical data from results files with the registration details in form files, and then checks whether all the results for each particular submission form are completed. When the latter situation exists the computer automatically produces a result report.

Finally the result report is attached to the submission form and returned to the client. Formerly, results were handwritten on to the submission form. The current procedure, whilst a vast improvement, is nevertheless an interim solution pending the introduction of a fertiliser recommendation scheme. When the latter is in operation a full report of results and recommendations will replace the current result report.

Full editing facilities are available at the minicomputer level, and in addition to the result report various others can be obtained to assist with laboratory work flow. Typical of these are extra test report, which produces lists of samples requiring extra analyses (phosphate retention, sulphate, etc.) and missing test report, which helps to check that all aspects of the laboratory work are reasonably in phase, and would highlight a situation where, for example, a result report was delayed by the completion of one result.

Conclusions

The mutal objectives of automation and computerisation have been achieved by a unique combination of on-line and off-line microcomputers servicing laboratory instrumentation and themselves hardwired to a minicomputer. The soil laboratory is now handling a 10% increase in samples with a 25% reduction in staff numbers. (The latter reduction in staff numbers has not been implemented by redundancies but by unfilled vacancies.) This has been achieved because of the advantages and benefits of the automation and data processing system, which are as follows.

1. Each laboratory analytical unit is independent.
2. Entry of registration details and results can proceed independently, unlike some systems where the laboratory cannot proceed until a computer provides work-lists.
3. The interim storage of results on floppy disks provides a 5–10 d buffer against breakdown or malfunction of the minicomputer.
4. Flexibility through the use of the same microcomputers throughout the laboratory.
5. Improvement in accuracy and precision.
6. Greatly increased staff morale by the introduction of sophisticated (yet easy to use) equipment and the removal of tedious routines.

This work has been achieved with a tremendous team effort by past and present Ruakura staff; particular appreciation is expressed to the staff of the Plant and Analytical Chemistry and Biometrics sections.

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Procedure for the Semi-quantitative Analysis of Chromatographic Traces Containing Overlapping Peaks

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A manual calculation procedure is described for estimating the amplitude of individual peaks in chromatographic traces where peak overlap occurs. The method is based on the assumptions that the peaks are Gaussian in shape and are reproducible, that small changes in elution volumes can be accommodated by a simple correction procedure and that individual protein elution curves can be summed to give the observed experimental curve. The reproducibility of peak height estimations for five consecutive repeat chromatographic separations ranged from 1.5% for major components to 15% for minor peaks.

Keywords: *Chromatographic traces; overlapping peaks; mathematical analysis*

There are considerable variations in the appearance of UV absorbance profiles of human plasma proteins separated using the ion-exchange gel filtration procedure we have described previously.[1] Some of the differences relate to the nature of the illness or to the treatment of the patient. A method of quantifying these differences was therefore sought to allow the maximum utilisation of the information available from the protein separations. Over the years, many relatively elaborate computer-based approaches have been reported[2-5]; however, the method to be described provides semi-quantitative data using simple manual computation, and is suitable when the throughput of analyses is small (about three per day).

The ideal shape of a chromatographic absorbance peak is Gaussian and may be represented by equation (1), where $h(V)$ is the signal at elution volume V, h_0 the amplitude of the peak, V_R the retention volume and $\Delta V_{\frac{1}{2}}$ the half-width at half-height of the peak. The precise shape of the experimental curves is complex, but even in the worst instance we found that an acceptable simulation was obtained using the Gaussian error curve. The object of the mathematical analysis is to determine the value of h_0 for the plasma samples.

$$h(V) = h_0 \times \exp\left[-0.693\frac{(V - V_R)^2}{\Delta V_{\frac{1}{2}}^2}\right] \quad \ldots \quad (1)$$

The elution patterns of plasma proteins, Fig. 1, are complex owing to the overlap of peaks arising from different proteins. Inspection of the patterns from many samples indicated that there were up to 11 peaks consistently present in all samples. The proposed mathematical analysis of the elution curve is based on the following assumptions: (1) the elution patterns are reproducible and small changes in the retention volume can be accommodated by a simple correction procedure; (2) the shape of a given protein elution curve is constant and its height is proportional to the protein concentration; and (3) the individual elution curves may be summed to give the observed experimental curve.

The retention volume and half-width at half-height of the individual protein peaks were deduced by an iterative "peak-stripping" procedure. In this procedure the retention volume, the width and the amplitude of the first peak were estimated and these values were inserted into equation (1). With the aid of tables of the Gaussian error curve the profile of the elution curve was calculated and subtracted from the experimental curve to reveal the second peak more clearly. The Gaussian curve parameters for this peak could then be estimated. The calculated values for the second peak were then subtracted from the experimental curve to reveal the third peak. This cycle for the estimation of the elution-curve parameters, calculation of the Gaussian curve and subtraction of the latter from the experimental curve was repeated for each of the 11 peaks. When parameters had been ascribed to all peaks, further slight changes were made to those values to achieve the best fit to the experimental curve. The final values are presented in Table 1.

An example of the analysis of an experimental curve into its constituent Gaussian components is shown in Fig. 1. The curve shown has been adopted as the standard curve. It was used to estimate the contribution of the overlap of the flanks of one peak on the amplitude of the absorbance curve at the position of the maxima of neighbouring peaks. The overlap contribution of one peak on another will always be a constant fraction of the height of the first peak. The correction is effected by multiplying the first peak height by the overlap factor and subtracting the resulting value from the experimental value of absorbance at the location of the second peak. The overlap factors for all peaks are presented in Table 2. Though most peaks suffer from overlap, in some instances, *e.g.*, peaks 2, 4 and 5, the amount is relatively small and has little effect on the correction due to those peaks on their neighbours. Where overlap is large, *e.g.*, peak 3, iterative calculation may be necessary to determine the amplitude of the peak.

The procedure for the analysis of an elution absorbance curve is as follows.

1. The locations of the following reference points on the elution trace are determined: (*a*) the instant of sample injection and (*b*) elution volumes from the instant of sample injection to: (*i*) the first rise in absorbance trace, V_0, and (*ii*) the positions of the maxima of peaks 5(V_5), 6(V_6) and 11(V_{11}), Fig. 1.

2. The elution volumes at which the absorbances corresponding to the 11 constituent peaks should be measured are calculated by multiplying the retention volumes of the peaks of the reference curve (Table 1) by the elution rate scaling

Table 1. Gaussian parameters of elution peaks of the reference curve (Fig. 1)

	Peak No.											
Parameter	0*	1	2	3	4	5	6	7	8	9	10	11
Retention volume†/ml . .	10.3	11.6	14.7	17.5	20.2	25.8	29.3	31.2	32.3	33.2	35.7	56.5
Half-width at half-height/ml	0	0.87	1.64	1.28	1.90	2.52	1.18	0.74	0.39	0.38	2.28	1.41

* This is the instant when the initial rise of the first peak is first detected.
† The origin for these measurements is the instant of sample injection.

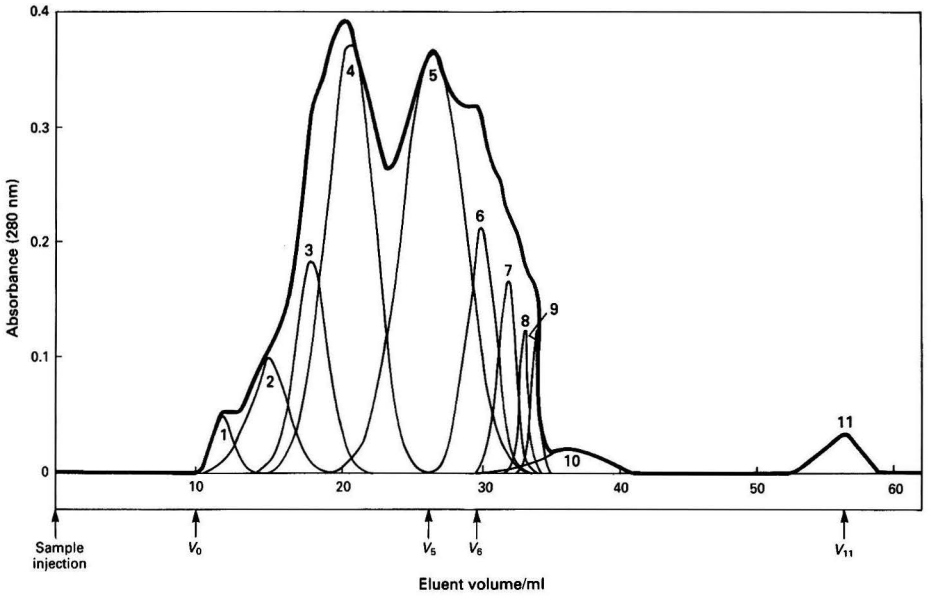

Fig. 1. Elution pattern of plasma proteins from a column of DEAE Sepharose CL6B (bold line). Constituent Gaussian components (thinner line): peaks 1–4, globulin; 5–7, albumin; 8–10, pre-albumin; and 11, low relative molecular mass component. Reference points on elution trace for compution of elution rate correction factor, ↑

Table 2. Correction factors for peak overlap

Measurement peak	Correction peak 1	2	3	4	5	6	7	8	9	10	11
1	—	0.083									
2		—	0.030								
3		0.120	—	0.294							
4			0.060	—	0.030						
5					—						
6					0.277	—	0.018				
7					0.050	0.201	—	0.003			
8						0.016	0.220	—	0.070	0.247	
9								0.095	—	0.463	
10										—	
11											—

factor, F. This factor, derived from the reference points identified in step 1, compensates for the effect of changes in the elution rate on the elution volumes of the peaks.

$$F = \frac{V_5 + \frac{1}{3}(2.51V_0 + 0.88V_6 + 0.46V_{11})}{2 \times (\text{elution volume of peak 5 of standard curve, } i.e.\ 25.8\ \text{ml})} \quad (2)$$

The numerical terms in equation (2) are weighting factors to ensure that equal significance is attributed to all reference points in calculating the scaling factor. The elution volume of peak 5 of the standard trace, 25.8 ml, is taken as the reference point and all other reference points are normalised to that value by their weighting factors, *e.g.*, 2.51 × (elution volume of V_0 on standard curve, 10.3 ml) = 25.8 ml. By this means an average elution rate for the separation is derived, which is then compared with that for the standard curve to give the scaling factor, F.

3. The amplitude of the elution curve at the predicted locations of the protein peaks is measured and these values are used to give the protein peak heights either directly or after correction for peak overlap (Table 2).

The application of the given procedure to the analysis of an experimental curve is illustrated in Table 3. Two features of the table are of interest. Firstly, the predicted value for the position of peak 11 does not agree closely with the observed value. However, as this peak is well isolated, in practice there is no difficulty in identifying its position. Secondly, the calculated values of peak height change by no more than 4% after iterative calculation.

The reproducibility of five consecutive chromatographic separations with semi-quantitative protein determinations are shown in Table 4. The precision for the major UV absorbing peaks 1–5 at 280 nm is good; however, errors in the measurement of smaller peaks are considerable but could be reduced by using increased instrumental sensitivity in that region of the chromatogram. Peak 5 was identified and measured as albumin by radial immunodiffusion. There was a good correlation (r = 0.999) between the amount of albumin placed on the column and the observed height of peak 5. Peak 4 was identified by radial immunodiffusion determinations of the column eluent to be immunoglobulin G (IgG). Semi-quantitative determinations for peak 4 by the procedure described in this paper were compared with immunoglobulin

Table 3. Specimen computation illustrating the procedure for the analysis of an elution absorbance curve

Parameter	Peak No. 0	1	2	3	4	5	6	7	8	9	10	11
Observed elution volume/ml . .	12.6					31.6	35.9					67.7
Predicted elution volume/ml . .	12.6	14.2	18.0	21.4	24.7	31.5	35.8	38.1	39.5	40.5	43.6	69
Total absorption at predicted peak position (A) . .	0	0.08	0.125	0.28	0.39	0.32	0.23	0.11	0.04	0.025	0.01	0.03
Overlap terms (Table 2) . .	0	0.083 × 0.125	0.030 × 0.28	0.120 × 0.117 0.294 × 0.39	0.060 × 0.151 0.030 × 0.32	0	0.277 × 0.32 0.018 × 0.11	0.050 × 0.32 0.201 × 0.139 0.003 × 0.04	0.016 × 0.139 0.220 × 0.66 0.070 × 0.025 0.247 × 0.01	0.095 × 0.19 0.465 × 0.010	0	0
Peak height (net absorption, A)—												
Initial calculation	0	0.07	0.117	0.151	0.371	0.32	0.139	0.066	0.019	0.019	0.01	0.03
1st iteration	0	0.07	0.120	0.157	0.371	0.32	0.140	0.066	0.019	0.019	0.01	0.03

$$\text{Scaling factor } (F) = \frac{31.6 + \frac{1}{3}(2.51 \times 12.6 + 0.88 \times 35.9 + 0.46 \times 67.7)}{2 \times 25.8} = 1.22$$

Table 4. Reproducibility of chromatographic peak heights (five consecutive runs on the same sample)

Peak No.	1	2	3	4	5	6	7	8	9	10	11
Coefficient of variation, %	6.3	4.1	1.4	1.5	3.7	23.1	10.8	10.8	15.3	10.2	14.1

G concentrations as determined by radial immunodiffusion. The correlation between the two methods was good (r = 0.91), but the peak-stripping procedure was not sufficiently selective to remove the effect of all overlapping peaks.

The chromatographic-analysis procedure outlined was designed to describe the shape of the elution profile in numerical terms using a simple manual calculation procedure. Although the absorbance profile is not calibrated in terms of protein concentrations, the separation procedure was sufficiently reproducible such that analysis of peak heights in arbitrary units provides valid information on variations in plasma protein profiles. The procedure has been in regular use in this laboratory for some time and has shown that information of considerable clinical significance may be obtained by relating individual protein peak heights, particularly peaks 1, 4 and 5 (IgM, IgG and albumin, respectively), to trace metal distribution patterns from the same separation.

We thank the MRC for a grant to one of us (D.C.C.).

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Voltammetric Behaviour of Phylloquinone (Vitamin K_1) at a Glassy-carbon Electrode and Determination of the Vitamin in Plasma Using High-performance Liquid Chromatography with Electrochemical Detection

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Cyclic voltammetry has been used to study the reduction of phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone) (vitamin K_1) at a planar glassy-carbon electrode. The reaction was found to be quasi-reversible with adsorption of the product, but not the reactant, taking place at the electrode surface. Liquid chromatography with electrochemical detection was used to determine endogenous levels of phylloquinone in human plasma. The normal range was found to be 0.08–1.24 ng ml^{-1}; the mean value was 0.41 ng ml^{-1}. The electrochemical assay was shown to be about three times as sensitive as the previous liquid chromatographic method in which the naphthoquinone was detected by its ultraviolet absorption. Agreement between the two methods was good when phylloquinone was added to plasma in the range 0–4.8 ng ml^{-1} ($r = 0.91$; $0.001 < p < 0.01$).

Keywords: *Phylloquinone; cyclic voltammetry; liquid chromatography; electrochemical detection*

Phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone) (vitamin K_1) is known to be involved in the formation of both blood clotting factors (II, VII, IX and X)[1] and a small protein, called osteocalcin, which is found in bone.[2] Therefore, the determination of endogenous plasma levels of phylloquinone may be of clinical importance, though until recently there had been no reports concerning this assay. The determination of endogenous phylloquinone levels in plasma has been achieved using high-performance liquid chromatography with UV detection (HPLC - UV). Using this technique, Shearer *et al.*[3] found that the mean fasting concentration of phylloquinone in human plasma was 0.26 ng ml^{-1} whereas Lefevere *et al.*[4] reported a mean value of 2.6 ng ml^{-1}.

The purpose of this study was to investigate the possibility of using HPLC with electrochemical detection (HPLC - EC) to improve the sensitivity of the phylloquinone assay developed by Shearer *et al.*[3] and to obtain further information concerning the physiological levels of the vitamin in plasma. It has been reported that HPLC - EC may be suitable for the determination of picogram amounts of certain organic species.[5,6] In addition, Ikenoya *et al.*[7] have used reductive HPLC - EC for monitoring phylloquinone in rat plasma following an oral dose of the vitamin. In this method a glassy-carbon working electrode was employed but only brief mention was made of the reduction process. Because we also intended to use a glassy-carbon working electrode, it was considered of interest to obtain further information on the nature of the electrode reaction occurring with this material and, in particular, to determine whether adsorption of reactant or product occurred at the electrode surface. This phenomenon can sometimes result in passivation of the electrode with consequent loss of sensitivity.

This paper consists of two main sections. The first section describes a study of the reduction of phylloquinone at a planar glassy-carbon electrode using cyclic voltammetry and the second section describes the application of HPLC - EC to the determination of physiological levels of phylloquinone in human plasma, including a comparison with a previously developed assay using HPLC - UV.

Experimental

Chemicals and Reagents

All chemicals were of analytical-reagent grade, unless stated otherwise. Phylloquinone (an isomer mixture containing 70% *trans* and 30% *cis*) was obtained from Sigma Chemical Company. Solvents used for HPLC were of HPLC grade and obtained from Rathburn Chemicals.

The methanolic acetate buffers which were used in the voltammetric and HPLC - EC studies were prepared by mixing a 5 M sodium acetate - acetic acid buffer solution (pH 3.0), methanol and distilled water to give a final electrolyte concentration of 0.05 M. Stock solutions of phylloquinone were prepared in ethanol or methanol and aliquots of these solutions were diluted with ethanol, or with methanol and acetate buffer (pH 3.0), to give the desired concentration. All glassware containing solutions of phylloquinone was protected from light during the course of the studies. The methanolic acetate buffers were de-aerated with oxygen-free nitrogen that had been continuously passed through vanadium(II) chloride and methanol. In order to keep the vanadium in the reduced state, zinc amalgam was added to the vanadium(II) chloride solution.[8] It was possible to minimise the time required for oxygen removal by prior de-gassing under vacuum, in a manner similar to that described by Senftleber *et al.*[9]

Apparatus

A Princeton Applied Research (PAR) Model 174A polarographic analyser, equipped with a PAR Model 174/70 drop-timer for a Servoscribe 1 s potentiometric recorder, were used for recording cyclic voltammograms. A three-electrode cell system was employed, which incorporated a glassy-carbon indicator electrode (Tokai GC-305; area 0.28 cm^2), a saturated calomel reference electrode and a platinum counter electrode.

HPLC - EC was performed with a constant-flow reciprocating pump (Model 300 from Applied Chromatography Systems) and a Bioanalytical Systems (BAS) electrochemical detector; this consisted of an LC 4B amperometric detector and a TL5 glassy-carbon flow-through cell containing an Ag - AgCl reference electrode. Sample injections were made through a syringe loading injection valve (Rheodyne Model 7125).

HPLC - UV was performed with the apparatus described previously.[10,11]

Cyclic Voltammetry

Cyclic voltammetry was performed on solutions containing 10^{-4} M phylloquinone dissolved in the appropriate methanolic acetate buffer solution, *i.e.*, 90, 95 or 99% methanol contain-

ing 0.05 M acetate buffer solution (pH 3.0). Voltammetric conditions were as follows: initial potential +1 V (held for 1 min prior to scanning); scan rate 10, 20, 50 and 100 mV s^{-1}; current range 0.02 mA. Between successive runs the indicator electrode was cleaned by washing with distilled water followed by acetone and then dried with a tissue-paper.

Optimisation of Conditions for HPLC - EC

Hydrodynamic voltammetry was performed by injecting fixed volumes of a standard solution of phylloquinone and varying the applied potential between −0.5 and −1.8 V in 50-mV steps. Between injections a cleaning potential of +0.7 V was applied for 3 min. Hydrodynamic voltammograms were constructed by plotting the recorded peak current against the applied potential. The optimum potential for phylloquinone determinations was found from the position of the plateau on the hydrodynamic wave.

The retention times and peak currents for vitamin K_1 were determined with mobile phases containing 94–99% methanol; the flow-rate was 1 ml min^{-1} and the applied potential was −1.0 V.

The TL5 glassy-carbon electrode was investigated for sensitivity using gaskets of 0.005 and 0.002 mm thicknesses.

Method for the Determination of Phylloquinone in Plasma by HPLC - EC

Aliquots of plasma (10 ml) were extracted with hexane as described previously.[3] Before HPLC - EC it was necessary to remove excessive and interfering lipids by a two-step procedure using firstly Sep-Pak silica cartridges (Waters Associates) and secondly semi-preparative HPLC on a silica column. In the first step the plasma - lipid extract was loaded on to a Sep-Pak silica cartridge in hexane (10 ml); the glassware was washed with a further 2 × 10 ml of hexane and the eluate discarded. A fraction containing phylloquinone was eluted from the cartridge with 3% diethyl ether in hexane (10 ml); the eluate was collected and evaporated to dryness in a water-bath at 60 °C, under a stream of nitrogen. Further purification of the phylloquinone fraction was achieved by HPLC on a column (25 cm × 5 mm i.d.) of Partisil-5 (Whatman) as described previously.[11]

The final stage of the assay with electrochemical detection was carried out with a Spherisorb (5 μm) octyl column (25 cm × 5 mm i.d.) and mobile phases of either 99% MeOH - 0.05 M acetate buffer (pH 3.0) or 95% MeOH - 0.05 M acetate buffer (pH 3.0). The flow-rate was 1 ml min^{-1} and the applied potential was −1.0 V. Sample injections were made by vortex mixing of the residues, after semi-preparative HPLC, in 70 μl of the mobile phase and injecting 50–60 μl via a 100-μl loop.

Calibration, Recovery and Precision of HPLC - EC

Calibration graphs were constructed by injecting between 1 and 10 ng of vitamin K_1 dissolved in the respective mobile phase. Peak heights were measured from the chromatograms and graphs of peak height *versus* amount injected were constructed.

Aliquots of plasma (10 ml) were spiked with various amounts of an ethanolic solution of phylloquinone so that final concentrations added were in the range 0–0.8 ng ml^{-1} of plasma. These solutions were diluted to 30 ml with ethanol and the above procedure was applied for extraction and measurement. The recovery of the vitamin for each plasma sample was then determined by reference to the calibration graph.

In order to determine the precision of the assay six 10-ml aliquots of plasma were spiked each with 0.5 ng ml^{-1} of phylloquinone and the above procedures for extraction and measurement were performed.

Results and Discussion

Cyclic Voltammetry

Cyclic voltammograms of phylloquinone in 90, 95 and 99% methanolic acetate buffers showed one peak on both forward and reverse scans. This is consistent with a two-electron reduction to produce the corresponding hydroquinone on the cathodic scan and re-oxidation to the quinone on the anodic scan. A typical cyclic voltammogram for a 10^{-4} M solution of the vitamin in 95% methanol - 0.05 M acetate buffer solution (pH 3.0) is shown in Fig. 1. The separation between the cathodic and anodic peaks is much greater than that predicted for a reversible reaction, *i.e.*, 59/*n* mV, where *n* is the number of electrons transferred; this indicates that the reduction process is quasi-reversible.

The quasi-reversible nature of the reduction process is also illustrated by the shift in the cathodic peak potential with scan rate and by the magnitude of the αn_a values (Table 1); these αn_a values were determined from the equation

$$\alpha n_a = \frac{0.048}{E_p - E_{p/2}}$$

where α is the transfer coefficient; n_a is the number of electrons involved in the rate-determining step; E_p is the peak potential; and $E_{p/2}$ is the peak potential at half peak current.[12]

Graphs of the current function $i_p/CV^{\frac{1}{2}}$ *vs.* $V^{\frac{1}{2}}$ (where i_p is the peak current in μA, C is the concentration in mmol l^{-1} and V is the scan rate in mV s^{-1}) were constructed for 10^{-4} M phylloquinone dissolved in the three different methanolic

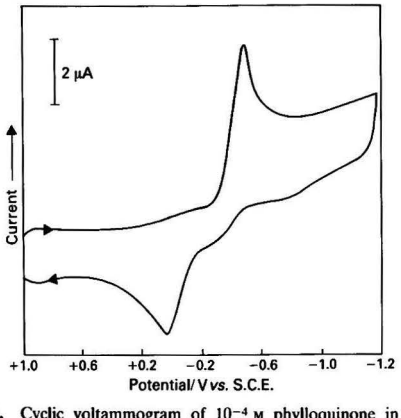

Fig. 1. Cyclic voltammogram of 10^{-4} M phylloquinone in 95% MeOH - 0.05 M acetate buffer solution (pH 3.0) using a planar glassy-carbon electrode. Initial potential, +1.0 V; and scan rate, 20 mV s^{-1}

Table 1. Variation of E_p and αn_a values with scan rate and percentage of methanol in the buffer solution for phylloquinone

	Scan rate/mV s^{-1}							
	10		20		50		100	
Methanol in solution, %	E_p	αn_a	E_p	αn_a	E_p	αn_a	E_p	αn_a
90	−0.440	0.6	−0.470	0.5	−0.535	0.4	−0.625	0.4
95	−0.470	0.5	−0.480	0.5	−0.585	0.4	−0.675	0.3
99	−0.470	0.6	−0.480	0.6	−0.560	0.5	−0.600	0.5

acetate buffer solutions (Fig. 2). In all three examples the current function decreased slightly with scan rate for cathodic currents but increased with anodic currents; this suggests that only the reduced form of phylloquinone adsorbs on to the surface of the glassy-carbon electrode. This is in contrast with the behaviour observed at a hanging mercury drop electrode where, under similar conditions, both the oxidised and reduced form of the vitamin were adsorbed and the reduction process was reversible.[13]

Optimisation of HPLC - EC Conditions

The hydrodynamic voltammograms of vitamin K_1 exhibited a plateau between −0.9 and −1.2 V (Fig. 3); therefore, −1.0 V was chosen as the applied potential in the HPLC - EC assay for vitamin K_1. When injecting fairly large amounts of phylloquinone (50–60 ng) it was necessary to reverse the polarity of the potential to +0.7 V for 3 min in order to remove adsorbed species from the working electrode; this adsorption process caused passivation of the electrode, which resulted in lowering of the peak height. As described earlier,

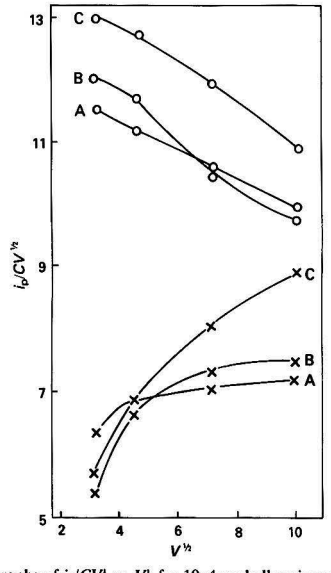

Fig. 2. Graphs of $i_p/CV^{\frac{1}{2}}$ *vs.* $V^{\frac{1}{2}}$ for 10^{-4} M phylloquinone in acetate buffered solutions: A, 90% methanol; B, 95% methanol; C, 99% methanol. O, Cathodic scan; ×, anodic scan

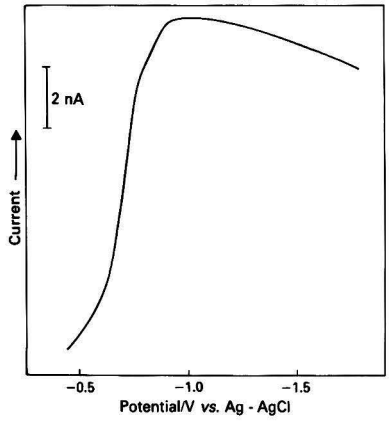

Fig. 3. Hydrodynamic voltammogram for phylloquinone in 99% MeOH - 0.05 M acetate buffer solution (pH 3.0)

the hydroquinone was found to adsorb on to the planar glassy-carbon electrode and it is probably this species that causes passivation in the HPLC - EC technique.

The variation of retention time of phylloquinone with percentage of methanol in the mobile phase is shown in Fig. 4. The shortest retention time occurred with a mobile phase containing 99% methanol - 0.05 M acetate buffer solution (pH 3.0); the largest peak currents were also obtained using this medium. When the mobile phase contained 95% methanol, a substantial decrease in sensitivity was found as shown in Fig. 5; this is probably a result of band spreading on the chromatographic column as indicated by the increase in peak width. The mobile phase containing 99% methanol therefore seemed the most suitable for the determination of endogenous phylloquinone levels in plasma; however, in several samples a large peak of unknown origin overlapped the peak due to the vitamin, making quantitative determinations impossible. To resolve the phylloquinone peak from the interfering peak we reduced the methanol concentration from 99 to 95%. Although this did improve the resolution between the two peaks, a decrease in the signal resulted; this was expected from earlier observations (Fig. 5). However, by merely changing the size of the gasket in the TL5 cell the signal was found to increase by almost 100% (Fig. 6). These conditions were then considered suitable for the assay of physiological levels of the vitamin in plasma.

Calibration, Recovery and Precision of the HPLC - EC Assay

A calibration graph of peak height *vs.* mass of phylloquinone injected was linear in the range 1–10 ng.

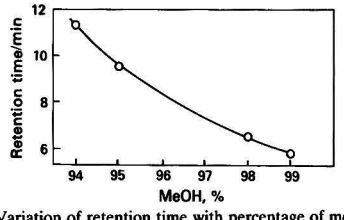

Fig. 4. Variation of retention time with percentage of methanol in the mobile phase

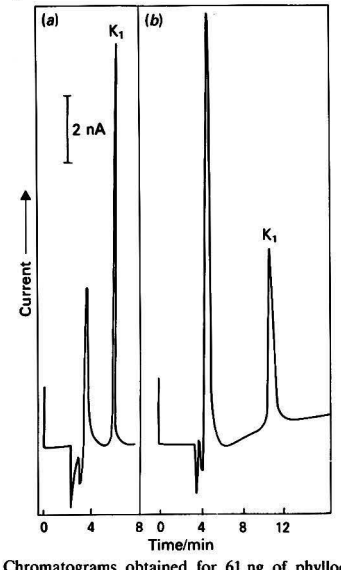

Fig. 5. Chromatograms obtained for 61 ng of phylloquinone in mobile phases containing (*a*) 99% MeOH - 0.05 M acetate buffer solution (pH 3.0); and (*b*) 95% MeOH - 0.05 M acetate buffer solution (pH 3.0)

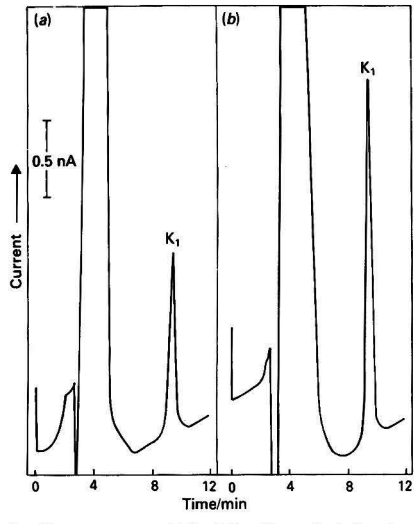

Fig. 6. Chromatograms obtained for 10 ng of phylloquinone by HPLC - EC using gaskets of (*a*) 0.005 mm and (*b*) 0.002 mm thicknesses. Mobile phase contained 95% MeOH.

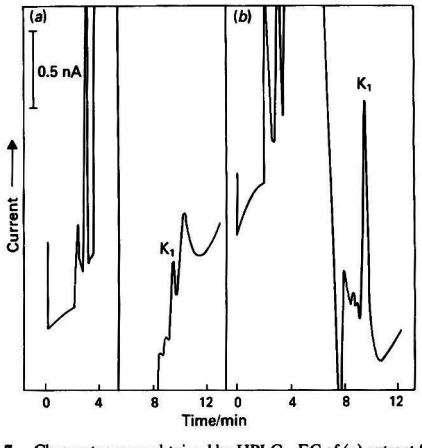

Fig. 7. Chromatograms obtained by HPLC - EC of (*a*) extract from 10 ml of control plasma; and (*b*) extract from 10 ml of plasma spiked with 0.8 ng ml^{-1} of phylloquinone. Plasma extracts were dissolved in 70 µl of mobile phase and 50 µl were injected

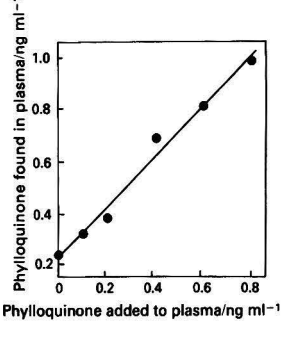

Fig. 8. Recovery of phylloquinone added to plasma

The chromatograms obtained for plasma samples spiked with phylloquinone all exhibited a well defined peak. Fig. 7 shows the chromatograms obtained for a plasma sample spiked with 0.8 ng ml^{-1} of the vitamin and for the control plasma itself. The endogenous concentration of phylloquinone in this plasma sample was only 240 pg ml^{-1}; this plasma was separated from expired blood obtained from a blood bank. The recovery of phylloquinone added to plasma in the range 0–0.8 ng ml^{-1} was linear (Fig. 8).

The coefficient of variation for six samples spiked with 0.5 ng ml^{-1} of phylloquinone was 8.9%.

Comparison of the Sensitivities of HPLC - EC and HPLC - UV

The sensitivity of HPLC - EC, for phylloquinone determinations, was compared with HPLC - UV by plotting graphs of peak height *vs.* mass of phylloquinone injected over the range 0–10 ng . Straight-line graphs passing through the origin were obtained in both instances and from the slopes of these graphs it was calculated that the sensitivity of HPLC - EC is about three times greater than that of HPLC - UV.

Comparison of the HPLC - EC and HPLC - UV Assays for Phylloquinone Added to Plasma

Duplicate aliquots of plasma (10 ml) were spiked with 0–4.8 ng ml^{-1} of phylloquinone and analysed by the HPLC - EC and HPLC - UV assays described previously. A linear relationship was found for the recoveries of phylloquinone determined by the two methods; the correlation coefficient (r) was calculated to be 0.91 ($0.001 < p < 0.01$).

Determination of the Stability of Phylloquinone in Whole Blood and Normal Levels in Plasma

Blood was drawn from a normal subject, divided into three aliquots and treated as follows: the first aliquot was centrifuged immediately; the second aliquot was left in the dark for 4 h before centrifuging; the third aliquot was left refrigerated for 24 h before centrifuging. The concentration of phylloquinone in the plasma samples was determined by the HPLC -EC assay and the results are presented in Table 2.

Table 2. Physiological plasma levels of phylloquinone in normal subjects

Subject's initials	Age	Sex	MeOH content of mobile phase (X)*	Phylloquinone concentration in plasma/ng ml^{-1}
M.J.S.	39	M	99	0.08
M.S.	27	M		0.17
J.C.†	59	M		0.40
J.C.‡	59	M		0.43
J.C.§	59	M		0.46
Donor A				0.30
Donor B			95	0.24
P.H.	23	M		0.44
I.F.	31	M		0.11
D.S.	39	M		0.58
F.C.	41	M		1.24
Z.B.	27	F		0.35
V.R.	28	F		0.44
L.F.	29	F		0.43
A.S.	57	F		0.46
Mean				0.41

* Mobile phase contained X% MeOH - 0.05 M acetate buffer solution (pH 3.0).

† Plasma separated immediately from whole blood.

‡ Plasma separated from whole blood after storing in the dark for 4 h at room temperature.

§ Plasma separated from whole blood after storing for 24 h in a refrigerator.

These results indicate that blood samples can be stored under the above conditions without degradation of the vitamin.

The HPLC - EC assay for the vitamin was applied to a few samples from normal subjects of various ages and the results are presented in Table 2. The mean phylloquinone concentration was found to be 0.41 ng ml^{-1} of plasma, which is in reasonable agreement with the value previously reported by Shearer *et al.*,[3] *i.e.*, 0.26 ng ml^{-1}. In the limited number of subjects studied in this work there did not appear to be a correlation between age and plasma phylloquinone concentration; however, a greater number of plasma samples are required before this can be more accurately established. It is envisaged that future work will involve the analysis of a greater number of normal individuals of different ages. In addition, the assay will be extended to subjects suffering from certain bone disorders.

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Organophosphorus Sulphides, Sulphoxides and Sulphones Part I. Determination of Residues in Fruit and Vegetables by Gas - Liquid Chromatography

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Conditions have been established for the routine quantitative determination of residues of demephion, demeton-S-methyl, disulfoton, fenamiphos, fensulfothion, fenthion, phorate, terbufos, thiometon and their toxic metabolites following conversion into the sulphones. Packed column gas chromatography - mass spectrometry demonstrated that most of the sulphoxides tended to decompose under the conditions required for gas chromatography, whereas most of the sulphides and sulphones were unaffected by such conditions. Temephos and its metabolites and the metabolites of vamidothion decomposed completely during gas chromatography. Some suggestions are given for assessing and avoiding the column priming and enhancement problems often observed in the gas chromatography of these and other polar organophosphorus pesticides.

Keywords: *Organophosphorus pesticides; metabolites; residues analysis; oxidation; gas chromatography*

Some of the most toxic and widely used of the organophosphorus pesticides possess an oxidisable sulphide (thioether) group. These sulphides are usually converted in plants and animals into the corresponding sulphoxides and sulphones,[1] although it is also possible for the sulphoxide to be biologically reduced to the sulphide.[2] Organophosphorus sulphoxides and sulphones are often more toxic and persistent than the parent sulphides and indeed some are formulated as pesticides. Thionophosphate (P=S) sulphides are also oxidised to the corresponding oxons (P=O) so that their toxic residues can include two sulphoxides and sulphones.

Residues of many organophosphorus pesticides are readily determined by gas-chromatographic methods, but the sulphones and, particularly, the sulphoxides can be difficult to assay by this technique because they are of low volatility, rather polar and prone to decomposition at elevated temperatures. Routine liquid-chromatographic determination of residues of these pesticides is limited to those compounds which also contain an aryl group, which can be satisfactorily detected by UV absorption; currently available detectors are not sufficiently sensitive or selective for analysis of the remainder.

Gas-chromatographic analysis would be simplified by completely converting residues either into the more readily chromatographed sulphides or into the sulphones. Unfortunately, quantitative reduction of the organophosphorus sulphones to the corresponding sulphides is virtually impossible because the phosphate moiety is more easily reduced than the very stable sulphone group. Reduction of sulphoxides is more practicable. The use of hydrochloric acid and potassium iodide in glacial acetic acid (derived from the method of Karaulova and Gal'pern[3]) provides a rapid and sensitive means of determining many organophosphorus sulphoxides in a wide range of fresh fruit and vegetables at residue levels (unpublished results). However, the sulphides produced are readily reoxidised if the solvents contain trace amounts of oxidants and, in addition, the sulphones still have to be determined separately. Oxidation of residues to the sulphones using potassium permanganate or 3-chloroperbenzoic acid is generally considered a more useful approach but the process is prone to losses of sulphone products. For the reasons outlined above, the gas-chromatographic determination of the sulphones can also be a source of errors. Several published methods of analysis employing these oxidants[4–7] were unreliable in our hands as a result of these problems. Similarly unreliable results were obtained using either minor modifications of these methods or alternative oxidants, whereas the techniques described here have proved satisfactory at this laboratory over a period of more than 2 years.

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Experimental

Extraction and Clean-up

The modified Watts *et al.* method[8] for extraction and clean-up of residues in fruits and vegetables was used to produce the results reported below but several other methods, both published[9] and unpublished, have been used with completely satisfactory results. However, extracts produced by the method of Abbott *et al.*[8] often contain too much oxidisable material for use with the oxidation procedure described in this paper. As yet we have not found any oxidation to be fully reliable when used in conjunction with extracts produced by the Abbott *et al.* method.

Oxidation

Reagents

All reagents were of analytical-reagent grade, and distilled water was used to prepare solutions.

Propylene glycol in acetone, 50% *V/V*. For use as a "keeper."
2-Methylpropan-2-ol.
Potassium permanganate solution, 0.2% *m/V*.
Dichloromethane.
Sodium sulphate, anhydrous crystalline granules.
Sodium sulphate solution, 5% *m/V*.

Oxidation of residues

Pipette an aliquot of the extract representing 10–25 g of crop into a 100-ml separating funnel (or any suitable container), add 0.1 ml of keeper and evaporate all of the volatile solvent, in the cold, with a stream of air. If the separator is rotated in the hands, evaporation with a low velocity air stream is rapid. Add 5 ml of 2-methylpropan-2-ol to the separator and completely dissolve the residue in it, keeping the liquid slightly warm to prevent freezing. Add 25 ml of potassium permanganate solution, shake the separator vigorously to mix the contents and then allow it to stand at room temperature for about 10 min. Add 25 ml of sodium sulphate solution and 50 ml of dichloromethane to the separator and shake the mixture

vigorously for about 1 min. Allow the layers to separate and run the lower layer through about 50 g of anhydrous sodium sulphate in a filter-funnel to dry it. Repeat the dichloromethane extraction twice more and then add 0.1 ml of keeper to the combined, dried extracts. Evaporate the solvent to a small volume and remove the last few drops, in the cold, using a stream of air. A sensitivity of 0.01 mg kg^{-1} is readily obtained if the residue is dissolved in acetone to the equivalent of 5 g of crop per ml of solvent for gas chromatography.

Gas Chromatography

Apparatus and reagents

Gas chromatograph. An all-glass system equipped for on-column injection and preferably designed so that the detector (nitrogen - phosphorus or flame-photometric) is connected directly to the end of the column.

Column tubing. Borosilicate glass, 1 m × 2 mm i.d., is adequate for most applications.

Packings. A loading of 5–10% of an intermediate-polarity silicone, *e.g*, OV-17, on a high-quality diatomaceous support, *e.g.*, 100–120-mesh Chromosorb W (HP), Supelcoport or Diatomite MQ.

Quartz-wool. FDK-960-E, 6-μm fibres (Gallenkamp, London).

Deacticol. Scientific Glass Engineering Pty. Ltd.

Column preparation

The column tubing should be cleaned thoroughly and silanised.[10] The column packing should be prepared by filtration[10] and the column plugged with silanised quartz-wool. With a carrier gas flow-rate of about 40 ml min^{-1} increase the column temperature slowly to 270 °C and condition it for 24 h. Reduce the column temperature to 220 °C and inject on to it, slowly and just behind the septum, ten portions of 10 μl of Deacticol at about 1-min intervals. Increase the column temperature to 270 °C again for 1–2 h before reducing it to 220 °C and continuing the conditioning for a minimum of a further 24 h. It is essential that efficient water and oxygen filters are used in the carrier gas line, at least when nitrogen is used.

Column assessment

To ensure reproducible quantitative results, the prepared column should perform adequately in the following tests.

(*i*) Compare the detector response obtained for 1–10 ng of demeton-S-methyl sulphone with that of a similar amount of malathion or parathion. A good column will provide a response for the sulphone of more than one third of that given by these very stable compounds. If less than one tenth of the response is obtained, the column is likely to show very marked effects in the following tests.

(*ii*) Compare the detector response obtained for 1–10 ng of demeton-S-methyl sulphone before and after injection of a crop extract that has not been oxidised (extracts produced by the modified Watts *et al.* method[8] are satisfactory). Ideally, there should be no change in response. If the crop extract induces a large increase in response the column may have to be "primed" regularly by injecting such extracts, but it may indicate that the carrier gas is contaminated with oxygen and water.

(*iii*) After priming of the column with crop extracts, if required [see (*ii*)], compare the detector response obtained for 1–10 ng of demeton-S-methyl sulphone dissolved in a crop extract with that for a similar amount dissolved in pure solvent. If the response to the sulphone in the spiked extract is more than a few per cent. greater than that in pure solvent, the carrier gas may be contaminated with oxygen and water or the column treatment with Deacticol may have been unsatisfactory and should be repeated.

Results and Discussion

Oxidation

Potassium permanganate does not oxidise thionophosphorus compounds to the corresponding oxons, which means that, as they will have been at least partly converted *in vivo*, residues of these compounds must be determined as the sum of the P=S and P=O sulphones produced. Complete conversion of such residues into the oxon sulphones can be achieved with 3-chloroperbenzoic acid but we, and others,[11,12] have found that variable losses of the sulphones can occur with this reagent, although some workers have been able to overcome this problem by the use of very specific reagent concentrations[6] or reaction time.[13] We have also found *tert*-butyl hydroperoxide and potassium permanganate in glacial acetic acid to give unreliable results. Aqueous permanganate oxidation methods commonly require acetone as co-solvent and relatively high concentrations (1–1.5% *m/V*) of oxidant[4,5,12,14,15] but, even when magnesium sulphate is used as a buffer,[5,12,14,15] the copious production of manganese dioxide that often occurs, even in the absence of crop extracts, suggests that the acetone is oxidised. Using acetone as the co-solvent we find that losses of demeton-S-methyl and its metabolites, ranging from negligible to complete, occur unpredictably. Using 2-methylpropan-2-ol as co-solvent it is evident that little oxidisable material is extracted from crops by the modified Watts *et al.*[8] and Becker[9] methods. Dornseiffen and Verwaal[11] have recently confirmed that recovery of the sulphone product can be satisfactory and reliable using much lower concentrations (0.1–0.2% *m/V*) of permanganate than are usually recommended.

A synopsis of recovery results obtained from crops, fortified at 0.1 mg kg^{-1} immediately prior to analysis, using the modified Watts *et al.*[8] method of extraction and cleanup is given in Table 1. Where appropriate, results are corrected for the gain in mass on oxidation. Values obtained for untreated crops ranged from <0.001 to <0.01 mg kg^{-1}, largely according to the prevailing detector sensitivity.

The level and variability of the recovery compare well with those found for other organophosphorus pesticides in a collaborative study of the modified Watts *et al.* method.[8] Similar recoveries have been obtained using the extraction and clean-up method of Becker[9] and unpublished rapid methods based on extraction of crops with ethyl acetate.

In the determination of terbufos residues, although oxidation of the sulphoxides is complete, that of the sulphides is not, at least in the presence of crop co-extractives. The unconverted sulphides can be readily determined from the same sample injection in gas chromatography, but this may mean that the total residue, as measured, is composed of three or four chromatographic peaks. The recovery of carbophenothion sulphones from carbophenothion and its metabolites was poor in the presence of wheat extracts prepared by the modified Watts *et al.* method,[8] although satisfactory through the oxidation procedure alone. The principal reason for this appeared to be the presence of an immiscible oily fraction, during the oxidation, into which the carbophenothion probably partitioned, rendering it inaccessible to the oxidant. In addition, we found the modified Watts *et al.* method[8] to be unsatisfactory for the extraction of some organophosphorus compounds from grains, so that an alternative extraction and clean-up method would be required for this crop.

Gas Chromatography

The column packings recommended have proved the best of many combinations of a wide range of stationary phases and supports assessed. Organophosphorus sulphones are moderately polar and tend to tail badly on packings consisting only of non-polar stationary phases. On the other hand, stationary phases of, or containing, polar liquids were mostly charac-

Table 1. Recovery of some pesticides and their metabolites from crop samples spiked at 0.1 mg kg^{-1}

Compound	Mean recovery, %	Standard deviation, %	*n*	Crop(s)
Demephion-S	88.5	—	2	Tomatoes
Demephion-S sulphone	85.2	—	2	Tomatoes
Demeton-S-methyl	82.4	6.6	10	Lettuce
Demeton-S-methyl sulphoxide	91.8	11.7	21	Lettuce, apples, peas, tomatoes
Demeton-S-methyl sulphone	88.2	9.9	6	Lettuce
Disulfoton	79.2	4.8	4	Lettuce
Disulfoton sulphoxide	85.8	6.9	4	Lettuce, apples
Disulfoton sulphone	83.4	5.1	4	Lettuce
Disulfoton oxon sulphoxide	96.5	—	2	Lettuce
Disulfoton oxon sulphone	90.0	—	2	Lettuce
Fenamiphos	93.2	—	2	Tomatoes
Fenamiphos sulphone	80.2	—	2	Tomatoes
Fenthion	79.8	—	2	Tomatoes
Fenthion sulphoxide	93.3	—	2	Tomatoes
Fenthion sulphone	97.5	—	2	Tomatoes
Fenthion oxon	102.7	—	2	Tomatoes
Fenthion oxon sulphone	71.8	—	2	Tomatoes
Fensulfothion	76.6	—	2	Tomatoes
Fensulfothion sulphone	75.7	—	2	Tomatoes
Fensulfothion oxon	97.2	—	2	Tomatoes
Fensulfothion oxon sulphone	71.3	—	2	Tomatoes
Phorate	93.7	—	2	Brussels sprouts
Phorate sulphoxide	100.7	17.0	11	Carrots, apples
Phorate sulphone	94.9	14.9	7	Carrots, Brussels sprouts
Phorate oxon	83.9	—	2	Brussels sprouts
Phorate oxon sulphoxide	78.7	7.7	11	Carrots, apples
Phorate oxon sulphone	95.9	10.6	7	Carrots, Brussels sprouts
Terbufos	98.7*	17.0	4	Tomatoes, lettuce
Terbufos sulphoxide	106.3	—	2	Tomatoes
Terbufos sulphone	95.0	—	2	Tomatoes
Terbufos oxon	107.0*	19.6	4	Tomatoes, lettuce
Terbufos oxon sulphoxide	82.3	—	2	Tomatoes
Terbufos oxon sulphone	94.4	—	2	Tomatoes
Thiometon	68.4	—	2	Lettuce
Thiometon sulphoxide	71.1	—	2	Lettuce
Thiometon sulphone	94.3	—	2	Lettuce

*Results include variable amounts (13.2–95.0%) recovered as unchanged sulphides.

terised by short working lives for the least stable sulphones (*e.g.*, demeton-S-methyl sulphone) even though they often remained satisfactory for the more stable sulphones (*e.g.*, phorate sulphones) and many other organophosphorus compounds. The columns recommended by Grant *et al.*[16] and McLeod *et al.*[17] seemed particularly prone to this defect. The use of diatomaceous supports deactivated with Carbowax 20M, both prepared by us and obtained commercially (*e.g.*, Ultrabond), similarly produced good columns, particularly when coated with liquids such as OV-17, but these were of short working life even though the nitrogen carrier gas was passed through several water and oxygen filters. The excellent results that have been obtained with such columns by others[18] may reflect their use of helium as the carrier gas, with its inherently lower concentration of reactive impurities. We have found silanised quartz-wool to be superior to silanised glass-wool for plugging columns, giving less decomposition of labile compounds. The quartz-wool that we used is not pure silica, but efforts to remove metallic impurities from the surface by acid treatments rendered the material too brittle to use, and therefore silanisation is the only preliminary deactivation recommended for it. Columns prepared as recommended have provided efficiencies of about 1 000 theoretical plates per metre[10] for demeton-S-methyl sulphone and service lives extending to many hundreds of injections of crop extracts.

Accurate quantification is a frequent problem in gas chromatography of the sulphones, and some other organophosphorus compounds, and this appears to be related to on-column catalytic decomposition and its inhibition, directly or indirectly, by crop co-extractives. Where it is necessary to prime the column (see *Column assessment*) before starting a series of injections, we have found that injection of a few microlitres of a suitable crop extract is much more efficient than injection of large amounts of the pesticides, which is sometimes recommended. We have not identified any of the compounds in crop extracts responsible for the priming effect, but they can be extracted from a wide range of crops using solvents of intermediate polarity and are largely destroyed by oxidation with permanganate. Although these compounds are of low volatility, they are removed from the gas-chromatographic column at a rate increasing with temperature, and therefore priming has to be repeated more frequently at high column temperatures.

The enhancement effects produced by crop extracts (see *Column assessment*) resemble short-lived priming effects and may or may not persist over several subsequent injections. Both oxidised and non-oxidised crop extracts produce these effects, which are also reproduced by injection of solutions of pesticides containing most free carboxylic acids of lower volatility than octanoic acid. The incorporation of Carbowax 20M, TPA[14] and FFAP, which contain free carboxyl groups, into stationary phases reduced or eliminated the observed enhancement effects produced by crop extracts, but we found that such packings were very sensitive to oxygen such that even changing the septum degraded this beneficial character and contributed to their short working lives. Gas chromatography - mass spectrometry of crop extracts produced by the modified Watts *et al.* method[8] usually shows the elution of appreciable amounts of free fatty acids, probably on-column decomposition products of triglycerides,[19] which

may account for the effects observed. Although it is sometimes recommended,[12] we consider that the use of pesticide standards made up in crop extracts is unsatisfactory as the degree of enhancement can vary considerably from extract to extract.

The use of Deacticol, a triethanolamine-based commercial preparation supposed to complex with metallic active sites on glass and other surfaces, and efficient water and oxygen filters in the nitrogen carrier gas lines usually results in the virtual elimination of both priming and enhancement effects. Treatment of packings or completed columns with triethanolamine, EDTA and some of their analogues (all of analytical-reagent grade) did not produce results as satisfactory as those achieved with Deacticol treatment. On-column injections of proprietary silanising reagents were similarly ineffective. The use of helium as the carrier gas may be advantageous when the "oxygen-free" nitrogen available is insufficiently pure.

Relative Retention Times

The data in Table 2 were produced using a 1 m × 2 mm i.d. column of 5% OV-17 on 100–120-mesh Diatomite MQ at 200 °C with a nitrogen flow-rate of 40 ml min^{-1}. Under these conditions, the sulphones had retention times about three to five times longer than those of the corresponding sulphides and the absolute and relative retention times were similar with either nitrogen or helium as the carrier gas. The relative retention times were affected considerably by changes in column temperature. Coupled gas chromatography - mass spectrometry was used to identify positively the peaks produced; details of the operating conditions used and the spectra produced will be published later.[20]

All of the sulphoxides that wholly or partially survived gas chromatography produced peaks with retention times fairly close to those of the corresponding sulphones. Where very short retention times have been reported for sulphoxides, relative to the sulphones,[14,21] we suggest that these may be due to sulphide impurities or dehydration products of sulphoxides. The latter are common thermal degradation products of sulphoxides and we have observed α-unsaturated demeton-S-methyl[22] in the gas chromatography of its sulphoxide. Those sulphoxides which partially or wholly decompose during gas chromatography tended to produce a pattern of product peaks that varied with the conditions. Only a few, or none, of the discrete peaks corresponded to phosphorus-containing compounds, these being mainly associated with groups of very broad, badly tailing peaks of, presumably, acidic compounds.

Table 2. Retention times of organophosphorus sulphides and sulphones relative to malathion (= 1.00)

Compound	Sulphide	Sulphone
Carbophenothion	3.30	10.0
Carbophenothion oxon	3.10	8.00
Demephion-S	0.25	1.20
Demephion-O	0.20	0.70
Demeton-S-methyl	0.25	1.35
Disulfoton	0.45	2.45
Disulfoton oxon	0.35	1.80
Fenamiphos	2.30	7.80
Fensulfothion	—	4.60
Fensulfothion oxon	—	4.00
Fenthion	1.10	4.10
Fenthion oxon	1.05	3.95
Phorate	0.30	1.30
Phorate oxon	0.25	0.90
Terbufos	0.30	1.50
Terbufos oxon	0.25	1.25
Thiometon	0.40	1.90
Vamidothion	2.90	Decomposed

Although reproducible chromatograms of the labile sulphoxides and their decomposition products can be obtained, at least over short time periods, they are unreliable in that they may be qualitatively and quantitatively altered by the presence of crop co-extractives.

Temephos and its metabolites appeared to decompose under our chromatographic conditions, although other workers have apparently been able to chromatograph the sulphide and sulphoxide intact.[23,24] Residues of this pesticide, in contrast to many of this group of compounds, can be readily determined by HPLC.[25] Although vamidothion and its sulphoxide appeared to be oxidised by permanganate to the sulphone, as observed by TLC, the product was totally decomposed by our gas-chromatographic systems even though the sulphide was perfectly stable. Available methods for vamidothion determination via oxidation[11,26] appear to depend upon the detection of an unidentified, non-phosphorus, electron-capturing decomposition product of the sulphone.

Conclusions

Using the methods described, the determination of residues of most of the organophosphorus sulphides, sulphoxides and sulphones has been consistently reliable. However, constant caution needs to be exercised in the interpretation of the results as many of the sulphones are rather labile under the conditions employed.

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Rapid Procedure for the Determination of Vitamins A and D in Fortified Skimmed Milk Powder Using High-performance Liquid Chromatography

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A rapid, high-performance liquid chromatographic (HPLC) method for the determination of vitamins A and D in fortified skimmed milk powder (SMP) is described. Either vitamin D_2 or D_3 is added as an internal standard, depending upon which formulation has been used for fortification, and the sample is saponified and extracted. Vitamin A can be determined directly (without further clean-up) in a portion of this extract using reversed-phase HPLC. Vitamin D is determined on the remainder of the extract after clean-up using a Sep-pak cartridge and the same HPLC system. Details of recoveries of added vitamins and the precision of the method are given.

Keywords: *Vitamin A determination; vitamin D determination; high-performance liquid chromatography; fortified skimmed milk powder*

The World Health Organization (WHO) emphasises the importance of an adequate dietary intake of vitamins A and D, particularly where high protein foods are provided for underdeveloped countries.[1] EEC Regulation No. 1354/83[2] states that all skimmed milk powder (SMP) if donated as part of the World Food Aid Programme must meet the following requirements: vitamin A content = 5 000–10 000 U per 100 g; and vitamin D content = 500–1 000 U per 100 g.

Fortified SMP is produced at a number of different creameries throughout Great Britain and Northern Ireland and is approved and registered by the Intervention Board for Agricultural Produce. Each batch of fortified SMP produced must be sampled and analysed quickly prior to its shipment to ensure that it complies with the EEC Regulation. Therefore a rapid and reliable laboratory method for determining vitamins A and D in these samples is required.

Several methods relating to the determination of vitamins A and D in a variety of different foodstuffs are described in reference 3. All these procedures, however, involve extensive clean-up stages, which make them unsuitable for routine use where large numbers of samples are involved. In recent years many workers have reported the separation and analysis of vitamin A and vitamins D_2 and D_3 using reversed-phase HPLC. Multi-vitamin procedures that determine all the lipid-soluble vitamins simultaneously[4,5] have also been reported but these were considered to be unsuitable for fortified SMP samples owing to the large difference between vitamin A and D levels, which would make precise quantification difficult.

Saponification and extraction of the unsaponifiable fraction are widely used and were found to be a useful way of separating the lipid-soluble vitamins from the lipid material. The extract could then be directly used for the HPLC determination of vitamin A, but further clean-up was found to be necessary before vitamin D could be determined. Several different clean-up techniques were evaluated, including sterol precipitation,[6] conventional column chromatography using alumina[7] and Sephadex,[8] thin-layer chromatography[9] and preparative HPLC.[10] Although most of these purification procedures produced some degree of success they increased the time taken for each analysis beyond an acceptable limit.

A method involving the use of Waters Associates Sep-pak cartridges to clean up samples of concentrated oils, pre-mixes and vitamin capsules prior to vitamin D analysis[11] has been applied to fortified SMP samples after saponification and extraction. This technique was found to be rapid and convenient and the resulting cartridge eluate could be used directly for the HPLC determination of vitamin D. Full details fo the method, which has been in use in this laboratory for more than 1 year, are presented together with precision and recovery data.

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Experimental

Apparatus

Saponification and extraction

A block of heating mantles with variable controls, a riffle sampler, a rotary evaporator and round-bottomed flasks (500 and 50 ml), round-bottomed flasks with side arms (250 ml) and separating funnels (500 ml) were used.

Vitamin D clean-up

Clean-up was performed with a glass syringe, Luer fitting (10 ml) and Sep-pak C_{18} reversed-phase cartridges from Waters Associates.

High-performance liquid chromatography

The HPLC system used consisted of a solvent reservoir, a high-pressure reciprocating pump (Altex Model 110A), an injection valve (Specac P/N 34000), an injection loop (50 μl), a guard column of stainless steel, 75 × 2.1 mm i.d., containing pellicular reversed-phase material (Chrompack UK), an analytical column of stainless steel, 250 × 4.6 mm i.d., containing CP Spherisorb C_{18} (5 μm) (Chrompack UK), a variable-wavelength UV detector (Kratos Spectroflow 773) and a 10 mV recorder.

Reagents

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

Saponification and extraction

Ascorbic acid.
Diethyl ether.
Ethanol. Absolute (99.8% ethanol).
Petroleum spirit, boiling-range 40–60 °C.
Phenolphthalein solution, 0.5% *in ethanol.*
Potassium hydroxide solution. Dissolve 60 g of potassium

hydroxide pellets in approximately 45 ml of distilled water, allow to cool and dilute to 100 ml.

Propan-2-ol.

Extracting solvent, diethyl ether - petroleum spirit (1 + 1).

Vitamin D clean-up

Methanol. HPLC grade.

Tetrahydrofuran.

Water. HPLC grade.

Mixed solvent, methanol - tetrahydrofuran - water (1 + 1 + 2).

Standard solutions

Vitamin A. Retinol acetate. All-*trans,* synthetic, crystalline (2 900 000 U g^{-1}*) obtained from Sigma Chemicals. This is the purest material commercially available and has been found to be free from the *cis*-isomer.

Retinol acetate (25.0 mg) is weighed out, saponified and extracted using the same procedure as described for the SMP. After evaporation to dryness the residue containing the free retinol is dissolved in propan-2-ol and diluted to 100 ml. This stock solution contains 250 μg ml^{-1} of retinol and is stable for 2–3 months if stored in a refrigerator. A working solution containing 5 μg ml^{-1} is prepared by diluting 2 ml of the stock solution to 100 ml with methanol. The working solution must be standardised daily using the UV procedure as described in reference 12.

The presence of any *cis*-isomer can be determined using the HPLC conditions described but with a mobile phase consisting of methanol - water (90 + 10).

Vitamin D. (i) Cholecalciferol. Activated 7-dehydrocholesterol; vitamin D_3 obtained from Sigma Chemicals. *(ii) Ergocalciferol.* Irradiated ergosterol; vitamin D_2 (40 000 000 U g^{-1}†) obtained from Sigma Chemicals.

For each standard 20.0 mg are weighed out, dissolved in ethanol and made up to 100 ml. This stock solution containing 200 μg ml^{-1} is stable for 1 week if stored in the refrigerator. A working solution is prepared daily, by diluting 2 ml of stock solution to 200 ml with ethanol (2 μg ml^{-1}). The exact concentration of this solution must be determined by measuring its absorbance at 365 nm, calculating the $A_{1cm}^{1\%}$ value and comparing the result with the given literature value (458.9 ± 7.5 and 473.2 ± 7.8 for D_2 and D_3, respectively).[13]

Method

Saponification and extraction

All manipulations should be carried out in subdued light and all glassware should be rinsed with methanol before use.

Weigh 10.0 g of a representative sub-sample of the SMP from a riffle sampler into a round-bottomed flask fitted with a side-arm. If vitamin D_3 is to be determined add a known amount of vitamin D_2 (12.5 μg is equivalent to 500 U per 100 g in the sample) as internal standard and *vice versa.* Add 1 g of ascorbic acid, 40 ml of ethanol and swirl to ensure that all the milk powder is wetted. Add 10 ml of potassium hydroxide solution, mix carefully and use a further 10 ml of ethanol to wash down the sides of the flask. Introduce a slow stream of nitrogen via the side-arm and boil gently under reflux for 30 min.

Cool the resultant solution and quantitatively transfer into a separating funnel using two 25-ml portions of distilled water to rinse out the flask. Add 125 ml of extracting solvent to the funnel and shake vigorously for 1 min. Add a further 125 ml of extracting solvent and re-shake vigorously for a further 1 min. Allow the layers to separate completely then discard the lower aqueous layer. Wash the organic layer carefully (gentle swirling to avoid the formation of emulsions) with successive 100-ml portions of distilled water until the washings are free from alkali and impart no coloration to phenolphthalein solution.

Transfer the extract into a 500-ml round-bottomed flask using 5 ml of methanol to rinse the separating funnel and evaporate to approximately 2 ml using a rotary evaporator at 50 °C. Complete the evaporation carefully using a gentle stream of nitrogen. Dissolve the residue in 25 ml of methanol. Remove a 5-ml aliquot and dilute to 15 ml with methanol. This solution can now be used for the vitamin A determination directly using HPLC. The remaining 20 ml of solution are transferred into a 50-ml round-bottomed flask, carefully evaporated to dryness and re-dissolved in 2 ml of ethanol. This solution is retained for the vitamin D determination.

Vitamin D clean-up

A glass syringe is used to prepare a Sep-pak cartridge by pumping through 2 ml of methanol followed by 6 ml of water. Add 1 ml of water to the ethanol solution. Swirl and pump on to the cartridge. Remove interfering compounds by washing with 15 ml of mixed solvent. Elute the vitamin D with 5 ml of methanol and retain for HPLC analysis.

High-performance liquid chromatography

A mobile phase of methanol - water (97.5 + 2.5) and a flow-rate of 1 ml min^{-1} are used, for both vitamin A and D determinations, and the detector wavelength is set at 325 nm (vitamin A) and 265 nm (vitamin D).

Inject 50-μl volumes for both sample and standard solutions. Vitamin A should have a retention time of approximately 6 min, vitamin D_2 approximately 17 min and D_3 approximately 18 min. The separation of D_2 from D_3 should be almost base line. The amounts of each vitamin in the sample are calculated using the following equations.

Vitamin A—

$$\text{Amount of vitamin A in the SMP sample (U per 100 g)} = \frac{\text{peak height of sample}}{\text{peak height of standard}} \times \frac{750}{1} \times \text{concentration of standard (U ml}^{-1}\text{)}$$

Vitamin D—

The relative responses of both D_2 and D_3 should be checked before each batch of samples by measuring the peak heights of the standard injections. The relative response factor is then calculated using the following equation:

$$\text{Relative response factor (RRF) of } D_3 = \frac{\text{peak height of } D_2}{\text{concentration of } D_2} \times \frac{\text{concentration of } D_3}{\text{peak height of } D_3}$$

$$\text{Amount of vitamin } D_3 \text{ in the SMP sample (U per 100 g)} = \frac{\text{peak height of } D_3}{\text{peak height of } D_2} \times \frac{10}{1} \times \frac{\text{concentration of}}{D_2 \text{ standard (U ml}^{-1}\text{)}} \times \text{RRF of } D_3$$

Results

Recovery of Vitamin A

Samples of unfortified SMP were spiked in triplicate with three different levels of retinol acetate and treated as described. The peak heights obtained from injections of the spiked samples were compared with the peak heights obtained from injections of standard retinol solutions and the percentage recoveries calculated. Duplicate samples of the unfortified SMP (unspiked) were also analysed to ensure that no vitamin A was present. The results are given in Table 1.

* 1 U of vitamin A = 0.3 μg.

† 1 U of vitamin D = 0.025 μg.

Table 1. HPLC determination of recoveries of retinol acetate added at different levels to unfortified SMP

Retinol acetate added/ U per 100 g	Recovery, %
5060	99.2
	98.2
	101.2
7775	96.6
	98.6
	99.2
9615	94.0
	90.3
	94.4

Table 2. Replicate determinations of vitamin A in fortified SMP

No.	Vitamin A* content/ U per 100 g
1	5640
2	5530
3	5790
4	5510
5	5490
6	5570
Mean	5588
Standard deviation ..	112

* Vitamin A as all-*trans*-retinol.

Table 3. HPLC determination of the recovery of vitamin D_3 relative to D_2 after complete analysis of spiked sample of SMP

Peak height D_2/peak height D_3		Recovery of D_3 relative to D_2
Standard solution	Spiked sample	
1.006	1.010	100.4
1.006	1.018	101
1.006	1.011	100.5

Table 4. Replicate determinations of vitamin D_3 in fortified SMP

No.	Vitamin D_3 content of SMP/ U per 100 g
1	520
2	515
3	565
4	540
5	520
6	535
Mean	532.5
Standard deviation ..	18.6

Precision of Vitamin A Determination

Six replicate determinations of a sample of SMP fortified by the dairy were carried out to assess the precision of the method and the results are shown in Table 2.

Recovery of Vitamin D

Before using vitamin D_2 as an internal standard for vitamin D_3 determinations and *vice versa*, the method was checked to ensure that the ratio of D_2:D_3 had not altered during the analysis.

A standard solution containing approximately equal amounts of vitamins D_2 and D_3 was prepared and analysed using HPLC. Aliquots of this standard solution were added to three samples of unfortified SMP and treated as described. The ratios of the peak heights (D_2:D_3) were calculated for both standard solutions and spiked samples. Duplicate samples of the unfortified SMP (unspiked) were also analysed to ensure that no vitamin D_2 or D_3 was present. The results are shown in Table 3.

Precision of Vitamin D Determination

Six replicate determinations of vitamin D_3 in a sample of SMP fortified by the dairy were carried out using vitamin D_2 as internal standard to assess the precision of the method and the results are shown in Table 4.

Typical HPLC traces of fortified skimmed milk powder extracts are shown in Fig. 1.

Discussion

This procedure is rapid, precise and simple to perform in comparison with standard methodology. It has been successfully employed to analyse more than 100 samples of fortified SMP. Several samples of fortified whole milk have also been analysed using this method. The determination of vitamin A was found to be satisfactory, but more extensive clean-up techniques involving sterol precipitation and preparative HPLC were necessary before vitamin D could be quantified precisely.

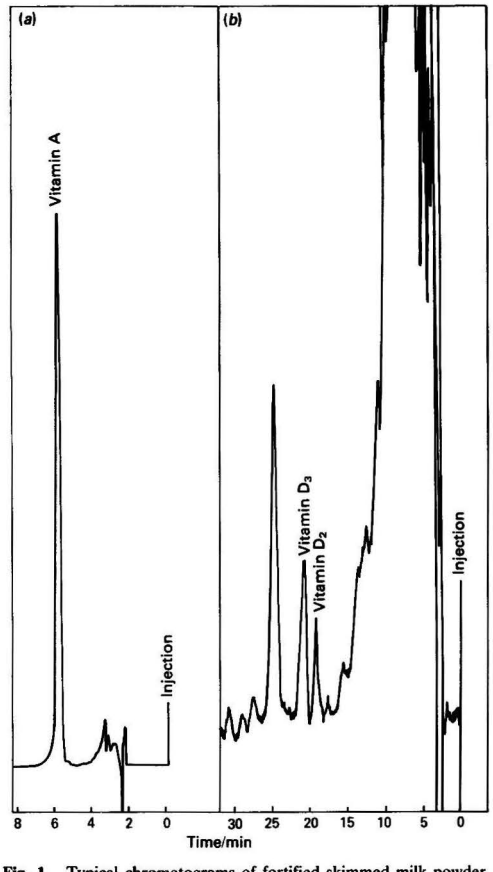

Fig. 1. Typical chromatograms of fortified skimmed milk powder extracts. (*a*) Fortified with vitamin A (325 nm); and (*b*) with vitamin D (265 nm)

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Separation of Dithiocarbamates by High-performance Liquid Chromatography Using a Micellar Mobile Phase

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A high-performance liquid chromatographic (HPLC) separation of salts of dithiocarbamates on a cyano-bonded column is described. The effect of variation of the content of organic modifier (methanol) and ionic strength on retention has been investigated. The retention mechanism depends on micelle formation in the mobile phase using cetyltrimethylammonium bromide (CTAB). An eluent containing CTAB has been used to separate five dithiocarbamates; these included sodium *N*-methyldithiocarbamate (metham-sodium) a widely used insecticide, and disodium ethylenebisdithiocarbamate (nabam) a suspected precursor of ethylenethiourea.

Keywords: *Micellar liquid chromatography; dithiocarbamates; high-performance liquid chromatography*

Dithiocarbamate (DTC) insecticides pose unique problems in residue analysis. Dithiocarbamate salts are highly polar, very soluble in water and are thermally unstable. In addition, dithiocarbamates are pH sensitive; it is well known[1-3] that rapid decomposition of dithiocarbamates occurs on acidification, the products usually being carbon disulphide and an amine. Moreover, dithiocarbamates are commonly metabolised to products whose presence in water and other samples are of toxicological significance[4]; such products would include ethylenethiourea, ethylenethiuram monosulphide and ethyleneurea.

Methods for the determination of dithiocarbamates have been reviewed elsewhere.[5-10] These methods are susceptible to interferences and require careful sample clean-up procedures prior to derivatisation before accurate and reproducible results may be obtained. These methods are specific only for the dithiocarbamate group and cannot distinguish between compounds with different *N*-alkyl substituents.

Smith *et al.*[11] reported the determination of dithiocarbamates by liquid chromatography using transition metal salts as ion-pairing reagents. Bond and Wallace[12] also used dithiocarbamates to detect copper by high-performance liquid chromatography and electrochemical detection.

This paper reports the separation of five dithiocarbamate compounds, two of which are in usage as commercial insectides; the procedure adopted employs the use of an appropriate surfactant, cetyltrimethylammonium bromide (CTAB), above its critical micelle concentration (c.m.c.) to achieve the separation by micellar chromatography.

Experimental

Apparatus

Liquid chromatography was carried out using a Waters Associates M6000A pump connected to a Waters μ-Bondapak CN column (3.9 mm × 30 cm) (10 μm particle size) fitted with a Rheodyne 7125 valve injector with a 20-μl loop. The eluates were detected with a 480 Lambda Max Variable UV detector (Waters Associates) operating at 253 nm. Retention times and peak areas were obtained with a recording integrator (Hewlett Packard Model 3390). The column temperature was maintained at 303 K by immersion of the column in a water-bath.

Reagents

Dithiocarbamate salts

The sodium salts of *N*-methyl- and *N,N*-dimethyldithiocarbamate were supplied by J. D. Cambell & Sons Ltd., Warrington, Fluorochem Ltd., Glossop and Robinson Bros. Ltd., West Bromwich.

Cetyltrimethylammonium bromide

Cetyltrimethylammonium bromide was supplied by Fluorochem Ltd., Glossop, and was used without further purification. Methanol was HPLC grade supplied by Rathburn Chemicals, Scotland. De-ionised water, distilled from glass and further de-ionised using a Waters I de-ioniser, was employed. Disodium hydrogen orthophosphate (analytical-reagent grade) was obtained from Fisons Apparatus, Loughborough. Potassium dihydrogen orthophosphate (AnalaR) was obtained from BDH Chemicals Ltd., Poole, Dorset.

Procedure

Solutions of dithiocarbamates (100 μg ml^{-1}) were injected on to the column. Different ratios of methanol - water were used as indicated. The mobile phases were buffered with phosphate at pH 6.8. The solutions were filtered using a 0.45-μm millipore filter. Individual solutes were chromatographed with a flow-rate of 2 ml min^{-1}. The column was conditioned by passage of the buffered mobile phase containing CTAB at 1.5 ml min^{-1} for 90 min. This gave reproducible retention times for the injected solutes. The capacity factor (k') was used as a measure of the degree of retention of the solute and is defined as $t_R - t_m/t_m$, where t_R is the elution time of the solute and t_m is the elution time of unretained solute.

Results and Discussion

Charged surfactants have been widely used as mobile phase modifiers to improve the partitioning characteristics of charged solutes in reversed-phase high-performance liquid chromatography. Other studies have focused on identifying the interactions that occur in the presence of the counter ions, and several retention mechanisms have been proposed. The first assumes an ion-exchange mechanism.[13] In this hypothesis the hydrophobic surfactant molecules are presumed to adsorb on to the bonded, non-polar stationary phase and cause the column to behave in a conventional ion-exchange manner. The second hypothesis assumes the formation of an ion-pair in the mobile phase prior to its adsorption on to the non-polar stationary phase.[14] Solvophobic theory[15] has been used to defend the ion-pair hypothesis. Bidlingmeyer proposed an "ion-interaction" model,[16] which does not require ion-pair formation in either phase and is not based on classical ion-exchange. In this model the hydrophobic surfactant is assumed to adsorb on to the stationary phase as a primary layer, while the counter ions occupy the secondary layer. Thus an electrical double layer is established. As the mobile phase also contains the hydrophobic salt, a dynamic equilibrium is

established between the double layer, the hydrophobic salt and the solute.

A micellar mobile phase differs from a conventional ion-pairing mobile phase in two important aspects. Firstly, micellar solutions can be regarded as microscopically heterogeneous, being composed of the micellar aggregate and the "bulk" surrounding medium. An ion-pairing mobile phase is homogeneous. Secondly, the concentration of surfactant in micellar chromatography is above its critical micelle concentration (c.m.c.), *i.e.*, the concentration above which micelle formation becomes appreciable.

The concentrations of CTAB used in this study were all above its c.m.c. (9.9×10^{-4} M). The ability to control "selectivity" in the mobile phase by the use of micelles results in a high degree of flexibility not available from other methods of ion chromatography. The importance of micelles in the mobile phase lies in their ability to participate in the partitioning mechanism. The three equilibria involved in micellar chromatography are schematically represented in Fig. 1.

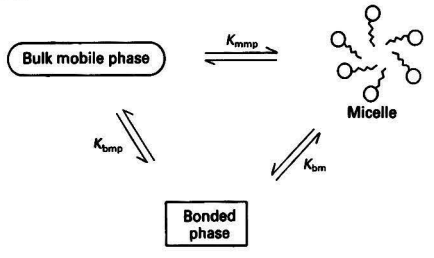

Fig. 1. Schematic representation of solute partitioning in micellar chromatography. The elution behaviour of a solute depends on three partition coefficients: K_{mmp}, the partition coefficient between mobile phase and micelle; K_{bm}, the partition coefficient between bonded phase and micelle; and K_{bmp}, the partition coefficient between bonded phase and bulk mobile phase

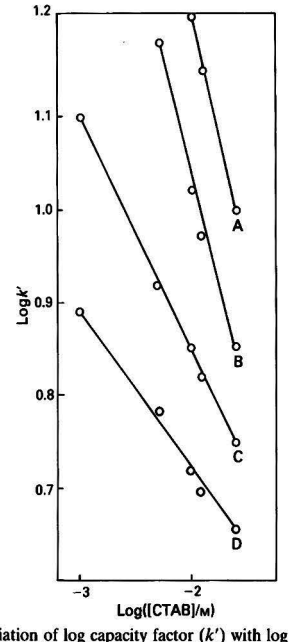

Fig. 2. Variation of log capacity factor (k') with log concentration of hexadecyltrimethylammonium bromide. (A) Sodium *N,N*-diethyldithiocarbamate; (B) ammonium tetramethylenedithiocarbamate; (C) sodium *N,N*-dimethyldithiocarbamate; and (D) sodium *N*-methyldithiocarbamate

The chromatographic properties of aqueous solutions of surfactants above their critical micelle concentrations have been studied by other workers.[17,18] In ion-pairing chromatography increasing the concentration of the hydrophobic surfactant increases the retention time for compounds that interact electrostatically with it. Fig. 2 shows that as the concentration of CTAB was increased, a decrease in retention of the dithiocarbamates was observed. This decrease in retention is expected when using a micellar mobile phase.[18] When log (capacity ratio) is plotted *versus* log (CTAB concentration), as in Fig. 2, an important feature of micellar chromatography becomes apparent. The linear graphs are not parallel but tend to converge. Thus not only the capacity, k', but also the separation factor, is observed to change. At low concentrations of CTAB ($<1 \times 10^{-3}$ M) injection of individual dithiocarbamates results in a double peak as shown in Fig. 3. The cause of this phenomenon is at present under study. Vacant peaks[19] and double peaks[20] are commonly observed phenomena in liquid chromatography; peak splitting in ion-pair high-performance liquid chromatography has also been reported.[21]

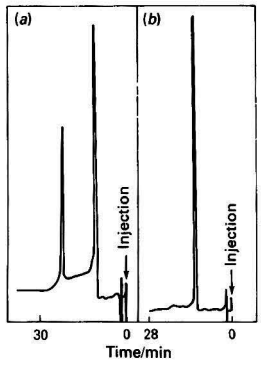

Fig. 3. Effect of variation of CTAB concentration on chromatogram obtained for sodium *N*-methyldithiocarbamate. (*a*) 0.001 M CTAB; (*b*) 0.01 M CTAB

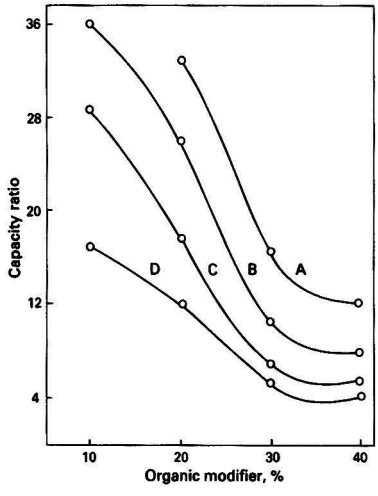

Fig. 4. Variation of capacity ratio (k') with concentration of organic modifier (methanol). (A) Sodium *N,N*-diethyl-dithiocarbamate; (B) ammonium tetramethylenedithiocarbamate; (C) sodium. *N,N*-dimethyldithiocarbamate; and (D) sodium *N*-methyldithiocarbamate

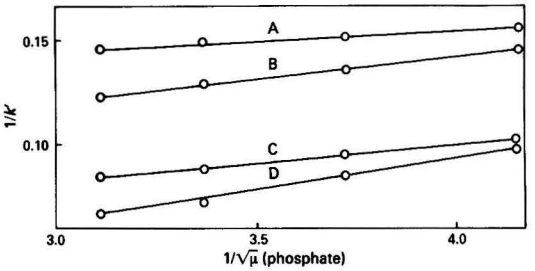

Fig. 5. Variation of capacity ratio with ionic strength. Mobile phase conditions: 70 + 30 *V/V* methanol - water; 1.25×10^{-2} M CTAB; flow-rate, 2 ml min^{-1}, pH, 6.8 (phosphate buffer 0.018 M); and ionic strength control with phosphate. A–D as in Figs. 2 and 4

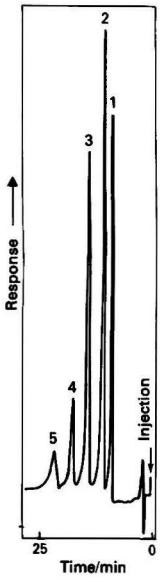

Fig. 6. Reversed-phase micellar partition chromatogram of dithiocarbamates. Solutes: 1, sodium *N*-methyldithiocarbamate; 2, sodium *N,N*-dimethyldithiocarbamate; (3) ammonium tetramethylenedithiocarbamate; (4) sodium *N,N*-diethyldithiocarbamate; and (5) disodium ethylenebisdithiocarbamate. Conditions: column, 300 × 3.9 mm; packing, 10 μm μ-Bondapak CN; eluent, 0.0125 M CTAB (pH 6.8), methanol - water (30 + 70); temperature, 30 °C; detector, UV photometer, 253 nm, 0.01 a.u.f.s.

The presence of non-aqueous phases can cause changes in partial molar volume and apparent relative molecular mass of a micelle. Guveli *et al.*[22] reported studies on the addition of aliphatic alcohols to micellar solutions. The effect of variation of the percentage of methanol in the mobile phase is illustrated in Fig. 4. A decrease in capacity ratio is observed with a corresponding increase in organic modifier concentration. For methanol - water (20 + 80) significant tailing on the signal peaks was observed. The micellar aggregates are possibly disrupted at this concentration of organic modifier; Weinberger *et al.*[17] reported similar effects. At concentrations between 20 and 40% *V/V* of methanol the retention mechanism appears normal and the capacity ratios decrease with increased methanol concentration.

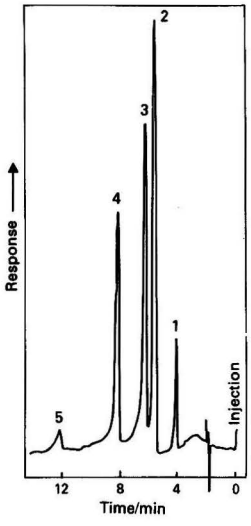

Fig. 7. HPLC of DTC salts in spiked pond water. Solutes: pond water filtered and purified by passage through C_{18} pre-column with (1) resorcinol (internal standard); (2) *N*-methyl DTC (10 mg l^{-1}); (3) *N,N*-dimethyl TC (10 mg l^{-1}); (4) TMDTC (10 mg l^{-1}); and (5) *N,N*-diethyl DTC (10 mg l^{-1}). Conditions: column, 250 × 5 mm; packing C_{18}, 5 μm; eluent, 30 + 70 *V/V* water - methanol, phosphate buffer (pH 6.8) and 1×10^{-2} M CTAB

In Fig. 5 variation of the inverse of capacity ratio *versus* the inverse of the square root of the ionic strength is shown; as the phosphate concentration increases, the capacity factor decreases. Iskandarini and Pietrzyk[23] have reported similar results and postulated that if a linear relationship exists between k'^{-1} *versus* $1/(\mu)^{\frac{1}{2}}$ the retention of the solute anion in the presence of a surfactant on a bonded phase results from a complex set of equilibria taking place within a double layer at the bonded-phase surface. Cantrell and Puon[24] have derived a quantitative explanation in terms of the Stern - Guoy - Chapman (SGC) theory of electrical double layer to account for the linear relationship between the reciprocal of the capacity ratio and the reciprocal of the square root of the ionic strength of the mobile phase. The linearity observed in Fig. 5 may indicate the establishment of dynamic equilibria between the dithiocarbamate salt and the electrical double layer on the bonded phase, and the electrical double layer on the micelle surface.

Fig. 6 illustrates the separation of five dithiocarbamates under optimum conditions, *i.e.*, 70 + 30 *V/V* water - methanol containing 2.5×10^{-2} M CTAB buffered to pH 6.8 with 0.018 M phosphate buffer.

Pond-water samples were used to assess the potential of micellar chromatography for the analysis of environmental samples. Pond water was collected and standard additions of four dithiocarbamates were made to this sample. The pond water was then filtered, passed through a C_{18}-bonded column to remove aromatic compounds and subsequently analysed by micellar chromatography; resorcinol was added as an internal standard. A typical chromatogram is illustrated in Fig. 7.

It is thus clear that micellar chromatography shows promise for the separation of labile dithiocarbamate species of the type whose metabolites are of toxicological significance, and which are commonly employed as insectides and hence may be present in industrial effluents.

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Identification of Major Perchloroaromatic Compounds in Waste Products from the Production of Carbon Tetrachloride and Tetrachloroethylene

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High-boiling residues from the chlorinolysis of propylene ("hex-waste") can be separated into two categories: (*i*) crude hexachlorobenzene and (*ii*) the mother liquor, which is subsequently processed and recycled. The composition of the mother liquor has been investigated. It was found to contain a large number of perchlorinated, higher aromatic compounds, *e.g.*, octachlorostyrene, decachlorobiphenyl, octachloronaphthalene, octachloroindene, octachloroacenaphthylene, decachlorofluoranthene and octachlorodibenzofuran. Results also confirmed that the only unambiguous method for the identification of these compounds was their separation by reversed-phase HPLC and comparison with authentic standards, most of which had to be synthesised for this work.

Keywords: *HPLC separation; perchloroaromatics; higher chlorocarbons; hex-waste*

Carbon tetrachloride and tetrachloroethylene, two important chlorinated solvents and chemical intermediates, are commercially produced using the Stauffer or Scientific Design processes.[1,2] Both of these processes require high-temperature chlorinolysis (550–600 °C) of propylene in an excess of chlorine. Occasionally, ethylene or other lower hydrocarbons are used as the feedstock. The reaction is also frequently used as a "sink" for waste products from other chlorination reactions, *e.g.*, waste streams from the production of vinyl chloride.

Calculated on the basis of chlorine used, the reaction yield can be as high as 96%, when the recovery of chlorine from resulting hydrogen chloride is included.[1] The rest of the chlorine is lost in the form of high-boiling residues after separation of carbon tetrachloride and tetrachloroethylene. The composition of these residues, "hex-waste," varies according to the production temperatures, feedstock character and product separation methods. Usually, the major components of hex-waste are hexachlorobenzene, hexachlorobutadiene and hexachloroethane, with some hexachlorocyclopentadiene, if the vinyl chloride waste is used as a part of the feedstock to the chlorination reactor. A large number of heavier compounds are also present in lower concentrations.

Hex-waste is subsequently treated in one of several possible ways: by burning, by landfill, simple storage, or by high-pressure, high-temperature chlorinolysis.[2]

Another procedure involves separating hexachlorobenzene by crystallising it from the hex-waste in tetrachloroethylene solvent. The mother liquor from the hexachlorobenzene crystallisation, which contains hexachlorobutadiene as the major component, is then catalytically chlorinated at 150–160 °C and the chlorination product recycled as a supplementary feedstock to the main reactor. In this catalytic chlorination, hexachlorobutadiene is converted into hexachloroethane, octachlorobutene and decachlorobutane, all of which, after introduction into the main reactor, are transformed, at 600 °C and an excess of chlorine, to carbon tetrachloride and tetrachloroethylene.[3] Hence, this treatment of the hex-waste eliminates the problem of hexachlorobutadiene disposal and the risk of possible environmental pollution by this "priority pollutant." Simultaneously, it increases the utilisation of any feedstock.

However, a new and different material distribution throughout the individual processing stages is established for minor components, which do not exactly follow the hexachlorobutadiene pattern of catalytic chlorination and decomposition. Eventually, a new balance is established, in which the newly formed part of undecomposed materials is continually withdrawn from the system in the form of impurities along with the precipitated hexachlorobenzene, while another part is being recycled through the system. The content of individual compounds in this recycle fluctuates with varying production conditions, especially with changes in the composition of feedstock. At times, the concentration of these minor waste products in the mother liquor, after precipitation of hexachlorobenzene, can be very high.

Several years ago, after the introduction of this hexachlorobutadiene conversion as an extension to an existing Scientific Design plant, a crystalline precipitate was observed in various places in the pipeline transporting the mother liquor from the hexachlorobenzene crystallisation to the catalytic chlorination of hexachlorobutadiene. This observation led to interest in the composition of the heavier part of hex-waste.

This paper describes a combined approach, consisting eventually in comparison of separated individual heavy components from hex-waste with pure standard compounds. Much of the separation procedure and all of the identification was based on HPLC, which has been found to be the only available method leading to unambiguous identification of individual higher chlorinated aromatic compounds.

Experimental and Results

Standards

Commercially available standards were purchased from Koch-Light (1), Aldrich (hexachloroethane, 2, 4, 10) and RFR Corp., Hope, Rhode Island, USA (7 and 9).* The remaining standards were synthesised according to published methods.

Octachlorostyrene (5). Synthesised from 2,6-dichlorostyrene,[4] m.p. 97–98 °C (99–100 °C,[5] 95–96 °C,[6] 93.5–97 °C[4]); MS, ^{13}C NMR and UV results were virtually identical with published data.[4,7,8]

Octachloroindene (6). Synthesised from indene by means of the BMC chlorination method, with subsequent treatment of the decachloroindane intermediate with $SnCl_2$-dioxane, as described by Ballester *et al.*,[9] for the preparation of octachloronaphthalene. The resulting octachloroindene (*ca.* 20% yield) had a melting-point of 131.5–132.5 °C (132 °C[10]) and the UV spectrum was identical with that obtained by Ballester and Castañer.[8]

* For numbering of individual compounds, see Table 1.

Octachloroacenaphthylene (8). BMC chlorination of acenaphthylene[9] resulted in decachloroacenaphthene as the major product with a small amount of several other compounds, including octachloroacenaphthylene (8). As the aim of the synthesis was to prepare only a small amount of a standard for comparative studies, no conversion of decachloroacenaphthene to (8)[9] was attempted. Instead, (8), already present in the product of the BMC chlorination, was concentrated by passing through a short column of alumina with carbon tetrachloride as eluent and purified by fractional crystallisation (acetone, ethanol) from the most concentrated fraction. The melting-point of the pure (8) was 380 °C (DTA) (380–385 °C[9]) and its UV spectrum was in agreement with that published by Ballester *et al.*[9]

Decachlorofluoranthene (15). Prepared previously by Ballester *et al.*,[9] as one of the products of the BMC chlorination of 1-phenylnaphthalene. In this work (15) was prepared by the BMC chlorination of fluoranthene, according to the general method for BMC chlorination,[9] in 60–65% yield. The final product (over 98% purity by HPLC) had m.p. 263 °C (DTA) (263–265 °C[9]) and a UV trace identical with the published one.[9]

Separation Methods

Distillation

Combination of normal and vacuum distillation (up to 180 °C and 20 Torr) was used to remove all solvent and most of the hexachlorobuta-1,3-diene from the mother liquor after hexachlorobenzene crystallisation. Usually 10–25% of solid residue was obtained.

Fractional extraction

A 100-g mass of solid distillation residue was extracted 16 times with 200-ml portions of 1 + 1 acetone - ethanol. After each addition of fresh mixture to the solids, the suspension was placed for 30 min in an ultrasonic bath and, after standing for 12–24 h, the solution was separated from undissolved solids. Fresh 200-ml amounts of acetone - ethanol were added to the solids and the procedure was repeated. After evaporation of the solvent from the extracts, 16 solid fractions and an extraction residue were collected. Even if the fractional extraction is only a crude separation method, it concentrated non-aromatic chlorocarbons (1–3) and octachloroindene (6) in the initial fractions, octachlorostyrene (5) in the middle fractions and hexachlorobenzene (4), decachlorobiphenyl (10) and other perchlorinated polynuclear aromatic compounds in the higher fractions or in the residue after extraction.

Fractional sublimation

This method was very effective in the separation and purification of gram amounts of octachlorostyrene (5) from the middle fractions of the acetone - ethanol extraction, which contained more than 80% of (5). Using the sublimer designed by Holba *et al.*,[11] pure (5) was separated at 85 °C and 0.1 Torr. The residue after sublimation of (5) was usually used later in the separation of smaller amounts of higher chlorocarbons by semi-preparative HPLC (Figs. 1 and 5).

Column chromatography on alumina

This method was used for the separation of heavier, less soluble fractions from previous fractional extractions with acetone - ethanol (Fig. 1). Columns up to 36 mm in diameter and 1.8 m long, containing up to 1300 g of alumina (aluminium oxide, 90 active, neutral for column chromatography, 70–230 mesh, obtained from Merck) were loaded with up to 900 mg of sample. The loading ratio of sample to alumina was kept between 1:1200 and 1:2000. The sample was introduced into a dry column as a concentrated solution in dichloromethane (12–13 mg ml^{-1}) and gradually eluted with gradients formed by means of petroleum spirit (b.p. range 40–60 °C), dichloromethane and methanol. Decachlorobiphenyl (10), hexachlorobenzene (4) and octachloronaphthalene (7) were usually eluted in the first fractions but were poorly separated. Only occasionally, when a slower gradient was applied, were some fractions significantly enriched with (7). However, it was more advantageous to neglect poor separation of (10), (4) and (7) and work with faster gradients,

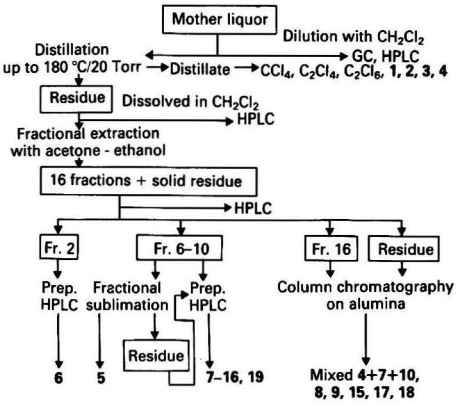

Fig. 1. Hex-waste: separation scheme for the mother liquor after crystallisation of hexachlorobenzene. Compounds numbered as in Table 1

Table 1. Compounds identified in the heavy fraction (>180 °C and 20 Torr) of hex-waste, after crystallisation of hexachlorobenzene

No.	Compound Name	Formula	Content*	Mode of identification†
1	Hexachlorobuta-1,3-diene	C_4Cl_6	5–10	F
2	Hexachlorocyclopentadiene	C_5Cl_6	0–5	F
3	Octachlorocyclopentene	C_5Cl_8	0–5	F
4	Hexachlorobenzene	C_6Cl_6	3–8	F
5	Octachlorostyrene	C_8Cl_8	100	F
6	Octachloroindene	C_9Cl_8	0.5–5	F
7	Octachloronaphthalene‡	$C_{10}Cl_8$	1–5	F
8	Octachloroacenaphthylene	$C_{12}Cl_8$	0.5–2	F
9	Octachlorodibenzofuran	$C_{12}Cl_8O$	0–0.01§	F
10	Decachlorobiphenyl	$C_{12}Cl_{10}$	5–12	F
11	Octachlorofluorenone	$C_{13}Cl_8O$		T
12	Decachlorofluorene	$C_{13}Cl_{10}$		T
13	Decachlorophenanthrene (anthracene)‡	$C_{14}Cl_{10}$		T
14	Not identified	$C_{15}Cl_8O$¶		T
15	Decachlorofluoranthene	$C_{16}Cl_{10}$	0.2–0.8	F
16	Dodecachloro-1-(2-)phenylnaphthalene	$C_{16}Cl_{12}$		T
17	Decachlorobenzo[*ghi*]-fluoranthene	$C_{18}Cl_{10}$		T
18	Decachlorocyclopenta[*cd*]-pyrene	$C_{18}Cl_{10}$		T
19	Not identified	$C_{18}Cl_{12}$¶		T

* Contents relative to octachlorostyrene = 100 (compare with Table 2).

† F = final (unambiguous); T = tentative.

‡ In some samples, the presence of "over-perchlorinated" compounds (*e.g.*, decachlorodihydronaphthalene, $C_{10}Cl_{10}$,[13] and dodecachlorodihydrophenanthrene or -anthracene, $C_{14}Cl_{12}$) was presumed, but not proved.

§ Contents of octachlorodibenzofuran varied from zero (at a 0.5 p.p.m. detection limit) to *ca.* 20 p.p.m.

¶ Determined by mass spectrometry.

which were still satisfactory for a successful one-run separation of several milligrams of pure octachloroacenaphthylene (8) and decachlorofluoranthene (15). In most runs, milligram amounts of a $C_{18}Cl_{10}$ chlorocarbon {tentatively assigned as decachlorobenzo[*ghi*]fluoranthene (17)} and sub-milligram amounts of another $C_{18}Cl_{10}$ (18?) were also separated. Octachlorodibenzofuran (9) of 80–90% purity was separated from some samples. Some components, which were only tentatively identified after semi-preparative HPLC (Table 1 and Fig. 5), were occasionally separated in amounts and purities that were insufficient for an unambiguous identification.

The elution did not follow the order encountered either in GC or in HPLC. The compound next eluted after (10), (4) and (7) was decachlorofluoranthene (15), followed by a $C_{18}Cl_{10}$ (probably 17), octachlorodibenzofuran (9), octachloroacenaphthylene (8) and eventually small amounts of another $C_{18}Cl_{10}$ (probably 18).

Gas chromatography

Gas chromatography on packed columns was suitable for the analysis of chlorocarbons up to C_8.[12] Further, because of its relative speed, it was still useful for some applications up to the C_{10}–C_{12} range [*i.e.* (7) or (10)]. It was also frequently used for detailed analyses in the region around octachlorostyrene (5) (Table 2). On our columns and with our temperature programme, the retention time for (5) was between 10.7 and 15.2 min, depending on the programme and the carrier-gas flow-rate.

As an example, conditions for one set of analyses and retention times of the major components were as follows: apparatus, Hewlett-Packard 5703A gas chromatograph, interfaced with a Hewlett-Packard 3390A recording integrator; detector, FID; column, 180 cm × 2 mm i.d. stainless steel, packed with 1.67% SE-30 on Chromosorb W; carrier gas, 29.3 ml min^{-1} nitrogen; temperature programme, 4 min isothermal at 80 °C, then 80–250 °C at a rate of 16 °C min^{-1}, then isothermal at 250 °C; injection, 1.5 µl of a sample solution in dichloromethane; elution times in minutes, hexachlorobutadiene (1) 2.6, hexachlorocyclopentadiene (2) 5.0, pentachlorobenzene (added as internal standard) 7.0, octachlorocyclopentene (3) 8.1, hexachlorobenzene (4) 8.6, octachlorostyrene (5) 10.8, octachloroindene (6) 12.4, octachloronaphthalene (7) 14.7, decachlorobiphenyl (10) 15.0 and octachloroacenaphthylene (8) > 20.

High-performance liquid chromatography

The analytical HPLC system consisted of an Altex 100A pump, an Altex 210 injection valve with a 20-µl injection loop, a Perkin-Elmer LC-75 variable-wavelength ultraviolet detector and a Perkin-Elmer 561 recorder. Most analyses were performed on Spherisorb S5 ODS (150 × 4.6 mm column with a 50 × 4.6 mm pre-column, from Chromatographic Systems, Melbourne). Occasionally, a reversed-phase S5 C_6 column was used for faster runs of higher chlorocarbons. All HPLC runs were isocratic. The most frequently used solvent was methanol which, on analytical columns, gave reasonably fast runs and satisfactory resolution of major components in the C_{10}–C_{16} range (Table 3 and Fig. 2). Aqueous methanol systems had to be used for good resolution of C_6–C_{10} compounds (Table 3, Fig. 3), while methanol - dichloromethane solvent systems were used for eluting heavier components or in accelerated runs on longer columns (Table 3, Fig. 4).

The ultraviolet detector was usually set on 220–230 nm, where all compounds examined were absorbed. Changes in recording wavelength were advantageous, when a particular wavelength was specific enough for a limited number of compounds, *e.g.*, 270 nm for quantification of (7)[13] (Fig. 3).

Table 2. Contents of hexachlorobuta-1,3-diene and major aromatic chlorocarbons in a sample of the mother liquor, after crystallisation of hexachlorobenzene*·†·‡

No.	Compound Name	Formula	Method of determination	Content
1	Hexachlorobuta-1,3-diene	C_4Cl_6	GC	31.3% *m/m*
4	Hexachlorobenzene	C_6Cl_6	GC	1.72% *m/m*
5	Octachlorostyrene	C_8Cl_8	GC	21.9% *m/m*
			HPLC§·¶	22.4% *m/m*
7	Octachloronaphthalene	$C_{10}Cl_8$	HPLC‖·**	0.27% *m/m*
				2680 p.p.m.
8	Octachloroacenaphthylene	$C_{12}Cl_8$	HPLC¶·††	0.10% *m/m*
				1045 p.p.m.
10	Decachlorobiphenyl	$C_{12}Cl_{10}$	HPLC¶·‖	1.17% *m/m*
15	Decachlorofluoranthene	$C_{16}Cl_{10}$	HPLC¶·††	0.05% *m/m*

* Mother liquor was sampled, when propylene was the only feedstock fed into the chlorination reactor.

† Tetrachloroethylene (*i.e.*, crystallisation solvent), together with smaller amounts of hexachloroethane, a few minor non-aromatic C_1–C_5 chlorocarbons and trace amounts of other aromatic chlorocarbons making the balance to 100%.

‡ No octachlorodibenzofuran (9) has been detected in this sample at the detection limit of 0.5 p.p.m.

§ Mobile phase, 85% aqueous methanol.

‖ Mobile phase, 95% aqueous methanol.

†† Mobile phase, 100% methanol.

¶ Detection at 230 nm.

** Detection at 270 nm.

Table 3. Capacity factors (k') of some identified compounds

		S5 ODS 150 × 4.6 mm column + 50 × 4.6 mm pre-column		S5 ODS 245 × 10 mm column + 50 × 10 mm pre-column	
	Compound	1 ml min^{-1} 85% aq. MeOH	1 ml min^{-1} 100% MeOH	4 ml min^{-1} 100% MeOH	4 ml min^{-1} 80% MeOH +20% CH_2Cl_2
No.	Name				
4	Hexachlorobenzene	3.48	1.31	2.12	1.72
5	Octachlorostyrene	4.12	1.31	2.12	1.72
6	Octachloroindene	7.67	—	—	—
10	Decachlorobiphenyl	13.08	2.13	3.42	2.26
7	Octachloronaphthalene	17.47	3.28	4.95	2.92
9	Octachlorodibenzofuran	—	4.25	6.26	3.50
8	Octachloroacenaphthylene	—	6.91	9.70	4.78
15	Decachlorofluoranthene	—	15.27	21.69	7.95

The semi-preparative HPLC system was similar to the analytical one except larger columns (200 × 7.7 mm or 245 × 10 mm with corresponding pre-columns) were used. Flow-rates were increased to 2.5–4.5 ml min^{-1} and injection loops were increased to 100–250 µl. More concentrated solutions (up to 10 mg ml^{-1}) were injected. Fractions were collected manually. Milligram amounts of all unambiguously identified aromatic chlorocarbons (4–8, 10, 15) were separated in high purity from pre-concentrated mixtures (Figs. 1, 4 and 5). As it is rather difficult to apply the GC - MS analysis to low-volatile compounds, it is suggested that semi-preparative HPLC should be the first step in any further identification attempts on chlorocarbon mixtures containing compounds over C_{10}.

Mass spectrometry

The MS of pure compounds separated by semi-preparative HPLC or column chromatography was used for the determination of their relative molecular masses, which was the first

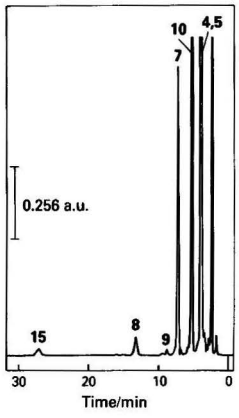

Fig. 2. HPLC of the 10th fraction from the acetone - ethanol extraction. Column: S5 ODS, 150 × 4.6 mm i.d. with a 50 × 4.6 mm i.d. pre-column. Injection: 20 µl. Solvent: 100% methanol, 1 ml min^{-1}, room temperature. Detector: 230 nm. Numbering of identified compounds as in Table 1

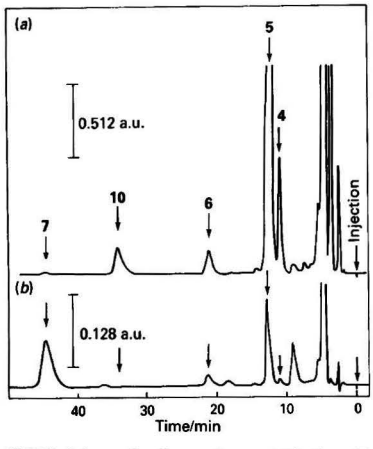

Fig. 3. HPLC of the mother liquor after crystallisation of hexachlorobenzene. Column: S5 ODS, 150 × 4.6 mm i.d. with a 50 × 4.6 mm i.d. pre-column. Injection: 20 µl as a 1% solution in dichloromethane. Solvent: 85% aqueous methanol, 1 ml min^{-1}, room temperature. Detectors: (*a*) 220 nm; (*b*) 270 nm. Numbering of compounds as in Table 1

step in their structural elucidation. In some instances, MS was used as supplementary evidence for the structural assignment, especially if the mass spectrum was very characteristic and known, *e.g.*, octachlorodibenzofuran (9) where comparison of the MS trace with that published[14] gave a definite indication of the presence of the compound.

Discussion

The crystalline precipitate obtained in the pipeline, while transporting the mother liquor after hexachlorobenzene crystallisation, was investigated by fractional extraction of the mother liquor solids with acetone - ethanol, purification of the concentrate by fractional crystallisation and determination of the properties of the pure compound obtained. On the basis of its mass spectrum,[7] ^{13}C NMR,[4] UV spectrum[8] and melting-point (96.5–98 °C), it was identified as octachlorostyrene (m.p. 99–100,[5] 95–96,[6] 93.5–97 °C[7]). This was later confirmed

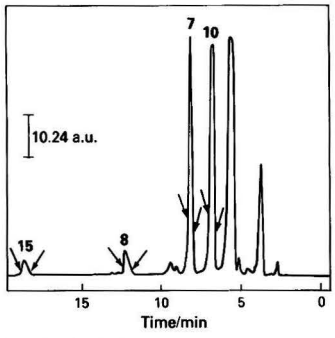

Fig. 4. Preparative HPLC of the 10th fraction from the acetone - ethanol extraction. Column: S5 ODS, 245 × 10 mm i.d. with a 50 × 10 mm i.d. pre-column. Injection: 250 µl. Solvent: methanol - dichloromethane (80 + 20), 4 ml min^{-1}, room temperature. Detector: 230 nm. Numbering of compounds as in Table 1

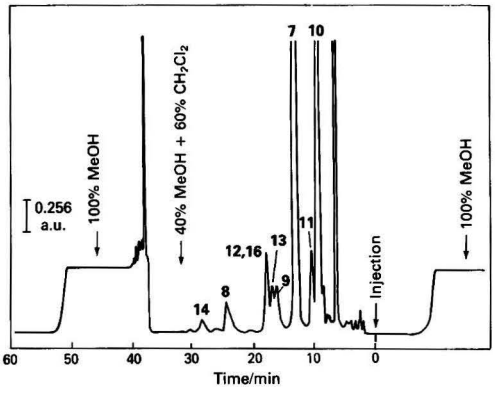

Fig. 5. Preparative HPLC of the residue after sublimation of octachlorostyrene (5) (octachlorostyrene was sublimed from the 6th acetone - ethanol extract; see Fig. 1). Column: S5 ODS, 245 × 10 mm i.d. with a 50 × 10 mm i.d. pre-column. Injection: 100 µl of dichloromethane solution (8.6 mg ml^{-1}). Solvent: 100% methanol, 4 ml min^{-1}, followed by flushing with 4 ml min^{-1} of methanol - dichloromethane (40:60), room temperature. Detector: 230 nm. Numbering of compounds as in Table 1

by comparison with a standard prepared from 2,6-dichlorostyrene.[4]

During the work on identification of octachlorostyrene, it was discovered that the mother liquor, after hexachlorobenzene crystallisation, also contained various amounts of other perchloro compounds. In early attempts to identify some of these compounds, it was found that HPLC could be used as a general method of analysis for any mixture of chlorocarbons. Its application to lower chlorocarbons, although feasible, was of limited practical value as GC was equally applicable and usually much faster. In the medium range (C_6–C_{10} or C_{12}), both GC and HPLC were found to be equally applicable and often mutually complementary; GC was faster, but HPLC more sensitive and easier to refine to any required degree of accuracy. Over the C_{10}–C_{12} range (octachloronaphthalene, decachlorobiphenyl), HPLC was the superior method.

Although it was possible to analyse solutions of the mother liquor after crystallisation of hexachlorobenzene or other waste streams directly (and identify and quantify the major components in this way), it was preferable in most instances to examine mixtures (fractions) prepared by means of some pre-concentration sequence, *e.g.*, distillation, followed by

fractional extraction of the distillation residue and separation of resulting fractions using column chromatography on alumina, or semi-preparative HPLC, as indicated in Fig. 1. Identification of individual compounds was based on the combination of several methods, usually starting with purification of the compound studied by preparative HPLC and determination of its relative molecular mass by mass spectrometry. Mass spectra infrequently led immediately to structural characterisation (as, for example, octachlorodibenzofuran), because, in the mass spectra of most aromatic chlorocarbons, the fragmentation pattern was similar. All positive identifications were based on comparisons with standards (HPLC, UV, MS, DTA). The standards were either purchased or synthesised by established methods. In the absence of a standard, any identification was considered only tentative.

The part of the mother liquor after crystallisation of hexachlorobenzene, which distilled over at up to 180 °C and 20 Torr, was briefly examined by GC. It usually consisted of the solvent (tetrachloroethylene with a small amount of carbon tetrachloride), hexachloroethane, hexachlorobutadiene, hexachlorocyclopentadiene, octachlorocyclopentene, hexachlorobenzene and several small unidentified components. The list of compounds identified in the distillation residue is given in Table 1, in which unambiguous and tentative identifications are indicated, the latter being used in instances where no comparative standard was available. As octachlorostyrene was always the principal component of the mixture, the mass proportions of other compounds have been determined on the basis of the octachlorostyrene content, which has been taken as 100. The investigation was performed up to the $C_{18}Cl_{10}$ compound, although minute amounts of unidentified higher chlorocarbons were also separated.

Quantitative determinations of hexachlorobuta-1,3-diene (1) and hexachlorobenzene (4) and (partly) of octachlorostyrene (5) were carried out by GC, by comparison with standard solutions, or using pentachlorobenzene (absent in all original samples) as an internal standard. Most of the octachlorostyrene (5) determinations and all quantitative determinations of higher chlorocarbons (6–8, 10 and 15) as well as of octachlorodibenzofuran (9) were carried out by reversed-phase HPLC,[13] by comparison with standard solutions.

The composition of the mother liquor, after crystallisation of hexachlorobenzene, varied frequently owing to variations in feedstock (depending on the amount of vinyl chloride production waste added to the propylene feedstock). Parameters such as temperature and crystallisation time also varied. A typical analysis of the mother liquor, sampled when only propylene was fed into the chlorination reactor, is given in Table 2.

It is worth mentioning that, according to literature data for similar mixtures, the presence of octachlorobiphenylene ($C_{12}Cl_8$)[15–17] and decachloropyrene ($C_{16}Cl_{10}$)[17] might have been expected. However, the only isolated $C_{12}Cl_8$ compound proved to be octachloroacenaphthylene (8).

The separated $C_{16}Cl_{10}$ chlorocarbon was identified as decachlorofluoranthene (15). Trace amounts of another $C_{16}Cl_{10}$ contamination were detected in some fractions in column chromatography, which probably were different to decachlorofluoranthene, yet their identity could not be established. Even if these traces were decachloropyrene, the content of decachloropyrene would be at least one order of magnitude lower than that of decachlorofluoranthene.

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Measurement of Sub-microgram Amounts of Nickel in Plant Material by Electrothermal Atomic-absorption Spectroscopy

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A sensitive method for the determination of nickel in small plant samples (100–200 mg dry mass) containing sub-microgram amounts of nickel is described. The samples are wet ashed with nitric and perchloric acids in a pressure decomposition vessel, and the nickel complexed with ammonium tetramethylenedithiocarbamate and extracted into a small volume of isobutyl methyl ketone. Nickel is then determined in the extracts by electrothermal atomic-absorption spectroscopy.

Keywords: *Nickel determination; plant material; ammonium tetramethylenedithiocarbamate - isobutyl methyl ketone extraction; electrothermal atomic-absorption spectroscopy; pressure decomposition*

Interest in the measurement of small amounts of nickel in plant material arises from recent work showing nickel to be an essential element for the growth of rats and chicks,[1] the demonstration that the urease from jackbean is a nickel metalloenzyme and the possibility that nickel may be an essential micronutrient for some plants.[2–4]

A variety of ashing procedures and methods for the subsequent determination of nickel have been reported but most have not been developed specifically for low-nickel plant material. In research on plants that accumulate high concentrations of nickel (>1 000 μg g^{-1} dry mass) Jaffre *et al.*[5] employed dry ashing at 500 °C, and protein samples containing >1 000 μg g^{-1} of nickel have been ashed in an atmosphere of oxygen at 450–460 °C.[6] However, Fudagawa and Kawase[7] concluded that wet ashing was preferable because of losses of nickel that can occur above 550 °C. Apparently satisfactory results have been obtained with HNO_3 - $HClO_4$ digestion of plant material,[7] but in preliminary research at our laboratory this method gave low (<71%) recoveries of nickel (unpublished results). Wet-ashing procedures developed for human and animal tissues have been applied successfully to the determination of nickel in plant material, although some are more complex than appears necessary for the digestion of plant samples. For example, Dornemann and Kleist[8] used a procedure in which the tissue was sequentially digested overnight at room temperature with HNO_3 - H_2SO_4, heated to 400 °C until clear, treated with 40–100 0.1-ml aliquots of an H_2O_2 - HNO_3 mixture while increasing the temperature to 420 °C, evaporated down to 0.1 ml (volume of H_2SO_4 added), taken up in HCl, then heated in a sealed flask at 350 °C for 20–30 min. Iron and copper were then removed by extraction with chloroform as cupferronates.

Microgram amounts of nickel in digests may be measured by UV spectrophotometry of the nickel dimethylglyoxime complex,[6] for example, but methods involving atomic-absorption spectroscopy (AAS) are more rapid[9] and more commonly used. As flame AAS has a relatively poor sensitivity to nickel, a prior concentration step is required. Nomoto and Sunderman[10] complexed the nickel in HNO_3 - H_2SO_4 - $HClO_4$ digests of human tissue homogenates and other biological materials with ammonium tetramethylenedithiocarbamate [ammonium pyrrolidinedithiocarbamate (APDC)], which was then extracted into isobutyl methyl ketone (IBMK). They used an acetylene - air flame and a Boling three-slot burner. However, most more recent studies have used electrothermal AAS.[7,8,11,12] Torjussen *et al.*[11] also used APDC - IBMK extraction when studying the nickel content of human tonsils, and Pedersen *et al.*[12] used APDC - xylene extraction of nickel from EDTA extracts of soils. Other extraction systems that have been used include dimethylglyoxime - chloroform - HCl,[9] dimethylglyoxime - *m*-xylene[7] and hexamethylenedithiocarbamidate - diisopropyl ketone - xylene.[8]

This paper reports an electrothermal AAS method developed for use with small amounts (<250 mg) of low-nickel plant material. It involves the use of a simple one-step ashing procedure in a pressure decomposition vessel followed by APDC - IBMK extraction.

Experimental

Equipment

The analyses were performed on a Varian Techtron AA6 atomic-absorption spectrophotometer fitted with a Model 63 graphite furnace.

Reagents

All aqueous solutions were prepared with ultrapure water prepared by glass distillation of triply de-ionised water. Reagents were of analytical-reagent grade unless indicated otherwise.

Nitric acid. This was purified in a Teflon still as described by Mattinson.[13]

Perchloric acid. BDH Chemicals Aristar-grade material was used throughout.

Ammonia solution. Ammonia solution at room temperature was distilled over into ultrapure water cooled to 3 °C in a refrigerated water-bath, using a Mattinson Teflon still. The final concentraion was approximately 10 M.

Ammonium tetramethylenedithiocarbamate solution, 1% m/V. This was prepared by the method of Timperley,[14] which is briefly as follows. A 25-ml volume of pyrrolidine was dissolved in 55.6 ml of ethanol in a 250-ml conical flask fitted with a reflux condenser, the flask being cooled in an ice-bath. After 30 min, three 5.5-ml aliquots of carbon disulphide were added through the condenser with gentle shaking of the flask and continued cooling, then 42 ml of 8 M ammonia solution were added. On standing, the product separated as white crystals, which were purified by recrystallisation from ethanol. The working solution was prepared by dissolving 1 g of the purified product in 100 ml of ultrapure water.

Isobutyl methyl ketone (IBMK). Atomic-absorption grade IBMK was saturated with water prior to use.

Potassium hydrogen phthalate buffer (pH 4.0). This was prepared as described by Timperley.[14] A 10.2-g amount of potassium hydrogen phthalate was dissolved in water, 1.4 ml of 1 M hydrochloric were added and the solution was diluted to 100 ml. The solution was then purified by adding 1 ml of APDC solution and extracting with IBMK.

Nickel stock solution, 100 μg ml^{-1}. A 0.100-g amount of nickel was dissolved in 10 ml of redistilled concentrated nitric acid and diluted to 1 l with ultrapure water.

Nickel working solutions. These were prepared by dilution of the nickel stock solution with ultrapure water.

Procedures

Sample digestion

A 100–200-mg amount of oven-dried plant material is weighed accurately into a 70-ml Teflon pressure decomposition vessel,[15] then 5 ml of nitric acid and 2 ml of perchloric acid are added and the vessel is sealed and heated in an oven at 140 °C for 7 h. After the vessel has cooled, the digest is transferred into a 25-ml calibrated flask. Use of a time-clock attached to the oven allows digestions to be conducted overnight with the vessels cool enough to open the following morning. Blanks are made up by adding 5 ml of nitric acid and 2 ml of perchloric acid to a 25-ml calibrated flask and thereafter treating them as digests. Standards are made up to contain the same amounts of nitric and perchloric acid as the blanks.

Extraction

Digests are titrated to approximately pH 4 with ammonia solution using bromophenol blue as the indicator. As the neutralisation reaction is exothermic, the calibrated flask is kept in an ice-bath during the addition of the first 5 ml of ammonia solution. The flask is then removed from the ice-bath, 1 drop of indicator added and the titration completed.

After the solution has returned to room temperature, 2 ml of phthalate buffer and 1 ml of APDC solution are added. The solution is then shaken for 30 s and diluted to 25 ml with ultrapure water. A 1-ml volume of IBMK is then added and the flasks are inverted and placed on a wrist-action shaker at approximately 10 Hz for 30 min. The flasks are then stood on the bench for approximately 1 min to allow phase separation prior to determination of nickel in the IBMK extract. Blanks and standards are treated similarly.

Atomic-absorption analysis

The graphite cups and rods are treated with tantalum to extend their working life[16] and given 12–15 firings for initial stabilisation. The furnace conditions are adjusted to minimise sample spitting and to ensure that each step of the sequence is completed before commencement of the next.

The following instrument parameters were found to be satisfactory: lamp current, 10 mA; band pass, 0.33 nm; wavelength, 232.0 nm; damping, A; drying cycle, time 60 s, voltage setting 7.0; ashing cycle, time 15 s, voltage setting 7.0; atomising cycle, time 3 s, voltage setting 8.3; nitrogen flow-rate, approximately 1 l min^{-1}; and sample volume, 10 µl.

Results and Discussion

Digestion

With 5 ml of nitric acid plus 2 ml of perchloric acid we obtained complete digestion of leaves and leguminous seeds and good recoveries of the nickel in NBS standard reference material (SRM) orchard leaves (97%). However, with nitric acid alone, with vanadate as catalyst or with only 1 ml of perchloric acid, the samples were solubilised but destruction of the organic matter was incomplete.

Effect of Extraction Time

Short extraction times have been found to be satisfactory for the extraction of trace metals from water samples with APDC and IBMK, *e.g.*, Parker[17] used a 2-min and Timperley[14] a 5-min extraction. Timperley cautioned against vigorous shaking for long periods because of the risk of forming a stable emulsion. With human serum, urine or tissue digests, Nomoto and Sunderman[10] applied mixing for 20 s on a vortex mixer, then placed the samples in an ice-bath for 10 min before centrifuging at 900 *g* for 5 min to ensure that the IBMK was free from water.

With plant digests, we found the recovery of added ^{63}Ni to be only 82% after shaking for 5 min, increasing to just over 97% after 30 min (Fig. 1). A 30-min extraction time was selected for routine use. No problems were encountered with separation of the phases.

Effect of Extraction pH

Nomoto and Sunderman[10] obtained maximum extraction of nickel in the region of pH 2.5 and Pierce *et al.*[18] recommended extraction at pH 2.8. In contrast, Brooks *et al.*[19] found a broad optimum pH range between 3.5 and 8.0 for the extraction of nickel and other heavy metals. With plant digests we also found a broad optimum pH range (Fig. 2), with a rapid decrease in nickel extraction below pH 2 and above pH 8. On the basis of these results, pH 4 was selected.

Accuracy

Over a 12-month period, 75 samples of NBS SRM 1571 (orchard leaves) with a certified nickel content of 1.30 ± 0.2 µg g^{-1} were interspersed among other samples analysed by the method, as a check on accuracy. The amounts of samples taken varied from 40 to 250 mg, corresponding to 52–326 ng of nickel per sample. Within the recommended sample mass range of 100–200 mg, the mean nickel concentration found was 1.264 µg g^{-1} (Table 1), corresponding to a recovery of just over 97%, in close agreement with the value obtained in the ^{63}Ni tracer experiments (Figs. 1 and 2). However, the data show that with amounts of sample below 100 mg, the nickel contents were slightly overestimated. Hence standardisation of sample masses within the recommended range is desirable. Further research is needed to find the cause of this apparent sample size effect.

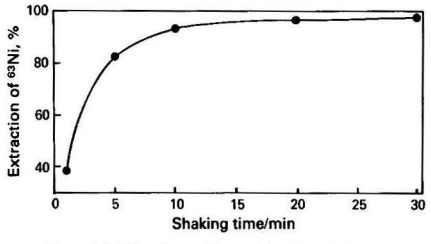

Fig. 1. Effect of shaking time with a wrist-action shaker at *ca.* 10 Hz on the extraction into the organic phase of ^{63}Ni added to plant digests. ^{63}Ni added at 7.6 kBq per sample; extraction pH, 4.0; and APDC - IBMK extracts counted in a 1 + 1 Triton - toluene mixture containing 0.5% *m/V* of 2,5-diphenyloxazole (PPO) on a Beckman LS100 liquid scintillation counter

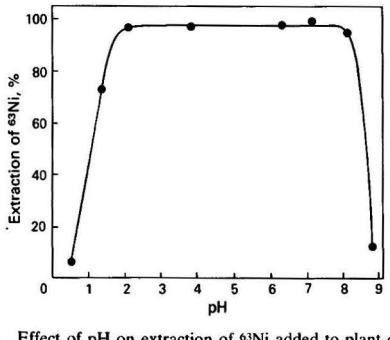

Fig. 2. Effect of pH on extraction of ^{63}Ni added to plant digests. Shaking time, 30 min. ^{63}Ni activity and counting procedure as in Fig. 1

Table 1. Results of the analysis of 75 samples of NBS SRM orchard leaves over a 12-month period

Sample mass/ mg	Number of samples	Nickel concentration found/μg g^{-1}	
		Mean	Standard error
<100	30	1.368	0.067
100–200	34	1.264	0.052
>200	11	1.204	0.053

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Determination of Lead in Gasoline by Atomic-absorption Spectrophotometry—Evaluation of Standard Methods

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Methods for measuring lead in gasoline have been reviewed and a modified procedure evaluated in an inter-laboratory test programme to determine its precision. The test samples are diluted with 4-methylpentan-2-one and the lead alkyls are reacted with iodine and a liquid anion exchanger (Aliquat 336). The lead content of the solution is determined by atomic-absorption spectrophotometry. The method is quantitative and is applied over the range 0.05 to 0.50 g l^{-1} of lead. It is rapid with good precision and has been adopted as a standard procedure by the Institute of Petroleum.

Keywords: *Lead determination; gasolines; liquid anion exchangers; atomic-absorption spectrophotometry; Institute of Petroleum standard method*

In recent years the Institute of Petroleum (IP) Standardization Committee has planned some of its work using the concept of economic significance. Two important factors that go to make up this concept are: (i) tests that lack precision; and (ii) tests that take too long and so delay the use of the product.

The published standard methods of analysis used in the determination of total lead in gasoline can be divided into two types: chemical and instrumental procedures, with methods falling into either of these categories. The chemical procedures based on an extraction with hydrochloric acid with either a gravimetric[1] or a titrimetric finish[2] are time consuming and not quantitative for all lead alkyl compounds.[3] One chemical method,[4] however, which involves reaction - extraction with iodine monochloride followed by a complexometric titration of lead(II) ions is rapid and has good precision.

The instrumental techniques are based on X-ray fluorescence spectrometry (XRF),[5] one method utilising bismuth as an internal standard, with the alternative method using the characteristic tungsten-tube radiation scattered by the sample as the internal standard. Although these techniques meet the requirements of speed, precision and accuracy, the equipment is very expensive and can only be justified economically for handling large numbers of samples. A new method based on non-dispersive XRF is very much cheaper and rapid, but is less precise.[6]

With the widespread use in recent years of atomic-absorption spectrophotometry (AAS) it appeared important that a procedure standardised on this technique should be devised and recommended by the IP. Several workers have reported the direct determination of lead in gasoline by AAS.[7–10] The main problem with all of these methods, however, was the variation in response that was obtained for the different lead alkyls. Kashiki *et al.*[11] recommended reaction of alkyllead with iodine to overcome the differences in response. Even so, small differences in response still occurred. Eventually, Du Pont[12] overcame the problem by the addition of a liquid anion exchanger, Aliquat 336 (trioctylmethylammonium chloride) which stabilised the response of the diiodolead compounds.

Several standardised AAS procedures and a method of The Associated Octel Company Limited use this fixing procedure.[13–16] A comparison of these methods and an alternative method, illustrating the main similarities and differences, can be seen in Table 1. Further consideration of the various features, including practical trials, has therefore been carried out in order to produce a standard procedure suitable for IP recommendation.

Table 1. Comparison of methods for lead in gasoline by atomic-absorption analysis

Parameter	Method			
	German Standard, DIN 51769, Part 7	European Standard, CEN 442E	Associated Octel, OP 77/1	Proposed IP Method
Method range/g l^{-1}	0.1—0.6	0.1—0.6	0.1—0.8	0.05—0.5
Sample range/mg of Pb	0.5—3.0	0.5—3.0	0.1—0.8	0.25—2.5
Sample size/ml	5.0	5.0	1.0	5.0
Lead standard	Lead acetate in 10% *V/V* acetic acid diluted with IBMK	Lead acetate in glacial acetic acid (100 ml l^{-1}) diluted with propan-2-ol	Lead chloride in 10% *m/V* Aliquat 336 diluted with IBMK	Lead chloride in Aliquat 336 (2 g l^{-1}) diluted with IBMK
Grade and purity,* %	AR, 99.5—103	AR, 99.5—103	LR, > 99	LR, > 99
Absorbance range	0.2—0.8	0.2—0.8	0.1—0.3	0.2—0.7
Wavelength/nm	283.3	283.3	283.3	283.3
Reagents				
Aliquat 336†	1% volume in IBMK, using 5 ml per sample	10 g l^{-1} in IBMK, using 5 ml per sample	1% *m/V* in IBMK, using 3 ml per sample	2 g l^{-1} in IBMK, using 25 ml per sample
Iodine	10 g l^{-1} in toluene, using 1 ml per sample	10 g l^{-1} in toluene, using 1 ml per sample	30 g l^{-1} in toluene, using 0.2 ml per sample	30 g l^{-1} in toluene, using 1 ml per sample
Reaction period/min	1	1	2	2

* AR = AnalaR; LR = laboratory-reagent grade.
† 1% volume Aliquat 336 = 0.88% *m/V* = 8.8 g l^{-1}.

Experimental and Results

Assessment of Lead Standards and their Stability

Different materials were specified in the existing procedures for preparing standard lead solutions. As a preliminary, therefore, these and other compounds were evaluated to find the most suitable reference material. Experiments showed that the material used in the German standard was not completely soluble in 4-methylpentan-2-one (isobutyl methyl ketone, IBMK) because of phase separation. Both the German (DIN) and European (CEN) methods used lead acetate that was of uncertain purity and probably not suitable as a primary standard. The use of AnalaR lead(II) oxide similarly dissolved in a glacial acetic acid - IBMK mixture was thought likely to overcome the latter problem. No technical objection was found, however, to exclude the use of lead(II) chloride dissolved in Aliquat 336 - IBMK mixtures as used by ASTM and others.

None of the methods gave information on the stability or shelf-life of their standard solutions; however, this was thought to be an important factor for a standardised procedure, because it affects the time required for analysis. Experiments were arranged, therefore, to examine the stability of some of these lead standards.

Test solutions were prepared, each containing 100 mg l^{-1} of lead, and stored either at room temperature or in a refrigerator for periods of up to 7 months, as follows.

1. European standard; made up from AnalaR lead(II) acetate with glacial acetic acid (100 ml l^{-1}) but dissolved in IBMK not propan-2-ol, stored at room temperature.
2. Proposed standard; made from AnalaR lead(II) oxide with glacial acetic acid (100 ml l^{-1}) dissolved in IBMK, originally stored at room temperature but later in a refrigerator.
3. ASTM standard; made from laboratory-reagent grade lead(II) chloride with 1–20 g l^{-1} Aliquat 336 dissolved in IBMK. Two solutions of each Aliquat 336 concentration were stored either at room temperature or in a refrigerator.

The concentration of lead in the solutions was measured at intervals, with the results shown in Table 2.

The data showed that the lead(II) acetate solution was unstable and decomposed, forming a crystalline precipitate after only 2–3 d. The lead(II) oxide solution appeared to be stable for 4 weeks at room temperature, but when subsequently stored in a refrigerator it precipitated progressively after a further 4 weeks storage and was, therefore, of doubtful stability. The lead(II) chloride solutions, containing from 1 to 20 g l^{-1} of Aliquat 336, were stable for at least 7 months, irrespective of the storage temperature.

The ASTM standard was, therefore, selected as the most appropriate for this determination at an Aliquat 336 concentration of 2 g l^{-1}.

Summary of Method

A known volume of gasoline is diluted with 4-methylpentan-2-one and shaken with a solution of iodine in toluene. The lead alkyls react to form dialkyllead iodides, which are stabilised by the addition of a quaternary ammonium salt (Aliquat 336). The lead content of the solution is determined by AAS at 283.3 nm using standards prepared from lead(II) chloride.

Calibration

Reference solutions were prepared from a standard lead(II) chloride solution covering a range of from 0 to 2.5 mg of lead per 100 ml of solution. These were mixed with the other reactants and measurements were made by the method designated IP 362/83.[17] The results showed that the analyser response was curvilinear with respect to lead concentration.

Calculation of Results

The method of calculating the lead content used in some of the existing procedures assumed that the response was linear. In these instances the calculations were made with results obtained from a reference solution chosen to have an

Table 2. Stablility of standard solutions

	Method			
	(A) Modified European*	(B) Proposed	(C) Modified ASTM†	(D) Modified ASTM†
Material	Lead(II) acetate	Lead(II) oxide	Lead(II) chloride	Lead(II)chloride
Lead concentration/mg l^{-1}	100	100	100	100
Storage condition	Room temperature	Room temperature	Room temperature	Refrigerator
Storage period	Lead found/mg l^{-1}			
Days / Weeks				
0	—	—	100	101
1	98.5	99.5	—	—
2	92.5	99	—	—
3	73.0	100	—	—
4	71.5	100.5	—	—
1	73.0	99.5	99	100.5
2	42.0	100	98.5	100
3	(Discontinued)	99	99	101
4		100	99	100.5
5		100.5 R ‡	—	—
8		95.5 R ‡	98	100.5
12		83.5 R ‡	99	100.5
17		84.5 R ‡	99.5	101.5
21		81 R ‡	100	102
26		84 R ‡	100	101
30		82 R ‡	100	101.5

*European standard diluted in IBMK not propan-2-ol.
†ASTM standard, $PbCl_2$ + 2 g l^{-1} Aliquat 336 in IBMK.
‡R, After 4 weeks testing the solutions were stored in a refrigerator.

absorbance close to that of the test sample. This procedure was seen to be invalid in the proposed method for test samples falling midway between two reference solutions. Quantitatively, differences of up to 0.025 g l^{-1} of lead were obtained, depending upon which reference solution was chosen for the calculation. Some typical results are given in Table 3.

An alternative calculation was used on the data, also shown in Table 3, and the results demonstrated that this avoided the problem. In this calculation, which is based on "the method of similar triangles,"[18] data from two reference solutions were selected with concentrations that closely bracketed that of the test sample. Over this range of concentration the error due to non-linearity was negligible and this calculation was adopted for the proposed method.

Modern instruments, however, usually incorporate some form of data processor that stores and/or updates calibration figures and automatically calculates results on unknown test samples. This presented two problems; firstly the recommended reference solutions were not quite appropriate and secondly, processors almost certainly use different mathematical methods of calculation.

To maintain wide application of the method two parts of the procedure were modified so that either type of instrument could be employed. Use of an alternative concentration lead standard and a defined calibration and analysis procedure using three reference solutions was arranged for analysers with data processors. Both types of instrument were then used to analyse the same set of test samples. The data showed that the instruments and procedures gave equivalent results.

Elasped Time

The time required for analysis was determined by simulating a routine analysis. This was carried out under conditions where all the reagents were equilibrated at room temperature and the analyser was switched on and set up but not optimised for the determination. Duplicate results were then obtained within 15 min.

Effect of Temperature

The existing chemical methods of analysis incorporate a volume correction factor so that samples may be analysed at any temperature. This validates the results so that they are expressed as "lead content, grams per litre at 15 °C." The currently used AAS methods utilising only 5-ml sample sizes do not employ such corrections. Consequently, it was decided to study the effect of differences in sample temperature and/or ambient temperature on the lead determination. Tests were made with the proposed method over a range of temperatures from 10 to 25 °C. The results showed that, providing the reagents, reference solutions (previously prepared at 15 °C as specified in the procedure) and test samples were equilibriated at the same temperature prior to analysis, there was no significant effect on the lead determination.

Testing the Method

Preliminary trials of the method in one laboratory were very encouraging and a working group was formed by the IP to test the method more fully. The test panel consisted of eleven laboratories, nine from the oil industry, one instrument manufacturer and one government quality assurance laboratory. Some of the laboratories had been using similar methods for several years and some had no experience of this determination. Initially, therefore, one test sample was sent to each laboratory for them to obtain experience of the method.

An inter-laboratory test programme was then designed involving the analysis of ten different samples spread across the range of application. The lead anti-knock compounds containing either tetraethyl- or tetramethyllead were blended into typical premium or regular grade basestock gasolines. The programme was vetted by the Institute's Precision Evaluation Panel and confirmed as satisfactory for derivation of precision estimates. A set of samples was sent to each participant along with a copy of the method, programme instructions and a reporting form for the results obtained. The samples were number coded and each laboratory was requested to analyse them in a pre-determined but random order. Each laboratory prepared its own lead standards and reagents and commercially available instruments of several different manufacturers and types were used. The data collected, therefore, covered all the aspects of "real-life" use and the method was tested under typical user conditions.

The results are given in Table 4 and they were processed statistically[19] by the Precision Evaluation Panel to produce the following over-all precision estimates: repeatability, $0.026M$; and reproducibility $0.076M$; where M is the mean lead content in grams per litre. Typical precision values are given in Table 5.

A comparison of the estimates with those of IP 270/77 (iodine monochloride method) showed that the new method gave a precision equal to or better than that of IP 270 at low lead levels but was slightly inferior at higher lead levels.

Conclusions

The proposed method is rapid and gives results of similar precision to those obtained with IP 270/77 and, therefore, satisfies the two original objectives.

Table 3. Methods of calculation

Sample number	Calculation method*	Reference solution/ mg of Pb	Lead present/ g l^{-1}	Lead found/ g l^{-1}	Difference/ g l^{-1}
1	DIN	1.50	0.350	0.339	−0.011
	DIN	2.00	0.350	0.361	+0.011
2	DIN	2.00	0.450	0.438	−0.012
	DIN	2.50	0.450	0.463	+0.013
1	Proposed	1.50 2.00	0.350	0.352	+0.002
2	Proposed	2.00 2.50	0.450	0.452	+0.002

* DIN: lead content of sample (g l^{-1}) $= \dfrac{\text{sample absorbance} \times \text{Pb in reference solution (g } l^{-1}) \times 20}{\text{absorbance of reference solution}}$

Proposed: lead content of sample (g l^{-1}) $= \left\{ \left[\dfrac{A_S - A_L}{A_H - A_L} \times (m_H - m_L) \right] + m_L \right\} \times 0.2$

where A_H = absorbance of high standard; A_L = absorbance of low standard; A_S = absorbance of test sample; m_H = mass of lead in high standard (mg); m_L = mass of lead in low standard (mg); and 0.2 is the reciprocal of the sample volume.

Table 4. Test results from precision evaluation programme; results are lead content in g l^{-1}

Laboratory	Sample number									
	1	2	3	4	5	6	7	8	9	10
1	0.091	0.109	0.193	0.208	0.290	0.309	0.391	0.394	0.486	0.505
	0.090	0.108	0.192	0.206	0.286	0.304	0.387	0.394	0.481	0.510
2	0.090	0.112	0.189	0.199	0.288	0.303	0.388	0.390	0.481	0.487
	0.089	0.108	0.191	0.199	0.288	0.300	0.391	0.390	0.485	0.490
3	0.093	0.109	0.192	0.206	0.286	0.304	0.392	0.400	0.480	0.494
	0.092	0.109	0.192	0.204	0.286	0.304	0.393	0.400	0.483	0.494
4	0.091	0.110	0.192	0.211	0.287	0.304	0.383	0.404	0.483	0.511
	0.090	0.110	0.190	0.210	0.287	0.304	0.388	0.389	0.491	0.507
5	0.089	0.108	0.188	0.206	0.288	0.303	0.388	0.395	0.482	0.500
	0.089	0.109	0.189	0.206	0.288	0.303	0.388	0.397	0.489	0.504
6	0.089	0.107	0.186	0.203	0.285	0.297	0.385	0.391	0.473	0.493
	0.089	0.107	0.186	0.203	0.299	0.299	0.386	0.396	0.478	0.494
7	0.090	0.096	0.185	0.189	0.291	0.305	0.388	0.380	0.475	0.490
	0.089	0.098	0.186	0.189	0.288	0.303	0.387	0.386	0.486	0.492
8	0.079	0.102	0.188	0.194	0.275	0.278	0.347	0.356	0.455	0.489
	0.079	0.104	0.188	0.197	0.264	0.280	0.349	0.352	0.458	0.486
9	0.089	0.100	0.191	0.196	0.276	0.288	0.395	0.389	0.504	0.504
	0.087	0.103	0.187	0.198	0.274	0.297	0.389	0.390	0.490	0.500
10	0.083	0.102	0.183	0.196	0.277	0.295	0.380	0.384	0.460	0.479
	0.083	0.104	0.187	0.200	0.280	0.294	0.388	0.383	0.473	0.472
11	0.088	0.107	0.190	0.200	0.290	0.309	0.386	0.391	0.483	0.492
	0.088	0.108	0.189	0.200	0.289	0.309	0.386	0.389	0.484	0.496

Table 5. Typical precision values

M/g l^{-1}	Repeatability	Reproducibility
0.1	0.003	0.008
0.2	0.005	0.015
0.3	0.008	0.023
0.4	0.011	0.030
0.5	0.014	0.038

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Some Problems Observed in the Determination of Lithium in Waste Waters by Atomic-absorption Spectrophotometry

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The atomic-absorption determination of lithium in waste waters using the air - acetylene flame is prone to chemical interference effects on some instruments. These effects are considered to be significant when the lithium tracer technique is used for flow monitoring. The use of the hotter dinitrogen oxide - acetylene flame is recommended to minimise these effects. For acceptable precision (a relative standard deviation of less than 1%) it is essential to employ an intense lithium source when using this hotter flame.

Keywords: *Lithium determination; atomic-absorption spectrophotometry; waste water analysis*

The lithium tracer technique is frequently employed in the water industry to determine the flow of raw or waste waters in open channels or pipes.[1-5] The results are often used for the calibration of flow meters. Basically, the technique consists of dosing a concentrated lithium chloride solution (typically containing 30–50 mg ml^{-1} of lithium) into a pipe or channel at a known constant flow-rate using a suitable flow controller. Samples are then taken at a point downstream where complete mixing of the added lithium is known to have occurred. A sample is also taken just prior to dosing the lithium in order to determine the background lithium level. All samples are then analysed for lithium and a simple mass balance calculation then gives the flow-rate in the pipe or channel.

Lithium is ideally suited for this procedure for the following reasons:

(*i*) the natural level of lithium in most raw and waste waters is less than 0.01 μg ml^{-1};
(*ii*) lithium is not significantly adsorbed on to any suspended solids in the flow and cannot be degraded;
(*iii*) lithium is non-toxic to aquatic flora and fauna at the typical concentrations employed; and
(*iv*) even for very high flows, the cost is not excessive.

The dosing rate of lithium is normally set to give a final lithium concentration in the flow of 0.5–2 μg ml^{-1}. Much effort has been expended on the design of accurate dosing systems and ensuring that complete mixing has taken place at the sampling site. However, the assumption is usually made that the flame atomic-absorption or flame emission determination step is bias free. It should be appreciated that the lithium tracer technique is often used for calibration of flow meters and a bias of less than 2% in the measured flow is desirable.[4] Almost all atomic-absorption literature (typical examples are references 6–9) suggests that lithium is not prone to significant chemical interference effects in the air - acetylene flame and that all solutions should be spiked with 1 000 μg ml^{-1} potassium to suppress ionisation. Recovery tests carried out using crude sewage and activated sludge samples as well as synthetic interference solutions indicated that some waste water lithium determinations could be prone to practically important chemical interference effects in the air - acetylene flame. These effects were not evident in the hotter dinitrogen oxide - acetylene flame. The results of these tests are summarised in this paper. Previous work has shown that the determination of chromium,[10] iron[11,12] and manganese[12] in raw and waste waters is more subject to chemical interference effects in the air - acetylene flame than was generally accepted and that the effects were instrument dependent.

It should be noted that although the results of this work relate to atomic absorption, similar chemical interference effects would be expected using the flame emission technique.

Experimental

Apparatus and Chemicals

An Instrumentation Laboratories (IL) 357 atomic-absorption spectrophotometer was used. Some measurements were also made on a Shandon Southern (SS) A3400 instrument. The light source was an IL lithium hollow-cathode lamp. Some measurements were also made using a less intense Cathodeon lithium hollow-cathode lamp. Both lamps had maximum current ratings of 10 mA.

Stock solutions of lithium chloride (1 000 μg ml^{-1} lithium) and potassium chloride (100 000 μg ml^{-1} potassium) were prepared.

Standard Method for Waste Waters

The sample was shaken well and 100 ml (±1 ml)* was poured into a 100-ml stoppered cylinder that had been rinsed previously with a small volume of the shaken sample. Then 1 ml (±0.02 ml) of nitric acid (70% *m/m*) and 1 ml (±0.02 ml) of 100 000 μg ml^{-1} potassium were added from suitable automatic dispensers. The contents were mixed and allowed to stand for 2 h at room temperature. If the sample contained suspended solids, 50 ml of the sample were then filtered through a medium porosity filter-paper that had been washed through previously with 10 ml of the treated sample. The filtrate was collected in a 50-ml polyethylene bottle. Standards were prepared by diluting a suitable aliquot of the stock solution of lithium to 200 ml in a calibrated flask and then adding 2 ml (±0.04 ml) of nitric acid (70% *m/m*) and 2 ml (±0.04 ml) of 100 000 μg ml^{-1} potassium. The standard flasks were selected with calibration marks well down the neck in order to accommodate the 4 ml of added reagents.

Standard Instrumental Conditions

These are given in Table 1.

Results

Recovery Tests on Crude Sewage and Activated Sludge Samples

Various crude sewage and activated sludge samples were spiked with 1 and 2 μg ml^{-1} lithium and the percentage recovery of the added lithium was then determined. On the IL 357 instrument, recoveries ranged from 92 to 100% and on the SS A3400, recoveries ranged from 95 to 101%. The lowest

* It should be noted that even if a volume of 102 ml of the sample was taken instead of 100 ml the relative error in the final lithium concentration would only be 0.038%

Table 1. Standard instrumental conditions. Numbers in parentheses refer to dinitrogen oxide - acetylene flame. Standard air and dinitrogen oxide - acetylene burners as supplied by the manufacturers were used

Parameter	Instrument: IL 357	Instrument: SS A3400
Lamp current/mA	6 (10)*	6
Spectral band pass/nm	0.5	0.6
Wavelength/nm	670.7	670.7
Flame conditions	Slightly fuel-lean* (3-mm red feather)	Verge of luminosity*
Burner height below grazing incidence/mm	0 (3)	0
Nebuliser uptake/ml min^{-1}	6.4	4.6

* Acetylene flow set to give maximum sensitivity. Oxidant flow-rate setting as recommended by the manufacturer.

Table 2. Effect of potassium on lithium in the air - acetylene flame* using the IL 357 instrument. Results are absorbance values of 1 μg ml^{-1} of lithium in various matrices

Flame condition	Matrix: Water	Matrix: 1% *V/V* HNO_3	Matrix: 1% *V/V* HNO_3 + 1000 μg ml^{-1} K
Lean	0.406	0.405	0.374
Stoicheiometric	0.363	0.362	0.336
Verge of luminosity	0.235	0.234	0.224

* All air - acetylene measurements were performed on standard path length air - acetylene burners.

Table 3. Effect of potassium on lithium in the air - acetylene flame* using the SS A3400 instrument. Results are absorbance values of 2 μg ml^{-1} of lithium in various matrices

Flame condition	Matrix: Water	Matrix: 1% *V/V* HNO_3	Matrix: 1% *V/V* HNO_3 + 1000 μg ml^{-1} K
Lean	0.200	0.200	0.202
Stoicheiometric	0.316	0.319	0.324
Verge of luminosity	0.346	0.345	0.346

* All air -acetylene measurements were performed on standard path length air - acetylene burners.

Table 4. Effect of potassium on lithium and copper in the air and dinitrogen oxide - acetylene flames* using the IL 357 instrument. Conditions: matrix, 1% *V/V* nitric acid (70% *m/m*); 1 μg ml^{-1} of lithium and 2 μg ml^{-1} of copper; copper 324.7-nm line; flame conditions as in Table 1

Potassium concentration/ μg ml^{-1}	Absorbance: Air - C_2H_2 Li	Air - C_2H_2 Cu	N_2O - C_2H_2 Li	N_2O - C_2H_2 Cu
0	0.395	0.437	0.224	0.080
500	0.382	0.435	0.277	0.080
1000	0.364	0.437	0.280	0.079
1500	0.346	0.435	0.278	0.080
2000	0.329	0.434	0.280	0.079

*All air - acetylene measurements were performed on standard path length air - acetylene burners.

Table 5. Standard deviation (SD) and relative standard deviation (RSD) of results in the air* and dinitrogen oxide - acetylene flames using an IL 357 instrument

Lamp	Current/mA	Flame	Lithium concn./ μg ml^{-1}	SD†/ng ml^{-1}	RSD,† %	Relative intensity
IL	10	N_2O	0	2.55	—	100‡
			1	5.62	0.56	100‡
			2	15.2	0.76	100‡
Cathodeon	10	N_2O	0	2.05	—	29.4
			1	13.8	1.38	29.4
			2	37.6	1.88	29.4
IL	6	Air	0	0.68	—	49.1
			1	3.88	0.39	49.1
			2	7.70	0.39	49.1
Cathodeon	6	Air	0	0.98	—	10.7
			1	4.46	0.45	10.7
			2	13.1	0.65	10.7

* All air - acetylene measurents were performed on standard path length air - acetylene burners.
† The mean of four separate determinations of SD and RSD, each being calculated from 10 consecutive 2-s integration periods.
‡ Arbitrary value.

recoveries were observed for the crude sewage and mixed liquor of a sewage works receiving a significant proportion of its load from an abattoir. The samples had a typical electrical conductivity of 3000–4000 μS cm^{-1}. This indicated that for some waste waters there are significant chemical interference effects. The magnitude of some of these recoveries was considered unacceptable for flow monitoring. Recoveries of 99–101% were observed in the dinitrogen oxide - acetylene flame on the IL 357.

Effect of Potassium on Lithium

It was predicted that adding 1000 μg ml^{-1} potassium to lithium solutions would result in a very slight enhancement in the air - acetylene flame because of suppression of ionisation.[6-9] Table 2 shows that for the IL 357 a significant depression of the lithium signal was observed under the optimum slightly fuel-lean flame conditions for maximum sensitivity. The relative depression decreased as the flame was made fuel-rich. Similar results were observed on an IL 257 instrument. The effect of potassium was not very dependent upon burner height. Table 3 shows that no depression of the lithium signal was observed with an SS A3400 instrument under all flame conditions tested. The optimum condition for maximum sensitivity was observed in a flame on the verge of luminosity. Under optimised flame conditions the sensitivity of the SS A3400 was 46% of that of the IL 357. The acetylene flow-rate required for maximum sensitivity on both instruments increased as the burner was lowered. The air - acetylene burner height for both instruments was set to grazing incidence by raising the burner until an absolute absorbance of 0.01 was observed with no flame. The variation in optical path through the flames of the two instruments could partly account for some of the variation in the flame conditions required for

optimum sensitivity, observed in Tables 2 and 3. The nebuliser and spray chamber designs are thought to be other important factors.

Table 4 shows the effect of increasing levels of potassium on the lithium response on the IL 357. In the air - acetylene flame, the depression was dependent upon potassium concentration over the range 0–2 000 μg ml^{-1}. The effect was thought to be a simple chemical interference rather than a solution transport effect because a similar effect was not observed with copper (Table 4). Table 4 also shows that in the dinitrogen oxide - acetylene flame the response was virtually independent of potassium concentration over the range 500–2 000 μg ml^{-1} of potassium. This indicated a negligible chemical interference effect in this hotter flame.

Determination of Lithium in the Dinitrogen Oxide - Acetylene Flame

Intense flame emission from the nebulised lithium solution can result in a significantly increased noise level when determining lithium by atomic-absorption spectrophotometry on the IL 357 using the dinitrogen oxide - acetylene flame. When a low intensity hollow-cathode lamp was employed, the relative standard deviations for lithium concentrations above about 0.5 μg ml^{-1} were found to increase significantly. In extreme instances very unstable negative absorbance signals were observed (*e.g.*, Cathodeon lamp operated at 6 mA). The increased noise level (and negative absorbance signals) were attributed to failure of the phase sensitive demodulator to cope with the high d.c. levels and the associated shot noise generated by the relatively intense lithium flame emission signal. Using the IL lamp the effect could be reduced to an acceptable level by increasing the lamp current to its maximum rating of 10 mA. However, this also resulted in a slightly less linear calibration graph at lithium concentrations above 1 μg ml^{-1}. Table 5 gives precision results obtained using the IL neon-filled lamp and also the less intense argon-filled Cathodeon lamp, both lamps run at their maximum current rating of 10 mA. It can be seen that acceptable relative standard deviations of less than 1% were observed with the IL lamp, but the less intense lamp gave unacceptable relative standard deviations. However, it should be noted that the standard deviations of the blank solution (no lithium emission from the flame) were similar for both lamps. This would indicate that both lamps had a similar output stability.

The results demonstrate that for acceptable precision (*i.e.*, RSDs less than 1%) in the dinitrogen oxide - acetylene flame, a relatively intense lithium source is essential. An emission breakthrough effect was also observed in a previous study on the determination of calcium in the dinitrogen oxide - acetylene flame.[13]

Table 5 also gives some precision results in the air - acetylene flame with both lamps operating at their normal current rating of 6 mA. It can be seen that for both lamps better precision is observed in this cooler flame than in the dinitrogen oxide - acetylene flame.

Table 6 shows that for an element such as copper, which is efficiently atomised in the cooler air - acetylene flame,[14] the absorbance is significantly decreased on changing from the air - acetylene flame to the hotter dinitrogen oxide - acetylene flame whilst for lithium an increase is observed. The ratio of the absorbances in the dinitrogen oxide - acetylene flame to that in the air - acetylene flame was 0.37 for copper and 1.45 for lithium (both flames being maintained on the same 50-mm path length burner). This would indicate that the lithium is poorly atomised in the air - acetylene flame. This is in agreement with the result of Halls[15] who reported a degree of atomisation of 0.26 for lithium in the air - acetylene flame. Elements with low degrees of atomisation would be expected to be more prone to chemical interference effects than elements such as copper that are completely atomised in the air - acetylene flame.

Effect of Other Substances

It is well known that major cation and anion concentrations in waste waters vary considerably. It has been recommended that methods of inorganic analysis for waters in the United Kingdom should be tested using specific maximum concentrations of various elements.[16] Table 7 gives some inter-element results based on these criteria. It can be seen from Table 7 that up to 11.3% suppression was observed in the air -acetylene flame using the IL 357, but less than 1% bias was observed using the dinitrogen oxide - acetylene flame. The maximum suppression observed with the SS A3400 was only 4.3% in the air - acetylene flame. Tests were not carried out in the dinitrogen oxide - acetylene flame on this instrument.

The difference in behaviour of the two instruments was partly attributed to the fact that the IL 357 instrument exhibited a high sensitivity (approximately twice that of the SS A3400 for lithium and copper) and this must be achieved by a higher sample transport rate into the flame resulting in

Table 6. Comparison of absorbances of lithium and copper in the air - acetylene and dinitrogen oxide - acetylene flames. All measurements were carried out on a 50-mm path length dinitrogen oxide - acetylene burner on the IL 357 instrument. The burner height was set to 3 mm below grazing incidence. Matrix, 1% *V/V* nitric acid (70% *m/m*) and 1 000 μg ml^{-1} potassium. Acetylene flow-rate was optimised for maximum lithium absorption

	Absorbance	
Flame	1 μg ml^{-1} lithium	2 μg ml^{-1} copper
Air - C_2H_2	0.195	0.210
N_2O - C_2H_2	0.282	0.078
N_2O - C_2H_2:air - C_2H_2 ratio	1.45	0.37

Table 7. Effect of other substances likely to be present in waste waters on the determination of lithium. All solutions contained 1% *V/V* nitric acid (70% *m/m*) and 1 000 μg ml^{-1} of potassium; lithium concentration, 1.0 μg ml^{-1}. If the other substances did not interfere, the effect would be expected (95% confidence) to be within the following ranges: IL 357, air - C_2H_2, 100.00 ± 0.40%; IL 357, N_2O - C_2H_2, 100.00 ± 0.71%; and A3400, air - C_2H_2, 100.00 ± 0.30%

			Recovery, %		
			IL 357		A3400:
Substance	Concentration/ μg ml^{-1}	Present as	Air - C_2H_2	N_2O - C_2H_2	air - C_2H_2
Ca	300	Cl	88.7	100.6	95.7
Mg	100	Cl			
Ca	300	Cl	92.8	99.8	96.0
PO_4	50	H_3PO_4			
Ca	300	Cl	92.6	100.6	99.9
SO_4	300	H_2SO_4			
Si	10	NH_4SiF_6	98.9	100.1	99.7

enhanced chemical interference effects for lithium in the air - acetylene flame. The difference in nebuliser, spray chamber and optical path through the flame are also thought to be critical factors.

Conclusions

The determination of lithium in waste water samples by atomic-absorption spectrophotometry in the air - acetylene flame can be prone to chemical interference effects on some instruments. These effects are considered to be significant when the lithium tracer technique is used for flow monitoring. The use of the hotter dinitrogen oxide - acetylene flame is recommended to minimise these effects. For acceptable precision (a relative standard deviation of less than 1%) it is essential to employ an intense lithium source when using this hotter flame.

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Determination of Trace Amounts of Mercury in Sea Water by Graphite Furnace Atomic-absorption Spectrophotometry

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A procedure for the determination of mercury(II) in inorganic and organomercury salts in sea water by carbon furnace atomic-absorption spectrophotometry at the nanogram level is described. Mercury(II) was concentrated using the ammonium tetramethylenedithiocarbamate (ammonium pyrrolidinedithiocarbamate, APDC) - chloroform system, and the chloroform extract was introduced into the graphite tube. A linear calibration graph was obtained for 5–1 500 ng of mercury in 2.5 ml of chloroform extract. Because of the high stability of the Hg(II) - APDC complexes, it is possible to evaporate the extract in order to obtain a crystalline powder to be dissolved with a few microlitres of chloroform. In this way Hg(II) can be detected at the sub-nanogram level.

Keywords: *Mercury determination; atomic-absorption spectrophotometry; ammonium tetramethylenedithiocarbamate; sea water*

In the study of the sea water pollution off the east Ligurian coast trace metal determination is performed by a pre-concentration step with ammonium tetramethylenedithiocarbamate (ammonium pyrlidinedithiocarbamate, APDC) in chloroform, followed by determination by graphite furnace atomic-absorption spectrophotometry (GFAAS).

The most widely used methods for the determination of trace amounts of mercury are cold vapour atomic-absorption[1] and atomic-fluorescence spectrophotometry.[2,3]

In this paper a method for determining low levels of mercury by GFAAS with preliminary pre-concentration using the APDC - chloroform system is described.

Previous workers have shown that losses of analyte occur during the thermal cycle, before atomisation, but that these losses can be reduced by matrix modification.[4,5] For example, absorbance values can be increased by addition of sulphide ion to the analyte solution; this effect has been attributed to the formation of stable mercury(II) sulphide at the ashing temperature. The aim of this work was to investigate the applicability of mercury(II) - APDC complexes to GFAAS.

Experimental

Instrumentation

The following apparatus was used: a Perkin-Elmer Model 300 atomic-absorption spectrophotometer, equipped with a deuterium background corrector, an HGA-2100 graphite furnace and a mercury hollow-cathode lamp; a Sargent-Welch Model SRG recorder for absorbance measurements; separating funnels, fitted with PTFE stopcocks and polypropylene plugs for sample extraction; and 5-ml glass cylinders with glass stoppers, for the collection of extracted samples.

Reagents

Water. Distilled, de-ionised water stored in a 5-l polyethylene bottles was used.

Nitric acid. Sub-boiling distilled nitric acid was prepared in an all-PTFE still.

Chloroform. Analytical-reagent grade chloroform (1 000 ml) was extracted three times with 50 ml of 1 M nitric acid and stored in a pre-cleaned brown-glass bottle.

APDC. A 1% *m/V* aqueous solution (100 ml) of APDC was extracted with six successive 5-ml portions of purified chloroform, then stored in a pre-cleaned 100-ml PTFE bottle.

Mercury (II) chloride stock solution, 1 000 mg l^{-1}. Mercury(II) chloride (0.1354 g) was dissolved in 100 ml of ethanol.

Mercury(II) iodide (α) stock solution, 1 000 mg l^{-1}. Mercury(II) iodide [α (tetragon) allotrope] (0.2267 g) was dissolved in 100 ml of ethanol.

Methylmercury(II) iodide stock solution, 1 000 mg l^{-1}. Methylmercury(II) iodide (0.1707 g) was dissolved in 100 ml of ethanol and stored at −10 °C.

Phenylmercury(II) acetate stock solution, 1 000 mg l^{-1}. Phenylmercury(II) acetate (0.1678 g) was dissolved in 100 ml of ethanol and stored at −10 °C.

Mercury working standard solutions, 1 mg l^{-1}. Working standards of inorganic and organomercury(II) compounds were freshly prepared daily by appropriate dilution of the stock solutions with distilled water.

Sea water. Surface sea water collected on May 23rd, 1983, at Punta Mesco station (44°06'N, 9°36'E) on the 100-m isobath, off the east Ligurian coast was used to check the efficiency of the method. The sea water was collected in a pre-cleaned 50-l polyethylene container and filtered through a 0.45-μm membrane filter. In addition, samples (1 l) were taken on the 50-m isobath off the east Ligurian coast in pre-cleaned polyethylene bottles and filtered through a 0.45-μm membrane filter. The samples were acidified on collection to pH 3 with sub-boiling nitric acid.

Procedure

A 500-ml portion of filtered sea water was transferred into a separating funnel and 1 M nitric acid was added in order to adjust the pH to 3.0 ± 0.1. A portion of mercury standard solution was added, followed by 1 ml of APDC solution and 5.0 ml of purified chloroform. The mixture was then equilibrated by shaking for 30 s and the phases were allowed 5 min to separate. About 2.5 ml of the clear extract was transferred into a pre-cleaned glass cylinder. The calibration graph was prepared by plotting the peak height against amount of mercury added to 500 ml of distilled water. The optimised experimental conditions are as follows: lamp current, 6 mA; wavelength, 253.63 nm; drying, 100 °C for 10 s; ashing, 200 °C for 10 s; atomisation, 2 000 °C for 3 s; and purge gas, nitrogen "stopped flow."

Results and Discussion

The chloroform extracts were analysed by GFAAS using 20-μl amounts.

Extraction of APDC Complex

Fig. 1 shows the recovery of mercury from sea water adjusted to various pH values and containing 500 ng of mercury(II) chloride. From these data we can easily conclude that optimum extraction conditions are achieved at pH 3 and this value was maintained throughout a subsequent investigation of recovery efficiency for inorganic and organomercury compounds. For this purpose 500 ng of mercury(II) added to 500 ml of distilled water were extracted three more times each after the addition of 1 ml of 1% APDC and 2.5 ml of chloroform. The four extracts were analysed separately and the results are shown in Table 1. The values are the means of ten repeated determinations.

After two extractions mercury recovery was almost complete, over 80% of the added mercury being extracted in the first step. In all subsequent determinations, one extraction alone was performed. This compromise allowed a short extraction time and minimised the possibility of sample contamination, a precaution of paramount significance in determining trace metals in sea water samples.

Precision and Accuracy

To check the precision of the method 500-ml portions of sea water and of distilled water were spiked with 500 ng of mercury as mercury(II) chloride and extracted as already described. The results obtained are listed in Table 2.

Table 1. Recovery of various mercury compounds in four successive extractions. Mercury (500 ng) was added to 500 ml of distilled water

	Recovery,*%			
Compound	1	2	3	4
Mercury(II) chloride	83.8	13.6	2.6	0
Mercury(II) iodide	84.8	12.9	2.3	0
Methylmercury(II) iodide	83.0	14.1	2.9	0
Phenylmercury(II) acetate	86.6	11.6	1.8	0

* Means of ten repeated determinations.

Table 2. Results of mercury determination. Mercury(II) chloride (500 ng) was added to 500 ml of water

	Hg(II) found/ng	
Analysis	Sea water*	Distilled water
1	517	465
2	539	500
3	508	535
4	517	508
5	517	500
6	539	—
7	548	—
8	517	—
9	548	—
10	539	—
Mean†	529	500

* Sea water collected at Punta Mesco station (44°06'N, 9°36'E).

† The mean results gave recoveries of 105.8 and 100% and coefficients of variation of 2.6 and 4.4% for sea water and distilled water, respectively.

Table 3. Melting-points of various mercury(II) - APDC complexes

Compound	Melting-point of APDC complex/°C
Mercury(II) chloride	283
Mercury(II) iodide	242
Methylmercury(II) iodide	284
Phenylmercury(II) acetate	280

The difference between the mean mercury content of the sea water and that of the distilled water represents the amount of mercury in 500 ml of sea water.

The calibration graph was linear over the range 5–1 500 ng of mercury.

Typical recorder tracings are shown in Fig. 2 for chloroform extracts introduced directly into the graphite furnace. The relative standard deviation of ten repeated determinations of 500 ml of distilled water containing 10 ng of mercury(II) as mercury(II) chloride was 17.4%. In the course of the sample contamination study no blank values other than zero were ever experienced.

Sensitivity of the Method

The mercury(II) - APDC complexes of inorganic and organomercury salts are very stable and can be easily recovered in crystalline form by evaporation of chloroform solutions.

The melting-points of these complexes are reported in Table 3. It is clear that under these circumstances the smallest amount of mercury detectable is determined by the volume of solvent used. In order to improve the limit of detection, 2.5 ml of the extract, from a series of aqueous solutions containing 10 ng of mercury as mercury(II) chloride, were evaporated by means of a gentle flow of nitrogen and the residues dissolved with variable but known volumes of solvent. The results in Fig. 3 show that increasing absorbance values are obtained with decreasing volumes of solvent (20 µl having been used for the analysis in all examples). It is evident that by employing a concentration step a detection limit of less than 1 ng can be achieved.

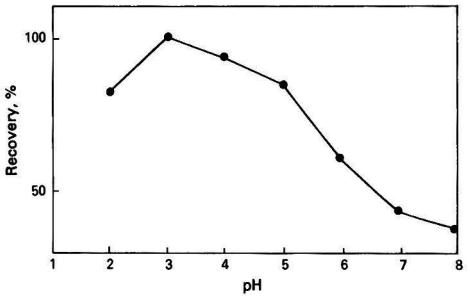

Fig. 1. Effect of pH on the efficiency of extraction of mercury from sea water

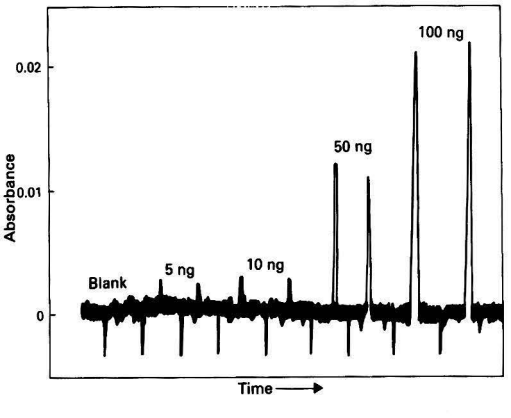

Fig. 2. Recorder tracings obtained from chloroform extracts with various mercury concentrations (20-µl aliquot in the furnace)

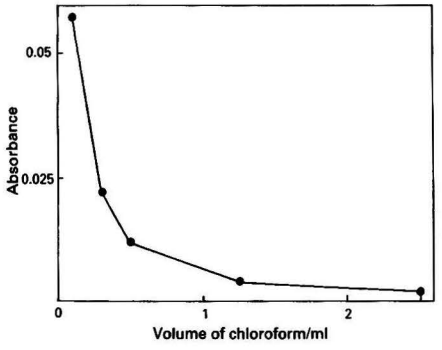

Fig. 3. Variation of the mercury absorption signal with the volume of solvent used for dissolution of the Hg - APDC complex

Table 4. Determination of the concentration of mercury in surface sea water samples by the proposed method

Sample*	Location	Amount of mercury/ ng l^{-1}
1	44°06′N, 9°36′E	60
2	44°05′N, 9°43′E	217
3	44°07′N, 9°40′E	156
4	44°08′N, 9°37′E	130
5	44°12′N, 9°30′E	165

* Sample 1 was taken on the 100-m isobath and samples 2, 3, 4 and 5 were taken on the 50-m isobath.

Analysis of Environmental Samples.

The proposed method was used to determine mercury in filtered sea water from the east Ligurian coast and the results are reported in Table 4.

Conclusion

The method described is extremely simple and allows nanogram levels of mercury(II) present as inorganic and organomercury salts to be determined in water samples by direct injection of a portion of the chloroform extract. It was found that the chloroform extracts can be stored for more than one week without mercury losses. In order to determine mercury at the sub-nanogram level it is necessary only to evaporate the chloroform from the extract and to re-dissolve the residue again with a few microlitres of chloroform and to transfer the solution quantitatively into the graphite furnace.

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Comparison of Photographic and Photoelectric Detection for Multi-element Analysis by Inductively Coupled Plasma Atomic-emission Spectrometry*

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The relative performances of two detecting and recording systems used in inductively coupled plasma atomic-emission spectrometry for multi-element analysis are compared. Whereas the more generally used scanning monochromator system has superior sensitivity and precision, the spectrographic system incorporating a computer-controlled microdensitometer provides a permanent record of the spectrum, requires no pre-selection of spectral lines, has a better ability to identify correctly emission lines and is more economic in terms of sample volume and time. The merits of both approaches are discussed in relation to multi-element analysis of diverse materials.

Keywords: *Inductively coupled plasma; optical emission spectrometry; detection systems*

During the last three decades, photoelectric recording of the spectra obtained in emission spectroscopy has dominated over photographic recording to the extent that very few laboratories use the latter and many instruments employing the former mode of detection are available commercially. When the number of elements being sought is not large and also when spectral line interferences are not significant, this approach is probably the more suitable. In this method,[1-3] specific pre-selected parts of the spectrum are scanned sequentially using a monochromator and the spectral lines are identified and quantified. However, when the number of elements being sought is large, and the range of matrix wide, this approach may not be totally practicable. In this event, resort is made to photographic recording[4,5] in which the whole emission spectrum is recorded permanently and examined subsequently using a computer-controlled microdensitometer.[6,7] In order to determine the most appropriate method of specific analyses, the relative performances of the two systems were assessed in terms of detection limits, precision, accuracy, sample economy, optical dispersion, optical resolution and reproducibility.

Instrumentation

Spectrographic Recording and Analysis

Spectra are recorded photographically using an Ebert 3.4-m spectrograph. The external optics consist of a 15-cm focal length condensing lens focusing on to a mask that allows emission from the plasma to be aligned on to the spectrograph slit by a 14-cm focal length cylindrical lens. Details of the plasma source, spectrograph and photographic plate processing are given in Table 1.

The recorded spectra are measured using a Joyce Loebl double-beam microdensitometer[7] interfaced to a PDP 11/24 computer. The position of any spectral line can be defined by its distance from a fiducial line, which is a line arising from an internal standard element added to each solution. The photographic plate is moved under computer control to the expected position of the line of interest and a scan of 96 steps over a wavelength range of 0.12 nm is recorded. After smoothing the raw data,[8] the line position and peak absorbance are calculated together with the background, which is defined as the mean of the eight lowest consecutive absorbance readings within the scan data. To be accepted as genuine, a peak must lie within ± 0.01 nm of its expected position. Using plate calibration data for the wavelength of the line measured the relative intensities of lines and backgrounds are calculated using the L transform.[9]

The microdensitometer acquires data for all the required elements for all of the standards and the computer constructs a linear calibration from which sample concentrations are subsequently calculated.

Spectrometric Recording

The system consists of a microcomputer-controlled scanning monochromator linked to an inductively coupled plasma (ICP) source. Light from the plasma is focused on to the entrance slit of the spectrometer as a 1:1 image. The computer contains all the necessary instructions and data for the interface to control the spectrometer drive mechanism. Spectral lines are scanned by slewing to 0.075 nm before the line of interest and then 150 0.001-nm steps are taken across the line, thereby recording and storing the integrated data points. Processing of the scan data is similar to that performed using the microdensitometer, and calibration plots for standards are obtained. Full instrumental and operating details are given in Table 1.

Experimental

The relative abilities of the two systems to identify correctly and quantify emission lines were determined in terms of their optical and mechanical performances, sensitivity, precision, accuracy and sample economy.

Optical Performance

Dispersion and practical resolution were compared by recording the titanium spectra of a 10 μg ml^{-1} solution. Recorded intensity scans for each method are shown in Fig. 1(*a*) and (*b*). With the scanning monochromator only that part of the spectrum in the range 322.700–322.950 nm was recorded, whereas for the spectrographic recording the whole spectrum was recorded but only the same wavelength region scanned using the microdensitometer. Dispersion and resolution are given in Fig. 1(*a*) and (*b*).

Mechanical Performance

Errors associated with mechanical operation, which can affect final accuracy and precision, were evaluated using replicate analyses for ten elements in solution. At each wavelength six

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Table 1. Instrumental methods for both the spectrographic and scanning monochromator systems

Function	Parameters	Spectrograph	Scanning monochromator
Plasma	Type	International Plasma Model 120–27	Plasma Therm Model HFP 2500D
	Frequency	27–12 MHz	27–12 MHz
	Torch type	Demountable Fassel	Demountable Fassel
	Forward power/kW	1.5	1.5
	Reflected power/W	5	5
	Coolant gas (Ar) flow-rate/l min^{-1}	15	15
	Auxiliary gas (Ar) flow-rate/l min^{-1}	0	1.4
	Injector gas flow-rate/l min^{-1}	1.1 at 15 lb in^{-2}	0.7 at 12 lb in^{-2}
	Observation height above work/mm	20–25	18–22
	Nebuliser	Pneumatic cross-flow: Plasma Therm Model TN-1	Pneumatic cross-flow: Plasma Therm Model TN-1
Optical	Type	3.4-m Ebert (Jarell-Ash)	1-m Spex 1802
	Grating	15 000 lines in^{-1} blazed at 300 nm	2400 mm^{-1} holographic
	Grating angle/°	5–85	–
	Wavelength range/nm	200–480	100–900
	Slit width/μm	20	20
	Slit height/mm	2	2
	Exposure time/s	120	–
Detection		Photographic plates (200–340 nm, spectrum analysis No. 1) (340–480 nm, spectrum analysis No. 3)	Photomultiplier (EMI-9802 operating at 1.67 kV)
Plate or signal processing		Plate	Signal
		Developer: Kodak D19	Computer: Apple II (48K) operating in DOS 3.3
		Developing time: 4 min	Language: Apple soft extended BASIC
		Developer temp.: 20 °C	Interface: JS Systems real-time Linkman
		Fixer: 20% Perfix + 5% S-type hardener	Central processing unit: 16K Mostek-Zilog 280
		Fixing time: 4 min	
		Fixing temp.: 20 °C	
		Washing time: 15 min	

Table 2. Comparison of mechanical performance

Element	True wavelength/nm	Mean difference from true wavelength*/nm: Microdensitometry	Mean difference from true wavelength*/nm: Scanning monochromator	Standard deviation/nm: Microdensitometry	Standard deviation/nm: Scanning monochromator
Mn	257.610	+0.005	−0.015	0.002	0.009
Fe	259.940	−0.001	0.000	0.001	0.006
Cr	283.563	−0.005	+0.009	0.002	0.014
Mg	285.210	−0.009	+0.008	0.002	0.004
Ni	305.082	0.000	−0.014	0.001	0.006
V	309.311	−0.004	+0.003	0.002	0.007
Ca	317.933	−0.001	−0.004	0.002	0.007
Cu	327.396	−0.001	+0.021	0.002	0.013
La	333.749	−0.003	+0.005	0.002	0.007
Ti	338.376	+0.002	−0.001	0.002	0.007

* Difference = true wavelength − measured wavelength.

measurements were performed, from which the mean and standard deviations about the mean shown in Table 2 were derived.

Sample Economy

Multi-element solutions were analysed by each method and the amounts of solution consumed and times for analyses determined.

Sensitivity

Limits of detection (L) were calculated from the expression

$$L = 3 \times (\text{RSD}) \times (I_b/I_a) \times C_A$$

where I_b and I_a are the mean background and analyte intensities, respectively, and RSD in the relative standard deviation of n measurements of the analyte at a concentration C_A. Limits of detection are given in Table 3.

Precision

Precision was determined by replicate analyses of multi-element standard solutions (0.1–10 μg ml^{-1}) prepared from BDH Spectrosol standards. Results on five replicate determinations on two solutions are given in Table 4.

Accuracy

The accuracy of each method was compared with expected values averaged from determinations by several other methods, such as flame atomic-absorption spectrometry and

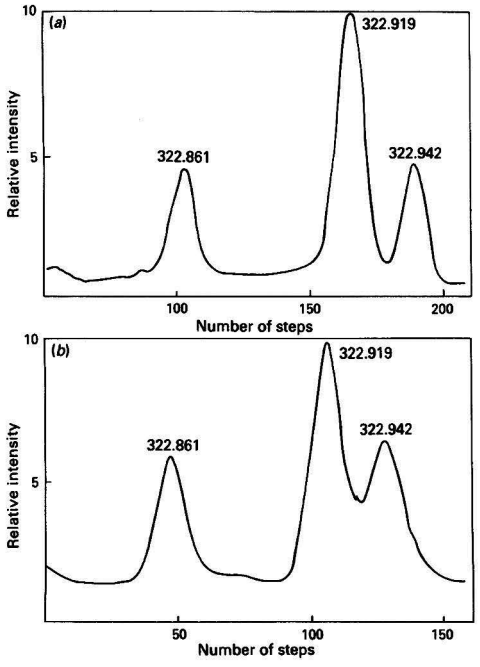

Fig. 1. Spectral scans of a 10 μg ml^{-1} titanium solution. (*a*) Recorded with the scanning monochromator: dispersion 0.42 nm mm^{-1}; theoretical resolution 82 500; and observed spectral band pass 0.022 nm. (*b*) Recorded with the spectrograph and microdensitometer: dispersion 0.50 nm mm^{-1}; theoretical resolution 228 000; and observed spectral band pass 0.031 nm. Wavelengths, in nanometres, are indicated on the peaks

Table 3. Comparison of sensitivites

Element	Wavelength/nm	Limit of detection/μg ml^{-1} Photographic detection	Photoelectric detection
Mn	257.610	0.041	0.003 9
Fe	259.940	0.091	0.005 9
Cr	283.563	0.023	0.006 6
Mg	285.210	0.011	0.001 3
Ni	305.082	0.282	0.034
V	309.311	0.053	0.002 5
Ca	317.933	0.164	0.010 2
Cu	327.396	0.094	0.004 1
La	333.749	0.31	0.027
Ti	338.376	0.069	0.001 9

epithermal neutron activation analysis. A boron control sample containing 5–100 p.p.m. of each impurity was used throughout, the chemical treatment of the sample being as follows. Three 2.0-g samples of boron were refluxed in 15 ml of nitric acid (Aristar grade) for 3 h. After transfer to platinum crucibles, the boric acid was dissolved in 40% *m/V* hydrofluoric acid, evaporated to dryness, the residue dissolved in 1 ml of perchloric acid (3% *V/V*) and diluted to 25 ml using de-ionised water. Reagent blanks were prepared similarly.

The results are summarised in Table 5.

Results and Discussion

Positive identification of spectral lines by wavelength requires good optical and mechanical performances of the instrumentation, thereby reducing the possibility of mis-identification to an acceptable minimum. As shown in Fig. 1 (*a*) and (*b*), the resolutions and dispersions of the two systems are similar, lines 0.02 nm apart can be resolved satisfactorily and the line half-widths correspond to the best resolution given by Boumans.[10] The microdensitometer locates lines more precisely than the scanning monochromator system, and as the criteria for line rejection are based on precision, the former is therefore the more suitable for correct identification of lines, especially in complex spectra.

Line mis-identification arises primarily from two causes. In the first the expected line is absent and a second line is incorrectly taken for measurement. If the difference between the wavelength of the expected line and that of the observed line is significant in comparison with the precision in wavelength setting, the second line will be rejected. In the second instance, where both the expected line and an interfering line are observed, the instrument will reject the interfering line provided it is resolved by the spectrometer, as the precision of wavelength reproducibility is better than the resolution of the spectrometer or spectrograph. When these two are not resolved, the peak of the combined line will shift in wavelength and if this shift is significant the line will be rejected.

Table 4. Comparison of precision and accuracy

Element	Expected concentration/ μg ml^{-1}	Precision* Photoelectric recording	Photographic recording
Mn	1.0	1.5	5.8
	2.0	0.7	6.4
Fe	1.0	0.8	6.1
	2.0	0.7	10.1
Cr	1.0	1.7	4.5
	2.0	1.5	10.1
Mg	1.0	0.6	5.1
	2.0	0.3	7.2
Ni	1.0	1.0	11.7
	2.0	1.0	12.6
V	0.5	1.9	5.5
	5.0	0.4	10.1
Ca	2.0	0.6	3.1
	5.0	0.5	10.5
Cu	1.0	0.7	10.6
	2.0	1.7	10.0
La	1.0	1.3	8.2
	2.0	2.4	10.4
Ti	1.0	0.9	2.8
	2.0	0.3	10.7

* Precision = $\frac{\text{standard deviation}}{\text{experimentally determined mean}} \times 100.$

Table 5. Analysis of boron control sample

Element	Expected value, p.p.m.	Spectrographic analysis, p.p.m. ($n = 3$)	Spectrophotometric analysis, p.p.m. ($n = 3$)
Al	295	280 ± 60	239 ± 18
Ca	250	270 ± 50	256 ± 29
Cu	29	29 ± 3	30 ± 3
Ni	33	36 ± 5	34 ± 2
Mo	161	165 ± 30	152 ± 10
Mg	110	110 ± 15	96 ± 10
Cr	44	41 ± 3	44 ± 3
La	5.5	6.0 ± 1.8	5.6 ± 0.8
Fe	1070	1100 ± 140	960 ± 120
Mn	670	680 ± 40	659 ± 28
Cd	<5	<100	<5
Pb	<2	<10	<2

Hence, as the primary cause of incorrect identification of a line arises when other more prominent lines appear within the wavelength limits that define the criteria for rejection, and which are based on the mechanical precision, and as the microdensitometer has the better performance, then this system is more suitable for examining complex and unpredictable spectra. With photographic detection the entire spectrum is permanently recorded and the system does not rely on accurate selection of interference-free lines prior to the analysis, as alternative lines can be examined at leisure. In contrast, the scanning monochromator system is less economic, because if additional lines are to be examined more sample is required.

Spectrographic determination of trace elements requires a single 2-min exposure, which permits all lines in the wavelength range 200–480 nm to be recorded permanently. Subsequent computer-controlled microdensitometer examination requires little manual involvement. Hence the total time for the spectrographic system relates mainly to spectra acquisition. In contrast, the scanning monochromator requires an additional volume of sample solution and, in the absence of a completely automated sample changing system, the analyst's time is increased for every additional element to be determined. Sample economy can be improved significantly by using a recirculating nebuliser,[11] which requires only one tenth of the sample volume required by other conventional nebulisers without degradation of performance.

Table 3 shows clearly that photoelectric detection is more sensitive than photographic detection, often by at least an order of magnitude. However, both methods are capable of detecting impurities at the sub-μg ml^{-1} level.

The precision of photoelectric recording is in the range 1–2% and is better than that of photographic recording, for which it is in the range 5–10%.

The relative over-all performances of both systems were assessed by using a boron sample typical of those often analysed in these laboratories and for which multi-element data are available from other techniques. The results given in Table 5 indicate that, within experimental error, both techniques are free from bias, apart from the possible exception of the spectrometric determination of aluminium. At present there is no reasonable explanation for this observation, and it will be investigated more fully in the future. Although the spectrographic recording system is better suited for interpreting complex spectra, once the major constituents have been identified spectrographically and the optimum spectral lines identified, the scanning monochromator system can then be used to give better precision. Also, owing to its better sensitivity, lower detection limits for elements not positively identified can be obtained. The poorer sensitivity for the spectrographic determination of cadmium arises from the inferior sensitivity of the photographic plate at this wavelength (228.802 nm).

Conclusions

Although during the last three decades photographic recording of emission spectra has been superseded largely by scanning and direct reading methods, there are instances, especially in trace multi-element analysis, where there are advantages in using the former. The main advantage is that all spectral data in the range 200–480 nm can be recorded permanently in a single exposure that can be interpreted later. Photographic recording has poorer sensitivity (×10) and precision (×5) than photoelectric recording, but provided these limitations are acceptable, the method, when combined with computer-controlled microdensitometry, provides an economic means of analysis. Both methods of recording are in regular use in the authors' laboratory, although for quantitative multi-element analyses of complex materials, spectrographic recording is more suitable.

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Determination of Marker Chromium in Faeces Using Inductively Coupled Plasma Emission Spectrometry

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Chromium is determined in chromium(III) oxide-labelled faeces using an inductively coupled plasma source and emission spectrometry. A nitric acid - perchloric acid mixture is used to destroy organic matter in the faecal material and to dissolve the chromium(III) oxide. The concentration of chromium in the diluted digest is determined using the 358-nm line. The procedure is manipulatively simple, requires minimum supervision and is suitable for high-throughput applications. The precision for a single determination is ±5.4%.

Keywords: *Marker chromium determination; chromum(III) oxide; faeces; inductively coupled plasma emission spectrometry*

Chromium(III) oxide (Cr_2O_3) is used as a marker substance to determine the feed intake of grazing animals, a constant oral infusion of chromium(III) oxide in glycerol is usually used. Chromium(III) oxide mixes uniformly with ingesta[1] and is inert to absorption by the animal,[2] and its faecal concentration depends on the concentration of the marker substance in the glycerol support medium, the rate of infusion of the marker substance and the digestibility and amount of the feed ingested by the animal. Labelled faeces may contain in excess of 2 000 μg g^{-1} of marker chromium. If digestibilities are known, intake can be calculated from the concentration of marker chromium in the faecal material.

A rapid procedure for the determination of marker chromium in faeces was required as part of an exploratory comparison[3] of the chromium(III) oxide - faecal output - forage indigestibility and the bite size - bite number methods for determining forage intake in cattle.

The convenience of simply pressing a pellet of the finely ground, labelled faeces with a boric acid backing coupled with the use of X-ray fluorescence spectrometry was most appealing. However, massive spectral interferences, particularly from iron, were observed with our spectrometer, which precluded the use of this technique.

Marked interferences have been encountered in the spectrophotometric and flame atomic-absorption spectrophotometric (AAS) determinations of chromium in solution. Preliminary testing of a spectrophotometric method[4] for the determination of chromium gave erratic and unreliable results for multiple re-analyses of the same sample material. Inductively coupled plasma emission spectrometry (ICPES) has good sensitivity and a wide dynamic range and is relatively free from chemical interferences.

Alkali fusion procedures[5] commonly used to solubilise chromium(III) oxide are slow and tedious. The simple, but not often used, perchloric acid procedure[6] for dissolving chromium(III) oxide was combined with ICPES to develop a simple, rapid and reproducible method for the determination of marker chromium in faeces.

Experimental

Instrumentation

A Labtest Plasmascan 700 inductively coupled plasma emission spectrometer was used. Its scanning monochromator had a focal length of 0.35 m and a grating blazed at 250 nm first order with 1 180 rulings mm^{-1}. The nebuliser was a modified cross-flow using the V-groove principle of Suddendorf and Boyer.[7] The plasma was run at 1.1 kW incident power and the viewing height was 11 mm above the load coil. An IP28 photomultiplier was used at 0.75 kV. The argon flow-rates were coolant gas 10.5 l min^{-1}, sample gas 0.9 l min^{-1} and plasma gas zero. The sample solution pump rate was 1.8 ml min^{-1}. A slit width of 0.1 mm was used with a wavelength of 358 nm.

Apparatus

Pyrex tubes (24 × 150 mm) calibrated with a 20-ml mark were used for sample digestion together with a 60-place aluminium heating block drilled to suit the digestion tubes and thermostatically controllable to 300 °C.

Reagents

Unless otherwise specified, all reagents were of analytical-reagent grade and the term "water" implies de-ionised or distilled water.

Nitric acid, concentrated, 70% *m/m.*

Perchloric acid, 70% *m/m.*

Potassium dichromate. Recrystallised three times from water.

Chromium(III) oxide.

Reagent blank solution. Dilute 66 ml of perchloric acid to 1 000 ml with water.

Standard solutions

Stock chromium solution. A solution containing 1 000 μg ml^{-1} of chromium was prepared using recrystallised potassium dichromate and the reagent blank solution as solvent.

Calibration standards. Prepare solutions containing 0, 25, 50, 75, 100 and 200 μg ml^{-1} of chromium in reagent blank solution from the stock chromium solution. An extended range of calibration standards up to a maximum of 500 μg ml^{-1} of chromium can be used if the marker chromium concentrations in the samples are exceptionally high.

Calibration

The Plasmascan employs a two-point calibration procedure. The upper calibration point was set using the most concentrated chromium standard solution in the selected set of standards. The reagent blank solution or, alternatively, the 25 μg ml^{-1} chromium standard solution was used to set the lower calibration point.

Intermediate standards were used to confirm the linearity of calibration over the working range of concentrations and as regular checks for instrument drift during the reading of sample digest solutions.

Procedure

Oven dry the sample overnight at 105 °C. Weigh 1.000 g of finely ground sample into a digestion tube, add 10 ml of nitric acid, ensuring that the acid wets the entire sample, and allow to stand overnight. Add 2 ml of perchloric acid and a few silica boiling chips, place the tube in a cold heating block, set the block thermostat to 50 °C and turn the mains power on. Heat the tubes at 50 °C for about 30 min, which allows initial frothing to subside.

Place a small glass funnel, about 30 mm in diameter, in each tube and increase the thermostat setting to 160 °C. Heat the samples at this temperature for 3–4 h, occasionally using small volumes of nitric acid to wash any chromium(III) oxide adhering to the upper walls of the tube down into the boiling solution, then increase the thermostat setting to 200–210 °C. Digestion is completed when all the nitric acid has boiled off (dense white fumes of perchloric acid fill the tube) and all green chromium(III) oxide has been converted into the soluble, orange, dichromate form. Remove the tubes from the block and, when they have cooled sufficiently, dilute the digest to the 20-ml mark with water. Mix the contents well and allow any insolubles to settle. After calibration of the plasma unit, analyse the diluted digests for chromium in solution using the previously described instrument operating parameters.

This procedure requires minimum operator supervision. The time required for digestion may be reduced considerably by boiling the solutions at 160 °C for only 2–3 h before raising the thermostat setting to 200–210 °C. If the reduced boiling time is used then regular supervision must be maintained once the thermostat setting has been raised to ensure that the solutions do not boil over. A small amount of nitric acid must be added to the digest if charring begins. The onset of charring is marked by a chocolate brown curdling effect in the solution.

Caution. The possible danger of explosion always exists with perchloric acid digests. All necessary precautions should be taken, a comprehensive list of which appears in reference 8.

Performance Characteristics of the Method

Samples

Marked and unmarked faecal samples were used to assess the performance characteristics of the method. These were collected from grazing cattle, some of which received a continuous oral infusion of chromium(III) oxide in glycerol.

Linearity of instrument response

Two separate sets of extended range calibration solutions containing 0, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450 and 500 μg ml^{-1} of chromium were prepared on each of two different days.

The linearity of the instrument response was assessed on each of the two days using the entire set of standard solutions. All combinations of low-point (reagent blank, 25 and 50 μg ml^{-1}) and high-point (200, 300, 400 and 500 μg ml^{-1}) calibrations were used. Duplicate instrument readings were made on each solution in the full standard set for each of the 12 different combinations of low-point - high-point calibrations. For each combination, a linear regression of instrument response on concentration of chromium in solution (μg ml^{-1}) was used to assess the linearity of instrument response. A lack-of-fit *F*-test was used with each assessment to test the suitability of the linear models.

Limit of quantitation

The detection limit determined from ten successive 10-s integrations of a near blank chromium solution was used to calculate the limit of quantitation,[9] which is defined as five times the detection limit.

Interferences

Spectral and ionisation effects due to 1 000 μg ml^{-1} of sodium, 1 000 μg ml^{-1} of potassium, 100 μg ml^{-1} of iron, 200 μg ml^{-1} of calcium and 100 μg ml^{-1} of magnesium in the presence of 200 μg ml^{-1} of chromium were monitored. These levels of interferents were much higher than the average levels found in faecal digest solutions.

Recovery of added chromium(II) oxide

Faecal material containing no marker chromium was ground in a beater cross mill fitted with a 1-mm sieve and the ground material was well mixed. Additions of 0, 4, 8, 12 and 16 mg of chromium as chromium(III) oxide were made to separate 10-g portions of ground faecal material. Each addition was replicated four times and then ground separately for 3 min in a large cast iron cup on a shatterbox mill. Four 1-g portions of each finely ground material were analysed for chromium. The recovery of added chromium was calculated from the slope of the linear regression of total chromium found (μg) on chromium added (μg). An *F*-test for the lack-of-fit of the linear model was also made using the regression data.

Repeatability and reproducibility

Three different faecal samples, each from different cattle treated with a continuous infusion of chromium(III) oxide, were analysed in triplicate on each of three days. A two-way analysis of variance of the data was used to obtain precision estimates for the method.

Results and Discussion

Linearity of Instrument Response

The instrument response was linear over the range 0–500 μg ml^{-1} of chromium for each of the low-point - high-point calibration combinations tested. The lack-of-fit *F*-test associated with each linear regression of instrument response *versus* concentration of chromium in solution (μg ml^{-1}) was not significant.

The variance of instrument response for the reagent blank solution was higher, but not significantly higher, than that for either the 25 or 50 μg ml^{-1} chromium solution. For this reason, the reagent blank solution is rarely used to set the low calibration point.

Limit of Quantitation

The detection limit was found to be 0.013 μg ml^{-1} of chromium. From this the limit of quantitation was calculated to be 1.4 μg g^{-1} of chromium.

Interferences

Duplicate determinations were used with each added interferent and the results are given in Table 1.

There were no significant spectral or ionisation effects due to the added interferents. These elements would not be expected to interfere in real samples as the level of spiking was considerably higher than that normally found in faecal digests.

Recovery of Added Chromium(III) Oxide

The linear regression of total chromium found (μg) on chromium added (μg) gave a least-squares slope of 0.993 6 with a standard deviation of 0.020 7 for 78 degrees of freedom. The recovery with its confidence interval ($P = 0.05$) of 99.4 ± 4.1% was not significantly different from 100%.

The *F*-test for lack-of-fit of the linear model to the regression data was not significant ($P = 0.05$).

Table 1. Effect of possible interferents on the determination of chromium using ICPES

Concentration/μg ml^{-1}	ICPES* result/μg ml^{-1} (duplicate determinations)
200 Cr + 1 000 Na	198.0, 198.4
200 Cr + 1 000 K	200.5, 203.7
200 Cr + 100 Fe	199.0, 199.8
200 Cr + 200 Ca	201.2, 201.0
200 Cr + 100 Mg	197.0, 199.1

* Calibration points were set using the 50 and 200 μg ml^{-1} standard solutions.

Table 2. Precision estimates for the determination of marker chromium in faeces using ICPES

(*a*) *Analytical data*—

Day	Replicated chromium/μg g^{-1} sample: Sample 1	Sample 2	Sample 3
1	1 240, 1 194, 1 292	1 712, 1 772, 1 732	2 212, 2 160, 2 168
2	1 270, 1 318, 1 270	1 782, 1 772, 1 780	2 226, 2 268, 2 160
3	1 244, 1 258, 1 200	1 846, 1 806, 1 826	2 192, 2 200, 2 314

(*b*) *Analysis of variance**—

Source	Degrees of freedom	Mean square	*F* ratio
Days	2	18.005 6	2.21
Samples	2	6 763.435 8	—
Day × sample interaction ..	4	8.156 767 5	1.62
Replicates	18	5.034 305 56	

Repeatability relative standard deviation[10] = 2.244%
Reproducibility relative standard deviation[10] = 2.678%

* A computer program was used to code the original data in (*a*) to have a grand mean of 100 and then to complete the analysis of variance on the coded data.

Repeatability and Reproducibility

Table 2 shows the replicated analyses for each sample for each day and the analysis of variance used to calculate precision estimates.

The precision, as a relative confidence interval ($P = 0.05$) about a mean determination, was calculated as ±4.7% from the repeatability relative standard deviation (18 degrees of freedom). The relative confidence interval ($P = 0.05$) about a single determination was ±5.4% calculated from twice the reproducibility relative standard deviation.

Note on Other Methods

Attempts were made to compare the ICPES method with a spectrophotometric method[4] following either perchloric acid digestion or alkali fusion of the faecal material. Erratic results were obtained with chromium-marked samples even though the calibration lines for the spectrophotometric procedures were linear and reproducible. No attempt was made to investigate the reasons for such poor sample results as the excellent recovery of the ICPES method was obtained using chromium(III) oxide mixed with faecal material in a manner similar to that of real samples.

Note on Sample Fineness

Chromium(III) oxide readily separates from dry powdered faecal material. For the highest precision, samples should be ground in a shatterbox-type mill. An acceptable but lower precision is obtained when a beater cross mill fitted with a 1-mm sieve is used.[11]

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SHORT PAPER

Determination of Nitrite and Nitrate in Meat Products by High-performance Liquid Chromatography

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Keywords: *Nitrite determination; nitrate determination; meat products; ion-pair high-performance liquid chromatography*

Nitrite gives meat a pleasing colour, which has been attributed to the decomposition to nitrogen oxide, NO, which reacts with haeme pigments to form the coloured nitrosohaeme. Nitrite also prevents the development of the bacterium *Clostridium botulinum*, which causes food poisoning due to botulism.

As a preservative, nitrate has a somewhat uncertain role; it is generally believed that it may be a reservoir for nitrite, which is formed by the reduction of nitrate. There is concern about the possibility of a reaction in processed foods or in the digestive system between nitrite and secondary amines to form nitrosamines, which are highly carcinogenic. For this reason accurate nitrite and nitrate determinations are of interest.

Under Dutch regulations controlling the use of preservatives in foods, the levels of nitrite and nitrate in meat products must not exceed 200 and 500 mg kg^{-1}, respectively, calculated as the sodium salt or calculated as the potassium salt.

There are many methods for the determination of nitrite and nitrate levels in meat. Colorimetric, fluorimetric, titrimetric and polarographic methods and the use of ion-selective electrodes are applicable to the determination of only a single species at one time. This drawback does not exist with chromatography, which offers separation, identification and quantification of more than one species successively in one run.[1]

Leuenberger *et al.*[2] described the determination of nitrate and bromide in foodstuffs by HPLC on an amino phase chemically bonded to silica gel. Unfortunately, nitrite could not be detected as it is eluted in the back of the solvent front. Several workers[3–11] have reported the determination of inorganic anions by reversed-phase ion-pair liquid chromatography on non-polar phases, coupled with UV detection. We adopted the technique of Iskandarani and Pietrzyk[6,7] with some modifications and successfully applied the chromatographic system to the determination of nitrite and nitrate in meat products.

Experimental

HPLC Equipment

Pump. Waters Model 6000A, with the flow-rate set at 1.0 ml min^{-1}.

Injector. Waters Model U6K (variable loop).

Detector. Spectra-Physics SP8440 variable-wavelength UV - visible detector with the wavelength set at 240 nm and time constant 0.1 s.

Integrator. Perkin-Elmer Sigma 15 Chromatography Data Station interfaced to a Model 3600 Chromatographics Intelligent Terminal.

Chromatography

Mobile phase. Acetonitrile - 5×10^{-3} M tetrapentylammonium bromide (25 + 75).

Stationary phase. Hamilton pre-packed PRP-1 column, 150 × 4.1 mm i.d. The column is operated at 30 °C and conditioned by recycling the eluent overnight.

A 75 × 2.1 mm i.d. guard column containing pellicular Bondapak C_{18}/Corasil (Waters) is used.

Sample Treatment

Transfer 10.0 g of finely comminuted and thoroughly mixed sample into a 250-ml calibrated flask, add 200 ml of hot water (90 °C) and allow the contents of the flask to cool, stirring occasionally. Dilute to volume with water and filter through Schleicher and Schüll No. 595.5, or an equivalent, filter-paper.

Add 1.0 ml of Carrez I [0.25 M potassium hexacyanoferrate(II)] and 1.0 ml of Carrez II (1.0 M zinc acetate + 0.5 M acetic acid) reagents to 50.0 ml of filtrate. Centrifuge the solution and filter the supernatant through a 0.5-μm filter. Inject 20 μl into the chromatograph.

Results

PRP-1 is a non-polar poly(styrene - divinylbenzene) adsorbent in the form of 10-μm spherical, rigid, macroporous particles, which tolerates exposure to extremely acidic (pH 1) or basic (pH 13) conditions.[12] The retention mechanism of inorganic anions on PRP-1 has been studied by Iskandarani and Pietrzyk.[6]

Fig. 1 shows a typical elution profile of a sample of roast beef before and after "spiking" with 200 mg kg^{-1} of $NaNO_2$ and 500 mg kg^{-1} of KNO_3. Chromatograms of samples of ham, bacon, roast beef and luncheon meat did not show interferences with the same retention volume as nitrite or nitrate. The chosen wavelength of 240 nm is a compromise between a clean chromatogram with low sensitivity and a crowded chromatogram with high sensitivity. Table 1 gives some quantitative results.

Chloride, phosphate and sulphite are optically transparent. The elution of ascorbate and nicotinic acid, respectively, does not interfere with the elution of either nitrite or nitrate.

Experiments with other chromatographic systems, described elsewhere, did not give satisfactory results. The cetrimide - cyano phase system[8] was lacking in stability and the octylamine - Partisil 10 ODS-3 system, suitable for the determination of iodide in iodised salt,[7] gave poor separations.

Table 1. Results for the chromatography of $NaNO_2$ and KNO_3

Compound	Retention time/s	Capacity factor (k')	HETP/ μm	Detection limit/ng
$NaNO_2$	447	2.63	37	17
KNO_3	665	4.41	29	103

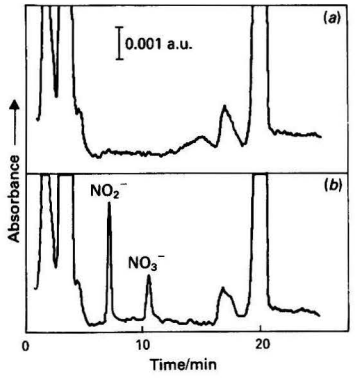

Fig. 1. Chromatogram of a sample of roast beef (*a*) before and (*b*) after spiking with 200 mg kg^{-1} of $NaNO_2$ and 500 mg kg^{-1} of KNO_3, respectively

Samples of ham, bacon, roast beef and luncheon meat were "spiked" with 200 mg kg^{-1} of $NaNO_2$ and 500 mg kg^{-1} of KNO_3 and the precision and accuracy of the described method were determined. The coefficient of variation, obtained under repeatability conditions,[13] appeared to be 4.1% for $NaNO_2$ and 4.5% for KNO_3, averaged over the various meat products. Recoveries of nitrite varied from 92 to 112% and of nitrate from 103 to 114%.

We conclude that reversed-phase ion-pair HPLC is suitable for application to the simultaneous determination of nitrite and nitrate in meat products.

Technical assistance from Mr. H. Gruppen, who performed some preliminary experiments, is gratefully acknowledged.

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COMMUNICATION

Material for publication as a Communication must be on an urgent matter and be of obvious scientific importance. Rapidity of publication is enhanced if diagrams are omitted, but tables and formulae can be included. Communications should not be simple claims for priority: this facility for rapid publication is intended for brief descriptions of work that has progressed to a stage at which it is likely to be valuable to workers faced with similar problems. A fuller paper may be offered subsequently, if justified by later work. Manuscripts are not subjected to the usual examination by referees and inclusion of a Communication is at the Editor's discretion

Enhanced Sensitivity in the Determination of Mercury by Inductively Coupled Plasma Atomic-emission Spectrometry

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Keywords: *Mercury determination; inductively coupled plasma; atomic-emission spectrometry; environmental analysis; pneumatic nebuliser*

At the present time the method most frequently used for the determination of trace amounts of mercury depends on atomic absorption in the cold vapour, by techniques based on that originally described by Hatch and Ott.[1] In this discrete-addition procedure, detection limits of about 1 μg l^{-1} are obtained, depending on the exact experimental conditions.

Following the introduction of inductively coupled plasma atomic-emission spectrometry (ICP-AES), it was shown that mercury could readily be determined by this method at a wavelength of 184.9 nm. The detection limit obtained, using a conventional pneumatic nebuliser for sample introduction, was 50 μg l^{-1}. This sensitivity is not high enough for many environmental applications and does not allow the simultaneous determination of mercury with other toxic metals. It was subsequently demonstrated that mercury could be reduced to the element and then transferred with high efficiency to the gas phase in a continuous-flow hydride generator coupled to an ICP-AES.[2] This method provided a detection limit of 0.1 μg l^{-1}, and has since been used in environmental analysis. However, only elements forming volatile hydrides can be determined simultaneously with mercury.

This paper reports a further method for enhancing the sensitivity of mercury determination by ICP-AES. Tin(II) chloride solution was added to the test solution containing Hg(II), and the mixture was passed immediately through a pneumatic nebuliser connected in the normal way to the plasma. The aerosol of the test solution was transported to the plasma with low efficiency (*i.e.*, less than 5%) while the remainder passed to waste, and a normal multi-element analysis was obtained. However, the violent agitation of the solution during its passage through the nebuliser and spray chamber transferred a large part of the elemental mercury into the gas phase, all of which passed into the plasma. This greatly increased transport efficiency for mercury allowed the element to be determined simultaneously with a number of other toxic elements.

Experimental

Instrumentation

The equipment used was a Bausch and Lomb (ARL) 34000 ICP-AES with lines for 36 elements including the Hg 184.9-nm line. Test solution was introduced by means of an unpumped concentric glass nebuliser (Meinhard Type TR-30-A3), with an uptake rate of 0.6 ml min^{-1}, discharging into a Scott-type double-pass spray chamber. Operational parameters were as used conventionally for aqueous solutions, *i.e.*, injector gas, 1 l min^{-1}; auxiliary gas, zero; coolant gas 12 l min^{-1} (all argon); viewing height, 14 mm above the load coil; forward power, 1250 W at 27 MHz; and integration period, 3 × 5 s after a stabilisation time of 60 s.

Reagent

Tin(II) chloride solution. Dissolve 10 g of AnalaR $SnCl_2.2H_2O$ in 100 ml of 1 M hydrochloric acid. Filter the solution and remove trace amounts of mercury by bubbling pure argon through it in a measuring cylinder until a negligible blank is obtained (about 15 min).

Procedure

Place 2.00 ml of test solution (*i.e.*, sample solution or mercury standard solution in 1 M hydrochloric acid) in a test-tube. Add 0.10 ml of tin(II) chloride solution and mix in gently with a glass rod. Immediately spray the mixture into the ICP-AES.

Results and Discussion

Calibration and Detection Limit

The effect of the addition of tin(II) chloride is shown in Fig. 1, where calibrations prepared from standard solutions of mercury, with and without the addition of the reducing agent, are compared. At concentrations below 1000 μg l^{-1}, the sensitivity was enhanced by a factor of about 15 by the addition of the tin(II) chloride. At higher concentrations the enhancement was progressively smaller, producing a marked curvature in the graph. This curvature must be attributed to a decrease in the transport efficiency of the elemental mercury at higher concentrations. The graph for mercury injected as an aerosol (*i.e.*, without reduction) is still linear in these ranges of instrument response (and therefore at equal concentrations of mercury in the injector gas). The instrumental detection limit was reduced from 50 μg l^{-1} in the unreduced solutions to 2 μg l^{-1} when tin(II) chloride was added.

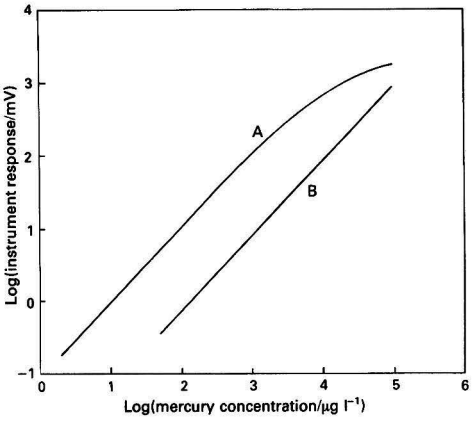

Fig. 1. ICP-AES calibrations for mercury, showing the enhancement in sensitivity produced by the addition to the test solution of tin(II) chloride. A, With 0.5% tin(II) chloride added; and B, normal nebulisation

Effect of Other Elements

Translational interferences (*i.e.*, owing to background enhancement or line overlap) on the Hg 184.9-nm line are small. Selectivity factors obtained by the conventional technique are as follows[3]: Mg, 2×10^{-5}; Ca, 1×10^{-5}; Al, 1.8×10^{-4}; Fe, 3×10^{-5}; Mn, 5×10^{-5}; and Ti, 5×10^{-5}. By employing the reduction technique, the selectivity factors are reduced further, because of the specific enhancement of the mercury sensitivity. Thus most translational interferences on mercury would be negligible in the analysis of environmental materials. The small effect of aluminium could be readily corrected by on-peak or off-peak methods, without degradation of the detection limit.

Interferences could also arise, in principle, from the effect of concomitant ions on the reduction of mercury. For instance, large concentrations of Fe(III) in the test solution could oxidise all of the Sn(II) and prevent the reduction of Hg(II). Because of this possibility the concentration of Sn(II) used in the proposed method was equivalent to more than five times the concentration of iron likely to be extracted from environmental samples (*e.g.*, soils and sediments). The effectiveness of this measure was confirmed by spiking with Hg(II) a variety of test solutions prepared from environmental samples and subjecting them to the given procedure. No low recoveries of mercury were observed. However, in tests with smaller concentrations of added Sn(II), [approximately equivalent to the Fe(III) present], considerably reduced responses for mercury were obtained.

It was also confirmed that the addition of the reducing agent had no effect on the determination of the other elements present in the test solution, which were injected by the normal transport of the aerosol. Thus the simultaneous determination of mercury with other toxic elements is a possibility.

Loss of Elemental Mercury from Solution

After the addition of tin(II) chloride, elemental mercury is rapidly lost from the test solution to the atmosphere. For instance, shaking the solution briefly in a test-tube transferred more than 95% of the mercury to the gas phase. Mercury is also lost from the still solution, although at a manageable rate. After 5 and 30 min standing, test solutions were found to have lost about 10 and 50%, respectively, of the mercury added. Thus it is necessary to mix the test solution and the Sn(II) solution with as little disturbance as possible, and to analyse the solution with the briefest subsequent delay.

Memory Effects

The response time of the nebuliser - spray chamber system was studied by recording the signal from the mercury channel with a fast chart recorder. It was found that both the signal stabilisation time and the clean-out time were increased by a factor of 2–3 when compared with the corresponding response times for elements transported as an aerosol. The prolonged memory effects were related to the time required for the liquid waste to be washed out of the spray chamber, rather than to the faster process of the aerosol being swept out by the injector gas. Transfer of the mercury from the aqueous into the gas phase was not at all quantitative and was still occurring as the waste liquid left the spray chamber. Because of this slow response time the pre-integration period was increased to 60 s from the normal 15 s.

Conclusions

By the addition of tin(II) chloride to the test solution, the sensitivity with which mercury could be determined by an ICP-AES equipped with a conventional nebuliser system was considerably increased. This allowed the determination of mercury simultaneously with a range of other toxic elements in environmental samples.

In the nebuliser system used, the transfer of mercury to the gas phase was incomplete. A spray chamber designed to maximise transfer of mercury into the gas phase and to speed up the drainage of the waste liquid would further increase the sensitivity, improve the precision and decrease memory effects.

Because of the rapid loss of elemental mercury from the reduced test solution, a flow injection type system for adding the tin(II) chloride continuously just before the nebuliser could be usefully deployed. This would both remove any tendency for loss of mercury to the atmosphere during mixing and also reduce the manipulations and liquid transfers in the analytical procedure.

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Paper A4/16
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Book Reviews

Computers in Analytical Chemistry
Philip G. Barker. *Pergamon Series in Analytical Chemistry, Volume* 6. Pp. xvi + 472. Pergamon. 1983. Price £37.50; $75. ISBN 0 08 024008 9.

The first three chapters of this book, totalling a little over 25% of its content, survey analytical chemistry. In the first chapter, "What is Analysis?," a fairly successful attempt is made to describe analytical science. This should be obligatory reading for all students of analytical chemistry as well as those chemists in other branches of the discipline who underestimate the professional skills of the analytical scientist.

In a survey of analytical techniques, wherever possible the author discusses the current use of computer technology and suggests ways in which it could be put to greater use. He includes thought-provoking sections on information handling, intelligent instruments, expert systems and robotics. When reviewing instrumentation the author summarises the essential principles and, wisely, refers readers to other sources for a comprehensive treatment. Once again emphasis is placed on the impact of computer technology, and how this has affected the design of instruments, GC - MS and a multi-channel continuous flow analyser being used as illustrations.

The description of computer architecture and operation is, of necessity, superficial but nevertheless gives a good over-all picture of the capabilities of micro-, mini- and mainframe computers. The mode of operation of a microprocessor is well illustrated by reference to the use of a National Semiconductor SC/MP and in the section on minicomputers considerable emphasis is put on the PDP/11 range. It is sad to note that in a listing of major mainframe manufacturers the author omits reference to any British companies.

The chapter on data collection is good on principles, especially the hazards involved in selecting data sampling rates, but less good on practice, as technology has advanced considerably since the book was written. The most recently dated reference in this chapter is 1980, which must represent work carried out at least a year earlier.

The chapter on interfaces and the principles of interfacing is a clear exposition of the types of interface standard and how they work. It contains down-to-earth advice on how to go about interfacing and, although the widely used RS-232C interface is treated rather briefly, a little more detail is supplied in the next chapter on communications, which ranges (perhaps too widely) from the use of couriers and the Post Office to satellite communication. It includes an interesting section on encryption.

The chapter on data-processing methods provides a useful overview with a large number of references for further reading. It treats both numeric and non-numeric methods and provides a brief introduction to chemometric techniques.

The final chapters on databases, information services and networks all serve as a useful introduction to information technology, but the serious reader will need access to more up-to-date sources in what is at present a very fast moving area of technology.

The author is Principal Lecturer in Computer Science at Teesside Polytechnic, but is also a chemist, writing for chemists. He has written a book, free from the unnecessary use of jargon, that serves as an excellent source for the analytical chemist who wishes to find out what computers can offer. Among its 835 literature references there is none to publications later than 1980, which means that its contents mostly predate the "microcomputer revolution." However, this seems to have resulted in a well balanced perspective.

The publishers state on the fly-leaf that the book has been reproduced from the author's original typescript for reasons of economy and speed. At £37.50 it is rather expensive for purchase by most individuals, and there is no evidence from the dates of the references that the publication process has been significantly accelerated. Although the book itself is well made, the quality of the reproduced typescript leaves a lot to be desired. One hopes that the author is producing a second edition that will incorporate technological advances in the subject, and that he is also adding a spelling - checking package to his word-processing program.

D. G. Porter

Analysis of Rubber and Rubber-like Polymers. Third Edition. W. C. Wake, B. K. Tidd and M. J. R. Loadman. Pp. xiv + 330. Applied Science Publishers. 1983. Price £36. ISBN 0 85334 215 6.

In the 14 years since the publication of the Second Edition of this treatise, the formulations of rubber compounds have, in general, become more complicated. Analysis techniques have had to keep up with this increasing complexity and many chemists now include a vast array of instrumental methods in their struggle to find out what was originally put into a rubber compound. Many papers and books are available on specific instruments or devoted to the analysis of individual raw materials.

This Third Edition of Wake's book brings together most of the analytical methods available today, including the instrumental techniques that were sadly lacking in the Second Edition. New chapters have been added that increase the scope of the work and include: "Sampling and Sample Preparation," which deals with the problem of composite samples, sample size and the analysis of the composition of localised portions; "Analysis of Extracts," which gives spot tests for many extractable materials but also includes UV and IR spectroscopy, TLC, NMR, HPLC and ion chromatography. "Instrumental Polymer Analysis" examines polymeric materials using IR, PGC, DTG, DSC, NMR and scanning electron microscopy (SEM). "Polymer Characterisation" deals with the determination of molar mass using end group analysis, osmometry, viscometry, light scattering, gel permeation chromatography and DSC. The microstructure of polymers is briefly discussed with reference to NMR, both proton and ^{13}C. A chapter on "Formulation Derivation and Calculation" discusses the determination of polymer content by pyrolysis and thermogravimetric analysis. Illustrations are given to show how the analytical data can be manipulated to provide the best estimate of the formulation actually used in the manufacture of the article.

Although there is now a large emphasis on the use of instrumentation there is obviously not enough room in one small volume to delve into detail on the use of the instruments. The book only gives examples of the type of results that can be obtained and how to use the information gained.

The use of non-instrumental methods, spot tests and burning tests, *etc.*, is not neglected, and much useful information can be had from the use of simple tests.

The book is well presented, very readable and packed with references to other publications and International Standards. It is a must for anyone who is involved in the analysis of rubber compounds, but I wish they had changed the title of the book. It involves much more than the analysis of rubber as such.

W. C. Thompson

Instrumentation for Environmental Monitoring. Volume 1, Radiation. Second Edition
Robert J. Budnitz, Anthony V. Nero, Donnie J. Murphy and Robert Craven. *Lawrence Berkeley Laboratory Environmental Instrumentation Survey.* Pp. xxii + 1130. Wiley-Interscience 1983. Price £142.50. ISBN 0 471 86880 9.

This book is almost too heavy to hold, as it weighs 2.2 kg. It is essentially a review of US experience in health physics involving ionising radiation, with a 500-page Appendix of radiation monitors on sale in January 1983. I cannot recommend it to workers outside the USA unless they are specialists in the field.

The main text, which covers sources, measurement and biological effects of ionising radiation, radiation hazards of the nuclear industry and the nuclides ^{3}H, ^{131}I, ^{85}Kr, ^{226}Ra, ^{222}Rn, ^{90}Sr together with plutonium, uranium and thorium falls between two stools. It treats the subjects, most of which have been discussed more fully in other works, in an encyclopaedic but rather uncritical manner. However, it rarely gives enough practical detail to be used in the laboratory or the field. For example, general methods for radiochemical separations of the elements listed above, together with Ac, Np, Pa and Po, are given in insufficient detail for direct practical use. ^{137}Cs is not treated at all, nor is much attention given to the problems of dissolving environmental materials prior to concentrating minute traces of radionuclides from them. The environment is an anthropocentric one, and effects of radiation on biota other than man are omitted; rather surprisingly, there is no discussion of instruments for whole-body monitoring. There are useful references to recent US literature, but very little to literature from other countries such as France, Japan, Scandinavia or the USSR.

The Appendix gives full technical descriptions of monitors for alpha, beta, gamma and X-rays as well as neutrons, culled from data sheets of about 50 US manufacturers plus two each from Britain and Canada. The information is not well organised from a user's point of view and deserves to be better indexed. Ideally such information should appear in annual buyers guides or on a computer tape, but here the buyer is being asked to pay nearly 13 pence a page for free advertising.

Minor faults include the use of many non-SI units such as Å, erg, curie, rad and rem along with modern units such as Bq, Gy and Sv. The title should include "ionising" before "radiation," as most environmental radiation is solar. Old-fashioned names such as Radium-B, Uranium-X and Thoron should be abandoned. Space could be saved by deleting numerous quotations from regulatory documents. Figs. 4.2 and 7.13 are identical, and Fig. 7.1 includes Fig. 4.1. Far too many acronyms are used, only about half of which are unravelled in Appendix C on pp. 1111–2. One is left floundering when trying to decipher such mysteries as ALI, BEIR, CTR, DAC, DOE, EPA, ERAMS, ICRU, PAEC and PMT. The standard of proof-reading is good for such a long book, but I found misprints on pp. 54, 383, 409, 459, 475, 477, 481, 501, 532 and 562. A useful feature is the summary after each chapter indicating whether current instrumentation is adequate or needs further development.

H. J. M. Bowen

ERRATA

The Forensic Analysis of Drugs of Abuse

Peter B. Baker and Geoffrey F. Phillips
Analyst, 1983, **108**, *777–807*

In our review[1] we were in error in stating (page 796) that the infrared spectra of the optically active pair of amphetamine mandelates described by Heagy[2] were mis-captioned. We had used a specimen described as D-mandelic acid, which is optically laevorotatory, and consequently obtained transposed spectra from those of Heagy who had used "*d*-mandelic acid," (which is dextrorotatory). Our error raises two points of emphasis.

(*i*) It is regrettable that optically active substances continue to be described by the terms *d* and *l*, particularly where the configuration based on the classical glyceraldehyde reference D or L structure may be of the opposite hand, *i.e.*, D-(*l*)- and L-(*d*)-, as with mandelic acid. The preferred absolute description would be *R*(−)- and *S*(+)- for *l*- and *d*-mandelic acid, respectively.

(*ii*) It also highlights the importance of using reference standards in (forensic) analysis. In this case the use[1] of configuration labelled mandelic acid enantiomer unexpectedly gave the opposite spectra compared with those previously published.[2] However, because authentic amphetamine enantiomer reference standard was simultaneously employed, the correct optical assignment was given to unknown amphetamine specimens.

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Studies with Immobilised Chemical Reagents Using a Flow Cell for the Development of Chemically Sensitive Fibre-optic Devices

Gordon F. Kirkbright, Ramaier Narayanaswamy and Neal A. Welti
Analyst, 1984, **109**, *15–17*

The second author's name should be as given above, and not Ramaies Narayanaswamy, as originally given.

The companies appearing on this page are able to offer scientific support to users of laboratory instrumentation. THE ANALYST will regularly publish specific Application Notes provided by their applications chemists.

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