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Spark-source Mass Spectrometry: Recent Developments and Applications

A Review

Jeffrey R. Bacon and Allan M. Ure
Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen, AB9 2QJ, UK

Summary of Contents

Keywords: *Review; spark-source mass spectrometry*

1. Introduction

About 50 years ago Dempster[1] introduced the idea of spark-source mass spectrometry (SSMS) as an analytical technique for elemental analysis and it is now some 25 years since the first commercial instruments became available. After a period of rapid development and application in the 1960s, activity has declined and the technique is now at a critical point in its lifespan. There are several reasons for this. Firstly, SSMS has generally suffered from the misconception that it was only a qualitative or, at the most, semi-quantitative technique. This probably arose because most of the original applications were in metals or materials research that needed quick-quantitative analyses. Further, the instrumentation is complex and expensive, and most commercial instruments in service are now becoming out-dated. There is virtually no active support from instrument manufacturers and at present there is only one instrument available commercially. It would appear that all development work is currently undertaken in users' laboratories.

That SSMS is still a very useful technique for certain applications is endorsed by the development work being undertaken and by the number of reported applications. The whole of the Periodic Table can be covered in one analysis

with little or no knowledge of the sample type or element concentrations. All elements exhibit relatively uniform sensitivity for all sample types with detection limits of 0.1 p.p.m. or less in the solid sample. About 25 mg of sample are required for analysis but this can be reduced by using tipped electrodes. Considerable efforts have been made to achieve quantitative analyses and, coupled with techniques such as isotope dilution, a precision of 5% can be attained. The combination of multi-element analysis with little, if any, sample pretreatment and the ability to achieve accuracy within an order of magnitude without calibration are particular strengths of the technique for certain applications. The major disadvantages of the technique are the generally poor precision, which in most applications cannot approach that of other techniques, long analysis time, complex instrumentation and expense. For the technique to survive and flourish it is essential that any major developments become available commercially and that simpler and cheaper instruments are produced.

This review is intended to cover all developments and applications reported since 1972. For earlier work the reader is directed to the book edited by Ahearn[2] published in 1972, while another recommended review of the principles and applications of SSMS is that by Deines.[3] A detailed review of mass spectrometry of solids up to 1970, including a bibliography, was given by Honig.[4]

Various general and more limited reviews have appeared, including some in less common languages. These include wider reviews of inorganic mass spectrometry and solids analysis,[5–8] over-all reviews of the SSMS technique and instrumentation[9–18] and, more specifically, the multi-element isotope dilution SSMS technique,[19] descriptions of "recent" developments and applications,[20–28] a discussion of systematic and statistical errors,[29] a theoretical discussion of plasma formation[30] and reviews of the application of SSMS for layer-by-layer analysis[31] and the analysis of metals,[32–34] geological samples,[35–37] coal and other fuels,[38,39] environmental samples,[40–43] biological materials,[44–45] forensic samples[46] and liquids.[47]

2. The Vacuum Spark Discharge

In the SSMS technique an electrical discharge is produced between two electrodes and in the process, material from one or both of the electrodes is atomised and ionised. Because this process of ion production shows approximately equal sensitivity for all elements, it is possible to use the technique for semi-quantitative analysis without regard to the actual changes in sensitivity that occur. For quantitative analysis, however, the ionisation processes need to be understood and controllable. One of the major factors in the poor precision of the technique is the irreproducible production of ions during the discharge. Considerable research has been made into understanding the fundamental processes involved and, although the mechanisms of ion formation are still not completely understood, real advances have allowed changes in instrumentation to be proposed.

There are two kinds of discharge commonly used—the pulsed r.f. spark and the triggered, low-voltage arc discharge. As most instruments use the former it follows that most research has been made into the r.f. spark source in which a pulsed 1-MHz r.f. voltage of up to 100 kV (peak to peak) is fed to the electrodes in vacuum. Various combinations of pulse length and repetition rate can be chosen.

There have been three different approaches to understanding the discharge—study of the voltage breakdown and plasma formation itself, study of the ion species formed during the discharge and study of the effects of spark parameters on ion production and sensitivity.

2.1 The Vacuum Spark Model

A detailed description of the physics of the electrical discharge is given by Franzen in Chapter 2 of reference 2. Since then, the research of Chupakhin and co-workers[48–58] has led to a model for the origin and development of the breakdown of the vacuum gap. Initially the breakdown was considered to consist of three distinct stages,[48,52] but later a fourth pre-breakdown stage in which little ionisation occurs was added.[58] The latter is considered to be relatively unimportant. The three principal stages can be summarised as follows.

1. Initiation of breakdown: from the appearance in vacuum of the current carrier to completion of formation of the discharge channel. The beams of auto-electrons emitted from points located on the cathode surface bombard the anode and discharge energy in the surface layer. The ion current increases irreversibly and a plasma cloud is formed in less than 10 ns.
2. Spark stage: a transitionary stage with a high-voltage discharge. The current increases at first and then decreases. The major ionisation process is electron bombardment of the anode.
3. Arc stage: a low-voltage discharge and quasi-stationary state. Here ionisation occurs by sputtering, *i.e.*, ion bombardment, of the cathode.

By modifying the spark circuit it has been found possible to cut off the spark discharge at any time and thus study the three principal stages independently.[48] In the first stage the population of multiply charged ions was high whereas that of multi-atomic ions (known also as polyatomic, cluster or molecular ions) was low. Compared with the later stages, relatively little of the electrodes was consumed. In the second stage the intensity of singly charged ions and multi-atomic species increased and electrode consumption was higher. Formation of ions in the final stage was determined by the properties of the element and condition of discharge, *i.e.*, it was variable and could affect the sensitivity. Consequently, it was suggested that if the ion current during the arc stage was gated out, the precision was improved, sensitivity increased and the intensity of multi-atomic species decreased.

The discharge model has also been considered in terms of the processes involved and the energy consumed.[53–55, 57] In this model the three stages were as follows: (a) atomisation, (b) ionisation and (c) plasma dispersal followed by ion detection.

(a) In the atomisation stage a weakly ionised plasma was formed and only 1% of the energy flux was used for atomising the sample.[56] There were two mechanisms for atomisation: firstly explosive, which was short and unselective, and secondly by evaporation, which was a longer process and was selective according to properties such as boiling-point and vapour pressure. In a short discharge of maximum power the explosive mechanism predominated, *i.e.*, all elements ionised with equal sensitivity.[53]

(b) During the ionisation stage, with an energy discharge of $1 \times 10^{-4} - 5 \times 10^{-3}$ J, material passing into the electrode gap was completely ionised.

(c) In the plasma dispersal stage intense recombination of ions occurred. This has been studied[55] using a computer model in which the degree of ionisation of particles escaping the plasma depended on their characteristic recombination path length. If this was greater than the initial plasma radius, all ions would escape before recombining. With a discharge energy in the range $10^{-4} - 10^{-3}$ J, all ions with three or more positive charges would recombine but doubly and singly charged ions would not. If after dispersal all the multiply charged ions were converted in this way to doubly and singly charged ions, the ratio of doubly charged to singly charged ions would be similar for all elements.

Atomisation and ionisation depend mainly on the power (W) liberated in a discharge and the duration of the discharge (t). These are not controllable in commercial instruments and

consequently the sensitivity is not equal for all elements. It is suggested[55] that a new spark source is needed in which it is possible to control W and t. With a short powerful discharge the explosive atomisation phase predominates and all elements are atomised, and consequently ionised, with equal sensitivity.

A similar model of the spark discharge has been proposed following the use of a time-resolved chopping device to study 5-μs periods of the spark discharge.[59, 60] In this the high-energy, high-temperature portion of the spark pulse showed the formation of a plasma of electrons and ions together with neutral atoms. In the first 1–5 μs this non-equilibrium plasma produced ions of all energy states, but higher energy states predominated. After a few microseconds the voltage and temperature dropped and a more stable, lower energy plasma existed, in which the singly charged ions predominated. Whereas Chupakhin *et al.*[48] recommended that this final arc stage should be gated out to achieve better reproducibility, Franklin and Dean[59,60] believed the spark stage should be gated out so as to accept only the more stable, lower energy arc stage (30–90 μs of a 100-μs pulse).

The latter strategy would effectively produce a triggered, low-voltage arc source as used in the Varian-MAT instrument. The advantages of the latter type of source were summarised by Radermacher and Beske[61] as a much lower energy distribution, a steady-state discharge with constant current, the production of ions of a higher ionisation state and a constant ratio of multiply to singly charged ions.

Demortier and co-workers[62–64] used X-ray spectroscopy of the hard X-rays produced in the r.f. spark discharge to study the processes of electron emission that gave rise to the ions. The spectra showed that the r.f. electron current was produced by field emission and was very low, the breakdown was initiated at sharp points on the electrode surface and actual breakdown involved thermoelectronic emission at high voltages (10–15 kV). This indicated that at the beginning of the breakdown highly multiply charged ions were produced at the electrode surface under the impact of energetic electrons.

Shelpakova *et al.*[65] have also demonstrated that admission of material to the plasma of the spark discharge is by an explosive mechanism in which atomisation of matrix and trace elements was equally probable, and that the predominant mode of ionisation was electron impact (*i.e.*, in the spark stage of the discharge). The dispersal of ions has been studied for various sparking procedures by fitting a detector on a ring placed around the electrodes at different positions within the source.[66] The material dispersed anisotropically and the distribution in space depended on the polarity of discharge and the impedance of the discharge circuit, but not on variable spark parameters such as pulse rate and length.

Muheim[67–71] has studied the ionisation processes involved in the spark to obtain information on electronic and lattice structure in the matrix. He concluded that "the intense r.f. spark electron bombardment on to the impact electrode probably gives rise to a positive space charge build-up, under the isotropic and instantaneous explosive action of which at least part of the atoms are being smoothly disassembled, preserving largely the microscopic electronic structure of the real solid."[67] The resulting gaseous spark plasma represented an almost perfect picture of the actual solid state. From measurement of the intensity of multiply charged ions[69] or multi-atomic ions[70] it was possible to derive information on electronic structures and valence. Similar studies have been reported by Joyes *et al.*[72]

2.2 Ion Formation

Three types of ion are produced during the spark discharge: multiply charged, singly charged and multi-atomic ions. Time-resolved studies have allowed the formation of the ion types to be timed. Use of beam-chopping devices showed that ion formation was not uniform throughout the pulse[50] and that the intensities of the multiply charged ions were at a maximum at 5–10 μs and then decreased to reach a minimum at 20 μs, whereas the singly charged ion intensities did not reach a maximum until 20–30 μs, after which ion production was relatively stable.[59,60] From measured and theoretical times of flight for ions produced by metal samples, it was shown that doubly charged ions and ions from volatile elements arrived at the detector about 250 μs before singly charged ions.[73,74] The evidence from the sparking of two dissimilar copper electrodes was that all these ions were produced in the vapour phase.

Measurement of the formation times for multi-atomic ions showed that multi-atomic carbon ions appeared about 300–500 ns after singly charged ions and that other multi-atomic ions also appeared at a later but not so well defined time.[27]

All of the preceding evidence, therefore, indicates that the first ions to be formed are multiply charged ions that form the singly charged ions at a later time. The multi-atomic ions are the last to appear. There is, however, conflicting evidence on whether the multi-atomic species are ejected as such from the electrodes or are formed by recombination processes in the vapour phase. All the earliest evidence suggested that they were ejected as molecules from the electrodes. The fragmentation patterns of metal carboxylate salt mixtures were studied and found to be consistent with rapid decomposition of polymeric radical ions with the same composition as the salt.[75,76] Recombination mechanisms, charge exchange and solid - solid reactions were considered to have minimum effect. The study of spectra produced by CaF_2 suggested that two groups of molecular ions were present, which represented two different mechanisms of formation.[77] One group was formed by evaporation from the electrodes and ionisation by high-energy particles whereas the other mechanism involved sputtering and/or thermal ionisation processes. In both mechanisms the multi-atomic species were released as such from the electrodes.

Most recent evidence has shown, however, that multi-atomic ions are formed in the vapour phase, predominantly by recombination processes. The simple fact that electrodes formed by mixing a metal oxide (MO) and carbon (C) will produce plentiful CO^+ but little MO^+ was stated by Stefani[78] as evidence for this. Analysis of alkali metal halides showed that M_2X^+ ions were formed by a gas-phase mechanism.[79] From the analysis of Al_xO_y species produced by alumina mixed with salts containing enriched ^{18}O, it was concluded that AlO and Al_2O, and probably other Al_xO_y species, were formed by single-atom reactions in the spark plasma.[80,81] Ramendik *et al.*[82] used electrodes made from erbium or its oxide to show that the dominant mechanism for the formation of multi-atomic ions was association.

Evidence from the analysis of metals is less definite, however, and the conclusions drawn are contradictory. Multi-atomic ions formed by copper - aluminium alloys showed abundant mixed Cu_xAl_y species that could only be formed by recombination, yet the authors[83] argued that large aggregates such as Al^{n+} could not be formed by the same mechanisms and suggested a mechanism in which multi-atomic ions are formed at an interface that separated the plasma and the solid crystalline phases. A complete rearrangement of atoms took place and the large aggregates could be formed and ejected. However, the analysis of copper - gold alloys led the same authors[84] to conclude that "plasma reactions are at most a minor cause of the appearance of polyatomic ions. Molecular ions can be produced by volatilisation of neutral complexes followed by the ionisation in plasma, or through sputtering processes."

It would appear from these studies that no single process can account for all the multi-atomic ions observed in spectra. The predominant mechanisms in most instances, however, will involve recombination processes, as many of the molecular species formed are chemically unstable and could not exist as such in the electrode material.

Various workers have studied the spectra of relatively pure materials to determine the ionic species which are formed and the relative abundance of each type. Metal samples studied include Cu - Al alloys,[83,85,86] Cu - Au alloys[84] and W metal.[87] In a study of AgBr crystals the ratios of Br_2^+, $AgBr^+$ and Ag_2^+ to Ag^+ were related to the mean positron lifetime.[88] A correction for the fraction of ions produced in the +2 and +3 ionisation states has been proposed.[89] In a study of the multi-atomic species formed in the analysis of CaF_2, two groups of multi-atomic ions were proposed, those whose intensity was related to the bulk composition and those which were not.[77] The ions produced in the spectra of various rare earth matrices have been studied and the author[90] remarked that "high-intensity molecular ions due to formation of almost any imaginable combination of individual atom with atoms of the pelleting medium are possible." The ratio of rare earth oxide to rare earth metal varied from 0.005 (for Eu and Tm) to 0.2 (for Y and La).

The existence of AlO_2 species, which is disputed on the evidence of other techniques, was shown in the analysis of alumina and the relative abundance of the Al_xO_y species were measured.[80] The multi-atomic species formed by Ga_2O_3 have also been studied.[91] The formation of the B_xC_y, and Si_xC_y species has been investigated by sparking electrodes of oxides mixed with graphite.[81] In the analysis of frozen drops of liquid samples the ratio MO/M was measured for various elements and those for the lanthanoid elements were in the range 0.5–1.[92] The analysis of SF_6 gas, using high-pressure SSMS with a quadrupole analyser, has shown the formation of a large number of multi-atomic species.[93,94] The intensities of multi-atomic ions[95] and multiply charged ions[96] have been measured for various elements to derive information on atomic structure.

It is generally assumed that multiply charged multi-atomic ions are either not formed or are negligible. The existence of these species has been demonstrated but their intensities in most analyses will be too weak to be significant. The existence of Si_3^{2+}, Si_4^{2+} and Si_6^{2+} was shown in the analysis of silicon samples[97] and the ratios of CaF^+ to CaF^{2+} [77] and ThO^+ to ThO^{2+} intensities[98] have been measured as 1000:1 and 3000:1, respectively.

The ion-beam profile for Cu^{n+} ions was relatively homogeneous in singly charged ions but became increasingly less so as n became greater.[99] Electrostatic repulsion forces resulted in beam expansion, the effect of which was greater for larger n. Other experiments on ion beam composition have shown the ion beam to be homogeneous over its full width, however.[100] The energy distribution curves have been measured for a number of elements in standard steels and were found to be similar for all elements with the single maximum shifting slightly to lower energy with increased m/z.[101] The width at half-maximum value was about 800 V for all elements.

In the counter probe technique a counter electrode of a pure conducting material is sparked against the sample electrode. The contribution of the counter electrode to the ion population has been measured and found to vary considerably according to the element used and the spark gap width.[102] In the frozen drop method the counter electrode contribution varied from 0.2% for W to 66% for Al.[103] No deterioration of absolute sensitivity was found as the width of the counter electrode was increased,[104] but a so-called "edge effect" resulted from material sputtered from the surface of the groove formed.[105]

2.3 Ion Production and Sensitivity

A number of investigations have been made into the effect of various source parameters on ion production. Increasing pulse voltage in the range 38–49 kV did not affect results in the analysis of frozen solutions[106] but caused transmission to decrease in the analysis of semiconductors.[107] The ion intensity of element lines was relatively independent of spark voltage, but the intensity of multi-atomic species decreased with increasing spark voltage, the optimum operating value of which was 40 kV.[108] Similarly, the intensity of multi-atomic species was found to decrease markedly with increasing spark breakdown voltage.[109] Changes in the discharge circuit parameters such as added capacitance have been found to change the relative populations of doubly charged ions and counter electrode ions considerably.[110,111]

As the distance of the electrodes from the slit increased so did the ratio of singly to doubly charged ions,[112] while the ion intensity decreased steeply for distances between 2 and 6 cm, then levelled out to the optimum position at 8 mm from the slit.[108] The relative ion populations changed significantly between 9 and 12 mm.[113] The effect of changing the electrode position in the other two planes was much less marked, however.[106,113]

It is generally considered that maintaining a constant spark gap is a critical factor in achieving good precision but, whereas the population of doubly charged ions was found to increase significantly with the spark gap,[114] the effect on the analytical results was considered to be small.[113] The shape of electrodes has also been considered for the counter probe technique and maximum transmission was obtained when the sample electrode was planar and the thin, pointed counter electrode was angled at 45° to it.[107] The production of CO^+, C^+ and O^+ ions was found to increase as the source pressure increased.[115] The ratio of singly to doubly charged ions was found to be dependent on the beam deflector setting but independent of element concentration.[112]

Some ions are formed not in the ion source but in the analyser section through a charge-transfer process, $M^{n+} \rightarrow M^{p+}$. Measurement of the shift in spectra of the position of these ions from their expected position gave evidence of a privileged location for the charge exchange and suggested that a collision-induced process is questionable.[116,117]

Relative sensitivitiy factors (RSFs), which are discussed in Section 3.10, are usually introduced in quantitative analysis to allow for the small but significant differences in elemental sensitivities from element to element and in different sample materials. In general they are determined from the analysis of standard materials in which the true element concentrations are known and RSFs can be considered as a correction factor, equal to (experimentally determined concentration)/(true concentration). Several workers have determined RSFs to study the effect of changing various source parameters.

From the measurement of RSFs in steel standards, Van Hoye and co-workers[118,119] concluded that changes in spark pulse repetition rate and length and accelerating voltage do not significantly affect the precision of analysis, and the same conclusion has been reached from studying the effects of photoplate emulsion sensitivity and electron multiplier sensitivity on RSFs.[101] In the analysis of aluminium and copper, however, the same authors found that RSFs did change with sparking conditions[120] and, in particular, elements with lower melting- and boiling-points than the internal standard showed increased sensitivity with more energetic sparking.[121]

Gross changes in RSFs between various matrices were attributed to non-homogeneity of samples and spark effects,[59] and RSFs for some elements are stated to vary with changing spark parameters.[122] More specifically, the spark parameters found to have the greatest effect on RSFs were spark gap,[123] spark gap and spark voltage,[124] spark gap and spark position relative to the exit slit,[125] the breakdown voltage and spark pulse frequency[126] and breakdown voltage.[65] This last parameter is only controllable in commercial instruments indirectly by changing the spark voltage and spark gap. Other uncontrollable parameters are discharge duration and power, which were also found to be critical in maintaining constant sensitivity.[55] In the analysis of silver halides, variations in

spark gap, spark voltage and electrode position affected the RSFs for Cl and Br but not that for I.[127]

It can be seen that changes in most spark parameters have been found to affect sensitivity in some samples and the only acceptable procedure is to maintain all parameters as constant as feasible during analysis.

2.4 Negative Ions

The formation of negative ions in the spark has been studied comprehensively by Kishi for a large number of elements.[128–131] Whereas negative ions were formed from the halogens and Group VIB elements, none were seen for the rare gases, alkaline earths, Zn, Cd, Hg, Sc, Ti, Mn, V, Ta, Re or the lanthanoid elements. Some negative multi-atomic ions were also observed. The negative ions observed in the spectra of SF_6 gas include F^-, F_2^-, SF_5^- and SF_6^-.[93]

Relative sensitivity factors have been determined for 16 negative ions.[132]

3. Analytical Procedures

The analysis of samples by SSMS involves a sequence of procedures: sample preparation, electrode preparation, sparking procedure, ion detection and calculation of results. Each of these steps has been studied in detail by various workers to optimise the operating procedures for different types of sample and to achieve easier, more precise and more accurate analysis. General descriptions of the principles and operation of the spark source mass spectrometer have been given,[133–136] but most procedures are concerned with a particular type of sample.

3.1 Specific Procedures

Metal samples are usually sparked directly and there have been no reported developments in the general procedures for metal analysis. A procedure for the single exposure analysis of ^{244}Cm and ^{252}Cf has been described in which small amounts of sample are mounted on gold electrodes.[137] Steel samples have been dissolved in acid, evaporated to dryness and mixed with pure graphite.[138] Procedures for the analysis of rare earth matrices have been described in full both for the metals and for their compounds.[90] Methods for the analysis of other pure oxide materials have been described.[139–141]

Whereas the analysis of metals is relatively simple and the procedures have become standardised, the analysis of non-conducting materials is more complex and generally requires the addition of a conducting powder to the sample. As a consequence, a number of different procedures have been developed. This is especially so for geological materials, to which the technique has been widely applied. A review of methods for geochemical and extraterrestrial samples has been given.[142] Comprehensive descriptions of procedures have been presented by a number of workers. In the methods of Taylor and Gorton[143,144] and Hintenberger[145] the sample was mixed with graphite as conducting material, and in that of Nicholls[13,122,146] the sample was fused to improve homogeneity and then mixed with graphite. The counter probe technique has been used by Chupakhin and co-workers,[147,148] with the probe electrode sparking against a thin disc of the sample, and by Ure and Bacon,[149,150] with an aluminium counter electrode sparking against an electrode pelletted from a mixture of the sample with aluminium powder as the conducting material. For the analysis of lunar samples graphite powder has been used.[151] A procedure has also been described for the analysis of geochemical materials using the low-voltage arc discharge.[152]

The analysis of biological materials is more difficult than that of geological samples in view of the problems of sample size, sample preparation, low concentration levels of trace elements and the higher concentrations of alkali and alkaline earth elements. A number of procedures for SSMS analysis of biological samples have been described, however, usually with special consideration of the sample preparation stage.[112,153–160] One procedure involved analysis of the lyophilised sample directly without ashing, but it was only applicable to the determination of the elements of high mass.[161]

Whereas most procedures described have used photoplate detection, electrical detection systems have also been employed for the analysis of non-conducting powdered samples.[162,163]

A review of the application of SSMS to environmental samples has been given by Cornides[164] and the use of a single spark method has been described for the analysis of thin conducting films.[165]

Gases have been analysed by SSMS. In one procedure, UF_6 was dissolved in water, which was then mixed with conducting powder and freeze-dried.[166] Arsine and monogermane gases, however, have been analysed by admitting them directly into the source chamber, where they were decomposed on thin electrically heated tungsten electrodes, which were subsequently sparked.[167]

3.2 Isotope Dilution Spark-source Mass Spectrometry

In this technique spikes of the elements to be determined but of different isotopic composition are added to the sample and from the altered isotope ratios the concentrations of elements can be calculated. It has the advantages of being very sensitive, precise, accurate and not dependent on quantitative chemical pre-treatment. Although thermal ionisation is more precise, SSMS can be used in this mode if the elements are involatile or have a high ionisation potential, and a multi-element analysis with a minimum pre-treatment is required. Reviews of isotope dilution SSMS (ID-SSMS) have considered both "wet" and "dry" techniques as well as extensions of the method.[19,39,168]

In the "wet" technique the sample and spiked isotopes are allowed to equilibrate in solution. Such a technique has been used with electrodeposition of a number of elements on to gold wire cathodes for the analysis of a wide range of sample types.[169–171] Procedures have also been described for sample solution equilibration with the spike solution followed by drying on to electrode powder.[172–174]

The "wet" technique has limitations imposed by the impurity of acids used, problems of losses during the drying phase and difficulties in complete dissolution of complex materials. A "dry" technique has been developed in which graphite powder is spiked with a solution of enriched isotopes, dried and mixed with the powdered sample.[175] Although no true equilibration between sample and spike isotopes occur, the technique is a compromise between the improved precision of ID-SSMS and the minimal pre-treatment required for direct SSMS. Similar procedures have been described by other workers for the analysis of geochemical samples.[173,176,177] The use of electrical detection systems is particularly suitable for ID-SSMS and one study showed peak switching to be the preferred approach.[178]

A double isotope dilution technique has been used to determine Cu in fish samples and sea water.[179] Copper enriched in ^{65}Cu was used as a tracer and copper enriched in ^{63}Cu as the SSMS spike. Sulphur has been determined in steels by dissolution, spiking with sulphur enriched in ^{34}S, precipitating the sulphur as $BaSO_4$ and mixing with graphite.[180]

A so-called isotope dilution method has been used for the direct analysis of gases in metals.[181,182] The sample was sparked against an electrode that contained certain elements enriched isotopically. If the sparking was controlled correctly, material from both electrodes was mixed in the plasma and isotopic equilibration was achieved.

An extension of the technique used elements enriched with stable isotopes as multiple internal standards.[183] This was intended particularly for monoisotopic elements that cannot be determined by ID-SSMS.

3.3 Sample Preparation

Any sample treatment required before analysis, especially of biological materials, is usually described as part of the over-all method, but a few studies have paid particular attention to this part of the procedure. Contamination is a problem in all trace element analyses, but especially so in the analysis of biological materials. Possible sources of contamination have been investigated[152] and ashing procedures discussed.[157,158,160] Oxidative acid digestion and high- and low-temperature dry ashing procedures are widely used, each having its own advantages and disadvantages. A procedure has been described for the acid digestion of powdered biological material in a digestion bomb followed by rotary evaporation on to graphite.[184] Methods for the homogenisation of powdered geological samples have been investigated[185,186] and sampling procedures for rare earth matrices, metallic and as oxide, have been described.[90]

3.4 Pre-concentration

Although a major advantage of SSMS is its ability to analyse samples with a minimum of pre-treatment, for certain applications it is necessary to pre-concentrate the analyte in the sample relative to the matrix, thus improving the detection limit. The consequent reduction in the concentration of the matrix elements minimises multi-atomic ion interferences produced by them. The matrix in which the elements are concentrated can often be made constant irrespective of the original sample matrix, and problems of sensitivity changes with matrix are consequently reduced. The homogenity of the new matrix can also be improved. The different techniques that have been used are illustrated by the following examples.

Evaporation

Fused silica was dissolved in HF and evaporated to give a concentration factor of about 150.[187]

Co-precipitation

Ammonia has been used to precipitate the lanthanoid elements from fused silicate materials[188] and to precipitate uranium from waters using iron as a carrier.[189] A number of elements were co-precipitated from soil extracts by quinolin-8-ol, tannic acid and thionalide (pH 2) using aluminium as carrier.[190] Selenium and tellurium were determined in copper standards by co-precipitation with gold (added as carrier) from solution by hypophosphorous acid.[191]

Co-crystallisation

Metals dissolved in geothermal water samples have been co-crystallised with 1-(2-pyridylazo)-2-naphthol (PAN) reagent.[192]

Ion-exchange chromatography

Cation-exchange chromatography has been developed for the separation of the lanthanoid elements as a group from rock matrices[193–195] but a comparison of this technique and mixed-solvent anion-exchange chromatography for the same purpose found the latter to be a preferable technique.[196,197]

Adsorption

Transition metals in solution have been chelated by quinolin-8-ol and subsequently adsorbed on to activated carbon.[198]

Cementation

In this technique a solution was passed through a small column of aluminium powder and a number of elements were collected by the method of spontaneous electrochemical displacement and deposition known as cementation.[190,199] This method is particularly suitable for the SSMS procedure[150] using aluminium powder electrodes as no further sample preparation other than drying and pelletting is required.

Electrodeposition

A number of elements can be electrodeposited on gold wire cathodes and the technique has been used with ID-SSMS.[170,171,200]

Solvent extraction

Elements can be extracted from iodide solution with 4-methylpentan-2-one,[201,202] back-extracted and evaporated on to alumina. Similarly, the platinum metals have been extracted with *N*,*N*-hexamethylene-*N*′-phenylthiourea and concentrated on CuO.[203] The platinum metals have also been concentrated using the fire assay technique and wet chemical methods, but no details were given.[204]

Disadvantages of pre-concentration and other pre-treatments are the added risk of contamination and the extended treatment time. This is compounded in some methods by the use of organic reagents, which thereby introduces an ashing step in the procedure. Losses resulting from digestion procedures or ashing can be minimised by using the isotope dilution technique, but this restricts the numbers of elements that can be determined.

3.5 Solutions

As SSMS requires a solid sample electrode, direct analysis of solutions is not possible. In the above pre-concentration procedures evaporation, coprecipitation, deposition and other methods are used to transfer elements from a liquid to a solid phase. Such methods can also be used for the SSMS analysis of solutions, whereby elements are concentrated in the process.

A brief review of methods for analysis of solutions has been given.[47] In the simplest procedure described the solution was dried on to the conducting material used for the analysis of acidic solutions of nuclear reactor fuels.[205] High-purity water and acids were analysed by evaporation to a small volume and transferring the drop to the end of silver or graphite electrodes, which were sparked against silver wire counter electrodes.[206] Metal chloride and nitrate solutions were analysed by applying them to the end faces of graphite electrodes that had been impregnated with a benzene solution of polythene to fix the sample in a thin surface film.[207]

An alternative procedure was to freeze the solution and spark against a counter electrode. This method has been investigated[106] and applied to the analysis of Antarctic ice.[92] For some samples it was necessary to deposit a thin gold film on the surface of the frozen sample to make it conducting.[208] Layers of semiconductor material have been analysed by removing with acid, freezing the solution and sparking against tantalum wire.[209,210] The freezing method has also been used for the analysis of high-purity tin, which was melted, frozen rapidly and sparked against tungsten wire.[103] Aluminosilicate catalyst has been analysed by dispersing in water, freezing, covering with a gold film and sparking against a gold counter electrode, but the sensitivity and the precision proved inferior to those obtained by analysing a pressed powder electrode.[211]

3.6 Electrode Preparation

The preparation of metallic samples is simple because, after cleaning, they can be sparked directly or, if in powder form, pressed into electrodes and sparked.

Procedures for non-conducting materials are more numerous and a review of such methods has been given.[212] For solid pieces an auxiliary electrode can be placed inside, around or close to the sample. In the analysis of magnesium oxide, gold wire was wound around the sample and sparked against a gold wire counter electrode[213]; pure quartz was analysed similarly with gold wire inserted into the sample[214] and monocrystals have been analysed by wrapping in purified aluminium foil.[89] Alternatively, the sample can be crushed or ground to a fine powder and analysed as for powdered samples, a procedure that was also used for quartz samples.[214] Boron fibres have been analysed by deposition on tungsten wire[215] and alumina, beryllia and boron have been analysed following vacuum deposition of copper or aluminium on the sample.[216]

In general there are two techniques for the analysis of non-conducting powders. In the first the sample is made conducting by mixing with a conducting powder and pressed into two electrodes, usually cylindrical, which are sparked together. The most commonly used conducting material is graphite, but aluminium, silver and gold powders have also found applications. The choice of conducting powder can be dictated by the application.

Several investigations have been made into the choice of conducting material. Gold powder was chosen because analysis was more sensitive than with graphite and drops of solution diffused deeper into the electrode,[217] because it presents fewer interference problems than graphite, aluminium or silver[197] and because the sample was not diluted atomically so much as with graphite.[218] In particular applications, such as the determination of silver as its sulphide, gold powder has also been used.[219] Silver wire was used as the cathode in a pre-concentration procedure using electrodeposition.[171,200,220] Silver powder was chosen instead of gold or graphite for the analysis of uranium hexafluoride because of impurities in the gold,[166] and was preferred to graphite because of faster and more sensitive analysis even though the silver gave problems of inhomogeneity.[221] Other examples of the use of silver powder include the analysis of calcium fluoride[77] and of *objets d'arts*.[222] In the analysis of cinnabar, gold was consumed too quickly, and detection limits and blanks were lower for graphite than for silver.[223] Similarly graphite was chosen instead of gold or silver for the analysis of fibre-optic glass because the analysis was easier and the cost lower.[224] It is for these reasons and the much higher purity of graphite that most workers use graphite as the conducting material, except in special situations in which interference effects and other considerations dictate otherwise. The addition of In and Re as internal standards to graphite by a slurry technique has been described.[225]

Other materials that have been used are copper,[226] which gives a cleaner spectrum than graphite under more energetic sparking conditions, and aluminium,[150] which is used in a hybrid technique with an aluminium wire counter electrode.

The second technique for non-conducting powdered materials is the counter probe technique, which can be used for analysing thin sample layers without addition of conducting materials and for localised micro-probe analysis. This technique has been developed by Chupakhin and co-workers,[51,211,227–230] who have used a number of different materials for the counter electrode including rhenium,[227] aluminium,[228,229] niobium[51] and tantalum.[51,211,230] The choice of counter electrode depends on the application and need not necessarily be that which gives the least contribution to the ions formed. In microprobe analysis, for example, the depth resolution was improved if less sample, as distinct from counter probe, material was consumed and for this application aluminium was used as this contributed more than the other 11 materials tested.[228] For most applications, however, the counter electrode consumption needs to be kept to a minimum and for this reason tantalum is widely used. The counter electrode should also be the cathode with a unipolar discharge to minimise its consumption.[126] Tantalum has also been used for the analysis of alumina, beryllia and glass,[214] of ionic crystals (in preference to tungsten)[231] and of semiconductor materials (in preference to aluminium).[110] For the analysis of high-purity tin by the frozen drop method, however, tungsten counter electrodes were chosen in preference to aluminium and tantalum.[103] Pure gold counter electrodes have been used as a probe for the localised analysis of vanadium metal[232] and silver counter electrodes for the analysis of high-purity water and acids deposited on silver or graphite powder.[206]

3.7 Sparking Procedures

From the investigations of the effect of changing spark and other conditions on sensitivity, ion production, precision and other parameters, it is possible to suggest optimum operating conditions. These conditions will depend largely on the type of sample being analysed and the purpose of the analysis. A fundamental principle must be that precision will be improved if all source conditions are held as constant as possible,[233] but the sensitivity will only be maximum under certain defined conditions.

Spark gap width

Several studies have found changes in sensitivity, some considerable, with changes in spark gap width.[114,123,125,132,234,235] A constant gap width is therefore desirable. At narrow gaps the ion energy distributions were broader[236] and the maximum shifted to higher energies,[132] but ion intensities were higher for wider gaps.[237] As the gap was increased the production of multiply charged ions and of multi-atomic ions increased.[63,84,132] The electrode temperature increased with increasing gap width and this could affect the analysis of volatile elements, especially mercury.[238] Generally, however, gap widths of 50 μm,[233] 40–50 μm[239] and 0.03–0.1 mm[56] have been recommended to improve precision, to reduce the counter electrode contribution and to optimise the discharge energy, respectively.

Electrode - slit distance

As the electrode - slit distance was increased the resolution increased,[99,132] the absolute intensities decreased,[74,99] the relative intensities or sensitivity changed[99,125,132,240] and the ion energy distribution became more asymmetric with the maximum shifting to a slightly higher value.[236] This parameter should therefore be kept constant, expecially if external standards are used, when both sample and reference electrodes must be sparked at the same distance from the slit.[112,156] Further, with an increased electrode - slit distance the consequent beam expansion resulted in a reduced number of multiply charged ions being sampled[99] and also the intensity of multi-atomic ions decreased.[85,132]

Electrode position

Although generally not thought to be an important parameter, it has been shown that off-axis sparking affected accuracy and so electrodes should be accurately aligned in front of the extraction slit.[114]

Electrode size and shape

These can affect the analytical sensitivity[125,241] and should be chosen so that masking of the slit is not significant.[114] Planar electrodes resulted in increased ion transmission.[107]

Electrode vibration

In addition to continually changing the spark gap, this also led to transfer of electrode material and the production of surface irregularities and should, if possible, be avoided.[114]

Extraction hole diameter

As this was reduced the beam intensity was also reduced.[74]

Spark duty cycle

Whereas some studies showed that pulse length and repetition frequency have little or no effect on analysis,[118,119,132,235,242] others showed an effect. As the duty cycle increased the sparking became more violent and hotter and could affect the intensities of some elements considerably,[122,237] especially for volatile elements[238] and those with boiling- and melting-points considerably lower than those of the matrix.[120] The pulse width and repetition frequency had no effect on the ion energy distribution.[236] These parameters should be kept constant during analysis and generally the recommended values are low[238] with, for example, a pulse length of 50 μs.[243] For metal analysis the precision was improved if the repetition frequency was fixed at 1000 or 3000 Hz,[242] and for rock analysis maximum sensitivity was obtained with a duty cycle of 3%.[241]

Spark voltage

There is evidence that this does not affect the analytical precision and sensitivity[118,242,243] except at values greater than 40 kV. It is suggested, therefore, that the optimum spark voltage should be the lowest that will maintain regular sparking.[243] However, a change in accuracy was found for oxygen in iron and steel[237] and definite changes in relative intensities have also been observed.[240] Multi-atomic ion intensities increased with increasing spark voltage.[85] The ion energy distribution changed,[236] but was dependent on whether the electrode at the accelerating voltage acted as anode or cathode, the energy spread being narrower in the former instance.[74] The transmission efficiency dropped with increased voltage.[107]

Breakdown voltage

This is a function of spark voltage, gap width, source pressure and electrode shape. For accurate analysis it should be kept constant within fairly narrow limits during all stages of a spark discharge.[244]

Discharge energy

If this is regulated by placing an additional capacitance in the electrode circuit,[230] the analytical precision is improved.[49,54] Optimum values for the discharge are a total energy flux of 10^8–10^9 W cm^{-2}, discharge duration 15–100 ns and inter-electrode distance 0.03–0.1 mm.[57]

Accelerating voltage

In the analysis of metals this was found to have no[119,242] or only a possible[118] effect on precision. For the AEI MS702 instrument the width of spectral peaks (at half-height) have a minimum at an accelerating voltage of 20.3 kV so a value of 20 kV is recommended.[223] Using the gold probe technique for analysis of high-purity compounds, an accelerating voltage of 24 kV consumed far less material than one of 16 kV, produced darker lines on the photoplate and produced a higher number of multiply charged ions.[245]

Source pressure

Pressures greater than 5×10^{-7} Torr have been found to affect the analysis of carbon, nitrogen and oxygen in metals.[239]

In the low-voltage discharge source, high currents and low graphite concentration resulted in transfer of material, which caused the spark discharge to become erratic and finally to be extinguished.[152]

In the analysis of thin layers by the counter probe technique, shallow craters are usually required. The best counter electrode to achieve this was aluminium[229] and the spark energy should be reduced by reducing gap width and modifying the spark circuit.[58] The depth of the layer removed increased with increasing breakdown voltage and surface roughness, but decreased with a wider counter electrode.[246] There is some disagreement over the ideal counter electrode dimensions, ranging from a point with zero thickness,[197] to 3.4–4.5 mm wide × 0.05–0.08 mm thick,[110] 3.5 mm wide[246] and up to 12 mm wide.[104] In a study of silicon, no clear relationship has been found between exposure and sparking area, thickness of layer removed and relative consumptions of sample and counter electrode.[247] It is clear that the choice of counter electrode shape is dictated by the lateral and depth resolution required.

Ion beam choppers improve the analytical precision by increasing the consumption of material for short exposures,[242] but can also be used to accept only ions produced during required parts of the spark discharge cycle. The arc discharge stage can be excluded by accepting only the initial part of the breakdown[50,51] or the arc stage alone may be accepted for 30–90 s of the discharge.[60] Both were claimed to increase the analytical precision.

To reduce the intensity of the multiply charged ions, the electrodes can be cooled.[87] For the analysis of thin layers of metal on cylindrical rods, the rods should be rotated during sparking to increase the layer volume available for consumption.[248]

3.8 Photoplate Evaluation

The photoplate is still the most widely used means of detection and some studies have been made to improve photoplate development. The most commonly used photoplate, Ilford Q2, has been compared with Kodak SWR and Ionomet JM plates.[249] The Ionomet and Kodak plates have considerably higher sensitivity than Ilford Q2 plates, but the Kodak plates have a reduced dynamic range. The Ilford Q2 plates have, however, a much lower background level and better line definition. The response of different types of ions has been studied.[250] Monatomic ions produce darker lines than multi-atomic ions of the same mass and energy, and for ions of the same mass the more compact ions produce darker images. The relationships between ion density, ion energy, ion mass and the line area have been investigated.[251]

The use of an internal developer gives more intense lines on the photoplate[103] and the background fog has been reduced by using a high-contrast phenidone developer[252] or a bleaching agent.[103] The latter was not entirely successful as the line intensities were weakened and the photoplate had less contrast. Modified development conditions have been described.[243]

The methods used for evaluating photoplates have been reviewed.[10,132,164,253,254] Three photoplate calibration methods have been compared in terms of reproducibility, speed and simplicity for manual processing.[255] The method of Kai and Miki[256] is the simplest and fastest but the precision is low, whereas the method of Hull[257] gives the highest precision but is tedious. A computer study of calibration methods[258] found the Churchill two-line method[259] and Mattauch and Ewald method[260] to be equally acceptable, but the latter was recommended.

New or modified photoplate calibration methods have been proposed by various workers. In the method of Taylor[261] isotope intensity ratios measured on one exposure were used to obtain an intensity *versus* density relationship using a Seidel function. The intensities at a chosen and fixed density reading were used to calculate element concentration. The calibration curve of Bouvy and Gauneau[214] used the equation

$$D = \{[\log 1 + aE(1 + abE)]/[1 + aE(1 + abE)10^{-c}]\}$$

where D is optical density, E is exposure and a, b and c are constants. Fergason and Young[262] used a mathematical procedure to calculate the number of ions that produced a particular line and included a background correction. In the analysis of thorium oxide, Childs[263] used a method in which the logarithm of peak area was plotted against the logarithm of

exposure for each charge state from Th^+ to Th^{7+}. Then the peak area equivalent to a 1 nCi exposure was plotted against ionisation potential for each state and three methods of element concentration determination were used, depending on the concentration level. The method used by Pearton[264] was basically the Schuy and Franzen expansion[265] of the Hull method, which introduced a grain distribution function. Calculated relative exposure levels, Q values, were introduced by Pilate and Adams[266] and their use and other factors in photoplate calibration have been discussed.[267]

A completely different approach, the so-called parabola method, has been developed by Radermacher and Beske for the low-voltage discharge source.[61,268–271] A plot of the logarithm of ion intensity against ionisation state was found to lie on a parabola that was specific for each element and was influenced by the matrix. From these a mean ion charge number (Zi) was calculated for each element and the matrix, and the aperture of each element parabola was altered to make the element Zi equal to that of the matrix Zi. The intensities of impurity lines were compared at the matrix Zi for concentration determination. This method requires the matrix spectral line intensities to be measured and so very short exposures must be taken.

The precision of photoplate densitometry and calibration has been studied[99,272] and found to improve if a correction for line width variation was made[233,273] or integrated line areas used.[272,274] Three different methods of measuring line area have been studied.[274] The evaluation of the same photoplate by eight different laboratories using well defined conditions showed a considerable range of analytical results.[275] There was no advantage in using the defined conditions in place of the laboratories' own methods. Calibration graphs have been found to change close to the matrix lines, but the reasons are not clear.[276] A study of the Ilford Q2 photoplate response to ion mass showed that response decreases proportionally to $M^{0.4}$ and not $M^{0.5}$ as is widely used.[273]

There is rarely any problem of the identification of elemental lines, but a method has been proposed that compares standard and sample spectra on the same plate.[277] Another method for assigning mass, used for the spectra of organic compounds, used the mass and position of calibration lines over small segments of the photoplate.[278]

3.9 Spectral Interferences

Before any line intensities can be used in concentration calculations they must be seen to be clear of superimposed interferences or corrected using other lines present in the spectrum. The problems of overlapping element lines are usually not great as all elements (except indium) have a unique isotope. The presence of multiply charged ions is seldom a hindrance for they often fall at fractions of masses and, in the spark source, are not so intense as the singly charged ions. The interfering species that give rise to most problems are the multi-atomic ions, which often, but not always, arise from the matrix element. Oxides, carbides and halides are often present and the combinations possible seem endless and are not restricted to normally stable chemical species. In the spectra of high-purity materials the interferences tend to be specific, on particular elements of interest, whereas in complex matrices the problems of interferences are much more widespread.

In many applications the occurrence of interferences is not discussed and this could account for some so-called matrix effects. One study claimed that "spectral interferences would be very slight or non-existent using mass spectrometry for the analysis of a complex mixture such as coal and coal ash."[279] However, the reports in which interferences have been identified or discussed are listed in Table 1 and these indicate that interferences do indeed exist for all types of matrices.

Table 1. Reports in which interferences have been identified and/or discussed

Matrix	References
General	13, 23, 122, 170, 274, 280–282
Metals	98, 103, 120, 135, 191, 283–287
Pure materials:	
Pure chemicals and crystals	77, 89, 108, 141, 166, 223, 288, 289
Semiconductors	115, 290–292
Rare earth matrices	90
Pure acids	293
Azides	294
Silicate matrices:	
Glasses	97, 113, 187, 214, 224, 295–297
Rocks	132, 143, 144, 149, 150, 174, 175, 185, 196, 197, 199, 241, 298–303
Coal	39, 160
Platinum metal ores	204
Environmental materials:	
Air particulates	164, 304
Water	305
Sewage sludge	288
Biological materials:	
General	9, 157
Plants	45, 158, 288
Animal tissue	45, 154, 158
Human tissue	159, 306, 307
Negative ions	130, 131

Although they are rarely a major problem and "spectral complications must not be over emphasised,"[102] they should never be ignored or underestimated, and chemical pre-concentration may be necessary to remove them.[150,187,190,199] There is a danger, however, that chemical pre-treatment may introduce new interferences, as in the dissolution of rock samples.[174]

The complexity of multi-atomic species formed is best illustrated by the study of complex materials such as rocks[150] and biological materials.[45,158,159,288] Combinations of calcium and aluminium with oxygen or carbon seem to form particularly easily and can spread beyond the high-mass elements.[143,144,288] The lanthanoid elements are of considerable interest, particularly in geochemical samples, and the interferences in this region have been well studied[90,143,144,149,175,196,197,294,298,300,302] and range from simple oxide and carbides to complex species such as $CaAlOC_{2n}$[143] and $BaC_xO_yN_z$.[294] Mathematical procedures have been proposed for correcting these interferences both on individual lanthanoid elements[143,175,298] and on the group as a whole.[149,302] A computer program has been used to identify multi-atomic species of the general formula $A_kB_lC_mD_n$, where A is the conducting matrix, B the cation, C the complex former (*e.g.*, Si) and D the anion.[282]

3.10 Sensitivity

For quantitative analysis the calculated results are usually corrected using sensitivity factors that are determined by the analysis of standards with the same or similar matrix as the sample and in which the element concentrations are known. Values for the correction factors have been presented by numerous workers and comparisons have been made between these studies. Such comparisons are difficult, however, as the definitions of the correction factors are not standardised and details of the exact means by which they have been calculated are often not reported. Given this, even the terminology is not consistent as two terms (relative sensitivity factor and relative sensitivity coefficent) are frequently used and appear at times to be interchangeable. The relative sensitivity factor (RSF) can be defined as $RSF = f_s f_t f_p$ where f_s, f_t and f_p are individual factors for the source, transmission and photoplate relative sensitivities,[308] *i.e.*, the RSF includes all the discrimination

effects involved, and if no allowance for these effects has been made in the calculations, the correction factor is the *RSF*.

The relative sensitivity coefficient (*RSC* or S_R) should be the term f_s only, *i.e.*, the relative ease of formation of ions, but is generally accepted to be f_s f_t, so that if photoplate, or detector, discrimination effects are included in the calculations, the correction factor is the *RSC*.[145] In some instances attempts have been made to allow for discrimination in ion formation before the calculation of *RSC*s.[150]

The basic formula for sensitivity factors as used by most workers is (measured concentration)/(true concentration),[99,309] but in a few instances the inverse of this relationship has been used.[158,205,296,310] When an internal standard is used in the calculation, the *RSF* or *RSC* is defined as $[(C_x/C_y)$ measured$/(C_x/C_y)$ true$]_z$, where C_x and C_y are the concentrations of the unknown element and internal standard, respectively, and z refers to the matrix.[118–120,221,308,309,311] Another equation used for the calculation of senstivity factors is

$$C_i = C_s \cdot \frac{E_s}{E_i} \cdot \frac{I_s}{I_i} \cdot \frac{A_i}{A_s} \cdot \frac{1}{RSC}$$

where C is concentration, E is the exposure needed for a defined blackening, I is the isotopic abundance, A is the relative atomic mass and the subscripts s and i refer to the internal standard and unknown element, respectively.[112,154] This equation has been modified in some studies by raising the mass term to the power 0.8 and making corrections for line width,[312] by leaving this term out of the relationship[139,298] or by replacing it with isotope intensity ratios.[313] Graphical methods have also been used to determine *RSF*s or *RSC*s.[86,264]

Reports that give calculated sensitivity coefficients are listed in Table 2 and include comprehensive studies of sensitivity, ranging from one element in one matrix to a large number of elements in several matrices. The matrix does not always refer to the type of sample being analysed, as it can be altered in the sample preparation stage as, for example, with water samples dried on to a graphite powder.

The variation of sensitivity factors with matrix is still a matter of debate and the evidence presented is contradictory. One major study concluded "that the *RSC* of an individual element does not change significantly from matrix to matrix,"[132,324] and this is supported by other studies.[122,309,336,347] In the analysis of the platinum group metals, the *RSF*s did not vary from one platinum metal to another.[326,328] Other studies, however, found that "for most elements relative sensitivity factors differ significantly with the type of matrix"[314] or that there were large changes with matrix.[59,139] It has been suggested that if all the charged states of the measured element are used then the *RSF*s would approach unity.[339]

The absolute sensitivities of the individual elements in any one matrix are different and attempts have been made to correlate these differences with physical and chemical properties of the elements. Whereas two studies have found no dependence of *RSC*s on the chemical forms of the element,[132,325] another found large differences dependent on whether the elements were in the oxide or fluoride form.[289] The chemical form could have an influence in certain samples, for example in the analysis of thorium in rock matrices, which, it was suggested, was dependent on the titanium content and the decomposition of $ThTiO_6$.[346] Most studies show that the *RSC*s are independent of element concentration,[118–120,207,221,286] but some changes in *RCS*s have been found at low concentrations.[320] The vaporisation terms that were best correlated with *RSC*s were the melting-point,[235] boiling-point,[143,311] vapour pressure,[55,308,320] heat of sublimation[139] and both melting-point and heat of sublimation.[316] Similarly, the correlation of *RCS*s with ionisation terms has been found to be best for ionisation potential[55,123,139,298] or ionisation cross-section,[65,272,308,312] whereas one study concluded that neither was satisfactory.[92]

Certain equations based on these physical properties have been proposed for calculating theoretical sensitivity factors and comparisons have been made of experimental and theoretical factors to determine the most satisfactory equation. Several workers[132,315,317,324] have found closest agreement with the equation of Goshgarian and Jensen[351]:

$$RSC_{x/y} = \left(\frac{CR_x}{CR_y}\right)^2 \left(\frac{\Delta H_y}{\Delta H_x}\right)\left(\frac{IP_y}{IP_x}\right)$$

where CR is the covalent radius, ΔH is the heat of sublimation at 298 K and IP is the first ionisation potential. Some agreement has also been found[132,315] with the equation of Socha and Masumoto[352]:

$$RSC_{x/y} = \left(\frac{IP_y}{IP_x}\right)^3 \left(\frac{\theta_y}{\theta_x}\right)^c$$

where θ is the temperature at which the vapour pressure is 10^{-8} Torr and c is an empirical constant. Other equations have been proposed by Billon[339]:

$$RSC_{x/y} = \left(\frac{CR_x}{CR_y}\right)^2 \frac{\Delta H_y}{\Delta H_x}\sqrt{\frac{M_y}{M_x}}$$

and by Itoh and Sata[87]:

$$RSC_{x/y} = \left(\frac{\Delta H_y}{\Delta H_x}\right)\left(\frac{IP_y}{IP_x}\right)$$

Table 2. Reported sensitivity coefficients

Matrix	References
Detailed studies	101, 118, 119, 132, 286, 308, 313–318
Metals	59, 65, 84, 86, 87, 101, 118–120, 123, 132, 138, 139, 152, 174, 196, 221, 226, 233, 235, 237, 264, 285, 286, 308, 310, 311, 314, 316, 317, 319–331
Pure materials:	
Graphite	123, 152, 194, 196, 205, 207, 221, 272, 314, 318, 332–335
Chemicals	55, 90, 127, 139, 188, 202, 217, 223, 288, 289, 308, 309, 312, 313, 315, 336–340
Semiconductors	115, 341–344
Glass	224, 296, 308
Frozen solutions	55, 92, 339
Rocks and related materials	17, 132, 143, 145, 148, 150, 188, 203, 298, 314, 324, 345–357
Biological materials	112, 114, 132, 153, 154, 158, 161, 307, 314, 324, 348, 349
Negative ions	129, 132, 350

4. Instrumentation

4.1 General

All the commercial SSMS instruments are now of dated design and only the JEOL instrument is still available. Consequently, a number of improvements in design have been suggested for improving the precision and ease of analysis and to implement improved analytical procedures. Descriptions have been given of a JEOL instrument that had been modified with an interchangeable Knudsen cell source,[353] of the Thomson - CSF TSN 212 instrument[10] and of the AEI MS702R instrument and the operating procedure for it.[135] Because of their age it is becoming necessary to upgrade the older instruments. An improved cold-trap filling system[334] and new solid-state power supplies[354,355] have been designed for the AEI MS7/702

instrument, and a complete upgrading of a CEC 21-110 instrument included new electronics, pumping systems, source unit and focusing system.[356] A totally different instrument using a time-of-flight mass analyser was designed for the analysis of micro-particles, but the instrument was only applicable to this specific purpose and had a very poor resolution.[357] Another specialised application was the high-pressure analysis of gases using a spark-source instrument combined with a quadrupole analyser.[93,244] There is a need for the development of new, simpler instruments or, as stated in one report, for "improved r.f. spark ion sources combined with relatively uncomplicated, rationally working and inexpensive analysers."[309]

4.2 Ion Source

The spark source unit has been redesigned to make it interchangeable with other types of ion source such as the Knudsen cell,[353] ion microprobe source,[358–360] hollow-cathode gas discharge source[361,362] and the laser source.[363] An AEI MS7 instrument has been redesigned with a low-voltage d.c. arc discharge source.[364]

For the specialised application to the analysis of radioactive materials the source unit must be redesigned for remote handling of samples by means of a glove-box.[365] For γ-emitting materials all loading of samples, sparking and even repairs must be carried out remotely with the aid of closed-circuit television.[366]

In some applications such as the determination of gases in semiconductor materials, the ion source pressure must be considerably reduced, most commonly by fitting a cryogenic pump using either liquid nitrogen[367] or liquid helium.[115,341,365,368,369] To remove hydrogen from the system it is necessary to retain the diffusion pump[341] or cover some of the cooling fins with activated carbon.[369] A cryosorption pump has been designed using activated charcoal cooled with liquid nitrogen and fitted with a heater to desorb the gases.[370] The combination of a mechanical cryopump and high-speed diffusion pump has been fitted to a modified source housing,[371,372] and stainless-steel grids were added at a later date to reduce the desorption of gases from the source walls.[373,374] A source unit has been modified to accommodate a 1200 l s^{-1} oil diffusion pump, cold-trap baffle and a gate valve.[375]

The time required for sample loading limits the possibility of reducing the analysis time. A probe-type sample changer has been used for the analysis of a large number of samples for one element, mercury.[376] An alternative approach has been to design multi-sample holders for two pairs of electrodes,[114,377] six samples[103] or up to twelve samples.[245]

A system for rotating vertically mounted cylindrical electrodes has been described for improving analytical precision.[378] Automatic scanning systems have been designed for the analysis of semiconductor surfaces using a counter electrode traversing over a rotating disc[379] and for layer-by-layer analysis using a probe scanning in two mutually perpendicular directions.[380]

Two methods have been used for the analysis of gases. In one the gas is heated and introduced into the spark gap through a small longitudinal hole drilled through one of the metal electrodes.[381,382] In the other method the source is filled with the gas under pressure and sparked between two tungsten electrodes.[93]

Various modifications have been made to the spark circuit electronics to improve sparking procedures. The output stages of the r.f. generator have been modified by the addition of a high-voltage kenotron and capacitor to make the discharge unipolar.[48,126,231,383–385] With the same aim, modifications have been described that allow only the portion of the beam that was formed with one electrode acting either as cathode or anode to be transmitted.[386,387] Using both systems the electrodes connected to the accelerating voltage should act as anode. The second system has been used with a spark circuit modified by the addition of resistors to give a self-triggered damped discharge.[73,388,389]

Other modifications to the spark circuit have been used to control the spark discharge. Cut-off capacitors in the discharge circuit reduce the duration of the spark discharge[148,230] and a new spark circuit discharge generator has been designed, although no details were given, to control the power and duration.[57] Ballast elements have been added to the circuit to reduce the discharge intensity and to decrease the sampling depth in layer analysis,[110,384] as well as to extend the lifetime of frozen drops.[209] Ballast capacitance has also been added to reduce the effects of stray capacitances.[111] An alternative modification provided single pulses for the analysis of thin films.[165] The energy distribution of the ion beam has been controlled with a new method of commutation of the electrodes,[390] for which no details were given, and by an ion-beam compression system.[391] Another device used to stabilise the matrix line intensities irrespective of spark gap width has been reported, but again without details.[111]

Ion-beam chopping devices have been designed to improve precision by consuming more material than is actually detected or by accepting the ion beam from only a part of the total discharge. The devices can be synchronous in which only a pre-selected time interval from each pulse is accepted[48,50,60,383,392,393] or asynchronous in which only a certain number of whole pulses are accepted.[392] Alternatively, the portions of the discharges accepted were totally random.[50,393]

Devices have been designed to control the spark gap width in order to improve precision. The AEI Autospark unit[394] has been modified to make it possible to select the mean electrode gap by controlling the r.f. voltage developed between the electrodes.[114,234,395] In a similar device a constant peak r.f. voltage was maintained.[396] Circuits have been given for measuring the breakdown voltage and current[385,397] and this breakdown voltage has been used to keep the spark gap constant.[109,244] The electrode position can also be controlled by keeping the ion-illumination angle constant. A device has been designed for keeping this and the gap width constant by intercepting the ion beam with two plates on either side of the beam and measuring the ratio of the signals.[398,399] A system for the self-vibration of electrodes used the attractive force experienced by the electrodes during the breakdown and the resilience of an elastic holder.[247]

4.3 Analyser

All the commercial instruments have a double-focusing analyser with Mattauch - Herzog geometry and such is the soundness of the design that there has been very little modification to the analyser section. Time-of-flight[93,400] and quadrupole[93] analysers have been used in special applications but the resolution was very poor. The quadrupole analyser has been investigated for SSMS and if the energy band width could be reduced the combination might be viable.[401,402]

New magnet power supplies have been designed for the AEI MS702.[243] Electrical detection requires accurate control of the magnet and systems have been described for the fitting of a Hall probe[403] and a temperature compensation network for a Hall probe.[404] The alignment of the ion beam on the optical axis[276] and determination of the position of the image plane[405] have been described. An internal shield of nickel-coated soft iron has been placed between the source and analyser to reduce the fringe magnetic field.[406]

Secondary electrons ejected from the ion collector by impinging ions cause non-reproducibility of the monitor reading and the ion collector design has been modified to reduce this.[407] A thin metallic shield has been fastened to the photoplate cassette in the position of the matrix lines to reduce background fogging caused by secondary emission, but with

the loss of some analysis lines.[103,408,409] The size of the α-slit has been reduced by welding thin strips of tantalum across the slit in order to increase the resolving power.[410]

4.4 Ion Detectors

Although still widely used, there has been very little development of the photographic plate detector. A simple method has been described to check that the plates lie in the focal plane.[411] A comparison of three detection systems found peak scanning to be the fastest and peak switching to be the most precise, but that photoplates still had the advantage of simultaneous detection.[218]

Most development work has been on electrical detection systems. The system developed for the JEOL instrument has been described[412] and used to determine the precision and accuracy of the method for the analysis of steels.[119] In the peak scanning mode precision was 18% whereas in the peak switching mode it was 10%. The peak scanning mode has been digitised to allow the data to be stored on magnetic tape and a signal averager used to sum the spectra.[413] One electrical detection system has been designed using peak scanning with a slow scanning speed.[321] Changes to the AEI electrical detection system included a new digital integrator system, a tapered collector slit, modified amplifier circuits and the use of magnetic peak switching with a Hall probe in place of electrostatic peak switching.[114] A multi-channel analyser has been used to record repetitive multiple scans in the scanning mode and to signal-average.[362,375,414] Peak switching systems have been designed with either electrostatic peak switching[240] or magnetic peak switching.[349] The latter is considered more precise but takes longer. A digital ratio circuit for the peak switching mode has been described.[396]

Automation of the electrical detection system has been described using an autoprogrammer that reads instructions from a paper tape[415]; the electrostatic peak switching mode was used. On-line computer-controlled electrical detection systems using both scanning and magnetic peak switching modes have been designed for the Nuclide[162,416] and JEOL instruments.[330] Automation of the AEI system in the peak scanning[417] and peak switching[418] modes has also been described. The computers control the mass spectrometer by setting the magnet current and electrostatic analyser voltage, and simultaneously acquire and reduce data.

A circuit has been described for the automatic attenuation of the signals to an electrometer so that very weak and very strong ion currents can be measured.[419] For the measurement of very small ion currents a detector has been used that monitors the rate at which charge accumulates across a calibrated capacitor.[420]

The major disadvantage of these electrical systems is that they are single-channel systems and the ion currents are measured sequentially and not simultaneously. A simultaneous ion beam collection has been described for isotope ratio work in which a Faraday collector with a slit was placed in the focal plane.[421] One isotope passed through the slit to a separate detector while the remaining isotopes fall on the Faraday collector. A multiple collector system, each movable relative to the others, has been described but no applications of the technique have been recorded.[422]

Attempts have been made to develop new electron - optical ion detector systems. In one system a channel electron multiplier array (CEMA) was used with three optical readout systems to assess their viability.[423–425] Twelve CEMAs would be required to replace a photographic plate and there would be considerable problems with the data readout. Resolution was poor and the response was non-linear with the high peak ion currents driving the CEMA into saturation. It was concluded that the CEMA was not suitable for a pulsed source and that the optical detectors were not sensitive enough.

An alternative concept used three channel plates in a chevron array and self-scanning silicon photodiode arrays.[426,427] Applications of this design have not been reported and there would be problems of cost and dynamic range.

The need for the development of new simultaneous electrical detection systems is still present and the possible approaches that have been suggested[28] are channel electron multiplier, charge-coupled devices, electrostatic build-up on insulated plates and Fourier transform mass spectrometry.

4.5 Data Processing

Manual processing of data for the photoplate is slow and tedious and much effort has been made to automate to various degrees the densitometry and calculation of results. This is highlighted by the number of computer-controlled systems (Table 3) that have been reported for the aquisition and subsequent processing of data from photoplates. An early detailed description of an on-line computer-controlled microdensitometer and data processing system also critically assessed methods of calibrating and reading photographic plates.[434] Different systems vary in their sophistication from small interactive microcomputers to the large totally automatic systems that only require initial setting up of the densitometer and the input of basic data. There are two basic design concepts that differ as to whether the computer is instructed which lines to measure or whether it detects all peaks and subsequently identifies them. The latter system needs much larger data storage. Other differences lie in the definition of peaks, the measurement of peak height, width or area, the photoplate calibration method used and the degree of automation of the densitometer. The trend is towards a two-step operation with a small computer used on-line for data acquisition and data reduction,and then a larger computer, either time-shared or off-line, used for data evaluation. Some systems use one dedicated computer for both steps. Such systems are, however, more expensive and often permit few operator decisions, and these disadvantages have dictated the design of a simpler system with no on-line computer.[439] The problems of interferences in spectra do not always seem to be considered but in one system the program identified interfering multi-atomic species.[282]

The modification required to automate a manually controlled densitometer have been described,[446,449] including in one report a system for maintaining the photographic plates in the focal plane.[449] Programs for the on-line computer control of a microphotometer have been given[451] and another system punches reduced data from photographic plates on to paper tapes.[452]

Simpler, generally earlier, systems have been described in which data from photographic plates are fed to the computer through the keyboard,[261] punched cards[453] or punched tapes.[20,435] In one system the densitometer is lined up manually on peaks to be measured and the digital output can be fed directly to the computer.[454] Desk-top computers[455] and programmable calculators with printers[456] have also been used for the data processing routines.

Systems have also been described for the processing of data from the electrical detection modes.[330,417,418,457,458]

5. Applications

The power of SSMS is demonstrated by the very wide range of sample types for which analyses have been reported. These are but the tip of the iceberg, for the number of unreported

Table 3. Reports giving details of computer systems for data acquisition and processing from photographic plates

References
20, 61, 264, 266, 268, 269, 274, 277, 282, 428–450

analyses will be much greater. The analyses range from the determination of the concentration of one element to a complete survey of the whole Periodic Table with detection limits given for those elements not detected.

5.1 Metals

The analysis of metals (Table 4) is generally for quality control, to check that products are up to specification, that purification methods are achieving their object and that the concentrations of certain elements are not above their critical level.

The procedures are usually straightforward as metal samples can be sparked directly without pre-treatment. Some metals, however, do require special methods if they are liquid, for example sodium,[488,489] or melt at a low temperature, for example tin.[103] In the latter instance the frozen drop technique can be used for analysis.[103,408,470] The main problem with metals is that internal standards cannot be introduced into the sample and usually a matrix isotope is used for this purpose. Ion implantation has been used to prepare standards.[329,461,511] One method of introducing an internal standard has been to spark a sample electrode with a reference electrode containing a known amount of internal standard and allowing the internal standard to distribute over the surface of both electrodes.[460]

5.2 Non-metallic High-purity Materials

This group of compounds (Table 5) is considered here to include non-metallic elements and simple compounds, a large number of which are semiconductor materials. As these materials are non-conducting the analysis often requires mixing of the sample with a conducting material such as graphite, the purity of which must also be determined. These analyses, as for those of metals, are generally made in order to ascertain whether impurity element concentrations are below the tolerated or specified levels.

Recent reviews on the analysis of semiconductor materials have underlined the importance of these impurity levels.[478,518] The electrical properties of 28 GaAs crystals manufactured by eight different companies by two techniques have been correlated with their trace element concentration.[524] A similar study related free electron or free hole concentrations to dopant concentrations in epitaxial layer and bulk crystal GaAs.[528]

The trace element concentrations are critical in materials that are used in other applications. Germanium single crystals are used as radiation detectors and the concentrations of Cu, Ni and O are critical.[517] Doped AgCl crystals are used as detectors of nuclear particles and the purity of synthetic crystals prepared by different methods has been determined.[108,537] Fused silica used in the manufacture of optical waveguide glasses[187] and semiconductor-grade silica used in the manufacture of solar cells[344,522] have been studied. Lead chalcogenide lasers have been applied to gas spectroscopy and pollution monitoring and their performance depends largely on the quality of the PbS substrate crystal.[546] Other examples of analysis for which quality control is important are monocrystals (*e.g.*, $PbMoO_4$[89]), oxide insulators (*e.g.*, MgO and Al_2O_3[213]) and pigments (*e.g.*, TiO_2[531]).

The materials analysed are not necessarily in the form of solids. Germanium and arsenic hydrides (gases) have been analysed following decomposition in the ion source on to tantalum wire electrodes.[167] Liquid samples can be analysed by conversion into solid compounds,[338] by freezing[208] or by evaporation on to metal wires[293,540] or powders.[203,206,544] The purity of waters and acids has been shown to be dependent on the materials from which beakers and other containers are manufactured.[206] The contamination that results from grinding graphite in different containers has also been investigated.[157]

Table 4. Reported analyses of metal samples

Metal	References
General	233, 459–461
Aluminium	10, 89, 120, 135, 287, 317, 329, 367, 461–465
Barium	466
Bismuth	408
Cadmium	408
Calcium	466
Cobalt	181
Copper	51, 58, 135, 178, 191, 201, 239, 243, 286, 310, 322, 330, 331, 377, 418, 462, 467, 468
Iron	118, 119, 181, 237, 239, 321, 469
Gallium	292, 470–472
Gold	89, 224, 239, 284, 295, 296, 473–477
Hafnium	478
Indium	292, 408, 479
Iridium	480
Lead	243, 248, 285, 292, 334, 481
Molybdenum	181, 370, 482, 483
Nickel	135, 191, 239, 484, 485
Niobium	478, 486
Platinum	284, 487
Ruthenium	135
Silver	224, 295, 327
Sodium	488, 489
Tantalum	453, 462, 478, 486
Thorium	98, 490
Tin	11, 103, 321, 487
Titanium	28, 181, 491
Tungsten	87, 371, 372
Uranium	292, 321, 474, 479, 492
Vanadium	232, 478, 493, 494
Yttrium	495
Zinc	292
Zirconium	276, 478, 496
Lanthanoids	90, 283, 495, 497–499
Transuranium elements	137, 217, 365, 366, 500–505
Alloys:	
Steels	32, 114, 125, 138, 173, 174, 182, 202, 219, 220, 237, 239, 243, 316, 320, 321, 349, 377, 482, 496, 506–508
Copper alloys	83, 85, 222, 239, 286, 418, 462, 482
Nickel alloys	320, 334, 509, 510
Zirconium alloys	396, 496, 511
Other alloys	22, 384, 487, 495, 499, 506

Table 5. Reported analyses of non-metallic high-purity materials

Material	References
Graphite, carbon	157, 224, 295, 296, 308, 512–514
Boron	215, 216, 515, 516
Semiconductor materials:	
Antimony	292
Arsenic	495
Germanium	10, 115, 290, 292, 341, 379, 380, 478, 541, 517–519
Silicon	104, 115, 209, 210, 290, 322, 341–344, 370, 379, 380, 391, 462, 478, 479, 520–522
Tellurium	384, 523
Gallium arsenide	104, 210, 246, 290, 291, 342, 368, 370, 379, 380, 463, 478, 518, 520, 524–528c
Others	115, 245, 290, 291, 369, 490, 528a, 528d
Simple compounds:	
Oxides	90, 91, 139–141, 187, 213, 216, 218, 226, 263, 296, 313, 337, 340, 487, 529–536
Halides	77, 88, 108, 127, 208, 231, 338, 537–541
Lanthanoid compounds	67, 90, 218, 226, 499, 510, 535, 539, 542, 543
Pure water and acids	202, 206, 293, 544
Others	10, 58, 89, 167, 294, 490, 545, 546

5.3 Other Manufactured Materials

This group (Table 6) is taken to include more complex manufactured materials not included in Sections 5.1 and 5.2, and also some specific applications that include samples from all three groups. Quality control is important in the manufacture of special glasses, such as those used in fibre optics.[187,296,549] Attenuation of the signal along the fibre is critically dependent on the concentration of certain trace elements. Such analyses have not always been successful,[549] however, probably owing to inhomogeneous distribution of the elements, and an alternative approach has been to use a pre-concentration procedure that gives a more homogeneous matrix and allows the determination of elements at the parts per billion level.[187] Some special glasses are treated with lanthanoid compounds whose concentration levels are important.[297]

Trace levels of certain elements cause discoloration in TiO_2 pigments and SSMS is an ideal technique for the analysis of such insoluble and refractory materials.[531] A number of facial cosmetics have been analysed and the elements Pb, As, Se and Cr were found to be above acceptable concentrations in some of them.[557] Catalysts used in a coal liquefaction process were found to be contaminated by trace elements associated with the coal.[558]

A brief review of the analysis of radioactive materials has been given.[562] Such materials, associated with nuclear reactors, include $^{233}UO_2$ used to make fuel,[140] acid solutions of irradiated uranium - plutonium fuel rods,[205,366] uranium and plutonium oxides,[530,536] insoluble residue left from fission dross (mainly silicon)[561] and waste sludges and supernatants from the processing of fission products.[559,560]

The analysis of glasses for forensic purposes has been discussed[46,113,555] and generally involves correlating two glass samples, one from the scene of the crime and one from the suspect. An over-all elemental analysis can differentiate glasses that are indistinguishable by physical methods. The types of glass studied include vehicle headlamp and auxiliary lamp glass,[548] window glass[550,552,553] and container glass.[554] Similar studies have been made of bullet lead[285,481] and copper wires.[468] Human liver samples have been analysed to assess the feasibility of using such analyses in forensic toxicology.[307]

Archaeological samples have been analysed to identify their origin, but such analyses have achieved only limited success. A number of pigments used in Indian paintings have been identified,[222] but it was not possible to distinguish between the red and grey shards used in Iranian ceramics.[556] Analysis of copper artifacts from North America showed differences in samples from the same site and so was of little use,[467] whereas the analysis of Peruvian copper artifacts showed changes in manufacturing methods in different eras.[310]

5.4 Environmental Materials

With the growing concern for the quality of the environment there has arisen a need for multi-element analysis of environmental samples ranging from air particulates and waters to sewage sludges. Analyses that have used SSMS are listed in Table 7.

A general scheme for the assessment of environmental samples, especially in relation to pollution, included SSMS analysis for 73 elements.[563,564] Air pollution analyses have been made of urban air samples,[23,188,327,565,566] laboratory and external air[157] and of samples taken within or in the vicinity of various industrial plants (see Table 7). Fly ashes are generally produced by coal-fired generating stations and by municipal incinerators.[573] The analyses of respirable dust and coal dust of respirable size were particularly comprehensive.[574] Airborne samples were usually collected on filters made of nitrocellulose[23,565] (which requires ashing), silver membrane[397] (which can be sparked directly), glass-fibre[304] or millipore filters.[160,578]

Wastes studied include the bottom ash from coal burning plants[567,573] and from refuse incinerators,[573] chars and tars from coal conversion plants,[579] sludges from nuclear power plants[559,560] and sewage sludges.[190,288,580] Industrial effluents from condensers, scrubbers and chillers and liquid wastes from processing plants[577,584,597] have been studied. Most water samples have been analysed by evaporation on to the electrode powder, which is usually graphite, but freeze-drying,[587] filtration on to Ag powder,[582] cementation on to Al powder,[199] collection on activated charcoal,[198] electrochemical deposition,[171] ion exchange[594] and solvent extraction[192,594] have all been used.

5.5 Fuels

The analysis of fuels (Table 8) has usually been for the purpose of monitoring possible pollution effects arising from the use of such fuels. Coal samples are usually ashed before analysis, but it has been shown that it is possible to obtain a comprehensive analysis with unashed samples.[574,598,600] Petrol could be a major source of environmental Pb, S, Cr, Ni, Cd, Mn and V.[605]

Table 6. Reported analysis of other materials and specific applications

Material	References
Glass	46, 113, 187, 188, 216, 224, 295–297, 487, 534, 547–555
Ceramics	337, 529, 556
Pigments	222, 531
Cosmetics	557
Catalysts	211, 558
Applications:	
Radioactive materials	140, 205, 217, 365, 366, 490, 530, 533, 536, 559–562
Forensic samples	46, 113, 285, 307, 468, 481, 548, 550, 552–555
Archaeological samples	222, 310, 467, 556

Table 7. Reported analyses of environmental materials

Material	References
General	563, 564
Air particulates:	
Fresh air	23, 157, 164, 188, 304, 327, 565, 566
Fly ash	22, 171, 188, 279, 567–573
Coal mine dust	22, 574, 575
Other industrial plants	22, 160, 171, 347, 571, 576–579
Wastes and sludges	190, 288, 559, 560, 567, 573, 579–581
Waters:	
General	189, 196, 582, 583
Purified waters	175, 333, 335
Tap/intake waters	157, 198, 199, 333, 573, 584
Lake and river waters	23, 198, 199, 305, 332, 333, 577, 585–590
Geothermal waters	192, 586, 590–592
Sea waters	544, 593–597
Industrial effluents	171, 573, 577, 579, 581, 584, 596, 597

Table 8. Reported analyses of fuels

Material	References
Coal	22, 160, 170, 171, 183, 279, 558, 566, 567, 570, 571, 573–576, 579, 598–604
Fuel oil	567, 570
Petrol	172, 335, 570, 605
Refuse	573
Nuclear fuels	140, 205, 606

5.6 Geological and Related Materials

This is the largest group of complex matrices that have been analysed by SSMS (Table 9). For geochemical research the technique has proved itself valuable for giving over-all multi-element analyses and is probably the only technique that can determine the whole lanthanoid group, at trace levels, simultaneously.

Such analyses allow conclusions to be made on the history of samples and consequently on the formation of the geosphere. Lanthanoid elements are immobile in most metamorphic conditions but mobility has been associated with uranium mineralisation.[634] There was little relative fractionation of the lanthanoid elements during the sedimentary process but the lanthanoid element pattern changed from Archean to post-Archean rocks.[303,636] Sharp increases in thorium and uranium were also associated with the Archean - Proterozoic boundary.[637] The analysis of lanthanoid elements has been used to discuss the origin of plutonic rocks from Nova Scotia.[614] Investigations into the origin of volcanic lavas and rocks have, in a number of instances, used SSMS analyses for lanthanoids and other trace elements.[617,618,629–633,640–644] Samples from the Oklo fossil reactor have been analysed to study migration of uranium fission products.[622–625]

Considering the large number of analyses of rock samples by SSMS it is perhaps surprising that relatively few analyses of similar soil samples have been reported. Whereas rocks are of geochemical interest, soils are also the first link in the food chain. Trace element deficiencies occurring in plants or animals can be correlated with low soil contents or low availability to plants and toxicity effects can occur where soil trace element contents are high. There has been only one comprehensive study of trace element contents in soil using SSMS,[150,199,656] but the technique has also been used to determine the lanthanoid contents of soils.[613,654] The analysis of soil extracts indicates the availability of some trace elements to plants.[150,190] Soil extracts have also been analysed to detect the presence of bromine- and fluorine-containing herbicides.[657]

In the analysis of meteorites, the wide element coverage of SSMS is particularly useful and a large number of samples have been analysed, either comprehensively or for a number of trace elements. Similarly, materials returned to earth by the Luna 16 and 20 and Apollo 11, 12, 14, 15 and 16 moon landing missions have been analysed. Comprehensive analyses can be made with the relatively small amount of material available. Analysis of Apollo 11 samples showed that organogenic elements are present in small amounts[677] and that the trace element composition was different from that of Apollo 12 samples.[679] The results for Apollo 12 samples suggested that the moon interior was heterogeneous on a small scale, that lunar material was heated to high temperatures before accretion and that tektites were not of lunar origin.[681]

5.7 Biological Materials

Of all the groups of materials, perhaps the greatest analytical challenge is given by samples of biological origin (Table 10). The matrices can be complex and widely varying from sample to sample. The relatively high levels of alkali and alkaline earth metals, halogens and phosphorus give rise to a large number of complex interfering species that can make analysis difficult. The concentrations of most trace elements are much lower than in, for example, rock matrices and, coupled with the necessity for ashing and possible losses, are much more sensitive to problems of blank levels and contamination. The problems of contamination[157] and sample preparation[688] have been discussed in detail.

The analyses of plant materials range from the determination of one element, for example boron, in radish leaves by isotope dilution,[699] to comprehensive analyses, for example of normal and deficient wheat.[697] Lanthanoid element contents have been determined in water lily,[658] lichens and mosses,[153,694] clubmoss,[693] horsetail,[692] dwarf shrubs and trees[694] and ferns[654] and compared with those for the soil or rocks on which the plants grew. The contents of other trace elements in lichens and mosses[348] and spruce samples[690] have also been reported. Other plants analysed include rice,[451,698] apple[691] and pear[696] samples. Usually photoplate detection has been used for the analysis of biological samples but electrical detection in the scanning mode has been used for some plant analyses.[692,693]

The analyses of marine samples have generally been for the detection of water pollution and include the determination of mercury in fish meal[700] and copper in plaice.[179] Multi-element analyses have been reported for mussel,[23,701] oyster[544] and trout[158] samples.

Mammalian samples analysed include liver,[108,144,158,170] kidney,[158] whole blood,[702] serum[108] and urine.[108] A detailed study has been made of the yttrium and lanthanoid element contents of various organs from rats fed on different diets.[154] Heavy metals can in some instances be determined in unashed samples by using a high sparking voltage to minimise the production of organic molecular ions above *m/z* 192.[108] The accumulation of Cd, Pb and Zn by earthworms has also been studied.[655]

Analyses of human materials are numerous and most internal organs and body fluids have been analysed. A particularly comprehensive survey by Hamilton *et al.*[688] gives contents for 28–53 elements in 10 different organs and blood. Most analyses attempted to correlate trace element contents with specific diseases. In a study on alcoholism the yttrium and lanthanoid element contents were determined in a large number of various internal organs from alcoholics and non-alcoholics.[154] Grossly contaminated miner's lung has been compared with normal tissue[22,160] and hilar lymph nodes have also been analysed in a large survey.[159] Patients with advanced carcinoma were found to have increased copper

Table 9. Reported analyses of geological and related materials

Material	References
Terrestrial:	
Standard rocks	18, 50, 122, 143, 144, 148, 150, 152, 163, 173, 177, 178, 188, 193–197, 199, 212, 299, 302, 303, 330, 346, 433, 607–616
Rock samples	18, 163, 203, 230, 300, 345, 346, 604, 608, 611, 614, 616–644
Minerals	57, 77, 160, 204, 212, 214, 223, 226, 230, 289, 310, 346, 358, 359, 477, 604, 610, 619, 626, 627, 629, 638, 645–653
Soils	149, 150, 188, 190, 199, 302, 613, 654–656
Soil extracts	150, 190, 657
Lake and river sediments	23, 585, 658
Extra-terrestrial:	
Meteorites	23, 142, 144, 145, 173, 174, 176, 185, 186, 241, 299, 659–676
Lunar materials	57, 132, 147, 148, 151, 185, 241, 261, 299, 473, 661, 677–687

Table 10. Reported analyses of biological materials

Material	References
General	45, 157, 158, 688, 689
Plants	153, 158, 169, 170, 184, 288, 348, 377, 451, 654, 658, 690–699
Marine	23, 158, 179, 544, 700, 701
Animal	108, 114, 154, 158, 170, 655, 702
Human	22, 23, 112, 154–156, 159, 160, 306, 307, 314, 688, 703–711
Other samples	160, 183, 712–715

content and lower zinc content in their blood serum.[23] Skin samples have been analysed[709,710] but no significant difference could be detected between mycetoma grains and normal dermis.[710] Hair and fingernail samples have been analysed for forensic applications[112,155,156,704,705] and accumulations of Hg, As and Pb in hair and As in fingernails have been found.[704] Similarly, the use of liver analysis for forensic toxicology has been investigated.[307] A large number of dental enamel samples from 17 American States have been analysed to establish the normal range for trace element concentrations.[706] The presence of sulphur in the biochemical Slow Reacting Substance was first indicated by SSMS.[711]

Other samples analysed include diet[160] and food samples,[187] raw and refined sugars,[712,713] honey[714] and the enzyme *Subtilisin carlsberg*.[715]

5.8 Miscellaneous Materials

Other analyses have been of wear-metals in lubricating oils,[23] polyheavy water,[716] azide explosives,[294] some polymers and plastics,[230,717] bromine- and fluorine-containing herbicides extracted from soil[657] and Trizma base used in subtilisin enzyme assay.[715]

Gaseous materials analysed by SSMS include uranium hexafluoride[166,381,718] and silicon hexafluoride.[93,94]

Although principally an elemental analytical technique, SSMS has been used to yield structural information from the fragmentation pattern of non-volatile metal carboxylates.[75,76]

5.9 Standard Reference Materials

A large number of certified reference materials (CRMs) have been analysed using SSMS and the list of reported analyses (Table 11) contains the more common ones for which analyses have been reported by two or more SSMS laboratories.

Table 11. Reported analyses of selected standard reference materials

Material	References
USGS:	
BCR-1	18, 143, 144, 148, 149, 152, 194, 195, 197, 212, 241, 299, 302, 330, 610–615, 628
AGV-1	148, 150, 194, 195, 212, 616
W-1	122, 145, 148, 152, 163, 173, 176, 193, 194, 212, 433, 608, 611, 615, 672
G-1	145, 163, 212, 616
G-2	150, 152, 188, 194, 195, 199, 212, 302, 608
GSP-1	188, 194, 195, 212, 612
PCC-1	194, 195, 212
Japanese:	
JB-1	196, 197, 612, 616
JG-1	196, 199, 612
NIM:	
D	609, 615
G	194–196, 609, 615
L	609, 615
N	196, 609, 615
P	609, 615
S	609, 615
NBS:	
SRM 1632 (coal)	170, 171, 183, 279, 566, 571, 602
SRM 1633 (fly ash)	171, 188, 279, 566, 571
SRM 1571 (orchard leaves)	158, 169, 183, 184, 332, 377, 658, 695
SRM 1577 (bovine liver)	158, 170
SRM 444 (stainless steel)	173, 377
SRM 461 (low-alloy steel)	243, 349, 506
SRM 685w (gold)	239, 284
SRM 1213 (zircalloy)	276, 396

6. Concluding Remarks

From the number of reported analyses that have been listed in this review and from the fact that some laboratories are investing in the updating of power supplies, vacuum systems, detectors, etc., it is evident that the technique is still attractive for certain applications. This is especially so when a multi-element survey analysis is required for which the accuracy of the technique is sufficient (generally 15–30% with calibration or within an order of magnitude without).

If SSMS is to be used for analysis, its cost and long analysis time have to be justified. Comparisons of the technique with various other instrumental techniques have been made[18,309,491,521,566,592,595,689,719–722] for the whole range of sample types. Factors considered include the number of detectable elements, whether analysis is simultaneous, detection limits, accuracy, precision, analysis time, matrix effects, charge-up and field problems, resolution (depth and lateral), beam-induced chemical changes and on-stream capability. The general conclusion can be summarised by those given in a review of twelve instrumental techniques used for water analyses[721]; SSMS is the most specific and the most comprehensive multi-element technique but the accuracy, about 30%, is very poor. Similarly, in the analysis of sea water SSMS had the best sensitivity and provided multi-element data, but was also expensive and slow.[595] SSMS is considered to be a "sensitive survey method" for the analysis of surfaces and thin films.[521]

The strengths of SSMS are its multi-element nature, reasonably uniform sensitivity and its high absolute sensitivity. Its detection limit has been compared with those for a number of other techniques.[9,28,45,134,199,544,580,723] In a comparison of nine techniques, SSMS detects the greatest number of elements to the lowest detection limit,[9] and for solid samples it gives the lowest detection limit.[28,199] It is superior to neutron activation analysis in that the detection limits by SSMS are constant for all elements.[45,544]

Two other types of ion source have been found to have the same advantages as the spark source but fewer of the disadvantages. These are the laser source[724–726] and the hollow-cathode ion source,[361,482] but neither has yet found wide applications. Recent commercial developments have included the coupling of an inductively coupled plasma source with a quadrupole mass spectrometer for the rapid analysis of solutions[727] and the use of a d.c. plasma discharge source for the analysis of solid materials.[728]

The expense of and limited demand for SSMS instruments will probably preclude the development of a new generation of instruments, and even simpler instruments would still not be cheap. It is more likely that users of SSMS instruments will modify and update present instruments to improve performance. Probably fewer than 200 instruments have been produced and the number still in use is decreasing each year. The technique is at the point where it will fall into decline unless instrumental developments become significant enough for increased use to be considered.

7. References

1. Dempster, A. J., *Proc. Am. Philos. Soc.*, 1935, **75**, 755.
2. Ahearn, A. J., *Editor*, "Trace Analysis by Mass Spectrometry," Academic Press, New York, 1972.
3. Deines, P., *Pa. State Univ. Coll. Earth Miner. Sci. Exp. Sta. Circ.*, No. 78, 1970.
4. Honig, R. E., *in* Ogata, K., and Hayakawa, T., *Editors*, "Recent Developments in Mass Spectroscopy," University Park Press, Baltimore, 1970, pp. 116–149.
5. Elser, R. C., *in* Winefordner, J. D., *Editor*, "Trace Analysis: Spectroscopic Methods for Elements," Wiley, New York, 1976, pp. 383–417.
6. Liebl, H., *Mikrochim. Acta*, 1978, **1**, 241.
7. Demortier, G., *Rev. Quest. Sci.*, 1979, **150**, 509.

8. Stefani, R., *Adv. Mass Spectrom.*, 1978, **7A**, 729.
9. Morrison, G. H., *in* Ogata, K., and Hayakawa, T., *Editors*, "Recent Developments in Mass Spectroscopy," University Park Press, Baltimore, 1970, pp. 158–168.
10. Vastel, J., and Valeriano, P., *Rev. Tech. Thomson - CSF (France)*, 1971, **3**, 489.
11. Bukowski, J. A., and Patryas, Z., *Pr. Nauk. Inst. Chem. Nieorg. Metal. Pierwiastkow Rzadkich Politech. Wroclaw.*, 1973, **18**, 29.
12. Morrison, G. H., *Bull. Soc. Chim. Belg.*, 1975, **84**, 581.
13. Nicholls, G. D., and Wood, M., *in* Nicol, A., *Editor*, "Physiochemical Methods of Mineral Analysis," Plenum, New York, 1975, pp. 195–229.
14. Cuna, C., Ioanoviciu, D., and Cuna, S., *Stud. Cercet. Fiz.*, 1976, **28**, 481.
15. Wang, T. S., *Fen Hsi Hua Hsueh*, 1978, **6**, 387.
16. Gauneau, M., *Spectra 2000*, 1979, **7**, No. 53, 55.
17. Dietze, H. J., *ZIF. Mitt.*, 1980, **28**, 39.
18. Beske, H. E., Gijbels, R., Hurrle, A., and Jochum, K. P., *Fresenius Z. Anal. Chem.*, 1981, **309**, 329.
19. Carter, J. A., Franklin, J. C., and Donohue, D. L., *ACS Symp. Ser.*, 1978, No. 70, 299.
20. Cornu, A., *Proc. 2nd Conf. Mass Spectrom., Ispra, Italy, Sept. 1971*, Euratom, Luxembourg, 1972, pp. 317–341.
21. Rigby, L. J., Report No. IR/R445/1971/155, Standard Telecommunication Laboratories, Harlow, Essex, 1971, 17 pp.
22. Brown, R., Jacobs, M. L., and Taylor, H. E., *Am. Lab.*, 1972, **4**, 29.
23. Bingham, R. A., and Vossen, P. G. T., *Lab. Pract.*, 1975, **24**, 233.
24. "L'Analyse Elementaire des Solides par Spectrometrie de Masse," collection of articles, *Spectra 2000*, 1979, **7**, No. 51, 29; No. 53, 45; No. 54, 61.
25. Stefani, R., *Trends Anal. Chem.*, 1981, **1**, 84.
26. Bacon, J. R., and Ure, A. M., *Anal. Proc.*, 1981, **18**, 356.
27. Adams, F., *Philos. Trans., R. Soc. London, Ser. A*, 1982, **305**, 509.
28. Ure, A. M., *Trends Anal. Chem.*, 1982, **1**, 314.
29. Cornides, I., and Gal, T., *Kem. Kozl.*, 1976, **46**, 41.
30. Ramendik, G. I., *Priroda (Moscow)*, 1977, 26.
31. Derzhiev, V. I., Liebich, V., Mai, H., and Ramendik, G. I., *Wiss. Ber. Akad. Wiss. DDR, Zentralinst. Festkörperphys. Werkstofforsch.*, 1979, **17**, 259.
32. du Bois, E. A., and Wrigley, K. B., *Proc. Chem. Conf., 25th*, British Steel Corp., Sheffield, 1972, pp. 97–102.
33. Wrigley, K. B., and Dubois, E. A., Report No. MG/CC/569/72, British Steel Corp., London, 1972, 13 pp.
34. Tunney, A. A., Report No. GS/TECH/479/1/75/C, British Steel Corp. Sheffield, 1976, 28 pp.
35. Herzog, L. F., *Int. J. Mass Spectrom. Ion Phys.*, 1970, **4**, 253.
36. Mapper, D., and Smales, A. A., *in* Wainerdi, R. E., *Editor*, "Analytical Chemistry in Space," Pergamon Press, Oxford 1970, pp. 209–275.
37. Cornides, I., *Miner. Slovaca*, 1973, **5**, 489.
38. Carter, J. A., Walker, R. L., and Sites, J. R., *in* Babu, S. P., *Editor*, "Trace elements in Fuel," American Chemical Society, Washington DC, 1975, pp. 74–83.
39. Carter, J. A., Donohue, D. L., Franklin, J. C., and Walker, R. L., *in* Karr Jr., C., *Editor*, "Analytical Methods for Coal and Coal Products," Volume 1, Academic Press, New York, 1978, pp. 403–420.
40. Roboz, J., *in* Ciaccio, L. L. *Editor*, "Water and Water Pollution Handbook," Volume 4, Marcel Dekker, New York, 1973, pp. 1617–1661.
41. Alford, A., *Biomed. Mass Spectrom.*, 1975, **2**, 229.
42. Alford, A., *Biomed. Mass Spectrom.*, 1977, **4**, 1.
43. Alford, A., *Biomed. Mass Spectrom.*, 1978, **5**, 259.
44. Lehmann, W. D., and Schulten, H.-R., *Agnew. Chem., Int. Ed. Engl.*, 1978, **17**, 222.
45. Morrison, G. M., *in* "Elemental Analysis of Biological Materials," Technical Reports Series, No. 197, International Atomic Energy Agency, Vienna, 1980, Chapter 10.
46. Butterworth, A., German, B., Morgans, D., and Scaplehorn, A., *Int. Microform J. Legal Med.*, 1972, **8**, card 5, 10 pp.
47. Leyder, F., *Spectra 2000*, 1979, **7**, No. 53, 64.
48. Chupakhin, M. S., Ramendik, G. I., Derzhiev, V. I., Tatsii, Yu. G., and Potapov, M. A., *Sov. Phys. Dokl.*, 1973, **18**, 430.
49. Ramendik, G. I., Derzhiev, V. I., Gushchin, V. N., and Chupakhin, M. S., *Sov. Phys. Dokl.*, 1975, **20**, 289.
50. Ramendik, G. I., Chupakhin, M. S., and Potapov, M. A., *J. Anal. Chem. USSR*, 1975, **30**, 564.
51. Ramendik, G. I., Derzhiev, V. I., Chupakhin, M. S., Liebich, V., and Mai, H., *J. Anal. Chem. USSR*, 1975, **30**, 1609.
52. Ramendik, G. I., and Derzhiev, V. I., *J. Anal. Chem. USSR*, 1978, **32**, 1197.
53. Ramendik, G. I., and Derzhiev, V. I., *J. Anal. Chem. USSR*, 1978, **32**, 1204.
54. Derzhiev, V. I., and Ramendik, G. I., *Sov. Phys. Tech. Phys.*, 1978, **23**, 186.
55. Ramendik, G. I., and Derzhiev, V. I., *J. Anal. Chem. USSR*, 1979, **34**, 647.
56. Derzhiev, V. I., Liebich, V., Mai, H., and Ramendik, G. I., *Wiss. Ber. Akad. Wiss. DDR, Zentralinst. Festkörperphys. Werkstofforsch.*, 1979, **17**, 265.
57. Ramendik, G. I., *Adv. Mass Spectrom.*, 1980, **8**, 408.
58. Derzhiev, V. I., Ramendik, G. I., Liebich, V., and Mai, H., *Int. J. Mass Spectrom. Ion. Phys.*, 1980, **32**, 345.
59. Franklin, J. C., *Thesis*, Tennessee University, Knoxville, TN, 1971, 121 pp.
60. Franklin, J. C., and Dean, J. A., *Anal. Lett.*, 1971, **4**, 857.
61. Radermacher, L., and Beske, H. E., *Spectrochim. Acta, Part B*, 1979, **34**, 105.
62. Demortier, G., and Lamberts, L., *Int. J. Mass Spectrom. Ion Phys.*, 1979, **31**, 135.
63. Demortier, G., Lamberts, L., and Mathieu, G., *Adv. Mass Spectrom.*, 1980, **8B**, 1934.
64. Demortier, G., *Spectra 2000*, 1980, **8**, No. 55, 50.
65. Shelpakova, I. R., Saprykin, A. I., Gerasimov, V. A., and Yudelevich, I. G., *J. Anal. Chem. USSR*, 1980, **35**, 413.
66. Gerasimov, V. A., Shelpakova, I. R., and Rudaya, N. S., *J. Anal. Chem. USSR*, 1979, **34**, 13.
67. Muheim, J. T., *Proc. Rare Earth Res. Conf., 10th*, Volume 1, National Technical Information Service, Washington, DC, 1973, pp. 208–217.
68. Muheim, J. T., *Helv. Phys. Acta*, 1976, **49**, 739.
69. Muheim, J. T., *Rare Earths Mod. Sci. Technol.*, 1980, **2**, 429.
70. Muheim, J. T., *Spectra 2000*, 1980, **8**, No. 55, 53.
71. Muheim, J. T., *Spectra 2000*, 1980, **8**, No. 55, 54.
72. Joyes, P., Leleyter, M., and Hoareau, A., *J. Phys. (Paris) Lett.*, 1979, **40**, 131.
73. Berthod, J., Cornu, A., and Stefani, R., *C.R. Acad. Sci., Ser. B.*, 1977, **285**, 195.
74. Berthod, J., Andreani, A. M., and Stefani, R., *Int. J. Mass Spectrom. Ion Phys.*, 1978, **27**, 305.
75. Hass, J. R., *Thesis*, University of North Carolina, Chapel Hill, NC, 1972, 222 pp.
76. Buck, R. P., and Hass, J. R., *Anal. Chem.*, 1973, **45**, 2208.
77. Luck, J., Möller, P., and Szacki, W., *Int. J. Mass Spectrom. Ion Phys.*, 1974, **13**, 25.
78. Stefani, R., *J. Phys. (Paris), Colloq.*, 1977, 19.
79. Rechsteiner, C. E., Youngless, T. L., Bursey, M. M., and Buck, R. P.,, *Int. J. Mass Spectrom. Ion Phys.*, 1978, **28**, 401.
80. Cornides, I., and Gal, T., *High Temp. Sci.*, 1978, **10**, 171.
81. Cornides, I., *JEOL News*, 1980, **16A**, 20.
82. Ramendik, G. I., Kryuchkova, O. I., Derzhiev, V. I., Stroganov, N. S., and Strel'nikova, E. B., *Dokl. Phys. Chem.*, 1979, **245**, 336.
83. Mathieu, G., and Lamberts, L., *Int. J. Mass Spectrom. Ion Phys.*, 1979, **31**, 125.
84. Mathieu, G., Lamberts, L., Thomas, M., and Demortier, G., *Int. J. Mass Spectrom. Ion Phys.*, 1981, **38**, 35.
85. Reding, A. M., Labar, C., Mathieu, G., and Lamberts, L., *Int. J. Mass Spectrom. Ion Phys.*, 1978, **26**, 421.
86. Mathieu, G., and Lamberts, L., *Bull. Soc. Chim. Belg.*, 1979, **88**, 21.
87. Itoh, N., and Sata, T., Report No. 6, Res. Lab. Eng. Mater., Tokyo Institute of Technology, Tokyo, 1981, pp. 131–138.
88. Muheim, J. T., and Surbeck, H., *Helv. Phys. Acta*, 1975, **48**, 446.
89. Lasne, C., *Spectra 2000*, 1979, **7**, No. 53, 59.
90. Conzemius, R. J., *in* Gschneidner, K. A., Jr., and Eyring, L., *Editors*, "Handbook on the Physics and Chemistry of Rare Earths," North-Holland, Amsterdam, 1979, Chapter 37C.
91. Cornides, I., and Gal. T., *High Temp. Sci.*, 1981, **14**, 71.
92. Chupakhin, M. S., Kryuchkova, O. I., and Sulimova, S. I., *J. Anal. Chem. USSR*, 1974, **28**, 1504.

93. Frees, L. C., Christophorou, L. G., Ellis, H. W., and Sauers, I., *Abstr. 28th Annu. Conf. Mass Spectrom. Allied Topics, New York, May 1980*, pp. 197–198.
94. Frees, L. C., Sauers, I., Christophorou, L. G., and Ellis, H. W., *Abstr. 29th Annu. Conf. Mass Spectrom. Allied Topics, Minneapolis, May 1981*, p. 145.
95. Muheim, J. T., *Mater. Res. Bull.*, 1972, **7**, 1417.
96. Muheim, J. T., *Helv. Phys. Acta*, 1977, **50**, 584.
97. Vidal, G., *Int. J. Mass Spectrom. Ion Phys.*, 1972/73, **10**, 204.
98. Vidal, G., Galmard, P., and Lanusse, P., *Int. J. Mass Spectrom. Ion Phys.*, 1969, **2**, 405.
99. Wadlin, W. H., *Thesis*, University of Virginia, Charlottesville, VA, 1969, 110 pp.
100. Boulin, Y., *Spectra 2000*, 1980, **8**, No. 55, 44.
101. Van Hoye, E., Gijbels, R., and Adams, F., *Anal. Chim. Acta*, 1980, **115**, 239.
102. Bedrinov, V. P., Ukrainskii, Yu. M., and Chupakhin, M. S., *J. Anal. Chem. USSR*, 1972, **27**, 1729.
103. Shabanova, L. N., Shelpakova, I. R., and Yudelivich, I. G., *J. Anal. Chem. USSR*, 1978, **33**, 885.
104. Bedrinov, V. P., Belousov, V. I., and Khromova, N. V., *J. Anal. Chem. USSR*, 1974, **28**, 2132.
105. Bedrinov, V. P., and Belousov, V. I., *J. Anal. Chem. USSR*, 1976, **31**, 1701.
106. Chupakhin, M. S., Kryuchkova, O. I., and Semenov, A. D., *J. Anal. Chem. USSR*, 1972, **27**, 1724.
107. Chupakhin, M. S., and Ramendik, G. I., *J. Anal. Chem. USSR*, 1972, **26**, 1241.
108. Fitchett, A. W., *Thesis*, University of North Carolina, Chapel Hill, NC, 1973, 137 pp.
109. Colby, B. N., and Morrison, G. H., *Anal. Chem.*, 1972, **44**, 1263.
110. Gerasimov, V. A., Saprykin, A. I., Shelpakova, I. R., and Yudelevich, I. G., *J. Anal. Chem. USSR*, 1978, **33**, 998.
111. Pustovit, A. N., and Sikharulidze, G. G., *Mikrochim. Acta*, 1981, **II**, 219.
112. Clemena, G. G., *Thesis*, University of Virginia, Charlottesville, VA, 1971, 165 pp.
113. German, B., and Scaplehorn, A. W., *J. Forensic Sci. Soc.*, 1972, **12**, 367.
114. Magee, C. W., *Thesis*, University of Virginia, Charlottesville, VA, 1973, 256 pp.
115. Clegg, J. B., and Millett, E. J., *Philips Tech. Rev.*, 1974, **34**, 344.
116. Stefani, R., *Adv. Mass Spectrom.*, 1980, **8B**, 1859.
117. Stefani, R., *Int. J. Mass Spectrom. Ion Phys.*, 1980, **34**, 317.
118. Van Hoye, E., Gijbels, R., and Adams, F., *Talanta*, 1976, **23**, 369.
119. Van Hoye, E., Adams, F., and Gijbels, R., *Talanta*, 1976, **23**, 789.
120. Van Hoye, E., Adams, F., and Gijbels, R., *Talanta*, 1978, **25**, 73.
121. Van Hoye, E., Adams, F., and Gijbels, R., *Int. J. Mass Spectrom. Ion Phys.*, 1979, **30**, 75.
122. Nicholls, G. D., *Proc. Br. Ceram. Soc.*, 1970, April, 85.
123. Magee C. W., and Harrison, W. W., *Anal. Chem.*, 1973, **45**, 852.
124. Oda, S., Aihara, H., and Kamada, H., *Bunseki Kagaku*, 1973, **22**, 757.
125. Yanagihara, K., Sato, S., Oda, S., and Kamada, H., *Anal. Chim. Acta*, 1978, **98**, 307.
126. Ramendik, G. I., Chupakhin, M. S., Tatsii, Yu. G., and Derzhiev, V. I., *J. Anal. Chem. USSR*, 1974, **29**, 202.
127. Aoki, M., Nishiyama, Y., and Aoyama, T., *Abstr. Pittsburgh Conf. Anal. Chem. Appl. Spectrosc., Cleveland, OH, 3–7 March 1975*, No. 172.
128. Makita, T., Kishi, H., and Kodera, K., *Shitsuryo Bunseki*, 1973, **21**, 293.
129. Kishi, H., *Bull. Chem. Soc. Jpn.*, 1981, **54**, 703.
130. Kishi, H., *Bull. Chem. Soc. Jpn.*, 1981, **54**. 1999.
131. Kishi, H., *Bull. Chem. Soc. Jpn.*, 1981, **54**, 2005.
132. Jaworski, J. F., *Thesis*, Cornell University, Ithaca, NY, 1974, 123 pp.
133. Chupakhin, M. S., *Ocherki Sovrem. Geokhim. Anal. Khim.*, 1972, 591.
134. Morrison, G. H., *Ann. N.Y. Acad. Sci.*, 1972, **199**, 162.
135. Pearton, D. C. G., and Sobiecki, A., Report. No. 1984, National Institute for Metallurgy, Randburg, South Africa, 1978, 22 pp.
136. Beske, H. E., Hurrle, A., and Jochum, K. P., *Fresenius Z. Anal. Chem.*, 1981, **309**, 258.
137. Chastagner, P., and Tiffany, B., *Int. J. Mass Spectrom. Ion Phys.*, 1972, **9**, 325.
138. Saito, M., and Sudo, E., *Trans. Natl. Res. Inst. Met.*, 1977, **19**, 178.
139. Kai, J., Ogata, Y., Ohi, K., and Watanabe, M., *in* Ogata, K., and Hayakawa, T., *Editors*, "Recent Developments in Mass Spectroscopy," University Park Press, Baltimore, 1970, pp. 314–318.
140. Franklin, J. C., Landau, L., Donohue, D. L., and Carter, J. A., *Anal. Lett.*, 1978, **11**, 347.
141. Sobiecki, A., Report, No. 2014, National Institute for Metallurgy, Randburg, South Africa, 1979, 7 pp.
142. Jochum, K. P., and Seufert, M., *Geol. Rundsch.*, 1980, **69**, 997.
143. Taylor, S. R., and Gorton, M. P., *Geochim. Cosmochim. Acta*, 1977, **41**, 1375.
144. Taylor, S. R., *in* Gschneidner K. A., Jr., and Eyring, L., *Editors*, "Handbook on the Physics and Chemistry of Rare Earths," North-Holland, Amsterdam, 1979, pp. 359–376.
145. Hintenberger, H., *Fortschr. Mineral.*, 1977, **54**, 141.
146. Nicholls, G. D., *Inst. Min. Metall. Trans., Sect. B*, 1971, **80**, 299.
147. Chupakhin, M. S., and Polyakov, A. L., *J. Anal. Chem. USSR*, 1972, **27**, 460.
148. Ramendik, G. I., *J. Anal. Chem. USSR*, 1977, **32**, 1578.
149. Ure, A. M., and Bacon, J. R., *Proc. Anal. Div. Chem. Soc.*, 1976, **13**, 124.
150. Ure, A. M., and Bacon, J. R., *Analyst*, 1978, **103**, 807.
151. Dietze, H. J., Freyer, K., Geisler, M., Haase, G., Hartmann, G., Maul, E., and Panse, H., *Gerlands Beitr. Geophys.*, 1976, **85**, 497.
152. Jackson, P. F. S., and Strasheim, A., *Analyst*, 1974, **99**, 26.
153. Erämetsä, O., and Yliruokanen, I., *Suom. Kem. B*, 1971, **44**, 121.
154. Sihvonen, M.-L., *Ann. Acad. Sci. Fennicae, Ser A2*, 1972, 168.
155. Harrison, W. W., Ryan, M. A., Copper, L. D., and Clemena, G. C., *in* Mertz, W., and Cornatzer, W. E., *Editors*, "Newer Trace Elements in Nutrition," Marcel Dekker, New York, 1971, Chapter 17.
156. Harrison, W. W., and Clemena, G. G., *Anal. Chem.*, 1972, **44**, 940.
157. Hamilton, E. I., Minski, M. J., and Cleary, J. J., *Sci. Total Environ.*, 1972, **1**, 1.
158. Wong, S. F., *Thesis*, Cornell University, Ithaca, NY, 1973, 83 pp.
159. Brown, R., and Taylor, H. E., Report No. PB-274206, National and Technical Information Services, Washington, DC, 1975, 55 pp.
160. Jacobs, M. L., Report No. 75–186, US Department of Health, Education, and Welfare, Washington, DC, 1975, 91 pp.
161. Fitchett, A. W., Buck, R. P., and Mushak, P., *Anal. Chem.*, 1974, **46**, 710.
162. Morrison, G. H., and Colby B. N., *Anal. Chem.*, 1972, **44**, 1206.
163. Nieminen, K., and Yliruokanen, I., *Bull. Geol. Soc. Finl.* 1974, **46**, 167.
164. Cornides, I., *in* Malissa, H., and Robinson, J. W., *Editors*, "Analysis of Airborne Particles by Physical Methods," CRC Press, West Palm Beach, 1978, pp. 73–91.
165. Liebich, V., and Mai, H., *Adv. Mass Spectrom.*, 1974, **6**, 655.
166. Journoux, J. P., Teulieres, P., and Boyer, R., *in* Karr, C., Jr., "Proceedings of Symposium on Analytical Methods in Nuclear Fuel Cycle, Vienna, Austria, 1971," IAEA, Vienna, 1972, pp. 315–328.
167. Gaivoronskii, P. E., Larin, N. V., and Chernikovich, M. P., *J. Anal. Chem. USSR*, 1978, **33**, 766.
168. Cesario, J., *Spectra 2000*, 1979, **7**, No. 54, 68.
169. Alvarez, R., *Anal. Chim. Acta*, 1974, **73**, 33.
170. Paulsen, P. J., *in* Mavrodineanu, R., *Editor*, "Procedures Used at the National Bureau of Standards to Determine Selected Trace Elements in Biological and Botanical Materials," National Bureau of Standards, Washington, DC, 1977, pp. 33–48.
171. Koppenaal, D. W., Lett, R. G., Brown, F. R., and Manahan, S. E., *Anal. Chem.*, 1980, **52**, 44.
172. Franklin, J. C., Carter, J. A., Donohue, D. L., and Stelzner, R. W., *Abstr. 23rd Annu. Conf. Mass Spectrom. Allied Topics, Houston, Texas, May 1975*, 355.

173. Jochum, K. P., Seufert, M., and Knab, H.-J., *Fresenius Z. Anal. Chem.*, 1981, **309**, 285.
174. Jochum, K. P., Seufert, M., and Best, S., *Fresenius Z. Anal. Chem.*, 1981, **309**, 308.
175. Jaworski, J. F., and Morrison, G. H., *Anal. Chem.*, 1975, **47**, 1173.
176. Knab, H.-J., and Hintenberger, H., *Anal. Chem.*, 1980, **52**, 390.
177. Dietze, H. J., *Isotopenpraxis*, 1979, **15**, 46.
178. Dietze, H. J., and Opauszky, I., *Isotopenpraxis*, 1979, **15**, 309.
179. Harvey, B. R., *Anal. Chem.*, 1978, **50**, 1866.
180. Saito, M., and Sudo, E., *Bunseki Kagaku*, 1981, **30**, 281.
181. Glavin, G. G., Kopetskii, Ch. V., Nikolaev, R. K., and Sikharulidze, G. G., *J. Less Common Met.*, 1972, **26**, 1.
182. Sikharulidze, G. G., and Glavin, G. G., *Metody Opred. Issled. Sostoyaniya Gazov Met., Vses Konf., 3rd*, 1973, **1**, 98.
183. Donohue, D. L., Carter, J. A., and Franklin, J. C., *Anal. Lett.*, 1977, **10**, 371.
184. Verbueken, A., Michiels, E., and Van Grieken, R., *Fresenius Z. Anal. Chem.*, 1981, **309**, 300.
185. Rothenberg, A. M., *Thesis*, Cornell University, Ithaca, NY, 1971, 101 pp.
186. Morrison, G. H., and Rothenberg, A. M., *Anal. Chem.*, 1972, **44**, 515.
187. Tong, S. S. C., Su, Y.-S., and Williams, J. P., *Anal. Chim. Acta*, 1976, **84**, 327.
188. Sugimae, A., *Bunseki Kagaku*, 1978, **27**, 169.
189. Callis, E. L., Report No. NBL-262, New Brunswick Laboratory, New Brunswick, NJ, 1972, pp. 52–55.
190. Welch, K. H., and Ure, A. M., *Anal. Proc.*, 1980, **17**, 8.
191. Paulsen, P. J., Dunstan, L. P., and Diaz, M. G., *Abstr. 25th Annu. Conf. Mass Spectrom. Applied Topics, Washington, DC, 1977*, pp. 703–704.
192. Blommaert, W., Vandelannoote, R., Van't Dack, L., Gijbels, R., and Van Grieken, R., *J. Radioanal. Chem.*, 1980, **57**, 383.
193. Strasheim, A., Strelow, F. E. W., and Jackson, P. F. S., *Proc. XVII Colloq. Spectrosc. Int., Florence*, 1973, **1**, 306.
194. Strelow, F. W. E., and Jackson, P. F. S., *Anal. Chem.*, 1974, **46**, 1481.
195. Strelow, F. W. E., Victor, A. H., and Weinert, C. H. S. W., *Geostand. Newsl.*, 1978, **2**, 49.
196. Van Puymbroeck, J., and Gijbels, R., *Bull. Soc. Chim. Belg.*, 1978, **87**, 803.
197. Van Puymbroeck, J., and Gijbels, R., *Fresenius Z. Anal. Chem.*, 1981, **309**, 312.
198. Vanderborght, B. N., and Van Grieken, R. E., *Talanta*, 1980, **27**, 417.
199. Ure, A. M., *in* "Proceedings of the VIth Czechoslovakian Spectroscopic Conference, September 1–5, 1980, Nitra," Czechoslovak Spectroscopic Society, Prague, 1981, pp. 61–76.
200. Koppenaal, D. W., Lett, R. G., Brown, F. R., and Manahan, S. E., *Abstr. 30th Pittsburgh Conf. Anal. Chem. Appl. Spectrosc., Cleveland, OH, 5–9 March 1979*, No. 437.
201. Zolotov, Yu. A., *Analyst*, 1978, **103**, 56.
202. Zolotov, Yu. A., Shakhova, N. V., Kryuchkova, O. I., Gronskaya, S. I., Spivakov, B. Ya., Ramendik, G. I., and Gushchin, V. N., *J. Anal. Chem. USSR*, 1978, **33**, 983.
203. Petrukhin, O. M., Zolotov, Yu. A., Shevchenko, V. N., Kryuchkova, O. I., Gronskaya, S. I., Gushchin, V. N., Ramendik, G. I., Dunina, V. V., and Rukhadze, E. G., *J. Anal. Chem. USSR*, 1979, **34**, 256.
204. Mastrangelo, F., Matteucci, E., and Restino, G., *Mem. Sci. Geol.*, 1979, **33**, 205.
205. Franklin, J. C., Landau, L., and Carter, J. A., *Radiochem. Radioanal. Lett.*, 1979, **41**, 217.
206. Mykytiuk, A., Russell, D. S., and Boyko, V., *Anal. Chem.*, 1976, **48**, 1462.
207. Gladskoi, V. M., Ivanova, G. A., Kuzovlev, I. A., and Kuz'min, N. M. *J. Anal. Chem. USSR*, 1971, **26**, 975.
208. Chupakhin, M. S., and Kryuchkova, O. I., *Khim. Svostva Soedin. Redkozemel. Elem., Dokl. Vses. Soveshch. Fiz.-Khim. Primen. Redkozemel. Elem., Ikh. Soedin. Splavov, 6th, 1969*, 1973, 82.
209. Yudelevich, I. G., Shelpakova, I. R., Shabanova, L. N., Gerasimov, V. A., and Korda, T. M., *J. Anal. Chem. USSR*, 1976, **31**, 474.
210. Yudelevich, I. G., and Shelpakova, I. P., *Mikrochim. Acta*, 1978, **I**, 547.
211. Chupakhin, M. S., Kogan, L. O., and Polyakova, A. A., *J. Anal. Chem. USSR*, 1974, **29**, 881.
212. Andreani, A. M., Brun, J. C., Mer, J. P., Fillot, A., and Stefani, R., *Methodes Phys. Anal.*, 1971, **7**, 258.
213. Nakano, M., Yamaguchi, G., and Saito, K., *J. Ceram. Soc. Jpn.*, 1971, **79**, 72.
214. Bouvy, G., and Gauneau, M., *Analusis*, 1971, **1**, 15.
215. Viala, J. C., Thevenot, F., and Duclot, J. C., *Analusis*, 1972, **1**, 485.
216. Orzhonikidze, K. G., Melashvili, V. A., Sikharulidze, G. G., and Glinskikh, V. M., *in* Ogata, K., and Hayakawa, T., *Editors*, "Recent Developments in Mass Spectroscopy," University Park Press, Baltimore, 1970, pp. 362–366.
217. Carter, J. A., Cameron, A. E., and Dean, J. A., *Anal. Lett.*, 1971, **4**, 337.
218. Bingham, R. A., and Vossen, P. G. T., *Int. J. Mass Spectrom. Ion Phys.*, 1974, **14**, 259.
219. Paulsen, P. J., and Burke, R. W., *Abstr. 29th Annu. Conf. Mass Spectrom. Allied Topics, Minneapolis, MN, 1981*, p. 235.
220. Paulsen, P. J., Alvarez, R., and Mueller, C. W., *Appl. Spectrosc.*, 1976, **30**, 42.
221. Kannapin, L., Luck, J., Möller, P., and Szacki, W., *Fresenius Z. Anal. Chem.*, 1974, **271**, 1.
222. Cairns, T., and Johnson, B. B., *in* Perkins, A. J., Grove, E. L., Kaeble, E. F., and Wetermeyer, J. E., *Editors*, "Developments in Applied Spectroscopy," Volume 10, Plenum Press, New York, 1972, pp. 3–14.
223. Gauneau, M., and Bouvy, G., *Analusis*, 1973, **2**, 314.
224. Ikeda, Y., Umayahara, A., Kubota, E., Aoyama, T., and Watanabe, E., *Abstr. 20th Annu. Conf. Mass Spectrom. Allied Topics, Dallas, Texas, 1972*, pp. 142–145.
225. Jaworski, J. F., Burdo, R. A., and Morrison, G. H., *Anal. Chem.*, 1974, **46**, 805.
226. Bulaceanu, R., and Dumitrescu, P., *Rev. Chim. (Bucharest)*, 1976, **27**, 606.
227. Chupakhin, M. S., Ramendik, G. I., and Kryuchkova, O. I., *J. Anal. Chem. USSR*, 1969, **24**, 249.
228. Chupakhin, M. S., Ramendik, G. I., and Kryuchkova, O. I., . *Anal. Chem. USSR*, 1969, **24**, 773.
229. Chupakhin, M. S., and Ramendik, G. I., *in* Ogata, K., and Hayakawa, T., *Editors*, "Recent Developments in Mass Spectroscopy," University Park Press, Baltimore, 1970, pp. 319–322.
230. Ramendik, G. I., Derzhiev, V. I., and Vasyuta, Yu. V., *J. Anal. Chem. USSR*, 1979, **34**, 1016.
231. Babushkina, T. V., Sikharulidze, G. G., and Nikolaev, R. K., *J. Anal. Chem. USSR*, 1975, **30**, 142.
232. Schmidt, F. A., Conzemius, R. J., Carlson, O. N., and Svec, H. J., *Anal. Chem.*, 1974, **46**, 810.
233. Konishi, F., *Shitsuryo Bunseki*, 1971, **19**, 284.
234. Haemers, J., *J. Phys. E: Sci. Instr.*, 1974, **7**, 294.
235. Ito, M., Sato, S., and Yanagihara, K., *Anal. Chim. Acta*, 1980, **120**, 217.
236. Oda, S., *JEOL News*, 1973, **11A**, 15.
237. Oda, S., Ohashi, Z., Furuya, K., and Kamada, H., *Talanta*, 1972, **19**, 779.
238. Harrison, W. W., and Donohue, D. L., *Anal. Chem.*, 1973, **45**, 1741.
239. Konishi, F., *Int. J. Mass Spectrom. Ion Phys.*, 1972, **9**, 33.
240. Colby, B. N., *Thesis*, Cornell University, Ithaca, NY, 1971, 79 pp.
241. Kashuba A. T., Jr., *Thesis*, Cornell University, Ithaca, NY, 1970, 104 pp.
242. Ito, M., and Yanagihara, K., *Denki Seiko*, 1972, **43**, 171.
243. Pearton, D. C., and Sobiecki, A., Report No. 2075, National Institute of Metallurgy, Randburg, South Africa, 1980, 15 pp.
244. Fursov, V. Z., and Chupakhin, M. S., *J. Anal. Chem. USSR*, 1975, **29**, 1449.
245. Socha, A. J., Masumoto, E. M., and Willardson, R. K., Report No. AD-728219, Bell and Howell Research Center, Pasadena, CA, 1970, 96 pp.
246. Dorokhov, A. N., Shelpakova, I. R., and Yudelevich, I. G., *J. Anal. Chem. USSR*, 1975, **30**, 672.
247. Gladshoi, V. M., Kuznetsov, Yu. N., Babenkov, L. M., Belousov, V. I., and Kuzovlen, I. A., *Ind. Lab.* 1975, **40**, 1796.
248. Vidal, G., Galmard, P., and Lanusse, P., *Int. J. Mass Spectrom. Ion Phys.*, 1969, **2**, 373.
249. Masters, J. I., Report No. NSF/RA-780507, Ionomet Co., Waban, MA, 1978, 36 pp.

250. Vouros, P., Desiderio, D. M., Leferink, J. G., Odiorne, T. J., and McCloskey, J. A., *Int. J. Mass Spectrom. Ion Phys.*, 1972, **10**, 133.
251. Grais, K. I., and Grais, G. S., *Indian J. Pure Appl. Phys.*, 1971, **9**, 127.
252. Dale, L. S., *Anal. Chem.*, 1976, **48**, 2040.
253. Blain, C., *Spectra 2000*, 1979, **7**, No. 53, 47.
254. Labar, Ch., and Lamberts, L., *Spectra 2000*, 1979, **7**, No. 51, 46.
255. Ordzhonikidze, K. G., Samadashvili, O. A., Glinskikh, Y. M., Yur'eva, O. K., and Karpenko, I. K., *J. Anal. Chem. USSR*, 1973, **27**, 2100.
256. Kai, J., and Miki, M., *Mitsubishi Denki Lab. Rep.*, 1964, **5**, 175.
257. Hull, C. W., *Abstr. Tenth Mass Spectrom. Confer., New Orleans, 1966*, p. 404.
258. Belousov, V. I., Bedrinov, V. P., Gladskoi, V. M., and Kuznetsov, Yu. N., *Ind. Lab.*, 1974, **40**, 535.
259. Churchill, J. R., *Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 653.
260. Mattauch, J., and Ewald, W., *Naturwissenschaften*, 1943, **41**, 487.
261. Taylor, S. R., *Geochim. Cosmochim. Acta*, 1971, **35**, 1187.
262. Fergason, L. A., and Young, R. C., *Appl. Spectrosc.*, 1972, **26**, 620.
263. Childs, B. G., Report No. PR-CMa-23, Chalk River Nuclear Laboratories, Chalk River, Ontario, 1973, pp. 79–81.
264. Pearton, D. C. G., Report No. 2062, National Institute for Metallurgy, Randburg, South Africa, 1980, 21 pp.
265. Schuy, K. D., and Franzen, J., *Fresenius Z. Anal. Chem.*, 1967, **225**, 260.
266. Pilate, A., and Adams, F., *Anal. Chim. Acta*, 1980, **122**, 57.
267. 'Verbueken, A., and Van Grieken, R., *Bull. Soc. Chim. Belg.*, 1981, **90**, 331.
268. Radermacher, L., and Beske, H. E., Report No. Jül-1325, Kernforschungsanlage Jülich, Jülich, 1976, 82 pp.
269. Radermacher, L., and Beske, H. E., Report No. Jül-Spez-7, Kernforschungsanlage Jülich, Jülich, 1978, 44 pp.
270. Radermacher, L., and Beske, H. E., *Adv. Mass Spectrom.*, 1978, **7A**, 545.
271. Radermacher, L., and Beske, H. E., *Fresenius Z. Anal. Chem.*, 1981, **309**, 319.
272. Labar, C., and Lamberts, L., *Analusis*, 1979, **7**, 175.
273. Konishi, F., and Nakamura, N., *Shitsuryo Bunseki*, 1971, **19**, 213.
274. Burdo, R. A., *Thesis*, Cornell University, Ithaca, NY, 1973, 155 pp.
275. Ailloud, P., and Allegre, J., *Spectra 2000*, 1977, **5**, No. 35, 16.
276. Andreani, A. M., Blanchard, B., Brun, J. C., Mermoud, J. P., Pellet, J., and Stefani, R., *Adv. Mass Spectrom.*, 1971, **5**, 534.
277. Owens, E. B., *Rev. Sci. Instrum.*, 1970, **41**, 636.
278. Weber, J. H., Earnshaw, D. G., Severin, D. W. K., and Decora, A. W., Report No. RI 7938, National Technical Information Service, Washington, DC, 1974, 14 pp.
279. Guidoboni, R. J., *in* Karr, C., Jr., *Editor*, "Analytical Methods for Coal and Coal Products," Academic Press, New York, 1978, pp. 421–434.
280. Jackson, P. F. S., and Strasheim, A., *Proc. XVIII Colloq. Spectrosc. Int., Grenoble*, 1975, **1**, 183.
281. Fuller, C. W., *Proc. Soc. Anal. Chem.*, 1974, **11**, 176.
282. Bürstenfinder, J., Luck, J., and Szacki, W., *Fresenius Z. Anal. Chem.*, 1981, **309**, 325.
283. Jordan, R. G., and Jones, D. W., *J. Less-Common Met.*, 1973, **31**, 125.
284. Beske, H. E., *Eurisotop Off. Inf. Bookl.*,, 1974, No. 72, 657.
285. Haney, M. A., and Gallagher, J. F., *Anal. Chem.*, 1975, **47**, 62.
286. Van Hoye, E., Gijbels, R., and Adams, F., *Talanta*, 1977, **24**, 625.
287. Sobiecki, A., Report No. 1985, National Institute for Metallurgy, Randburg, South Africa, 1978, 9 pp.
288. Bacon, J. R., and Ure, A. M., *Adv. Mass Spectrom.*, 1980, **8**, 362.
289. Luck, J., Möller, P., and Szacki, W., *Fresenius Z. Anal. Chem.*, 1973, **267**, 186.
290. Andreani, A. M., Brun, J. C., Mermoud, J. P., and Stefani, R., *Methodes Phys. Anal.*, 1971, **7**, 269.
291. Gauneau, M., Rupert, A., Minier, M., Regreny, O., and Coquille, R., *Anal. Chim. Acta*, 1982, **135**, 193.
292. Marugaiyan, P., *Pure Appl. Chem.*, 1982, **54**, 835.
293. Kuehner, E. C., Alvarez, R., Paulsen, P. J., and Murphy, T. J., *Anal. Chem.*, 1972, **44**, 2050.
294. Ribaudo, C., Semel, S., and Blosser, E. R., Technical Report 4014, Picatinny Arsenal, Dover, NJ, 1970, 43 pp.
295. Ikeda, Y., and Umayahara, A., *JEOL News*, 1973, **11A**, 22.
296. Gaunaeu, M., *Analusis*, 1975, **3**, 368.
297. Bulaceanu, R., and Dumitrescu, P., *Rev. Chim.(Bucharest)*, 1976, **27**, 707.
298. Roaldset, E., *Talanta*, 1970, **17**, 593.
299. Morrison, G. H., Kashuba, A. R., and Rothenberg, A. M., *Adv. Mass Spectrom.*, 1971, **5**, 520.
300. Nance, W. B., and Taylor, S. R., *Geochim. Cosmochim. Acta*, 1976, **40**, 1539.
301. Ure, A. M., and Bacon, J. R., *Adv. Mass Spectrom.*, 1978, **7A**, 549.
302. Bacon, J. R., and Ure, A. M., *Anal. Chim. Acta*, 1979, **105**, 163.
303. McLennan, S. M., and Taylor, S. R., *Chem. Geol.*, 1980, **29**, 333.
304. Daughtrey, E. H., and Bostick, J. K., *Abstr. 28th Annu. Conf. Mass Spectrom. Allied Topics, New York, May 1980*, pp. 433–434.
305. Wahlgren, M. A., Edgington, D. N., Rawlings, F. F., and Rawls, J. L., *in* "Proceeding of 15th Conference on Great Lakes Research," International Association for Great Lakes Research, Ann Arbor, MI, 1972, pp. 298–305.
306. Gregory, N. L., *Anal. Chem.*, 1972, **44**, 231.
307. Locke, J., Boase, D. R., and Smalldon, K. W., *Anal. Chim. Acta*, 1979, **104**, 233.
308. Sasamoto, T., Itoh, Y., Hara, H., and Sata, T., *Bull. Tokyo Inst. Technol.*, 1975, **126**, 91.
309. Ehrlich, G., Stahlberg, U., and Scholze, H., *Spectrochim. Acta, Part B*, 1982, **37**, 45.
310. Friedman, A. M., and Lerner, J., *Adv. Chem. Ser.*, 1978, No. 171, 70.
311. Opauszky, I., and Nyary, I., Report No. KFKI-1981-09, Hungarian Academy of Sciences, Budapest, 1981, 12 pp.
312. Sasamoto, T., Hara, H., and Sata, T., *Shitsuryo Bunseki*, 1976, **24**, 121.
313. Haaland, J., and Stijfhoorn, D. E., *in* Michelsen, O. B., *Editor*, "Analysis and Application of Rare Earth Materials," Universitetsforlaget, Oslo, 1973, pp. 153–164.
314. Hamilton, E. I., and Minski, M. J., *Int. J. Mass Spectrom. Ion Phys.*, 1972, **10**, 77.
315. Conzemius, R. J., and Svec, H. J., *Talanta*, 1973, **20**, 575.
316. Itoh, M., and Yanagihara, K., *Bunseki Kagaku*, 1973, **22**, 10.
317. Van Hoye, E., Adams, F., and Gijbels, R., *Talanta*, 1979, **26**, 285.
318. Vanderborght, B., and Van Grieken, R., *Talanta*, 1979, **26**, 461.
319. Billon, J. P., Report No. CEA-R-3883, Commissariat à l'Energie Atomique, Paris, 1970, 59 pp.
320. Bratton, W. D., and Wood, C. H., *Appl. Spectrosc.*, 1970, **24**, 509.
321. Blanchard, B., *Methodes Phys. Anal.* 1971, **7**, 280.
322. Beske, H. E., Frerichs, G., and Melchers, F. G., *Fresenius Z. Anal. Chem.*, 1973, **267**, 99.
323. Billon, J. P., *Adv. Mass Spectrom.*, 1974, **6**, 649.
324. Jaworski, J. F., and Morrison, G. H., *Anal. Chem.*, 1974, **46**, 2080.
325. Billon, J. P., *Proc. XVIII Colloq. Spectrosc. Int., Grenoble*, 1975, **3**, 819.
326. Jackson, P. F. S., Strasheim, A., and Dixon, K., *Procs. XVIII Colloq. Spectrosc. Int., Grenoble*, 1975, **1**, 164.
327. Oda, S., Kubo, H., and Kamada, H., *Bunseki Kagaku*, 1975, **24**, 66.
328. Jackson, P. F. S., Strasheim, A., and Dixon, K., "Extended Abstracts, International Symposium on the Analytical Chemistry of Exploration, Mining and Processing Materials, 1976," CSIR, Pretoria, South Africa, 1977, pp. 553–556.
329. Matus, L., Opauszky, I., Nyary, I., and Pasztor, E., *Adv. Mass Spectrom.*, 1980, **8B**, 1962.
330. Dale, L. S., Liepa, I., Rendell, P. S., and Whittem, R. N., *Anal. Chem.*, 1981, **53**, 2288.
331. Winkler, P., *Fresenius Z. Anal. Chem.*, 1981, **309**, 266.
332. Wahlgren, M. A., Edgington, D. N., and Rawlings, F. F., *in* Vogt, J. R., *Editor*, "Nuclear Methods in Environmental Research," Proceedings, American Nuclear Society, University of Missouri, Columbia, MO, 1971, pp. 97–103.

333. Hamilton, E. I., and Minski, M. J., *Environ. Lett.*, 1972, **3**, 53.
334. Crocker, I. H., and Knight, C. H., Report No. PR-CMa-23, Atomic Energy of Canada Ltd., Chalk River, Ontario, 1973, pp. 45–48.
335. Carter, J. A., Donohue, D. L., Franklin, J. C., and Stelzner, R. W., *Proc. 9th Annu. Conf. Trace Substances in Environ. Health, Colombia, MO*, 1975, 303.
336. Griffith, D. A., Conzemius, R. J., and Svec, H. J., *Talanta*, 1971, **18**, 665.
337. Leipold, M. H., *in* Kirkendale, G. A., *Editor*, "Analytical Methods for Materials Investigation," Gordon and Breach, New York, 1971, pp. 145–162.
338. Howard, O. H., *Anal. Chem.*, 1972, **44**, 1482.
339. Kryuchkova, O. I., Derzhiev, V. I., Ramendik, G. I., Stroganova, N. S., and Strel'nikova, E. B., *Sov. Phys. Dokl.*, 1979, **24**, 924.
340. Kudryavtsev, V. N., and Vlasov, V. N., *USSR Pat.*, 1979, No. 673903.
341. Clegg, J. B., Gale, I. G., and Millett, E. J., *Analyst*, 1973, **98**, 69.
342. Clegg, J. B., and Millett, E. J., *Proc. Soc. Anal. Chem.*, 1974, **11**, 52.
343. Hurrle, A., and Dietl, J., *Proc. Electrochem. Soc.*, 1980, **80-85**, 106.
344. Hurrle, A., and Dietl, J., *Fresenius Z. Anal. Chem.*, 1981, **309**, 277.
345. Bulaceanu, R., and Dumitrescu, P., *Rev. Chim. (Bucharest)*, 1976, **27**, 894.
346. Luck, J., Szacki, W., and Pluhar, E., *Fresenius Z. Anal. Chem.*, 1981, **309**, 281.
347. Pilate, A., and Adams, F., *Fresenius Z. Anal. Chem.*, 1981, **309**, 295.
348. Erämetsä, O., and Yliruokanen, I., *Suom. Kem.*, 1971, **44B**, 372.
349. Magee, C. W., and Harrison, W. W., *Anal. Chem.*, 1974, **46**, 474.
350. Makita, T., Kodera, K., and Kishi, H., *in* Ogata, K., and Hayakawa, T., *Editors*, "Recent Developments in Mass Spectroscopy," University Park Press, Baltimore, 1970, pp. 329–333.
351. Goshgarian, B. B., and Jensen, A. V., *Abstr. 12th Annu. Conf. Mass Spectrom. Allied Topics, Montreal, 1964*, p. 350.
352. Socha, A. J., and Masumoto, E. M., *Abstr. 15th Annu. Conf. Mass Spectrom. Allied Topics, Denver, 1967*, p. 179.
353. Watanabe, E., and Naito, M., *in* Ogata, K., and Hayakawa, T., *Editors*, "Recent Developments in Mass Spectroscopy," University Park Press, Baltimore, 1970, pp. 249–256.
354. Dabbs, M. D. G., *Lab. Pract.*, 1978, **27**, 560.
355. Pearton, D. C. G., and Hulse, N. D., Report, No. 2040, National Institute for Metallurgy, Randburg, South Africa, 1980, 15 pp.
356. Herzog, L. F., Eskew, T. J., Marshall, D. J., and Underwood, K. H., *Abstr. 29th Annu. Conf. Mass Spectrom. Allied Topics, Minneapolis, 1981*, pp. 98–99.
357. Auer, S., and Berg, O. E., *Rev. Sci. Instrum.*, 1975, **46**, 1530.
358. Banner, A. E., Bateman, R. H., Halliday, J. S., and Willdig, E., *Abstr. 20th Annu. Conf. Mass Spectrom. Allied Topics, Dallas, 1972*, pp. 150–155.
359. Banner, A. E., and Stimpson, B. P., *Vacuum*, 1974, **24**, 511.
360. Blattner, R. J., Baker, J. E., and Evans, C. A., *Anal. Chem.*, 1974, **46**, 2171.
361. Harrison, W. W., and Magee, C. W., *Anal. Chem.*, 1974, **46**, 461.
362. Mattson, W. A., *Thesis*, University of Virginia, Charlottesville, VA, 1975, 201 pp.
363. Bingham, R. A., and Salter, P. L., *Anal. Chem.*, 1976, **48**, 1735.
364. Franzen, J., *in* Ogata, K., and Hayakawa, T., *Editors*, "Recent Developments in Mass Spectroscopy," University Park Press, Baltimore, 1970, pp. 296–299.
365. Billon, J. P., *Analusis*, 1973, **2**, 137.
366. Warmack, R. J., Landau, L., Christie, W. H., and Carter, J. A., Report No. CONF 811025-21, Oak Ridge National Laboratory, Oak Ridge, 1981, 9 pp.
367. Degreve, F., *Adv. Mass Spectrom.*, 1971, **5**, 529.
368. Andreani, A. M., *Spectra 2000*, 1979, **7**, No 54, 61.
369. Jansen, J. A. J., and Witmer, A. W., *Fresenius Z. Anal. Chem.*, 1981, **309**, 262.
370. Glinskikh, V. M., Melashvili, V. A., Ordzhonikidze, K. G., and Samadashvili, O. A., *J. Anal. Chem. USSR*, 1974, **29**, 89.
371. Dugger, D., and Oblas, D., *Abstr. 22nd Annu. Conf. Mass Spectrom. Allied Topics, Philadelphia, 1974*, pp. 414–416.
372. Dugger, D. L., and Oblas, D., *Talanta*, 1977, **24**, 447.
373. Dugger, D. L., and Oblas, D. W., *Abstr. 24th Annu. Conf. Mass Spectrom. Allied Topics, San Diego, 1976*, pp. 682–684.
374. Dugger, D. L., and Oblas, D. W., *J. Vac. Sci. Technol.*, 1980, **17**, 826.
375. Harrison, W. W., and Mattson, W. A., *Anal. Chem.*, 1974, **46**, 1979.
376. Carter, J. A., and Sites, J. R., *Anal. Lett.*, 1971, **4**, 351.
377. Magee, C. W., Donohue, D. L., and Harrison, W. W., *Anal. Chem.*, 1972, **44**, 2413.
378. Haemers, J., *J. Phys. E*, 1978, **11**, 897.
379. Clegg, J. B., and Millett, E. J., *Acta Electron.*, 1975, **18**, 27.
380. Ramendik, G. I., Tatsii, Yu. G., and Chupakhin, M. S., *J. Anal. Chem. USSR*, 1973, **28**, 653.
381. Callis, E. L., Report No. NBL-265, New Brunswick Laboratory, New Brunswick, NJ, 1972, pp. 53–54.
382. Callis, E. L., Report No. NBL-267, New Brunswick Laboratory, New Brunswick, NJ, 1973, p. 93.
383. Chupakin, M. S., *Usp. Anal. Khim.*, 1974, 44.
384. Potapov, M. A., Chupakhin, M. S., Shtanov, V. I., and Zlomanov, V. P., *J. Anal. Chem. USSR*, 1978, **33**, 820.
385. Gerasimov, V. A., Chupakhin, M. S., and Shelpakova, I. R., *J. Anal. Chem. USSR*, 1980, **35**, 138.
386. Berthod, J., *Ger. Offen.*, 2719243, 1977.
387. Berthod, J., *Br. Pat.*, 1576453, 1980.
388. Berthod, J., Alexandre, B., and Stefani, R., *Int. J. Mass Spectrom. Ion Phys.*, 1973, **10**, 478.
389. Berthod, J., *Adv. Mass Spectrom.*, 1974, **6**, 421.
390. Zelenin, A. E., Pustovit, A. N., and Sikharulidze, G. G., *Sov. Phys. Tech. Phys.*, 1980, **25**, 1374.
391. Saprykin, A. I., Shelpakova, I. R., and Yudelevich, I. G., *Izv. Sib. Otd. Akad. Nauk SSR, Ser. Khim. Nauk*, 1979, **6**, 123.
392. Schuy, K. D., and Frazen, J., *US Pat.* 3809896, 1974.
393. Warmack, R. J., and Futrell, T. L., *Anal. Lett.*, 1980, **13**, 965.
394. Bingham, R. A., Powers, P., and Wolstenholme, W. A., *in* Ogata, K., and Hayakawa, T., *Editors*, "Recent Developments in Mass Spectroscopy," University Park Press, Baltimore, 1970, pp. 339–350.
395. Magee, C. W., and Harrison, W. W., *Anal. Chem.*, 1973, **45**, 220.
396. Eskew, T. J., and Smith, A. J., *Abstr. 20th Annu. Conf. Mass Spectrom. Allied Topics, Dallas, 1972*, pp. 194–199.
397. Gerasimov, V. A., and Saprykin, A. I., *Instrum. Exp. Tech.*, 1977, **20**, 809.
398. Conzemius, R. J., and Svec, H. J., *Talanta*, 1973, **20**, 477.
399. Conzemius, R., *US Pat.*, 3911272, 1975.
400. Auer, S., Report No. N74-31920, Goddard Space Flight Centre, Greenbelt, MD, 1974, 12 pp.
401. Fujii, T., and Fuwa, K., *Shitsuryo Bunseki*,. 1980, **28**, 151.
402. Fujii, T., and Fuwa, K., *Int. J. Mass Spectrom. Ion Phys.*, 1980, **33**, 325.
403. Degreve, F., Figaret, R., Le Goux, J. J., and Calavrias, L., *Int. J. Mass Spectrom. Ion Phys.*, 1974, **14**, 183.
404. Vozenilek, E. F., *Anal. Chem.*, 1976, **48**, 1654.
405. Berthod, J., and Stefani, R., *Int. J. Mass Spectrom. Ion Phys.*, 1976, **22**, 121.
406. Andreani, A. M., Berthod, J., Mercadier, D., and Mermoud, J. P., *Analusis*, 1979, **7**, 172.
407. Gal'burg, V. A., Glavin, G. G., and Zelenin, A. E., *J. Anal. Chem. USSR*, 1975, **29**, 1223.
408. Shabanova, L. N., Shelpakova, I. R., and Yudelevich, I. G., *Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk*, 1979, **6**, 126.
409. Fuller, C. W., and Whitehead, J., *Analyst*, 1971, **96**, 779.
410. Jacobs, M. L., Sweeney, S. L., and Kerfoot, E. G., *Abstr. 24th Annu. Conf. Mass Spectrom. Allied Topics, San Diego, 1976*, p. 504.
411. Brouwer, G., Meeuwsen, Th. P. M., and Witmer, A. W., *Int. J. Mass Spectrom. Ion Phys.*, 1973, **12**, 397.
412. Arai, M., Naito, M., Takagi, T., and Takeuchi, M., *Abstr. 21st Annu. Conf. Mass Spectrom. Allied Topics, San Francisco, 1973*, pp. 507–511.
413. De Ceuninck, F., Van Hoye, E., and Adams, F., *Proc. Anal. Div. Chem. Soc.*, 1978, **15**, 104.

414. Mattson, W. A., and Harrison, W. W., *Anal. Chem.*, 1975, **47**, 967.
415. Conzemius, R. J., Rhinehart, W. A., and Svec, H. J., *Talanta*, 1972, **19**, 1147.
416. Morrison, G. H., Colby, B. N., and Roth, J. R., *Anal. Chem.*, 1972, **44**, 1203.
417. Giltrap, J. W., Mapper, D., and Farren, J., Report No. AERE R-8866, UK Atomic Energy Research Establishment, Harwell, 1978, 20 pp.
418. Giltrap, J. W., Mapper, D., and Farren, J., Report No. AERE R-9583, UK Atomic Energy Research Establishment, Harwell, 1979, 15 pp.
419. Conzemius, R. J., and Sleege, G. A., *Talanta*, 1976, **23**, 556.
420. Herzog, L. F., *Abstr. 20th Annu. Conf. Mass Spectrom. Allied Topics., Dallas, 1972*, pp. 295–305.
421. Conzemius, R. J., and Svec, H. J., *Talanta*, 1974, **21**, 171.
422. Powers, P., *US Pat.*, 3522428, 1970.
423. Donohue, D. L., *Thesis*, University of Tennessee, Knoxville, TN, 1979, 124 pp.
424. Donohue, D. L., Carter, J. A., and Mamantov, G., *Int. J. Mass Spectrom. Ion Phys.*, 1980, **33**, 45.
425. Donohue, D. L., Carter, J. A., and Mamantov, G., *Int. J. Mass Spectrom. Ion Phys.*, 1980, **35**, 243.
426. Radermacher, L., Beske, H. E., Holzbrecher, H., and Maeckelburg, D., *Int. J. Mass Spectrom. Ion Phys.*, 1976, **20**, 333.
427. Radermacher, L., and Beske, H. E., *Adv. Mass Spectrom.*, 1980, **8A**, 378.
428. Franzen, J., Schönfeld, W., and Stüwer, D., *Adv. Mass Spectrom.*, 1971, **5**, 322.
429. Frazer, J. W., Carlson, L. R., Kray, A. M., Bertoglio, M. R., and Perone, S. P., *Anal. Chem.*, 1971, **43**, 1479.
430. Hunt, M. H., Nikula, J. V., Posen, H., and Vickers, V. E., *Abstr. 20th Annu. Conf. Mass Spectrom. Allied Topics, Dallas, 1972*, pp. 409–413.
431. Matsumoto, R., Suzuki, R., Sato, K., and Yamaguchi, N., *Shitsuryo Bunseki*, 1972, **20**, 63.
432. Frisch, M. A., and Reuter, W., *Anal. Chem.*, 1973, **45**, 1889.
433. Burdo, R. A., Roth, J. R., and Morrison, G. H., *Anal. Chem.*, 1974, **46**, 701.
434. Millett, G. J., Morice, J. A., and Clegg, J. B., *Int. J. Mass Spectrom. Ion Phys.*, 1974, **13**, 1.
435. Ordzhonikidze, K. G., Orlova, L. A., Samadashvili, O. A., and Yur'eva, O. K., *J. Anal. Chem. USSR*, 1974, **29**, 684.
436. Woodward, W. S., Ridgway, T. H., and Reilley, C. N., *Analyst*, 1974, **99**, 838.
437. Stelzner, R. W., Sites, J. R., McKown, H. S., *Abstr. 169th National ACS Meeting, Philadelphia, 1975*, No. ANAL72.
438. Van Hoye, E., Adams, F., and Gijbels, R., *Bull. Soc. Chim. Belg.*, 1975, **84**, 595.
439. Conzemius, R. J., Adduci, D. J., Foss, G. O., and Svec, H. J., *Anal. Chem.*, 1976, **48**, 1647.
440. Dale, L. S., and Whittem, R. N., *Appl. Spectrosc.*, 1976, **30**, 469.
441. Naudin, G., Report No. CEA-N-1825, Commissariat à l'Energie Atomique, Paris, 1976, 26 pp.
442. Stüwer, D., *Int. J. Mass Spectrom. Ion Phys.*, 1976, **20**, 387.
443. Nakamura, N., Kai, Y., and Konishi, F., *Shitsuryo Bunseki*, 1977, **25**, 67.
444. Hoyland, J. R., and Alexander, C. A., *Abstr. 26th Annu. Conf. Mass Spectrom. Allied Topics, St. Louis, 1978*, pp. 647–649.
445. Vanderborght, B., and Van Grieken, R., *Anal. Chim. Acta*, 1978, **103**, 223.
446. Desantis, R. R., Lett, R. G., and Plankey, F., *Abstr. 31st Pittsburgh Conf. Anal. Chem. Appl. Spectrosc., Atlantic City, 1980*, No. P15.
447. Knab, H. J., *Z. Naturforsch, Teil A*, 1980, **35**, 18.
448. Udalov, V. I., and Gaivoronskii, P. E., *J. Anal. Chem. USSR*, 1980, **35**, 1203.
449. Naudin, G., Report No. CEA-CONF-5773, CEA Centre d'Etudes de Bruyeres-le-Chatel, Montrouge, France, 1981, 16 pp.
450. Pearton, D., and Heron, C., Report No. 2117, National Institute for Metallurgy, Randburg, South Africa, 1981, 8 pp.
451. Warashina, M., Mori, Y., Shishido, T., Kato, Y., Kondo, M., Nishihara, T., Fujii, M., Yamashita, T., and Kato, M., *Seikatsu Eisei*, 1978, **22**, 145.
452. Babushkina, T. V., Glavin, G. G., Zelenin, A. E., and Kostenko, T. A., *J. Anal. Chem. USSR*, 1977, **32**, 856.
453. Chupakhin, M. S., and Khusainova, B. N., *J. Anal. Chem. USSR*, 1971, 26, 971.
454. Woolston, J. R., *Abstr. 21st Annu. Conf. Mass Spectrom. Allied Topics, San Francisco, 1973*, pp. 514–517.
455. Viczian, M., Kada, T., and Rethati, R., *Publ. Hung. Min. Inst.*, 1976, No. 19, 219.
456. Daughtrey, E. H., and Bostick, J. K., *Abstr. 26th Annu. Conf. Mass Spectrom. Allied Topics, St. Louis, 1978*, pp. 623–625.
457. Bingham, R. A., Brown, R., and Powers, P., *Adv. Mass Spectrom.*, 1971, **5**, 326.
458. Marushia, P., and Delmore, J., *Abstr. 29th Annu. Conf. Mass Spectrom. Allied Topics, Minneapolis, 1981*, pp. 462–463.
459. Ailloud, P., *Spectra 2000*, 1979, **7**, No. 53, 50.
460. Gaivoronskii, P. E., and Chernikovich, M. P., *J. Anal. Chem. USSR*, 1979, **34**, 1429.
461. Beske, H. E., Frerichs, G., Jahn, M. D., and Melchers, F. G., *Proc. Int. Conf. Anal. Non-Met. Met., 1980*, 1981, 107.
462. Beske, H. E., *Fresenius. Z. Anal. Chem.*, 1971, **252**, 103.
463. Bedrinov, V. P., Ukrainskii, Yu. M., Fursov, V. Z., and Chupakhin, M. S., *J. Anal. Chem. USSR*, 1972, **27**, 535.
464. Beske, H. E., Welter, J. M., Frerichs, G., and Melchers, F. G., *Fresenius Z. Anal. Chem.*, 1981, **309**, 269.
465. Matus, L., Opauszky, I., Nyary, I., and Pasztor, E., *Fresenius Z. Anal. Chem.*, 1981, **309**, 316.
466. Evers, J., Weiss, A., Kaldis, E., and Muheim, J., *J. Less-Common Met.*, 1973, **30**, 83.
467. Crocker, I. H., and Knight, C. H., Report No. PR-CMa-24, Atomic Energy of Canada Ltd., Chalk River, Ontario, 1973, pp. 48–50.
468. Locke, J., Boase, D. R., and Smalldon, K. W., *Med. Sci. Law*, 1980, **20**, 175.
469. Burke, R. W., Paulsen, P. J., Maienthal, E. J., and Lambert, G. M., *Talanta*, 1982, **29**, 809.
470. Shabanova, L. N., Shelpakova, I. P., Gerasimov, V. A., and Yudelevich, I. G., *Izv. Sib. Otd. Nauk SSSR, Ser. Khim. Nauk*, 1977, **14**, 55.
471. Kurthy, J., *Acta Chim. Acad. Sci. Hung.*, 1978, **96**, 209.
472. Nyary, I., and Opauszky, I., *Fresenius Z. Anal. Chem.*, 1981, **309**, 274.
473. Socha, A. J., Updegrove, W. S., and Oro, J., *Abstr. 18th Annu. Conf. Mass Spectrom. Allied Topics, San Francisco, 1970*, pp. B222–B226.
474. Golightly, D. W., *Methodes Phys. Anal.*, 1971, **7**, 275.
475. Strasheim, A., and Jackson, P. F. S., *J. S. Afr. Inst. Min. Metall.*, 1971, **72**, 121.
476. Vasile, M. J., and Malm, D. L., *Anal. Chem.*, 1972, **44**, 650.
477. Krendelev, F. P., Teplov, S. N., Karmanov, N. S., and Yablokova, S. V., *Geol. Rudn. Mestorozhd.*, 1980, **22**, 89.
478. Karpov, Yu. A., and Alimarin, I. P., *J. Anal. Chem. USSR*, 1979, **34**, 1085.
479. Mahalingham, T. R., Murugaiyan, P., Sonni, K. S., and Venkateswarlu, C., Report No. BARC-797, Bhabha Atomic Research Centre, Bombay, 1975, 7 pp.
480. Callis, E. L., Report No. NBL-272, New Brunswick Laboratories, New Brunswick, NJ, 1974, pp. 62–65.
481. Haney, M. A., and Gallagher, J. F., *J. Forensic Sci.*, 1975, **20**, 484.
482. Colby, B. N., and Evans, C. A., Jr., *Anal. Chem.*, 1974, **46**, 1236.
483. Maciejewska, A., and Substyk, D., *Pr. Nauk. Inst. Chem. Nieorg. Metal. Pierwiastkow Rzadkich, Politech. Wroclaw.*, 1975, **32**, 67.
484. Graham, M. J., and Caplan, D., *J. Electrochem. Soc.*, 1973, **120**, 769.
485. Pauwels, J., Report No. EUR 6304, Commission of the European Communities, Brussels, 1979, 14 pp.
486. Surowiec, J., *Pr. Nauk. Inst. Chem. Nieorg. Metal. Pierwiastkow Rzadkich, Politech. Wroclaw.* 1975, **32**, 103.
487. Fowkes, A. J., *Br. Ceram. Res. Assoc. Spec. Publ.*, 1975, No. 86, 82.
488. Berkey, E., and Hickam, W. M., *Adv. Mass Spectrom.*, 1971, **5**, 543.
489. Berkey, E., Sweeney, G. G., and Hickam, W. M., *Trans. Am. Nucl. Soc.*, 1971, **14**, 616.
490. Crocker, I. H., Armstrong, S. V., and Taylor, J. G. V., Report No. PR-CMa-40, Atomic Energy of Canada Ltd., Chalk River, Ontario, 1977, p. 48.
491. Bunus, F., Dumitrescu, P., Rodeanu, T., and Spiridon, S., *J. Radional. Chem.*, 1978, **42**, 463.

492. Northrup, C. J. M., Jr., *J. Phys. Chem.*, 1975, **79**, 726.
493. Conzemius, R. J., and Svec, H. J., *Abstr. 22nd Annu. Conf. Mass Spectrom. Allied Topics, Philadelphia, 1974*, pp. 420–423.
494. Van Deventer, E. H., Renner, T. A., Pelto, R. H., and Maroni, V. A., *J. Nucl. Mater.*, 1977, **64**, 241.
495. Taylor, J. B., Calvert, L. D., Despault, J. G., Gabe, E. J., and Murray, J. J., *J. Less-Common Met.*, 1974, **37**, 217.
496. Beske, H. E., Frerichs, G., Jahn, M. D., and Melchers, F. G., *Mikrochim. Acta, Suppl.*, 1979, **8**, 493.
497. Wrigley, K. B., *Proc. Soc. Anal. Chem.*, 1972, **9**, 298.
498. Spedding, F. H., Sanden, B., and Beaudry, B. J., *J. Less-Common Met.*, 1973, **31**, 1.
499. Taylor, J. B., Calvert, L. D., Utsunomiya, T., Wang, Y., and Despault, J. G., *J. Less-Common Met.*, 1978, **57**, 39.
500. Buffereau, M., Vierney, J., De La Graviere, M., and Pichotin, B., *in* Karr, C., Jr., *Editor*, "Analytical Methods in the Nuclear Fuel Cycle," IAEA, Vienna, 1972, pp. 77–99.
501. Chastagner, P., and Tiffany, B., *Adv. Mass Spectrom.*, 1971, **5**, 539.
502. Ganivet, M., Genty, C., Huart, A., and Pichotin, B., *in* Karr, C., Jr., *Editor*, "Analytical Methods in the Nuclear Fuel Cycle," IAEA, Vienna, 1972, pp. 101–113.
503. Billon, J. P., *Bull. Inf. Sci. Tech., Commis. Energ. At.* (Fr.), 1975, **200**, 93.
504. Billon, J. P., Buffereau, M., and Deniaud, S., Report No. CEA-N-1790, Commissariat à l'Energie Atomique, Paris, 18 pp.
505. Billon, J. P., Buffereau, M., and Deniaud, S., *Proc. XVIII Colloq. Spectrosc. Int., Grenoble*, 1975, **3**, 820.
506. Guidoboni, R. J., and Evans, C. A., Jr., *Anal. Chem.*, 1972, **44**, 2027.
507. Sato, K., Uzuki, Y., Suzuki, R., and Yamaguchi, N., *Tetsu To Hagane*, 1972, **58**, 1495.
508. Van Craen, M., Verlinden, J., Gijbels, R., and Adams, F., *Talanta*, 1982, **29**, 773.
509. Stefani, R., Andreani, A. M., Berthod, J., and Eloy, J. F., *Abstr. 22nd Annu. Conf. Mass Spectrom. Allied Topics, Philadelphia, 1974*, pp. 425–427.
510. Crocker, I. H., Armstrong, S. V., and Taylor, J. G. V., Report No. AECL-5851, Atomic Energy of Canada Ltd., Chalk River, Ontario, 1977, pp. 37–39.
511. Christie, W. H., Carter, J. A., Eby, R. E., and Landau, L., *Anal. Lett.*, 1979, **12A**, 1123.
512. Vanderborght, B., and Van Grieken, R., *Anal. Chim. Acta*, 1977, **89**, 399.
513. Siu, D. P., and Immorlica, A. A., Jr., Report No. ERC 41013.3RF, Rockwell International, Thousand Oaks, CA, 1980, 54 pp.
514. Jansen, J. A. J., and Witmer, A. W., *Fresenius Z. Anal. Chem.*, 1981, **309**, 305.
515. Cueilleron, J., Thevenot, F., and Viala, J. C., in Tavadze, F. N., *Editor*, "Boron. Production, Structure and Properties," Izdatel'stvo Nauka, Moscow, 1974, pp. 27–32.
516. Thevenot, F., and Viala, J. C., *Analusis*, 1975, **3**, 76.
517. Crocker, I. H., and Wray, L. W., *Can. Spectrosc.*, 1971, **16**, 77.
518. Gauneau, M., *Spectra 2000*, 1979, **7**, No. 53, 52.
519. Saprykin, A. I., Shelpakova, I. R., Yudelevich, I. G., and Kanter, Yu. O., *Izv. Sib. Otd. Akad. Nauk SSR, Ser. Khim. Nauk*, 1979, **4**, 100.
520. Huber, A. M., and Moulin, M., *J. Radioanal. Chem.*, 1972, **12**, 75.
521. Honig, R. E., *Thin Solid Films*, 1976, **31**, 89.
522. Lindstrom, R. M., Fleming, R. F., and Paulsen, P. J., *Nat. Bur. Stand. (US) Spec. Publ.*, 1980, No. 572, 61.
523. Gaivoronskii, P. E., Larin, N. V., and Chernikovich, M. P., *Poluch. Anal. Veshchestv. Osoboi Chist. (5th Dokl. Vses. Konf. Moscow, USSR, 1976)*, 1978, 165.
524. Look, D. C., *J. Appl. Phys.*, 1977, **48**, 5141.
525. Dorokhov, A. N., Shelpakova, I. R., Yudelevich, I. G., and Christanova, S. T., *Izv. Sib. Otd. Akad. Nauk SSR, Ser. Khim. Nauk*, 1977, 61.
526. Brozel, M. R., Clegg, J. B., and Newmann, R. C., *J. Phys. D*, 1978, **11**, 1331.
527. Huber, A. M., Morillot, G., Merenda, P., and Linh, N. T., *in* Goldanskii, V. I., Gomer, R., Schafer, F. P., and Toennies, J. P., *Editors*, "Proceedings of the 2nd International Conference on Secondary Ion Mass Spectrometry (SIMS II)," Springer-Verlag, Berlin, 1979, pp. 91–94.
528. Clegg, J. B., Grainger, F., and Gale, I. G., *J. Mater. Sci.*, 1980, **15**, 747.
528a. Blackmore, G. W., Clegg, J., Hislop, J., and Mullin, J., *J. Electron. Mater.*, 1976, **5**, 401.
528b. Martin, G. M., Jacob, G., Hallais, J., Grainger, F., Roberts, J. A., Clegg, J. B., Blood, P., and Poiblaud, G., *J. Phys. C.*, 1982, **15**, 1841.
528c. Clegg, J. B., *in* Makram-Ebeid, S., and Tuck, B., *Editors*, "Semi-insulating III-V Materials," Shiva Publishing, Nantwich, 1982. p. 80.
528d. Clegg, J. B., Mullin, J., Timmins, K., Blackmore, G., Everett, G., and Snook R., *J. Electron. Mater.*, 1983, **12**, 879.
529. Tong, S. S. C., and Williams, J. P., *J. Am. Ceram. Soc.*, 1970, **53**, 58.
530. Ko, R., Report No. HEDL-SA-392, Hanford Engineering Development Laboratory, Richland, WA, 1972, 9 pp.
531. Beveridge, R. L., *Appl. Spectrosc.*, 1973, **27**, 271.
532. Gladskoi, V. M., and Belousov, V. I., *Tr. Khim. Khim. Tekhnol.*, 1973, 110.
533. Billon, J. P., Buffereau, M., Sanson, C., and Vienney, J., Report No. CEA-N-1788, CEA Centre d'Etudes de Bruyers-le-Chatel, Montrouge, France, 1975, 15 pp.
534. Williams, J. P., Su, Y.-S., and Wise, W. M., *Microchim. Acta*, 1977, **II**, 527.
535. Allegre, J., *Spectra 2000*, 1979, **7**, No. 53, 61.
536. Alkire, G. J., Report No. HEDL-6798, Hanford Engineering Development Laboratory, Richland, WA, 1981, 6 pp.
537. Fitchett, A. W., and Buck, R. P., *Anal. Chem.*, 1973, **45**, 1027.
538. Kaushik, S. B., Gupta, S. R., Chatterjee, S. N., and Laroia, K. K., *Indian J., Pure Appl. Phys.*, 1974, **12**, 389.
539. Spedding, F. H., Beaudry, B. J., Henderson, D. C., and Moorman, J., *J. Chem. Phys.*, 1974, **60**, 1578.
540. Barnes, I. L., Moore, L. J., Machlan, L. A., Murphy, T. J., and Shields, W. R., *J. Res. Natl. Bur. Stand., Sect. A*, 1975, **79**, 727.
541. Dietze, H. J., and Zahn, H., *ZIF. Mitt.*, 1982, **46**, 60.
542. Kowalczyk, J., Lukas, W., and Tomasik, Z., *Pr. Nauk. Inst. Chem. Nieorg. Metal. Pierwiastkow Rzadkich Politech. Wroclaw.*, 1975, **32**, 17.
543. Crocker, I. H., Schruder, J. A., Bokwa, S. R., and Hildebrandt, B. W., Report No. AECL-5851, Atomic Energy of Canada Ltd., Chalk River, Ontario, 1977, pp. 39–40.
544. Russell, D. S., *Chem. Can.* 1979, **31**, 13.
545. Garner, E. L., Murphy, T. J., Gramlich, J. W., Paulsen, P. J., and Barnes, I. L., *J. Res. Natl. Bur. Stand., Sect. A*, 1975, **79**, 713.
546. Maier. H., Daniel, D. R., Herkert, R., and Luck, J., *J. Mater. Sci.*, 1978, **13**, 297.
547. Bergholz, J., Luck, J., Möller, P., and Szacki, W., *Fresenius Z. Anal. Chem.*, 1974, **269**, 121.
548. Butterworth, A., German, B., Morgans, D., and Scaplehorn, A., *J. Forensic Sci. Soc.*, 1974, **14**, 41.
549. Fuller, C. W., Himsworth, G., and Whitehead, J., *Analyst*, 1971, **96**, 177.
550. Dabbs, M. D. G., German, B., Pearson, E. F., and Scaplehorn, A. W., *J. Forensic Sci. Soc.*, 1973, **13**, 281.
551. Ikeda, K., *Bunseki Kiki*, 1973, **11**, 456.
552. Drayton, J. V., *Chem. Br.*, 1975, **11**, 439.
553. Haney, M. A., *J. Forensic Sci.*, 1977, **22**, 535.
554. German, B., Morgans, D., Butterworth, A., and Scaplehorn, A., *J. Forensic Sci. Soc.*, 1978, **18**, 113.
555. Locke, J., Boase, D., and Smalldon, K. W., *J. Forensic Sci. Soc.*, 1978, **18**, 123.
556. Stefani, R., Berthod, J., Pellet, J., Bourguillot, R., Cornu, A., and Bouchez, R., Report No. CEA-CONF-2406, Commissariat à l'Energie Atomique, Saclay, 1973, 16 pp.
557. Jacobs, M. L., Sweeney, S. L., and Rogowski, M. F., *Abstr. 27th Pittsburg Conf. Anal. Chem. Appl. Spectrosc., Cleveland, OH, 1976*, No. 220.
558. Sharkey, A. G., Jr., *in* Freudenthal, R. I., and Jones, P. W., *Editors*, "Aromatic Hydrocarbons: Chemistry, Metabolism and Carcinogenesis," Raven Press, New York, 1976, pp. 341–347.
559. Kelley, J. A., *Radiochem. Radioanal. Lett.*, 1973, **14**, 95.
560. Gray, L. W., and Ondrejoin, R. A., Report No. DP-MS-74-45, E. I. Du Pont de Nemours, Aiken, SC, 1974, 22 pp.
561. Bodnar, L. Z., Report No. NBL-277, New Brunswick Laboratories, New Brunswick, NJ, 1976 pp. 14–19.
562. Naudin, G., *Spectra 2000*, 1979, **7**, No. 54, 65.

563. Briden, F., Dorsey, J., and Johnson, L., *Int. J. Environ. Anal. Chem.*, 1981, **9**, 189.
564. Briden, F., and Lewis, D., *Int. J. Environ. Anal. Chem.*, 1981, **9**, 249.
565. Brown, R., and Vossen, P. G. T., *Anal. Chem.*, 1970, **42**, 1820.
566. Daughtrey, E. H., and Bostick, J. K., *Abstr. 27th Annu. Conf. Mass Spectrom. Allied Topics, Seattle, 1979*, pp. 410–411.
567. Carter, J. A., Walker, R. L., and Sites, J. R., *Am. Chem. Soc., Div. Fuel. Chem., Prepr.*, 1973, **18**, 78.
568. Davison, R. L., Natusch, D. F. S., Wallace, J. R., and Evans, C. A., Jr., *Environ. Sci. Technol.*, 1974, **8**, 1107.
569. Natusch, D. F. S., Wallace, J. R., and Evans, C. A., Jr., *Science*, 1974, **183**, 202.
570. Von Lehmden, D. J., Jungers, R. H., and Lee, E. R., Jr., *Anal. Chem.*, 1974, **46**, 239.
571. Daughtrey, E. H., Jr., *Abstr. 24th Annu. Conf. Mass Spectrom. Allied Topics, San Diego, 1976*, pp. 636–640.
572. Linton, R. W., Loh, A., Natusch, D. F. S., Evans, C. A., Jr., and Williams, P., *Science*, 1976, **191**, 852.
573. Trischan, G. M., *Abstr. 27th Annu. Conf. Mass Spectrom. Allied Topics, Seattle, 1979*, pp. 406–409.
574. Kessler, T., Sharkey, A. G., and Friedel, R. A., Report No. USBM-TPR-42, US Bureau of Mines, Pittsburgh, 1971, 15 pp.
575. Sharkey, A. G., Jr., Kessler, T., and Friedel, R. A., *Adv. Chem. Ser.*, 1975, No. 141, 48.
576. Bolton, N. E., Carter, J. A., Emery, J. F., Feldman, C., Fulkerson, W., Hulett, L. D., and Lyon, W. S., *Am. Chem. Soc., Div. Fuel Chem., Prepr.*, 1973, **18**, 114.
577. Taylor, C. E., Report No. EPA/660/2-74-078, Environmental Protection Agency, Athens, GA, 1974, 41 pp.
578. Brown, R., Taylor, H. E., and Mesich, F., *Abstr. Pittsburgh Conf. Anal. Chem. Appl. Spectrosc., Cleveland, OH*, 1975, No. 1.
579. Gasior, S. J., Lett, R. G., Strakey, J. P., and Haynes, W. P., *Am. Chem. Soc., Div. Fuel Chem., Prepr.*, 1978, **23**, 88.
580. Ure, A. M., *Anal. Proc.*, 1980, **17**, 409.
581. Taylor, C. E., and Taylor, W. J., Report No. EPA-660/2-74-001, Environmental Protection Agency, Athens, GA, 1974, 30 pp.
582. Moody, J. R., Paulsen, P. J., Rains, T. C., and Rook, H. I., *in* Lafleur, P. D., *Editor*, "Accuracy in Trace Analysis: Sampling, Sample Handing, Analysis," Volume 1, National Bureau of Standards, Gaithersburg, MD, 1976, pp. 267–273.
583. Paulsen, P. J., and Moody, J. R., *Abstr. 24th Annu. Conf. Mass Spectrom. Allied Topics, San Diego, 1976*, pp. 501–503.
584. Ribaudo, C., Campbell, C., Bulusu, S., Fisco, W., Chen, T., Kramer, H., and Castorina, T., Report No. ARLCD-TR-81028, US Army Armament Research and Development Command, Dover, NJ, 1981, 61 pp.
585. Brown, R., and Vossen, P. G. T., *in* Westley, B., *Editor*, "Identification and Measurement of Environmental Pollutants," National Research Council of Canada, Ottawa, 1971, pp. 427–431.
586. Klose, M., *Fresenius Z. Anal. Chem.*, 1971, **254**, 195.
587. Crocker, I. H., and Merritt, W. F., *Water Res.*, 1972, **6**, 285.
588. Wahlgren, M. A., Edgington, D. N., and Rawlings, F. F., Report No. ANL-7860 (Pt. 3), Argonne National Laboratory, Argonne, IL, 1972, pp. 55–63.
589. Crocker, I. H., *Am. Soc. Test. Mater. Spec. Tech. Publ.*, 1975, STP573, 71.
590. Vandelannote, R., Blommaert, W., Gijbels, R., and Van Grieken, R., *Fresenius Z. Anal. Chem.*, 1981, **309**, 291.
591. Klose, M., *Fresenius Z. Anal. Chem.*, 1971, **254**, 7.
592. Watson, J. C., Report No. PLN-MA-572, Batelle Pacific Northwest Laboratories, Richland, WA, 1978, 406 pp.
593. Sturgeon, R. E., Berman, S. S., Desaulniers, A., and Russell, D. S., *Anal. Chem*, 1979, **51**, 2364.
594. Mykytiuk, A. P., Russell, D. S., and Sturgeon, R. E., *Anal. Chem.*, 1980, **52**, 1281.
595. Sturgeon, R. E., Berman, S. S., Desaulniers, J. A. H., Mykytiuk, A. P., McLaren, J. W., and Russell, D. S., *Anal. Chem.*, 1980, **52**, 1585.
596. Walters, D. B., and Russell, R. B., *Abstr. 24th Southeastern Regional ACS Meeting, Birmingham, AL, 1972*, No. 92.
597. Pellizzari, E. D., Bursey, J. T., Smith, D. J., Castillo, N. P., and Willis, S. L., *Abstr. 26th Annu. Conf. Mass Spectrom. Allied Topics, St. Louis, 1978*, pp. 42–45.
598. Kessler, T., Sharkey, A. G., and Friedel, R. A., Report No. PB-204151, US Bureau of Mines, Pittsburgh, 1971, 19 pp.
599. Guidoboni, R. J., *Anal. Chem.*, 1973, **45**, 1275.
600. Kessler, T., Sharkey, A. G., Jr., and Friedel, R. A., Report No. RI-7714, US Bureau of Mines, Pittsburgh, 1973, 8 pp.
601. Pollock, E. N., *Am. Chem. Soc., Div. Fuel Chem., Prepr.*, 1973, **18**, 92.
602. Daughtrey, E. H., Jr., and Bostrick, J. K., Jr., *Abstr. 25th Annu. Conf. Mass Spectrom. Allied Topics, Wahsington, DC, 1977*, pp. 700–702.
603. Lyon, W. S., Lindberg, S. E., Emery, J. F., Carter, J. A., Ferguson, N. M., Van Hook, R. I., and Raridon, R. J., "Nuclear Activation Techniques in the Life Sciences," IAEA, Vienna, 1978, pp. 615–625.
604. Kronberg, B. I., Brown, J. R., Fyfe, W. S., Peirce, M., and Winder, C. G., *Fuel*, 1981, **60**, 59.
605. Jungers, R. H., Carter, J. A., Clay, D. A., and Bumgarner, J. E., Report No. CONF-760401-4, Oak Ridge National Laboratory, Oak Ridge, TN, 1976, 22 pp.
606. Alkire, G. J., Anderson, H. J., Delvin, W. L., Hartcorn, L. A., Matsumoto, W. Y., and Moore, R. L., *in* Karr, C., Jr., *Editor*, "Analytical Methods in the Nuclear Fuel Cycle," IAEA, Vienna, 1972, pp. 295–313.
607. Christie, O. H. J., *Talanta*, 1975, **22**, 1048.
608. Howarth, R., and Rankin, P. C., *Chem. Geol.*, 1975, **15**, 239.
609. Jackson, P. F. S., and Strelow, F. W. E., *Chem. Geol.*, 1975, **15**, 303.
610. Maksimovic, Z., and Roaldset, E., *in* "Travaux du Comite International pour l'Etude des Bauxites, de l'Alumine et de l'Aluminium," Academie Yougoslave des Sciences et des Arts, Zagreb, 1976, No. 13, pp. 199–220.
611. Roaldset, E., *Geochim. Cosmochim. Acta*, 1975, **39**, 455.
612. Rankin, P. C., *Chishitsugaku Zasshi*, 1976, **82**, 215.
613. Rankin, P. C., and Childs, C. W., *Chem. Geol.*, 1976, **18**, 55.
614. De Albuquerque, C. A. R., *Geochim. Cosmochim. Acta*, 1977, **41**, 1.
615. Jackson, P. F. S., and Strasheim, A., "Extended Abstracts, International Symposium on the Analytical Chemistry of Exploration, Mining and Processing Materials, 1976," CSIR, Pretoria, South Africa, 1977, pp. 606–610.
616. Rankin, P. C., *N. Z. Dep. Sci. Ind. Res. Bull.*, 1977, **218**, 110.
617. Taylor, S. R., Capp, A. C., Graham, A. L., and Blake, D. H., *Contrib. Mineral. Petrol.*, 1969, **23**, 1.
618. Jakes, P., and Gill, J., *Earth Planet. Sci. Lett.*, 1970, **9**, 17.
619. Roaldset, E., and Rosenqvist, I. Th., *Lithos*, 1971, **4**, 417.
620. Roaldset, E., *Lithos*, 1973, **6**, 349.
621. Cook, E. W., *Chem. Geol.*, 1973, **11**, 321.
622. Neuilly, M., Bussac, J., Frejacques, G. N., Vendryes, G., and Yvon, J., *C. R. Acad. Sci., Ser. D*, 1972, **275**, 1847.
623. Hagemann, R., Nief, G., and Roth, E., *Bull. Inf. Sci. Technol., Paris*, 1974, **193**, 65.
624. Frejacques, C., Blain, C., Devillers, R., and Ruffenach, J. C., "The Oklo Phenomenon," IAEA, Vienna, 1975, pp. 509–524.
625. Walton, R. D., Jr., and Cowan, G. A., "The Oklo Phenomenon," IAEA, Vienna, 1975, pp. 499–507.
626. Roaldset, E., and Christie, O. H. J., *Schweiz. Mineral. Petrogr. Mitt.*, 1975, **55**, 191.
627. Neiva, A. M. R., *Chem. Geol.*, 1975, **16**, 153.
628. Glikson, A. Y., *Geochim. Cosmochim. Acta*, 1976, **40**, 1261.
629. Kyle, P. R., and Rankin, P. C., *Geochim. Cosmochim. Acta*, 1976, **40**, 1497.
630. Peccerillo, A., and Taylor, S. R., *Earth Planet. Sci. Lett.*, 1976, **32**, 121.
631. Peccerillo, A., and Taylor, S. R., *Contrib. Mineral. Petrol.*, 1976, **58**, 63.
632. Gorton, M. P., *Geochim. Cosmochim. Acta*, 1977, **41**, 1257.
633. Taylor, S. R., and Hallberg, J. A., *Geochim. Cosmochim. Acta*, 1977, **41**, 1125.
634. McLennan, S. M., and Taylor, S. R., *Nature (London)*, 1979, **282**, 247.
635. Taylor, S. R., and McLennan, S. M., *Geochim. Cosmochim. Acta*, 1979, **43**, 1551.
636. Bavinton, O. A., and Taylor, S. R., *Geochim. Cosmochim. Acta*, 1980, **44**, 639.
637. McLennan, S. M., and Taylor, S. R., *Nature (London)*, 1980, **285**, 621.
638. Neiva, A. M. R., *Chem. Geol.*, 1980, **29**, 49.
639. Jenner, G. A., *Chem. Geol.*, 1981, **33**, 307.
640. Bhatia, M. R., and Taylor, S. R., *Chem. Geol.*, 1981, **33**, 115.
641. Irving, A. J., and Price, R. C., *Geochim. Cosmochim. Acta*, 1981, **45**, 1309.

642. Taylor, H. E., and Lichte, F. E., *Geophys. Res. Lett.*, 1980, **7**, 949.
643. McCulloch, M. T., and Perfit, M. R., *Earth Planet. Sci. Lett.*, 1981, **56**, 167.
644. Whitford, D. J., White, W. M., and Jezek, P. A., *Geochim. Cosmochim. Acta*, 1981, **45**, 989.
645. Cornu, A., and Brun, J. C., *Dosage Elem. Etat Traces Roches Autres Subst. Miner. Natur., Actes Colloq., 1968*, 1970, 215.
646. Hunt, M. H., *Abstr. 18th Annu. Conf. Mass Spectrom. Allied Topics, San Francisco, 1970*, pp. 240–244.
647. Kopp, O. C., and Statts, P. A., *J. Phys. Chem. Solids*, 1970, **31**, 2469.
648. Nalbantoglu, M., *Dosage Elem. Etat Traces Roches Autres Subst. Miner. Natur., Actes Colloq., 1968*, 1970, 223.
649. Agiorgitis, G., Panagos, A., and Marien, R., *Chem. Erde*, 1976, **35**, 199.
650. Viczian, M., *Foldt. Kozl.*, 1978, **108**, 47.
651. Kronberg, B. I., Couston, J. F., Stilianidi Filho, B., Fyfe, W. S., Nash, R. A., and Sugden, D., *Econ. Geol.*, 1979, **74**, 1869.
652. Ramendik, G. I., and Lisitsyn, A. E., *Geochem. Inter.*, 1979, 117.
653. Cesbron, F., and Drin, N., Report No. CEA-R-5118, Centre d'Etudes Nucleaires de Fontenay-aux-Roses, France, 1981, 110 pp.
654. Erämetsä, O., and Haukka, M., *Suom. Kem.*, 1970, **B43**, 189.
655. Van Hook, R. I., *Bull. Environ. Contam. Toxicol.*, 1974, **12**, 509.
656. Ure, A. M., Bacon, J. R., Berrow, M. L., and Watt, J. J., *Geoderma*, 1979, **22**, 1.
657. Tong, S. C., Gutenmann, W. H., St. John, L. E., and Lisk, D. J., *Anal. Chem.*, 1972, **44**, 1069.
658. Ure, A. M., and Bacon, J. R., *Geochim. Cosmochim. Acta*, 1978, **42**, 651.
659. Hintenberger, H., and Berghof, W., *in* Ogata, K., and Hayakawa, T., *Editors*, "Recent Developments in Mass Spectroscopy," University Park Press, Baltimore, 1970, pp. 657–667.
660. Mason, B., and Graham, A. L., *Smithson. Contrib. Earth Sci.*, 1970, **3**, 1.
661. Smales, A. A., Mapper, D., Webb, M. S. W., Webster, R. K., and Wilson, J. D., *Science*, 1970, **167**, 509.
662. Morrison, G. H., Nadkarni, R. A., Potter, N. M., Rothenberg, A. M., and Wong, S. F., *Radiochem. Radioanal. Lett.*, 1972, **11**, 251.
663. Allen, R. O., Jr., and Mason, B., *Geochim. Cosmochim. Acta*, 1973, **37**, 1435.
664. Hintenberger, H., Jochum, K. P., and Seufert, M., *Earth Planet. Sci. Lett.*, 1973, **20**, 391.
665. Hintenberger, H., Jochum, K. P., and Seufert, M., *in* Kiesl, W., and Malissa, H., Jr., *Editors*, "Analyse extraterrestrichen Materials," Springer-Verlag, Vienna, 1974, pp. 125–145.
666. Jochum, K. P., Seufert, M., and Hintenberger, H., *Meteoritics*, 1974, **9**, 355.
667. Jacques, A. L., Lowenstein, P. L., Green, D. H., Kiss, E., Nance, W. B., Taylor, S. R., and Ware, N. G., *Meteoritics*, 1975, **10**, 289.
668. Shima, M., Jochum, K. P., Sighinolfi, G. P., and Hintenberger, H., *Meteoritics*, 1974, **9**, 199.
669. Jochum, K. P., Hintenberger, H., and Buchwald, V. F., *Meteoritics*, 1975, **10**, 419.
670. Mason, B., Nelen, J. A., Muir, P., and Taylor, S. R., *Meteoritics*, 1975, **11**, 21.
671. Hintenberger, H., Jochum, K. P., Braun, O., Christ, P., and Martin, W., *Earth Planet. Sci. Lett.*, 1978, **40**, 187.
672. Knab, H. J., and Hintenberger, H., *Meteoritics*, 1978, **13**, 522.
673. Li, Z.-H., Liang, Z.-C., Zhou, H., Li, Y.-G., Zhao, D.-Z., and Zhang, M.-L., *Ti Ch'iu Hua Hsueh*, 1979, 249.
674. Jochum, K. P., Grais, K. I., and Hintenberger, H., *Meteoritics*, 1980, **15**, 31.
675. Jochum, K. P., Seufert, M., and Bergmann, F., *Z., Naturforsch., Teil A*, 1980, **35**, 57.
676. Knab, H. J., *Geochim. Cosmochim. Acta*, 1981, **45**, 1563.
677. Oro, J., Updegrove, W. S., Gilbert, J., McReynolds, J., Gil-av, E., Ibanez, J., Zlatkis, A., Flory, D. A., Levy, R. L., and Wolf, C. J., *in* Levinson, A. A., *Editor*, "Proceedings of the Apollo 11 Lunar Science Conference," Volume 2, Pergamon Press, Oxford, 1970, pp. 1901–1920.
678. Bouchet, M., Kaplan, G., Voudon, A., and Beroletti, M.-J., *in* Levinson, A. A., *Editor*, "Proceedings of the 2nd Lunar Science Conference," Volume 2, MIT Press, Cambridge, MA, 1971, pp. 1247–1252.
679. Morrison, G. H., Gerard, J. T., Potter, N. M., Gangadharam, E. V., Rothenberg, A. M., and Burdo, R. A., *in* Levinson, A. A., *Editor*, "Proceedings of the 2nd Lunar Science Conference," Volume 2, MIT Press, Cambridge, MA, 1971, pp. 1169–1185.
680. Smales, A. A., Mapper, D., Webb, M. S. W., Webster, R. K., Wilson, J. D., and Hislop, J. S., *in* Levinson, A. A., *Editor*, "Proceedings of the 2nd Lunar Science Conference," Volume 2, MIT Press, Cambridge, MA, 1971 pp. 1253–1258.
681. Taylor, S. R., Rudowski, R., Muir, P., Graham, A., and Kaye, M., *in* Levinson, A. A., *Editor*, "Proceedings of the 2nd Lunar Science Conference," Volume 2, MIT Press, Cambridge, MA, 1971, pp. 1083–1089.
682. Strasheim, A., Jackson, P. F. S., Coetzee, J. H. J., Strelow, F. W. E., Wybenga, F. T., Gricus, A. J., Kokot, M. L., and Scott, R. H., *in* Heymann, D., *Editor*, "Proceedings of the 3rd Lunar Science Conference," Volume 2, MIT Press, Cambridge, MA, 1972, pp. 1337–1342.
683. Taylor, S. R., Kaye, M., Muir, P., Nance, W., Rudowski, R., and Ware, N., *in* Heymann, D., *Editor*, "Proceedings of the 3rd Lunar Science Conference," Volume 2, MIT Press, Cambridge, MA, 1972, pp. 1231–1249.
684. Taylor, S. R., Gorton, M. P., Muir, P., Nance, W. B., Rudowski, R., and Ware, N., *Geochim. Cosmochim. Acta*, 1973, **37**, 2665.
685. Taylor, S. R., Gorton, M. P., Muir, P., Nance, W., Rudowski, R., and Ware, N., "Proceeedings of the 4th Lunar Science Conference," Volume 2, Pergamon Press, New York, 1973, pp. 1445–1459.
686. Hubbard, N. J., Ramendik, G. I., Gronskaya, S. I., Gubina, I. Ya., and Gushchin, V. N., *Geokhimiya*, 1975, 803.
687. Hubbard, N. J., Vinogradov, A. P., Ramendik, G. I., and Chupakhin, M. S., "The Soviet American Conference on Cosmochemistry of the Moon and Planets," Part 1, NASA Special Publication SP-370, 1977, pp. 259–262.
688. Hamilton, E. I., Minski, M. J., and Cleary, J. J., *Sci. Total Environ.*, 1972/73, **1**, 341.
689. Morrison, G. H., *ICP Inf. Newsl.*, 1977, **3**, 165.
690. Erämetsä, E., Erämetsä, O., and Yliruokanen, I., *Suom. Kem.*, 1972, **B45**, 311.
691. Cornides, I., Pais, I., and Viczian, M., in *Proc. XVII Colloq. Spectrosc. Int., Florence*, 1973, **1**, 301.
692. Erämetsä, O., Haarala, A.-R., and Yliruokanen, I., *Suom. Kem.*, 1973, **B46**, 234.
693. Erämetsä, O., Viinanen, R., and Yliruokanen, I., *Suom. Kem.*, 1973, **B46**, 355.
694. Yliruokanen, I., *Bull. Geol. Soc. Finl.*, 1975, **47**, 71.
695. Yliruokanen, I., *Ann. Acad. Sci. Fenn., Ser. A2*, 1975, **176**, 28 pp.
696. Chamel, A., Andreani, A. M., and Mercadier, D., *Commun. Soil Sci. Plant Anal.*, 1979, **10**, 1311.
697. Leonhardt, J. W., Dahn, E., Dietze, H. J., Freyer, K., Geisler, M., Hartmann, G., Jung, K., and Schelhorn, H., "Nuclear Activation Techniques in the Life Sciences," IAEA, Vienna, 1979, pp. 91–102.
698. Ramaswamy, K., Perumal, R., and Pandurangan, S. V., *Appl. Spectrosc.*, 1979, **33**, 516.
699. Chamel, A. R., Andreani, A. M., and Eloy, J. F., *Plant. Physiol.*, 1981, **67**, 457.
700. Ball, D. F., Barber, M., and Vossen, P. G. T., *Sci. Total Environ.*, 1973, **2**, 101.
701. Ball, D. F., Barber, M., and Vossen, P. G. T., *Sci. Total Environ.*, 1975, **4**, 193.
702. Ball, D. F., Barber, M., and Vossen, P. G. T., *Biomed. Mass Spectrom.*, 1974, **1**, 365.
703. Erämetsä, O., and Sihvonen, M. L., *Ann. Med. Exp. Biol. Fenn.*, 1971, **49**, 35.
704. Harrison, W. W., Clemena, G. G., and Magee, C. W., *J. Assoc. Off. Anal. Chem.*, 1971, **54**, 929.
705. Harrison, W. W., and Clemena, G. G., *Clin. Chim. Acta*, 1972, **36**, 485.
706. Losee, F., Cutress, T. W., and Brown, R., *in* Hemphill, D. S., *Editor*, "Trace Substances in Environmental Health," University of Missouri, Columbia, MO, 1973, pp. 19–24.

707. Gooddy, W., Williams, T. R., and Nicholas, D., *Brain*, 1974, **97**, 327.
708. Gooddy, W., Hamilton, E. I., and Williams, T. R., *Brain*, 1975, **98**, 65.
709. Christie, O. H. J., Dinh-Nguyen, N., Vincent, J., Hellgren, L., and Pimlott, W., *J. Invest. Dermatol.*, 1976, **67**, 587.
710. Findlay, G. H., and Vismer, H. F., *Br. J. Dermatol.*, 1977, **97**, 497.
711. Murphy, R. C., *Abstr. 28th Annu. Conf. Mass Spectrom. Allied Topics, New York, 1980*, p. 208.
712. Hamilton, E. I., and Minski, M. J., *Sci. Total Environ.*, 1972/73, **1**, 375.
713. Pommez, P., and Clarke, M. A., Report No. ARS-S-51, US Agricultural Research Service, Southern Region, New Orleans, LA, 1975, pp. 40–46.
714. Tong, S. S. G., Morse, R. A., Bache, C. A., and Lisk, D. J., *Arch. Environ. Health*, 1975, **30**, 329.
715. Locke, J., Carpenter, R., and Osselton, M. D., *Med. Sci. Law*, 1981, **21**, 123.
716. Davis, R. E., *in* Shirley, D. A., *Editor*, "Electron Spectroscopy," North Holland, Amsterdam, 1972, pp. 903–908.
717. Aoki, M., and Kurosaki, K., *Kobunshi Kagaku*, 1972, **29**, 442.
718. Callis, E. L., Report No. NBL-272, New Brunswick Laboratories, New Brunswick, NJ, 1974, p. 61.
719. Cristy, S. S., and Annis, B. K., Report No. Y-Da-5554, Oak Ridge Y-12 Plant, Oak Ridge, TN, 1973, 27 pp.
720. Braier, H. A., and Eppolito, J., *in* Yen, T. F., *Editor*, "Role of Trace Metals in Petroleum," Ann Arbor Science Publishers, Ann Arbor, MI, 1975, pp. 65–87.
721. Baudin, G., *J. Radioanal. Chem.*, 1977, **37**, 119.
722. Morrison, G. H., *in* Lafleur, P. D., *Editor*, "Accuracy in Trace Analysis: Sampling, Sample Handling, Analysis," Volume 1, National Bureau of Standards, Gaithersburg, MD, 1976, pp. 65–77.
723. Kane, P. F., *Chem. Technol.*, 1971, 532.
724. Bingham, R. A., and Salter, P. L., *Int. J. Mass Spectrom. Ion Phys.*, 1976, **21**, 133.
725. Conzemius, R. J., and Svec, R. J., *Abstr. 24th Annu. Conf. Mass Spectrom. Allied Topics, San Diego, 1976*, pp. 323–326.
726. Opauszky, I., *Pure Appl. Chem.*, 1982, **54**, 879.
727. Date, A. R., and Gray, A. L., *Analyst*, 1981, **106**, 1255.
728. Cantle, J. E., Hall, E. F., Shaw, C. J., and Turner, P. J., *Int. J. Mass Spectrom. Ion Phys.*, 1983, **46**, 11.

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Evaluation of an Electrothermal Atomisation Procedure for the Determination of Lead in Potable Water

John Webster*
Department of Water and Drainage, Lothian Regional Council, 6 Cockburn Street, Edinburgh, EH1 1NZ, UK

and Andrew Wood
Napier College, Edinburgh, UK

An electrothermal atomisation procedure using a lanthanum-impregnated tube was investigated for its suitability for the routine determination of lead in potable water. The precision and bias were found to be acceptable but to ensure adoption of the procedure for routine use a detailed comparison was made between the results and those obtained using two other common methods involving concentration followed by atomic-absorption spectrophotometry. The first involved concentration by evaporation and the second extraction of the ammonium tetramethylenedithiocarbamate complex into isobutyl methyl ketone. A statistical comparison of data from all three procedures was made.

Keywords: *Lead determination; potable water; atomic-absorption spectrophotometry; electrothermal atomisation; lanthanum-treated tube*

Electrothermal atomisation procedures are widely used in many fields for the direct determination of trace metals in a variety of materials. The determination of lead in water has been the subject of many papers but has not been widely accepted because of reports of serious matrix interference effects; various reagents have been suggested for eliminating or reducing such effects or for improving the precision.[1,2]

In addition, L'vov[3] has recommended the use of a modified tube design to ensure isothermal heating of the sample at atomisation and to give increased sensitivity and improved precision of analysis, a technique adopted by Sturgeon *et al.*[4] for the analysis of marine sediments. More recently, it was reported that either the addition of lanthanum to samples or impregnation of the tube overcame interferences in the determination of lead in a variety of potable waters and met the requirements recommended by the Water Research Centre for analytical methods by having a total standard deviation of not more than 1.5 μg l^{-1} or 5% of the concentration and a bias of not more than 5 μg l^{-1} or 10% of the concentration, whichever was the greater.[5]

It was decided to investigate this procedure using an impregnated tube[6] as part of the work to be undertaken by a student from Napier College, Edinburgh, on a 6-month work experience secondment to the Lothian Region's Water and Drainage Department.

The background to the project lay in the need to analyse comparatively large numbers of household tap waters as part of a programme to identify areas of high lead content (>100 μg l^{-1}) within the City of Edinburgh originating from the use of lead tanks or piping within older properties. The water supplied to the City comes from upland sources and is relatively unpolluted and of low hardness. In some areas the raw water may be coloured by humic acids, but this is much reduced by treatment. During the course of the investigation a range of raw and treated waters from within the Region were used for tests and the chemical characteristics of four of these, selected to cover a range of hardness and organic carbon content, are given in Table 1. Most of the work was carried out on samples originating from Alnwickhill and Fairmilehead, as these supplies provide the bulk of the water to the City, particularly in the areas where elevated lead levels would be expected.

The statistical performance of the electrothermal atomisation procedure was evaluated after optimising the instrumental conditions, and the data produced by this procedure were compared with lead levels measured routinely in the laboratory using flame AAS after concentration by evaporation. In addition, a number of samples were analysed in replicate by extraction of the ammonium tetramethylenedithiocarbamate (ammonium pyrrolidinedithiocarbamate; APDC) complex into a solvent, again followed by flame AAS.

Experimental

Reagents

Nitric acid, 70% *m/V*. Aristar grade (BDH Chemicals).
Lanthanum nitrate, $La(NO_3)_3$. Analytical-reagent grade.

Apparatus

All glassware was soaked in 10% nitric acid for 24 h before use. Measurements were made using a Perkin-Elmer 272 atomic-absorption spectrophotometer with a Perkin-Elmer HGA 2200 graphite furnace accessory, which allowed the use of pre-set drying, ashing and atomising temparature cycles but with no ramping. Background correction was made by deuterium arc and a lead hollow-cathode lamp (IL) was used at 283.3 nm.

Signals were recorded on a 10-mV recorder as peak heights. Twenty-microlitre aliquots of sample and standard were used for all measurements, delivered by a Perkin-Elmer autosampler into pyrolytically coated graphite tubes (Perkin-Elmer) held in the light path by end-clamps, which also served as electrical contacts. The tubes were impregnated with lanthanum as described below. Disposable, acid-soaked, 4-ml polystyrene cups were used with the autosampling accessory.

Electrothermal Atomisation

A tube was soaked in saturated lanthanum nitrate solution overnight, wiped dry, then subjected to a series of heating runs in the range 110–500 °C, wiping any crystals from the outside of the tube each time with a tissue. Finally, a programmed run with atomisation at 2200 °C was made at least twice or until no signal was observed during the run. It was important to follow this procedure with a new tube in

* To whom correspondence should be addressed.

Table 1. Summary of major ion concentrations in waters used during tests. All results in milligrams per litre except where stated

Property	Alnwickhill	Fairmilehead	Pateshill	Harburnhead
Conductivity/μS cm^{-1}	95–120	70–110	100–130	250–280
Total solids	60–80	40–60	85–105	150–180
Hardness ($CaCO_3$)	35–50	30–45	45–70	120–150
Chloride (Cl)	8–11	5–8	6–12	7–9
Sodium (Na)	5	4	5	6
Potassium (K)	0.5	0.3	0.6	1.3
Magnesium (Mg)	2–4	1–2.5	2–3.5	3.5–4.8
Total organic carbon (C)	2–3	2–4	8–13	1–3

Table 2. Recovery data for lead by the APDC procedure

Sample	Lead added/μg l^{-1}	Lead found*/μg l^{-1}
Distilled water	25.0	28.7
	75.0	70.0
	100.0	98.7
Alnwickhill supply	0	1
	50	52
Fairmilehead supply	0	5
	50	52
Castle Moffat (raw)	0	1
	50	45.5

* Duplicate measurements.

order to avoid short-circuiting of the power or physical damage.

The conditions of analysis were optimised according to the procedures outlined in the manufacturer's manual using a sample of raw water comparatively high in organic carbon (11 mg l^{-1}) and spiked with 50 μg l^{-1} of lead.

The optimised conditions for subsequent analysis were as follows:

Drying temperature	110 °C; time	50 s
Charring temperature	500 °C; time	30 s
Atomisation temperature	2200 °C; time	5 s

The argon flow used to purge the tube during a run was set at 30 units on the meter (300 ml min^{-1}) with a flow stop time of 3 s at atomisation.

All samples and standards were acidified with 10 ml of concentrated nitric acid per litre of sample.

Concentration by Evaporation Procedure

A 200-ml volume of acidified sample was transferred into a beaker, then concentrated to about 20 ml on a hot-plate before transfer into a 25-ml calibrated flask and dilution to the mark, giving an 8-fold concentration. The lead level was measured at 283.3 nm by flame AAS on a second instrument (IL 151) with background correction from a deuterium hollow-cathode lamp.

Extraction of APDC Complex Procedure

The method employed was essentially that described in "Methods for the Examination of Water and Associated Materials."[7] One modification was introduced to avoid having to aspirate isobutyl methyl ketone into the AAS instrument.

Lead was back-extracted from the solvent into 25 ml of 50% nitric acid then determined by flame AAS. Excellent recoveries were obtained for standards treated in this way and for spiked water supply samples, as can be seen from Table 2. The APDC method was used as an independent comparison to test if results from the furnace gave statistically acceptable values for lead.

Results and Discussion

Performance Characteristics of the Electrothermal Atomisation Procedure

Fig. 1 shows a calibration graph, corrected for the blank due to trace amounts of lead in the nitric acid. The blank was equal to about 1–2 μg l^{-1} in the samples. No scale expansion was employed during the runs; typically a 100 μg l^{-1} lead standard gave a peak height of about 120 mm, which was equivalent to an absorbance of 0.5.

The calibration in this instance included five points in the 1–10 μg l^{-1} range and the linearity is excellent (correlation coefficient = 1.0) with the line passing through the origin. Some performance characteristics are given below.

Substance determined	Lead		
Type of sample	Tested on raw and treated water		
Calibration graph	Linear to at least 100 μg l^{-1}		
Standard deviation (within batch)	The following are pooled estimates:		
	15 μg l^{-1}	1.54 μg l^{-1}	13 D.F.*
	39	2.02	45 D.F.
	126	5.81	28 D.F.
Criterion of detection	Estimated at 3.2 μg l^{-1} (17 D.F.)		
Bias	Typically less than 5 μg l^{-1} or 10% of the concentration (see Table 3)		

In practice the calibration was defined in each run using six standards in the range 10–100 μg l^{-1} inclusive, with the result that any between-batch random errors caused by calibration were minimised. The within-batch standard deviation was calculated by pooling estimates of standard deviation obtained from 15 runs over a period of 2 months to give the values reported above.

The criterion of detection was calculated from replicate analyses of a blank solution consisting of acidified distilled water as used in the preparation of standards. The value of 3.2 μg l^{-1} was calculated from $2.33S_w$ for the blank.

Tests for Bias

Bias was tested from the difference between the "true" result as measured by the standard additions method and that obtained from direct analysis using a lanthanum-impregnated tube. Confidence limits were calculated on the result obtained by direct analysis and used with the difference between the results from the two procedures to see if this exceeded 10% of the concentration found by standard addition or 5 μg l^{-1}, whichever was the greater. Table 3 presents some of the results obtained.

Only the result from supply sample B was unacceptable, and this was associated with a higher than usual standard deviation attributed at the time to a tube nearing the end of its useful

* Degrees of freedom.

Table 3. Test for bias by standard additions

Sample	"True" result from standard additions	Direct electrothermal analysis	Difference*	Max. allowable difference
Raw Alnwickhill (spiked)	81.0	80.8	0.17 ± 0.62	8.1
Raw Fairmilehead (spiked)	96.0	94.5	1.5 ± 0.45	9.6
Tap water A	133	134.5	1.5 ± 0.69	13.3
B	61	54.2	6.8 ± 1.21	6.1
C	40	36.8	3.2 ± 0.34	5.0
D	91	88.8	2.2 ± 0.62	9.1

* With 90% confidence limits.

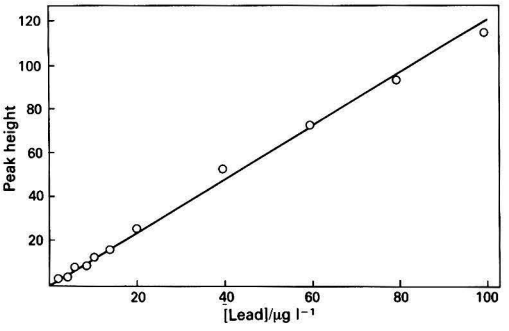

Fig. 1. Lead calibration graph

life. Experience indicated that a treated tube could be used for about 200 cycles before deterioration became evident by loss of precision. Examination of the tube suggested that the trouble was due to the build-up of deposits within the tube, perhaps resulting in occlusion of the sample and subsequent difficulty at the atomisation stage.

In general, however, the results obtained by direct determination are not significantly different from those by standard additions and, with one exception, meet the requirements of a bias of less than 10% of the concentration or 5 μg l^{-1}.

In view of the acceptable results obtained from these tests, a detailed comparison was made of results obtained by the concentration by evaporation and flame AAS procedure used routinely by the laboratory and direct electrothermal analysis.

Comparison with Results from Concentration by Evaporation - Flame AAS

During the project, measurements were made on many of the samples routinely received by the laboratory so that a comparison could be made between the two procedures. Over a period of 2 months, 86 pairs of results were obtained in this way.

The differences between the lead values found by each procedure were statistically compared, on the assumption that both procedures should yield the same result. The actual differences should therefore in theory be normally distributed with a mean of zero and a test made as to whether the mean difference is significant.

When all 86 results were considered and the calculated value for the *t*-statistic was compared with the tabulated value, there was a significant difference at the 95% confidence level ($t = 5.9$ compared with 1.96). However, inspection of the data showed that early runs gave poorer agreement than later runs, possibly owing to greater experience with the electrothermal atomisation technique and increased care on the part of analysts in general. To test this, later sets of results for two runs about a week apart were compared in the same way. The data contained 33 pairs and on this occasion gave a calculated *t*-statistic of 1.82 compared with the tabulated value of 2.01, confirming that more recent measurements by the two procedures were not statistically different.

Table 4. Comparison of results of concentration by evaporation - AAS and electrothermal atomisation procedures

Tap water sample	Concentration/μg l^{-1} AAS	Electrothermal atomisation	Difference (D)
A	32	40	+8
B	134	131	−3
C	97	89	−8
D	5	13	+8
E	93	86	−7
F	23	26	+3
G	5	11	+6
H	10	7	−3
I	32	31	−1
J	32	32	0
K	51	53	+2
L	46	38	−8
M	42	36	−6
N	139	136	−3
O	87	84	−3

Number of samples $(N) = 15$

$\Sigma D = -15$

$(\Sigma D)^2 = 225$

$\Sigma D^2 = 427$

$$S = \sqrt{\frac{\Sigma D^2 - (\Sigma D)^2/N}{N-1}} = 5.4$$

$$t = \frac{(\Sigma D/N) - 0}{S/\sqrt{N}} = 0.71$$

Table 4 shows a complete batch of results obtained by the two procedures from a routine survey of tap waters from various sources within the Region. The agreement is generally good and a *t*-test showed no statistically significant difference ($t = 0.71$ compared with 2.14 for 14 degrees of freedom). The most obvious discrepancy occurs for very low levels of lead, in the 5–15 μg l^{-1} range, which were easily measured by the electrothermal atomisation procedure but were at the limit of detection for the flame AAS procedure even after concentration of the samples.

Closer examination of the latter procedure indicated a high noise level on the recorder trace (2–3%) with resulting uncertainty in drawing a base line. Background correction on the instrument (an IL 151) was poor owing to the condition of the deuterium lamp, which required high currents when in use, with a resulting increase in noise. The detection limit of the flame AAS procedure was estimated at 0.07 mg l^{-1}, so that the concentrations in many samples, even after 8-fold concentration, were too close to this for adequately precise results to be obtained.

Table 5. Comparison of APDC and electrothermal atomisation results (μg l^{-1}) using the test for non-homogeneous standard deviations

	APDC method		Electrothermal atomisation method			
Sample	Mean ($\bar{x}_A$)	Standard deviation (S_A)	Mean ($\bar{x}_E$)	Standard deviation (S_E)	Tan θ* = $S_{\bar{x}A}/S_{\bar{x}E}$	$\frac{\lvert\bar{x}_A - \bar{x}_E\rvert}{\sqrt{S_{\bar{x}A}^2 + S_{\bar{x}E}^2}}$
Alnwickhill (spiked)	31.0	5.33	35.3	1.93	2.77	1.85
Fairmilehead (spiked)	31.0	3.52	29.1	0.93	3.79	1.26
Tap water A	45.0	2.19	51.0	4.05	0.54	3.19
B	158.2	6.50	134.5	0.84	8.50	8.10
C	47.2	12.07	54.2	1.47	8.98	1.28
D	164.8	24.02	172.3	1.37	19.3	0.70
E	37.2	4.60	36.8	0.41	12.33	0.18
F	130.0	6.68	122.2	0.98	8.33	2.33
G	87.2	8.87	82.5	0.55	17.71	1.18
H	76.7	6.41	80.8	0.75	8.52	1.58
I	95.8	5.88	94.5	0.55	10.7	0.55

* The values of tan θ represent angles ranging from 70 to 87°. Critical values at the 5% level of significance and for 6 degrees of freedom are 60° = 2.435, 75° = 2.440 and 90° = 2.447. The value of $|\bar{x}_A - \bar{x}_E|/\sqrt{S_{\bar{x}A}^2 + S_{\bar{x}E}^2}$ should therefore be less 2.4 for no statistically significant difference between the means for the two procedures.

Concentration by Extraction of APDC Complex Followed by AAS

The modified APDC procedure described earlier was used on a number of samples over the period of the investigation. Each sample was analysed in replicate (usually five or six times) by both the APDC and electrothermal atomisation procedures. The mean values were then compared to see if the difference was statistically significant. Table 5 gives the results for 11 sets of results.

Table 5 shows very high standard deviations for the APDC procedure, but confirms the excellent precision of the electrothermal atomisation method. The marked difference in standard deviation invalidated the use of *t*-testing so the means were compared by a test for non-homogeneous variances.[8] The ratio of the standard deviations for the two sample means (tan θ) was used to determine the significance of the difference between the means as summarised in Table 5. Only one result was highly significant on this criterion, with the APDC method giving a mean of 158.2 compared with 134.5 by the electrothermal atomisation procedure. The standard additions technique, when used with the electrothermal atomisation procedure on this sample, gave 133 μg l^{-1}, so it seems likely that the APDC data were less accurate in this instance.

In general, however, the direct electrothermal atomisation procedure gave results comparable to those obtained by the APDC method but with a much superior precision, in many instances by a factor of 10, although this could probably be improved with more experience of the APDC procedure.

Conclusions

The work reported confirms that the use of lanthanum either to impregnate the tube, as in this instance, or added to solutions prior to the atomisation, as reported by Bertenshaw *et al.*,[5] can eliminate matrix interferences in the determination of lead in potable waters. The precision and bias were acceptable and excellent recoveries were obtained from a range of raw and treated waters.

The results obtained by direct electrothermal atomisation showed no statistically significant difference from those obtained by flame AAS after concentration. In addition, generally acceptable agreement was observed between the mean values obtained by replicate analysis by the electrothermal atomisation and an APDC - AAS procedure.

Waters from the Lothian Region are low in dissolved salts compared with supplies from many parts of the UK and may therefore be expected to suffer less from matrix effects. Such effects were nevertheless present when analysis was attempted using an untreated tube (*e.g.*, only 72% recovery for 50 μg l^{-1} added to Pateshill water) and lanthanum treatment effectively improved the recovery figures, giving a simple and rapid procedure for determining low levels of lead.

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References

1. Regan, J. G. T., and Warren, J., *Analyst*, 1978, **103**, 447.
2. Hodges, D. J., *Analyst*, 1977, **102**, 66.
3. L'vov, B. V., *Spectrochim. Acta, Part B*, 1978, **33**, 153.
4. Sturgeon, R. E., Desaulniers, J. A. H., Berman, S. S., and Russell, D. S., *Anal. Chim. Acta*, 1982, **134**, 283.
5. Bertenshaw, M. P., Gelsthorpe, D., and Wheatstone, K. C., *Analyst*, 1981, **106**, 23.
6. Thompson, K. C., and Reynolds, R. J., "Atomic Absorption, Fluorescence and Flame Emission Spectrophotometry," Charles Griffin, London, 1978, p. 231.
7. "Lead in Potable Waters by Atomic Absorption Spectrophotometry," Methods for the Examination of Waters and Associated Materials, HM Stationery Office, London, 1976.
8. Kennedy, T. B., and Neville, A. M., "Basic Statistical Methods for Engineers and Scientists," Second Edition, Harper International, New York, 1976, p. 213.

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Collaborative Study of a Graphite-furnace Atomic-absorption Screening Method for the Determination of Lead in Infant Formulas*

Robert W. Dabeka
Food Research Division, Food Directorate, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada K1A 0L2

Blind duplicates of three infant formulas and one evaporated milk were analysed by nine laboratories. Lead levels in the samples ranged from 29 to 200 ng g^{-1}. Only one of the laboratories obtained good agreement with reference values for all samples; two laboratories were outliers. Analysis of the data indicated that the remaining laboratories had instrumentation problems, the main cause of which was inadequate simultaneous background correction. The mean over-all sample reproducibility (relative standard deviation; RSD) was 66.8% for the data as submitted and 18.7% after adjustment for background correction. The mean precision between blind duplicates was 38.9% (RSD) for the submitted data and 6.6% after adjustment and rejection of statistical outliers.

The results imply that for matrices and elements for which moderate (0.2 absorbance unit) non-specific background can be expected, most laboratories with conventional background correctors will have difficulty in obtaining accurate results using graphite-furnace atomic-absorption spectrometry.

Keywords: *Lead determination; graphite-furnace atomic-absorption spectrometry; infant formulas; collaborative study*

A study conducted by the International Atomic Energy Agency for the inter-laboratory determination of trace elements in a single lot of powdered milk[1] revealed that the world status of methodology for lead in milk is very poor. Twelve laboratories reported lead values for the sample ranging from 0.017 to 246 μg g^{-1}, a range of over four orders of magnitude.

The toxicological importance of lead has prompted several countries to promulgate regulations specifying the maximum allowable level of lead in canned milks and infant formulas. As a result, many samples require accurate and precise analyses for lead. As compliance methods are usually time consuming and expensive for regulatory laboratories, rapid screening methods, which can considerably reduce the number of unnecessary compliance analyses, provide an inexpensive means of assessing the approximate levels of lead in formulas, whilst simultaneously increasing the number of samples that can be analysed.

A collaborative study of one such graphite-furnace atomic-absorption spectrometric method,[2] which has the advantages of using a single unwashed polystyrene test-tube for each sample and inexpensive reagents, is described in this paper. The purpose of the collaborative study was to evaluate the performance of the method in different laboratories. This paper analyses the results of the study, detailing the instrumental difficulties encountered when graphite-furnace atomic-absorption spectrometric methods progress from one laboratory to many.

Experimental

Design of Study

Four samples were chosen: one evaporated milk [2% butter fat (B.F.)], one milk-free formula (concentrated liquid) and two milk-base formulas (ready-to-use). The lead levels in all samples were background levels, ranging from 29 to 200 ng g^{-1}, and no samples were spiked. All samples had been purchased commercially and were in lead-soldered cans. Some had been stored in the laboratory for several years, explaining the high lead levels.

The lead levels in the formulas were determined using a reference method[3] and the rapid screening method being tested. The latter method was also used to test sample homogeneity, which was about ±5% of the confirmed lead levels for all samples for the sample sizes sent to collaborators.

Samples (blind duplicates randomly numbered) and instructions were sent to 14 collaborators. Samples were sent in their digestion tubes. No practice sample was included in the study. In addition, each collaborator received empty unwashed test-tubes, for preparation of standards and reagent blanks, a bottle of modification solution (0.5% citric acid, 0.5% hydrogen peroxide and 0.25% ammonium dihydrogen phosphate), two washed pipettes and two lead stock standard solutions (0.2 and 0.4 μg ml^{-1}) for preparation of working standards. The stock standards had been prepared from US National Bureau of Standards certified lead nitrate and were in 1% nitric acid.

Collaborators had to supply their own concentrated nitric acid (about 30 ml in total) for digestions, and skim milk for standards. It was requested that the skim milk be purchased locally from a retail outlet, and the concentrated nitric acid contain less than 10 ng g^{-1} of lead.

Procedure

To 2 ml of ready-to-use or 1 ml of concentrated-liquid formula was added 1 ml of concentrated nitric acid. The test-tubes were tightly capped and heated at 58–63 °C for 6–16 h in a water-bath. For reagent blanks, two test-tubes containing 1 ml of water and 1 ml of concentrated nitric acid were digested as above. For standards and standard blanks, seven test-tubes containing 2 ml of skim milk were digested as above. After digestion, four of the skim-milk digests were spiked with two lead stock standard solutions to yield final lead concentrations of 10, 20, 30 and 40 ng ml^{-1}. All sample, standard and blank digests were then diluted to 10 ml with modification solution, and the tubes were capped with their original caps and shaken briefly.

Determinations were made using graphite-furnace atomic-absorption spectrometry. The instrument conditions requested were as follows: aliquot volume, 10 μl for the HGA-400, HGA-500, HGA-2200, IL-455 and Hitachi 170-70, 20 μl for the HGA-2100 and HGA-2000 and 2 or 5 μl for the CRA-90 and CRA-63 instruments; drying temperatures adjusted so

* Collaborators: G. R. Cutris, G. G. Durany, F. Estiandan, D. A. Gooden, J.-P. Hanchay, L. J. Hart, C. D. Howes, P. A. Keller, M. J. Lingenfelser, L. H. Marion, R. A. Moffitt, L. I. Rivera, K. K. Rousey, T. L. Shannon, M. K. Tikkanen, N. G. Webb and R. W. Woods

Table 1. Sample description and collaborator results (ng g^{-1}) as submitted

Sample No.*	Sample description	Laboratory No. and values†										
		0‡	1	1A§	2	3	4	5	6	7	8	9
1(13)	Evaporated milk	57	48.1	44.2	62.0	44.4	25.0	29.4	265	286.8	34.4	105
2(32)	(2% B.F.)		50.9	42.3	59.3	31.5	3.7	59.1	167	36.3	59.9	109
3(14)	Conc. liquid	200	246	199	139	168	—	85.0	281	194.0	440.3	282
4(24)	soya base formula		244	184	140	187	236.8	101.7	308	189.5	296.6	277
5(12)	Ready-to-use	53	66.8	48.9	39.4	42.3	62.5	22.5	93	88.3	79.4	64
6(17)	milk base formula A		59.6	48.9	37.0	28.8	65.9	20.1	100	83.5	44.2	79
7(15)	Ready-to-use	29	27.4	23.9	8.9	10.6	20.2	11.9	78	17.9	4.7	9
8(34)	milk base formula B		31.7	26.8	8.9	10.6	22.1	18.6	75	16.2	44.2	34

* Numbers in parentheses were submitted to collaborators.
† Blind duplicate values for each sample.
‡ Reference value from this laboratory.
§ Values obtained by laboratory 1 using peak-area measurements. These values were not included in the statistical evaluation.

that 2-, 5-, 10- and 20-μl samples dry in about 5, 8, 12 and 20 s, respectively; ashing temperature and time, 650 °C for 20 s; recorder mode, continuous to monitor background noise during the atomisation cycle; background correction, simultaneous; gas-interrupt mode, if available, used during the atomisation step; graphite tubes, uncoated, if available; measurements, peak height; and other parameters, manufacturers' specifications.

Three replicate determinations were made for each solution. The measurement sequence was fixed in the following general pattern: 20 ng ml^{-1} standard - all blanks - all standards - all samples (any order) - all standards.

Results and Discussion

Raw Data

Nine laboratories participated in the study. The results, as submitted, are listed in Table 1. Only laboratory 1, which analysed the samples using both peak-height and peak-area measurements, obtained good agreement with reference values for all samples (Table 1). Most of the other laboratories obtained values for some samples that appeared reasonably acceptable; however, the results were generally poor.

Laboratory 4 reported that sample 3 was broken during shipping, and that losses were suspected during the digestion for samples 1 and 2. The value for the lost sample was set at 237 ng g^{-1}, that obtained for the blind duplicate, and was used in all subsequent statistical manipulations.

Identification of Problems

The raw data indicated that most of the collaborators experienced problems with the method. Two possible reasons for this were considered: contamination and instrumentation.

As the method was simple and there was little chance of contamination, and as most collaborators obtained good agreement between blind duplicates, contamination was excluded as the prime factor influencing the quality of results.

Instrumentation was investigated as the second reason for the poor results, and subsequent examination of the method using a new atomic-absorption spectrometer (Varian Model 775-ABQ) in our laboratory revealed that inadequate simultaneous background correction could cause accuracy problems. The non-specific background at the wavelength of 283.3 nm was about 0.13 absorbance unit using an ashing temperature of 650 °C. At the 217.0-nm line, the background was a factor of 2 greater and, using the Varian instrument at this wavelength, could not be adequately corrected. This problem was not detected when the method was first developed because, for the spectrometer used (Perkin-Elmer Model 403), only the wavelength of 283.3 nm recommended by the manufacturer was used, and extensive care was always taken to assure proper lamp alignment for background correction.

Table 2. Adjusted values (ng g^{-1}). A constant was added to all the reported solution concentrations so that a mean value of 29 ng g^{-1} was obtained for samples 7 and 8

Sample No.	Ref. value*	Laboratory†						
		1	2	3	4	6	7	9‡
1	57	47	101	80	40§	173¶	310¶	95
2	57	50	98	67	19§	75	59	98
3	200	245	198	204	251	189	218	208
4	200	243	178	223	251	217	212	203
5	53	66	60	61	70	45	100¶	55
6	53	59	57	47	74	53	95¶	65
7	29	27	29	29	28	30	30	22¶
8	29	31	29	29	30	28	28	36¶

* Composite using rapid screening method and extraction method.[3]
† Results from laboratories 5 and 8 excluded owing to poor instrument performance.
‡ Data based on linear least-squares fit for standards as well as adjustment.
§ Laboratory reported possibility of losses during digestion.
¶ Statistical outliers at 95% confidence level (Dixon or Cochran test).

On examining the recorder tracings submitted by six of the nine laboratories, it became obvious that inadequate background correction, revealed by the presence of negative peaks, or positive peaks or shoulders that occurred during atomisation and could not be attributed to lead, could indeed be one of the factors affecting the quality of the results. Assisting in the evaluation was the observation of differences between the time - absorbance profiles of blanks for standards containing skim milk and reagents without milk.

Adjustment of Data for Background Correction

The possible effects of background on the data was numerically evaluated by assuming that for any one laboratory uncorrected background absorption was identical for all sample solutions, regardless of tube age, and that any uncorrected background absorption did not affect the calibration slope. If these approximations are correct and if background is the main factor causing the poor results, it should be possible to improve the results by adjusting, for each collaborator, the reported solution concentrations by a constant factor so that the mean value for one of the samples equals the reference value. The sample chosen as a reference for this adjustment was ready-to-use milk-base formula B and its value was set at 29 ng g^{-1}. For laboratory 9, a calibration problem was detected and, prior to the background adjustment, a linear least-squares treatment of the calibration graph was made.

In spite of the crudeness of the approximations made, the results of the adjustments (Table 2) showed a significant

improvement for most of the laboratories, supporting the hypothesis that incomplete background correction was the major cause of the poor inter-laboratory performance of the method.

Laboratory 5 appeared to have a general accuracy problem, which revealed itself by erratic changes in sensitivity for some standards run before and after samples. The sensitivity increased 33% for the 10 ng ml^{-1} standard whereas, for the 40 ng ml^{-1} standard, it decreased by 8.7%. Based on linear least-squares fits for the two groups of standards (10, 20, 30 and 40 ng ml^{-1} in each group), the slopes of the calibration graphs before and after sample determinations changed by 22%, and the intercept with the x-axis changed by 0.079 absorbance unit, equivalent to a blank uncertainty range of about 6.3 ng ml^{-1}. Based on the author's experience with the CRA-63 and CRA-90 atomisers, the problem was probably due to unstable electrode - graphite tube contact, inappropriate drying temperature or too great an aliquot volume (5 µl). Although 2–5 µl was the recommended aliquot volume for the furnace, recent studies showed that 2 µl is the maximum aliquot volume that can be reliably used with the furnace.[4]

For laboratory 8 the instrument sensitivity was low and, after submission of results, a weak lead electrodeless discharge lamp was reported. This may have caused the poor precision between blind replicates [mean 55% (RSD)].

Owing to the above difficulties, laboratories 5 and 8 were treated as outliers.

Statistical Evaluation of Data

About 6 months after this collaborative study was run, our laboratory organised a workshop in which seven analysts from five Field Laboratories of the Health Protection Branch, Health and Welfare Canada, participated. One of the methods studied was the rapid screening method for lead in infant formulas, and the analysts were given the same samples and standards that were used in this collaborative study. Each analyst performed the sample analysis independently, although solutions were analysed using the same well aligned instrument. Results for the workshop were good and no procedural, homogeneity or contamination problems were encountered.[5] The results are summarised in Table 3, together with the collaborative study data.

For the adjusted data base (Table 2), six values were individual outliers at the 95% level of significance (Dixon and Cochran tests), and losses were suspected for two other samples. These values encompassed 14% of the data. In comparison, for the workshop results only one value was a statistical outlier.[5]

The mean levels for all collaborators agreed reasonably well between workshop and collaborative study results (Table 3); the major difference between the two studies was that the precision for the latter was much poorer. The mean RSD was 3.8% for the workshop and 38.9% for the submitted values for the collaborative study. Adjustment of data and rejection of outliers and samples suspected of losses reduced the latter value to 6.6%.

The mean over-all sample reproducibility (RSD) was 7.0% for the workshop and 66.8% for the collaborative study. The latter was reduced to 18.7% (range 11.1–30.2%) when the data were adjusted.

Instrument Performance

The collaborative study results demonstrated that accurate application of graphite-furnace atomisers is difficult to achieve on an inter-laboratory basis. As a fairly representative population of spectrometers, atomisers and autosamplers were used (Table 4), the problems did not appear to be limited to any specific model or design of spectrometer or atomiser. A discussion of instrument performance, diagnosed using precision and background correction parameters, is given to illustrate the nature of the problems encountered and the steps that can be taken to overcome them.

Table 3. Statistical evaluation of data

Parameter	Sample No.	Data base: Workshop values*	Data base: Submitted values (all labs.)	Data base: Adjusted values†
Mean levels/ng g^{-1}	1	60.0	82.0	77.0
	2	215.2	225.2	217.1
	3	57.6	59.8	59.3
	4	29.3	25.0	29.0‡
Standard deviation between replicates/ng g^{-1}	1	1.8	64.4	5.0
	2	7.0	35.0	10.7
	3	2.5	10.0	6.0
	4	1.3	11.2	1.5
RSD between replicates, %	1	3.0	78.4	6.2
	2	3.3	15.6	4.9
	3	4.3	16.7	10.1
	4	4.6	44.9	5.3
	1–4	3.8	38.9	6.6
Reproducibility (standard deviation)/ng g^{-1}	1	3.6	80.7	24.0
	2	14.6	87.9	23.9
	3	2.6	21.9	8.9
	4	3.1	21.6	1.1
Reproducibility (RSD), %	1	6.1	98.4	30.2
	2	6.8	39.0	11.0
	3	4.5	42.4	15.0
	4	10.5	87.5	3.7
	1–4	7.0	66.8	18.7§

* Data include 1 outlier out of 56 values in total.[5]

† Values adjusted according to Table 2. Laboratories 5 and 8 not included. Replicate pairs containing a statistical outlier and samples for which losses were suspected were not included in the calculations.

‡ As a result of adjustment, values meaningless for comparative purposes.

§ Value does not include sample 4.

Table 4. Instrumentation and performance characteristics

Lab. No.	AA spectrometer	Furnace	Autosampler	Aliquot volume/µl Used	Aliquot volume/µl Recommended	λ/nm	Instrument precision (mean % RSD) Standards	Instrument precision (mean % RSD) Sample	Mean RSD between duplicates, % Submitted	Mean RSD between duplicates, % Adjusted	Observed non-specific absorbance	Adjustment made for background/ng ml^{-1}	Standard blank−reagent blank/ng ml^{-1}	Analyst experience with GFAAS (months) General	Analyst experience with GFAAS (months) Lead detn.
1	IL-951	IL-551	IL-254	20	10	283.3	2.8	5.2	5.8	4.0	—	0.6	0.3	6–12	6–12
2	PE-503	HGA-2100	AS-1	20	20	283.3	1.6	11.4	2.0	2.3	Negative peaks	−2.9	1.1	<6	<6
3	PE-5000	HGA-500	AS-40	10	10	283.3	9.1	8.9	14.6	6.6	Positive peaks	7.8	−1.8	6–12	6–12
4	PE-603	HGA-400	AS-1	10	10	217.0	6.6	17.7	28.8	3.1	Positive peaks	−1.6	−0.5	>12	>12
5	Varian 475	CRA-90	ASD-53	5	2–5	217.0	9.7	12.6	24.8	—	Negative peaks	—	2.0	6–12	6–12
6	PE-306	HGA-2100	AS-1	20	20	283.3	2.3	2.6	11.6	6.2	Positive peaks	16.1	5.6	>12	>12
7	PE-305B	HGA-2100	AS-1	20	20	283.3	2.2	1.5	30.6	2.4	Positive peaks	3.9	0.2	>12	>12
8	PE-5000	HGA-500	None	20	10	217.0	9.8	12.1	55.1	—	—	—	−1.7	>12	>12
9	PE-306	HGA-2100	None	20	20	283.3	3.8	14.9	25.2	3.7	—	4.5	4.3	<6	<6
10	Varian 775	HGA-400	AS-1	10	10	283.3	1.3	2.4	3.8	—	—	0.0	—	—	—

For the lead levels determined in the collaborative study, all the instruments used, when properly optimised, should yield an instrumental precision better than 5% (RSD). Failure to achieve this is indicative of problems. For collaborators, instrument precision for samples [range 1.5–17.7% (RSD)] and standards [range 1.6–9.8% (RSD)] varied widely. The mean precision for the standards [5.3% (RSD)] was better than for the samples [9.7% (RSD)], and both were high in comparison with the respective workshop values of 1.3 and 2.4% (RSD). While laboratories 1, 6 and 7 obtained good precisions for both samples and standards, the precision for laboratories 3, 5 and 8 was generally poor (Table 4). The causes of poor general precision are traceable to inadequate background correction, too large an aliquot volume and instrument noise (caused by faulty lamps, electronic noise and poor optical throughput). For laboratories 2, 4 and 9, the precision for standards was reasonable [range 1.6–6.6% (RSD)]; however, for samples, the RSD was an average of 4.6 times higher. This effect is indicative of problems with furnace parameters (drying temperature, aliquot volume) and, to a smaller extent, with background correction deficiencies.

The ranges of aliquot volumes recommended by manufacturers for their furnaces usually include aliquot volumes too large to be handled accurately on a routine basis by any but highly skilled analysts. Large aliquot volumes can cause precision and accuracy problems owing to distribution effects within the furnace[4] (and for this method, significant background absorbance). As no publications have appeared on this topic, less experienced analysts tend to use aliquot volumes that are unsuitable for their furnaces in order to improve the detection limits. Although the author attempted to overcome this potential problem by specifying aliquot volumes for each model of furnace used, his recommendations of 20 µl for the HGA-2100 and 5 µl for the CRA-63 and CRA-90 were, in retrospect, too large for the furnaces. This may have had a significant impact on the collaborative study results because the above furnaces were used by half of the collaborators.

Regarding simultaneous background correction, the adjustments required for correction of inaccuracy due to non-specific absorbance varied from −2.9 to 16.1 ng ml^{-1}, indicative of both positive and negative background effects (Table 4). The submitted recorder tracings were ineffective in determining the sign of the background effect. While the effects of wavelength and aliquot volume on background correction were discussed, proper lamp alignment is just as important a parameter. For example, laboratory 3, using an appropriate wavelength and aliquot volume, still required a background adjustment of 7.8 ng ml^{-1}, equivalent to a 78 ng g^{-1} error for a 1-g sample. The most probable reason for this was improper alignment of the deuterium and lead lamps, the beams of which should be coincidental with respect to size and shape as they pass through the furnace. Because, for the specific spectrometer model, the deuterium lamp must be aligned by the manufacturer and the instrument allows little flexibility with respect to lead lamp alignment owing to the type of lamp mount, the analyst can do little to ensure proper background correction. All other spectrometers used by collaborators do allow analysts to make proper lamp alignment.

Conclusions

The collaborative study of the method was a failure, primarily owing to inadequate simultaneous background correction on the instruments used. This was substantiated by (a) significant improvement of the data adjusted for background, and (b) by the comparatively good results obtained by seven analysts using the same samples and standards in a workshop situation where only one well aligned instrument was used.

The importance of the results, however, extends beyond this particular method. The non-specific background for other elements and matrices often exceeds that for lead in milk. For instance, the background for cadmium at 228.8 nm is roughly twice that for lead at 283.3 nm for most of the food matrices studied in our laboratory, and even after dilution factors of 10, has been observed to exceed 1 absorbance unit. Thus, it can be predicted that no graphite-furnace cadmium method applicable to all foods is likely to achieve good inter-laboratory accuracy unless more powerful background-correction systems, such as Zeeman or Smith - Heifje, are used or the method includes the separation of cadmium from the bulk of the inorganic matrix.

The implications of this for future collaborative studies are multiple, relating both to the choice of methods and to verification of the adequacy of background correction.

For situations when background correction is applied but

not tested for adequacy, organisers of collaborative studies would be wise to choose methods for which the maximum non-specific background will not exceed roughly 0.05 absorbance unit, a level significantly smaller than that which can be conveniently corrected with well optimised instrumentation. This approach would ensure wide applicability of the method without the analyst spending excessive time optimising the background corrector of the spectrometer. Once a method has been chosen, samples giving the highest background levels that can be expected for the method should be included in the study.

For collaborative study of methods so convenient that meticulous instrument adjustment for compensation of moderate (0.1–0.5 absorbance unit) background is warranted, it is necessary to include, as part of the method, a method for ensuring adequacy of background correction. Unfortunately, there is currently no published graphite-furnace atomic-absorption method available whereby the analyst can guarantee that non-specific background is being corrected by the spectrometer. Approximations can be made, however, and the simplest way to do this would be to include, again as part of the method, analysis of a practice sample. Such a practice sample, to be valid and useful, would be unlike those commonly suggested for collaborative studies, for several reasons. Firstly, it would have to be accessible to all analysts wishing to use the method, *i.e.*, it would have to be a standard reference material such as those supplied by the US National Bureau of Standards. Secondly, the level of analyte in the sample would have to be certified and below or near the detection limit of the method. Thirdly, the level of background for the sample, after digestion, etc., would have to exceed that of the "worst" sample to which the method would be applied. Fourthly, the matrix composition of the test sample would have roughly to approximate that of real samples to avoid unexpected interferences and to match the source of the background. Using the method of interest, the sample is analysed twice, once unspiked and once spiked to give a signal roughly 10 times the detection limit of instrumentation. If the results for both the spiked and unspiked sample correspond to the true levels, it can be assumed that the instrument, as aligned, can adequately correct for the background of unknown samples.

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References

1. Dybczynski, R., Veglia, A., and Suschny O., "Report on the Intercomparison Run A-11 for the Determination of Inorganic Constituents of Milk Powder," IAEA/RL/68, International Atomic Energy Agency, Vienna, 1980.
2. Dabeka, R. W., "Rapid-screening Determination of Lead in Canned Milks and Infant Formulas Using Graphite-furnace Atomic-absorption Spectrometry," Procedure LPFC-114, Health Protection Branch Laboratory, Ottawa, Canada, 1980.
3. Dabeka, R. W., *Anal. Chem.*, 1979, **52**, 902.
4. Dabeka, R. W., "Drying Temperature Studies Using Graphite-furnace Atomic-absorption Spectrometry," 8th Annual Meeting, Federation of Analytical Chemistry and Spectroscopy Societies, Philadelphia, PA, Sept. 20–25, 1981.
5. Dabeka, R. W., *J. Assoc. Off. Anal. Chem.*, 1982, **65**, 1005.

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Low-pressure Evaporation Concentration Prior to Discrete Nebulisation Flame Spectroscopic Analysis

Fiona A. Robertson, Anthony C. Edwards and Malcolm S. Cresser
University of Aberdeen, Department of Soil Science, Meston Walk, Old Aberdeen, AB9 2UE, UK

The use of low-pressure evaporation in a vacuum desiccator for small-volume sample solutions is described. The procedure utilises hydrophobic plastic AutoAnalyzer cups without heating, which reduces deposition on the container walls, the final volume is assessed by weighing and therefore no dilution is required, no reagents are necessary and the system is closed to atmospheric contamination during the evaporation stage.

Keywords: *Pre-concentration; evaporation; atomic-absorption spectroscopy; atomic-emission spectroscopy*

Numerous pre-concentration techniques have been employed in analytical atomic spectroscopy over the years. In flame spectroscopy, where improved detectability is essential, solvent extraction is most widely used.[1] However, solvent extraction is not without problems for trace analysis. Substantial amounts of solvent and reagents of adequate purity are required, and the solvents used are invariably flammable. Moreover, a considerable input of skilled operator time is necessary, unless an expensive automated system is available. Cation exchange is generally regarded as a less popular alternative to solvent extraction but purified reagents are still required and manual sample processing is still very time consuming. Also, samples are unavoidably diluted upon elution unless the resin is dissolved or analysed as a solid.

Concentration by evaporation has never been very popular. At elevated temperatures, evaporation in glass vessels often leads to loss of sample by deposition or absorption on to the glass walls above the liquid surface. Evaporation is slow if the samples are covered, but open samples are prone to contamination from the atmosphere. A slow air bleed through the samples at reduced pressure speeds up the evaporation, but again may introduce contamination.

This paper describes the use of low-pressure evaporation in a vacuum desiccator for small-volume sample solutions. Hydrophobic plastic AutoAnalyzer sample cups are used without heating to reduce deposition on the container walls. The final volume is assessed by weighing, so no dilution is necessary. No reagents are required, and the system is closed to atmospheric contamination during the evaporation stage.

Experimental

Apparatus

A 240-mm i.d. vacuum desiccator was used with a sample holder made from a 182 × 106 mm rubber block containing five rows of ten 16-mm diameter holes, to hold 50 Technicon AutoAnalyzer standard sample cups. When more uniform evaporation was required, a 160-mm diameter disc sample cup holder was used. It was made from expanded polystyrene, with 24 equally spaced holes drilled out, their centres being 64 mm from the disc centre. Each desiccator was fitted with a sintered-glass filter in the extraction - air inlet line, to prevent dust entrainment on releasing the vacuum.

Procedure

Pipette 1.5 ml of each sample or standard into a sample cup, and weigh the cup to ±1 mg. Load the samples into a sample tray and place the tray in a vacuum desiccator over granular calcium chloride. Grease the lid thoroughly with vacuum grease, close and evacuate using a water pump until there is no change in the sound of the water pump when the desiccator tap is closed. This generally takes 10–15 min. Close the tap and leave to stand until the required degree of evaporation is observed. For 24 samples in a normal sized vacuum desiccator, 10-fold concentration is achieved in 10–24 h, depending on the efficiency of the water pump and the number of times the calcium chloride has already been used. Allow air to re-enter slowly, via a filter. Weigh the sample cups and contents to ±1 mg and calculate the mass of water evaporated, and hence the volume of solution remaining.

Determine the element of interest by flame atomic-absorption or -emission spectroscopy, using the discrete nebulisation technique,[2] reading the absorbance or emission intensity peak from a suitable chart recorder. Use matrix-matched standards, allowing for the concentration of matrix components if necessary, or run suitable standards and blanks through the evaporation procedure. Calculate the concentration factors, based on the volumes of solution remaining, and hence the amount of analyte in the original sample solutions.

Results and Discussion

Choice of Desiccant

The net removal of water from the sample surface, and hence the rate of concentration, depends on the rate at which water molecules escape from the surface of the sample solution, the rate of diffusion of water molecules to the desiccant surface and the rate of absorption of water molecules by the desiccant.

At normal atmospheric pressure, five desiccants were studied, *viz.*, granular calcium chloride, phosphorus pentoxide, concentrated sulphuric acid, anhydrous calcium sulphate (freshly ignited) and silica gel. Four sample cups, each containing 1.5 ml of distilled water, were weighed and placed in each of a series of 75-mm i.d. desiccators containing the five desiccants. The sample cups and contents were re-weighed after 18, 23, 40, 46 and 64 h, with a view to comparing the trapping efficiencies of the five desiccants, and to seeing if their efficiency deteriorated as they became wetter.

The efficiency of the desiccators decreased in the order sulphuric acid (most efficient) > calcium chloride > phosphorus pentoxide ≈ silica gel > calcium sulphate. Calcium chloride was used in subsequent work, because it was only marginally inferior to sulphuric acid and is safer to use under vacuum. For calcium chloride and sulphuric acid, the desiccant efficiency remained constant as the desiccants became wetter. For the other three desiccants, the absorption efficiency decreased with increasing time and wetness. Without evacuation, the samples lost only about 500 µl each over 60 h. The evaporation rates were greatly accelerated at low pressure, indicating that the evaporation of water was limited by the rate at which water molecules escaped from the sample solution surface and/or their rate of diffusion to the desiccant, as well as the trapping efficiency of the desiccant.

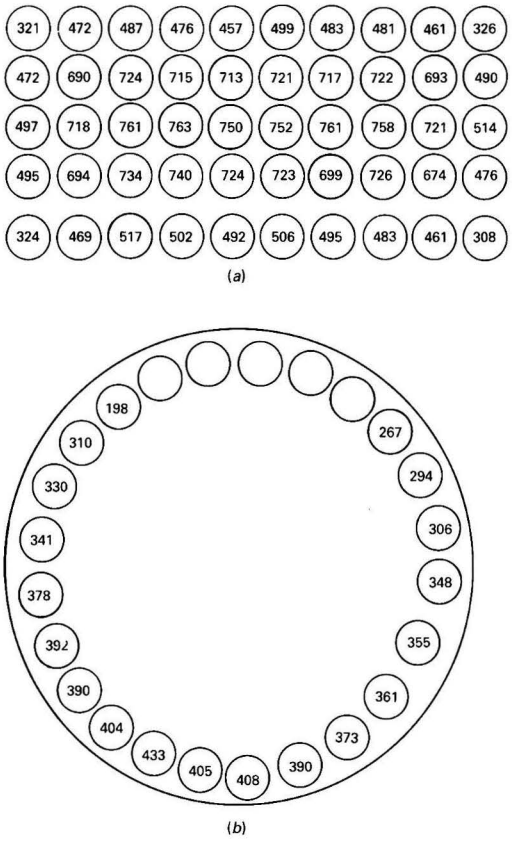

Fig. 1. Volumes (μl) remaining after evaporation from 1.5-ml sub-samples. (*a*) 50 water samples after 8 h; (*b*) 19 dilute hydrochloric acid extracts of plant ash after 8 h. Values obtained depend on pump efficiency and prior use of desiccant

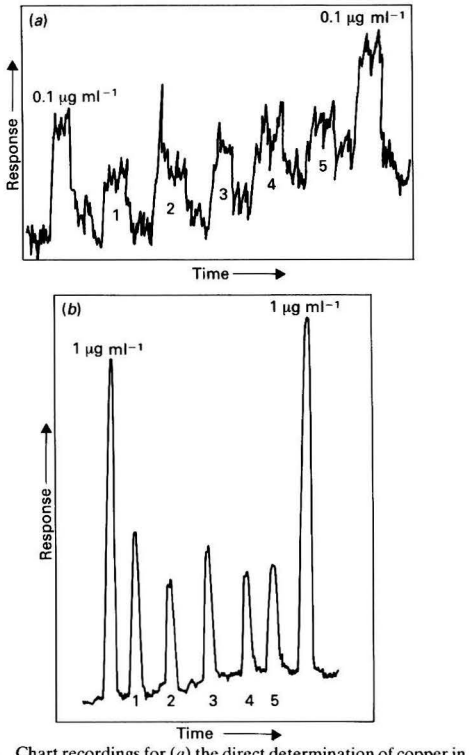

Fig. 2. Chart recordings for (*a*) the direct determination of copper in dilute hydrochloric acid extracts of five plant ash samples; and (*b*) for the same solutions after evaporation pre-concentration

Evaporation Rates at Reduced Pressure

Using a rectangular block containing five rows of ten sample cups, the rate of evaporation was very uneven, as shown in Fig. 1(*a*). For 1.5-ml samples, the evaporation was greatest at the block corners and lowest at the centre of the block. If a more uniform evaporation rate is required, a symmetrical, circular pattern of sample cups is necessary. A 162-mm disc containing 24 equally spaced cups around its circumference gave a uniform 10-fold concentration over 10–24 h, depending on pump efficiency and desiccant age. The desiccator should not be left permanently connected to the water pump once a suitable low pressure has been attained, because the water vapour pressure in the desiccator does not then fall below the saturation vapour pressure at the operating temperature of the pump, and the evaporation rate is thus reduced. It should be noted that if the disc sample holder is not completely filled, the evaporation rate is no longer uniform from sample to sample. The pattern shown in Fig. 1(*b*) for 0.06 M hydrochloric acid solutions from ashed plant samples for a batch of 19 samples is typical.

Examples of Applications

The technique has been applied successfully to the atomic-absorption determination of iron and manganese in natural fresh water samples. For iron, contamination problems were encountered initially. These were traced to minute but significant inputs of dust on releasing the vacuum. Using the rectangular sample block, contamination was greatest at the centre of the block, immediately under the desiccator tap, and lowest, but still a problem, at the edges. The effect was eliminated by placing a fine glass sinter in the air input line and slowing down the rate of vacuum release.

Reduced-pressure evaporation has also been applied to the determination of copper in 0.06 M hydrochloric acid solutions from 6 M acid extracts of ashed plant samples. Typical results with and without the evaporation concentration step are shown in Fig. 2. Copper was determined by atomic-absorption spectroscopy using a Baird A3400 spectrometer and an air - acetylene flame. The improved detectability and precision are immediately apparent. A 10-fold concentration of ten replicate 1.5-ml portions of a single hydrochloric acid digest gave a result of 0.080 μg ml^{-1} for copper in the ash extract, with a relative standard deviation of 1.9%.

Conclusions

Evaporation concentration in a vacuum desiccator provides a simple procedure for up to 10-fold pre-concentration of analyte elements prior to determination by discrete nebulisation flame atomic-absorption or -emission spectroscopy. Because the system is closed, and addition of reagents is generally unnecessary, contamination is minimal. Because no heating is used, inexpensive, hydrophobic plastic sample cups may be employed, and solid deposition on to the evaporation vessel walls, which in our experience may be a problem with heated glass systems, appears to be negligible. The conical shape of the bottom of the Technicon sample cups is ideal for discrete nebulisation of 100–200-μl samples.

Although in this work the technique has been used prior to flame atomic-absorption spectroscopy, it is also perfectly well suited to other techniques that may be successfully completed using small volumes of sample solution. If concentration factors greater than 10-fold are required, a second 1.5-ml portion of sample may be added after the first evaporation and the evaporation repeated to achieve 20-fold concentration. The use of mass change to measure final volume is very precise and eliminates the need to dilute to a fixed, small volume.

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References

1. Cresser, M. S., "Solvent Extraction in Flame Spectroscopic Analysis," Butterworths, London, 1978.
2. Fry, R. C., Northway, S. J., and Denton, M. B., *Anal. Chem.*, 1978, **50**, 1719.

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Combined Reagent Purification and Sample Dissolution (CORPAD) Applied to the Trace Analysis of Silicon, Silica and Quartz

V. J. Phelan and R. J. W. Powell
GEC Research Laboratories, Hirst Research Centre, Wembley, Middlesex, HA9 7PP, UK

A simple and reliable method is described for the determination of impurities in very high-purity single-crystal silicon and in siliceous materials of importance to the electronics industry. Reagent purification and sample dissolution take place simultaneously within a closed PTFE vessel; the analyses are completed by carbon furnace atomic-absorption spectrometry. The very low reagent blank levels enable aluminium, chromium, copper and iron to be determined at levels as low as 5 ng g^{-1}.

Keywords: *Silicon, silica and quartz analysis; reagent purification - sample dissolution; atomic-absorption spectrometry; trace metal determination*

The relevant desirable properties of some materials used in high-technology industries are seriously affected by trace metal impurities. The concentration above which an impurity has a deleterious effect on the behaviour of the material is often below the microgram per gram level. Three examples of such materials are given in Table 1 with indications of the impurity elements of interest, the range of interest and the properties affected by the impurity.

The detection and determination of metals at the required level can be achieved by a variety of techniques such as mass spectrometry, neutron activation and carbon furnace atomic-absorption spectrometry (CFAAS). The first two techniques require complex and costly equipment that is not widely available. Mass spectrometry also requires standard samples of known concentration for calibration purposes. For iron determination, difficulty may be experienced because of the confusion between Si_2^+ and Fe^+, both of which have ions with a mass number of 56. CFAAS provides adequate sensitivity for the elements of interest and in the work described here it has been the method of choice for the provision of quantitative results. It can be operated using either solid samples or solutions. The use of solid samples avoids the problem of impurities in the dissolving acid. However, it suffers from the necessity of weighing repeatedly and accurately small amounts of sample and the difficulty of calibrating the method.[1] With siliceous materials there would also be the problem of releasing the impurities from a relatively large amount of refractory material in the furnace.

In view of the above problems associated with the direct examination of solid samples, attention has been concentrated on solution techniques. For silicon or silica samples this approach has a particular advantage in that attack with hydrofluoric acid leads to the formation of silicon tetrafluoride, which is volatile and conveniently leaves the impurities in an acidic solution. Matrix effects are thus largely eliminated.

The dissolution of samples does, in general, have a major drawback in that impurities in the dissolving acid contribute a blank to the determination. One method of achieving low reagent blank values is the separate purification of reagents by sub-boiling distillation followed by conventional dissolution. The use of this method has been reported by Stewart and Newton.[2] However, we did not adopt this approach for several reasons: a platinum still was not available to us; the construction of a sub-boiling dissolution unit from PTFE would be at least as complex as the making of our equipment; there are numerous difficulties in handling and storing high-purity acids once they have been prepared[3]; and further clean space is required in which to carry out the dissolution procedure.

The principle made use of in the procedures discussed here is that of isopiestic distillation in which vapour from concentrated volatile acids is absorbed in high-purity water surrounding the sample. The concentrated acid and sample are in separate vessels inside one sealed container. The transferred acid vapour effects the dissolution but does not contain the impurities present in the concentrated acid as these do not transfer with the vapour.

Experimental

Apparatus

The analyses reported here were carried out using a PTFE vessel based on a design by Wooley.[4] The design was modified so that relatively large samples could be accommodated with minimum handling (*i.e.*, crushing, grinding and transferring of solutions). Figs. 1 and 2 show the vessel and the four sample cups that it contains. Features of the modified design are as follows.

(*i*) For "as-received" samples up to 1 g can be accommodated. The relatively large volume of the sample cups allows each sample to be made up to 10 ml *in situ*.
(*ii*) All four sample cups may be readily removed simultaneously.
(*iii*) Chamfers on the top edges of the cups reduce the risk of condensed acid running into them.

Table 1. Effect of impurities on three materials used in high-technology industries

Material	Property affected	Impurities of relevance	Range of interest/ ng g^{-1}
Silicon single crystal	. . Electrical breakdown of devices	Fe	1–100
High-purity synthetic quartz crystal	. . Radiation hardness	Al,Fe	5–100
Optical fibres (high-purity silica) . .	. . Optical transmission	Cu,Cr,Ni,Fe	5–100

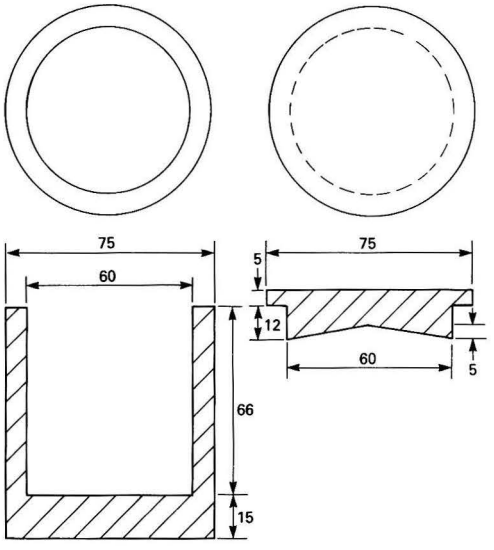

Fig. 1. PTFE vessel and lid. All dimensions in millimetres

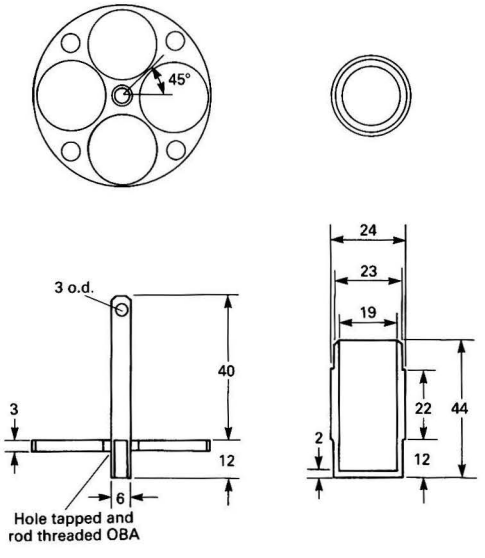

Fig. 2. PTFE cradle and cup. All dimensions in millimetres

(*iv*) Shoulders on the outside of the cups ensure that they do not lean against the wall of the vessel.

(*v*) The four-cup design allows the dissolution of up to three samples and one blank or more commonly two samples and two blanks.

The atomic-absorption measurements were obtained using a Perkin-Elmer Model 280 spectrometer fitted with an HGA 500 graphite furnace. The samples were introduced using an AS-40 autosampler. The spectrometer readout was recorded on a Perkin-Elmer Model 56 chart recorder. Standard conditions for each element were listed in the users' manual.[5] The graphite tubes used were all supplied by Perkin-Elmer and no problem with residual blanks was encountered in any of the analyses reported.

Sample Preparation

The silicon samples are received as slices of approximately 50 mm diameter (other common sizes are 75, 100 and 125 mm). These are cleaved into strips prior to cleaning. Samples for analysis as-received are washed with ethanol and then rinsed in de-ionised water. If bulk analysis is required the silicon is heavily etched using a mixture of hydrofluoric and nitric acids (acids of the highest purity available are used). The samples are thoroughly rinsed in de-ionised water before dissolution.

This procedure was found to be unsatisfactory for bulk iron analysis. A commonly recognised cleaning procedure (RCA clean) for removing surface contamination employs a mixture of hydrochloric acid, hydrogen peroxide and de-ionised water.[6,7] Cleaning is followed by thorough rinsing of the sample with de-ionised water.

Quartz

The quartz samples are received as blocks and are washed with ethanol prior to a light etch in hydrofluoric acid. This etch is followed by cleaning with hydrochloric acid, hydrogen peroxide and water as described for silicon preparation. The sample is finally rinsed thoroughly with de-ionised water.

Optical Fibres

These samples are received as collapsed tubes. The inner core is removed by trepanning and etched in hydrofluoric acid to remove any remaining outer tube and trepanning residues. The core is then cleaned in a mixture of hydrochloric acid, hydrogen peroxide and water and finally the sample is rinsed thoroughly in de-ionised water.

The optical fibre, silicon or quartz crystal samples are weighed, cleaned and placed in the sample cups. The acids used for dissolution are placed in the bottom of the large vessel (1 + 1 *V/V* hydrofluoric acid - nitric acid for silicon; hydrofluoric acid for quartz and silica. Aristar grade acids are used). The four sample cups are then placed in the rack in the top of the vessel.

The apparatus is sealed with the PTFE lid and the whole is clamped together between brass plates and placed in an oven at approximately 110 °C. The assembly is usually left in an oven overnight. When the vessel is removed from the oven it is spun about its axis on a turntable at approximately 30 rev min^{-1} for a few minutes to encourage any condensation on the roof of the vessel to run back into the bulk acid. The pot is cooled, the clamps are released and the pot is transferred to a clean environment before opening. Although silicon tetrafluoride has been generated there is no build-up of pressure and the lid has to be gently prised open. There is some advantage in cooling below room temperature when silicon has been dissolved as this reduces the evolution of oxides of nitrogen from the bulk solution.

All of the samples examined are siliceous and therefore most of the silicon is removed as a vapour during dissolution leaving a liquid residue containing only the fluorides and/or nitrates of the impurities associated with the sample. It was found that leaving the samples to dissolve for a longer time resulted in a larger liquid residue in the sample cups. To facilitate manipulation of the residues and remove any residual fluorides, which could cause interference problems in subsequent furnace analysis,[8] 50 µl of Aristar sulphuric acid are added to each cup. The cups are then heated at approximately 100 °C on a hot-plate. This leaves a residue of 50 µl containing all the impurities associated with the samples. The liquid is then made up to volume (typically 1 ml) using a micropipette to dispense the de-ionised water (Elgastat Spectrum RO1). The samples are subsequently analysed against standards matched in acid concentration.

The analyses of standards and samples were carried out in triplicate. The standard deviation (δ) of results on 30-µl

Table 2. Impurities sought in single-crystal silicon. Sample mass, *ca.* 1 g; initial sample volume of 5% H_2SO_4, 1 ml

Sample	Impurity/ng g^{-1} Fe	Cr	Ni	Cu	Pb
As received from manufacturer	200*	7	10	15	20
After first HF - HNO_3 etch	40	40	10	10	
After second HF - HNO_3 etch	40	5	3	10	
RCA clean on 2	17				
After heavy HF - HNO_3 etch followed by RCA clean on as-received silicon	5				
Blank assuming 1 g of sample	3	10	3	10	5

* Sample diluted 100-fold for Fe analysis.

Table 3. Impurities sought in quartz crystals. Sample mass, *ca.* 1 g; initial sample volume of 5% H_2SO_4, 1 ml

Quartz sample	Impurity/ng g^{-1} Fe	Al
Commercially available quartz (1)	100*	9000*
Commercially available quartz (2)	500*	11000*
Commercially available quartz (3)	100*	9000*
Pure quartz A	50	30
Pure quartz B	5	10
Blanks assuming 1 g of sample	5	5

*Samples diluted 100-fold for analysis.

samples of a 0.01 p.p.m. iron solution was 0.0005 (based on ten replicate determinations). Using the same procedure the standard deviation for the analysis of replicate samples was similar. It has been possible to maintain the blank levels at 3–8 ng of iron per sample.

A similar method of sample preparation has also been used to provide matrix-free samples for spark-source mass spectrometry (SSMS). In this method the residue, or a portion of the residue, is dried on to graphite (Ringsdorff RW-A/Total), which is pelleted prior to analysis. SSMS is a semi-quantitative method of trace analysis which, in these applications, is limited by the large number of complex ions of different mass produced by silicon isotopes in combination with carbon and oxygen. Using residues from the present method of dissolution, where silicon has been removed, allows more elements to be detected at lower levels than before. However, the sulphuric acid treatment has to be omitted from the procedure to prevent the formation of complex ions based on sulphur and oxygen.

In the studies reported here, SSMS results have been used to show which elements should be measured quantitatively by atomic-absorption spectrometry.

Results and Discussion

Analysis of Silicon

A high iron content is expected with the as-received slice owing to the unavoidable presence of surface impurities. This is well known in the semiconductor industry and it is standard practice in device technology to oxidise the silicon (approximately 100 nm) and remove this layer by dip-etching in hydrofluoric acid.

The initial results from the analysis of the silicon crystal slices confirmed the high iron content of as-received slices. In order to determine a bulk iron content for the silicon the samples were pre-etched in a mixture of hydrofluoric and nitric acids to remove the silicon surface. Although this procedure did lead to lower levels of iron being detected they were still higher than expected, possibly because iron can be plated back on to the fresh silicon surface. After heavy etching followed by an RCA clean the iron level was reduced to 5 ng g^{-1}, a level close to but distinguishable from the current blank level.

The results in Table 2 show that there is a high chromium content obtained after the first etch. As this was higher than both the "as-received" chromium content and the "subsequently cleaned" chromium content it was thought that it was most likely to have been due to some particulate contamination introduced during the cutting - polishing procedures. This is supported by other "as-received" analyses where samples from the same slice can give widely differing iron contents.[9]

Table 4. Impurities sought in optical fibres. Sample size, 1 g; initial sample volume of 5% H_2SO_4, 1 ml

Sample	Impurity/ng g^{-1} Cr	Ni	Cu	Fe
Fibre B118: end 1	8	50	130*	70
end 2	7	80*	140*	10
Fibre B120	5	10	10	140
Fibre B111	10	5	160*	60
Commercial silica (used for preform tubing)	2	10	10	240
Blanks assuming 1 g of sample	2	5	2	10

* Samples diluted 10-fold for analysis.

Analysis of Quartz

The results (Table 3) from the analysis of the two high-purity quartz crystals (A and B) showed that the iron and aluminium contents of these crystals are considerably lower than those found in commercially available material. With respect to aluminium these crystals are purer by nearly three orders of magnitude. This is of great significance as aluminium is known to be the most important impurity limiting the so-called "radiation hardness" of quartz crystals.[10]

Analysis of Silica Optical Fibres

The analysis of silica optical fibres is a requirement arising from the necessity for extremely low levels of potential colouring elements, to minimise attenuation of the optical signal transmitted along fibres that may be several kilometres in length. The results obtained (Table 4) have been of value in locating the presence of such impurities and in evaluating the degree of success of attempts to achieve lower impurity levels at various stages in the development of fibre-producing processes.

Conclusion

In this work the results obtained indicate that the immediate requirements in the determination of concentration of metallic impurities in high-purity silicon and silica have been met. With the quartz crystals and silica optical fibres, the limits of detection now attained are adequate for the foreseeable future. However, for silicon, advances in technology will probably require higher purities than are currently available and hence will generate a need for analytical methods with even lower limits of detection.

Employing the dissolution process described, the detection limit is set by the blank value of the sulphuric acid used to remove residual fluoride. It is planned that improvement be sought by several different means. These include use of high-purity sulphuric acid, a larger sample size (up to 5 g could be used) with the same amount of sulphuric acid, or use a smaller volume of acid.

With the success obtained in application of the isopiestic distillation combined with dissolution as a means of lowering blank levels on siliceous samples, it is intended to pursue similar techniques for other materials. Semiconductor technology has generated a demand for high-purity materials such as gallium arsenide, indium phosphide and cadmium mercury telluride and there is thus a need for means of analysis. Whereas with these materials there will not be the advantage of loss of matrix effects from volatilisation of silicon tetrafluoride, the ability to effect solution with very low reagent blanks will enable quantitative analysis to be carried out at lower levels than is easily possible at present. It is expected that the results of investigations with such materials will be the subject of a further paper.

References

1. Fuller, C. W., *Anal. Chim. Acta*, 1972, **62**, 261.
2. Stewart, D. A., and Newton, D. C., *Analyst*, 1983, **108**, 145.
3. Moody, J. R., and Beary, E. S., *Talanta*, 1982, **29**, 1003.
4. Wooley, J. F., *Analyst*, 1975, **100**, 896.
5. "Perkin-Elmer Users Manual for HGA 500 Graphite Furnace," Perkin-Elmer, Norwalk, CT, 1978.
6. Kern, W., and Puotinen, D. A., *RCA Rev.*, 1970, **31**, 187.
7. Kern, W., *RCA Rev.*, 1970, **31**, 234.
8. L'vov, B. V., *Spectrochim. Acta, Part B*, 1978, **33**, 153.
9. Ward, P. J., *J. Electrochem. Soc.*, 1982, **129**, 2573.
10. Martin, J. J., Doherty, S. P., Halliburton, L. E., Marks, M., Koumvakalis, N., Sibley, W. A., Brown, R. N., and Armington, A., "Proceedings of the 33rd Annual Symposium on Frequency Control, 1979," US Army Electronics Command, Fort Monmouth, NJ, 1979, pp. 134–147 (copies available from the Electronic Industries Association, 2001 Eye Street, N.W., Washington, DC, 2006.

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Determination of the Flow-point of Lubricating Greases and Petroleum Waxes Including Petrolatum

Dhoaib Al-Sammerrai, Hazim Al-Najjar and Wejdan D. Selim
Petroleum Research Centre, P.O. Box 10039, Jadiriyah, Baghdad, Iraq

The flow-point has been determined for lubricating greases, petroleum waxes, including petrolatum, and protectives and the results obtained have been compared with the corresponding drop-points. The flow-point can be used as a characterisation parameter in the identification of these products.

Keywords: *Flow-point determination; lubricating greases; petroleum waxes*

It is important to be able to identify lubricating greases, petroleum waxes, including petrolatum, and protectives for the purpose of classifying them and quality control. Methods of identification of these products include the determination of their drop- and melting-points, which can be defined as the temperature at which the product passes from a solid or semi-solid to a liquid state under the conditions of the test. Many standardised methods are available for such determinations.[1-3]

In the work described in this paper, the flow-point, which is usually defined as the temperature at which a plug of sample slides down to a complete halt from one leg into the bend of a specimen U-tube,[4] has been determined for lubricating greases, petroleum waxes, including petrolatum, and a protective. The results obtained have been compared with the corresponding drop-points, measured according to standard procedures.

It was observed that the flow-point was of a slightly higher value than the drop-point and that it can be used as a characterisation parameter in the identification and classification of these products.

Experimental

The instrument used to obtain the flow-points is the Büchi Model 510 melting-point determination apparatus. The instrument consists essentially of two parts, the control unit and the apparatus. The control unit contains all operating elements and the electronic temperature controller with a control range of 0–300 °C. Control is effected by a proportional-action controller.

The U-shaped oil container, mounted on top of the control unit, is capable of accepting the specimen tubes. A specially designed stirrer ensures an intense circulation and mixing of the silicone oil used. This produces uniform temperature distribution around the specimen table and the thermometer. The glareless lighting and adjustable magnifying glass permits observation of the specimen with both eyes.

For the temperature rise during the determinations, temperature programmes with heating rates of 0.2, 0.5, 1, 2, 3 and 5 °C min^{-1} are available. A thermometer (0–360 °C) is used for measuring the temperatures (see Fig. 1). The specimen glass U-tubes supplied with the Büchi 510 have the dimensions shown in Fig. 2.

The specimen glass U-tubes were filled with lubricating greases by inserting one leg of the tube about 10 mm into the product to be tested and pushing the plug of product downwards with a filling wire until it is about 10 mm above the bend of the specimen tube. It was found from experience that repeated insertions are required in order to ensure that a sufficient amount of sample (*ca.* 15–20 mg) had been pushed into the specimen tube. It is also advisable to place the tube with the pushed-out plug of product into a refrigerator for a short time so that the plug can then be pushed downwards much easier and to avoid the formation of air bubbles. This cooling procedure does not cause any changes in the structure of the grease, as nowadays most lubricating greases are for multi-purpose use and can withstand very low temperatures.

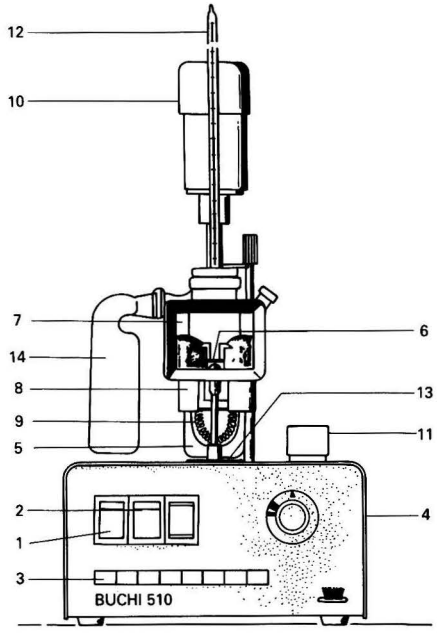

Fig. 1. Flow-point determination apparatus. 1, Main switch; 2, switch for lighting and cooling fan; 3, selector switches for temperature programme; 4, set-point potentiometer; 5, oil container; 6, specimen table; 7, adjustable magnifying glass; 8, lighting; 9, heating coil; 10, stirrer motor; 11, holder for spare capillary tubes; 12, thermometer; 13, cooling fan; and 14, expansion vessel

Petroleum waxes, including petrolatum, and protectives are added to the specimen tubes by inserting one leg of the tube about 10 mm into a hot melt of the product (approximately 10 °C above its drop-point). The U-tube is then heated on a hot-plate, tilted slowly until the product flows downwards to about 10 mm above the bend of the U-tube and the product is then allowed to solidify on cooling.

If the flow-point is not known, an orientation determination is carried out by turning the set-point potentiometer to increase the oil temperature rapidly until the sample flows and the approximate temperature is immediately recorded by the thermometer. The set-point potentiometer is then adjusted to

a temperature 10–15 °C below the approximate reading. The silicone oil is allowed to cool to the set temperature and a new specimen is placed in the determination apparatus. The desired temperature rise is then achieved by pressing the appropriate programme button.

The U-tubes can be cleaned and used for more than one determination by flushing out the plug of grease sample with a strong fine jet of light petroleum followed by a jet of soapy water and finally acetone. As for wax samples, the U-tubes are heated on a hot-plate and inverted allowing the wax melt to flow out, the tubes are cleaned with a hot solvent (toluene), then acetone and dried.

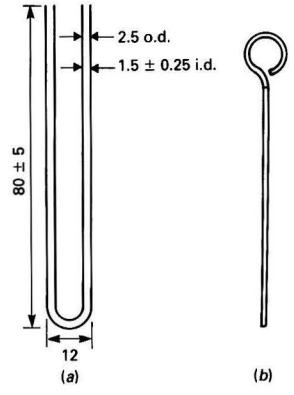

Fig. 2. (*a*) Specimen U-tube; and (*b*) filling wire. All dimensions in millimetres

Results and Applications

Flow-point determinations were all conducted by the same operator at a recommended[5] temperature programme of 0.5 °C min^{-1}, starting 10–15 °C below the approximate reading. No trial determination was run as the drop- and melting-points of all the samples were known.

Five lubricating greases prepared from mineral-based oils and different thickeners were chosen for our investigation. The data shown in Table 1 are for a set of ten flow-point determinations for each sample. Means and standard deviations are presented.

Betonite grease exhibited no flow and began to decompose at a temperature of about 250 °C, *i.e.*, degradation of the oil liquid phase.

The data shown in Table 2 are for a set of ten flow-point determinations on three petroleum-based waxes, a petrolatum and a protective grease. Means and standard deviations are presented.

The results in Tables 1 and 2 show that the repeatability for samples with a wide range of drop- and melting-points is good.

Table 1. Flow-points for lubricating greases at a temperature programme of 0.5 °C min^{-1}

	Flow-point*/°C				
Determination No.	Calcium (98 °C)	Sodium (144 °C)	Lithium hydroxystearate (198 °C)	Betonite (no drop-point)	Calcium complex (290 °C)
1	99	145.5	200	Decomp. 253	292.5
2	99	145	200	Decomp. 253	292
3	99	145	200	Decomp. 250	292
4	98.5	145	200.5	Decomp. 250	292.5
5	99	145	199.5	Decomp. 252	292.5
6	99	145.5	199.5	Decomp. 253	293
7	99	145	199.5	Decomp. 252	292.5
8	99	145	199.5	Decomp. 252	292.5
9	99	145.5	200	Decomp. 252	292.5
10	98.5	145	199.5	Decomp. 253	293
Mean	98.9	145.1	199.8		292.5
Standard deviation	0.2	0.23	0.33		0.32

* The results in parentheses are the drop-points of the grease samples measured according to ASTM standard methods.

Table 2. Flow-points for petroleum waxes, petrolatum and a protective grease at a temperature programme of 0.5 °C min^{-1}

	Flow-point*/°C				
Determination No.	Microcrystalline wax (A) (73 °C)	Microcrystalline wax (B) (71 °C)	Paraffin wax (64 °C)	Petrolatum (59 °C)	Protective (63 °C)
1	73.5	71.5	64.5	60	63.5
2	73.5	71.5	64.5	59.5	63.5
3	73.5	71.5	64.5	59.5	63.5
4	74	71.5	65	59.5	63.5
5	73.5	71.5	64.5	59.5	63.5
6	74	72	64.5	59.5	64
7	74	71.5	64.5	60	64
8	74	72	65	59.5	63.5
9	73.5	72	64.5	59.5	63.5
10	73.5	71.5	64.5	59.5	63.5
Mean	73.7	71.6	64.6	59.6	63.6
Standard deviation	0.24	0.23	0.2	0.2	0.2

* The results in parentheses are melting-points for the samples measured according to ASTM standard methods.

The slight increase in the flow-point, over the drop- and melting-points is due to the time it takes the melt to flow from one leg of the specimen U-tube into the bend, and this will vary according to the type of product to be tested and the viscosity of its melt.

Conclusions

It has been shown that the flow-point of a product is slightly higher than its drop- or melting-point and that it can be used for the identification of lubricating greases and petroleum waxes, including petrolatum. However, the method is limited to a temperature of 300 °C when using silicone oil as the heating medium. A flow-point determination is a less time-consuming procedure than a drop- or melting-point determination and requires only a minute amount of sample. For a lubricating grease exhibiting no drop-point, it is possible to record the temperature of decomposition of the product when using a flow-point determination apparatus.

References

1. "Standard Method for Measurement of Dropping Point of Lubricating Greases over Wide Temperature Range, Designation D-2265, 1982 Book of ASTM Standards," American Society for Testing and Materials, Philadelphia, 1982.
2. "Standard Method for Measurement of Dropping Point of Lubricating Greases, Designation D-566, 1982 Book of ASTM Standards," American Society for Testing and Materials, Philadelphia, 1982.
3. "Standard Method for Measurement of Drop Melting Point of Petroleum Wax including Petrolatum, Designation D-127, 1982 Book of ASTM Standards," American Society for Testing and Materials, Philadelphia, 1982.
4. "Instruction Manual for Büchi 510 Melting Point Determinator," Büchi Laboratoriums-Technik AG, Flawil, Switzerland.
5. Al-Sammerrai, D., unpublished work.

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Analytical Uses of Charge-transfer Complexes: Determination of Pure and Dosage Forms of Piperazine*

U. Muralikrishna
Department of Chemistry, Andhra University, Postgraduate Extension Centre, Nuzvid 521 201, India
and Mannam Krishnamurthy and N. Someswara Rao
Analytical Chemistry Laboratories, School of Chemistry, Andhra University, Waltair 530 003, India

The charge-transfer interaction of piperazine with benzoquinone and its halo derivatives was used for the simple spectrophotometric determination of piperazine in chloroform medium. The sensitivity of the method and the time stability of the charge-transfer complexes were found to depend on the electron affinity of the quinones. The drug-base in different dosage forms was extracted into chloroform after alkali treatment and the piperazine was assayed. The method is sensitive and rapid and the base tolerates a wide variation of reagent concentration. The results compare well with the official BP method.

Keywords: *Charge-transfer complexes; piperazine determination; benzoquinone; spectrophotometry; tablets*

Piperazine, a pyrazine derivative, is one of the most potent drugs and is used as an anthelmintic for the treatment of threadworms and roundworms in humans and animals.

Sodium tetraphenylborate,[1] ammonium reineckate,[1-3] bromothymol blue,[4] Folin - Ciocalteu reagent,[5] sodium 1,2-naphthoquinone-4-sulphonate,[6,7] 2,5-dichloro-1,4-naphthoquinone,[8] 1,4-benzoquinone,[9-11] dichloro-1,4-benzoquinone[12] and 3,5-dichlorobenzoquinone chlorimide[13] have been proposed as reagents for the determination of piperazine and its formulations. However, these methods are either time consuming or indirect or suffer from the disadvantage of maintaining a narrow range of pH.

The molecular interactions between electron donors and acceptors are generally associated with the formation of intensely coloured charge-transfer complexes,[14] which absorb radiation in the visible region. The photometric methods based on these interactions are usually simple and convenient,[15] because of the rapid formation of the complexes. Piperazine is a good n-electron donor and will form charge-transfer complexes[16,17] with organic π-acids such as benzoquinones. These types of complexes are used in the determination of the components of the complexes.[17,18] Belal *et al.*[19] and Rizk *et al.*[20] reported the determination of piperazine using tetrachloro-1,4-benzoquinone as reagent, but in dioxane and butanol as solvents. Dioxane[21] and alcohols[22] are known to interact with quinones and their utility for quinones is stated to be non-ideal.[23]

In continuation of earlier work on the analytical uses of charge-transfer complexes,[17,18,24-26] we have now examined the utility of benzoquinone and its halo derivatives as reagents for the determination of piperazine.

Materials and Methods

Apparatus

A Carl Zeiss Spekol spectrophotometer was used for absorbance measurements with matched, stoppered cells of 1-cm path length.

Reagents

A saturated solution of each quinone in chloroform was prepared from the purified samples. Piperazine (Fluka, puriss grade sample) was recrystallised twice from cyclohexane solution and a 3×10^{-3} M stock solution was prepared in chloroform.

Procedure for Colour Development

Place an aliquot, ranging from 0.3 to 4.0 ml, of piperazine in a 10-ml calibrated flask, add 2 ml of the reagent and dilute to the mark with chloroform. Measure the absorbance of the solution at the wavelength of maximum charge transfer (λ_{CT}) after the appropriate time indicated in Table 1. Calculate the unknowns from Beer's law graphs. The results are given in Table 1.

Procedure for the Assay of Salts and Dosage Forms of Piperazine

Salts and tablets

Weigh an amount of the salt or powdered tablet containing 25 mg of piperazine into a suitable vessel and add 15–20 ml of distilled water. Shake, filter, wash with two 5-ml aliquots of water and combine the filtrate and washings. Add 10 ml of 10% sodium hydroxide solution. Extract the drug base with three 30-ml volumes of chloroform, transfer the combined extracts into a 100-ml calibrated flask and dilute to the mark with chloroform. Take an aliquot and develop the colour as described under Procedure for Colour Development.

Solutions and syrups

Dilute a volume of piperazine hydrate solution or syrup containing 25 mg of piperazine to 25–30 ml with water. Add the alkali and extract the drug base as described under *Salts and tablets*. Develop the colour as described under Procedure for Colour Development.

The results of the assay of salts and dosage forms of piperazine using tetrachloro- and tetrabromo-1,4-benzoquinones are compared with the official method[27] in Table 2.

Results and Discussion

Piperazine is a fully saturated molecule and the electrons that are responsible for the charge-transfer transition are the non-bonded electrons on the nitrogen atom. Benzoquinones are well known π-acceptors and hence the nature of the excitation in these interactions is an n–π* transition. The absorption bands of the complex are well separated from those of either of the components.

* Presented at the National Symposium on Absorption Spectrometry, Bhabha Atomic Research Centre, Bombay, India, February, 1984.

Table 1. Analytical data for the determination of piperazine

Sample No.	Reagent	Electron affinity*	λ_{CT}/nm	Time to attain maximum absorbance	Beer's law range/ μg ml^{-1}	Sandell's sensitivity/ μg cm^{-2}	Time stability/ h
1	1,4-Benzoquinone	0.77	506	3 h	13–120	0.128	3
2	Monochloro-1,4-benzoquinone	1.00	527	1 h	11–110	0.108	12
3	2,5-Dichloro-1,4-benzoquinone	1.15	538	30 min	10–100	0.098	20
4	2,6-Dichloro-1,4-benzoquinone	1.20	546	20 min	10–90	0.092	24
5	2,3,5,6-Tetrachloro-1,4-benzoquinone	1.37	575	15 min	7–70	0.078	36
6	2,3,5,6-Tetrabromo-1,4-benzoquinone	1.37	576	10 min	7–70	0.076	36
7	3,4,5,6-Tetrachloro-1,2-benzoquinone	1.55	588	5 min	6–60	0.064	48

* Values from reference 30.

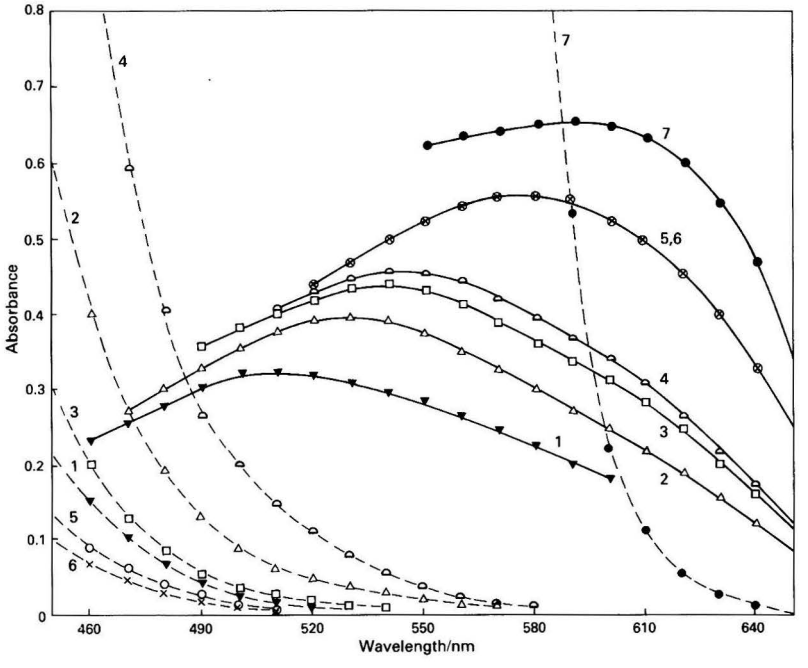

Fig. 1. Absorption spectra of charge-transfer complexes of piperazine (5×10^{-4} M) with benzoquinones (numbers refer to compounds indicated in Table 1). The broken lines represent the absorption of benzoquinones (saturated solutions diluted five-fold)

The continuous variations[28] and the molar ratio[29] methods show that the composition of the complexes is 1 : 1, except that for tetrachloro-1,2-benzoquinone, where a ratio of 1 : 2 (acceptor to donor) is observed. This indicates that only a single nitrogen is involved in the formation of charge-transfer complexes, although piperazine is a twin-site donor.

From Table 1 it can be seen that the reaction is rapid with tetrahalo-1,4-benzoquinones. No reagent blank is necessary in measuring the absorbance of the complexes (*cf.*, Fig. 1).

The complexes were found to be stable. The results also indicate that the method is sensitive to microgram amounts of piperazine. The time stability and the sensitivity were found to have a direct relationship with the electron affinity of the quinones (Fig. 2).

The results for the variation of reagent concentration (Table 2) indicate that 1×10^{-3} to 4×10^{-2} M of tetrachloro- or tetrabromo-1,4-benzoquinone will not affect the determination of piperazine. The higher concentrations of the reagent may, on the other hand, be useful for rapidly reaching equilibrium, thus minimising the time required to attain the maximum absorbance at λ_{CT}.

The assay of dosage solutions involves extraction of the drug base into chloroform. A single extraction is capable of extracting 93.8% of piperazine and thus three batch extractions are sufficient, instead of the five reported earlier.[20]

Piperidine and *N*-methyl- and *N*-(aminoethyl)piperazines interfere at all concentrations, but pyridine, pyrazine and 2,5-dimethylpiperazine (up to a 5-fold excess), triethanol-

Table 2. Assay of salts and dosage forms of piperazine using three different methods

Piperazine product	Recovery,* % of label claim				
	Official method[27]	Tetrachloro-1,4-benzo-quinone	(C.V.)	Tetrabromo-1,4-benzo-quinone	(C.V.)
Hexahydrate	99.5	99.8	(0.39)	99.4	(0.28)
Hydrate solution	100.4	100.2	(0.29)	100.4	(0.65)
Phosphate BPC	99.7	99.3	(1.07)	99.3	(0.83)
Phosphate tablets	101.2	101.8	(0.65)	102.0	(0.65)
Citrate BP	99.6	99.7	(0.57)	100.0	(0.65)
Citrate tablets T_1	100.8	100.2	(0.39)	100.4	(0.56)
Citrate tablets T_2	99.3	99.8	(0.40)	99.2	(0.57)
Citrate syrup S_1	101.7	102.1	(0.80)	102.1	(0.65)
Citrate syrup S_2	98.2	97.8	(0.40)	98.6	(0.29)
Dihydrochloride	99.0	99.0	(0.76)	98.6	(0.29)
Adipate BP	98.9	98.4	(0.48)	98.8	(1.13)
Adipate tablets	99.3	99.0	(0.58)	99.6	(0.57)

* The coefficient of variation (C.V.) values are derived from three determinations for each product.

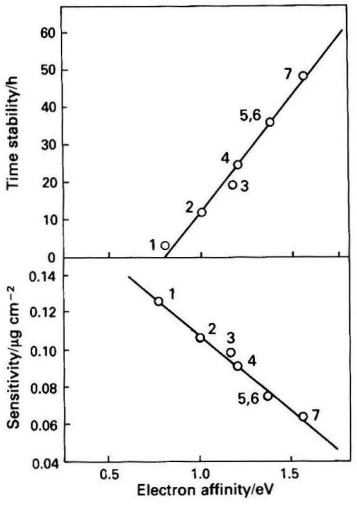

Fig. 2. Correlation of the sensitivity of the method and the time stability of the charge-transfer complexes with the electron affinity of the acceptors (numbers refer to compounds indicated in Table 1)

amine (up to a 0.3-fold excess) and ethylenediamine (up to a 0.2-fold excess) do not interfere.

The assay results on dosage forms of piperazine using the present method compare well with those obtained using the official BP method. Further, the results (coefficients of variation in Table 2) indicate a good precision for the method.

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References

1. Masse, M., *Pharm. Acta Helv.*, 1958, **33**, 80.
2. Pankratz, R. E., *J. Pharm. Sci.*, 1961, **50**, 175.
3. Shirsat, P. D., *Indian J. Pharm.*, 1975, **37**, 101.
4. Das Gupta, V., *Am. J. Hosp. Pharm.*, 1976, **33**, 283.
5. Rao, G. R., Kanjilal, G., and Mohan, K. R., *Analyst*, 1978, **103**, 993.
6. Hanana, S., and Tang, A., *J. Pharm. Sci.*, 1973, **62**, 2027.
7. Dessouky, Y. M., and Ismaiel, S. A., *Analyst*, 1974, **99**, 482.
8. Abou-Ouf, A. S., Taha, A., and Saidhom, M. B., *J. Pharm. Sci.*, 1973, **62**, 1700.
9. Cavett, J. W., and Heotis, J. P., *J. Assoc. Off. Agric. Chem.*, 1958, **41**, 323.
10. Parlmulter, S. H., *J. Assoc. Off. Agric. Chem.*, 1958, **41**, 506.
11. Loucks, M. F., and Nauer, L., *J. Assoc. Off. Anal. Chem.*, 1967, **50**, 268.
12. Wachsmuth, H., and Koeckhoven, L. V., *J. Pharm. Belg.*, 1962, **17**, 220.
13. Baggi, T. R., Surinder, N. M., and Rao, G. R., *J. Assoc. Off. Anal. Chem.*, 1974, **57**, 1144.
14. Mulliken, R. S., and Person, W. B., "Molecular Complexes," Wiley-Interscience, New York, 1969.
15. Townshend, A., *Proc. Soc. Anal. Chem.*, 1973, **10**, 39; 1976, **13**, 64.
16. Muralikrishna, U., Seshasayi, Y. V. S. K., and Krishnamurthy, M., *J. Indian Chem. Soc.*, 1983, **60**, 447.
17. Muralikrishna, U., and Krishnamurthy, M., *Indian J. Chem.*, 1982, **21A**, 1018.
18. Muralikrishna, U., and Rao, N. S., *Indian J. Chem.*, 1978, **16A**, 993.
19. Belal, S., Elsayed, M. A., Abdel-Hamid, M. E., and Abdine, H., *J. Pharm. Sci.*, 1981, **70**, 127.
20. Rizk, M. S., Walash, M. I., and Ibrahim, F. A., *Analyst*, 1981, **106**, 1163.
21. Kobashi, H., Tomioka, Y., and Morita, T., *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1568.
22. Kuboyama, A., *Bull. Chem. Soc. Jpn.*, 1960, **33**, 1027.
23. Thomson, R. H., "Naturally Occurring Quinones," Academic Press, London, 1971, p. 44.
24. Muralikrishna, U., Rao, N. S., and Ramanadham, G. V., *Curr. Sci.*, 1975, **44**, 534.
25. Muralikrishna, U., Rao, N. S., and Krishnamurthy, M., *Indian J. Pharm. Sci.*, 1983, **45**, 28.
26. Muralikrishna, U., and Krishnamurthy, M., *Microchem. J.*, in the press.
27. "British Pharmacopoeia," Volume 1, Pharmaceutical Press, London, 1980, p. 352.
28. Job, P., *Ann. Chim. (Paris)*, 1928, **9**, 113.
29. Yoe, J. H., and Jones, A. L., *Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 111.
30. Briegleb, G., *Angew. Chem., Int. Ed. Engl.*, 1964, **3**, 617.

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Spectrophotometric Determination of Nitrite with Composite Reagents Containing Sulphanilamide, Sulphanilic Acid or 4-Nitroaniline as the Diazotisable Aromatic Amine and *N*-(1-Naphthyl)ethylenediamine as the Coupling Agent

George Norwitz and Peter N. Keliher*
Chemistry Department, Villanova University, Villanova, PA 19085, USA

Composite diazotisation - coupling reagents containing sulphanilamide, sulphanilic acid and 4-nitroaniline as the diazotisable aromatic amine and *N*-(1-naphthyl)ethylenediamine (NED) as the coupling agent have been used for the spectrophotometric determination of nitrite. The proposed procedure consists in adding 10 ml of the composite reagent to the sample and diluting to 50 ml in a calibrated flask. The recommended composition of the composite reagents is equivalent to the following: 0.25% sulphanilamide, 0.0060% NED and 5.42% hydrochloric acid; 0.50% sulphanilic acid, 0.0050% NED and 0.29% hydrochloric acid; 0.25% 4-nitroaniline, 0.010% NED and 15.0% sulphuric acid. A minimum and maximum amount of NED and large excess of the aromatic amine must be present at an optimum acidity in order to avoid interfering side-reactions, especially the nitrosation of NED. The same results are obtained over the range 7.5–49.0 ml of sulphanilamide - NED or sulphanilic acid - NED reagent and 3.0–49.0 ml of 4-nitroaniline - NED reagent per 50 ml; this would indicate that the ratios of aromatic amine, NED and acid are just as important as the specific amounts of these substances. The sensitivity, accuracy and precision of the methods decrease in the order 4-nitroaniline - NED > sulphanilamide - NED > sulphanilic acid - NED.

Keywords: *Nitrite determination; spectrophotometry; sulphanilamide, sulphanilic acid or 4-nitroaniline reagent; N-(1-naphthyl)ethylenediamine coupling agent*

Nitrite can be determined spectrophotometrically by treating the sample (at a suitable acidity) with a solution of a diazotisable aromatic amine such as sulphanilamide or sulphanilic acid, allowing the diazotisation to proceed for 3–10 min, adding a solution of a coupling agent such as 1-naphthylamine or 1-naphthol-3,6-disulphonic acid or *N*-(1-naphthyl)ethylenediamine (NED) to produce an azo dye, and measuring the absorbance after 10–60 min.[1–3] As the technique is time consuming, some investigators have proposed the use of composite reagents containing the aromatic amine, the coupling agent and the requisite amount of acid in the same solution. The first composite reagent (sulphanilic acid - 1-naphthylamine - acetic acid) was proposed by Lunge in 1889 for the detection of nitrite.[4] Subsequently, composite reagents were applied to the quantitative determination of nitrite[5–12] (Table 1). Surprisingly, no comprehensive study has been made of the many interlocking factors affecting the use of composite reagents. It was the purpose of this work to make such a study and to develop improved composite reagents containing sulphanilamide, sulphanilic acid and 4-nitroaniline as the diazotisable aromatic amines and NED as the coupling agent. NED is advantageous as a coupling agent because it couples in a fairly strong acidic solution. Also, unlike 1-naphthylamine, it is non-carcinogenic (although as with other aromatic amines precautions should be taken to avoid skin contact).[13] 4-Nitroaniline has not been used previously as a diazotisable aromatic amine to any appreciable extent in the spectrophotometric determination of nitrite.

Table 1. Composite diazotisation - coupling reagents for the spectrophotometric determination of nitrite

Aromatic amine, %*	Coupling agent, %	Ratio of amine to coupling agent	Acid, %	Technique	Reference
Sulphanilic acid, 0.16	1-Naphthylamine, 0.031	5.2:1	HOAc, 14.5	3.0 ml† to 20-ml sample, 5 min (6.5/50)‡	Yoe[5]
Sulphanilic acid, 0.15	1-Naphthylamine, 0.030	5:1	HOAc, 4.7	2.0 ml to 100-ml sample, 1 h (0.9/50)	Wattenberg[6]
Sulphanilic acid, 0.16	1-Naphthylamine, 0.031	5.2:1	HOAc, 14	2.0 ml to 50-ml sample, 1 h (1.9/50)	AOAC[7]
Sulphanilic acid, 0.40	1-Naphthylamine. HCl, 0.098	4.1:1	HOAc, 14	10.0 ml to sample, dil. to 50 ml, 25 min (10/50)	Hamilton[8]; AOAC[9]
Sulphanilic acid, 0.050	1-Naphthol, 0.040	1.3:1	HOAc, 10 + NH_3, 1.8	10.0 ml to 10-ml sample, 30 min (25/50)	Hart and Fisher[10]
Sulphanilamide, 1.0	NED, 0.050	20:1	H_3PO_4, 10	3.0 ml to 10-ml sample, 15 min (11.5/50)	ASTM[11]
Sulphanilamide, 1.0	NED, 0.10	10:1	H_3PO_4, 10	2.0 ml to 50-ml sample, 10 min (1.9/50)	US EPA[12]
Sulphanilamide, 1.0	NED, 0.10	10:1	HCl, 21 + NaOAc. $3H_2O$, 27	2.0 ml to 50-ml sample, 15 min (1.9/50)	US EPA[12]
Sulphanilamide, 0.25	NED, 0.0060	41.7:1	HCl, 5.42	10 ml to sample, dil. to 50 ml, 15 min (10/50)	This work
Sulphanilic acid, 0.50	NED, 0.0050	100:1	HCl, 0.29	10 ml to sample, dil. to 50 ml, 30 min (10/50)	This work
4-Nitroaniline, 0.25	NED, 0.010	25:1	H_2SO_4, 15	10 ml to sample, dil. to 50 ml, 10 min (10/50)	This work

* The aromatic amine and coupling agent are calculated on an *m/V* basis and the acid on a *V/V* basis.
† Volume of composite reagent.
‡ Volume (ml) of composite reagent present in 50 ml of solution.

* To whom correspondence should be addressed.

Experimental

Apparatus and Reagents

A Bausch and Lomb Model 70 spectrophotometer (1-cm cell) was used.

All reagents were of analytical-reagent grade.

Standard nitrite solution A (1 ml = 100 μg NO_2-*N*). Dissolve 0.4926 g of sodium nitrite in water and dilute to 1 l with water in a calibrated flask.

Standard nitrite solution B (1 ml = 1.00 μg NO_2-*N*). Prepare fresh daily by diluting a 10-ml aliquot of standard nitrite solution A to 1 l with water in a calibrated flask.

NED solution, 0.20%. Dissolve 0.400 g of *N*-(1-naphthyl)ethylenediamine dihydrochloride ($C_{10}H_7NHCH_2$-$CH_2NH_2.2HCl$) in water and dilute to 200 ml. Store in a brown bottle in a refrigerator. This reagent is stable for 3 weeks.

Note—The abbreviations NED, NEDA, NNEDA and N-na have been used by various investigators to designate *N*-(1-naphthyl)ethylenediamine dihydrochloride. NED is the abbreviation that has been used most frequently in dealing with the spectrophotometric determination of nitrite, and is used here.

Sulphanilamide - NED reagent. Dissolve 2.50 g of sulphanilamide [4-$(H_2N)C_6H_4SO_2NH_2$] in 650 ml of 1 N hydrochloric acid at room temperature, add 30 ml of 0.20% NED solution and dilute to 1 l with water.

Sulphanilic acid - NED reagent. Dissolve 5.00 g of sulphanilic acid [4-$(H_2N)C_6H_4SO_3H.H_2O$] in a mixture of 750 ml of water and 35 ml of 1 N hydrochloric acid by heating on a hot-plate, cool to room temperature, add 25 ml of 0.20% NED solution and dilute to 1 l with water.

4-Nitroaniline - NED reagent. Dissolve 2.50 g of 4-nitroaniline in 300 ml of sulphuric acid (1 + 1) by heating on a hot-plate, dilute to about 900 ml with water, cool to room temperature, add 50 ml of 0.20% NED solution and dilute to 1 l with water.

Note—Store the sulphanilamide - NED and sulphanilic acid - NED reagents in brown bottles in a refrigerator. Store the 4-nitroaniline - NED reagent in a brown bottle at room temperature. The reagents are stable for several months.

Preparation of Calibration Graphs

Transfer the following aliquots of standard nitrite solution B into 50-ml calibrated flasks: 0, 2, 4, 5, 6 and 7 ml for the sulphanilamide - NED; 0, 2, 4, 6, 7 and 8 ml for the sulphanilic acid - NED; and 0, 2, 3, 4, 5 and 6 ml for 4-nitroaniline - NED. Add 10 ml of composite reagent from a graduated cylinder, dilute to the mark with water and mix. Measure the absorbance against the reagent blank at the following wavelengths and after the following times: sulphanilamide - NED, 542 nm and 15 min; sulphanilic acid - NED, 541 nm and 30 min; and 4-nitroaniline - NED, 542 nm and 10 min. Plot absorbance against micrograms of NO_2-N per 50 ml.

Procedure

Transfer an aliquot of the sample (up to 35 ml) containing a maximum of 7, 8 and 6 μg of NO_2-N for the sulphanilamide - NED, sulphanilic acid - NED and 4-nitroaniline - NED methods, respectively, into a 50-ml calibrated flask and proceed as described for the preparation of the calibration graphs.

Table 2. Effect of amount of NED (5.00 μg of NO_2-N per 50 ml)

Amine	Volume of 0.20% NED solution/ml	Absorbance after 2 min	8 min	15 min	20 min	30 min	60 min
Sulphanilamide*	0.10	0.32	0.41	0.41		0.41	0.40
	0.20	0.36	0.42	0.42		0.42	0.42
	0.25	0.36	0.41	0.42		0.42	0.42
	0.30	0.36	0.42	0.425		0.425	0.42
	0.40	0.37	0.41	0.42		0.42	0.42
	0.50	0.36	0.41	0.41		0.41	0.42
	1.00	0.34	0.38	0.38		0.38	0.39
	2.00	0.32	0.33	0.33		0.33	0.33
	5.00	0.23	0.24	0.23		0.23	0.23
	10.00	0.16	0.17	0.17		0.17	0.17
Sulphanilic acid†	0.10		0.22		0.30	0.34	0.34
	0.15		0.29		0.35	0.36	0.36
	0.20		0.29		0.35	0.36	0.36
	0.30		0.32		0.36	0.36	0.36
	0.35		0.30		0.34	0.35	0.355
	0.40		0.30		0.34	0.35	0.36
	0.50		0.32		0.34	0.35	0.35
	1.00		0.32		0.33	0.33	0.34
	2.00		0.20		0.28	0.28	0.28
	5.00		0.17		0.18	0.19	0.19
	10.00		0.11		0.12	0.12	0.12
4-Nitroaniline‡	0.10	0.22	0.33	0.37		0.45	0.46
	0.20	0.41	0.49	0.50		0.50	0.50
	0.35	0.45	0.50	0.50		0.50	0.50
	0.50	0.48	0.50	0.50		0.50	0.50
	0.75	0.49	0.50	0.50		0.50	0.50
	1.00	0.43	0.48	0.46		0.49	0.49
	2.00	0.42	0.46	0.46		0.49	0.49
	5.00	0.38	0.39	0.41		0.41	0.41
	10.00	0.32	0.33	0.34		0.34	0.45

* 25.0 mg of sulphanilamide and 5.0 ml of 1 N HCl per ml.
† 50 mg of sulphanilic acid and 0.5 ml of 1 N HCl per 50 ml.
‡ 25.0 g of 4-nitroaniline and 2.0 ml of 1 + 1 H_2SO_4 per 50 ml.

Results and Discussion

Preliminary Investigation

The factors affecting the constitution of the composite reagents were studied by placing amounts of aromatic amine solution (1.00%), NED solution (0.20%) and acid in 50-ml beakers, washing into 50-ml calibrated flasks containing 5.00 μg of NO_2-N, diluting to the mark and measuring the absorbances. The 1.00% sulphanilamide and 4-nitroaniline solutions were methanolic solutions, whereas the 1.00% sulphanilic solution was an aqueous solution.

It was established by preliminary work that the 50-ml volumes in which the colours were developed should contain about 5 ml of 1 N hydrochloric acid, 0.5 ml of 1 N hydrochloric acid and 2 ml of 1 + 1 sulphuric acid for the sulphanilamide - NED, sulphanilic acid - NED and 4-nitroaniline - NED methods, respectively. All absorbance readings were made at 540 nm. The possible interference from the yellow colour of the 4-nitroaniline was eliminated by setting the spectrophotometer with a blank solution.

Effect of Amount of Aromatic Amine and NED

A very large excess of the aromatic amine (25 mg of sulphanilamide, 50 mg of sulphanilic acid and 25 mg of 4-nitroaniline) must be present, otherwise inadequate colour development will occur. The results obtained for the effect of amount of NED in the presence of the above amounts of sulphanilamide, sulphanilic acid and 4-nitroaniline are shown in Table 2. The results represent the averages of two runs (averages were also used in the study of the other factors).

The results obtained for the effect of amount of NED in the presence of 50 mg of sulphanilamide or 50 mg of 4-nitroaniline (not shown) were essentially the same as for 25 mg of these compounds. The results obtained for the effect of the amount of NED in the presence of 25 mg of sulphanilic acid (not shown) were essentially the same as for 50 mg, except that the absorbance at the maximum was about 0.02 units lower. It is concluded from Table 2 that the colours from the sulphanilamide - NED, sulphanilic acid - NED and 4-nitroaniline - NED reagents should be measured after 15, 30 and 10 min, respectively (using 4-nitroaniline the colour is completely developed after 8 min). It is seen that the permissible ranges of 0.20% NED solution per 50 ml in the presence of 25 mg of sulphanilamide, 50 mg of sulphanilic acid and 25 mg of 4-nitroaniline are 0.20–0.40, 0.15–0.30 and 0.35–0.75 ml, respectively. The recommended amounts of 0.20% NED solution per 50 ml are 0.30, 0.25 and 0.50 ml, respectively.

The ratios of aromatic amine to NED (41.7 : 1, 100 : 1 and 25 : 1 for the sulphanilamide - NED, sulphanilic acid - NED and 4-nitroaniline - NED reagents, respectively) are much higher than the ratios used by previous investigators, as can be seen from Table 1. The comparative amounts of 0.20% NED solution and ratios of aromatic amine to NED recommended in the proposed composite reagents are a clear indication of the tendency of nitrite to react with the NED (an undesirable characteristic noted by previous investigators). It can be concluded that this tendency to react with the NED decreases in the presence of sulphanilic acid, sulphanilamide and 4-nitroaniline, in that order. The reason is that NO_2 is a stronger electron-donating group than SO_3NH_2, which in turn is a stronger electron-donating group than SO_3H. The stronger the electron-donating group in the aromatic amine, the more readily will the aromatic amine diazotise and couple with the NED, thus decreasing the likelihood of a reaction between nitrite and NED.

An interesting aspect of the data in Table 2 is that in general the colour does not increase in intensity after the recommended development time, no matter how much NED is present. This indicates that nitrosated NED does not react with NED to produce a dye or that all the NO_2^- has been used in the nitrosation reactions.

Effect of Amount of Acid

The results obtained on the effect of the amount of acid on the colours, using the recommended amounts of aromatic amine and NED, are shown in Table 3. It can be seen that the permissible range of acid for the sulphanilamide - NED and sulphanilic acid - NED reagents is 5–10 and 0.20–0.50 ml of 1 N hydrochloric acid, respectively. For the 4-nitroaniline - NED reagent, the permissible acidity covers the range from 3 ml of 1 N hydrochloric acid to 5 ml of 1 + 1 sulphuric acid. The recommended amounts of acid are 6.5 ml of 1 N hydrochloric acid, 0.35 ml of 1 N hydrochloric acid and 3 ml of 1 + 1 sulphuric acid, respectively. The pHs obtained on adding these amounts of acid were all below 1.5.

The effect of acidity on the 4-nitroaniline - NED method was unusual in that the colour seemed to change from orange - red to violet - red as the amount of acid increased. At first it was thought that this might be due to a change in the colour of the dye with increasing acidity. In fact, however, the colour change was due to a decrease in the colour of the 4-nitroaniline with increasing acidity. A solution containing 25 ml of 1 + 1 sulphuric acid and 25 mg of 4-nitroaniline alone was found to be almost colourless. With 3 ml of 1 + 1 sulphuric acid, the yellow colour from the 4-nitroaniline showed no absorption at the wavelength used for measuring the absorbance of the dye. Because sulphanilic acid is a moderately strong acid ($K = 3.89^{14}$), the optimum acidity for the sulphanilic acid - NED reagent is very low and considerable diazotisation - coupling takes place when no other acid is present (Table 3).

The optimum acidity for the spectrophotometric determination of nitrite is different from that for the spectrophotometric determination of the aromatic amine by the diazotisation - coupling technique using an excess of nitrite and NED. For example, for the determination of sulphanilimide, the permissible range of acidity was 1.0 ml of 1 N hydrochloric acid to 10 ml of concentrated hydrochloric acid per 50 ml.[15] We conducted some experiments on the use of phosphoric acid and acetic acid in the sulphanilic acid - NED reagent (using 25 mg of the sulphanilic acid per 50 ml). We found that the optimum range of phosphoric acid was 0.15–0.25 ml of concentrated acid per 50 ml, and concluded that this acid offered no advantage over hydrochloric acid. The optimum range of acetic acid was found to be 3–10 ml of concentrated acid per 50 ml. If 7 ml of acetic acid were selected as optimum, the amount of this acid needed for the preparation of 1 l of composite reagent (assuming that 10 ml of the composite reagent would be used per determination) would be 700 ml. As acetic acid is moderately expensive, its use would seem disadvantageous for reasons of cost.

Non-interference of Methanol

Obviously, it was necessary to establish whether methanol had any effect on the colours, as that solvent had been used to prepare the sulphanilamide and 4-nitroaniline solutions. For this investigation, up to 15 ml of methanol were placed in 50-ml beakers containing the recommended amounts of aromatic amine, NED and acid, the solutions were washed into 50-ml calibrated flasks containing 5.00 μg of NO_2-N and the solutions were diluted to the mark. In no instance was there any significant change in absorbance produced by the added methanol. Also, the methanol had no effect on the stability of the colours.

Calculation of the Constitution of the Composite Reagents

On the basis that 10 ml of composite reagent would be used in the method, it was calculated that 1 l of composite reagent should contain the following: 2.50 g of sulphanilamide, 30 ml

Table 3. Effect of amount of acid (5.00 μg of NO_2-N per 50 ml)

Amine	Acid	Volume/ml	Absorbance after 2 min	8 min	15 min	20 min	30 min	60 min
Sulphanilamide*	—	—	0.00	0.00	0.00		0.00	0.00
	1 N HCl	0.25	0.20	0.30	0.35		0.36	0.36
		0.50	0.25	0.34	0.39		0.40	0.40
		1.0	0.32	0.40	0.41		0.42	0.42
		5.0	0.38	0.42	0.42		0.42	0.42
		10.0	0.41	0.42	0.42		0.42	0.42
	H_2SO_4 (1 + 1)	1.0	0.26	0.38	0.39		0.40	0.40
		3.0	0.18	0.30	0.35		0.38	0.39
		5.0	0.13	0.24	0.28		0.36	0.39
		10.0	0.10	0.16	0.20		0.25	0.26
Sulphanilic acid†	—	—		0.20		0.29	0.30	0.34
	1 N HCl	0.10		0.27		0.33	0.34	0.34
		0.20		0.29		0.35	0.36	0.36
		0.30		0.30		0.355	0.36	0.36
		0.40		0.30		0.355	0.36	0.36
		0.50		0.30		0.35	0.36	0.36
		1.0		0.29		0.34	0.35	0.35
		2.5		0.28		0.33	0.34	0.34
		5.0		0.27		0.31	0.33	0.33
	H_2SO_4 (1 + 1)	2.0				0.23	0.31	0.32
		5.0				0.12	0.21	0.29
		10.0				0.05	0.09	0.10
4-Nitroaniline‡	—	—	0.00	0.00	0.00		0.00	0.00
	1 N HCl	1.0	0.45	0.47	0.47		0.49	0.50
		3.0	0.46	0.495	0.50		0.50	0.50
	H_2SO_4 (1 + 1)	0.5	0.48	0.50	0.50		0.50	0.50
		1.0	0.48	0.50	0.50		0.50	0.50
		5.0	0.48	0.50	0.50		0.50	0.50
		10.0	0.30	0.48	0.49		0.49	0.50
		20.0	0.09	0.15	0.27		0.27	0.27

* 25.0 mg of sulphanilamide and 0.30 ml of 0.20% NED solution per 50 ml.
† 50.0 mg of sulphanilic acid and 0.25 ml of 0.20% NED solution per 50 ml.
‡ 25.0 mg of 4-nitroaniline and 0.50 ml of 0.20% NED solution per 50 ml.

of 0.20% NED and 650 ml of 1 N hydrochloric acid; 5.00 g of sulphanilic acid, 25 ml of 0.20% NED solution and 35 ml of 1 N hydrochloric acid; and 2.50 g of 4-nitroaniline, 50 ml of 0.20% NED solution and 300 ml of 1 + 1 sulphuric acid. With the aromatic amines and NED calculated on a *m/V* basis and the acid calculated on a *V/V* basis, this would be equivalent to the following percentages: 0.25% sulphanilamide, 0.0060% NED and 5.42% hydrochloric acid; 0.50% sulphanilic acid, 0.0050% NED and 0.29% hydrochloric acid; and 0.25% 4-nitroaniline, 0.0100% NED and 15.0% sulphuric acid. As the aromatic amines can be dissolved directly in the acid, the methanol (which would add considerably to the cost of the reagent) can be omitted.

The sulphanilamide - NED and sulphanilic acid - NED reagents should be stored in brown bottles in a refrigerator; the 4-nitroaniline - NED reagent should be stored in a brown bottle but not in a refrigerator. If stored at room temperature for more than a few days, the sulphanilamide - NED and sulphanilic acid - NED reagents give lower results and high blanks. We believe that the reason for the difference in stability of the reagents at room temperature is that NED deteriorates at room temperature in the dilute hydrochloric acid solutions used in the sulphanilamide - NED and sulphanilic acid - NED reagents but does not deteriorate in the moderately concentrated sulphuric acid solution used in the 4-nitroaniline - NED reagent. It was found that a solution of NED in 15% sulphuric acid solution remained colourless at room temperature for several months, whereas solutions of NED in 5.42 and 0.29% hydrochloric acid darkened after a few days. Apparently, NED (a weakly basic aromatic amine) requires a large excess of acid, preferably sulphuric acid, to form the stable salt. In assessing the importance of the deterioration of the NED, it should be borne in mind that this deterioration would cause the amount of NED to fall below the minimum critical amount necessary for full development of the colour.

Wavelength of Maximum Absorption

The colours obtained with sulphanilamide - NED and sulphanilic acid - NED reagents are violet - red. The 4-nitroaniline - NED reagent produces the same sort of colour, but it is affected by the yellow colour of the excess of 4-nitroaniline, as indicated earlier. The wavelength of maximum absorption ($\lambda_{max.}$) of the colours obtained with the sulphanilamide - NED, sulphanilic acid - NED and 4-nitroaniline - NED reagents, as determined using a Cary Model 519 recording spectrophotometer, were 542, 541 and 542 nm, respectively. The wavelength setting in the analytical method is not critical. At an absorbance of about 0.5, the same readings were obtained with a Bausch and Lomb Model 70 spectrophotometer over a range ±2 nm of the $\lambda_{max.}$. At lower absorbances, the permissible range would, of course, be even greater, as the peaks are broader.

Effect of Temperature on Colour Development

Cooling in an ice-bath to about 5–10 °C increased the time required for development of the colour but had no other effect. Heating to 50–70 °C after adding the reagent speeded the colour development; however, as time would be required for cooling the solution before the colour measurement, heating would not be worthwhile. Heating the solution to

incipient boiling or vigorous boiling after adding the reagent caused the results to be 25–40% low. Heating the solution before the addition of reagent destroyed the nitrite to varying extents (depending on the temperature and time of heating) and hence caused low results. The colours were not light-sensitive.

Effect of Amount of Composite Reagent

The effect of the volume of composite reagent used per determination was studied by adding volumes of composite reagent covering the range 1.0–49.0 ml to 50-ml calibrated flasks containing 5.00 μg of NO_2-N (Table 4). The colours developed more rapidly with larger amounts of composite reagent (greater than 35 ml), but the results reported are for the previously recommended development times. The results are surprising in that there is no significant change of absorbance over the range 7.5–49.0 ml of sulphanilamide - NED and sulphanilic acid - NED reagent and 3.0–49.0 ml of 4-nitroaniline - NED reagent. The results indicate that the proportions of aromatic amine, NED and acid are just as important as their actual amounts. The use of 10 ml of composite reagent is recommended. The reagent can be added with a graduated cylinder, as the volume is not critical. Also, it is obvious that it is immaterial whether the reagent is added to the sample and the volume adjusted to 50 ml or if some water is first added to the sample, the reagent added and the volume then adjusted to 50 ml.

Table 4. Effect of volume of composite reagent (5.00 μg of NO_2-N per 50 ml)

Volume of composite reagent/ml	Absorbance*		
	Sulphanilamide - NED	Sulphanilic acid - NED	4-Nitroaniline - NED
49.0†	0.42	0.36	0.50
35.0‡	0.425	0.36	0.50
20.0‡	0.42	0.35	0.50
15.0‡	0.42	0.36	0.50
10.0‡	0.42	0.36	0.50
7.5‡	0.42	0.36	0.50
5.0‡	0.41	0.32	0.50
3.0‡	0.27	0.21	0.50
1.0‡	0.10	0.28	0.47

* The absorbances were measured after 15, 30 and 10 min for the sulphanilamide, sulphanilic acid and 4-nitroaniline reagents, respectively.

† 1.00 ml of a standard nitrite solution containing 5.00 μg of NO_2-N per ml was used.

‡ 5.00 ml of standard nitrite solution B was used.

Determination of Larger Amounts of Nitrite

In determining larger amounts of nitrite by previous diazotisation - coupling techniques, the dye may precipitate or low results may be obtained on dilution to a high volume with water. This is not true in the present methods if a large excess of composite reagent is used. For example, on treating 140, 160 and 120 μg of NO_2-N with 100 ml of sulphanilamide - NED, sulphanilic acid - NED and 4-nitroaniline - NED reagent, respectively, and diluting to 1 l with water in a calibrated flask, the recoveries obtained were 142, 156 and 122 μg, respectively. In these experiments, the 100 ml of sulphanilamide - NED and sulphanilic acid - NED reagents were allowed to come to room temperature before use. The above dilution method, although useful under certain circumstances, is wasteful of reagent and ordinarily it would be simpler to take a small aliquot and proceed as in the regular method.

Accuracy and Precision of the Recommended Methods

The calibration graphs for all three reagents followed Beer's law. The methods decrease in sensitivity in the order 4-nitroaniline - NED > sulphanilamide - NED > sulphanilic acid - NED (the ratio of the sensitivities is 1.00 : 0.84 : 0.72). The accuracy and precision of the methods were tested by analysing four aliquots of standard nitrite solution B on five different days. The results (Table 5) indicate that the accuracy and precision decrease in the order 4-nitroaniline - NED > sulphanilamide - NED > sulphanilic acid - NED; however, the accuracy and precision of all three methods are satisfactory.

Use of Other Aromatic Amines and Coupling Agents

We tested several aromatic amines, including 2-aminobenzoic acid, 2- and 4-chloroaniline, 2- and 4-methylaniline, 2- and 4-methoxyaniline, 2- and 4-dinitroaniline, 2,4,6-trichloroaniline and *m*- and *p*-phenylenediamine. None was found to give better results than sulphanilamide, sulphanilic acid or 4-nitroaniline. The results for the phenylenediamines were found to be particularly low, probably because the two NH_2 groups present a special situation.[16] We found that NED (in acidic solution) was such an excellent coupling agent that we did not conduct any experiments with other coupling agents.

References

1. Boltz, D. F., and Howell, J. A., "Colorimetric Determination of Nonmetals," Wiley, New York, 1978, pp. 216–220.
2. Williams, W. J., "Handbook of Anion Determination," Butterworths, London, 1979, pp. 147–151.
3. Lange, B., and Vejdělek, Z. J., "Photometrische Analyse," Verlag Chemie, Weinheim and Deerfield Park, FL, 1980, pp. 369–371.
4. Lunge, G., *Z. Angew. Chem.*, 1889, No. 23, 666; *Analyst*, 1890, **15**, 17.

Table 5. Accuracy and precision of the results for nitrite

NO_2-N added/ μg	NO_2-N recovered/μg*								
	Sulphanilamide - NED			Sulphanilic acid - NED			4-Nitroaniline - NED		
	Average	Difference	Std. dev.	Average	Difference	Std. dev.	Average	Difference	Std. dev.
1.00	1.04	0.04	±0.061	0.98	0.02	±0.033	1.05	0.05	±0.066
3.00	2.93	0.07	±0.061	3.11	0.11	±0.113	3.03	0.03	±0.045
5.00	4.98	0.02	±0.055	5.07	0.07	±0.098	4.98	0.02	±0.036
6.00	5.98	0.02	±0.070	5.94	0.06	±0.066	6.04	0.04	±0.049

* Average of five determinations. The average differences for all the runs for the sulphanilamide - NED, sulphanilic acid - NED and 4-nitroaniline - NED reagents were 0.038, 0.065 and 0.035 μg, respectively, and the average standard deviations were 0.062, 0.078 and 0.049 μg, respectively.

5. Yoe, J. H., "Photometric Chemical Analysis," Volume I, Wiley, New York, 1928, pp. 308–309.
6. Wattenberg, H., *Cons. Int. Explor. Mer. Raap. Proc. Verb.*, 1937, **103**, 1.
7. "Official Methods of Analysis of the Association of Official Analytical Chemists," Twelfth Edition, Association of Official Analytical Chemists, Washington, DC, 1975, Method 24.037–24.038.
8. Hamilton, J. E., *J. Assoc. Off. Anal. Chem.*, 1976, **59**, 284.
9. "Official Methods of Analysis of the Association of Official Analytical Chemists," Thirteenth Edition, Association of Official Analytical Chemists, Washington, DC, 1980, Method 24.041–24.042.
10. Hart, F. L., and Fisher, H. J., "Modern Food Analysis," Springer-Verlag, New York, 1971, pp. 195–196.
11. "Standard Test Methods for Nitrite - Nitrate in Water," American Society for Testing and Materials, Philadelphia, PA, 1983, D3867–79.
12. "Methods for Chemical Analysis of Water and Wastes," US Environmental Protection Agency, Cincinnati, OH, 1974, pp. 201–216.
13. "Bioassay of *N*-(1-Naphthyl)ethylenediamine Dihydrochloride for Possible Carcinogenicity," National Cancer Institute Carcinogenic Test Program, Bethesda, MD, Report No. 79–1724 (PB289733), 1978.
14. Weast, R. C., *Editor*, "CRC Handbook of Chemistry and Physics," Sixty-third Edition, CRC Press, Cleveland, OH, 1982, p. D172.
15. Norwitz, G., and Keliher, P. N., *Anal. Chem.*, 1982, **54**, 807.
16. Norwitz, G., and Keliher, P. N., *Anal. Chem.*, 1983, **55**, 1226.

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Extraction and Spectrophotometric Determination of Bismuth(III) with *N-m*-Tolyl-*p*-Chlorobenzohydroxamic Acid and Xylenol Orange

Yadvendra K. Agrawal and Vinay J. Bhatt
Analytical Laboratories, Pharmacy Department, Faculty of Technology and Engineering, M.S. University of Baroda, Kalabhavan, Baroda-390001, India

A solvent extraction and spectrophotometric method for the determination of microgram amounts of bismuth(III) is described. The bismuth(III) is extracted at pH 4.5 with *N-m*-tolyl-*p*-chlorobenzohydroxamic acid and forms a mixed complex with xylenol orange, which gives a red colour, $\lambda_{max.}$ 530 nm and molar absorptivity 2.0×10^4 l mol^{-1} cm^{-1}. Bismuth has been determined in alloy and pharmaceutical samples.

Keywords: *Bismuth determination; N-m-tolyl-p-chlorobenzohydroxamic acid; xylenol orange; spectrophotometry; micro-determination*

Bismuth is a toxic metal obtained as a by-product from tin, lead and copper ores and used in the manufacture of alloys, mirror silvers, low-melting solders and heat-sensitive devices.[1] Trivalent bismuth salts are used medicinally to control diarrhoea and other gastrointestinal distresses. Bismuth salts are also used as radio-contrast agents. The toxic effects of bismuth on man consist of decreased appetite, weakness, rheumatic pain, diarrhoea and dermatitis.

Various reagents, *viz.*, dithizone, cupferron, diethyl dithiocarbamate, 8-hydroxyquinoline, xylenol orange, etc.,[2] have been used for the extraction and spectrophotometric determination of bismuth and, generally, interferences from Hg^{2+}, Pb^{2+} and Ag^{2+} were observed. It has been pointed out that the hydroxamic functional group has a considerable ability to form complexes with several metal ions.[3–5] *N*-Phenylbenzohydroxamic acid (PBHA) has been used for the gravimetric[6] and polarographic[7] determination of bismuth. In this investigation, bismuth was extracted into chloroform using *N-m*-tolyl-*p*-chlorobenzohydroxamic acid and determined spectrophotometrically using xylenol orange. The metals that interfere in the direct determination of bismuth with xylenol orange can be eliminated by first extracting the bismuth with hydroxamic acid.

Experimental

Reagents and Chemicals

All the chemicals used were of AnalaR or general-reagent grades from BDH Chemicals and E. Merck, respectively, unless specified otherwise.

N-m-*Tolyl*-p-*chlorobenzohydroxamic acid.* Synthesised as described elsewhere.[8] Its purity was checked by a melting-point determination, TLC and its IR and UV spectra. A 0.2% solution was prepared in chloroform.

Xylenol orange solution, 0.1% in ethanol. Prepared by dissolving 100 mg of xylenol orange in 100 ml of ethanol.

Standard bismuth solution. Prepared by dissolving 0.0242 g of bismuth nitrate in 250 ml of distilled water. A few drops of nitric acid were added to prevent hydrolysis. The final concentration was determined titrimetrically.[9]

Apparatus

The spectral measurements were made on a VSU2-P spectrophotometer (Carl Zeiss, Jena, GDR). The pH adjustments were made on a Systronics digital pH meter equipped with glass and calomel electrodes.

A Varian Techtron Model AA-6 atomic-absorption spectrophotometer equipped with an HCN - Bi hollow-cathode lamp was used. The 223.6-nm bismuth line and an air - acetylene flame were used.

Procedure

Take 2 ml of bismuth solution (2.0×10^{-4} M) in a separating funnel and dilute to 10 ml with water. Adjust the pH to 4.5 with acetic acid and sodium acetate buffer. Add 10 ml of reagent solution, shake the contents for 10 min and allow the phases to separate. Transfer the chloroform layer, after drying over anhydrous sodium sulphate, into a 25-ml calibrated flask. Wash the sodium sulphate with 2.5 ml of chloroform and add 10 ml of 0.1% ethanolic xylenol orange solution to the bismuth - hydroxamate complex. Dilute the extract to 25 ml with ethanol and measure the absorbance at 530 nm against a blank solution similarly prepared but without bismuth.

The method for the determination of bismuth by AAS is essentially the same as that described by Hofton and Hubbard.[10] The extracts were aspirated into the flame either directly or after back-extraction with 0.1 M HCl.

Results and Discussion

Effect of pH

The extraction of bismuth(III) was carried out at various pH values in the range 2.0–7.0. Quantitative extraction of bismuth into the organic phase was observed from pH 4.2 to 4.8. Outside this range the extraction was incomplete; hence all extractions were carried out at pH 4.5 (Table 1).

Table 1. Effect of pH on the extraction of bismuth(III). Bismuth(III) concentration, 3.34 p.p.m. Solvent, chloroform - ethanol (1 + 1). *N-m*-Tolyl-*p*-chlorobenzohydroxamic acid solution, 10 ml (0.2%) in chloroform; colour, red. Xylenol orange solution, 10 ml (0.1%) in ethanol. $\lambda_{max.}$ = 530 nm

pH	Absorbance	Extraction, %	Molar absorptivity/ l mol^{-1} cm^{-1}
2.0	0.040	12.5	2.7×10^3
3.0	0.240	75.0	1.5×10^4
3.5	0.260	81.3	1.6×10^4
4.0	0.300	93.8	1.9×10^4
4.2	0.320	100.0	2.0×10^4
4.5	0.320	100.0	2.0×10^4
4.8	0.320	100.0	2.0×10^4
5.0	0.280	87.5	1.7×10^4
5.5	0.250	78.1	1.6×10^4
6.0	0.230	71.9	1.4×10^4
7.0	0.100	31.3	6.2×10^3

Table 2. Effect of *N-m*-tolyl-*p*-chlorobenzohydroxamic acid concentration. Bismuth(III) concentration, 3.34 p.p.m. Solvent, chloroform - ethanol (1 + 1). Xylenol orange solution, 10 ml (0.1%) in ethanol; colour, red. pH = 4.5. $\lambda_{max.}$ = 530 nm

N-m-Tolyl-*p*-chlorobenzohydroxamic acid solution			
Concentration, %	Volume added/ ml	Absorbance	Molar absorptivity/ l mol^{-1} cm^{-1}
0.10	2.0	0.240	1.5×10^4
	3.0	0.260	1.61×10^4
	5.0	0.300	1.7×10^4
0.20	5.0	0.320	2.0×10^4
	8.0	0.320	2.0×10^4
	10.0	0.320	2.0×10^4

Table 3. Effect of xylenol orange concentration. Bismuth(III) concentration, 3.34 p.p.m. Solvent, chloroform - ethanol (1 + 1). *N-m*-Tolyl-*p*-chlorobenzohydroxamic acid solution, 10 ml (0.2%) in chloroform; colour, red. pH = 4.5. $\lambda_{max.}$ = 530 nm

Xylenol orange (0.1%)/ml	Absorbance	Molar absorptivity/ l mol^{-1} cm^{-1}
0.5	0.09	5.6×10^3
1.0	0.18	1.2×10^4
2.0	0.22	1.4×10^4
5.0	0.29	1.8×10^4
6.0	0.32	2.0×10^4
10.0	0.32	2.0×10^4

Effect of Reagent Concentration

Bismuth was extracted at pH 4.5 with various reagent concentrations. The results showed that 5 ml of 0.2% *N-m*-tolyl-*p*-chlorobenzohydroxamic acid solution was adequate for the quantitative extraction of bismuth. Lower concentrations gave incomplete extraction. Larger amounts of reagent could be used and had no effect on the absorbance of the extracted species (Table 2). Hence, all the extractions were carried out with 10 ml of 0.2% reagent solution.

Effect of Xylenol Orange Concentration

The effect of xylenol orange concentration on the extraction of bismuth(III) was studied. It was observed that 10 ml of 0.1% xylenol orange solution gave the maximum colour intensity (Table 3).

Effect of Shaking Time and Stability of the Colour

It was confirmed that manual shaking for 5–10 min was sufficient for the complete extraction of bismuth. However, a longer shaking time has no effect on the equilibrium system. The complex is stable under optimum conditions; the absorbance remains unchanged for several days.

Validity of Beer's Law

Beer's law is obeyed within the range 0.334–16.700 p.p.m. of bismuth with *N-m*-tolyl-*p*-chlorobenzohydroxamic acid. The molar absorptivity of the extracted species was 2.0×10^4 l mol^{-1} cm^{-1} at 530 nm. The Beer's law plot of absorbance against concentration was a straight line passing through the origin with a slope of 0.0958. The correlation coefficient was calculated to be 1.0006.

Table 4. Effect of cations and anions on the extraction of bismuth(III). Bismuth(III) concentration, 3.34 p.p.m. pH = 4.5. *N-m*-Tolyl-*p*-chlorobenzohydroxamic solution, 10 ml (0.2%) in chloroform. Solvent, chloroform - ethanol (1 + 1). Xylenol orange, 10 ml (0.1%) in ethanol; absorbance = 0.320. $\lambda_{max.}$ = 530 nm

Ion	Added as	Amount added/mg	Absorbance
Ag^+	$AgNO_3$	30	0.320
Ba^{2+}	$BaCl_2$	50	0.325
Sr^{2+}	$SrCl_2$	50	0.325
Ca^{2+}	$CaCl_2$	40	0.320
Cu^{2+}	$CuSO_4$	40	0.320
Pb^{2+}	$Pb(NO_3)_2$	40	0.320
Cd^{2+}	$CdSO_4$	40	0.320
Mg^{2+}	$MgSO_4$	40	0.320
Fe^{3+}	$FeCl_3$	40	0.320
Sn^{2+}	$SnCl_2$	40	0.325
Sb^{3+}	$K(SbO)CuH_4O_6.½H_2O$	40	0.320
Hg^{2+}*	$HgCl_2$	30	0.320
Ti^{4+}†	TiO_2	30	0.320
Zr^{4+}	$ZrOCl_2$	30	0.325
Hf^{4+}	$HfOCl_2$	30	0.320
Ce^{4+}*	$(NH_4)_4Ce(SO_4)_4.2H_2O$	30	0.320
Nb^{5+}	Nb_2O_5	30	0.320
Ta^{5+}	Ta_2O_5	30	0.325
V^{5+}	NH_4VO_3	30	0.325
Mo^{6+}	Na_2MoO_4	40	0.325
U^{6+}*	$UO_2(CH_3COO)_2$	40	0.320
Al^{3+}	$Al(NO_3)_3$	40	0.320
Zn^{2+}	$ZnCl_2$	40	0.320
SO_4^{2-}	Na_2SO_4	50	0.320
Cl^-	NaCl	40	0.320
F^-	NaF	40	0.320

* Masked with $SnCl_2$.
† Masked with sodium fluoride.

Table 5. Extraction and determination of bismuth(III) in a pharmaceutical preparation, pectomycin. Label amount of bismuth(III) in pectomycin = 13.97 mg ml^{-1}. Note: the Indian Pharmacopeia and British Pharmacopoeia do not describe any method for the determination of bismuth in pharmaceuticals

Label amount of bismuth in pectomycin taken, p.p.m.	Bismuth found, p.p.m.		Relative Standard deviation, *%
	AAS	Present method	
1.91	1.90	1.89	1.58
3.83	3.81	3.81	0.78
5.74	5.74	5.74	0.35
9.55	9.54	9.53	0.21
13.37	13.35	13.36	0.15

* Six determinations.

Effect of Diverse Ions

Different amounts of diverse ions were added to a fixed amount of bismuth and the bismuth was extracted according to the procedure. Most of the metal ions associated with bismuth do not interfere, except for Ce^{4+} (>5 mg), U^{6+} (>100 μg), Hg^{2+} (>5 mg) and Ti^{4+} (>3 mg). The interference from large amounts of Ce^{4+}, U^{6+} and Hg^{2+} can be overcome by masking them with tin(II) chloride and Ti^{4+} with sodium fluoride. The data are given in Table 4.

Analysis of a Pharmaceutical Sample

Pectomycin (Manufactured by Lyka Labs., India) containing bismuth carbonate as an active ingredient was analysed by the proposed method. A 0.5-ml volume of pectomycin syrup was dissolved in 5 N H_2SO_4 (25 ml) and centrifuged. The aqueous layer was diluted to 250 ml with doubly distilled water, then

Table 6. Determination of bismuth in standard alloy, steel and brass samples

Sample	Composition, %	Bismuth content, %	Bismuth found, %		Relative error, %	Relative standard deviation, %
			AAS	Present method*		
Sn - Bi alloy (BCS 216)	Sn + Bi (25.00)	25.00	25.50	25.00	0.0	−0.01, −0.01, +0.01, +0.03, −0.02
Pb - Bi alloy (BCS 139)	Pb + Bi (15.40)	15.40	15.36	15.00	2.6	+0.30, +0.40, −0.40, −0.42, −0.28
NBS 33b cast iron	C (2.25); Si (2.00); P (0.011); S (0.03); Mn (0.064); Ni (2.25); Cr (0.61); Mo (0.40); Fe (91.7); Bi (0.502)	0.50	0.50	0.49	2.0	−0.01, +0.01, +0.02, −0.01, −0.02
Brass (BCS 41)	Pb (2.33); Zn (40.45); Cu (56.57); Fe (0.009); Bi (0.497)	0.497	0.507	0.497	0.0	+0.01, −0.03, −0.01, +0.02, +0.01

* Average of five determinations.

bismuth was extracted from an aliquot of this solution according to the recommended procedure. The results obtained were in good agreement with the calculated value (Table 5).

Determination of Bismuth in Standard Alloys, Steel and Brass

Dissolve 0.5 g of alloy, steel or brass as described elsewhere[11] and make up to a standard volume. Extract an aliquot as in the recommended procedure. The results for some alloys and synthetic mixtures are shown in Table 6.

References

1. Oehme, O. W., "Toxicity of Heavy Metals in the Environment, Part II," Marcel Dekker, New York, 1979.
2. Patke, S. K., *PhD Thesis*, M. S. University of Baroda, 1981.
3. Agrawal, Y. K., *Rev. Anal. Chem.*, 1980, **5**, 3.
4. Agrawal, Y. K., *Anal. Chem.*, 1975, **47**, 940.
5. Agrawal, Y. K., and Patel, S. A., *Rev. Anal. Chem.*, 1980, **4**, 232.
6. Das, J., and Shome, S. C., *Anal. Chim. Acta*, 1961, **24**, 31.
7. Chwastowska, J., and Minczewski, J., *Chem. Anal. (Warsaw)*, 1964, **9**, 791.
8. Agrawal, Y. K., and Tandon, S. G., *J. Chem. Eng. Data*, 1971, **16**, 371.
9. Vogel, A. I., "A Text-Book of Quantitative Inorganic Analysis," Longmans, London, 1961, p. 442.
10. Hofton, M. E., and Hubbard, D. P., *Anal. Chim. Acta*, 1972, **62**, 311.
11. Shapiro, L., and Brannock, W. W., "Rapid Analysis of Silicate, Carbonate and Phosphate Rocks," Geological Survey Bulletin, 1144–A, United States Government Printing Office, Washington, DC, 1962.

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Spectrophotometric Determination of Tellurium after Separation by Coprecipitation of Its Trifluoroethylxanthate with Naphthalene

Md. Farid Hussain, Bal Krishan Puri* and Raj Kumar Bansal
Department of Chemistry, Indian Institute of Technology, New Delhi-110 016, India
and Masatada Satake
Faculty of Engineering, Fukui University, Fukui 910, Japan

Tellurium reacts with potassium trifluoroethylxanthate (potassium *O*-trifluoroethyl dithiocarbonate) to form a water-insoluble 1 : 2 (metal to ligand) complex in the pH range 0.5–2.5. This complex is easily coprecipitated with microcrystalline naphthalene from its acetone solution. It absorbs in the range 405–415 nm; Beer's law is obeyed over the concentration range 1.5–20.0 p.p.m. of Te; and the molar absorptivity and Sandell sensitivity are 6.698×10^3 l mol^{-1} cm^{-1} and 0.0185 μg cm^{-2} of Te, respectively. Ten replicate determinations on samples containing 37 μg of Te gave a mean absorbance of 0.200 with a standard deviation of 0.0022 and a relative standard deviation of 1.1%. The interference of various ions was studied and optimum conditions were developed for the determination of tellurium in various copper - tellurium alloys and synthetic samples.

Keywords: *Tellurium determination; spectrophotometry; potassium trifluoroethylxanthate; coprecipitation; alloys*

Xanthates have been widely used as analytical reagents for the determination of metal ions using various techniques.[1–4] A survey of the literature revealed that only simple xanthates have been used for the extraction and spectrophotometric determination of metal ions but no attempt has been made to determine metal ions using substituted xanthates. Preliminary observations have revealed that halogen-substituted xanthates are reasonably sensitive and selective as complexing agents for the determination of metal ions. A new extraction technique, that is, solid - liquid separation after liquid - liquid extraction, has been developed and applied successfully to the determination of various metal ions using different complexing agents.[5–8] However, although there are many advantages of this method over the usual liquid - liquid extraction, it cannot be applied to thermally unstable complexes. Recently, we have found that most of the metal complexes in aqueous solution can easily be coprecipitated with microcrystalline naphthalene. This method is more convenient (it is carried out at room temperature) rapid (naphthalene does not require heating), economical (only 0.4 g of naphthalene is required) and sensitive and can be applied to many types of complexes. In this paper, potassium trifluoroethylxanthate (potassium *O*-trifluoroethyl dithiocarbonate) is recommended as a complexing agent for the quantitative determination of tellurium after coprecipitation of its trifluoroethylxanthate with microcrystalline naphthalene. Various parameters such as the effect of pH, reagent concentration, digestion time, naphthalene concentration, shaking time and aqueous phase volume have been studied and the optimum conditions have been established for the determination of tellurium in copper - tellurium alloys and synthetic samples. The method has been found to be reasonably sensitive as only 0.4 g of naphthalene is required for coprecipitation of the metal complex from 80 ml of aqueous phase.

Experimental

Equipment

An Elico pH meter and Pye Unicam SP-500 and SP-700 spectrophotometers were used.

Reagents

Distilled water and analytical-reagent grade chemicals were used throughout.

Potassium trifluoroethylxanthate. Prepare by the method given by DeWitt and Roper.[9] For 0.2 and 0.5% solutions prepare in 2 ml of dimethylformamide and distilled water.

Standard tellurium solution. Dissolve 1.785 g of $K_2TeO_3.3H_2O$ in distilled water, dilute to 500 ml and standardise[10] gravimetrically by reducing with hydrazine sulphate.

Perchloric acid solution, 1 and 10 M.

Ammonia solution, 1 M.

Naphthalene solution, 20%. Prepare in acetone.

Naphthalene. Check the purity spectrophotometrically before use.

General Procedure

Transfer about 40 ml of sample solution containing 15–200 μg of tellurium into a 100-ml Erlenmeyer flask with a tightly fitting stopper. Add 2 ml of 0.2% potassium trifluoroethylxanthate and adjust to pH 0.5–2.5 with perchloric acid and ammonia solution. Mix the solution well, allow it stand for 2 min, then add 2 ml of 20% naphthalene solution and shake vigorously for 1 min. Filter through a filter-paper (*e.g.*, No. 5c, Toyo Roshi Co.) placed flat on a filter-plate in a funnel. Dissolve the solid in chloroform, dilute to 10 ml and measure the absorbance in a 1-cm cell at 410 nm against a reagent blank prepared similarly.

Results and Discussion

Absorption Spectra

A sample solution containing 37 μg of tellurium and 2 ml of 0.2% potassium trifluoroethylxanthate at pH 1.5 was prepared by the described procedure, and coprecipitation of the complex was carried out. Fig. 1 shows the absorption spectra of the reagent blank and that of the tellurium complex in naphthalene - chloroform solution. The tellurium complex shows absorption in the range 405–415 nm, the absorption of the reagent blank being negligible at this wavelength. Thus, in every instance the absorption was measured at 410 nm.

*To whom correspondence should be addressed.

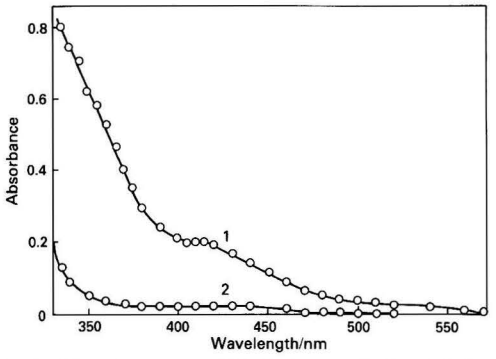

Fig. 1. Absorption spectra of (1) tellurium complex in naphthalene - chloroform solution, measured against a reagent blank; and (2) reagent blank, measured against water. Conditions: Te, 37 μg; 0.2% potassium trifluoroethylxanthate, 2 ml; 20% naphthalene solution, 2 ml; chloroform, 10 ml; pH 1.5; and $\lambda_{max.}$ = 405–415 nm

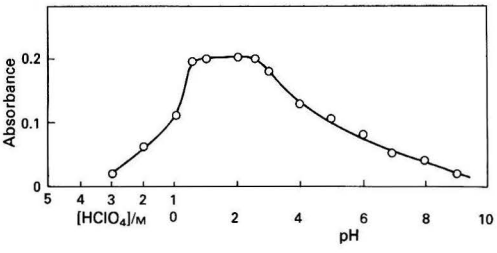

Fig. 2. Effect of acidity on absorbance of the Te complex. Conditions as in Fig. 1 except wavelength 410 nm and variable acidity

Effect of Acidity

The pH of sample solutions containing 37 μg of tellurium and 2 ml of 0.2% potassium trifluoroethylxanthate was adjusted to the desired values with perchloric acid and ammonia solution and coprecipitation of the chelate was then carried out. The result is shown in Fig. 2. Coprecipitation of the chelate started at 3 M perchloric acid and reached a maximum at pH 0.5 and thereafter it remained constant over the pH range 0.5–2.5. Therefore, the pH of the solution was adjusted to 1.5 for subsequent coprecipitation of the chelate.

Effect of Reagent

Various amounts of 0.2% trifluoroethylxanthate were added to the solution containing 37 μg of tellurium at pH 1.5 and the procedure as given above was followed. The absorbance remained constant when volumes of the reagent solution between 0.5 and 6.0 ml were used. Therefore, 2.0 ml of 0.2% reagent were preferred throughout the experiment.

Effect of Digestion Time

A solution containing 37 μg of tellurium(IV) and 2.0 ml of 0.2% reagent at pH 1.5 was digested at room temperature and then the general procedure for coprecipitation was followed. It was observed that the absorbance increased with increase in digestion time up to 2 min, then remained almost constant between 2 and 10 min. Therefore, a digestion time of 5 min was chosen for the coprecipitation of the chelate.

Effect of Naphthalene

Coprecipitation of the chelate was carried out by changing the volume of 20% naphthalene solution added to the solution containing tellurium chelate. The absorbance increased with increase in the amount of naphthalene up to 0.5 ml of 20% naphthalene solution and then remained almost constant between 0.5 and 5.0 ml. Therefore, 2.0 ml of 20% naphthalene solution were used for coprecipitation of the chelate.

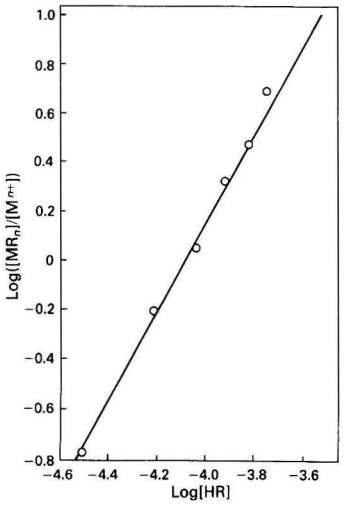

Fig. 3. Logarithmic method of formula determination for the tellurium trifluoroethylxanthate complex. Initial tellurium concentration, 3×10^{-4} M; potassium trifluoroethylxanthate concentration, 3×10^{-4} M; pH, 1.5; other conditions as in Fig. 1

Table 1. Effect of anions on the determination of tellurium. Conditions: Te, 74 μg; 0.5% potassium trifluoroethylxanthate, 5 ml; 20% napthalene solution, 2 ml; and pH, 1.5

Salt added	Amount added/mg	Absorbance at 410 nm
—	—	0.400
Sodium fluoride	80	0.380
Potassium chloride	100	0.400
Potassium bromide	80	0.390
Potassium iodide	60	0.400
Sodium carbonate	100	0.380
Potassium sulphate	100	0.400
Sodium phosphate	100	0.400
Potassium thiocyanate	100	0.390
Sodium acetate	100	0.400
Sodium citrate	100	0.400
Sodium oxalate	100	0.380
Ammonium tartrate	100	0.400
Disodium EDTA	40	0.380

Effect of Shaking Time

A 2-ml volume of 20% naphthalene solution was added to a sample containing the tellurium complex at pH 1.5 and the mixture was shaken vigorously for periods of time ranging from 5 to 100 s. The results showed that the tellurium complex was coprecipitated quantitatively with microcrystalline naphthalene on vigorous shaking during that period. Therefore, a 1-min shaking time was preferred.

Effect of Standing Time

The coprecipitated naphthalene mixture was dissolved in chloroform and the effect of standing time on the absorbance was investigated. The results indicated that the tellurium complex in chloroform was stable for only 2 h. Therefore, a 15-min standing time was selected for all measurements.

Table 2. Effect of cations on the determination of tellurium. Conditions as given in Table 1

Metal salt added	Metal ion added/μg	Absorbance at 410 nm		Remarks
—	—	0.400		
Aluminum nitrate	1000	0.400		
Ammonium metavanadate	200	0.415		
Ammonium molybdate	300	0.315	0.390	After masking with 2 ml of 5% ascorbic acid
	200	0.360	0.395	
Antimony(III) chloride	250	0.385		
Bismuth nitrate	100	0.410		
Cadmium chloride	500	0.390		
Chromium(III) nitrate	500	0.395		
Cobalt(II) nitrate	250	0.160	0.395	After masking with 10 ml of 5% NaCN solution
	25	0.375	0.400	
Copper(II) chloride	100	—	0.415	
	10	0.410	0.410	
Indium(II) sulphate	500	0.394		
Iridium(III) chloride	100	0.460	0.400	After masking with 10 ml of 5% NaCN solution
	10	0.400	0.400	
Iron(III) chloride	300	0.224	0.385	After masking with 2 ml of 5% ascorbic acid
	100	0.340	0.390	
	50	0.380	0.395	
Lead (II) nitrate	250	0.390		
Manganese(II) acetate	1000	0.395		
Mercury(II) chloride	350	0.170	0.380	After masking with 2 ml of 5% KI solution
	100	0.280	0.385	
	25	0.370	0.400	
Nickel chloride	500	—	0.420	After masking with 10 ml of 5% NaCN solution
	100	0.440	0.410	
Osmium tetraoxide	1000	0.395		
Palladium chloride	100	0.190	0.390	After masking with 10 ml of 5% NaCN solution
	5	0.380	0.395	
Platinum(IV) chloride	500	0.400	—	
Potassium selenite	500	0.110	0.370	After pre-coprecipitation with 2 ml of 20% naphthalene solution in 4 M $HClO_4$
	100	0.390	0.395	
Rhodium(III) chloride	500	0.400		
Silver(I) nitrate	200	0.390		
Sodium arsenite(III)	500	0.390		
Sodium tungstate	500	0.400		
Thallium(I) nitrate	500	0.400		
Thorium(IV) nitrate	500	0.400		
Uranyl acetate	250	0.385		
Zinc nitrate	200	0.390		
Zirconyl choride	500	0.400		

Effect of Aqueous Phase Volume

The volume ratio of the organic to aqueous phase is an important factor for the coprecipitation of the tellurium complex with naphthalene. Therefore, the effect of volume ratio on the absorbance was examined. The absorbance remained almost constant up to 80 ml of the aqueous phase. With further increase in the volume of the aqueous phase, the absorbance decreased.

Choice of Organic Solvent

Various organic solvents were examined for dissolving the coprecipitated tellurium complex with microcrystalline naphthalene. The complex mixture is soluble in chloroform, benzene, carbon tetrachloride, isobutyl methyl ketone, dimethylformamide, chlorobenzene, 1,2-dichloroethane, *o*-xylene and acetonitrile. Chloroform was found to be the best solvent for this system.

Composition of the Complex

The composition of tellurium trifluoroethylxanthate was established by Job's method. A sharp peak at a 0.30 molar fraction suggested that $Te(CF_3CH_2OCS_2)_2$ was coprecipitated with microcrystalline naphthalene under these conditions, probably owing to the reduction of tellurium(IV) to tellurium(II) by the xanthate in acidic media. Further, the composition was also ascertained by the logarithmic method.[11] The required values of $\log[MR_n]/[M^{n+}]$ and $\log[HR]$ for the logarithmic method were calculated and a graph was plotted (Fig. 3). A straight line of slope 1.86 was obtained, which confirmed the formation of $Te(CF_3CH_2OCS_2)_2$ under these conditions.

Beer's Law and Sensitivity

A calibration graph was plotted under the optimum conditions described above. The graph obeys Beer's law over the

Table 3. Simultaneous determination of tellurium and selenium in synthetic samples. Conditions: 0.5% potassium trifluoroethylxanthate, 5 ml; 20% naphthalene solution, 2 ml for each successive coprecipitation; acidity, 4 M $HClO_4$ for Se and pH 1.5 for Te; wavelength, 410 nm for Te and 430 nm for Se exchanged Cu

Amount present/ µg		Amount found/ µg		Average/ µg		Relative standard deviation, %	
Te	Se	Te	Se	Te	Se	Te	Se
46.0	150.5	45.45	150.35	45.77	150.99	0.52	0.38
		45.70	150.85				
		45.85	151.90				
		46.10	150.75				
		45.75	151.10				
130.5	22.5	129.30	22.10	130.63	22.21	0.49	1.46
		130.90	22.70				
		130.75	21.80				
		131.10	22.25				
		130.90	22.20				
19.3	60.0	19.05	60.20	18.91	59.84	0.88	0.56
		18.90	59.60				
		19.10	60.10				
		18.90	59.49				
		18.70	59.90				

Table 4. Determination of tellurium in high copper concentrate alloys. Conditions: 0.5% potassium trifluoroethylxanthate, 5 ml; 20% naphthalene solution, 2 ml for each successive coprecipitation; acidity, 7 M $HClO_4$ and pH 1.5; wavelength, 410 nm

Alloy	Composition, %	Amount of Te present/µg	Amount of Te found/µg	Average/ µg	Relative standard deviation, %
Copper - tellurium	Cu, 98.88; Te, 1.12	84.7	84.95	84.87	0.41
			85.10		
			84.80		
			84.30		
			85.20		
Copper - tellurium	Cu, 99.515; Te, 0.485	41.5	41.10	41.84	1.07
			42.30		
			41.90		
			42.05		
			41.85		
Copper - tellurium	Cu, 99.905; Te, 0.095	25.0	25.60	25.42	1.13
			25.80		
			25.10		
			25.40		
			25.20		

Table 5. Determination of tellurium in synthetic mixtures. Conditions as in Table 4

Amount of metal ions present/µg	Amount of Te present/µg	Amount of Te found/µg	Average/µg	Relative standard deviation, %
Al, 500, Sb, 500; Bi, 250; Cr, 500; Cu, 500; Fe, 500; Sn, 1000 (in 7 M $HClO_4$) . .	55.0	55.70	55.49	0.83
		56.05		
		55.15		
		54.90		
		55.65		
	30.5	31.45	30.81	1.27
		30.60		
		30.45		
		30.65		
		31.90		
Se, 500; Pt, 500; Os, 400; Rh, 500 (in 4 M $HClO_4$) . .	35.0	34.85	34.85	0.75
		35.00		
		34.95		
		34.40		
		35.05		
	20.5	20.68	20.40	1.16
		20.03		
		20.46		
		20.39		
		20.46		

concentration range 1.2–20.0 p.p.m. of tellurium. Ten replicate determinations on samples containing 37 μg of tellurium gave a mean absorbance of 0.200 when measured in a 1-cm cell with a standard deviation of 0.0022 and relative standard deviation of 1.1%. The molar absorptivity was calculated to be 6.898×10^3 l mol^{-1} cm^{-1} at 410 nm and the sensitivity was 0.0185 μg cm^{-2} of tellurium for an absorbance of 0.001.

Effect of Diverse Ions

The interference of various ions was studied in detail. Various amounts of the salts of the anions and metal ions were added individually to an aliquot containing 74 μg of tellurium. None of the anions examined (Table 1) interfered. Of the cations examined (Table 2), only Mo(VI), Co(II), Cu(II), Ir(III), Fe(III), Hg(II) Ni(II), Pd(II) and Se(IV) interfered. The interference of Mo(IV) and Fe(III) was eliminated by masking with 2 ml of 5% ascorbic acid and that of Hg(II) with 2 ml of 5% KI solution; Co(II), Cu(II) and Ni(II) were masked with 10 ml of 5% NaCN solution. For copper, if the concentration exceeded more than a 2-fold excess, the masking with cyanide was not successful. In such instances, copper has to be eliminated by pre-coprecipitation of its trifluoroethylxanthate with microcrystalline naphthalene at a high acid concentration (7 M $HClO_4$). The interference of selenium was eliminated in a similar way to that of copper in 4 M perchloric acid media and then tellurium could be determined in the aqueous phase by using the general procedure.

Simultaneous Determination of Tellurium and Selenium

Both tellurium and selenium react with this reagent giving coloured and colourless complexes, respectively. An aliquot containing appropriate amounts of Te (15–200 μg) and Se (20–260 μg) was taken, 5 ml of 0.5% reagent solution being added, and the acid concentration was adjusted to 4 M perchloric acid. Under these conditions only selenium formed the complex, which was separated by coprecipitation with microcrystalline naphthalene. It was dissolved in 10 ml of chloroform and then exchanged with 10 ml of 0.001 M copper nitrate solution in a separating funnel in order to develop the colour in the chloroform layer. The chloroform layer was poured on to anhydrous sodium sulphate (2 g) in a beaker in order to remove the last traces of water. The absorbance of this solution was measured at 430 nm[12] and referred to a calibration graph prepared under similar conditions. From the filtrate, tellurium was determined by the general procedure. The results are given in Table 3.

Determination of Tellurium in High Copper Concentrate Alloy

A 1.0-g sample of a copper - tellurium alloy was placed in a beaker and 5–10 ml of nitric acid were added. The mixture was heated on a hot-plate until the mixture dissolved completely and the volume of the solution was reduced to 1–2 ml. It was diluted to 100 ml with distilled water, filtered and the final volume was made up to 500 ml in a calibrated flask. To an aliquot of this solution were added 5 ml of 0.5% reagent solution and the acid concentration was adjusted to 7 M with perchloric acid. A 2-ml volume of 20% naphthalene solution was added and copper was coprecipitated. It was filtered off and to the filtrate were added 2 ml of 0.5% reagent and the pH was re-adjusted to 0.5–2.5 with ammonia solution. Tellurium was then determined by the general procedure. The results are given in Table 4.

Determination of Tellurium in Synthetic Samples

The developed conditions were applied to the determination of tellurium in some synthetic samples and the results are given in Table 5.

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References

1. Donaldson, E. M., *Talanta*, 1976, **23**, 411.
2. Donaldson, E. M., *Talanta*, 1982, **29**, 663.
3. Rao, A. L. J., and Singh, S., *J. Indian Chem. Soc.*, 1974, **51**, 503.
4. Rao, A. L. J., and Singh, S., *Fresenius Z. Anal. Chem.*, 1971, **257**, 133.
5. Puri, B. K., Gautam, M., Kumar, A., Wasey, A., Hussain, M. F., and Sethi, C. L., *Chem. Scr.*, 1983, **22**, 19.
6. Gautam, M., Bansal, R. K., and Puri, B. K., *Bull. Chem. Soc. Jpn.*, 1981, **54**, 3178.
7. Satake, M., Puri, B. K., Yuh, J. C., and Chang, Lih-Fen, *Mem. Fac. Eng. Fukui Univ.*, 1982, **30**, 63.
8. Kumar, A., Sethi, C. L., Satake, M., and Puri, B. K., *Bunseki Kagaku*, 1983, **32**, E61.
9. DeWitt, C. C., and Roper, E. E., *J. Am. Chem. Soc.*, 1932, **54**, 444.
10. Vogel, A. I., "A Text Book of Quantitative Inorganic Analysis," Third Edition, Longmans, London, 1969.
11. Zolotov, Yu. A., "Extraction of Chelate Compounds," Ann Arbor Science Publishers, Ann Arbor, MI, 1970, p. 122.
12. Hussain, M. F., Bansal, R. K., and Puri, B. K., unpublished work.

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Kinetic - Spectrofluorimetric Determination of Nanogram Levels of Manganese in Wines by the 2-Hydroxynaphthaldehyde Thiosemicarbazone - Hydrogen Peroxide System

D. Pérez-Bendito
Department of Analytical Chemistry, Faculty of Sciences, University of Córdoba, Córdoba, Spain
and J. Peinado and F. Toribio
Department of Biochemistry, Faculty of Veterinary Science, University of Córdoba, Córdoba, Spain

A kinetic method is described for the determination of nanogram amounts of manganese(II) based on its catalytic effect on the hydrogen peroxide oxidation of 2-hydroxynaphthaldehyde thiosemicarbazone. Several wine and brandy samples containing 130–550 μg l^{-1} and 42–140 μg l^{-1} of manganese, respectively, from some Spanish wine-growing areas have been analysed by this method without any previous ashing of the sample. The preparation and properties of the reagent and the kinetic parameters of the catalysed reaction are also reported.

Keywords: *Manganese determination; kinetic - fluorimetric technique; wine; brandy; 2-hydroxynaphthaldehyde thiosemicarbazone*

Manganese is an essential component in the metabolism of plants and acts as the activator of the nitrite reductase. Its content in grapes is a characteristic of the wine-growing area. For equal masses the pips are 3-fold richer in manganese than the skin and 30-fold more than the pulp. In general, the content in wine varies between 400 and 200 μg l^{-1}.

Traditionally, the determination of this metal in wines has been carried out spectrophotometrically by the oxidation to MnO_4^- with $K_2S_2O_8$ in the presence of Ag^+ as a catalyst; however, this method is not sensitive enough. Recently, manganese(II) has been determined by complexing with aldoxime[1] or by atomic-absorption spectrophotometry.[2] In this paper, the determination of manganese(II) in wine by a kinetic method is proposed.

The method is based on the catalytic effect of manganese(II) on the oxidation of 2-hydroxynaphthaldehyde thiosemicarbazone (HNTS) (shown overleaf) by H_2O_2 and the reaction rate is followed spectrofluorimetrically. In an earlier paper, the determination of manganese(II) in water and food using the 2-hydroxybenzaldehyde thiosemicarbazone (HBTS) - H_2O_2 system was reported.[3] By using HNTS the limit of detection for manganese(II) is 0.75 μg l^{-1}, whereas with HBTS it is 2 μg l^{-1}. The method is also very selective and it makes the determination of manganese(II) possible in wine and brandy samples without any pre-treatment of the sample. The results are comparable to those obtained by flame atomic-absorption spectrophotometry.

In this paper, the synthesis of the compound and the kinetic study of the catalysed reaction are also described.

Experimental

Synthesis of 2-Hydroxynaphthaldehyde Thiosemicarbazone

The reagent has been previously synthesised[4] from 2-hydroxynaphthaldehyde and thiosemicarbazide. The following procedure is a modification of the general method of synthesis for thiosemicarbazones.

2-Hydroxynaphthaldehyde (0.5 g) was dissolved in 50 ml of ethanol and thiosemicarbazide (0.3 g) was dissolved in 25 ml of hot water. Several drops of dilute hydrochloric acid were added to reduce the pH to approximately 3.5 and the mixture was shaken for 30 min. The yellow precipitate was filtered and washed with hot water, then purified by successive recrystallisations from ethanol - water - acetone(1+1+1).

The result of the elemental analysis for $C_{12}H_{11}N_3OS$ was as follows: calculated, C 58.8, H 4.5, N 17.1, S 13.1%; found, C 58.3, H 4.3, N 16.9, S 12.8%.

Reagents

All solvents and reagents were of analytical-reagent grade.

Standard manganese(II) solution. Manganese metal (1.0 g) was dissolved in a minimum volume of hydrochloric acid (1+1) and diluted to 1.0 l with hydrochloric acid (1+20) in a calibrated flask. The stock solution was diluted as required immediately before use.

2-Hydroxynaphthaldehyde thiosemicarbazone. A 0.015% *m/V* solution in ethanol was prepared.

Apparatus

The following apparatus was used: an Aminco-Bowman Model J4-8961 spectrofluorimeter equipped with recorder and thermostat for kinetic measurements and 10-mm quartz cells; a Beckman Model 24 spectrophotometer with 10-mm quartz cells; and a Perkin-Elmer Model 380 atomic-absorption spectrophotometer, used with an air - acetylene flame.

All spectrofluorimetric measurements were made with 5.5-nm band widths for excitation and emission monochromators. Under these conditions a 1.0 μM quinine sulphate solution gave a fluorescence signal of 9.2% full-scale deflection.

Procedure for the Determination of Manganese

To a 10-ml calibrated flask add, in this order, 1.5 ml of 0.015% *m/V* HNTS solution in ethanol, a sample containing between 7.5 and 48 ng of manganese(II), 1.0 ml of ethanol, 0.5 ml of 1+5 ammonia solution and 0.5 ml of 1+1000 *V/V* hydrogen peroxide solution (0.097 M). Dilute to the mark with redistilled water. Mix and transfer a portion into a 10-mm quartz fluorimeter cell maintained at 40 ± 0.1 °C. Wait for 2 min before recording the fluorescence intensity ($\lambda_{ex.}$ 390 nm, $\lambda_{em.}$ 450 nm) as a function of time.

Follow the uncatalysed reaction under similar conditions, but with no manganese. The reaction rate is calculated from the difference in the slopes of the fluorescence *versus* time graphs. The methods of determination of manganese(II) are described below.

Determination of Manganese in Wines and Brandies

(a) With ashing of the samples

A 20-ml volume of white or rosé wine or 150 ml of brandy are evaporated to dryness in a porcelain crucible and the residue is heated for 2 h in an electrically heated furnace at 425±25 °C. The cold ash is treated with 1 ml of concentrated hydrochloric acid and diluted to the mark with re-distilled water in a 250-ml calibrated flask. Aliquots of 0.25 and 0.50 ml of these dilutions are taken and, to mask iron(III) interference, 750 μg l^{-1} of sodium fluoride are added.

(b) Without ashing of the samples

Volumes of samples (without prior concentration) of between 25 and 100 μl are taken, so that the amount of manganese is in the determination range of the method proposed.

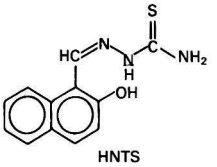
In all instances the standard additions method is used.

The determination procedure indicated above is used for both (*a*) and (*b*).

Results and Discussion

Analytical Properties of the Reagent

At room temperature HNTS is soluble in dimethylformamide (20 g l^{-1}), slightly soluble in acetone (5 g l^{-1}) and ethanol (0.5 g l^{-1}) and insoluble in water.



HNTS

The infrared spectrum of HNTS was obtained using potassium bromide discs. The selected infrared absorption bands were assigned to stretching vibrations of the NH_2 bond (3500–3010 cm^{-1}), the OH bond (3180 cm^{-1}), the C=N bond (1615–1530 cm^{-1}), the C=S bond (1100 cm^{-1}) and the C(S)–NH_2 bond (1580 cm^{-1}).

The absorption and the fluorescence spectra of HNTS at different pH values are shown in Fig. 1. HNTS does not fluoresce in acidic and neutral media. In an alkaline medium it has a faintly green fluorescence ($\lambda_{ex.}$ 410 nm; $\lambda_{em.}$ 475 nm). The pK values were calculated by the Stenström and Goldsmith method[4] from photometric and fluorimetric measurements. The results agreed and gave $pK_1 = 8.8$, which can be ascribed to the ionisation of the hydroxynaphtholic group, and pK = 12.5, which can be ascribed to the deprotonation of the thiol group.

The ethanolic solutions are stable for at least 1 week at room temperature. Reducing agents at moderate concentrations, *e.g.*, hydroxylamine, ascorbic acid and hydrazine, do not alter the absorption and emission spectra of HNTS. Ammoniacal solutions of HNTS in the presence of oxidising agents, such as hydrogen peroxide, potassium peroxidisulphate and sodium periodate, experience disappearance of the absorption maximum and a change in the fluorescence of the reagent ($\lambda_{ex.}$ 390 nm; $\lambda_{em.}$ 450 nm) (Fig. 2). When sodium periodate is used the reaction rate is higher than that obtained with hydrogen peroxide or potassium persulphate.

Reactions with Cations

The photometric and fluorimetric behaviour of the reagent with 40 cations at various pH values has been investigated. The samples were prepared in 25-ml calibrated flasks containing between 2000 and 10 000 μg l^{-1} of metallic ion, 5 ml of 0.015% HNTS solution in ethanol and 5 ml of buffer at different pH values, and diluted to the mark with re-distilled water. The absorption in the visible region and the excitation - emission spectra were recorded. The most interesting results are shown in Table 1, as other cations do not form coloured complexes or have a very low molar absorptivity. From a fluorimetric point of view, Al(III), Be(II), Ti(IV) and Ca(II) cause quenching of the reagent and not of the oxidation product.

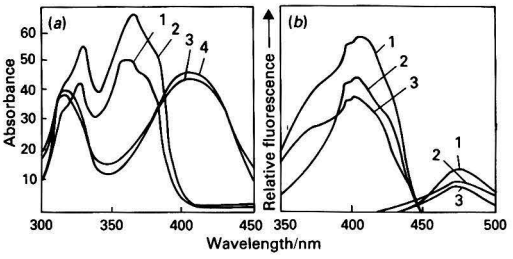


Fig. 1. (*a*) Absorption spectra of HNTS (7×10^{-5} M) at different pH values. Curves 1–4 correspond to pH 2.2, 7.2, 10.0 and 13.2, respectively. (*b*) Excitation and emission spectra of HNTS (9×10^{-5} M) at different pH values. Curves 1–3 correspond to pH 9.1, 9.8 and 12.5, respectively

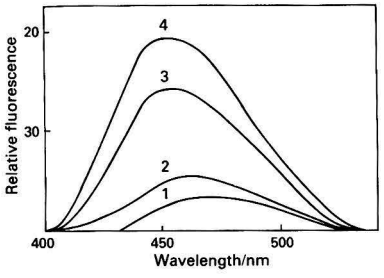


Fig. 2. Effect of hydrogen peroxide (4.8×10^{-3} M) on the emission spectra of HNTS (9×10^{-5} M). Ammoniacal solution and temperature 25 °C. Curves 1–4 correspond to 5, 10, 30 and 45 min after preparation of the samples, respectively

Table 1. Photometric reactivity of HNTS with metallic ionic species

Ion	pH	λ/nm	ε × 10^{3}*/l mol^{-1} cm^{-1}
Co(II) ..	4.0	425	12.6
	9.6	450	6.9
Ni(II) ..	9.6	484	8.6
Mo(VI) ..	1.5	410	16.7
Cu(II) ..	4.1	420	7.2
Os(VIII) ..	9.6	530	14.4
In(III) ..	4.5	416	11.6
Mn(II) ..	9.5	484	4.0

* Calculated against a reagent blank corresponding to the same value of pH.

Table 2. Influence of the volume of ethanol added (%) and the ionic strength on the reaction rate

Ethanol, %	Tan α × 10^{3}	I*/M × 10^{-3}	Tan α × 10^{3}
—	54.0	—	77.0
5	65.0	2	65.0
10	76.5	10	60.0
15	88.0	20	56.0

* Adjusted with 0.2 M KCl solution.

Catalytic Effect of Manganese(II) on the Oxidation Reaction

The fluorimetric characteristics of HNTS in an ammoniacal medium are altered in the presence of nanogram amounts of manganese(II) and the green fluorescence ($\lambda_{ex.}$ 410 nm; $\lambda_{em.}$ 475 nm) of the reagent changes to blue ($\lambda_{ex.}$ 390 nm; $\lambda_{em.}$ 450 nm). The last two spectral characteristics are identical with those of the oxidation product.

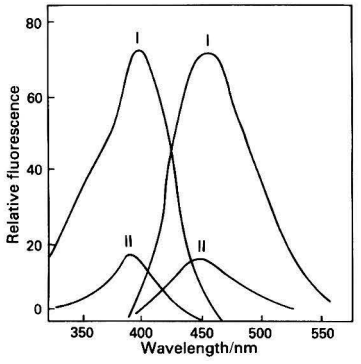


Fig. 3. Catalytic effect of manganese(II) on the excitation and emission spectra of the oxidation product of HNTS. I, In the presence of 1.5 ng ml^{-1} of manganese; II, in the absence of manganese. Curves recorded after 15 min. Conditions as described in the procedure

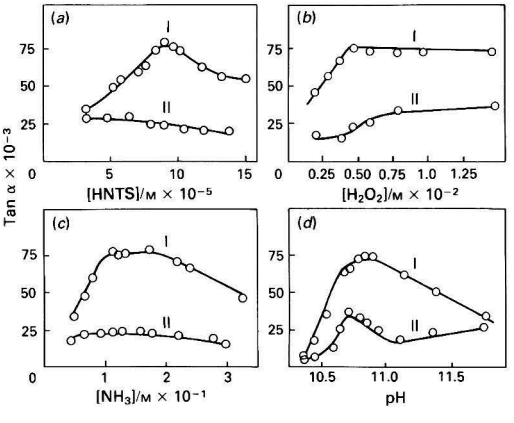


Fig. 4. Effect of variables on the reaction rate. I, Reaction catalysed by 1.5 ng ml^{-1} of manganese(II); II, uncatalysed reaction. Conditions as described in the procedure

The HNTS oxidation is instantaneous in the presence of $NaIO_4$. In the presence of H_2O_2 and $K_2S_2O_8$ it is not as fast, but speeds up considerably if small amounts of manganese(II) are present, owing to the catalytic effect of this ion. This effect is greatly enhanced when hydrogen peroxide in an ammoniacal solution is used (Fig. 3).

Effect of Reaction Variables

The temperature was varied over the range 25–50 °C; the reaction was faster at higher temperatures but remained constant above 40 °C. This temperature was judged to be the optimum for the final procedure. The activation energy calculated from the reaction rates using the Arrhenius equation in the 25–40 °C range is 17.5 kJ mol^{-1}.

It has been observed that the oxidation reaction increases considerably with the volume of ethanol added to the sample (the addition of 1.0 ml of ethanol is recommended) and decreases slightly with increasing ionic strength (Table 2).

The order of addition of the reagents has very little influence of the oxidation reaction rate, the order recommended in the experimental section being the one that gives the best results.

The influence of the HNTS, H_2O_2, NH_3 and H^+ concentrations has been studied on both the catalysed and uncatalysed reactions for the purpose of obtaining optimum conditions for the Mn(II) determination and stabilising the kinetic dependence of each of these variables.

The effect of these variables is shown in Fig. 4(*a*)–(*d*) in the ranges indicated in Table 3; from these have been selected the optimum conditions described under Experimental on the basis of choosing the concentration that offers the maximum difference between the catalysed and uncatalysed reactions, and on the fact that small variations in the reagent concentrations do not affect the reaction rate.

The influence of the pH on the reaction rate is shown in Fig. 4(*d*). The rate of the reaction increases up to pH 10.6, and then remains almost constant up to pH 10.8. The rate then begins to decrease at higher values. The zone of minor variation (between pH 10.6 and 10.8) corresponds to an ammonia concentration range of 0.1–0.19 M.

The initial rate of reaction varies linearly with the manganese(II) concentration tested, in the range 0.75–4.8 μg l^{-1}, which is the basis of one of the proposed methods for the determinaton of low levels of manganese(II).

Determination of the Partial Orders of Reaction

The partial orders for each of the variables, from the data shown in Fig. 4, by means of the graphical representation of the logarithm of the tan α *versus* the logarithm of the concentration of the tested variable, have been calculated (Table 3).

Table 3. Summary of kinetic data for the HNTS - H_2O_2 system in the presence and absence of Mn(II)

	Uncatalysed reaction		Catalysed reaction	
Variable	Concentration range/M	Partial reaction order	Concentration range/M	Partial reaction order
HNTS ..	3.0×10^{-5} – 6.0×10^{-5}	0	3.0×10^{-5} – 9.5×10^{-5}	1/2
	6.0×10^{-5} –13.7×10^{-5}	−1	9.5×10^{-5} –15.3×10^{-5}	−3/4
H_2O_2 ..	1.9×10^{-3} – 3.8×10^{-3}	0	1.9×10^{-3} – 4.5×10^{-3}	1/2
	3.8×10^{-3} – 6.6×10^{-3}	1	4.5×10^{-3} –14.5×10^{-3}	0
	6.6×10^{-3} –14.0×10^{-3}	0		
NH_3	2.2×10^{-2} – 6.7×10^{-2}	1/3	2.2×10^{-2} –10.0×10^{-2}	1
	6.7×10^{-2} –18.0×10^{-2}	0	10.0×10^{-2} –19.0×10^{-2}	0
	18.0×10^{-2} –33.0×10^{-2}	−1	19.0×10^{-2} –33.0×10^{-2}	−1
NH_4^+ ..	4.9×10^{-3} – 7.0×10^{-3}	2	4.9×10^{-3} – 7.5×10^{-3}	−1/2
H^+	1.6×10^{-11}– 2.3×10^{-11}	0	1.6×10^{-11}– 2.5×10^{-11}	0

Table 4. Dependence of the catalysed reaction on the NH_4^+ and H^+ concentrations

Initial [HCl]*/ mol l^{-1} × 10^{-3}	pH	Actual [NH_4^+]†/ mol l^{-1} × 10^{-3}	Tan α × 10^3‡	Tan α × 10^3/ [NH_4^+]$^{-\frac{1}{2}}$
—	10.80	4.92	75.0	5.26
3.1	10.75	5.52	75.6	5.61
6.2	10.70	6.18	68.8	5.40
12.5	10.64	7.05	65.4	5.49
25.0	10.61	7.52	64.5	5.59

* Initial ammonia concentration, 0.178 M.
† Calculated from pH, known concentration and pK_b for NH_3.
‡ After subtraction of rate of uncatalysed reaction.

Table 5. Linear concentration ranges, sensitivities and relative standard deviations for the three methods of calculation for the kinetic determination of manganese

Method	Range/ng ml^{-1}	Sensitivity	Relative standard deviation, %
Tangent	0.75–4.8	4.9×10^7 I_f min^{-1} M^{-1}	1.12
Variable-time ..	1.0 –4.5	2.3×10^7 min^{-1} M^{-1}	1.36
Fixed-time ..	0.75–4.8	9.2×10^8 I_f M^{-1}	1.97

Table 6. Tolerance levels of interferents in the kinetic determination of 1.5 μg l^{-1} of manganese(II) by the tangent method

Tolerance level, [ion]/[Mn^{2+}]	Ions added
500	Oxalate, F^-, Pb(II)
200	Os(VIII), V(V), Sb(III), Ga(III), Ca(II), Co(II), Na(I), $S_2O_8^{2-}$, PO_4^{3-}, ClO_4^-, NO_3^-, I^-, citrate
150	Fe(III),* Sn(II)
100	Mo(VI), W(VI), Pt(IV), As(III), Be(II), Bi(III), Hg(II), Cd(II), Zn(II), Cu(II), Sr(II), Tl(I), S^{2-}, IO_4^-, tartrate, $S_2O_3^{2-}$, Cl^-, $P_2O_7^{2-}$
50	Ti(IV), Se(IV), Sb(V), Cr(VI), In(III), K(I), Ni(II), Mg(II), Pd(II), Ba(II), Br^-
25	Cr(III), SCN^-, EDTA
10	Al(III), Ag(I), Fe(III)

* In the presence of 750 μg l^{-1} of sodium fluoride.

Table 7. Determination of manganese in wines and brandies

Sample	Manganese found*/μg l^{-1} Kinetic method† Ashed sample	Kinetic method† Untreated sample	AAS method
Wines—			
Oloroso (Montilla)‡ ..	257±4.7	255±2.9	256
Rama (Montilla)‡ ..	297±6.3	298±3.5	301
Manzanilla (Jerez)‡ ..	—	130±1.7	131
White (Valdepeñas)‡ ..	—	265±3.2	263
White (Jumilla)‡ ..	—	544±1.5	545
Rosé (Rioja)‡	—	556±3.4	551
Brandies—			
Jerez‡	47±1.3	42±1.7	44
Villafranca de Penedés‡	—	144±2.8	146

* Average values of six separate determinations and their standard deviations.
† Using a standard additions determination.
‡ Spanish wine-growing area.

The partial reaction orders in hydrogen and ammonium ions were calculated simultaneously because both species are closely related. At constant concentrations of other variables tan α/($[H^+]^x[NH_4^+]^y$) = K_1. Thus, the x and y values (partial reaction orders in hydrogen and ammonium ions, respectively) were calculated by testing pairs of values that gave values of K_1 in different experiments with the minimum standard deviation. These partial reaction orders were calculated by using a computer program to give $x = 0$, $y = -0.5$ for the catalysed reaction (see Table 4) and $x = 0$, $y = 2$ for the uncatalysed reaction.

From the partial reaction orders calculated above, the following kinetic equation for the over-all oxidation reaction is suggested under the conditions proposed for the manganese(II) determination:

$$\frac{d[HNTS]_{ox.}}{dt} = K_o[HNTS]^{-1}[H_2O_2][NH_4^+]^2 + K_1[HNTS]^{1/2}[NH_4^+]^{-1/2}[Mn^{2+}]$$

$[HNTS]_{ox.}$ is the concentration of the oxidised form of the reagent, and K_o and K_1 are the conditional rate constants for the uncatalysed and catalysed reactions, respectively.

Calibration Graphs

Three methods have been used for the determination of small amounts of manganese and their performances compared, *viz.*, the tangent, the fixed-time and the fixed-fluorescence (or variable-time) methods. For the fixed-time method, the fluorescence was measured 4 min after beginning the recording of the fluorescence intensity *versus* time curves and was plotted against the manganese concentration. For the intensity of fluorescence method, the inverse of the required time was plotted to obtain a relative fluorescence intensity of 40% against the manganese concentration.

The calibration graphs are linear in the concentration ranges indicated in Table 5. The calculated sensitivity in terms of the slope of the calibration straight line[6] and the coefficient of variation for each of the methods are also given in Table 5. The tangent method is recommended. The percentage error calculated for the determination of 1.5 μg l^{-1} of manganese(II) by the tangent method is 0.7% (for $n = 11$ and at a 95% confidence limit).

Interferences

The effect of various ions on the reaction rate was examined, and the proposed system is reasonably selective. The tolerance levels for the ions investigated are given in Table 6. From the cations, many basic metals such as Hg(II), Cd(II), Zn(II) and Cu(II) can be present in 100-fold excess concentrations without having any observable effect on the reaction rate. The most important interference is from iron(III), because it acts as a catalyst of the oxidation reaction. However, the tolerance can be increased to 225 μg l^{-1} of iron(III) by the addition of fluoride as a masking reagent.

Determination of Manganese(II) in Wines and Brandies

The procedure described under Experimental was applied to the determination of manganese in wines and brandies.

Because of the high tolerance to interference from species in the method, the determination of manganese in wine and brandy samples was also carried out without any pretreatment and the same results were obtained as for treated samples.

The similarity of the results is due to the low concentration of free iron(III) present in wines and brandies (0.1–1.0 μg l^{-1}); although the total iron varies between 7 and 14 mg l^{-1} it exists in the form of hardly dissociated complex salts such as tartrates, malates, citrates and pyrophosphates,[7] depending on the type of wine and its fermentation process.

In Table 7 the results obtained for the determination of manganese for ashed and untreated samples are shown, and compared with those obtained by atomic-absorption spectrophotometry, giving good agreement (the brandies checked by atomic-absorption spectrophotometry required preconcentration of the sample). Thus, the ashing of wine and brandy samples is not necessary for the determination of their manganese content.

References

1. Cunha Ramos, M., and Guedes Gomes, L., *Feuill. Verts Office Int. Vin*, 1970, No. 359.
2. Ribereau-Gayon, J., Peyneaud, E., Sudraud, P., and Ribereau-Gayon, P., "Sciences et Techniques du Vin," Volume 1, Dunod, Paris, 1976.
3. Moreno, A., Silva, M., Pérez-Bendito, D., and Valcárcel, M., *Talanta*, 1983, **30**, 107.
4. Sah, P. T., and Daniel, T. C., *Recl. Trav. Chim. Pays-Bas*, 1950, **69**, 1545.
5. Stenström, W., and Goldsmith, N., *J. Phys. Chem.*, 1926, **30**, 1683.
6. Mottola, H. A., *Crit. Rev. Anal. Chem.*, 1975, 225.
7. Sanfourche, A., *C.R. Acad. Sci.*, 1932, 195.

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Kinetic - Spectrophotometric Determination of Manganese and Vanadium + Manganese by Oxidation of Pyrogallol Red

A. Sevillano-Cabeza, J. Medina-Escriche and M. de la Guardia-Cirugeda
Department of Analytical Chemistry, Faculty of Chemical Sciences, Valencia University, Burjasot (Valencia), Spain

Spectrophotometric methods for the determination of manganese and the simultaneous determination of vanadium + manganese, based on the oxidation of Pyrogallol Red (PGR), are described. Manganese or vanadium + manganese are determined by the decrease in the absorbance of the characteristic band at 490 nm at pH 4. At pH 4, an ionic strength of 0.1 M, a temperature of 25 °C and a wavelength of 490 nm, there is a linear relationship between the absorbance of PGR (6×10^{-5} M), at a fixed time, and the concentration of manganese(VII) over the range 0–1.10 p.p.m. The limit of detection of manganese is 0.03 p.p.m. Studies were made of the selectivity of the method with respect to possible interferences from 22 species, which have been classified according to their possible mechanism of interference. The precision and accuracy of the method using a fixed time of 5 min were also determined. The simultaneous determination of vanadium in the range 0.20–1.83 p.p.m. and manganese(VII) in the range 0.11–1.10 p.p.m. by using a system of equations in which the absorbance at a fixed time is considered to be proportional to the total concentration of the two elements and their effects on the absorbance of PGR are additive, is described.

Keywords: *Manganese determination; vanadium determination; kinetic - spectrophotometric method; Pyrogallol Red oxidation*

Pyrogallol Red (PGR) has been widely used in analytical chemistry both as a metallochromic indicator for complexometric titrations and as a spectrophotometric reagent.[1] The determination of trace amounts of lead[2,3] and iodide[4] by means of their catalytic effect on the oxidation of PGR by persulphate and hydrogen peroxide, respectively, has been described. We have studied a spectrophotometric method for the determination of vanadium by oxidation of PGR.[5] In this paper, we describe spectrophotometric methods for the determination of manganese and vanadium + manganese, based on the oxidation of PGR.

Experimental

Reagents

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

Manganese(VII) stock solution, 2×10^{-2} M. Dissolve 0.8 g of potassium permanganate in distilled water (boil gently for 30 min, allow to stand overnight and filter through a G4 sintered-glass filter crucible), then dilute to 250 ml. Standardise the solution with iron(II) ethylenediammoniumsulphate (general-purpose reagent grade) volumetric standard.

Prepare a working solution (10^{-3} M) by appropriate dilution of the stock solution.

Vanadium(V) stock solution, 10^{-2} M. Dissolve 1.232 g of sodium vanadate ($NaVO_3$) in distilled water (by warming) and then dilute to 1 l. Standardise the solution with iron(II) ethylenediammoniumsulphate (general-purpose reagent grade) volumetric standard.

Prepare a working solution (10^{-4} M) by appropriate dilution of the stock solution.

Buffer solutions. 2.2 M acetic acid - 0.5 M sodium acetate buffer of pH 4, 0.2 M acetic acid - 0.5 M sodium acetate buffer of pH 5, and 0.4 M potassium dihydrogen orthophosphate - 0.05 M sodium hydroxide buffer of pH 6.

Pyrogallol Red solutions, 10^{-3} *and* 8×10^{-4} M. Dissolve 0.100 and 0.080 g, respectively, of PGR (Merck) in 250 ml of methanol. These solutions are stable for at least 1 month.

Apparatus

All the spectrophotometric measurements were made on a Pye Unicam SP 8–100 spectrophotometer using 1-cm cells. A Crison-501 pH meter, equipped with a Metrohm EA-121 Ag - AgCl electrode system, was used for measuring the pH of the solutions. The pH should be measured to an accuracy of ±0.01 pH unit. A Frigedor Selecta-398 refrigerator unit for use in a water-bath and a Termotronic Selecta-389 immersion thermostat capable of maintaining the temperature within ±0.05 °C, were also used.

Results and Discussion

Influence of pH and Calibration Graph for the Manganese(VII) - PGR System

Into series of 25-ml calibrated flasks, hydrochloric acid or sodium hydroxide solution or 5 ml of the appropriate buffer solution were added to obtain solutions of different acidities. Then 10^{-3} M manganese(VII) solution was added and the solution was diluted to *ca.* 20 ml with distilled water. This solution was shaken gently while 1 or 2 ml of 10^{-3} or 8×10^{-4} M PGR solution, respectively, were added. The stop-watch was turned on when the last drop was added and the solution was diluted to the mark with distilled water. As the rate of disappearance of the characteristic band of PGR is quicker than the rate of appearance of a new band at 390 nm, the decrease in absorption was measured at the maximum wavelength of PGR at the corresponding pH, against water as a reference blank, at times of 1.5 and 2 min and then at 1-min intervals for up to 20 min.

This study was performed over the pH range 1–6 using hydrochloric acid and sodium hydroxide for pH 1, 2 and 3 and the appropriate buffer solution for pH 4, 5 and 6. Solutions of pH 7 and higher were not tested because of instability of the reagent. It was not possible to study the optimum working acidity from the rate decrease in the absorbance values of the characteristic band of PGR at each pH because it is too fast, so it was studied by means of the maximum amount of 4×10^{-5} M PGR oxidised by 0.22 p.p.m. of Mn(VII). The results are

shown in Table 1. The maximum amount of PGR oxidised at pH 4, 5 and 6 for times between 5 and 15 min was obtained. From these results, pH 4 was selected as this acidity is the optimum for V(V) - PGR[5] and Cr(VI) - PGR systems.

In order to demonstrate the possible analytical applications of the Mn(VII) - PGR system, the regression between the absorbance values of 4×10^{-5} M PGR solutions (which provided an absorbance in an intermediate range of minimum photometric error) or at its upper limit of 6×10^{-5} M at a fixed time and different concentrations of manganese(VII) (c_{Mn}) up to 1.32 p.p.m. was studied at a pH of 4, an ionic strength of 0.1 M, a temperature of 25 °C and at a wavelength of 490 nm. The results are shown in Table 2. From this study, a PGR concentration of 6×10^{-5} M was chosen as the optimum working concentration because the slope (S) of the calibration graph increased by approximately 7%. The most suitable measuring time chosen was 5 min, because S remains almost constant with time.

For this time and a numerical factor $k = 2$ or 3 (confidence level), the theoretical limit of detection, c_L (reference 6), is 0.03 or 0.05 p.p.m., respectively, of manganese(VII) for 11 independent solutions of 6×10^{-5} M PGR with a standard deviation (s) of 4.08×10^{-3} absorbance unit. The experimental limit of detection is 0.03 p.p.m. of Mn(VII).

Influence of Temperature

The study of the influence of temperature on the oxidation of PGR in the presence of different concentrations of manganese(VII) at pH 4.0 was performed at 15,20,25,30,35 and 40 °C (Fig. 1). The rate constant (K) and the order of reaction (n) with respect to manganese(VII) were calculated by the differential method and determining the initial rate of reaction (v_o) (Table 3).

In order to calculate the activation energy (E) and the frequency factor (A), the Arrhenius equation was applied. The graph of ln K against $1/T$ is a straight line, $\ln K = (-91.705/T) - 5.080$, with a correlation coefficient $r = -0.9998$ when $E = 0.182$ kcal mol^{-1} and $A = 0.006$ s^{-1}.

From this study, it can be concluded that at the temperatures studied, the Arrhenius equation was followed, except at 15 °C, and the influence of temperature is small. On this basis, 25 °C was chosen as the working temperature.

Interferences

The influence of 22 different species on the oxidation of 6×10^{-5} M PGR in the presence of 0.55 p.p.m. of manganese(VII) was studied. The results are shown in Table 4.

In order to establish the effect of each species on the manganese(VII) - PGR system, the spectra at different concentrations of possible interfering species were printed, starting from a time of 5 min. From this study it can be concluded that, for higher concentrations than those in Table 4, the interfering processes may be classified as follows: (a) complex formation with PGR was observed by a bathochromic shift and a hyperchromic shift in the absorption spectrum of the system in the presence of arsenic, iron, chromium(III), boron, zirconium, niobium, antimony, aluminium, tungsten molybdenum, tin and titanium; (b) possible complex formation at non-optimum pH 4 with nickel, cobalt and copper; and (c) acceleration of the oxidation of PGR by manganese(VII) in the presence of lead, manganese(II), vanadium and chromium(VI).

Precision and Accuracy

A study of the precision was performed with five independent solutions of various concentrations of manganese(VII) and a fixed concentration of PGR. The results are shown in Table 5. The study of the accuracy[7–10] was performed over the range 0.11–1.10 p.p.m. of manganese(VII) with 6×10^{-5} M of PGR, at a fixed time of 5 min.

The homogeneity of the variances of the analysed samples was confirmed by application of Bartlett's test. The linear regression of the values obtained for each analysis of each sample and the corresponding real values were obtained. The statistical t-test was applied to the study of the slope,and the intercept of the straight line was obtained. From this study we conclude that the proposed method does not present a constant-type error (a slope equal to unity) and it does not need blank correction (an intercept equal to zero).

Recommended Procedure

Samples containing between 0.75 and 27.50 μg of manganese(VII) were placed in 25-ml calibrated flasks, 5 ml of 2.2 M acetic acid - 0.5 M sodium acetate buffer solutions were

Table 1. Variation of number of moles of PGR oxidised by 1 mol of manganese(VII). Conditions: [PGR], 4.0×10^{-5} M; [Mn(VII)], 0.22 p.p.m.; temperature, 25 °C; and pairs of pH/$\lambda_{max.}$ (nm) values, 1.67/466, 2.36/466, 3.25/466, 4.04/490, 5.01/510 and 6.01/520

pH	Time/min	No. of moles of PGR oxidised by 1 mol of manganese(VII)
1.67	5.0	0.75
	10.0	0.75
	15.0	0.75
2.36	5.0	0.75
	10.0	0.75
	15.0	0.75
3.25	5.0	0.75
	10.0	1.00
	15.0	1.00
4.04	5.0	1.00
	10.0	1.00
	15.0	1.00
5.01	5.0	1.00
	10.0	1.00
	15.0	1.00
6.01	5.0	1.00
	10.0	1.00
	15.0	1.00

Table 2. Calibration graphs. Conditions: pH, 4.0; ionic strength, 0.1 M; temperature, 25 °C; and $\lambda_{max.}$, 490 nm

Time/min	[PGR] 4.1×10^{-5} M; [Mn(VII)] 0–1.10 p.p.m.		[PGR] 6.2×10^{-5} M [Mn(VII)] 0–1.10 p.p.m.	
	Calibration graph	r	Calibration graph	r
1.5	$y = -0.242c + 0.534$	−0.999	$y = -0.263c + 0.801$	−0.999
3.0	$y = -0.248c + 0.534$	−0.999	$y = -0.266c + 0.802$	−0.999
5.0	$y = -0.250c + 0.534$	−0.999	$y = -0.268c + 0.802$	−0.999
10.0	$y = -0.252c + 0.534$	−0.999	$y = -0.269c + 0.802$	−0.999
15.0	$y = -0.252c + 0.534$	−0.999	$y = -0.271c + 0.802$	−0.999

Table 3. Dependence of rate constant (K) and reaction order (n) on temperature (T). Conditions: [PGR], 6.5×10^{-5} M; [Mn(VII)], 0.22–1.10 p.p.m.; pH, 4.0; ionic strength, 0.1 M; and $\lambda_{max.}$, 490 nm

T/K	$K\times10^{-3}$/s^{-1}	n
293	4.55	1.14
298	4.57	1.11
303	4.59	1.07
308	4.62	1.02
313	4.64	1.00

Table 4. Classification of interferents. Conditions: [PGR], 6.4×10^{-5} M; [Mn(VII)], 0.55 p.p.m.; pH, 4.0; $\lambda_{max.}$, 490 nm; temperature, 25 °C; and time, 5 min. Maximum tolerance, ±2% of the original absorbance (0.674)

Species	Concentration of species, p.p.m.	Ratio of [Mn(VII)] to [species]	Absorbance (490 nm)
$C_6H_5O_7^{3-}$ ($Na_3C_6H_5O_7.2H_2O$)	10000	1:18182	0.683
As(V) ($Na_2HAsO_4.7H_2O$)	7500	1:13636	0.677
P (Na_2HPO_4)	5000	1:9091	0.687
F^- (NaF)	4000	1:7273	0.682
Fe(III) [$Fe(NO_3)_3.9H_2O$]	560–780	1:1018–1418	0.661–0.659
Cr(III)[$Cr(NO_3)_3.9H_2O$)	440	1:800	0.661
Ni(II) [$Ni(NO_3)_2.6H_2O$]	320	1:582	0.659
B ($Na_2B_4O_7.10H_2O$)	200	1:364	0.687
Pb(II) [$Pb(NO_3)_2$]	125	1:227	0.662
Co(II) [$Co(NO_3)_2.6H_2O$]	35	1:64	0.662
Mn(II) [$MnSO_4.H_2O$]	11	1:20	0.662
Zr(IV) ($ZrOCl_2.8H_2O$)	4	1:7.3	0.684
Nb(V) (Nb_2O_5)	4	1:7.3	0.689
Sb(V) ($SbCl_5$)	2.5	1:4.5	0.685
Cu(II) [$Cu(NO_3)_2.5H_2O$]	2	1:4	0.665
Al(III) [$Al(NO_3)_3.9H_2O$]	1.2	1:2.2	0.681
W(VI) [$Na_2WO_4.2H_2O$]	0.3	1:0.5	0.685
Mo(VI) [$(NH_4)_6Mo_7O_{24}.4H_2O$]	0.2	1:0.4	0.687
V(V) ($NaVO_3$)	0.2	1:0.4	0.662
Sn(IV) ($SnCl_4$)	0.1	1:0.2	0.686
Ti(IV) ($TiOSO_4$)	0.1	1:0.2	0.684
Cr(VI) ($K_2Cr_2O_7$)	0.1	1:0.2	0.660

Table 5. Precision data for the method for the determination of manganese. Equation of calibration graph: $y = 0.811 - 0.267c_{Mn}$ ($r = -0.9999$). Pairs of values A/c_{Mn} (0.809/0.00; 0.783/0.11; 0.722/0.33; 0.664/0.55; 0.577/0.88; and 0.515/1.10). Conditions: [PGR], 6.3×10^{-5} M; pH, 4.0; ionic strength, 0.1 M; $\lambda_{max.}$, 490 nm; temperature, 25 °C; and time, 5 min

True concentration of Mn(VIII), p.p.m.	Mean concentration of Mn(VIII) found, p.p.m.	Standard deviation, p.p.m.
0.11	0.102	0.004
0.33	0.332	0.004
0.55	0.550	0.007
1.10	1.110	0.007

Table 6. Precision data for the method for the simultaneous determination of vanadium and manganese. Conditions: [PGR], 6.4×10^{-5} M; pH, 4.0; ionic strength, 0.1 M; $\lambda_{max.}$, 490 nm; and temperature, 25 °C

True concentration, p.p.m.		Mean concentration found and standard deviation, p.p.m.			
c_V	c_{Mn}	$\bar{c}_V$	s	$\bar{c}_{Mn}$	s
0.20	0.11	0.21	0.02	0.10	0.03
0.20	0.55	0.22	0.04	0.57	0.04
0.20	1.10	0.21	0.04	1.12	0.04
0.61	1.10	0.67	0.05	1.11	0.04
1.02	1.10	1.03	0.05	1.10	0.04
1.83	0.55	1.83	0.04	0.51	0.03
1.83	1.10	1.74	0.03	0.96	0.01

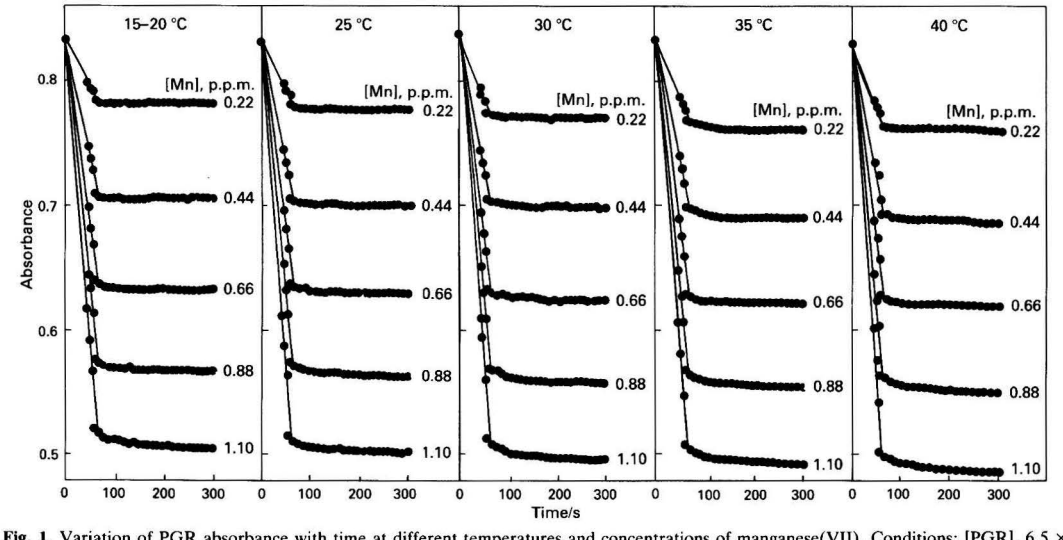

Fig. 1. Variation of PGR absorbance with time at different temperatures and concentrations of manganese(VII). Conditions: [PGR], 6.5×10^{-5} M; pH, 4.0; ionic strength, 0.1 M; and $\lambda_{max.}$ 490 nm

added and the contents were diluted to 20 ml with distilled water. This solution was shaken gently while 2 ml of 8×10^{-4} M PGR were added. The stop-watch was turned on as the last drop fell and the solution was diluted to the mark with distilled water. The absorbance was measured at 490 nm and 25 °C against water as a reference, at a time of 5 min. The corresponding manganese concentration was calculated from the equation of the calibration graph.

Description of Reaction

The absorbance of the wide band of PGR solutions between 10^{-5} and 8×10^{-5} M (490 nm at pH 4) in the presence of manganese(VII) at concentrations between 0.03 and 1.32 p.p.m. decreased and a new band at 390 nm was obtained (Fig. 2). The rates of disappearance of the band at 490 nm and the appearance of that at 390 nm were dependent on the concentration of the manganese(VII) present, and the final intensity of the latter band was proportional to the initial concentration of PGR.

On the one hand, the decomposition of PGR by manganese(VII) is an oxidation process because its spectrum agrees with that observed for V(V) - PGR,[5] Cr(VI) - PGR and Ce(IV) - PGR systems. On the other hand, the spectrum of the Mn(VII) - PGR system also agrees with the spectra observed in the treatment of PGR with small amounts of persulphate or hydrogen peroxide at 50 °C.

The decomposition of the reagent is rapid during the first few minutes owing to the oxidation of PGR and the reduction of manganese(VII) to manganese(II), because it was shown that the manganese(VII) was immediately discoloured in the treatment with PGR. However, the rates of disappearance of the band at 490 nm and the appearance of that at 390 nm are very slow for longer times. This second decomposition is analogous to that which occured in the PGR (8×10^{-5} M and pH 4), which is unstable for times longer than 7 h.

Simultaneous Determination of Vanadium and Manganese by Oxidation of PGR

The similarity between the V(V) - PGR and Mn(VII) - PGR systems makes possible the simultaneous determination of vanadium and manganese by using the following system of equations, where the absorbance at a fixed time is considered to be proportional to the concentration of the two elements and their effects on the absorbance of PGR are additive:

$$A_{t_1}=A_{PGR}+m_{1c_V}c_V+m_{1c_{Mn}}c_{Mn}$$
$$A_{t_2}=A_{PGR}+m_{2c_V}c_V+m_{2c_{Mn}}c_{Mn}$$

where A_{t_1} and A_{t_2} are the absorbances of the sample at two fixed times chosen from the most suitable measuring times for each element individually (1–5 and 2–10 min), A_{PGR} is the mean value of the intercepts of the four calibration graphs used and m is the value of the corresponding slope of the calibration graph for each element and time.

The equations of the calibration graphs obtained for each element individually are as follows: $y_1=0.827-0.157c_V$ ($r=-0.9988$); $y_2=0.825-0.175c_V$ ($r=-0.9986$); $y_1=0.825-0.249c_{Mn}$ ($r=-0.9998$); and $y_2=0.825-0.250c_{Mn}$ ($r=-0.9998$). The following conditions were used: PGR, 6.4×10^{-5} M; pH, 4.0; ionic strength, 0.1 M; $\lambda_{max.}$, 490 nm; and temperature, 25 °C.

Samples containing between 5.00 and 45.75 μg of vanadium(V) and 2.75–27.50 μg of manganese(VIII) were placed in 25-ml calibrated flasks, 5 ml of 2.2 M acetic acid - 0.5 M sodium acetate buffer solution were added and the mixture was diluted to 20 ml with distilled water. The solution was shaken gently while 2 ml of 8×10^{-4} M PGR were added. The stop-watch was turned on as the last drop fell and the solution was diluted to the mark with distilled water. The absorbance was measured at 490 nm and 25 °C against water as a reference, at fixed times of 5 and 10 min. The corresponding vanadium and manganese concentrations were calculated by using the system of equations above or a simplification of it, as the oxidation of PGR by manganese(VII) finishes at 5 min, from which $m_{1c_{Mn}}=m_{2c_{Mn}}$.

A study of the precision was performed with five independent solutions of various concentrations of vanadium(V) + manganese(VII) and a fixed concentration of PGR. The results are shown in Table 6. The study of the accuracy[7–10] was carried out over the range 0.20–1.83 p.p.m. of vanadium(V) and 0.11–1.10 p.p.m. of manganese(VII) with 6×10^{-5} M of PGR. From this study we conclude that the proposed method does not present a constant-type error and does not need a blank correction.

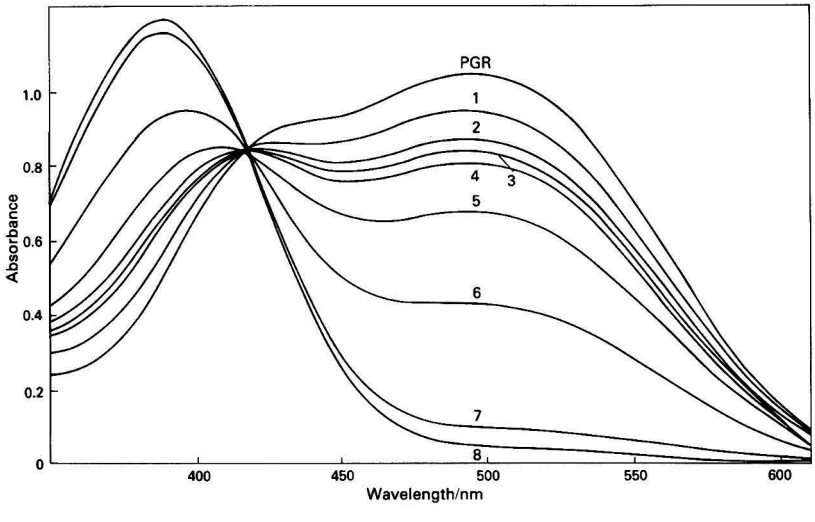

Fig. 2. Variation of PGR spectrum with time in the presence of Mn(VII). 1, 3 min; 2, 2 d; 3, 3 d; 4, 4 d; 5, 7 d; 6, 14 d; 7, 30 d; and 8, 39 d. Conditions: [PGR], 8×10^{-5} M; [Mn(VII)], 0.44 p.p.m.; pH, 4.0; ionic strength, 0.1 M; and temperature, 25 °C

References

1. Antonovich, V. P., and Suvorova, E. N., *Fiz. Khim. Metody Anal.*, 1978, **3**, 9.
2. Jasinskiene, E., and Kalesnikaite, S., *Liet. TSR Aukst. Mokyklu Moskslo Darb., Chem. Chem. Technol.*, 1970, **11**, 51; *Zh. Anal. Khim.*, 1970, **25**, 87.
3. Anderson, R. G., and Brown, B. C., *Talanta*, 1981, **28**, 368.
4. Jasinskiene, E., and Umbraziunaite, O., *Zh. Anal. Khim.*, 1973, **28**, 2025; *Chem. Abstr.*, 1974, **80**, 66402r.
5. Medina Escriche, J., Sevillano Cabeza, A., de la Guardia Cirugeda, M., and Bosh Reig, F., *Analyst*, 1983, **108**, 1402.
6. IUPAC, "Compendium of Analytical Nomenclature," Pergamon Press, Oxford, 1978.
7. Mandel, J., and Linnig, F. J., *Anal. Chem.* 1957, **29**, 743.
8. Commissariat à l'Energie Atomique, "Statistique Appliquée à l'Exploitation de Mesures," Masson, Paris, 1978.
9. de la Guardia, M., Salvador, A., and Berenguer, V., *An. Quim.*, 1981, **77**, 129.
10. de la Guardia, M., Salvador, A., and Berenguer, V., paper presented at 5° Encontro Anual da Sociedade Portuguesa de Química, 1982.

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Equivalence-point Location in the Titration of a Weak Acid with Sodium Hydroxide Solution: A Comparison of Photometric and Potentiometric Methods. Influence of Titrant Carbonation on the Accuracy and Precision of End-points

Colin G. Halliday, Theresa J. Kelly and Michael A. Leonard
Department of Analytical Chemistry, The Queen's University of Belfast, Belfast, BT9 5AG, UK

Using standard laboratory equipment a statistical comparison was carried out of several photometric methods (normal and various Higuchi plots) and potentiometric methods (point of inflection and Gran) for equivalence-point location in an acetic acid - sodium hydroxide solution titration. All methods exhibited similar precision but were divided into two groups with respect to accuracy. With increasing carbonate concentration in the titrant there was a decrease in the linear range for Higuchi and Gran plots and a decrease in accuracy for all methods.

Keywords: *Acid - base titration; photometric titration; end-point error; Higuchi plots; carbonation of basic titrant*

A photometric end-point location can bring very high precision to weak acid - strong base (or *vice versa*) titrations with dyestuff indicators. Therefore, a Brinkmann Probe Absorptiometer was applied to this determination to compare the results with glass-electrode methods of potentiometric end-point location.

Theoretical

If V_A cm^3 of a weak acid HA with a dissociation constant K_a and an original concentration of C_A mol dm^{-3} is diluted to a volume of V_0 cm^3 and titrated with a mixture of volume fraction $1 - f$ of sodium hydroxide solution of concentration C_B mol dm^{-3} and a volume fraction f of sodium carbonate solution of concentration C_C, the relevant equations are

$$[H^+][OH^-] = K_w = 10^{-14} \quad (1)$$
$$[H^+][A^-] = K_a[HA] \quad (2)$$
$$[H^+][HCO_3^-] = K_1[H_2CO_3] \quad (3)$$
$$[H^+][CO_3^{2-}] = K_2[HCO_3^-] \quad (4)$$
$$[H^+]^2[CO_3^{2-}] = K_1K_2[H_2CO_3] \text{ (not independent)} \quad (5)$$

where K_1 and K_2 are the stepwise dissociation constants of carbonic acid.

On balancing the charges we obtain equation (6).

$$[H^+] + [Na^+] = [OH^-] + [A^-] + [HCO_3^-] + 2[CO_3^{2-}] \quad (6)$$

On mass balancing after the addition of V cm^3 of base titrant equations (7)–(11) are obtained (assuming the "normality" of the alkaline titrant remains essentially constant).

$$[Na^+] = \frac{C_BV(1-f)}{V+V_0} + \frac{2C_CVf}{V+V_0} \quad (7)$$

$$[A^-] = \frac{K_aC_AV_A}{([H^+]+K_a)(V+V_0)} \quad (8)$$

$$[OH^-] = \frac{K_w}{[H^+]} \quad (9)$$

$$[HCO_3^-] = \frac{C_CVf}{\left(\frac{[H^+]}{K_1} + 1 + \frac{K_2}{[H^+]}\right)(V+V_0)} \quad (10)$$

$$[CO_3^{2-}] = \frac{C_CVf}{\left(\frac{[H^+]^2}{K_1K_2} + \frac{[H^+]}{K_2} + 1\right)(V+V_0)} \quad (11)$$

Substituting equations (7), (8), (9), (10) and (11) into equation (6) and collecting all the terms on to one side:

$$[H^+] + \frac{C_BV(1-f)}{V+V_0} + \frac{2C_CVf}{V+V_0} - \frac{K_w}{[H^+]} - \frac{K_aC_AV_0}{([H^+]^2+K_a)(V+V_0)} - \frac{C_CVf}{\left(\frac{[H^+]}{K_1} + 1 + \frac{K_2}{[H^+]}\right)(V+V_0)} - \frac{2C_CVf}{\left(\frac{[H^+]^2}{K_1K_2} + \frac{[H^+]}{K_2} + 1\right)(V+V_0)} = 0 \quad (12)$$

Hence $[H^+]$ can be determined as a function of V at various f values.

Higuchi Plots

The basic theory of Higuchi linearised plots[1] for indicated photometric titrations is as follows:

$$[H^+][In_B] = K_{In}[In_A] \quad (13)$$

$$A^- + In_A \rightleftharpoons HA + In_B \quad (14)$$

$$K = \frac{[In_B][HA]}{[In_A][A^-]} = \frac{K_{In}}{K_a} \quad (15)$$

If the system has a total amount of HA equivalent to V_e cm^3 of a standard base and if V is the volume of base added,

$$K = \frac{[In_B]}{[In_A]}\left(\frac{V_e - V}{V}\right) \quad (16)$$

hence

$$\frac{[In_A]}{[In_B]} = \frac{1}{K}\left(\frac{V_e - V}{V}\right) \quad (17)$$

and

$$\frac{[In_A]}{[In_B]} = \frac{A_B - A}{A - A_A} \quad (18)$$

where A_B is the absorbance of the solution with the indicator fully in its basic form; A_A is the absorbance with the indicator fully in its acidic form; and A is the absorbance of the solution during titration.

A graph of $[In_A]/[In_B]$ *versus* $1/V$ is linear with an intercept on the abscissa of V_e^{-1}; this is a Higuchi Type II plot and is used for values of $K > 0.05$. If $K < 0.05$ a graph of $[In_A]/[In_B]$

Table 1. End-point volumes for seven parallel titrations using the named methods for the titration of 10.01 cm³ of 0.1044 M acetic acid with 0.099 67 M sodium hydroxide solution. Results are in cm³

	Method*					
Titration No.	Photometric	Higuchi Type I	Higuchi Type II	Modified Higuchi I	Potentiometric	Gran
1	10.486	10.456	10.466	10.464	10.485	10.467
2	10.485	10.470	10.471	10.471	10.486	10.471
3	10.471	10.456	10.456	10.456	10.475	10.453
4	10.467	10.457	10.456	10.457	10.467	10.457
5	10.468	10.455	10.456	10.456	10.467	10.451
6	10.492	10.467	10.477	10.476	10.494	10.479
7	10.482	10.466	10.468	10.466	10.484	10.465
Average	10.479	10.464	10.464	10.464	10.480	10.463
Standard deviation	0.0099	0.0079	0.0085	0.0079	0.0103	0.0102

* The photometric end-point refers to the intersection illustrated in Fig. 1. All potentiometric points of inflection were determined by the graphical construction of Ebel described in references 3 and 4. Grand mean = 10.469; and grand standard deviation = 0.0113.

Table 2. ANOVA (analysis of variance) from Table 1

Source of variation	Sum of squares	Degrees of freedom	Variance estimate*
Between methods	2.250×10^{-3}	5	4.50×10^{-4}
Within methods	3.027×10^{-3}	36	8.41×10^{-5}
Total	5.277×10^{-3}	41	1.29×10^{-4}

* Variance ratio (F) = $4.50 \times 10^{-4}/8.41 \times 10^{-5} = 5.35$.

versus V in the region where V approaches V_e is linear with an intercept on the abscissa of V_e. This is a Higuchi Type I plot. A graph of $V[In_A]/[In_B]$ *versus* V is linear with an intercept on the abscissa of V_e. This is suggested as a modification to the Higuchi Type I plot and should give an extended linear region.

Gran Plot[2]

From equation (2)

$$[H^+] = \frac{K_a[HA]}{[A^-]} = \frac{K_a(V_e - V)}{V} \quad \ldots \quad (19)$$

$$10^{-pH} = \frac{K_a(V_e - V)}{V} \quad \ldots \quad (20)$$

Hence a graph of $V \times 10^{-pH}$ *versus* V is linear with an intercept on the abscissa of V_e.

Experimental

Apparatus

A Sybron Brinkmann PC 801 fibre-optic absorptiometer (Chem-Lab Instruments Ltd., Hornchurch, Essex) fitted with a 2-cm light path polycarbonate probe was used to give digital indication of absorbance. pH measurements were made using an EIL Vibret 46A meter in conjunction with a Philips G210 glass electrode and an EIL saturated calomel reference electrode. Two grade A 10-cm³ pipettes and one grade B 1-cm³ burette were used.

Reagents

All chemicals used were of AnalaR grade and all solutions were prepared using boiled-out distilled water cooled under nitrogen. Hydrochloric acid (0.1 M) and sodium hydroxide solution (0.1 M) were prepared from concentrated volumetric ampoules. Other prepared solutions were sodium carbonate (0.050 00 M), acetic acid (0.1 M), sodium nitrate (0.1 M) (for ionic strength adjustment) and bromothymol blue (5×10^{-4} M) in ethanol. The hydrochloric acid was checked potentiometrically against the sodium carbonate solution, the sodium hydroxide solution against the hydrochloric acid and the acetic acid against the sodium hydroxide solution.

Procedure

A 10.01 cm³ volume of 0.1044 M acetic acid and a 39 cm³ volume of 0.1 M sodium nitrate solution were pipetted into a 100-cm³ beaker held in a water-bath at 22 °C. The wavelength of the absorptiometer was set to 620 nm. The fibre-optic probe, the glass electrode and the calomel electrode, all mounted in a rubber stopper, were introduced into the solution. The stirrer was activated, the absorbance set to zero and 1.0 cm³ of bromothymol blue solution was introduced. The pre-mixed base titrant was introduced under nitrogen using a 10.01-cm³ pipette and the titration was completed with the 1-cm³ burette.

Results

The results of the precision test are shown in Tables 1 and 2. The F value (variance ratio) (experimental) is 5.35 while the F value from statistical tables is 3.35 at a level of significance of 1%. This means that the difference between methods is highly significant.

The standard deviations of all methods are statistically identical: standard deviation of pipette 1 = 0.0053 cm³; standard deviation of pipette 2 = 0.0059 cm³. Therefore, the standard deviation of two pipettings is 0.0079 cm³, which is statistically identical with the precision of all the methods. This illustrates the very high precision inherent in the titrations themselves. Results of the titrant carbonation test are shown in Table 3.

Equation (12) was solved numerically using the Newton - Raphson method on a microcomputer to produce titration curves for the range of carbonate contents used experimentally. Taking $K_a = 1.78 \times 10^{-5}$ ($V_A = 10.01$ cm³), $K_1 = 4.17 \times 10^{-7}$ ($V_0 = 50.0$ cm³), $K_2 = 4.17 \times 10^{-11}$, $K_w = 1.00 \times 10^{-14}$, $C_A = 0.1044$ M; $C_B = 0.099\,67$ M and $C_C = 0.050\,00$ M yielded the theoretical end-points for the potentiometric and Gran plots shown in Table 4.

Some qualitative effects of increased titrant carbonation were observed: (1) the photometric titration curves showed a gradual decrease in the slope at the approach to the end-point (Fig. 1); (2) all the linear methods showed a gradually decreasing slope and region of linearity (Fig. 2); and (3) the potentiometric titration curves showed a decreasing quality of the inflection point (Fig. 3). All these observations could be duplicated by theoretical curves and plots derived from equation (12).

Table 3. Effect of percentage conversion into carbonate ($f \times 100$) in the titrant on the end-point for the titration of 10.01 cm³ of 0.1044 M acetic acid with 0.099 67 M sodium hydroxide solution. Results are in cm³

Conversion into carbonate, %	Method*					
	Photometric	Higuchi Type I	Higuchi Type II	Modified Higuchi I	Potentiometric	Gran
0	10.485	10.468	10.469	10.468	10.486	10.469
1.0	10.543	10.525	10.529	10.528	10.543	10.523
2.0	10.626	10.602	10.601	10.603	10.628	10.601
3.0	10.685	10.665	10.666	10.666	10.685	10.656
4.0	10.735	10.706	10.708	10.707	10.727	10.705
5.0	10.786	10.748	10.750	10.750	10.779	10.747
6.0	10.840	10.815	10.816	10.816	10.830	10.807
7.0	10.897	10.863	10.865	10.865	10.889	10.856

* Each result is the average of two titrations.

Table 4. Theoretical end-points for the effect of titrant carbonation on potentiometric and Gran titrations. Conditions as for Table 3, results in cm³

Conversion into carbonate, %	Method*	
	Potentiometric	Gran
0	10.473	10.475
1	10.525	10.521
2	10.577	10.567
3	10.634	10.617
4	10.687	10.662
5	10.742	10.714
6	10.795	10.760
7	10.852	10.816

* The equations of lines for the end-points (EP) are as follows for the potentiometric and Gran methods, respectively: EP = 0.054 18%C + 10.471 and EP = 0.048 43%C + 10.472, where %C is the percentage conversion.

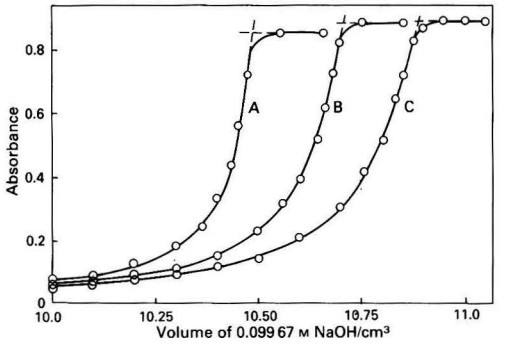

Fig. 1. Photometric titration of 10.01 cm³ of 0.1044 M acetic acid with sodium hydroxide solution using 1.0 cm³ of 5×10^{-4} M bromothymol blue as indicator. Starting volume, 50.0 cm³; ionic strength, 0.1 M; wavelength, 620 nm; and temperature, 22 °C. (A) Carbonate-free titrant; (B) 3% conversion into carbonate; and (C) 7% conversion into carbonate

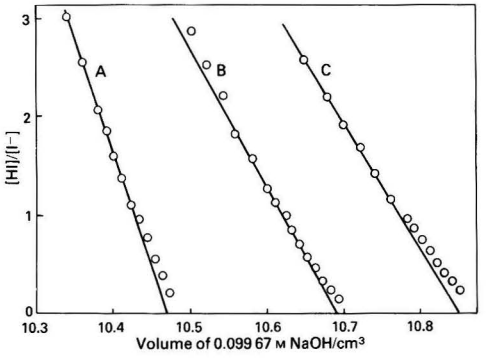

Fig. 2. Photometric Higuchi Type I plots for the titrations described in Fig. 1. From (A) $K = 4.2 \times 10^{-3}$ and hence $K_{In} = 7.5 \times 10^{-8}$ at ionic strength 0.1 (*cf.*, $K_{In} = 7.9 \times 10^{-8}$ in water)

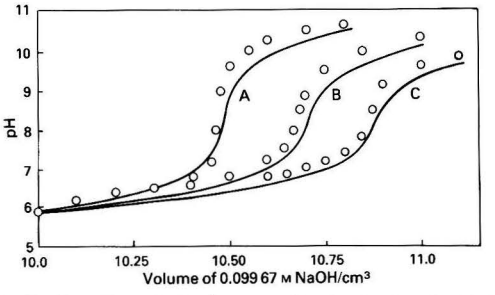

Fig. 3. Potentiometric titration curves for the titrations described in Fig. 1. ○, Theoretical points calculated from equation (12)

Discussion

The six methods of end-point location displayed a statistically identical precision (coefficient of variation *ca.* 0.10%), which was limited largely by the precision of pipetting the acid and base. However, the accuracies of the methods were very significantly different. Direct examination of the photometric and potentiometric titration curves gave one result, while the Higuchi and Gran linear intercept methods gave a lower result. Intra-group variation was insignificant. This shows the importance of standardising titrants by a method closely related to that subsequently used for samples.

The effect of conversion of the basic titrant into carbonate was found experimentally to be 0.056 cm³ per 1% conversion; the theoretical value is 0.054 cm³. All the qualitative effects of carbonate noted experimentally were borne out by theory, although we cannot explain the small discrepancy between the direct potentiometric and Gran results. The need to exclude carbon dioxide or at least to standardise the titrant at the same time as titrating unknowns is clearly shown.

We thank Dr. G. Svehla for help with the statistical analysis.

References

1. Higuchi, T., Rehm, C., and Barnstein, C., *Anal. Chem.*, 1956, **28**, 1506.
2. Gran, G., *Analyst*, 1952, **77**, 661.
3. Svehla, G., "Automatic Potentiometric Titrations," Pergamon Press, Oxford, 1978, pp. 196–197.
4. Ebel, S. *Fresenius Z. Anal. Chem.*, 1969, **245**, 108.

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Determination of Zinc Dialkyldithiophosphate Additives in Lubricating Oil by Photometric Titration

Stanisław Płaza
Department of Chemical Technology and Environmental Protection, University of Łódź, Nowotki 18, Łódź, Poland

A photometric titration, using *p*-dimethylaminophenylmercury(II) acetate with Michler's thioketone as indicator, is described for the determination of zinc dialkyldithiophosphates in lubricating oils. The method is simple, rapid, reproducible and accurate and samples do not require separation or other preliminary stages prior to their analysis. The method is suitable for determining additives in oils at concentrations above 0.01%.

Keywords: *Zinc dithiophosphate determination; photometric determination; thiomercurimetric titration; lubricating oil additives*

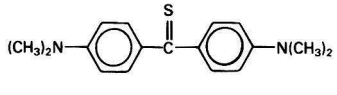
O,O'-Disubsituted dithiophosphates (phosphorodithionates), particularly zinc salts (ZDDPs), are commonly used in lubricating oils as multi-functional additives. The esters of dithiophosphoric acid have been found to be an important class of analytical reagents for the separation and determination of metal ions; they are also used as flotation agents.

The blending of zinc dialkyldithiophosphates with lubricants to give a closely specified product has presented a problem in process control analysis that has persisted for many years. Numerous methods of zinc and phosphorus determination have been proposed for the control analysis of zinc dialkyldithiophosphates as lubricating oil additives. Infrared spectroscopy[1] has been used for this control, but because of the low concentration of ZDDPs in oil and because some other additives present in oil interfere in the determination by changing the intensity of absoprtion of the P=S bond this technique is not completely satisfactory. Most of the methods for dithiophosphate determination require the separation of oil and sometimes other additives, which can be achieved, for example, by dialysis, silica gel chromatography and liquid chromatography. However, because such methods are time consuming and can give misleading results, there still remains the need for a rapid, selective, simple and inexpensive procedure.

Greenhow and Spencer[2] determined milligram amounts of dithiophosphates by a catalytic thermometric iodimetric titration. Busev and Ivanyutin[3] used *p*-dialkylaminophenylmercury(II) acetate reagent for a direct titrimetric determination of dialkyldithiophosphate ions. Dithizone has been used in this method as an indicator, but unsuccessfully for the determination of zinc and lead dithiophosphates as these metals form coloured dithizonates similar to organic mercury(II) compounds.

Wroński and Goworek[4] recommended Michler's thioketone as an indicator for the thiomercurimetric determination of some vulcanisation accelerators.

This paper describes the use of *p*-dimethylaminophenylmercury(II) acetate with Michler's thioketone as an indicator for the determination of zinc dialkyldithiophosphates. The indicator has the formula



Additive concentrates, unused oils and especially used oils that contain some additives are coloured; therefore, a visual end-point location is unsuitable and a photometric determination of the end-point in the titration mixture is proposed, thus eliminating the subjectiveness of the visual procedure.

Experimental

Reagents and Solutions

All chemicals and solvents were of analytical-reagent grade except the reagent, indicator and zinc dialkyldithiophosphates, which were prepared as described below.

Lubricating oils, hydrocarbon oil and additive concentrates of ZDDPs were of technical grade.

Preparation of p-*dimethylaminophenylmercury(II) acetate (APM)*

Pure APM was synthesised by the procedure developed by Pesci[5] as follows: 24.2 g of freshly distilled *N,N*-dimethylaniline were added to a filtered solution of 31.8 g of mercury(II) acetate in 300 ml of ethanol - water and 5 ml of acetic acid. After a few minutes a white precipitate was formed, which was allowed to settle for about 1 h and filtered to extract the crystalline solid, which was recrystallised twice from ethanol; 35 g of the product, of melting-point 165 °C, were obtained.

Preparation of Michler's thioketone (MT)

Michler's thioketone was prepared in accordance with a previously described thioketone synthesis[6]. 4,4-Bis-(dimethylamino)benzophenone (6 g), phosphorus pentasulphide (10 g) and toluene (100 ml) were heated under reflux for 6 h and the solvent was decanted whilst hot. The residue was extracted with hot toluene (3 × 30 ml). The solvent and extracts were concentrated under reduced pressure and filtered. The crude material was crystallised from ethanol, yielding 3.5 g of red crystals melting at 202 °C.

Zinc dialkyldithiophosphates were prepared from the corresponding alcohol according to Brazier and Elliot.[7]

Solutions

A 0.01 M APM solution was prepared by dissolving 3.797 g of reagent in 10 ml of DMF, adding 2 ml of acetic acid and diluting to 1 l with absolute ethanol. This solution was further diluted with absolute ethanol to obtain the required concentrations. The solution can be used directly as a working standard without standardisation. A stock solution of APM is stable for at least 3 months if stored in a dark bottle.

A 0.05% *m/V* indicator solution of Michler's thioketone was freshly prepared in absolute ethanol. Solutions of APM and MT are most stable in anhydrous solvents.

Hydrocarbon oil solutions of zinc dithiophosphates were prepared by dissolving an additive in a technical-grade mineral-based oil.

Instrumentation

The end-point was measured with a Spekol spectrophotometer (Carl Zeiss, Jena, FRG) in a 10-mm bore test-tube.

Titration Procedure

Determination of pure zinc dialkyldithiophosphate

The procedure was calibrated by titrating various amounts of zinc diisobutyldithiophosphate as follows.

To 0.5–2.0 g of oil sample containing more than 50 μg of additive, which was diluted with chloroform to a total volume of 5 ml in a 15 mm diameter test-tube, 0.2–0.5 ml of indicator solution was added. The mixture was then titrated with APM and measured photometrically at a wavelength of 580 nm. A concentration of APM in the range 1×10^{-2}–2×10^{-4} M is recommended. The measurements were plotted and the equivalence point was read from the graph. Fig. 1 shows a typical photometric titration graph.

Determination of zinc dialkyldithiophosphates in unused oils, used oils and additive concentrates

Zinc dialkyldithiophosphates in unused oils and additive concentrates were determined according to the procedure described. Before the determination, used oils were diluted with hexane (1+3 *V*/*V*), centrifuged at 15 000 rev min^{-1} for 30 min and titrated using the recommended procedure. When greater amounts of oil were present in solution the mixture became non-homogeneous during titration owing to the formation of an alcoholic solution of the reagent. More chloroform was added to obtain a clear solution. In a benzene solution of oils (containing zinc dithiophosphates) the end-point was less sharp than in chloroform.

In both the procedures the blank solution of indicator for the titrations with 5×10^{-3}—2×10^{-4} M reagents is subtracted from the amount of reagent when analysing the results.

APM reacts with zinc dithiophosphonate in a 2:1 molar ratio. Some used oils were so dark that the location of the end-point was impossible even by a photometric method and therefore the determination of zinc dithiophosphate was carried out by two-phase titration, in which the end-point was indicated by the visual change of ethanol layer from yellow to blue - violet. The time of titration is prolonged to about 20 min.

Results and Discussion

The titration graph for zinc diisobutyldithiophosphate is shown in Fig. 1. For the dithiophosphate determination the precision of the proposed method was investigated. The results for dithiophosphate in hydrocarbon oil solutions are presented in Table 1. For nine determinations (when the additive is present in amounts higher than 0.5 mg) the recoveries are near to 100% and coefficients of variation are in the range 0.18–0.35%. The coefficient of variation increased to 2.00% at 0.075 mg.

An application of the method presented is illustrated in Fig. 2, which shows the depletion graph of zinc diisooctyldithiophosphate in lubricating oil during engine work.

The recommended photometric titration method is generally satisfactory for the determination of more than 10^{-7} M of zinc dialkyldithiophosphates. Thus, a concentration as low as 0.01% in 1-ml or 1-g samples of used and unused oil solutions can be determined with the same precision as the results shown in Table 1. A method of this sensitivity is useful for determining additives in lubricating oils, where concentrations are usually about 1% and, as the sensitivity is good, samples of oils may be diluted prior to analysis.

Lubricating oils may contain other additives (with associated impurities) in addition to technical-grade dithiophosphates. Most of these compounds do not interfere in the determination, except for sulphydryl compounds and dithiocarbamates, which react with *p*-dimethylaminophenylmercury(II) acetate.

Table. 1 Determination of zinc diisobutyldithiophosphate in hydrocarbon oil solution

Amount taken/mg	Amount found*/mg	Coefficient of variation, %
10.94	10.98	0.21
1.09	1.10	0.18
0.54	0.54	0.35
0.110	0.108	1.28
0.075	0.073	2.03

* Mean concentration of nine determinations.

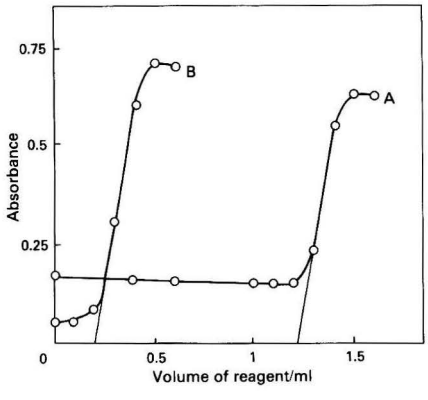


Fig. 1. A, Photometric titration graph for a chloroform solution of zinc diisobutyldithiophosphate and B, with 0.5 ml of indicator and 5 ml of chloroform, titrated with a 2×10^{-3} M solution of APM. Wavelength of measurement, 580 nm

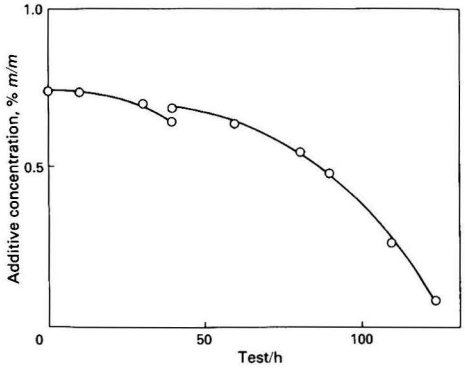


Fig. 2. Depletion of zinc diisooctyldithiophosphate in engine lubricating oil during engine work; after 40 h a portion of fresh engine oil was added

Conclusions

The determination of zinc dialkyldithiophosphates by the photometric titration described is far more rapid than other procedures. The method is also simple, reproducible and accurate and samples do not require preliminary separation or another intermediate stage prior to their analysis. Only for used oil samples containing solid particles is a preliminary separation required prior to analysis, and this is achieved by centrifugation.

In view of the results obtained and the simplicity of the analytical procedure, the proposed method can be recommended for the determination of zinc dialkyldithiophosphates in oil additive concentrates and used and unused oils. Further, although a method with this sensitivity may not be required in the assay of fungicide formulations it is useful for determining additives in rubber compositions and plastics formulations.

References

1. Fodor, G. E., and Newman, F. M., *ASLE Trans.*, 1979, **22**, 389.
2. Greenhow, E. J., and Spencer, L. E., *Analyst*, 1976, **101**, 777.
3. Busev, A. J., and Ivanyutin, M. J., *Zh. Anal. Khim.*, 1956, **11**, 523.
4. Wroński, M., and Goworek, W., *Chem. Anal. (Warsaw)*, 1975, **20**, 113.
5. Pesci, E., *Gazz. Chim. Ital.*, 1899, **23**, 521; *Chem. Zentralbl.*, 1901, **1**, 452.
6. Baker, W., Harborne, J. B., and Ollis, W. D., *J. Chem. Soc.*, 1952, 1303.
7. Brazier, A. D., and Elliott, J. S., *J. Inst. Pet., London*, 1967, **53**, 63.

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Two-point Gran Titration of Chloride in Natural Waters by Using a Silver - Sulphide Ion-selective Electrode

Hirokazu Hara
Laboratory of Chemistry, Faculty of Education, Shiga University, Otsu, Shiga 520, Japan
and Satoshi Okazaki
Department of Chemistry, Faculty of Science, Kyoto University, Kyoto 606, Japan

A simple, rapid and precise determination of chloride in a stream or a lake water was performed without any sample pre-treatment by using the precipitation titration - Gran plot procedure. A good correlation was observed between the results obtained by potentiometry and by spectrophotometry or ion chromatography. The effects of various experimental factors on the accuracy and precision in Gran titration were examined. It was demonstrated that two titration points were sufficient to obtain a maximum precision of 0.2%, provided that the potential difference between them was chosen to exceed 20 mV. The two-point Gran titration method was applied to the analysis of streams and lake water with an error of less than 2% and compared with the usual multi-point Gran titration method.

Keywords: *Gran titration method; potentiometric precipitation titration; chloride determination; natural water; silver - sulphide ion-selective electrode*

In water analysis, potentiometry with ion-selective electrodes is well known as a simple and rapid method.[1] More than 20 papers have been published that deal with the application of ion-selective electrodes to the determination of chloride in natural waters.[2] Various potentiometric methods were utilised, such as a known addition and a potentiometric titration with silver nitrate solution and direct potentiometry with a chloride ion-selective electrode.[3–5]

In preliminary studies, direct potentiometry with an Orion solid-state chloride ion-selective electrode gave higher values for chloride in a stream and a lake water compared with those obtained by a spectrophotometric method with mercury(II) thiocyanate. The observed electrode potential shifted to a more negative value very slowly owing to interferences from some species, including bromide. This result indicates that it is not possible to use direct potentiometry or a known addition method without suitable pre-treatment of the sample.

In Japan, the concentration level of chloride in streams and lake water is usually below 10 mg l^{-1} unless the water is polluted by industrial or domestic sewages. In Shiga Prefecture is the largest lake in Japan, Lake Biwa, and over 100 streams flow into it. In order to elucidate the mixing process of stream water flowing into lake water by monitoring chemical species, chloride is the most suitable as an indicator ion because it does not undergo chemical reactions. For this purpose, however, a rapid and precise determination of chloride is required because there are many samples and the concentration difference between the lake and stream waters is usually small.

Potentiometric titration of chloride is a precise enough method for examining mixing processes, but the conventional method is generally tedious and time consuming. However, the Gran titration method can provide a rapid and precise determination because it utilises only a small part of the titration curve. Jagner and Åren[6] used the Gran plot method for the determination of the total halide concentration in sea water. So far, the application of the Gran titration method to the determination of chloride in natural waters has not been properly evaluated.

In this work, Gran titration of chloride in natural waters was evaluated with a silver - sulphide ion-selective electrode. Interferences and the detection limit were examined and the effects of temperature and the number of titration points on the accuracy and precision were also studied. The Monte Carlo simulation method was used to determine the precision of the method.

The two-point Gran titration method is recommended for the simple and precise determination of chloride in natural waters.

Experimental

An Orion 94–16 silver - sulphide ion-selective electrode together with an Orion 90–02 double-junction reference electrode was used as an indicator electrode in the precipitation titration with silver nitrate.[7] Electrode potentials were measured with an Orion 701A Ionalyzer to 0.1 mV. When considering measurements in the field, the temperature of sample solutions was not controlled by a thermostated bath. Instead, the water temperature was measured immediately after the titration was completed and the corresponding Nernstian slope was estimated.

A spectrophotometric procedure with mercury(II) thiocyanate and ammonium iron(III) sulphate was performed by measuring the absorbance at 460 nm with a filtered sample.[8]

An ion chromatograph (Toyo Soda Model HLC-601) was used for the analysis of stream water samples, which were filtered with a Millipore filter.[9]

All calculations, including the Gran plot by means of a least-squares method and Monte Carlo simulation, were carried out on a personal computer (PC 8801, produced by NEC) with an N88 disk BASIC system.

The concentration of silver nitrate titrants were 2.9×10^{-3} and 5.8×10^{-3} mol l^{-1} for the lake water (containing 7.5–8.5 mg l^{-1} of chloride) and stream water (containing 10–30 mg l^{-1} of chloride) samples, respectively. These titrants were standardised by titrating the chloride primary standard solutions of 10 or 20 mg l^{-1} from 6 to 9 ml with 0.5-ml portions. In the titration of samples, the first and the last points were fixed so as to take the same potential range as for the titration of standard chloride solutions.

The Monte Carlo simulation method was used for the determination of precision.[10–12] The calculations were carried out on the basis of the experimental data with the addition of a randomised error in the potential readings or volume of titrant.

Results and Discussion

Electrode Response

The silver - sulphide electrode showed a nearly Nernstian response for silver ion from 10^{-2} to at least 10^{-6} mol l^{-1}. In the titration of chloride, the effect of the solubility of silver chloride precipitates could be ignored because the concentration of silver ion was 7×10^{-5} mol l^{-1} after the first addition of silver nitrate titrant, which was about five times larger than the square root of the solubility product of silver chloride. The linearity of the Gran plot was so good that the correlation coefficient was higher than 0.9999. The electrode response was so rapid that the potential could be recorded immediately after the addition of titrant, except for the first addition, in which it took about 0.5–1 min to complete the precipitation and obtain a stable potential.

Interferences

Species that react with silver ion may possibly interfere. The possible inorganic interferents are bromide, iodide, sulphide, thiosulphate and cyanate. However, the concentration levels of the last four species were so low in the streams and lake waters examined that they caused no problems. A preliminary experiment with a bromide ion-selective electrode gave the concentration of bromide as 0.4 mg l^{-1} at most, although the bromide electrode showed a higher value because of the interference by the large amount of chloride. The error caused by the bromide was estimated to be less than 2%. It is more practical to consider that the total concentration of chloride and bromide is measured by the method.

Suspended solids such as a plankton or silt did not interfere because the values for filtered and unfiltered samples agreed completely with each other. Dissolved organics in a stream water containing domestic sewage also did not exert a noticeable effect on the determination, because no appreciable difference was observed between the results obtained by potentiometry and ion chromatography. The correlation coefficient between the results obtained by these methods was 0.998 for 21 different stream waters that contained chloride at levels from 10.9 to 46.6 mg l^{-1}. Fair agreement was also observed between the results of potentiometry and spectrophotometry (the correlation coefficient was 0.991 for 14 stream waters containing 13.1–38.2 mg l^{-1} of chloride). Spike tests with a stream water containing 24.0 mg l^{-1} of chloride gave an average recovery of 101.9%.

It is concluded that no serious problems from interferences arose with the method because a large excess of silver ion was present when the potentials were measured.

Detection Limit

Gran *et al.*[13] reported a method for the calculation of the equivalence volume in precipitation titrations and applied it to the determination of low levels of chloride. The over-all errors in the determinations were 0.1–0.2% at chloride concentrations down to 3.5 mg l^{-1}, but at lower concentrations the relative errors were greater. In our experiment, reliable results were obtained down to 5 mg l^{-1} of chloride with the simple Gran titration.[7] In a practical sense, this limit should be sufficient because the concentration of chloride in natural waters is usually above 5 mg l^{-1}.

It is also possible to measure lower concentration levels of chloride. In this instance a calibration graph, which is made up by equivalence volumes of standard chloride solutions obtained by the Gran plot, is indispensable for an accurate determination because it does not always pass through the origin. Fig. 1 serves as an example.

The reason why the equivalence volume showed inaccurate values may be explained by the fact that the solubility of precipitates was not allowed for in this simple Gran method.

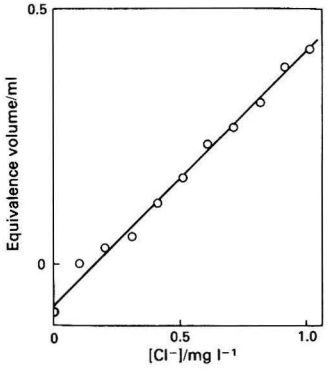

Fig. 1. Calibration graph for a low level of chloride. $V_e = -0.078 + 0.504[Cl^-]$; $r = 0.997$

Table 1. Reproducibility of response slope of silver ion-selective electrode. Temperature: 20.5 ± 1.5 °C

	$[AgNO_3]$/mol l^{-1}	
No.	2.90×10^{-3}	5.84×10^{-3}
1	57.9	59.0
2	59.0	59.1
3	58.6	59.1
4	59.6	57.9
5	59.2	57.6
Mean:	58.9	58.5
RSD, %	1.1	1.2

In fact, a linear calibration graph that passed through the origin was obtained by using the linear titration plot devised by McCallum and Midgley[14] for the determination of 0–10 mg l^{-1} of chloride. However, curvature of the Gran plot, as was pointed out by McCallum and Midgley, was not observed even in a solution of 0.1 mg l^{-1} of chloride ($r > 0.9999$ was usual) because of the large excess of silver ion present in the solution. The other possible reason for the discrepancy is the overestimation of the response slope, which is discussed later. In any event, such a low concentration of chloride was determined by using a calibration graph with a simple Gran method. It took about 15 min to obtain a stable potential after the first addition for the titration of 0.1–1 mg l^{-1} chloride. Rain water containing about 0.5 mg l^{-1} of chloride was analysed by this method with a relative standard deviation of 10%.

Accuracy

The equivalence volume calculated from the Gran plot is affected by the degree of over- or underestimation of the true value of the electrode slope. The larger the value of the slope, the smaller is the value of the equivalence volume, that is, the equivalence volume has a negative slope coefficient. The slope (S) in turn depends on the temperature through a Nernstian equation ($S=RT/F$). When the potential jump throughout the titration was about 27 mV with 3×10^{-3} mol l^{-1} silver nitrate solution, the temperature coefficient of the equivalence volume was 7–8 $\times 10^{-3}$ ml $°C^{-1}$, irrespective of the chloride concentration. For the Gran plot, the correct value of the response slope, which is usually determined experimentally beforehand, is required. However, there seems to be no evidence that the slope thus obtained remains constant throughout the experiment. Ivaska[15] devised a means of calculating the response slope and the equivalence volume

simultaneously, but it is not an easy procedure to carry out with a simple calculator. In this work, the theoretical Nernstian slope was used to compensate for the effect of temperature changes during the measurements by taking into account the difficulties of controlling the temperature in field work. As shown in Table 1, the reproducibility of the response slopes in five successive measurements was about 1%. The error caused by the deviation from the real slope value of the electrode response was estimated to be within 2% for the 10 mg l^{-1} chloride solution.

Precision

In general, the precision of potentiometric titrations with ion-selective electrodes depends not only on the precision of the potential readings and the volume of titrant, but also on the number of titration points. Buffle *et al.*[16] examined the error in the Gran additions method in detail. They stated that the number of additions should be greater than ten. Burden and Euler[17] also examined the dependence of the Gran titration error of acid - base reactions on the number of points. When a river water was measured ten times with the seven-point Gran titration procedure, the mean value was 9.75 mg l^{-1} with a relative standard deviation of 0.21%.

The effect of the number of titration points was examined on the basis of these data. In Table 2, the mean values of chloride concentration and the relative standard deviations are listed for all the combinations possible when points 1 and 7 were always included. The number of titration points did not influence the precision. The result indicated that the precision of Gran titration with two points could be the same as that of the multi-point Gran titration method. In Fig. 2, the dependence of the precision of the two-point Gran titration on the potential difference between them is shown in contrast with the results obtained by Monte Carlo simulation with a random error of 0.1 mV in the potential reading and 0.005 ml in the volume of titrant. The precision depends strongly on the potential difference when it is smaller than 10 mV, but becomes insensitive to the potential difference when it is greater than 20 mV. The same tendency was observed with the results of Monte Carlo simulation. Therefore, it was concluded that the precision could not become better than 0.2%.

These results indicate that two points are sufficient to obtain a maximum precision of 0.2%. In fact, the two-point Gran titration of the river water gave a mean value of 9.11 mg l^{-1} for ten successive measurements with a relative standard deviation of 0.15% when the potential difference between them was 23.6 or 23.7 mV.

The dependence of the measurement precision on the precision of the readings of potential or the burette was estimated by Monte Carlo simulation and the results are shown in Table 3. When the errors of the readings of potential and the burrette were ±0.1 mV and ±0.005 ml, respectively, which was the case with our measuring system, a measurement precision of 0.2–0.3% was obtained. The measurement precision was also estimated when the precision of the readings of potential or the burette declined to only one tenth of the original values. The dependence of the precision on the error in potential readings was found to be greater than that on the error in the volume of titrant.

Two-point Gran Titration of Chloride in Natural Water Analysis

The precision of the two-point Gran titration method has already been discussed. This method was applied to the determination of chloride in streams and lake water and the results are shown in Table 4 in comparison with those of the seven-point method. The agreement between the methods

Table 2. Effect of number of titration points on precision calculated from ten results of seven-point Gran titration. ΔE = 27.7 mV

No. of points	[Cl^-]*/mg l^{-1}	RSD,* %
2	9.77 (n = 1)†	0.21
3	9.76 (n = 5)	0.21
4	9.76 (n = 10)	0.20
5	9.76 (n = 10)	0.20
6	9.75 (n = 5)	0.20
7	9.75 (n = 1)	0.20

* Mean values for all the combinations possible if the first and the last points were always included.
† Number of combinations.

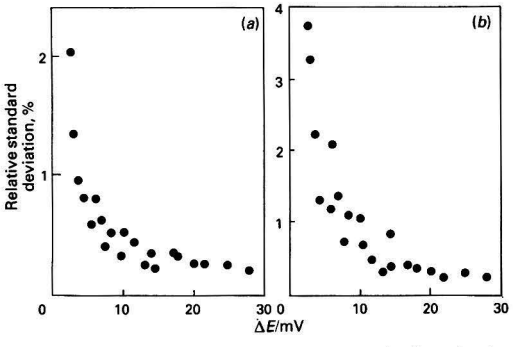

Fig. 2. Dependence of the precision of the two-point Gran titration method on the potential difference. (*a*) Calculated on the basis of experimental data; and (*b*) Monte Carlo simulation with a randomised error of 0.005 ml in the volume of titrant and 0.1 mV in the potential reading

Table 3. Precision of seven-point Gran titration method estimated by Monte Carlo simulation

	V ± 0.005 ml, E ± 0.1 mV		V ± 0.005 ml, E ± 1 mV		V ± 0.05 ml, E ± 0.1 mV	
No.	[Cl^-]*/mg l^{-1}	RSD, %	[Cl^-]*/mg l^{-1}	RSD, %	[Cl^-]*/mg l^{-1}	RSD, %
1	9.79	0.18	9.79	2.06	9.81	1.12
2	9.75	0.24	9.81	1.79	9.76	0.86
3	9.74	0.19	9.74	1.81	9.72	0.94
4	9.74	0.29	9.77	2.27	9.73	0.65
5	9.80	0.27	9.77	2.24	9.76	0.75
6	9.74	0.33	9.72	2.50	9.74	0.87
7	9.76	0.28	9.68	2.09	9.73	0.89
8	9.73	0.24	9.77	3.05	9.74	0.68
9	9.74	0.29	9.77	1.95	9.76	0.84
10	9.75	0.20	9.73	1.66	9.75	0.78
Mean	9.75	0.25	9.76	2.14	9.75	0.84

* Each figure is the mean value of ten independent calculations.

Table 4. Comparison of two-point with seven-point Gran titration of chloride in samples from Lake Biwa and streams

	[Cl⁻] in lake water/mg l⁻¹			[Cl⁻] in stream water/mg l⁻¹		
No.	Two-point method	Seven-point method	Difference, %	Two-point method	Seven-point method	Difference, %
1	7.82	7.83	−0.13	23.5	23.6	−0.42
2	7.83	7.91	−1.1	19.7	19.8	−0.51
3	7.82	7.80	0.26	11.2	10.9	2.8
4	7.88	7.79	1.2	17.2	17.0	1.2
5	7.87	7.84	0.38	14.9	14.7	1.4
6	7.90	7.87	0.38	14.2	14.2	0.0
7	7.87	7.82	0.64	22.8	22.6	0.88
8	7.92	7.97	−0.63	20.2	20.2	0.0
9	8.06	8.08	−0.25	19.7	19.7	0.0
10	8.29	8.22	0.85	13.6	13.6	0.0
11	9.33	9.43	−1.1	12.5	12.6	−0.79
12	8.06	8.05	0.12	12.0	12.2	−1.6
13	8.33	8.28	0.60	46.2	46.6	−0.86
14	8.19	8.15	0.49	18.8	18.9	−0.53
15	8.38	8.35	0.36	16.4	16.3	0.61

was excellent in both instances. As a simplified titration, Horvai *et al.*[18] suggested a single-point potentiometric titration procedure, in which a known amount of titrant was added and the resulting solution was analysed by direct potentiometry. Although their method is easy to perform, the two-point Gran titration method seems to be simpler because it does not require a procedure for calibration.

In conclusion, the precipitation titration - Gran plot procedure is useful for natural water analysis and the two-point Gran titration is a simple, rapid and accurate method. This titration process can easily be automated. The application of this method to flow titration is now being studied.

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References

1. Midgley, D., and Torrance, K., "Potentiometric Water Analysis," Wiley, New York, 1978.
2. Hulanicki, A., and Trojanowicz, M., *Ion-Sel. Electrode Rev.*, 1979, **1**, 207.
3. Matuszewski, W., Zytnikowski, A., and Trojanowicz, M., *Chem. Anal. (Warsaw).*, 1980, **25**, 897.
4. Stoyanov, A. S., Simeonov, V. D., and Andreev, G. A., *Dokl. Bolg. Acad. Nauk*, 1980, **33**, 219.
5. Sekerka, I., Lechner, J. F., and Wales, R., *Water Res.*, 1975, **9**, 663.
6. Jagner, D., and Årén, K., *Anal. Chim. Acta*, 1970, **52**, 491.
7. *Orion Newsl.*, Nov./Dec. 1970.
8. Utsumi, S., *Nippon Kagaku Zasshi*, 1952, **73**, 835.
9. Okada, T., and Kuwamoto, T., *Bunseki Kagaku*, 1983, **32**, 595.
10. Horvai, G., and Pungor, E., *Anal. Chim. Acta*, 1980, **113**, 295.
11. Kamakura, Y., *Bunseki Kagaku*, 1983, **32**, T49.
12. Efstathiou, C. E., and Hadjiioannou, T. P., *Anal. Chem.*, 1982, **54**, 1525.
13. Gran, G., Johansson, A., and Johansson, S., *Analyst*, 1981, **106**, 1109.
14. McCallum, C., and Midgley, D., *Anal. Chim. Acta*, 1973, **65**, 155.
15. Ivaska, A., *Talanta*, 1980, **27**, 161.
16. Buffle, J., Parthasarathy, N., and Monnier, D., *Anal. Chim. Acta*, 1972, **59**, 427.
17. Burden, S. L., and Euler, D. L., *Anal. Chem.*, 1975, **47**, 793.
18. Horvai, G., Tóth, K., and Pungor, E., *Anal. Chim. Acta*, 1979, **107**, 101.

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Phenothiazine Drug Poly(vinyl Chloride) Matrix Membrane Electrodes and Their Use in Pharmaceutical Analysis

Vasile V. Coşofreţ* and Richard P. Buck†
Department of Chemistry, University of North Carolina, Chapel Hill, NC 27514, USA

The construction and performance characteristics of ion-selective membrane electrodes for phenothiazine drugs based on their ion-pair complexes with tetraphenylborate and dinonylnaphthalene sulphonate in a poly(vinyl chloride) matrix are described. The electrodes show a near-Nernstian response over various ranges depending on the nature of the phenothiazine drug. The selectivity of these electrodes to a number of amino acids, alkaloids, neurotransmitters, quaternary ammonium compounds and other drugs or pharmaceutical excipients is reported. The standard additions method and potentiometric titrations are used to determine the phenothiazine drugs in pharmaceutical preparations with satisfactory results.

Keywords: *Phenothiazine drug electrodes; poly(vinyl chloride) membranes; standard additions method; potentiometric titration; drug analysis*

Ion-selective membrane electrodes are finding considerable use with many applications in pharmaceutical analysis,[1–5] even though not all of them provide acceptable sensitivity and selectivity for the drug of interest. Much effort is required in the development of a rapid, simple and sensitive method for the determination of a certain drug, as it may be found in complex pharmaceutical preparations or in human biological fluids, not in simple pure forms. Usually, the potentiometric methods can be simple and fast for pharmaceutical analyses when a suitable sensor is available.

In this paper, the performance characteristics of phenothiazine drug membrane electrodes are described. The electroactive materials of the membranes are either phenothiazine drug - tetraphenylborate or phenothiazine drug - naphthalene sulphonate ion pairs in a poly(vinyl chloride) (PVC) matrix. The electrodes exhibit useful analytical characteristics for the direct or indirect determination of protonated phenothiazine drugs either in the pure form or in pharmaceuticals.

Phenothiazine drugs are compounds with well known neuroleptic activity. Chlorpromazine remains the most widely used antipsychotic drug throughout the world and continues to serve as a standard with which other neuroleptics are compared.[6] Other phenothiazine analogues possess an antidepressive rather than a neuroleptic action and are widely used for the treatment of depressive states. Of the various phenothiazines the piperazine derivatives were found to be potent antiemetics.

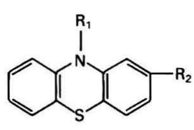
The structures and therapeutic category of the investigated phenothiazine drugs are given in Table 1.

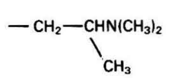
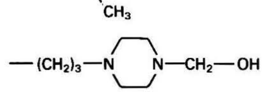
Experimental

Reagents and Materials

All reagents were of analytical-reagent grade. Chlorpromazine hydrochloride, promethazine hydrochloride and perphenazine were supplied by Sigma; other materials were sodium tetraphenylborate(III) (NaTPB) (Aldrich), 2-nitrophenyl octyl ether (2-NPOE) (Fluka), dinonylnaphthalene sulphonic acid (DNNS) (Pfaltz and Bauer) and PVC of high relative molecular mass (Aldrich). Pharmaceutical preparations were purchased from local drugstores and were of USP quality.

Table 1. Phenothiazine drugs examined



Drug	R_1	R_2	Therapeutic category
Chlorpromazine ..	$-(CH_2)_3N(CH_3)_2$	–Cl	Tranquilliser, sedative, antiematic
Promethazine ..		–H	Tranquilliser, antihistaminic
Perphenazine ..		–Cl	Tranquilliser, antiemetic

* On leave from the Institute of Chemical and Pharmaceutical Research, Bucharest, Romania.
† To whom correspondence should be addressed.

Apparatus

Potentiometric measurements were carried out using an Orion Model 701A digital pH/millivolt meter. The respective phenothiazine drug membrane electrodes were used with an Orion 91–01 double-junction reference electrode containing 10% *m/V* Na_2SO_4 solution in the outer compartment. pH measurements were performed with an Orion 91–02 combination glass electrode.

Phenothiazine Drug - TPB Ion Pairs

The respective phenothiazine drug - TPB ion pairs were precipitated by mixing 20 ml of 10^{-2} M chlorpromazine hydrochloride, promethazine hydrochloride or perphenazine monohydrochloride with 20 ml of 10^{-2} M NaTPB solution. The white precipitates obtained were filtered on a G4 sintered Gooch crucible, washed with distilled water and dried at room temperature.

Construction of Electrodes

The PVC membranes and the electrodes based on phenothiazine drug - TPB ion pairs were constructed according to the method of Moody and Thomas.[7] Powdered PVC, the ion exchanger and plasticiser - mediator were dissolved in a volatile solvent such as tetrahydrofuran. The membrane was cast on a glass sheet and was formed as the solvent evaporated. Punched circular membranes were attached to a PVC tube electrode body; compositions are given in a later section. In all instances the electrode body was filled with a 10^{-3} M solution of the respective phenothiazine drug hydrochloride - 10^{-1} M NaCl mixture. The electrodes were pre-conditioned for 10 min by soaking them in their respective 10^{-3} M phenothiazine drug hydrochloride solution. The PVC membranes and the electrodes based on phenothiazine drug - DNNS ion pairs were constructed as described elsewhere,[8] with the exception that the membrane composition was 4.0% *m/m* in DNNS and the plasticiser used was *o*-nitrophenyl octyl ether (64.0% *m/m*). These electrodes were pre-conditioned for 24 h by soaking in their respective 10^{-2} M phenothiazine drug hydrochloride solution. Between use all phenothiazine drug membrane electrodes were stored in the same solution as the internal solution.

Electrode Characteristics

The performances of the electrodes were investigated by measuring the e.m.f. values of between 10^{-2} and 10^{-6} M of the respective phenothiazine drug hydrochloride solutions. Potentials were recorded when stable readings were obtained (normally within 15 s).

Direct Potentiometric Measurements of Phenothiazine Drugs

The appropriate phenothiazine drug electrode and the Orion double-junction reference electrode were immersed in the respective phenothiazine-containing aqueous solution (50 ml) of ionic strength 0.1 M (adjusted with NaCl) and at the appropriate pH (see Effect of pH, under Results and Discussion). They were allowed to equilibriate with stirring, and their e.m.f. values were recorded and compared with the calibration graph. As an alternative, the standard additions method was used and for this purpose 5.0 ml of standard solution (10^{-2} M phenothiazine drug hydrochloride solution) were added. The change in the millivolt reading was recorded and used to calculate the concentration of the respective phenolthiazine drug.

For the direct potentiometric assay of tablets, typically five tablets containing chlorpromazine hydrochloride or promethazine hydrochloride as the active principle were finely powdered and transferred with 0.1 M NaCl solution into a 500-ml calibrated flask. A 25-ml aliquot of this solution was pipetted into a 100-ml beaker; then, 25 ml of 0.1 M NaCl solution were added and the appropriate indicator electrode and Orion double-junction reference electrode were immersed in it, as described above.

Potentiometric Titration of Phenothiazine Drugs

A 10-ml aliquot of the respective phenothiazine drug hydrochloride solution (containing 1–10 mg) was pipetted into a 100-ml beaker. About 30 ml of 0.1 M NaCl solution were added and the resulting solution was titrated with a 10^{-2} M standard solution of NaTPB, using an appropriate phenothiazine drug membrane electrode as the indicator. The volume of titrant at the equivalence point was obtained in the usual way. For tablet analysis, 25–50-ml aliquots from stock tablet solutions were pipetted into a 100-ml beaker and potentiometric titrations were carried out as described.

Results and Discussion

Membrane Materials

Phenothiazine drugs, as well as other organic amines, are well known for reacting with NaTPB and DNNS, forming stable ion-pair complexes. The TPB complexes were first isolated from aqueous solutions and later embedded in a PVC matrix, and DNNS complexes were obtained *in situ*, by soaking the DNNS - PVC membranes in the appropriate phenothiazine drug solution (hydrochloride salt). 2-NPOE showed, as well as other plasticisers, good behaviour regarding response time and reproducibility of e.m.f. values of the electrodes. The membrane compositions were: 3.2% *m/m* phenothiazine drug - TPB, 64.5% *m/m* 2-NPOE and 32.3% *m/m* PVC; and 4.0% *m/m* DNNS, 64.0% *m/m* 2-NPOE and 32.0% *m/m* PVC, respectively.

Electrode Response

The critical response characteristics for the various electrodes based on phenothiazine drug ion-pair complexes are summarised in Table 2. The data indicate that a near-Nernstian response to all three phenothiazine drugs for both TPB- and DNNS-based membranes is obtained over a relatively large range of concentrations. This linear range depends on the nature of phenothiazine drug.

Both ion-pair extracting agents seem to form complexes with various phenothiazines of the same solubility. The linear range of perphenazine is shorter than that obtained for chlorpromazine and promethazine. The detection limits are similar for all electrodes, except that of the perphenazine - DNNS-based membrane, where a detection limit of 4.47×10^{-6} M is obtainable. This is probably because DNNS forms a less soluble ion-pair complex with perphenazine than with other phenothiazine drugs. The calibration graphs for the individual electrodes were found to be reproducible from day to day provided that the electrodes were stored in the appropriate phenothiazine drug solution between measurements.

In addition, all electrodes exhibited a near-Nernstian response to the TPB anion in the range 10^{-2}–10^{-4} M and an over-Nernstian response in the range 10^{-4}–10^{-6} M. Similar behaviour has been observed for a quinidine electrode[4] and for plastic electrodes, prepared by coating a PVC film on to a graphite support.[9]

Effect of pH

The effect of pH on the potential readings of the phenothiazine drug electrodes was checked by recording the e.m.f. of a cell of the type Ag/AgCl/10^{-3} M phenothiazine drug

Table 2. Response characteristics for phenothiazine drug membrane electrodes

Parameter	Chlorpromazine electrode		Promethazine electrode		Perphenazine electrode	
	TPB-based membrane	DNNS-based membrane	TPB-based membrane	DNNS-based membrane	TPB-based membrane	DNNS-based membrane
Slope (mV/log a)	54.80 ± 0.32*	54.17 ± 0.56	55.60 ± 0.43	54.64 ± 0.45	52.50 ± 0.29	54.64 ± 0.65
Intercept/mV	232 ± 2.6†	228 ± 1.7	232 ± 2.3	228 ± 2.1	223 ± 2.9	231 ± 2.6
Linear range/M‡	8.0×10^{-3} -2.5×10^{-5}	8.0×10^{-3} -2.5×10^{-5}	1.0×10^{-2} -5.0×10^{-5}	1.0×10^{-2} -5.0×10^{-5}	2.0×10^{-3} -2.5×10^{-5}	2.0×10^{-3} -2.5×10^{-5}
Useable range/M§	10^{-2}–10^{-5}	10^{-2}–10^{-5}	10^{-2}–10^{-5}	10^{-2}–10^{-5}	5.0×10^{-3} -10^{-5}	5.0×10^{-3} -3.0×10^{-6}
Detection limit/M	1.00×10^{-5}	1.25×10^{-5}	1.42×10^{-5}	1.66×10^{-5}	1.86×10^{-5}	4.47×10^{-6}

* Standard deviation of average slope value for multiple calibrations.
† Standard deviation of values recorded during one month.
‡ Region of Nernstian response.
§ Total response range including sub-Nernstian region.

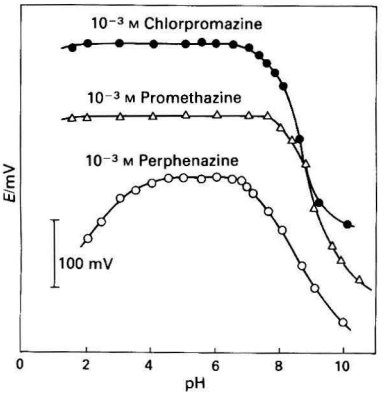


Fig. 1. Effect of pH on the response of the phenothiazine - TPB-based membrane electrodes. The graphs are displaced vertically for clarity; similar graph shapes were recorded for DNNS-based membrane electrodes

hydrochloride - 10^{-1} M NaCl (inner solution) ‖ plastic membrane ‖ 10^{-3} M phenothiazine drug hydrochloride - 10^{-1} M NaCl (outer solution) ‖ Orion double-junction reference electrode with 10% m/V Na_2SO_4 in the outer ccmpartment, with varying acidity by the addition of very small volumes of hydrochloric acid and/or sodium hydroxide solution (0.1 or 1.0 M from each). The latter is added to the right-hand or "outer solution." The graphs presented in Fig. 1 show that the linearity of potential (E) *versus* pH functions depends on the nature of the phenothiazine drug. For chlorpromazine and promethazine the electrode responses are not affected by pH from an acidic medium up to pH 6.8 and 7.6, respectively. For perphenazine, a linear potential versus pH graph was observed only in the pH range 4.5–6.5. At lower pH values the perphenazine electrodes become progressively sensitive to the diprotonated perphenazine species and the e.m.f. readings decrease with pH decrease. At higher pH values the perphenazine, as well as the chlorpromazine and promethazine free bases, precipitate in the test aqueous solutions, and consequently the concentration of unprotonated species gradually increases. As a result, lower e.m.f. readings were recorded.

The potential *versus* pH graphs in Fig. 1 have been used to evaluate the basicity constants (K_b) of each of the phenothiazine drugs. The acidity constant, pK_a, is equal to the pH value where the initial concentration of protonated phenothiazine drug is halved, *i.e.*, when the potential of the respective electrode decreases by 0.30S mV (S = electrode slope). For perphenazine, the second basicity constant (K_b') was evaluated by taking into account the fact that the respective acidity constant (pK_a') is equal to the pH value where the initial concentration of monoprotonated perphenazine, [Perph H$^+$], is also halved, *i.e.*, when the potential of the perphenazine electrode decreases by the same value (0.30S). The pK_b values obtained for the investigated phenothiazine drugs are given in Table 3.

Table 3. Basicity constants of examined phenothiazine drugs evaluated from potential *versus* pH graphs. Each is the average of two values obtained by using both types of membrane electrodes

Phenothiazine drug	K_b	K_b'
Chlorpromazine	1.76×10^{-7}	—
Promethazine	1.25×10^{-6}	—
Perphenazine	9.46×10^{-8}	3.57×10^{-11}

Selectivity of the Electrodes

The selectivity of an ion-pair complex-based membrane electrode depends on the selectivity of the ion-exchange process at the membrane - sample solution interface, the mobilities of the respective ions in the membrane, and hydrophobic interactions between the primary ion and the organic membrane. The selectivity of the phenothiazine drug membrane electrodes is related to the free energy of transfer of the phenothiazine drug cation between aqueous and organic phases. The response of the electrodes towards different substances has been checked and the selectivity coefficients, $K^{Pot}_{Phe^+,\,J^{z+}}$, were used to evaluate the degree of interference. The values given in Table 4 were obtained using the separate solution method and equation (1):

$$\text{Log } K^{Pot}_{Phe^+,\,J^{z+}} = (E_2 - E_1)/S + \log [Phe^+] - \log [J^{z+}]^{1/z} \quad (1)$$

where E_1 is the potential of the electrode in a phenothiazine drug solution (as hydrochloride) of 10^{-3} M concentration and I=0.1 M (adjusted with NaCl), and E_2 is the potential of the same electrode in a solution containing [Phe$^+$] = 0 and [J^{z+}] = 10^{-3} M at the same ionic strength and pH; J represents the interferent.

The substances listed in Table 4 were chosen as representatives of potentially low-level contaminants in phenothiazine drug pharmaceutical preparations or in biological fluids. The bulk of the excipient in a pharmaceutical tablet, usually consisting of lactose or glucose diluent and corn starch or gelatin binders, does not show any interference. The same lack of effect is shown by maltose, mannitol, sugar and by 2-chlorophenothiazine, the main metabolite of chlorpromazine and perphenazine. The data in Table 4 show that all

Table 4. Selectivity coefficients for the phenothiazine drug membrane electrodes

	Selectivity coefficient (log $K^{Pot}_{Phe^+, J^{z+}}$)					
	Chlorpromazine electrode		Promethazine electrode		Perphenazine electrode	
Interferent, J	TPB-based membrane	DNNS-based membrane	TPB-based membrane	DNNS-based membrane	TPB-based membrane	DNNS-based membrane
Chlorpromazine	1	1	0.62	0.58	0.98	0.78
Promethazine	−0.69	−0.59	1	1	0.19	0.09
Perphenazine	−0.80	−0.67	−0.23	−0.16	1	1
L-Arginine	−2.00	−2.22	−1.91	−2.16	−1.91	−2.49
Glycine	−1.89	−2.15	−1.96	−2.05	−2.47	−2.71
L-Histidine	−1.87	−2.31	−1.87	−2.25	−1.98	−2.42
L-Epinephrine	−1.71	−2.94	−2.07	−2.29	−1.83	−2.27
Dopamine	−1.93	−2.22	−1.85	−2.02	−1.66	−1.77
Isoniazid	−2.07	−2.50	−1.80	−1.96	−1.94	−2.47
Acetylcholine	−2.22	−2.67	−1.98	−2.44	−2.06	−2.67
Quinidine	−1.82	−1.63	−1.48	−1.38	−1.30	−1.35
Quinine	−1.89	−1.72	−1.59	−1.49	−1.43	−1.49
$(CH_3)_4N^+$	−2.20	−2.80	−2.04	−2.44	−2.09	−2.64
$(C_2H_5)_4N^+$	−2.11	−2.67	−1.82	−2.27	−1.62	−2.24
$(C_4H_9)_4N^+$	0.40	0.02	1.14	0.78	1.62	1.16

Table 5. Potentiometric determination of phenothiazine drugs in pure solutions with phenothiazine drug membrane electrodes

		Recovery, *%		Standard deviation, %	
Phenothiazine drug	Taken/mg (range)	Potentiometric titration†	Standard additions method‡	Potentiometric titration	Standard additions method
Chlorpromazine hydrochloride	1.78–8.90	99.1	100.8	0.88	1.72
Promethazine hydrochloride	1.61–8.05	100.6	101.0	0.47	1.91
Perphenazine hydrochloride	0.88–4.40	101.1	99.2	0.83	2.14

* With respect to amount taken; all values are the averages of 6–10 determinations.

† In all instances TPB-based membrane electrodes were used in potentiometric titrations; the perphenzaine - DNNS-based membrane was used only in the standard additions method for perphenazine determination.

‡ Volume (total) = 50 ml (I = 0.1 M, adjusted with NaCl); volume (sample) = 2, 4 or 5 ml; and concentration (sample) = 10^{-2} M solution of the respective phenothiazine drug hydrochloride.

Table 6. Potentiometric determination of phenothiazine drugs in tablets using phenothiazine drug membrane electrodes*

			Result, % of nominal		Standard deviation, %	
Product	Active principle	Sample	Potentiometric titration†	Standard additions method‡	Potentiometric titration	Standard additions method
Thorazine (Smith Kline & French Labs.), 25.0 mg per tablet	Chlorpromazine hydrochloride	1	99.2	100.7	0.80	2.60
		2	100.5	101.7	0.71	1.55
		3	100.6	102.3	0.94	1.41
Phenergan (Wyeth Labs.), 12.5 mg per tablet	Promethazine hydrochloride	1	100.7	100.0	0.64	1.56
		2	98.8	99.4	0.85	1.64
		3	99.0	100.4	0.73	1.04

* In all instances TPB-based membrane electrodes were used.

† All values are the averages of 4 determinations.

‡ All values are the averages of 6–10 determinations.

electrodes are negligibly affected in their response by the presence of a number of amino acids, neurotransmitters, alkaloids and quaternary ammonium compounds containing less than four carbon atoms in each side-chain. As expected by inspection of the phenothiazine drug structures, selectivity decreases in the order chlorpromazine, promethazine and perphenazine.

The greater selectivity of the chlorpromazine electrodes over promethazine is due to the fact that chlorpromazine is 34.4 atomic mass units larger than promethazine. This correlation between mass and hydrophobicity is not valid for perphenazine, which contains an OH hydrophilic group in the aminic chain. This result supports the fact that selectivity is determined primarily by the partition coefficient of the

protonated amine between organic and aqueous phases.[8] Generally, as can be seen in Table 4, the phenothiazine drug - DNNS-based membrane electrodes show greater selectivity than phenothiazine drug - TPB-based membrane electrodes.

Response Time

The response time of the chlorpromazine and promethazine electrodes of both types, and the perphenazine - TPB-based electrode was fast, being nearly instantaneous in the 10^{-2}–10^{-4} M range, and requiring less than 1 min with 10^{-5} M solution. For the perphenazine - DNNS-based membrane electrode, a response time of about 4–5 min was recorded over the 10^{-5}–10^{-6} M range. Otherwise, the reproducibility of e.m.f. readings was good enough in this range also.

Analytical Applications

All phenothiazine drug electrodes proved useful in the potentiometric determination of the respective phenothiazine drugs in pure forms or in pharmaceutical preparations, both by direct potentiometry and by potentiometric titrations (Tables 5 and 6). Larger potential breaks were obtained with DNNS-based membrane electrodes, but after one potentiometric titration the calibration graphs shifted by several millivolts. The original response was restored by soaking the electrodes in a 10^{-3} M solution of the appropriate phenothiazine drug for about 2 h. From these observations we recommend for use in potentiometric titrations the TPB-based membrane electrode. In these examples, after one potentiometric titration the calibration graph is maintained.

Table 5 shows the results of potentiometric determination of phenothiazine drugs examined in pure solutions, by both the standard methods and potentiometric titrations with 10^{-2} M NaTPB solution and Table 6 gives the results of the potentiometric analyses of two common phenothiazine pharmaceutical preparations.

Better results for precision and accuracy were obtained by potentiometric titrations. The standard additions method is also recommended for its simplicity and rapidity. In contrast to the 2 h approximately required for assay by the official method,[10] an electrode assay can be accomplished within 15 min. This rapidity using membrane electrodes makes them practical for performing the procedure on a single tablet, so that tablet to tablet variation can be followed if desirable.

References

1. Coşofreţ, V. V., *Ion-Sel. Electrode Rev.*, 1980, **2**, 159.
2. Coşofreţ, V. V., "Membrane Electrodes in Drug -Substances Analysis," Pergamon Press, Oxford, New York, 1982.
3. Pungor, E., Fehér, Z., Nagy, G., and Tóth, K., *Anal. Proc.*, 1982, **19**, 79.
4. Coşofreţ, V. V., and Buck, R. P., *Ion-Sel. Electrode Rev.*, 1984, **6**, 59.
5. Pinkerton, T. C., and Lawson, B. L., *Clin. Chem.*, 1982, **28**, 1946.
6. Kaiser, C., and Settler, P. E., *in* Wolff, M. E., *Editor*, "Burger's Medicinal Chemistry," Fourth Edition, Wiley, New York, 1981, Part III, Chapter 56.
7. Moody, G. J., and Thomas, J. D. R., *in* Freiser, H., *Editor*, "Ion Selective Electrodes in Analytical Chemistry," Plenum Press, New York, 1978, Chapter 4, pp. 287–309.
8. Martin, C. R., and Freiser, H., *Anal. Chem.*, 1980, **52**, 562.
9. Campbell, M. J. M., Demetrion, B., and Jones, R., *Analyst*, 1980, **105**, 605.
10. "United States Pharmacopeia, Twentieth Revision," US Pharmacopeial Convention Inc., Rockville, MD, 1980, pp. 142–144 and 669–670.

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Minimisation of Salicylic Acid Formation During Preparation of Aspirin Products for Analysis by High-performance Liquid Chromatography

Roger N. Bevitt, John R. Mather and Derek C. Sharman

Beecham Products Research Department, St. George's Avenue, Weybridge, Surrey, KT13 0DE, UK

The simultaneous separation of aspirin and its degradation product salicylic acid, together with caffeine and paracetamol, can be achieved by reversed-phase high-performance liquid chromatography. The accuracy of measurement of salicylic acid is reduced by conversion of aspirin into salicylic acid during sample preparation. This is a serious limitation when other components of aspirin preparations, such as carbonates, catalyse the conversion or when sample solutions are prepared in advance for use with an autoinjector. A sample solvent containing acetic anhydride, oxalic acid and acetic acid has been found to reduce hydrolysis to about 5% of that of a conventional solvent (methanol).

Keywords: *Minimisation of aspirin hydrolysis; aspirin, salicylic acid, paracetamol and caffeine analysis; high-performance liquid chromatography*

The traditionally accepted dominant degradation reaction of aspirin is the hydrolysis to salicylic and acetic acids.[1-3] Recent studies[4-7] have shown that under certain conditions a large number of other products can be formed and, according to an extensive survey of aspirin tablets,[4] levels of acetylsalicylic acid can be similar to those of salicylic acid. However, salicylic acid remains a valuable indicator of aspirin degradation in stability studies on new products or packs, and is the only compound that is specifically limited in aspirin-containing tablets in the current British Pharmacopoeia.

Reversed-phase high-performance liquid chromatography (HPLC) provides a convenient method for monitoring salicylic acid simultaneously with aspirin and other active ingredients, but conversion of aspirin into salicylic acid during sample preparation can limit the accuracy of the determination. Salicylic acid may be formed by hydrolysis in aqueous solvents or by transesterification in non-aqueous solvents.[3,8] Various other materials used in aspirin products accelerate this degradation.[3,8,9] Reported means of minimising or correcting errors from this source are injection of samples within a few minutes of preparation,[10] extrapolation of results back to time zero,[9] preparation of calibration standards with matching amounts of aspirin,[5] selection of solvents to minimise salicylic acid formation[8,11] and separation of aspirin from product ingredients that accelerate its degradation using adsorption on to and elution from chromatographic siliceous earth.[11]

The aim of this work was to establish a sample solvent that would minimise hydrolysis rates, thus allowing accurate salicylic acid levels to be obtained on formulations that promote rapid aspirin degradation in most solvents, and the use of a simple autoinjector to analyse sample solutions outside working hours.

Aspirin degradation in solution is promoted by moisture and the presence of basic materials in both aqueous and non-aqueous systems.[3,8] It appeared probable that the degradation could be minimised using a solvent containing acetic acid to exert a law of mass action effect, an acid anhydride to remove any moisture present and an excess of a relatively strong acid to give a rapid removal of basic components.

Several such systems were therefore chosen for initial trials and a mixture of acetic acid - acetic anhydride - oxalic acid gave encouraging results. An evaluation of this solvent, compared with others taken from the literature[6,10] that were compatible with a reversed-phase system, is described here.

Experimental

Apparatus

The HPLC system consisted of an Altex 110 pump, a Rheodyne 7010 injector with a 20-μl loop and a Pye Unicam LC3 variable-wavelength detector. The 250 × 4.5 mm stainless-steel column was packed with Spherisorb 10 ODS. Peak areas were determined with a Trivector Trilab II plus graphics data system.

Reagents and Chemicals

Acetylsalicylic acid (aspirin), salicylic acid, paracetamol and caffeine were of European Pharmacopoeia grade, hydrochloric acid and anhydrous oxalic acid were of reagent grade and acetonitrile and methanol were of HPLC grade. Other chemicals were of analytical-reagent grade.

Procedure

Preparation of eluent

Dissolve sodium dihydrogen orthophosphate dihydrate (31.2 g) in water (1400 ml) and add methanol (600 ml) and acetonitrile (10 ml). Adjust the pH to 3.75 ± 0.05 with concentrated hydrochloric acid. Degas before use.

Preparation of sample solvents

A. Dissolve anhydrous oxalic acid (20 g) in glacial acetic acid (*ca.* 1500 ml). Add acetic anhydride (50 ml) and make up to 2 l with glacial acetic acid. **Caution**—This solvent mixture is toxic and corrosive. Avoid breathing vapour and contact with skin. Any splashes on skin should be washed off immediately.

B. Methanol.

C. Methanol - 2.5% aqueous acetic acid (54 + 46).

Preparation of sample solutions

Blend the product by grinding briefly in a pestle and mortar. Weigh accurately into a conical flask an amount equivalent to approximately 0.25 g of aspirin and add 50 ml of sample solvent. Stopper and allow to stand with occasional shaking until the sample has dissolved or for 5 min if the product contains insoluble excipients.

Preparation of standard solutions

Calibration graphs for paracetamol, caffeine, aspirin and salicylic acid are sometimes slightly curved. Therefore, the concentrations of these compounds in the standard solutions should be chosen to correspond approximately to those in the sample solutions.

Aspirin, paracetamol and caffeine standard solutions. Weigh accurately amounts of aspirin, paracetamol and caffeine approximately equal to the amount expected in the sample into a 50-ml conical flask. Add sample solvent (50 ml) and allow the sample to dissolve. This solution must be kept stoppered. With solvent A it is stable for 24 h.

Salicylic acid standard solutions. The salicylic acid standard solution cannot be prepared with the above standard solution owing to the presence of some salicylic acid in all aspirin samples. Therefore, accurately weigh approximately 250 mg of salicylic acid into a 100-ml calibrated flask, dissolve and make up to the mark with sample solvent. Dilute with the sample solvent to give standard solutions of salicylic acid over the range found in the sample solutions.

Chromatography

If necessary filter the sample solution. Inject 20 µl and chromatograph at an eluent flow-rate of 2.0 ml min^{-1} with the detector set to 299 nm to give a maximum reponse for salicylic acid. The eluent composition may need adjusting slightly to achieve the type of separation shown in Fig. 1. Adjust the pH to resolve salicylic acid. Decreasing the pH over the range 5–2 tends to increase its separation from paracetamol but decreases its separation from aspirin. Increase the acetonitrile content to give a selective decrease in caffeine retention time.

Calculate the concentration of aspirin (or paracetamol or caffeine) in the sample by multiplying the mass in the standard solution by the ratio of the peak area of sample to standard, and express it (for the purpose of this study) as a percentage of the amount added. Calculate the concentration of salicylic acid in the same way using a standard of similar concentration and express the results as parts per million (milligrams per kilogram) of the added aspirin level.

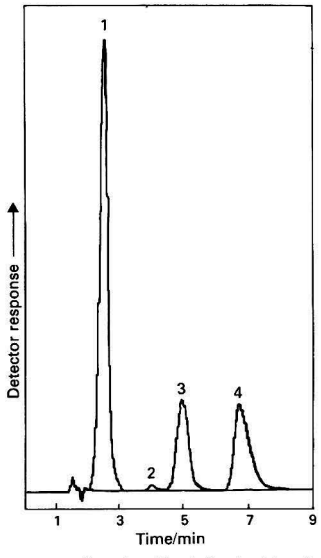

Fig. 1. Chromatogram of product No. 4 dissolved in solvent A. Peaks: 1, paracetamol; 2, salicyclic acid; 3, aspirin; and 4, caffeine

Table 1. Composition of products 1–4

Component	Concentration, % *m/m*			
	1	2	3	4
Aspirin	69.8	51.7	25.0	42.4
Caffeine	10.7	—	—	6.0
Paracetamol	—	—	—	30.3
Calcium carbonate	—	15.6	—	—
Sodium hydrogen carbonate	—	—	43.7	—
Sodium carbonate	—	—	2.8	—
Citric acid	—	5.2	25.9	—
Stearic acid	0.4	—	—	1.9
Other excipients	19.1	27.5	2.6	19.4

Results and Discussion

The reversed-phase HPLC separation has been applied with minor variations over a period of several years to the analysis of a wide variety of formulations. Common excipients and acetylsalicylsalicylic acid give no interfering peaks. To investigate the effect of the sample solvent on the rate of conversion of aspirin into salicylic acid four products were chosen. Their composition is given in Table 1.

The products were selected to cover a wide range of possible formulations. Products 1 and 2 are conventional formulations, similar to Aspirin and Caffeine Tablets BP and Dispersible Aspirin Tablets BP, respectively. Product 3 is a soluble effervescent aspirin formulation and 4 a combination product of aspirin, paracetamol and caffeine.

They were each prepared for this study by grinding an aliquot of the same blended batch of aspirin containing a measured (HPLC) level of 740 p.p.m. salicylic acid with a mix of the remaining product components just before dissolution in the sample solvent. The sample solutions were kept at 30 °C during the experiment to simulate the maximum laboratory temperature that is likely to be encountered. As has been previously observed in the reversed-phase HPLC of analgesic compounds,[12] the sample solvent composition affects the peak shapes. In this study it was noted that solvent B (methanol) in particular gave broader peaks but that resolution was still adequate and that the areas of corresponding peaks were similar with all three sample solvents. Analyses obtained after various time intervals are shown in Tables 2 and 3.

Product mixes with the aspirin omitted were dissolved in each of the solvents and chromatographed showing that any interference with the salicylic acid peak is equivalent to not more than 150 p.p.m. The results in Table 2 show the following:

(*i*) The clear superiority in minimising salicylic acid formation of the proposed solvent A over the conventional solvents, with methanol (B) being the better of the other two, but still giving hydrolysis rates about 20 times that of A.

(*ii*) That, except for the highly reactive product 3, solvent A stabilises the salicylic acid level close to that found in freshly prepared solutions of the aspirin raw material (740 p.p.m.) for at least 1 h. From this it may be predicted that for the majority of products at typical laboratory temperatures (20–25 °C), solvent A will stabilise sample solutions for several hours and permit the accurate analysis of salicylic acid to be performed out of working hours using an autoinjector, and substantially increase the throughput of samples on the HPLC.

(*iii*) That with the reactive product 3, the hydrolysis rate in solvents B and C is too great to permit accurate salicylic acid measurement, even with minimum sample preparation times.

(*iv*) That any acetylation of salicylic acid back to aspirin by solvent A is insufficient to cause a decrease in measured salicylic acid levels. The absence of appreciable acetylation by solvent A was confirmed in a separate experiment in which a solution of salicylic acid alone in solvent A was stored at room temperature for 2 d and then re-analysed. No formation of aspirin or change in the salicylic acid concentration was detected.

Table 2. Levels of aspirin and salicylic acid found at various times after the addition of solvent A, B or C

		Aspirin, % of amount added			Salicylic acid, p.p.m. with respect to added aspirin level		
Product	Time	A	B	C	A	B	C
1	5 min	99	103	105	560	950	1 360
	1 h	99	104	101	620	1 930	4 840
	24 h	105	101	87	1 190	22 300	89 700
2	5 min	99	101	106	700	1 350	3 470
	1 h	98	101	99	710	2 200	34 900
	24 h	106	100	32	1 500	29 000	519 000
3	5 min	97	95	97	690	3 510	8 860
	1 h	96	92	87	1 180	40 500	126 000
	24 h	92	21	0	28 500	610 000	819 000
4	5 min	98	102	103	640	1 880	1 730
	1 h	101	100	97	740	2 600	4 320
	24 h	102	94	89	960	32 100	95 800

Table 3. Levels of caffeine and paracetamol found at various times after the addition of solvent A, B or C

		Caffeine, % of amount added			Paracetamol, % of amount added		
Product	Time	A	B	C	A	B	C
1	5 min	106	103	105	—	—	—
	1 h	109	104	104	—	—	—
	24 h	101	103	104	—	—	—
4	5 min	105	98	101	103	100	98
	1 h	108	103	102	103	99	100
	24 h	103	100	97	101	99	103

Tables 2 and 3 together show that the HPLC system yields results close to the theoretical values for aspirin, paracetamol and caffeine in combination with any of the sample solvent systems with no degradation over the 24-h period (apart from those examples with solvents B and C where a significant proportion of the aspirin has been lost by conversion into salicylic acid).

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References

1. Florey, K., *in* Florey, K., *Editor*, "Analytical Profiles of Drug Substances," Volume 8, Academic Press, New York, 1979, p. 1
2. Connors, K. A., Amidon, G. L., and Kennon, L., "Chemical Stability of Pharmaceuticals," Wiley, New York, 1979, p. 151.
3. Kelley, C. A., *J. Pharm. Sci.*, 1970, **59**, 1053.
4. Kirchhoefer, R. D., Reepmeyer, J. C., and Juhl, W. E., *J. Pharm. Sci.*, 1980, **69**, 550.
5. Taguchi, V. Y., Cotton, M. L., Yates, C. H., and Millar, J. F., *J. Pharm. Sci.*, 1981, **70**, 64.
6. Mroso, P. V., Li Wan Po, A., and Irwin, W. J., *J. Pharm. Sci.*, 1982, **71**, 1096.
7. Reepmeyer, J. C., *J. Pharm. Sci.*, 1983, **72**, 322.
8. Bundgaard, H., *Arch. Pharm., Chem. Sci. Ed.*, 1976, **4**, 103.
9. Baum, R. G., and Cantwell, F. F., *J. Pharm. Sci.*, 1978, **67**, 1066.
10. Kirchhoefer, R. D., and Juhl, W. E., *J. Pharm. Sci.*, 1980, **69**, 548.
11. Galante, R. N., Visalli, A. J., and Grim, W. M., *J. Pharm. Sci*, 1984, **73**, 195.
12. Williams, K. J., Li Wan Po, A., and Irwin, W. J., *J. Chromatogr.*, 1980, **194**, 217.

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Identification of Monensin, Narasin, Salinomycin and Lasalocid in Pre-mixes and Feeds by Thin-layer Chromatography

Penny J. Owles*
Lilly Research Centre Limited,† Erl Wood Manor, Windlesham, Surrey, UK

A procedure is described for the qualitative identification of the four ionophores monensin, narasin, salinomycin and lasalocid in pre-mixes and feeds at levels from 3 to 100 mg kg^{-1}. These ionophores are extracted with 90% methanol, separated by thin-layer chromatography and detected either by spraying with vanillin reagent or, for low levels, by bioautography.

Keywords: *Ionophores; monensin, narasin, salinomycin and lasalocid identification; animal feeds; thin-layer chromatography; bioautography*

Monensin, narasin, salinomycin and lasalocid are all polyether, monocarboxylic acid ionophorous antibiotics.[1] A general characteristic of these compounds is that they possess unique structural properties making them capable of acting as carriers of cations across biological membranes.[2] These compounds are used prophylactically in animal husbandry as anticoccidial agents[3] and growth promoters.[4]

The increasing use of this class of compound and the introduction of narasin and salinomycin have produced a requirement for a qualitative test to recognise the presence of each ionophore and to distinguish one from another. The present methods available for monensin,[5,6] narasin and salinomycin[7] identification are based on either their reaction with an acidified vanillin reagent to yield coloured compounds or bioautography with *Bacillus subtilis* ATCC 6633 as the detecting organism.

Experiments undertaken at the Lilly Research Centre have shown these methods to be unsuitable for the purpose of identifying the four ionophores in current formulations used in the European animal feed industry. The reaction of lasalocid with vanillin is neither sufficiently sensitive nor selective, as the yellow product is frequently masked by a co-extracted feed excipient with a similar R_F value. In order to achieve adequate sensitivity for the analysis of samples at the lower inclusion levels, it is necessary to concentrate the extract. Several feeds, particularly those of northern European origin, incorporate a high level of animal fat derivatives, which has been found to interfere in the previously published methods.[5,7]

Experimental

Ionophore pre-mixes were added to a range of blank broiler and cattle rations to give samples with levels from 3 to 100 mg kg^{-1} of active ingredient. These levels of ionophores reflect those found in commercial use.

Reagents and Materials

Analytical-reagent grade reagents were used unless stated otherwise.

Monensin and Narasin standards. Analytical Development Division, Eli Lilly and Company, Indianapolis, IN, USA.

Salinomycin standard. Dr. K. Heil, Hoechst Ag, Pharma Qualitatskontrolle, Mikrobiologie, Frankfurt am Main, F.R.G.

Lasalocid standard. Roche Products Limited, Welwyn Garden City, Hertfordshire, UK.

* Present address: Lilly Industries Limited,† Kingsclere Road, Basingstoke, Hampshire, RG21 2XA, UK.
† Lilly Research Centre Limited and Lilly Industries Limited are subsidiaries of Eli Lilly & Company, Indianapolis, IN, USA.

Table 1. Sample masses

Formulation	Concentration range	Sample mass/g
Pre-mix	60–125 g kg^{-1}	1.0
	50–125 mg kg^{-1}	10.0
Final feeds ..	30–50 mg kg^{-1}	25.0
	<30 mg kg^{-1}	50.0

Vanillin reagent. 3% *m/V* vanillin in 2% *V/V* concentrated sulphuric acid in methanol.

Bacillus subtilis. ATCC 6633 spore suspension, 10^6 organisms ml^{-1}.

Bioautographic medium. Dissolve 0.69 g of dipotassium hydrogen phosphate, 0.45 g of potassium dihydrogen phosphate, 2.50 g of yeast extract, 10 g of glucose and 12 g of agar No. 3 (Oxoid) in 1 l of distilled water. Adjust the pH to 6.0 with 1 N HCl. Autoclave at 15 lb in^{-2} for 15 min.

MTT reagent. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 0.25% *m/V* in water.

Alumina. Laporte UGI 100S or Merck 1076 activity II.

Extraction

As the formulations vary in concentration, the masses are taken as indicated in Table 1.

Extract the sample with 100 ml of methanol - water (9 + 1) by shaking for 30 min. Dilute the lasalocid pre-mix extract to 10 μg ml^{-1} and the other pre-mix extracts to 100 μg ml^{-1} with methanol. The feed extracts of samples containing lasalocid or other active ingredients below 50 mg kg^{-1} are used directly. For feed samples containing no lasalocid or above 50 mg kg^{-1} of active ingredient, the following fat-removing clean-up procedure is used: shake a 5-ml extract with 750 mg of alumina for 5 min, decant off the extract, evaporate to dryness and dissolve the residue in 0.5 ml of methanol.

Standard Solutions

Prepare standard solutions in methanol at concentrations of 10 and 100 μg ml^{-1}.

Chromatography

Shake 3.0 ml of water with 100 ml of ethyl acetate until a single phase is produced and allow the solution to equilibrate in a lined developing tank for at least 30 min. *Note*: for optimum separation, use freshly prepared solvent.

Apply 10 μl of each of the reference standard solutions, the 100 μg ml^{-1} standard for plate A and the 10 μg ml^{-1} standard for plate B. Spot the sample volumes as shown in Table 2.

Table 2. Sample spotting volumes

Plate A—vanillin detection:			
Pre-mixes and feeds >50 mg kg^{-1}, excluding lasalocid formulations			
Formulation concentration . .	60–125 g kg^{-1}	50–125 mg kg^{-1}	
Spotting volume	1 µl	10 µl	
Plate B—bioautography:			
Lasalocid formulations and other formulations <50 mg kg^{-1}			
Formulation concentration . .	60–125 g kg^{-1}	30–125 mg kg^{-1}	<30 mg kg^{-1}
Spotting volume	10 µl	10 µl	20 µl

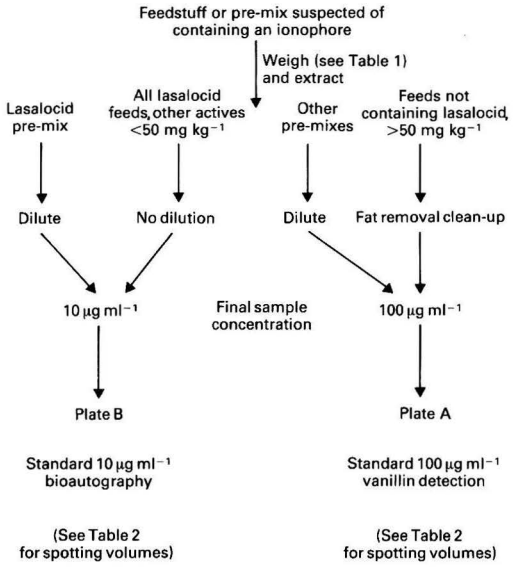

Fig. 1. Schematic diagram showing a summary of the procedure

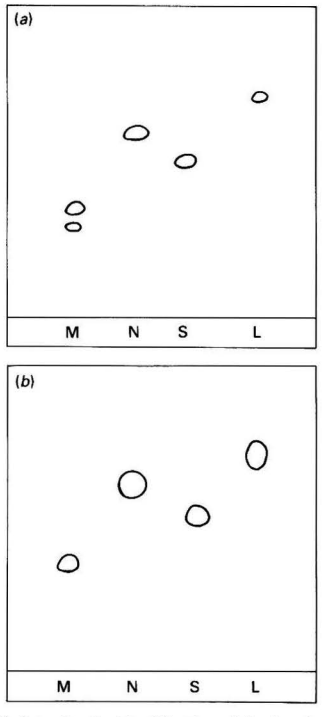

Fig. 2. TLC plates for the identification of the four ionophores: (*a*) vanillin detection; and (*b*) bioautography detection. M = Monensin; N = narasin; S = salinomycin; and L = lasalocid

Detection Methods

Plate A

Spray the dried plate with the vanillin reagent, air dry and then place the plate in an oven at 60 °C for 5–10 min. Monensin gives two pink spots and narasin and salinomycin give a single purple spot (see Fig. 2).

Plate B

Place the dried plate in a 21 × 21 cm sterile plastic bioassay plate. Spray a thin layer of uninoculated bioautography medium over the surface of the plate, ensuring the edges are well sealed, and allow it to set. Add 1% of magnesium chloride to the remaining medium, inoculate with 1% *Bacillus subtilis* ATCC 6633 spore suspension and pour 100 ml into the assay plate. Incubate overnight at 37 °C. Clear inhibition zones identify the ionophores. To enhance these inhibition zones, spray the plate with MTT reagent.

A summary of the procedure is presented in Fig. 1, and Fig. 2 shows typical plates for the identification of the four ionophores by both detection methods.

Results

No difficulty was found in identifying any of the ionophores in the feed examined.

The vanillin reagent reacts on heating with these ionophores to give colours. The two monensin factors A and B are distinguished and both give a pink colour. Narasin or salinomycin are identified by a purple spot. Lasalocid standard reacts with the vanillin to give a pale yellow colour. Some of the feed components also react with the vanillin reagent, but monensin, narasin and salinomycin are easily distinguished.

The bioautography method gives four or five easily identifiable, clear zones of inhibition that match the R_F values of the compounds detected by the vanillin reagent (monensin, 0.38; salinomycin, 0.51; narasin, 0.60; and lasalocid, 0.71). With monensin, factor B can sometimes be seen at higher loading levels.

Interferences

Other antibiotics commonly used in animal feed formulations, *e.g.*, avoparcin, bacitracin, flavomycin, tylosin and virginiamycin, were checked for potential interference on the TLC systems using bioautographic detection. The R_F values were as follows: avoparcin, 0; bacitracin, 0; flavomycin, 0; tylosin, 0; and virginiamycin, 0.17.

Discussion

The method described identifies the four ionophores in pre-mixes and feeds down to a level of 3 mg kg^{-1}. It is important that the chromatography solvent is prepared freshly because, if the ratio of water to ethyl acetate is changed, the separation is adversely affected. Other workers have used water-saturated ethyl acetate,[8] but we found more consistent results using 3% water in ethyl acetate. This is especially important with the separation of narasin and salinomycin. The temperature to which the plate is heated to react the vanillin reagent with the ionophores is crucial because, at higher temperature (100 °C), other feed components react with the vanillin reagent and can obscure the ionophores.

The use of two detection systems is required to enable lasalocid and the wide range of concentration levels to be detected. Each detection method has its advantages.

The vanillin detection method is far quicker and simpler and is selectively reactive to give characteristic colours with monensin, narasin and salinomycin. The bioautographic method is not affected by interference from feed components, whereas the vanillin may react with some of them and so mask an ionophore. This problem is exemplified with lasalocid, which, with vanillin, gives a pale yellow colour that is totally masked in some animal feed samples. A disadvantage of the vanillin procedure is that the feed samples require concentration and interfering fats must be removed as they will interfere in the chromatography. Alumina is used to remove interfering excipients but retains lasalocid completely.

Conclusions

The lowest level of detection with the vanillin method is 30 mg kg^{-1} of active ingredient for monensin, narasin and salinomycin. The bioautographic system limit, without concentration or clean-up procedures, is 3 mg kg^{-1} for the four ionophores but is not specific to these, as it will also detect other antibacterials. Therefore, the best procedure is to use the results from the two systems together to confirm the identity of the ionophore present.

I thank a number of colleagues within the Lilly organisation who have contributed to this study, especially at Lilly Research Centre: Mr. G. F. Snook, Dr. M. Spink, Mr. P. F. Washbrooke and Mr. S. A. Webb.

References

1. Westley, J. W., "Antibiotics (Polyethers)," *in* "Kirk-Othmer Encyclopaedia of Chemical Technology," Third Edition, Volume 3, 1978, pp. 47–64.
2. Pressman, B. C., *Annu. Rev. Biochem.*, 1976, **45**, 501.
3. Shumard, R. F., and Callender, M. E., "Proceedings of the Annual Conference on Antimicrobial Agents and Chemotherapy, 1967," 1968, pp. 369–407.
4. Raun, A. P., Colley, C. O., Potter, E. L., Rathmore, R. P., and Richardson, L. F., *J. Anim. Sci.*, 1976, **43**, 670.
5. Haney, M. E., and Hoehn, M. M., "Proceeding of the Annual Conference on Antimicrobiol Agents and Chemotherapy, 1967," 1968, pp. 349–352.
6. Donoho, A. L., and Kline, C. M., "Proceedings of the Annual Conference on Antimicrobiol Agents and Chemotherapy, 1967," 1968, pp. 736–766.
7. Berg, D. H., and Hamill, R. L., *J. Antibiot.*, 1978, **31**, 1.
8. Goodey, R., *et al.*, personal communication.

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Determination of α-Chloralose Residues in Vertebrate Tissues by Gas - Liquid Chromatography

Edward M. Odam, Hilary P. Wardall and Sidney Bailey
Agricultural Science Service, Tolworth Laboratory, Ministry of Agriculture, Fisheries and Food, Hook Rise South, Tolworth, Surrey, KT6 7NF, UK

and Elizabeth Findlay
Department of Agriculture and Fisheries for Scotland, Agricultural Science Service, East Craigs, Edinburgh, EH12 8NJ, UK

A method is described for the analysis of residues of the narcotic α-chloralose in vertebrate tissues. Following solvent extraction and gel permeation clean-up, residues are converted into the trimethylsilyl (TMS) derivative for the analysis by gas - liquid chromatography. Recoveries are greater than 65% and the limit of determination is between 0.2 and 1.0 mg kg^{-1}, depending on the type of tissue analysed. Methods for the confirmation of α-chloralose residues using thin-layer chromatography of underivatised α-chloralose or mass spectrometry of the chloralose - TMS derivative are described.

Keywords: *Chloralose determination; solvent extraction; gas - liquid chromatography; vertebrate tissue analysis*

Chloralose is the British Standards Institution common name for α-chloralose [*R*-1,2-*O*-(2,2,2-trichloroethylidene)-α-D-glucofuranose], also known as glucochloralose. It has two approved uses in pest control, either as a rodenticide or as a narcotic agent used under licence to control avian pests. In order to determine whether α-chloralose can be implicated in the death of non-target species, a reliable and sensitive method for detecting α-chloralose in vertebrate tissues is necessary.

Despite the extensive use of α-chloralose as an anaesthetic in veterinary practice, little work has been undertaken on the analysis of residues in body tissues and the existing methods are generally suitable only for the analysis of blood and urine. The methods of Cheramy,[1] Mascre *et al.*,[2] Mirjolet *et al.*,[3] Fauran *et al.*[4] and Lespagnol *et al.*[5] were found unsuitable for the analysis of tissues, from the point of view of both recovery and sensitivity. In seeking a suitable procedure, a modification of the Sweeley *et al.*[6] derivatisation technique was used, in which chloralose was converted into the trimethylsilyl compound for its determination by gas - liquid chromatography (GLC) using an electron-capture detector.[7] This derivatisation has also been used for the determination of α-chloralose in rodenticide formulations by Theobald[8] utilising a flame-ionisation detector. Daenens[9] also used the trimethylsilyl derivative to assay α-chloralose in biological samples using GLC with flame-ionisation detection. In this instance, a Florisil clean-up procedure was used.

Results obtained using the method of Sweeley *et al.*[6] with the pure compound were satisfactory but it was necessary to develop a method for removing substances from animal tissue extracts prior to derivatisation, which interfered with the chromatography. Originally, paper chromatographic separation was used, but recoveries of α-chloralose were inconsistent. Thin-layer chromatography using silica-gel plates gave some improvement, as reported by Knapp and Russel,[10] but column chromatography using Sephadex LH-20 proved to be a more efficient clean-up procedure. The method in regular use in this laboratory utilises a small disposable gel permeation column, although the size of the column may be scaled up to allow larger sample sizes to be analysed.

As one of the major uses of α-chloralose is as a narcotic for pigeon control, the method was applied to the analysis of residues in tissues from pigeons orally dosed with α-chloralose.

Experimental

Reagents

α-Chloralose. BDH Chemicals Ltd.
Methanol. HPLC grade. Rathburn Chemicals (Walkerburn) Ltd.
Sephadex LH-20. Pharmacia (GB) Ltd.
Tri-Sil in pyridine. Pierce & Warriner (UK) Ltd.
2-Phenoxyethanol. AnalaR grade. BDH Chemicals Ltd.
Acetone. AnalaR grade. BDH Chemicals Ltd.
Silver nitrate. AnalaR grade. BDH Chemicals Ltd.
Chloroform. AnalaR grade. BDH Chemicals Ltd.

Apparatus

Homogeniser. Ultra-Turrax Model K45, capable of macerating 1–5 g of tissue in 10–50 ml of solvent.
Rotary evaporator. Corning Model 349/2.
Vacuum filtration equipment. Buchner funnel (Hartley type) (10 cm) connected to a 150-ml round-bottomed flask via a vertical receiver adaptor fitted with a vacuum connection (Quickfit & Quartz RA 13/23).
Clean-up column. All-glass syringe barrel (10 ml) (80 mm × 15 mm i.d.) with central outlet hole and fitted with a sinter.
Syringes. With capacities of 1 and 100 μl [Scientific Glass Engineering (UK) Ltd.]
Dry glove box. Laboratory made.
Graduated test-tubes. Capacity 10 ml with joint and stopper.
Thin-layer chromatography plates. Silica gel G pre-coated, with a layer thickness of 250 μm (Anachem Ltd.).
UV lamp. Wavelength setting, 254 nm.
Gas - liquid chromatography. Pye Unicam 104 chromatograph, fitted with a 10-mCi ^{63}Ni electron-capture detector.

Procedures

Preparation and calibration of Sephadex columns

Using a mass of 1.5 g of dry gel per sample, place sufficient Sephadex LH-20 in a beaker with an excess of methanol and allow the gel to swell for at least 2 h.

Take the barrel of an all-glass syringe (10 ml) and place a sinter at the bottom. Pour in swollen Sephadex to obtain a settled gel bed of 50 mm height. Wash the Sephadex column with methanol (20 ml) before use and allow the level of methanol to fall to 2 mm above the Sephadex bed before introducing a sample.

To calibrate the Sephadex column, introduce 0.5 ml of 1 mg ml^{-1} standard α-chloralose in methanol on to one column from the batch and allow this to permeate into the gel before adding further methanol. Use a fraction collector to collect the eluate as 20 1-ml fractions. Reduce each fraction to 0.1 ml under N_2 and load on to a silica gel thin-layer chromatographic plate (250-μm thick). Do not run the plate, but spray with the silver nitrate reagent as described under *Confirmation by thin-layer chromatography*. Place under an ultraviolet lamp at 254 nm and allow the spots to develop. It is normal to find most of the α-chloralose contained in fractions 8–13. Once the system has been calibrated, only occasional checks of the Sephadex columns are required.

Extraction

Weigh accurately up to 5 g of tissue into a macerating tube and homogenise with 25 ml of methanol. Filter the extract under vacuum through a Hartley funnel containing a Whatman No. 1 filter-paper that has previously been washed with solvent. Retain the extract in the collecting vessel. Return the solid fraction to the macerating tube and homogenise again with 25 ml of methanol. Filter through the same apparatus, combining the two solvent extracts. Reduce the volume of the filtrate slightly using a rotary evaporator before quantitative transfer into a 50-ml calibrated flask. Dilute to volume with methanol.

Clean-up

Take 1 ml of extract and place in a pre-weighed vial. Evaporate to dryness under nitrogen. Weigh and calculate the amount of extracted material in the total extract. To avoid overloading the Sephadex column, take an aliquot of the extract as described under *Extraction* containing less than 40 mg of extracted material and place in a tapered graduated tube. Evaporate under nitrogen until the volume is reduced to approximately 0.5 ml. Transfer the concentrated sample on to the prepared Sephadex column and commence collection of the eluate in a graduated receiver. Wash the sample tube twice with 2 ml of methanol and add the washings carefully to the column. Add methanol to the column as required ensuring that the gel is covered with methanol at all times. Collect a volume of eluate in a graduated receiver according to the calibration above. Change the collector to a graduated test-tube at the appropriate stage to collect the α-chloralose in the sample. Discard the first fraction as this contains most of the unwanted co-extracted materials. Evaporate the fraction containing α-chloralose to dryness under a stream of nitrogen. The sample is now ready for derivatisation.

The Sephadex columns can be used up to three times, provided that they are washed with at least 20 ml of methanol between samples, although, it has been our practice to discard the Sephadex after each sample to eliminate any risk of cross-contamination.

Derivatisation

Place the dried, cleaned-up extracts into a dry glove-box containing silica gel. The box should also contain stoppers for the graduated test-tubes, sufficient ampoules of Tri-Sil for derivatisation, a 100-μl syringe and a set of graduated test-tubes containing 200–2000 ng of α-chloralose. Leave overnight to ensure complete dryness. It is essential to ensure that the sample is dry before adding the silylating agent or incomplete derivatisation will occur.

Open an ampoule and add 100 μl of Tri-Sil to each tube and stopper firmly before removing from the dry box. Seal the stoppers with adhesive tape and heat at 80 °C in a water-bath for 10 min, agitating the tubes occasionally.

Add methanol to all of the graduated test-tubes to give a volume of 10 ml.

Gas - liquid chromatographic determination

Inject 1 μl of solution on to the chromatograph fitted with a 1.5-m glass column (4 mm i.d.) packed with 100–120-mesh Supelcoport coated with a 3% *m/m* SP2401 and 3% *m/m* SP2100 mixed stationary phase. Maintain the column at a temperature of 180 °C with the carrier gas (oxygen-free nitrogen) having a flow-rate of 60 ml min^{-1}. The chromatograph is operated with an injector temperature of 210 °C, a detector temperature of 300 °C, the electron-capture supply with a pulse space of 150 μs and an amplifier attenuation setting of ×200. Under these conditions, the α-chloralose-TMS derivative has a retention time of 7.5 min.

Confirmation by thin-layer chromatography (TLC)

Take a 20 × 20 cm silica-gel thin-layer chromatographic plate and activate it by heating for 2 h at 100 °C. As the α-chloralose is confirmed in the underivatised form, a second aliquot from the original methanol extract is cleaned up using Sephadex LH-20.

Concentrate the eluate and, if possible, apply to the plate an amount containing 5–10 μg of α-chloralose. A range of standards (0.5–10 μg) should also be loaded on to the plate for a semi-quantitative appraisal.

Equilibrate a chromatography tank (lined with paper) with 100 ml of 10% methanol in chloroform (AnalaR grade) prior to the development of the plate. The solvent takes between 45 and 60 min to travel approximately 17 cm, at which point the plate is removed and allowed to air dry.

Prepare the spray by dissolving 0.4 g of silver nitrate in 1 ml of distilled water. Add 1.5 ml of 2-phenoxyethanol and dilute to 50 ml with acetone. This solution must be freshly prepared and kept in the dark even for the short periods of time between sprays. When the plate has been lightly sprayed place under a UV lamp at 254 nm for approximately 45 min to develop. The presence of α-chloralose is shown by black spots at an R_F of 0.31.

Confirmation of silylation by mass spectrometry

The α-chloralose-TMS standard was examined by GC - mass spectrometry using a Kratos MS30 instrument coupled to a DS50 data system. The mass spectrometer was interfaced to the gas chromatograph with an all-glass jet separator and ionisation was produced by electron impact. Spectra were obtained at operating voltages of 20 and 70 eV at a source temperature of 200 °C. The spectra were obtained from the DS50 data system with a magnification of ×20, but an increase in magnification to ×100 was necessary to observe the ions with high *m/e* values owing to their weak intensities. The mass spectrum showed that the three free hydroxy groups of α-chloralose had been derivatised (Fig. 1). This technique can also be applied to confirming the presence of α-chloralose in tissue samples.

Results

The reproducibility of derivative formation was found to be good, as standards derivatised on different days yielded responses on the gas chromatograph within 5% of each other. Care, however, must be taken to ensure the stability of the α-chloralose-TMS. Compared with a stable external standard of DDE, the α-chloralose derivative was found to be stable for at least one week, provided that it was stored at 4 °C in a refrigerator. However, when left in strong sunlight, or in a warm environment overnight, the chromatographic response decreased significantly. The linear GLC response to α-chloralose-TMS was obtained using α-chloralose standards in the range 20–640 mg ml^{-1} and a calibration graph was prepared, although the normal working range of the standard was 20–200 ng ml^{-1}.

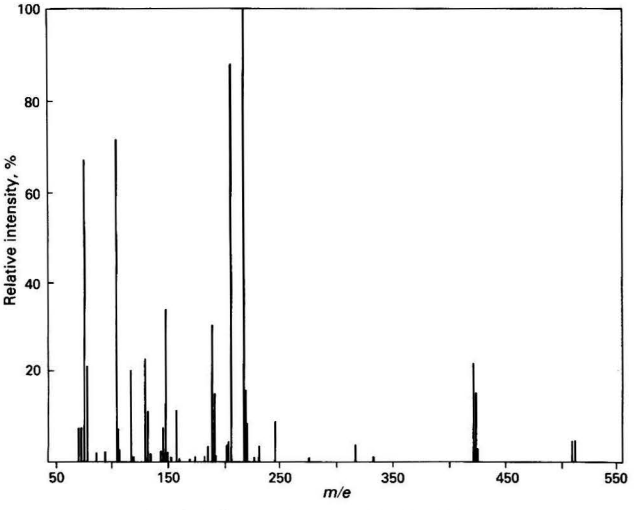

Fig. 1. Mass spectrum of α-chloralose-TMS

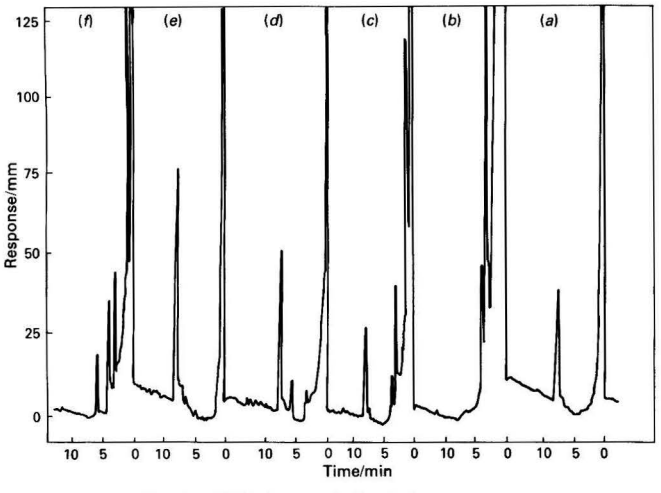

Fig. 2. Typical gas chromatogram of α-chloralose-TMS for standard solutions and fortified tissue extracts: (*a*) α-chloralose-TMS, 80 pg; (*b*) control pigeon muscle; (*c*) pigeon muscle fortified with α-chloralose; (*d*) fortified pigeon kidney; (*e*) α-chloralose-TMS, 160 pg; and (*f*) control pigeon kidney

The recovery of standard α-chloralose following the clean-up and derivatisation procedures was 80–85%. To ascertain the recovery of α-chloralose from tissues, samples of liver, kidney, muscle and brain from pigeons (*Columba livia*) were spiked at 0.2, 1.0 and 10.0 mg kg^{-1}. Tissues were injected with solutions of α-chloralose in methanol and were stored at 4 °C for 16 h prior to analysis. The results are shown in Table 1 and typical chromatograms are shown in Fig. 2.

The method of analysis has been applied to tissues from pigeons that were fed α-chloralose. Six pigeons were orally dosed with gelatine capsules containing α-chloralose, three at 130 mg kg^{-1} body mass and three at 260 mg kg^{-1} body mass. At the lower dose one pigeon died approximately 5 h after dosing and the other two recovered after being comatose for 24 h. These were killed by cervical dislocation 2 d after administration of α-chloralose. Two of the pigeons dosed at the higher level died after approximately 2.5 h and the other recovered after 3 d and was killed 2 d later. The muscle,

Table 1. Recovery of α-chloralose from spiked pigeon tissues

	α-Chloralose added		α-Chloralose recovered	
Tissue	mg kg^{-1}	μg	μg	%
Kidney	0.2	0.36	0.29	81
	1.0	1.8	1.75	97
	10.0	18.0	13.50	75
Muscle	0.2	1.0	0.7	70
	1.0	5.0	4.5	90
	10.0	50.0	42.5	85
Brain	0.2	0.4	0.28	69
	1.0	20.0	14.60	73
Liver	0.2	0.98	*	*
	1.0	4.80	2.46	72
	10.0	48.0	36.48	76

* The limit of determination for pigeon liver is greater than 0.2 mg kg^{-1} because of interfering peaks.

kidney, liver and brain were analysed and the results, not corrected for recoveries, are shown in Table 2.

Table 2. Tissue residues from pigeons fed with α-chloralose

Pigeon	Dose/mg	Remarks	α-Chloralose residues found/mg kg^{-1} Muscle	Liver	Brain	Kidney
1	43.5	Died	33.9	36.9	44.1	75.0
2	39.5	Recovered	N.d.*	N.d.	N.d.	N.d.
3	42.6	Recovered	N.d.	N.d.	N.d.	N.d.
4	91.4	Died	36.1	40.0	21.8	76.7
5	82.6	Recovered	N.d.	N.d.	N.d.	N.d.
6	73.8	Died	35.7	31.3	20.0	82.0

* N.d. = none detected; limit of determination = 0.2 mg kg^{-1}, except liver = 1.0 mg kg^{-1}.

Discussion

Recoveries from samples fortified with α-chloralose indicate that this analytical method is suitable for all of the four tissues studied. The limit of determination in the brain, kidney and muscle is 0.2 mg kg^{-1} but in the liver, because of interference from co-extracted material, the limit is 1 mg kg^{-1}.

The experimental data (Table 2) showed that pigeons that had died contained residue levels of α-chloralose well above the limits of determination for this method. Hence this is a convenient method of analysis as it is quick, reliable and an individual column clean-up can be used per sample, eliminating the possibility of cross-contamination. Residue levels greater than 2 mg kg^{-1} can be confirmed by the TLC method described.

The method of analysis has been successfully used for several years in these laboratories to investigate the involvement of α-chloralose in cases of poisoning in mammals and birds and the results have been discussed elsewhere.[11,12]

References

1. Cheramy, P., *J. Pharm. Chim.*, 1940, **9**, 233.
2. Mascre, M., Maillard, G., and Loiscan, J., *Bull. Sci. Pharmacol.*, 1940, **47**, 281.
3. Mirjolet, M., Semars, M., de Ren, G., and Petiet, G., *Bull. Soc. Pharm. Nancy*, 1969, **83**, 7.
4. Fauran, F., Pelissier, S., Fabre, S., and Gras, G., *Prod. Probl. Pharm.*, 1970, **25**, 881.
5. Lespagnol, A., Paris, J., and Merville, R., *Bull. Soc. Chim. Biol.*, 1942, **24**, 117.
6. Sweeley, C. C., Bentley, R., Makita, M., and Wells, W. W., *J. Am. Chem. Soc.*, 1963, **85**, 2497.
7. Ministry of Agriculture, Fisheries and Food, "Pest Infestation Control 1968–70," HM Stationery Office, London, 1973, p. 47.
8. Theobald, J. J., *J. Chromatogr.*, 1976, **129**, 444.
9. Daenens, P., Bruneel, N., and Van Boven, M., *in* Kovatsis, A., *Editor*, "Toxicological Aspects. Proceedings of the 9th International Congress of the European Association of Poison Control Centres," Michalopoulas, Salonika, 1980, p. 204.
10. Knapp, M., and Russel, H. A., *Dtsch. Tieraerztl. Wochenschr.*, 1973, **80**, 573.
11. Hamilton, G. A., Ruthven, A. D., Findlay, E., Hunter, K., and Lindsay, D. A., *Biol. Conserv.*, 1981, **21**, 315.
12. Brown, P. M., Bunyan, P. J., and Stanley, P. I., *J. Forensic Sci. Soc.*, 1977, **17**, 211.

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Application of Gas - Liquid Chromatography to the Analysis of Essential Oils

Part X.* Fingerprinting of Specified Essential Oils by Standardised Procedures

Analytical Methods Committee†
Royal Society of Chemistry, Burlington House, Piccadilly, London, W1V 0BN, UK

This introductory paper reports the background to a collaborative study of the GLC of various authentic essential oils. The GLC fingerprints of the essential oils will appear as individual monographs in subsequent papers.

Keywords: *Essential oils; gas - liquid chromatography; fingerprints; collaborative study*

The Analytical Methods Committee has received and approved for publication the following report from its Essential Oils Sub-Committee.

Report

The constitution of the Sub-Committee responsible for the preparation of this Report was Mr. A. M. Humphrey (Chairman), Mr. D. J. Bevis, Mr. E. Cummings, Dr. D. Farley, Mr. D. M. Forshaw, Mr. J. R. Harris, Mr. W. S. Matthews, Miss D. M. Michalkiewicz, Mr. M. Milchard, Mr. D. A. Moyler, Mr. A. Osbiston, Mr. J. Ridlington and Mr. D. Silvester, with Mr. J. J. Wilson as Secretary.

Introduction

The provenance and quality of an essential oil is often established using a range of analytical techniques varying from physico-chemical methods, through non-specific chemical methods to chromatographic methods. The physico-chemical and non-specific chemical characteristics are defined in a range of National and International Standards and the methods used to determine them have been studied extensively in the past by this Sub-Committee. These methods will be published in the Third Edition of the "Official, Standardised and Recommended Methods of Analysis" (1985). Although essential oils are, by their nature, ideally suited to analysis by gas - liquid chromatography, the publication of reliable methods in the past has been confined to the quantitative determination of specific components only.

Following the publication of Parts VII[1] and VIII[2] in this series, giving recommended procedures for the fingerprinting of essential oils using temperature-programmed gas - liquid chromatography with packed columns, the Sub-Committee has undertaken the collaborative examination of a number of authentic essential oils with a view to publication of their fingerprints.

Having overcome the problems of defining the GLC procedures in such a way as to make them reproducible, there are also the difficulties of locating samples of essential oils for examination which are at the same time authentic and typical. In turn, it then becomes necessary to phrase a suitably precise definition for them. Whilst it is clear that any definition should minimise the inclusion of oils of a fraudulent character, it should be realistic and not specifically exclude oils that have been made commercially desirable by a simple fractionation. It must include details of the complete method of preparation and the provenance and description of the vegetable material used.

All true essential oils are secondary metabolites of plant products and in some instances the oil produced in one part of a plant is different from that produced in another. In some instances the oils are stored in specialised oil cells, whilst in others they are not specifically located. Nor are the essential oils the only secondary metabolites that may be present and the loose definition of the volatile part is usually applied. This character of their volatility forms the basis of their isolation from vegetable material by some forms of water or steam distillation processes, although for those oils which occur in specialised oil cells, such as in the pericarp of citrus fruits, they may be isolated by cold expression. In these instances, the oils are of different characters to those produced by distillation and are often considered superior.

In addition to these processes, it is now becoming clear that the appropriate application of fluid-phase extractions with compressed sub- or super-critical gases can also yield volatile oils that are substantially free from the non-volatile components normally associated with extractions by conventional organic solvents. Such oils may subsequently form the subjects of investigations by the Sub-Committee.

In the commercial isolation of essentail oils, many of their constituents may undergo chemical changes. These may occur during the storage or preparation of the raw plant material, the isolation procedure itself or the subsequent storage of the finished product. The changes may include polymerisation, oxidation, hydrolysis or thermal decomposition. Further, essential oils are not totally immiscible with water and some of the more highly oxygenated and polar components may have considerable solubilities in water, thereby giving the potential for changes in composition resulting from partition fractionation.

It can therefore be seen that essential oils of nominally similar descriptions may have different compositions, but whose character may be determined by analyses. It is the intention that the publication of standardised and collaboratively agreed GLC fingerprint tracings for a range of authentic essential oils produced under defined conditions should form a series of valuable analytical references.

Following this preamble, the Sub-Committee proposes that the following definition should be applied:

> "Essential oils are the substantially volatile, water-immiscible portions of the secondary metabolites of plant products. They are normally prepared either by water or steam distillation processes from specified parts of plants of defined origin and species, or by cold expression from the pericarp of citrus fruits, where the essential oil will contain proportions of non-volatile products. The natural composition of essential oils may vary slightly from sample to sample

* For Part IX of this series, see *Analyst*, 1981, **106**, 456.

† Correspondence should be addressed to the Secretary, Analytical Methods Committee, Analytical Division, Royal Society of Chemistry, Burlington House, Piccadilly, London, W1V 0BN, UK.

and, in addition, some essential oils may have been subjected to a simple fractionation or purification procedure, such as distillation, crystallisation or washing. Details of such additional processing must be included in the descriptive title of the essential oil."

Experimental

For the purpose of the present studies, only those oils which have been obtained under the personal supervision of a Sub-Committee member and have not been subjected to further treatment following the primary production were chosen for use.

Samples of different oils were distributed to all members of the Sub-Committee with instructions to prepare standardised fingerprint chromatograms for each using polyethylene glycol 20M and methyl polysiloxane stationary phases, according to the methods described in Parts VII and VIII.[1,2] Each member was asked to return the chromatograms for the oils on each of the columns, together with the completed column operating check sheets, giving details of the analytical conditions. The check sheets were studied to ascertain the absence of anomalies and the chromatograms reduced photographically to a standard size for comparison purposes.

The identification of the components of the oils and their relative peak area percentages was carried out in a separate exercise using a coupled GC - MS system equipped with support-coated open-tubular (SCOT) capillary columns. The transfer of information from this exercise to the agreed standardised chromatograms using packed columns was achieved by inspection.

The usual analytical tests were applied as appropriate to each oil to establish its conformity with published standards.

Results

Results for individual essential oils will be published as separate monographs in subsequent papers in this series. The published fingerprint tracings are representative of those obtained by the individual Sub-Committee members using the two different stationary phases. Each chromatogram will be presented with simultaneous tracings at two sensitivities, adjusted in such a way that an equivalent volume of sample containing 5% hexadecane gives approximately 100% and 10% of full-scale deflections in the two instances. The identification of the major peaks and positions of the reference *n*-alkanes will also be shown.

These compounds will further be tabulated for the two stationary phases, giving their identities, relative retention indices with reference to the appropriate *n*-alkanes and quantitative presence in the oils as determined by the method of peak-area normalisation and without the application of response factor corrections. Relative retention indices determined on poorly resolved peaks are less reliable and will be marked with asterisks.

A summary of the collaboratively determined column operating parameters from six different laboratories is shown in Table 1.

Discussion

The work of the Sub-Committee to date has shown that reproducible fingerprint tracings can be obtained using packed columns, but in contrast, the reproducibility using capillary columns is less satisfactory. Although the greatly increased resolving power of capillary columns can be used in conjunction with mass spectrometry to identify a large number of components that would otherwise remain unresolved on packed columns, the Sub-Committee is of the opinion that reproducibility should not be sacrificed thereby. Accordingly, the indentity and quantification of the peaks to be shown in the published chromatograms were obtained by careful reference to analyses on SCOT columns and it has thus been possible in some instances to identify the individual components of compound peaks shown in the packed column fingerprints. The relative retention indices, however, were measured directly from the packed column fingerprints and it will be found that these values are subject to small variations in different oils owing to experimental error, but all should fall within the limits given in Parts VII and VIII[1,2] in respect of the test mixture components. It must be noted that relative retention indices are temperature dependent and may be compared only under reproducible conditions of elution. Hence it is not possible to compare relative retention indices, determined on a packed column, with relative retention indices determined on a capillary column with the same stationary phase, as the elution temperatures of the components will be different.

The quantitative determinations of the components were made by peak-area normalisation and are subject to the errors normally associated with the technique, *e.g.*, no corrections made for relative response factors or for the presence of non-volatile or degradable components. The figures are given as a guide only and have only been applied to peaks that have been positively identified. Thus, the sum total of the percentages given may not reach 100%. Further, the figures given for any one compound on the two stationary phases may also differ owing to experimental error and some compounds identified on one stationary phase may not have been identified on the other.

Table 1. Average column operating parameters

Column	Properties	Compound	Relative retention index
Methyl polysiloxane	*g*-pack = 1.003 ± 0.0014	Limonene	1027 ± 0.8
	RRI (275 °C) = 2394 ± 9	Acetophenone	1041 ± 1.7
	Resolution > 95%	Linalol	1085 ± 1.8
	Programme time = 100.4 ± 1.3 min	Naphthalene	1172 ± 2.3
		Linalyl acetate	1242 ± 1.9
		Cinnamyl alcohol	1279 ± 2.4
Polyethylene glycol 20M . .	*g*-pack = 1.276 ± 0.0014	Limonene	1212 ± 2.2
	RRI (225 °C) = 2399 ± 6	Linalol	1540 ± 2.9
	Resolution > 95%	Linalyl acetate	1559 ± 2.3
	Programme time = 75.6 ± 0.9 min	Acetophenone	1652 ± 3.1
		Naphthalene	1746 ± 5.3
		Cinnamyl alcohol	2260 ± 8.2

Each monograph to be presented in later papers will include a brief description of its derivation and general characteristics, the two chromatograms with peak identities, tables of the relative retention indices and approximate concentrations of identified components and a table of published standards.

In the tables giving details of published standards, these are drawn from International Standards, British Standards, Essential Oil Association of America Standards and British Pharmacopoeia Standards. The actual figures obtained for the oils used for the collaborative fingerprinting studies are listed under AMC/EO sample.

References

1. Analytical Methods Committee, *Analyst*, 1980, **105**, 262.
2. Analytical Methods Committee, *Analyst*, 1981, **106**, 448.

NOTE—References 1 and 2 are to Parts VII and VIII of this series, respectively.

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Application of Gas - Liquid Chromatography to the Analysis of Essential Oils

Part XI.* Monographs for Seven Essential Oils

Analytical Methods Committee†
Royal Society of Chemistry, Burlington House, Piccadilly, London, W1V 0BN, UK

Fingerprint tracings for essential oils of citronella (Sri Lanka), clove bud (Malagasy Republic), juniper berry (Hungary), lemon (Sicily), lime (Cuba), nutmeg (East Indies) and black pepper (Sarawak) are presented.

Keywords: *Essential oils; gas - liquid chromatography; fingerprints; collaborative study*

The Analytical Methods Committee has received and approved for publication the following report from its Essential Oils Sub-Committee.

Report

The constitution of the Sub-Committee responsible for the preparation of this Report was: Mr. A. M. Humphrey (Chairman), Mr. D. J. Bevis, Mr. E. Cummings, Dr. D. Farley, Mr. D. M. Forshaw, Mr. J. R. Harris, Mr. W. S. Matthews, Miss D. M. Michalkiewicz, Mr. M. Milchard, Mr. D. A. Moyler, Mr. A. Osbiston, Mr. J. Ridlington and Mr. D. Silvester, with Mr. J. J. Wilson as Secretary.

Introduction

In accordance with the programme outlined in Part X,[1] GLC fingerprint chromatograms of seven authentic essential oils, namely oils of citronella, clove bud, juniper berry, lemon expressed, lime distilled, nutmeg East Indian and black pepper, were obtained in a collaborative study and are presented here.

Experimental

Samples of seven different oils were distributed to all members of the Sub-Committee with instructions to prepare standardised fingerprint chromatograms for each using polyethylene glycol 20M and methyl polysiloxane stationary phases, according to the methods described in Parts VII[2] and VIII.[3] The seven oils were citronella, clove bud, juniper berry, lemon expressed, lime distilled, nutmeg East Indian and black pepper. Each member was asked to return the chromatograms for the oils on each of the columns, together with the completed column operating check sheets, giving details of the analytical conditions. The check sheets were studied to ascertain the absence of anomalies and the chromatograms reduced photographically to a standard size for comparison purposes.

The identification of the components of the oils and their relative peak-area percentages was carried out in a separate exercise using a coupled GC - MS system equipped with support-coated open-tubular (SCOT) capillary columns. The transfer of information from this exercise to the agreed standardised chromatograms using packed columns was achieved by inspection.

The usual analytical tests were applied as appropriate to each oil to establish its conformity with published standards.

Results

The results for each essential oil are presented in the form of monographs, each including a brief description of its derivation and general characteristics, the two chromatograms with peak identities, tables of the relative retention indices and approximate concentrations of identified components and a table of published standards. Where any essential oil chromatogram was found to have finished before the elution of the C_{24} alkane, it has been shortened in order to avoid publication of a blank trace.

The published fingerprint tracings are representative of those obtained by the individual Sub-Committee members using the two different stationary phases. Each chromatogram is presented with simultaneous tracings at two sensitivities, adjusted in such a way that an equivalent volume of sample containing 5% hexadecane gives approximately 100% and 10% of full-scale deflections in the two instances. The identifications of the major peaks and positions of the reference *n*-alkanes are also shown.

The identifications of the peaks are mostly given by their accepted trivial names, although some examples exhibit mixtures of nomenclatures, but which nevertheless conform to common practice, *e.g.*, 1-terpinen-4-ol is reduced to terpinen-4-ol.

These compounds are further tabulated for the two stationary phases giving their identities, relative retention indices (RRI) and quantitative presence in the oils as determined by the method of peak-area normalisation (RPA) and without the application of response factor corrections. Relative retention indices determined on poorly resolved peaks are less reliable and are marked with asterisks.

Tables 1 and 2 list the determined relative retention indices for all the compounds identified in all seven essential oils using the two different columns. As these figures were determined from the agreed chromatogram from one laboratory, a comparative set of relative retention indices determined by all the collaborating laboratories in respect of selected compounds is shown in Table 3. This table includes some compounds that have been found in only one of the oils, as well as some other compounds which have been more widely found.

* For Part X, see p. 1339.

† Correspondence should be addressed to the Secretary, Analytical Methods Committee, Analytical Division, Royal Society of Chemistry, Piccadilly, London, W1V 0BN, UK.

Table 1. Relative retention indices on methyl polysiloxane stationary phase

Peak identity	Citronella	Clove	Juniper	Lemon	Lime	Nutmeg	Pepper
α-Thujene			919	917		921	916
α-Pinene	930		930	928	929	931	930
Camphene	944		944	945	942	948	944
Sabinene	968		965	966		971	
β-Pinene				976	973	979	975
Myrcene	981		979		978		
α-Phellandrene	1004		1000			1000	
Δ^3-Carene							1009
α-Terpinene			1014	1016	1007	1013	
p-Cymene	1017		1014	1016	1014		
Limonene	1026		1026	1032	1029	1028	1025
β-Phellandrene			1026				
cis-Ocimene	1039						
γ-Terpinene			1051	1055	1052	1052	1050
2-*p*-Tolylpropene			1077				
Terpinolene	1084		1081	1083	1081	1083	1082
Fenchol					1104		
Terpinen-1-ol					1122		
cis-β-Terpineol					1130		
Citronellal	1133			1131			
trans-β-Terpineol					1148		
Borneol	1157				1157		
Terpinen-4-ol	1168		1165		1165	1170	1169
α-Terpineol	1178		1176	1176	1178	1180	1179
γ-Terpineol					1184		
Citronellol	1210						
Neral				1217			
Nerol				1217			
Geranial				1245			
Geraniol	1237			1245			
Safrole						1269	
Geranyl formate	1277						
Bornyl acetate	1277		1278				
4-Pentylanisole						1290	
Chavicol		1294					
Terpinyl acetate	1316						
Citronellyl acetate	1336						
Eugenol		1341				1335	
Neryl acetate				1341			
δ-Elemene							1344
α-Cubebene			1356				1359
Geranyl acetate	1360			1359			
α-Copaene		1388	1384			1382	1385
β-Elemene	1393		1391				1394
Isoeugenol						1423	
β-Caryophyllene	1427	1431	1429	1431	1430		1430
α-Bergamotene	1439			1439	1439	1436	
α-Humulene		1466	1461				1462
trans-Methylisoeugenol	1465						
Eugenyl acetate		1492					
Myristicin						1497	
trans, trans-α-Farnesene					1498		
β-Bisabolene				1508	1508		
δ-Cadinene	1521	1525	1523			1521	1525
Elemol	1540						
Caryophyllene alcohol		1559					
Caryophyllene oxide	1576	1577					

Table 2. Relative retention indices on polyethylene glycol 20M stationary phase

Peak identity	Citronella	Clove	Juniper	Lemon	Lime	Nutmeg	Pepper
Tricyclene	1016						
α-Pinene	1027		1035	1023	1026	1033	1027
α-Thujene			1035				
Camphene	1066		1076	1068	1063	1072	1068
β-Pinene			1114	1111	1113	1117	1110
Sabinene	1118		1124	1123		1122	
Δ^3-Carene						1155	1156
Myrcene	1159		1165	1154	1147	1155	
α-Phellandrene						1172	1173
α-Terpinene			1195	1188	1174	1189	
Limonene	1209		1213	1211	1204	1210	1210
β-Phellandrene	1220		1226	1232		1219	
cis-Ocimene	1236						
γ-Terpinene			1256	1250	1244	1250	1248
trans-Ocimene	1251						
p-Cymene	1275		1282	1272	1269	1275	1268
Terpinolene	1289		1293	1284	1283	1288	1292
2,6-Dimethylhept-2-en-1-al	1356						
Nonanal				1394			
2-*p*-Tolylpropene			1448		1444		
α-Cubebene			1473				
δ-Elemene							1480
Citronellal	1485			1481			
Bicycloelemene					1482		
Decanal					1506		
α-Copaene		1505	1510			1503	1510
β-Elemene							1540
Linalol	1547	1541		1541	1542	1540	
Isopulegol	1564						
Terpinen-1-ol					1576		
Bornyl acetate	1578						
Fenchol					1585		
α-Bergamotene	1600			1590	1605		
Terpinen-4-ol	1615		1612		1614	1614	
β-Caryophyllene	1615	1614	1600	1607	1597		1614
cis-β-Terpineol					1628		
Citronellyl acetate	1671						
trans-β-Terpineol					1672		
Neral				1678			
α-Humulene		1688	1687				1686
α-Terpineol	1689			1689	1698	1693	
Borneol	1704						
Germacrene D			1724				
Geranial				1730			
β-Bisabolene				1730	1737		
Neryl acetate				1735			
Geranyl acetate	1764			1753			
trans, trans-α-Farnesene					1754		
α-Farnesene		1754					
Citronellol	1764						
Terpinyl acetate	1764						
δ-Cadinene	1775	1771	1770				1771
Nerol	1800			1788			
Geraniol	1845			1836			
p-Cymen-8-ol					1846		
Safrole						1883	
Geranyl butyrate	1901						
Caryophyllene oxide	2000	2000					1992
Elemol	2078						
cis-Methylisoeugenol	2091						
Eugenol		2161					
trans-Methylisoeugenol	2173						
α-Cadinol			2224				
Eugenyl acetate		2253					
Myristicin						2264	
Chavicol		2373					

Table 3. Average relative retention indices

Column	Compound	Citronella	Clove	Juniper	Lemon	Lime	Nutmeg	Pepper	Σ	σ
Methyl polysiloxane	α-Pinene	√	—	√	√	√	√	√	931	4.1
	γ-Terpinene	—	—	√	√	√	√	√	1053	3.6
	α-Terpineol	√	—	√	√	√	√	√	1178	3.4
	β-Caryophyllene	√	√	√	√	√	—	√	1432	4.3
	cis-Ocimene	√	—	—	—	—	—	—	1040	1.2
	cis-β-Terpineol	—	—	—	—	√	—	—	1137	3.0
	Citronellol	√	—	—	—	—	—	—	1211	0.9
	Safrole	—	—	—	—	—	√	—	1272	2.4
	Bornyl acetate	—	—	√	—	—	—	—	1278	0.4
	Chavicol	—	√	—	—	—	—	—	1293	1.9
	Eugenol*	—	—	—	—	—	√	—	1337	2.4
	Eugenol*	—	√	—	—	—	—	—	1340	2.4
	Neryl acetate	—	—	—	√	—	—	—	1343	4.1
	δ-Elemene	—	—	—	—	—	—	√	1344	2.3
	α-Cubebene	—	—	√	—	—	—	—	1359	2.4
Polyethylene glycol 20M	α-Pinene	√	—	√	√	√	√	√	1028	3.1
	γ-Terpinene	√	—	√	√	√	√	√	1252	3.5
	Terpinolene	√	—	√	√	√	√	√	1289	3.2
	α-Terpineol	√	—	—	√	√	√	√	1692	3.0
	cis-Ocimene	√	—	—	—	—	—	—	1237	0.5
	Nonanal	—	—	—	√	—	—	—	1397	2.4
	α-Cubebene	—	—	√	—	—	—	—	1473	1.1
	δ-Elemene	—	—	—	—	—	—	√	1478	1.3
	cis-β-Terpineol	—	—	—	—	√	—	—	1625	3.1
	Citronellyl acetate	√	—	—	—	—	—	—	1671	0.8
	Borneol	√	—	—	—	—	—	—	1699	5.0
	Geranial	—	—	—	√	—	—	—	1729	2.0
	trans, trans-α-Farnesene	—	—	—	—	√	—	—	1751	2.3
	Safrole	—	—	—	—	—	√	—	1876	3.9
	Eugenol	—	√	—	—	—	—	—	2154	5.4
	δ-Cadinol	—	—	√	—	—	—	—	2215	10.9
	Myristicin	—	—	—	—	—	√	—	2254	7.3
	Chavicol	—	√	—	—	—	—	—	2369	4.0

* Compares the average difference when measured in two different essential oils.

In the tables giving details of published standards, these are drawn from International Standards, British Standards, Essential Oil Association of America Standards and Britsh Pharmacopoeia Standards. The actual figures obtained for the oils used for the collaborative fingerprinting studies are listed in the AMC/EO sample columns.

Essential Oil of Citronella, Sri Lanka

Essential oil of citronella is prepared by the steam distillation of the fresh or partially dried leaves of citronella grass. In Sri Lanka the variety of grass is *Cymbopogon nardus* Rendle and the oil produced from it can be differentiated from oil distilled in Java using a different variety of grass. The yield of oil is about 0.5% in both instances, but the Sri Lankan oil contains 25–40% of geraniol and 5–15% of citronella, which are lower levels than those normally found in the Java type.

The Sri Lankan oil is a pale yellow to yellowish brown liquid with a characteristic odour of citronella. Its principle use is in inexpensive perfumery applications for household products. In commercial trade, the description citronella Ceylon is still used.

The quality and value of the oil are traditionally assessed by a determination of "total alcohols" by the standard method of acetylation, but this procedure is known to give erroneously high results because of interference from the citronellal. However, the results by this method are precise and as the levels of citronellal tend to follow those of the alcohols, the results give a useful guide. A more accurate determination of the geraniol can be achieved by GLC and details of this specific determination are given in Part III of this series.[4]

Citronella oil is subject to changes during storage resulting from polymerisation. The presence of polymers will not be revealed by any GLC fingerprint procedure which applies the method of peak-area normalisation, but is more readily revealed by a decrease of solubility in 80% ethanol and an increase in the specific gravity. Because of the possible presence of polymeric material, any quantitatively accurate measurements of components by GLC must be made using the method of internal standards.

Typical chromatograms for the Sri Lankan type oil, distilled in Sri Lanka, are shown in Figs. 1 and 2 and the identification of components and their approximate proportions in the oil are given in Tables 4 and 5; Table 6 shows the physico-chemical standards that are applied.

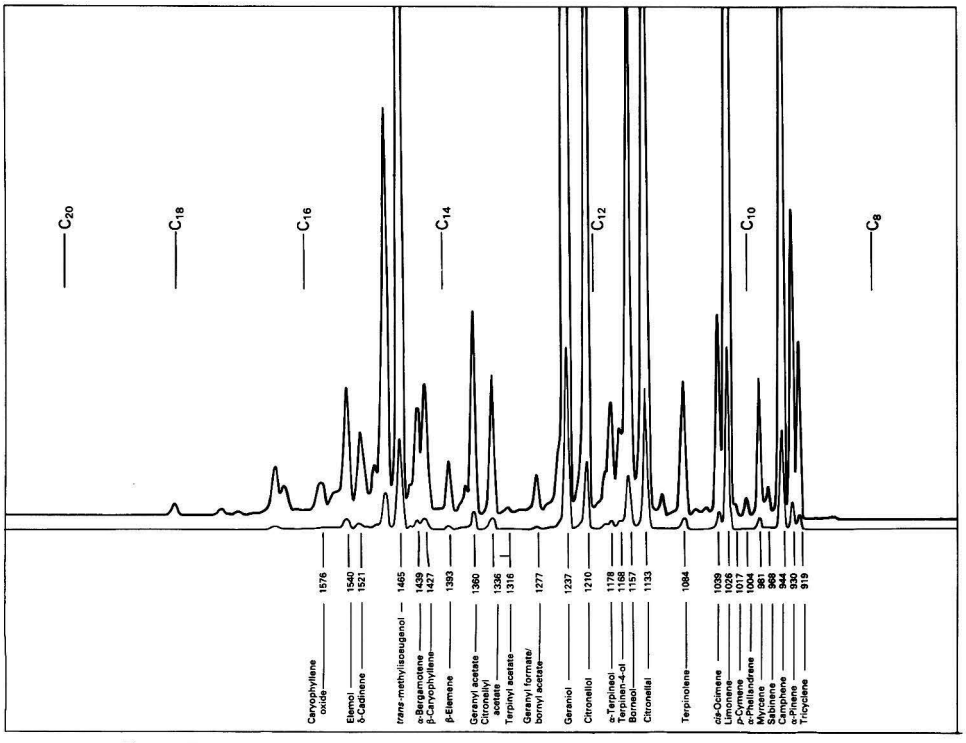

Fig. 1. Essential oil of citronella (Sri Lanka) on methyl polysiloxane (non-polar) stationary phase

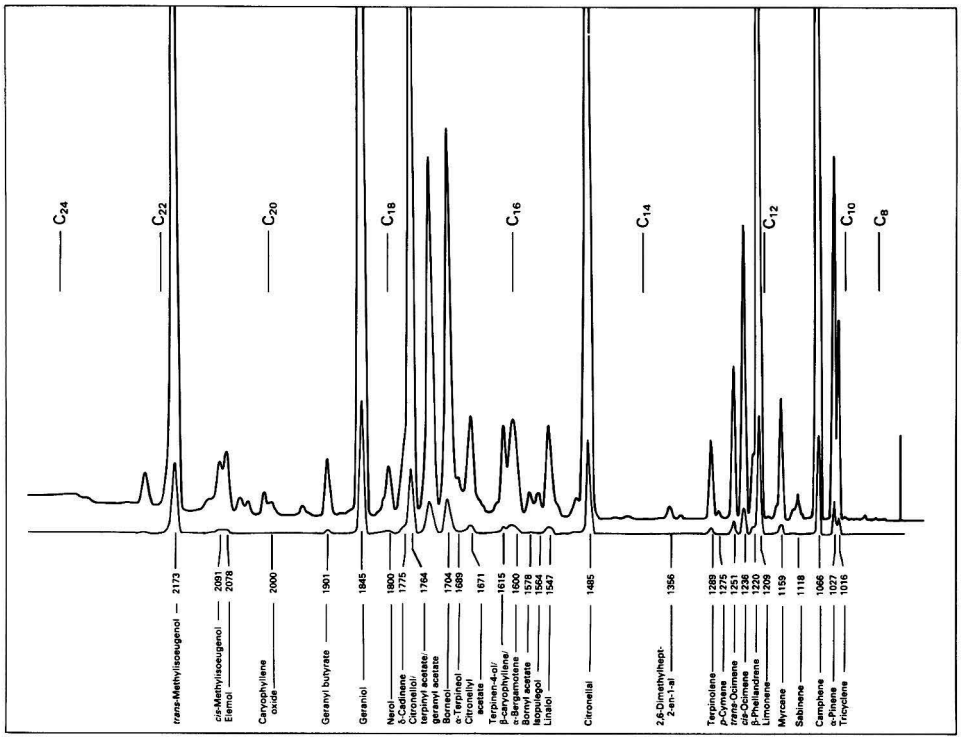

Fig. 2. Essential oil of citronella (Sri Lanka) on polyethylene glycol 20M (polar) stationary phase

Table 4. Essential oil of citronella, Sri Lanka: methyl polysiloxane (non-polar) stationary phase

Peak identity	RRI	RPA, %
Tricyclene	919	1.2
α-Pinene	930	2.2
Camphene	944	7.6
Sabinene	968	0.3
Myrcene	981	0.8
α-Phellandrene	1004	0.1
p-Cymene	1017	0.1
Limonene	1026	11.3
cis-Ocimene	1039	2.1
Terpinolene	1084	0.6
Citronellal	1133	14.7
Borneol	1157	4.8
Terpinen-4-ol	1168	0.3
α-Terpineol	1178	0.5
Citronellol	1210	6.2
Geraniol	1237	17.0
Bornyl acetate	1277	0.5
Geranyl formate	1277	0.2
Terpinyl acetate	1316	0.4
Citronellyl acetate	1336	1.1
Geranyl acetate	1360	2.1
β-Elemene	1393	0.7
β-Caryophyllene	1427	1.0
α-Bergamotene	1439	0.8
trans-Methylisoeugenol	1465	10.1
δ-Cadinene	1521	0.6
Elemol	1540	0.7
Caryophyllene oxide	1576	0.3

Table 5. Essential oil of citronella, Sri Lanka: polyethylene glycol 20M (polar) stationary phase

Peak identity	RRI	RPA, %
Tricyclene	1016	1.2
α-Pinene	1027	2.2
Camphene	1066	7.6
Sabinene	1118	0.2
Myrcene	1159	0.8
Limonene	1209	11.3
β-Phellandrene	1220*	0.4
cis-Ocimene	1236	2.1
trans-Ocimene	1251	1.1
p-Cymene	1275	0.1
Terpinolene	1289	0.7
2,6-Dimethylhept-2-en-1-al	1356	0.2
Citronellal	1485	13.3
Linalol	1547	0.5
Isopulegol	1564	0.4
Bornyl acetate	1578	0.5
α-Bergamotene	1600*	1.0
β-Caryophyllene	1615*	0.9
Terpinen-4-ol	1615*	0.4
Citronellyl acetate	1671	1.2
α-Terpineol	1689	1.1
Borneol	1704	5.2
Geranyl acetate	1764*	2.1
Terpinyl acetate	1764*	0.4
Citronellol	1764*	6.5
δ-Cadinene	1775*	0.6
Nerol	1800	0.6
Geraniol	1845	17.5
Geranyl butyrate	1901	0.6
Caryophyllene oxide	2000*	0.1
Elemol	2078	0.7
cis-Methylisoeugenol	2091*	0.4
trans-Methylisoeugenol	2173	11.3

Table 6. Essential oil of citronella, Sri Lanka: published standards

Property	BS 2999/18 (1972)	EOA No. 12	AMC/EO sample
Apparent density (mass per ml) at 20 °C: min.	0.893	0.891*	0.902
max.	0.910	0.904*	
Refractive index at 20 °C: min.	1.479	1.479	1.484
max.	1.485	1.485	
Optical rotation at 20 °C: min.	−18°	−18°	−15°
max.	−9°	−9°	
Carbonyl value (free hydroxylamine): min.	25	7%†	26
max.	55	15%	
Ester value after acetylation: min.	185	55%‡	191
max.	201	65%	
Miscibility with 80% *V/V* ethanol at 20 °C	1 in 2 1 in 4, remaining clear for 24 h	1 in 2, remaining clear when diluted to 10	Passes tests

* Sp. gr. 25/25 °C.
† Calculated as citronellal
‡ Calculated as geraniol.

Essential Oil of Clove Bud, Malagasy Republic

Essential oil of clove bud is prepared by the water distillation of the dried flower bud of *Syzigium aromaticum* L. This is an evergreen tree and and is native to the tropical islands of Molucca, Zanzibar, Reunion, the Malagasy Republic, Indonesia and Comores. The best quality clove buds are harvested only when they are fully developed, but before they open. They are then sun-dried under conditions that prevent fermentation and mould growth. Other parts of the tree, such as twigs, stems and leaves, will also yield essential oils on distillation, but these oils are of an inferior quality. Further, the finest quality oil of clove buds is obtained by water distillation of whole or lightly crushed buds, rather than steam distillation, which introduces an undesirable harsh odour and flavour.

The oil is a pale yellow, highly refracting and slightly viscous liquid with a characteristic warm, spicy odour and flavour of clove, without any harshness. The colour may vary according to the degree of any bleaching in the light or contact with any trace metals, such as iron or copper, which cause considerable darkening. Essential oil of clove bud is used in the flavouring, perfumery and pharmaceutical industries.

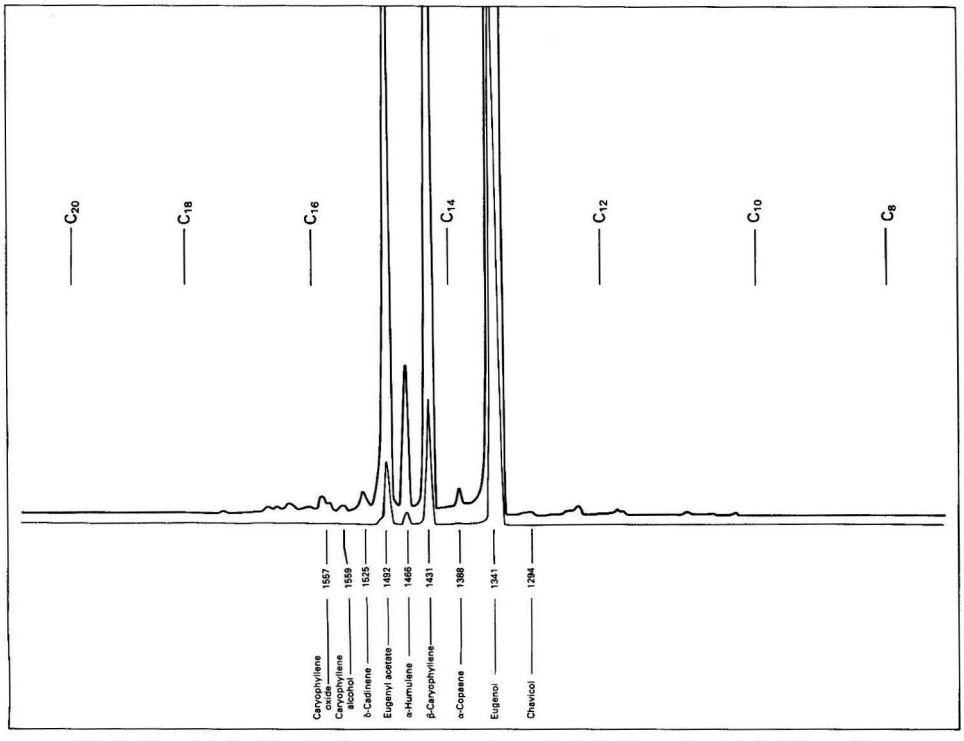

Fig. 3. Essential oil of clove bud (Malagasy Republic) on methyl polysiloxane (non-polar) stationary phase

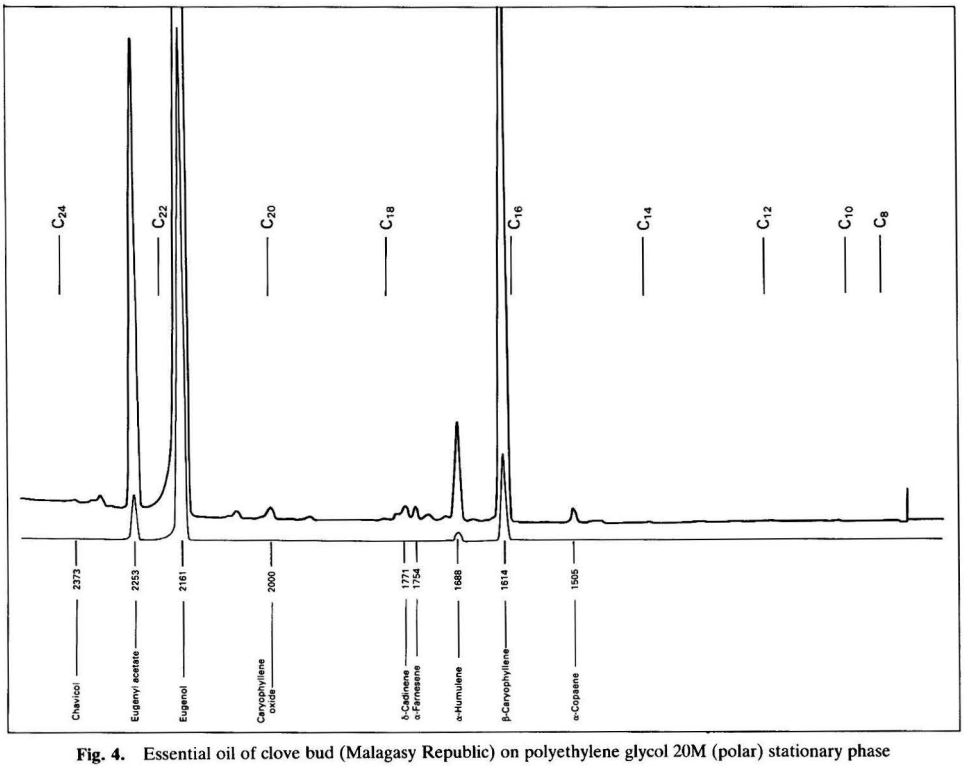

Fig. 4. Essential oil of clove bud (Malagasy Republic) on polyethylene glycol 20M (polar) stationary phase

The clove buds yield between 16 and 21% of essential oil, containing typically 90% of total phenols, principally eugenol and including eugenyl acetate. The fine odour and flavour of the best oils are attributed to very small amounts of other oxygenated compounds that are difficult to detect by GLC. Some clove oils, particularly those from Comores, may have an even higher content of total phenols.

Adulteration of clove bud oil is usually by the addition of clove leaf or clove stem oil, or by the addition of clove terpenes obtained as residues from the extraction of eugenol from the poorer qualities of clove oil. These types of adulteration are difficult to detect by GLC and an organoleptic evaluation may be a preferable guide.

Typical chromatograms are shown in Figs. 3 and 4 for an English distilled oil and the identified components and their approximate proportions in the oil are given in Tables 7 and 8; Table 9 shows the physico-chemical standards that are applied.

Table 7. Essential oil of clove bud, Malagasy Republic: methyl polysiloxane (non-polar) stationary phase

Peak identity	RRI	RPA, %
Chavicol	1294	0.2
Eugenol	1341	72.8
α-Copaene	1388	0.2
β-Caryophyllene	1431	15.7
α-Humulene	1466	1.6
Eugenyl acetate	1492	7.8
δ-Cadinene	1525	0.2
Caryophyllene alcohol	1559	0.2
Caryophyllene oxide	1577	0.3

Table 8. Essential oil of clove bud, Malagasy Republic: polyethylene glycol 20M (polar) stationary phase

Peak identity	RRI	RPA, %
α-Copaene	1505	0.2
β-Caryophyllene	1614	15.8
α-Humulene	1688	1.6
α-Farnesene	1754	0.2
δ-Cadinene	1771	0.2
Caryophyllene oxide	2000	0.3
Eugenol	2161	73.3
Eugenyl acetate	2253	6.3
Chavicol	2373	0.2

Table 9. Essential oil of clove bud, Malagasy Republic: published standards

Property		ISO 3142 (1974)	BS 2999/20 (1972)	BP (1980)	AMC/EO sample
Apparent density (mass per ml) at 20 °C:	min.	1.044	1.041	1.041	1.044
	max.	1.057	1.054	1.054	
Refractive index at 20 °C:	min.	1.528	1.528	1.528	1.532
	max.	1.538	1.538	1.537	
Optical rotation at 20 °C:	min.	−1.5°	−1.5°	−1.5°	−0.4°
	max.	0°	0°	0°	
Total phenols:	min.	85%	85%	85%	86%
	max.	93%	93%	90%	
Miscibility with 70% *V/V* ethanol at 20 °C		1 in 2	1 in 2	1 in 2	1 in 4

Essential Oil of Juniper Berry, Hungarian

Essential oil of juniper berry is obtained by the distillation with wet steam of the fruits of *Juniperus communis* L. This shrub grows wild in the mainly dry areas of Europe, Asia and the Americas, but there are several geographical varieties. The essential oil from the European form (var. *erecta*) is considered superior to that from the North American form (var. *depressa*). The oil is a colourless to faint greenish or yellowish liquid, with a characteristic odour and flavour. The odour is described as balsamic and resembling pine needles, whilst the flavour has an aromatic, bitter taste that persists even at very low concentrations. The berries may be ripe or partially ripe, dried or partially dried, and are usually crushed prior to distillation with wet steam. The yield of oil varies from 0.5 to 2.5%, according to the quality and condition of the berries. Its main constituent is α-pinene, with a wide range of other mono- and sesquiterpene hydrocarbons and minor, but important, amounts of oxygenated compounds.

The production of the essential oil by the direct distillation of the berries accounts for only a minor proportion of the so-called essential oil of commerce, most of which is obtained as a by-product from the distillation of gin or from the distillation of fermented berries. These oils are deficient in the oxygenated compounds and are inferior in quality. Such oils are not covered by the general description of essential oils, but are often confused with, or used to adulterate, the genuine article. In addition, the oils may be adulterated with Juniper wood oil, turpentine or other terpene fractions. GLC fingerprinting can be a useful guide to adulteration, particularly if attention is paid to the level and presence of the trace amounts of oxygenated compounds, but organoleptic testing and the physico-chemical properties are also important guides. On ageing, the oil becomes viscous and denser, and develops an odour recalling that of old turpentine. Its solubility in aqueous ethanol decreases.

Essential oil of juniper berry is used in perfumery and pharmaceutical products, but in flavouring it is usually used after the partial removal of the mono- and/or sesquiterpene hydrocarbons in order to increase its solubility.

Typical chromatograms are shown in Figs. 5 and 6 for an oil obtained in England by the direct distillation with wet steam of crushed Hungarian berries, *Juniperus communis* L. var. *erecta*. Identified components and their approximate proportions in the oil are given in Tables 10 and 11; Table 12 shows the physico-chemical standards that are applied.

Table 10. Essential oil of juniper berry, Hungarian: methyl polysiloxane (non-polar) stationary phase

Peak identity	RRI	RPA, %
α-Thujene	919	2.8
α-Pinene	930	35.0
Camphene	944	0.3
Sabinene	965	5.0
Myrcene	979	9.3
α-Phellandrene	1000	0.4
α-Terpinene	1014	2.1
p-Cymene	1014	1.2
Limonene	1026	3.7
β-Phellandrene	1026	0.7
γ-Terpinene	1051	3.7
2-*p*-Tolylpropene	1077*	0.3
Terpinolene	1081	1.8
Terpinen-4-ol	1165	9.6
α-Terpineol	1176	0.9
Bornyl acetate	1278	0.3
α-Cubebene	1356	0.4
α-Copaene	1384	0.5
β-Elemene	1391	1.9
β-Caryophyllene	1429	1.9
α-Humulene	1461	1.5
δ-Cadinene	1523	2.8

Table 11. Essential oil of juniper berry, Hungarian: polyethylene glycol 20M (polar) stationary phase

Peak identity	RRI	RPA, %
α-Thujene	1035	2.9
α-Pinene	1035	35.1
Camphene	1076	0.3
β-Pinene	1114	1.9
Sabinene	1124	5.0
Myrcene	1165	9.5
α-Terpinene	1195	2.2
Limonene	1213	3.7
β-Phellandrene	1226	0.7
γ-Terpinene	1256	3.7
p-Cymene	1282	1.3
Terpinolene	1293	1.8
2-*p*-Tolylpropene	1448	0.3
α-Cubebene	1473	0.4
α-Copaene	1510	0.4
β-Caryophyllene	1600	1.9
Terpinen-4-ol	1612	9.5
α-Humulene	1687	1.9
Germacrene D	1724	2.7
δ-Cadinene	1770	2.9
α-Cadinol	2224	0.7

Table 12. Essential oil of juniper berry: published standards

Property		EOA No. 113	Food Chem. Codex, USA	AMC/EO sample
Apparent density (mass per ml) at 20 °C:	min.	0.854*	0.854*	0.871
	max.	0.879*	0.879*	
Refractive index at 20 °C:	min.	1.4740	1.4740	1.480
	max.	1.4840	1.4840	
Optical rotation at 20 °C:	min.	−15°	−15°	−4.8°
	max.	0°	0°	
Miscibility with 95% *V/V* ethanol at 20 °C		1 in 4 with turbidity	Not given	1 in 4 with turbidity

* Sp. gr. 25/25 °C.

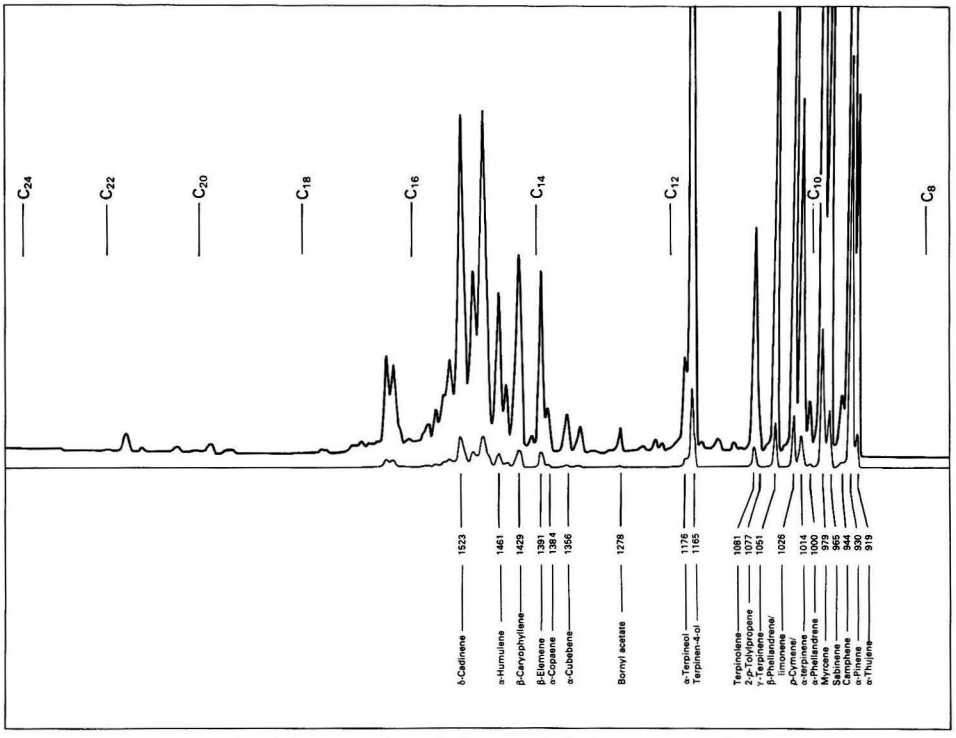

Fig. 5. Essential oil of juniper berry (Hungarian) on methyl polysiloxane (non-polar) stationary phase

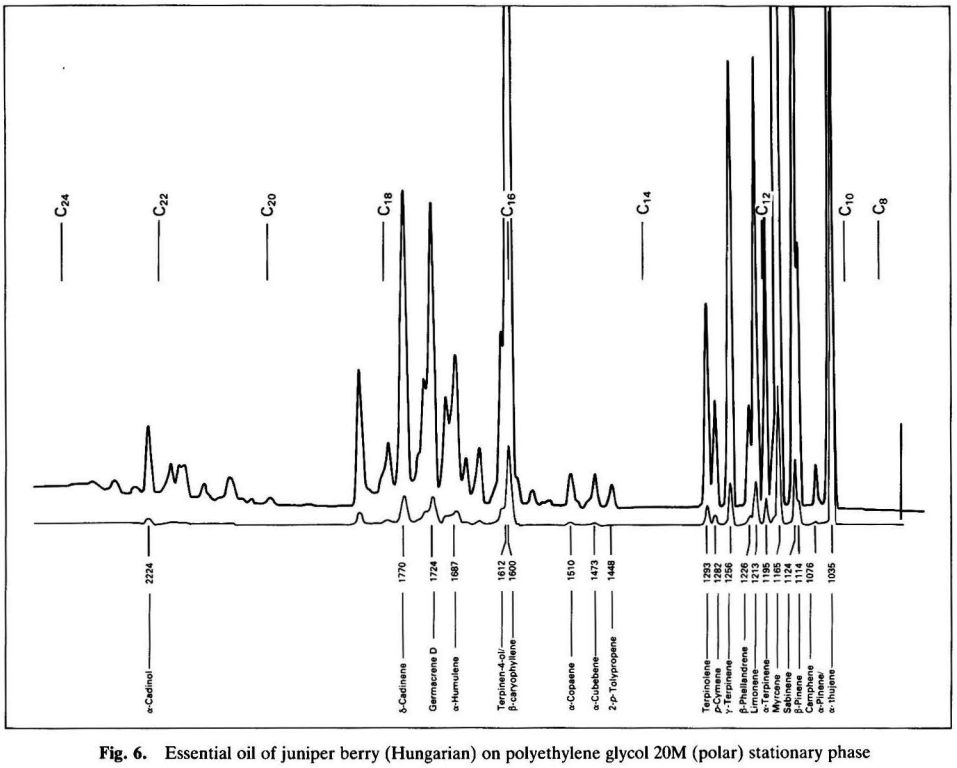

Fig. 6. Essential oil of juniper berry (Hungarian) on polyethylene glycol 20M (polar) stationary phase

Essential Oil of Lemon, Sicilian, Obtained by Expression

This oil is prepared by the cold expression from the fresh pericarp of the ripe fruit of *Citrus limon* L. and is native to the Far East. Its cultivation has spread to such Mediterranean regions as Spain, Italy, Israel and Greece and in America to California and Florida.

Isolation of the oil by cold expression was traditionally achieved by the "sponge" process, but although this yields the finest quality of oil, it is not capable of mechanisation and alternative mechanical devices have been developed to produce a similar product. These mechanical processes have developed separately in different countries and as they yield oils of slightly different characteristics, it is necessary to define an oil by its country of origin and method of production.

The underlying reason for these differences in characteristics lies in the change induced in the oil by the extent of its contact with the lemon juice, which is acidic and enzymically active. In addition, the oil is affected by its contact with water, which preferentially dissolves the oxygenated components, and contact with air, which induces oxidation and polymerisation. The combined effect of these factors particularly reduces the citral content, which should normally be of the order of 3.5% when determined as total aldehydes.

The cold pressed essential oil of lemon is a pale to deep yellow or greenish yellow liquid with a characteristic odour recalling that of fresh lemon peel. The principal constituent of the oil is limonene, but the important flavour components are the isomeric citrals, together with other minor oxygenated components. The oil contains a proportion of dissolved waxes and other non-volatile components which may separate as flock or sediment on storage, particularly under cold conditions.

Traditional analyses of the oil depend on determination of the citral content and the non-volatile residue, as well as a range of physical constants. A list of applied standards is given in Table 15, but the application of GLC gives far more detailed information about the composition of the volatile portion of the oil, whilst spectrophotometric analyses and various forms of liquid chromatography can be applied to the non-volatile part.

The adulteration of lemon oil is facilitated by the availability of a range of by-products arising from the further processing of genuine oil in order to reduce its terpene content and improve its solubility. These "terpenes" are often added back to genuine oil and the aldehyde balance adjusted by the addition of citral from external sources. Similarly, the non-volatile residue content can be adjusted by the addition of other

Table 13. Essential oil of lemon, Sicilian, obtained by expression: methyl polysiloxane (non-polar) stationary phase

Peak identity	RRI	RPA, %
α-Thujene	917	0.4
α-Pinene	928	1.8
Camphene	945	0.1
Sabinene	966	1.9
β-Pinene	976	12.1
α-Terpinene/*p*-cymene	1016	0.5
Limonene	1032	67.0
γ-Terpinene	1055	8.6
Terpinolene	1083	0.4
Citronellal	1131	0.1
α-Terpineol	1176	0.2
Neral/nerol	1217	1.1
Geranial/geraniol	1245	1.8
Neryl acetate	1341	0.5
Geranyl acetate	1359	0.5
β-Caryophyllene	1431	0.2
α-Bergamotene	1439	0.4
β-Bisabolene	1508	0.5

Table 14. Essential oil of lemon, Sicilian, obtained by expression: polyethylene glycol 20M (polar) stationary phase

Peak identity	RRI	RPA, %
α-Pinene	1023	2.2
Camphene	1060	0.1
β-Pinene	1111	12.2
Sabinene	1123	1.9
Myrcene	1154	1.5
α-Terpinene	1188	0.2
Limonene	1211	66.5
β-Phellandrene	1232	0.3
γ-Terpinene	1250	8.8
p-Cymene	1272	0.3
Terpinolene	1284	0.4
Nonanal	1394	0.1
Citronellal	1481	0.1
Linalol	1541	0.2
α-Bergamotene	1590	0.4
β-Caryophyllene	1607	0.3
Neral	1679	0.9
α-Terpineol	1689	0.2
Geranial/β-bisabolene	1730	2.0
Neryl acetate	1735	0.7
Geranyl acetate	1753	0.7
Nerol	1788	0.1
Geraniol	1836	0.1

Table 15. Essential oil of lemon, Sicilian, obtained by expression: published standards

Property		ISO 855 (1981)*	BP (1980)	AMC/EO sample
Apparent density (mass per ml) at 20 °C:	min.	0.849	0.850	0.852
	max.	0.858	0.856	
Refractive index at 20 °C:	min.	1.474	1.474	1.475
	max.	1.476	1.476	
Optical rotation at 20 °C:	min.	+57°	+57°	+60°
	max.	+65°	+65°	
Residue on evaporation:	min.	1.6%	2%	2.2%
	max.	3.9%	3%	
Acid value		3 max.	Not given	2
Carbonyl value:	min.†	11	3.5%	3.6%
	max.	17	(as citral)	(as citral)
Miscibility with 90% *V/V* ethanol at 20 °C		Not given	1 in 12	Passes BP test

* In addition, the ISO specification includes two spectrophotometric standards.
† Carbonyl value × 0.2713 gives percentage as citral.

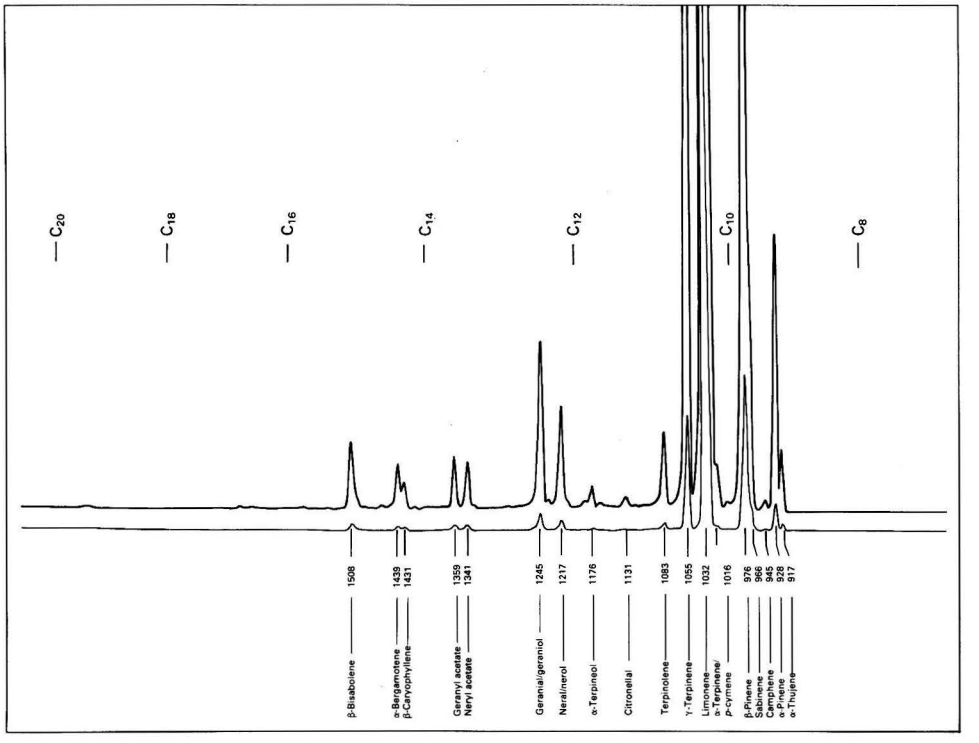

Fig. 7. Essential oil of lemon (Sicilian, obtained by expression) on methyl polysiloxane (non-polar) stationary phase

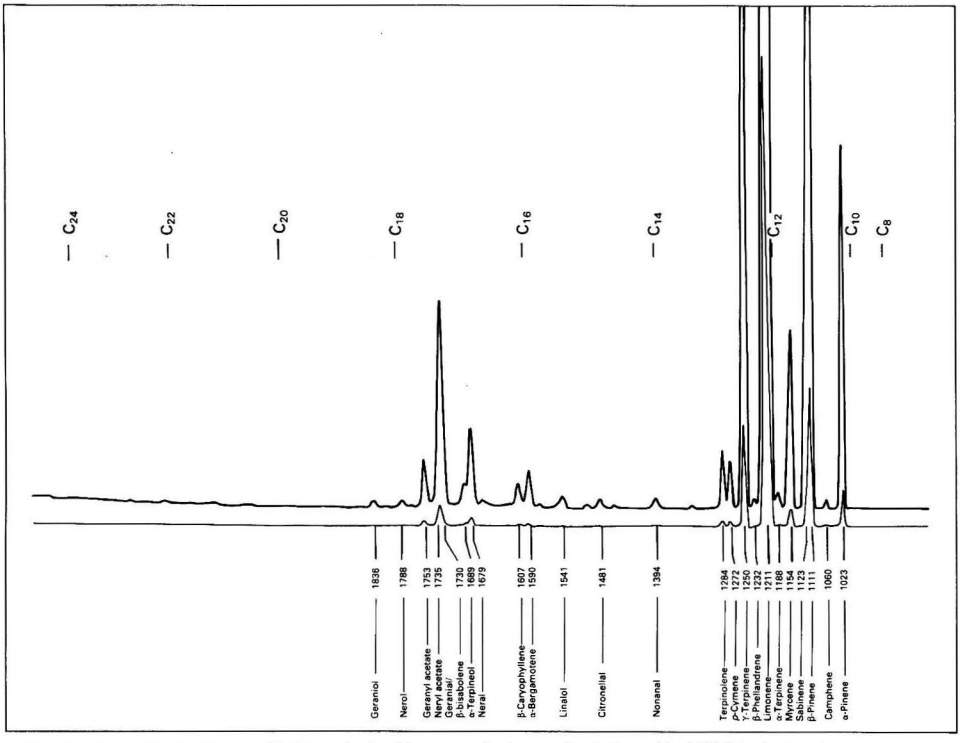

Fig. 8. Essential oil of lemon (Sicilian, obtained by expression) on polyethylene glycol 20M (polar) stationary phase

natural or synthetic waxes possessing the correct physical characteristics. These types of adulteration are difficult to detect by conventional analyses, even though organoleptic testing may give clear indications of a poor quality of oil. The situation is further complicated because very minor changes in the composition of the oil may give rise to major changes in the organoleptic qualities and may arise not only from adulteration, but also from a poor manufacturing process or poor storage of an otherwise good oil.

Notwithstanding these difficulties, an authentic sample of winter crop lemon oil from Barchelona in Sicily and prepared by the "Sfumatrice" process was used for the chromatograms shown in Figs. 7 and 8, which are therefore representative of an unadulterated top quality oil from this region. A list of the identified components and their approximate proportions in the oil is given in Tables 13 and 14; Table 15 shows the physico-chemical standards that are applied.

It should be particularly noted that a polyethylene glycol 20M stationary phase was used, and not one of its close equivalents, such as those terminated with terephthalic acid, which are known to decompose aldehydes at elevated temperatures. This does not pose a problem when these phases are used in capillary columns, because the elution temperatures are lower and decomposition of the aldehyde is reduced.

Essentail Oil of Lime, Cuban, Distilled

Essential oil of lime is prepared by the steam distillation of the unclarified juice obtained from strained fruit. The limes are of the "acid" variety, *Citrus aurantifolia* Swingle, and are widely grown throughout the tropics. This particular type of lime oil should not be confused with that obtained on a lesser scale from the "sweet" lime or from those obtained by cold expression from either variety of limes. Essential oil of distilled lime finds extensive use as a flavouring, particularly in soft drinks.

In the original commercial processing of "acid" limes, the distilled oil was a by-product from the production of lime juice and citric acid, but the market demand for lime oil favoured the distilled oil in preference to the cold pressed oil up to the point where contemporary production now treats the distilled oil and the juice as equally desirable products. The oil is a colourless to greenish yellow liquid with a characteristic odour. The best qualities are obtained from the slow, prolonged steam distillation of the juice from green limes. The yield of oil may be as high as 0.3%, of which the main constituents are limonene and other monoterpene hydrocarbons, with smaller amounts of sesquiterpene hydrocarbons and oxygenated compounds, the principal oxygenated compound being α-terpineol. Better quality oils are characterised by a higher density and a lower optical rotation, but adulteration is rife, the usual adulterants being lime terpenes,

Table 16. Essential oil of lime, Cuban, distilled: methyl polysiloxane (non-polar) stationary phase

Peak identity	RRI	RPA, %
α-Pinene	929	0.8
Camphene	942	0.4
β-Pinene	973	0.9
Myrcene	978	1.1
α-Terpinene	1007	1.8
p-Cymene	1014	3.4
Limonene	1029	46.6
γ-Terpinene	1052	8.7
Terpinolene	1081	7.0
Fenchol	1104	1.1
Terpinen-1-ol	1122	1.1
cis-β-Terpineol	1130	0.9
trans-β-Terpineol	1148	0.3
Borneol	1157	0.6
Terpinen-4-ol	1165	1.3
α-Terpineol	1178	8.7
γ-Terpineol	1184	1.4
β-Caryophyllene	1430	0.7
α-Bergamotene	1439	0.9
trans,trans-α-Farnesene	1498	1.0
β-Bisabolene	1508	1.2

Table 17. Essential oil of lime, Cuban, distilled: polyethylene glycol 20M (polar) stationary phase

Peak identity	RRI	RPA, %
α-Pinene	1026	0.8
Camphene	1063	0.4
β-Pinene	1103	1.2
Myrcene	1147	1.1
α-Terpinene	1174	1.9
Limonene	1204	46.9
γ-Terpinene	1244	8.9
p-Cymene	1269	3.5
Terpinolene	1283	7.3
2-*p*-Tolylpropene	1444	0.3
Bicycloelemene	1482	0.1
Decanal	1506	0.2
Linalol	1542	0.3
Terpinen-1-ol	1576	1.2
Fenchol	1585	1.4
β-Caryophyllene	1597	0.9
α-Bergamotene	1605	0.9
Terpinen-4-ol	1614	0.8
cis-β-Terpineol	1628	0.9
trans-β-Terpineol	1672	0.3
α-Terpineol	1698	9.0
β-Bisabolene	1737	1.5
trans,trans-α-Farnesene	1754	1.0
p-Cymen-8-ol	1846	0.4

Table 18. Essential oil of lime, Cuban, distilled: published standards

Property	ISO 3519 (1976)	BS 2999/45 (1972)	EOA No. 78	AMC/EO sample
Apparent density (mass per ml) at 20 °C: min.	0.856	0.856	0.855*	0.860
max.	0.865	0.862	0.863*	
Refractive index at 20 °C: min.	1.474	1.475	1.4745	1.476
max.	1.478	1.477	1.4770	
Optical rotation at 20 °C: min.	+34°	+34°	+34°	+38°
max.	+45°	+45°	+47°	
Residue on evaporation	2.5% max.	3% max.	Not given	2.2%
Carbonyl value†	1.5% min. as citral	1.5% min, 5.5 max.	0.5% min., 2.5% max.	4.0%
Miscibility with 90% *V/V* ethanol at 20 °C	Not given	Not given	1 in 5	

* Sp. gr. 25/25 °C.

† Carbonyl value × 0.2713 gives percentage as citral.

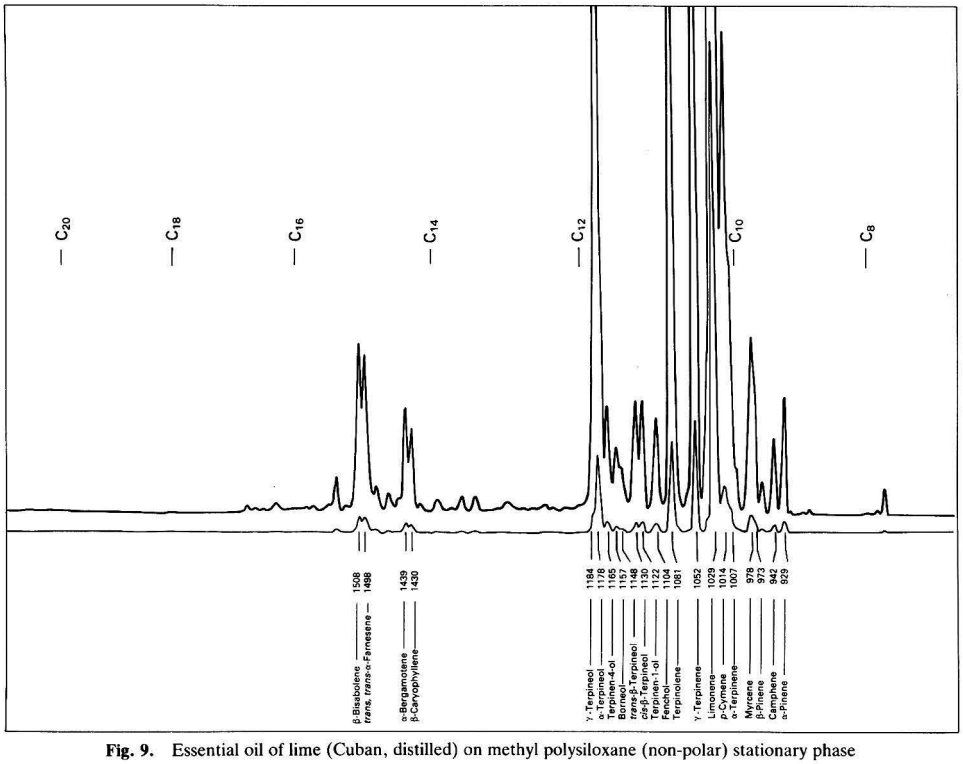

Fig. 9. Essential oil of lime (Cuban, distilled) on methyl polysiloxane (non-polar) stationary phase

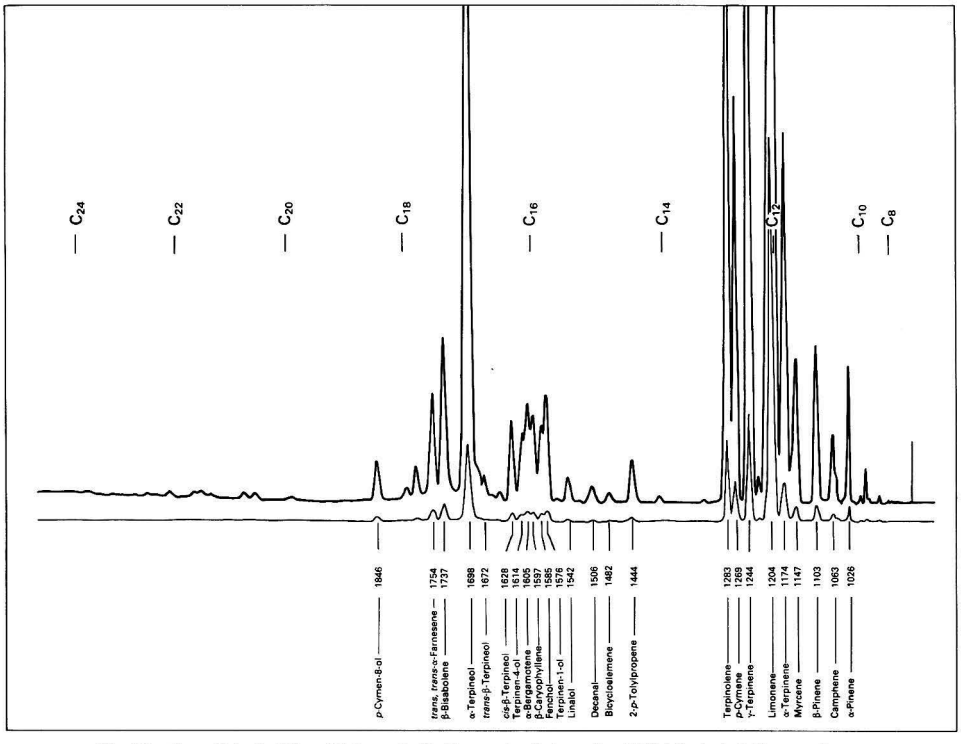

Fig. 10. Essential oil of lime (Cuban, distilled) on polyethylene glycol 20M (polar) stationary phase

obtained from the manufacture of "terpeneless" oils, and α-terpineol, obtained synthetically. The addition of these has the effect of reducing the level of the other minor components, which can serve as a guide to the level of adulteration. In particular, the levels of sesquiterpene hydrocarbons are difficult to adjust by adulteration.

Typical chromatograms, for an oil distilled in Cuba, are shown in Figs. 9 and 10 and the identified components and their approximate proportions in the oil are given in Tables 16 and 17; Table 18 shows the physico-chemical standards that are applied.

Essential Oil of Nutmeg, East Indian, Padang

Nutmegs are dried, ripe seeds of the fruit from a large evergreen tree, *Myristica fragrans* Houttuyn, native to Indonesia, but now cultivated in both the East and West Indies. When the fruit is ripe, it falls naturally from the tree and, in so doing, splits along a lateral groove. The fallen fruits are collected and the seeds, which are covered with scarlet arillode (mace), are removed. After drying, the mace is detached from the shells, which are then further dried until the inner kernel rattles within the shell. The brittle shell is then removed by cracking to reveal the nutmeg of commerce. Essential oil of nutmeg finds extensive use in flavouring, perfumery and pharamceutical products.

The dried nutmegs not only contain the essential oil, but also considerable amounts of seed fat, mainly composed of trimyristin. The essential oil may be recovered from ground nutmegs by steam distillation, but the oil is obtained only in low yield, because of the "fixative" effect of the seed fat, and prolonged distillation hydrolyses this fat to give an essential oil of poor quality and containing considerable amounts of free fatty acids. Improved qualities of essential oil are obtained in higher yield from ground nutmegs that have had their seed fat content reduced. In commercial practice, the dried nutmegs are very susceptible to insect attack, which consumes a proportion of the fat, whilst leaving the essential oil unchanged, and it is these nutmegs, described as "distillation grade," that are used for essential oil production. Yields of oil vary considerably from 7 to 16% and the oil obtained from East Indian nutmegs is significantly different from that obtained from West Indian nutmegs. The main differences lie in the lower monoterpene content of the East Indian oil, with a correspondingly higher content of oxygenated compounds, notably safrole and myristicin. The oil is a colourless or pale yellow liquid, having an odour and flavour characteristic of nutmegs. Adulteration is usually confined to the addition of monoterpene hydrocarbons, or turpentine, which is difficult to detect unless gross amounts are added.

Typical chromatograms for an East Indian oil prepared in Padang from distillation grade nutmegs are shown in Figs. 11 and 12 and the identified components and their approximate proportions in the oil are given in Tables 19 and 20; Table 21 shows the physico-chemical standards that are applied.

Table 19. Essential oil of nutmeg, East Indian, Padang: methyl polysiloxane (non-polar) stationary phase

Peak identity	RRI	RPA, %
α-Thujene	921	2.2
α-Pinene	931	22.8
Camphene	948	0.3
Sabinene	971	18.6
β-Pinene	979	15.7
α-Phellandrene	1000	2.4
α-Terpinene	1013	3.4
Limonene	1028	3.7
γ-Terpinene	1052	5.1
Terpinolene/linalol	1083	1.7
Terpinen-4-ol	1170	7.7
α-Terpineol	1180	1.0
Safrole	1269	2.1
4-Pentylanisole	1290	0.3
Eugenol	1335	0.2
α-Copaene	1382	0.2
Isoeugenol	1423	0.2
α-Bergamotene	1436	0.1
Myristicin	1497	5.3
δ-Cadinene	1521	0.3

Table 20. Essential oil of nutmeg, East Indian, Padang: polyethylene glycol 20M (polar) stationary phase

Peak identity	RRI	RPA, %
α-Pinene	1033	22.6
Camphene	1072	0.3
β-Pinene	1117	15.4
Sabinene	1122	18.5
Myrcene/Δ^3-carene	1155	3.2
α-Phellandrene	1172	0.9
α-Terpinene	1189	3.5
Limonene	1210	3.9
β-Phellandrene	1219	2.4
γ-Terpinene	1250	5.1
p-Cymene	1275	1.1
Terpinolene	1288	2.4
α-Copaene	1503	0.2
Linalol	1540	0.4
Terpinen-4-ol	1600	8.0
α-Terpineol	1693	1.0
Safrole	1883	2.0
Myristicin	2264	6.9

Table 21. Essential oil of nutmeg, East Indian, Padang: published standards

Property	ISO 3215 (1974)	BS 2999/37 (1971)	BP (1980)	AMC/EO sample
Apparent density (mass per ml) at 20 °C: min.	0.883	0.885	0.885	0.892
max.	0.917	0.915	0.915	
Refractive index at 20 °C: min.	1.475	1.475	1.475	1.479
max.	1.488	1.488	1.488	
Optical rotation at 20 °C: min.	+8°	+8°	+10°	+15°
max.	+25°	+25°	+25°	
Residue on evaporation	Not given	3% max.	3% max.	2.5%
Miscibility with 90% *V/V* ethanol at 20 °C	1 in 5 (1 in 3 or 4 for fresh oils)	1 in 3	1 in 3	1 in 3

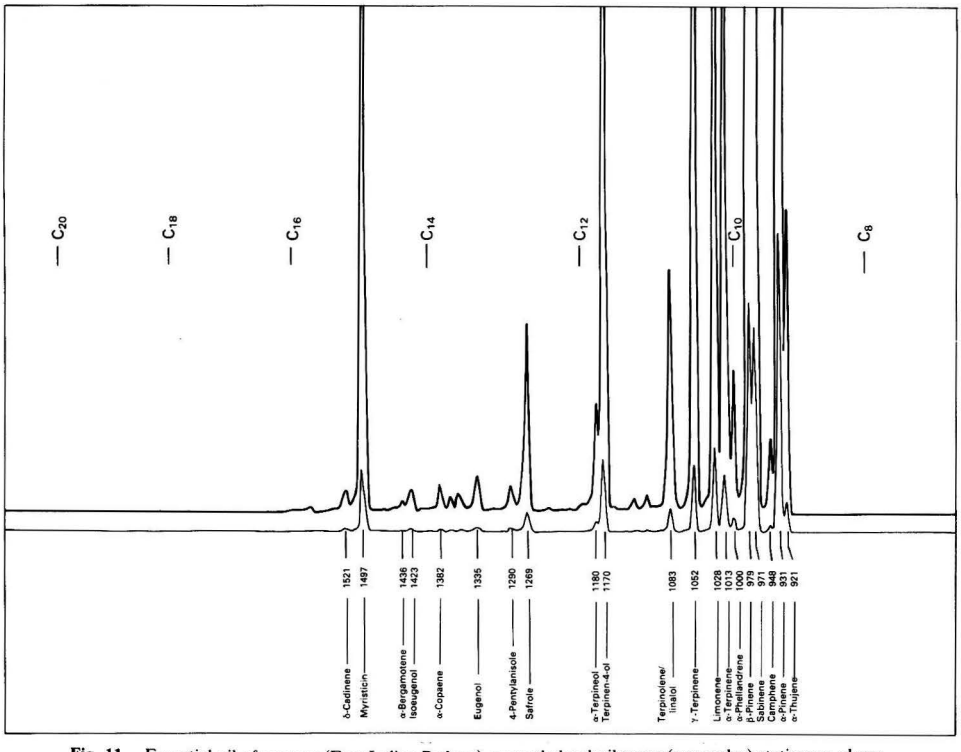

Fig. 11. Essential oil of nutmeg (East Indian Padang) on methyl polysiloxane (non-polar) stationary phase

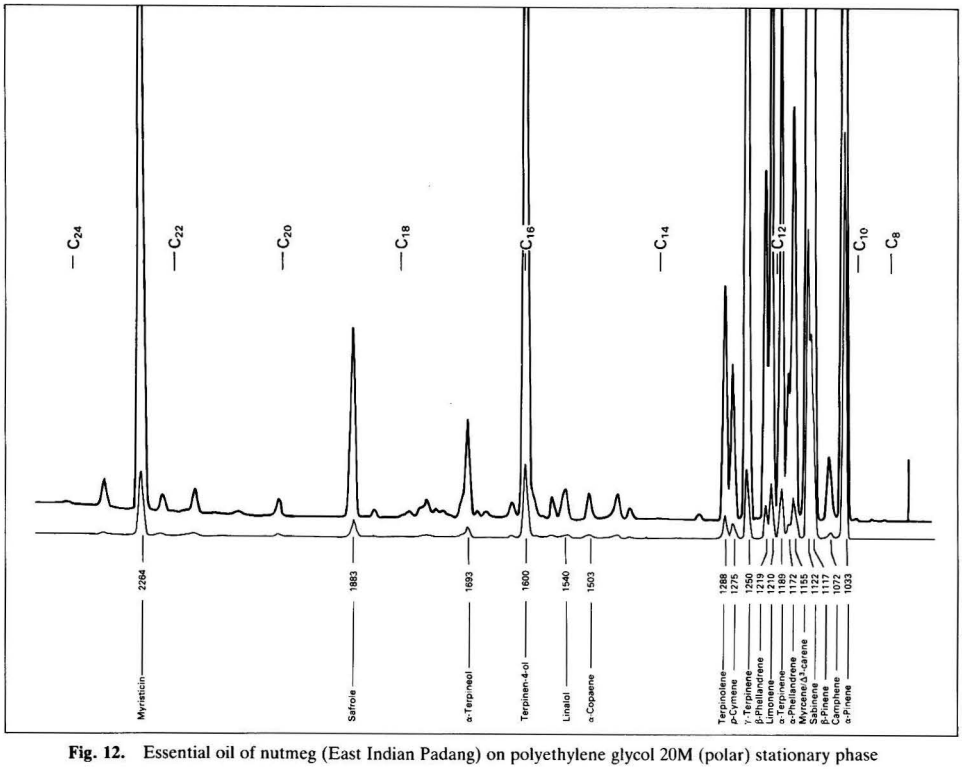

Fig. 12. Essential oil of nutmeg (East Indian Padang) on polyethylene glycol 20M (polar) stationary phase

Essential Oil of Black Pepper, Sarawak

Essential oil of black pepper is prepared by the steam distillation of the dried immature berries of *Piper nigrum* L. The main exporting countries are India, Sri Lanka, Indonesia, Brazil and Malaysia, the source of the berries being designated by the port from which they were exported or the region where they were grown. The pepper plant is a perennial climbing vine and is extensively cultivated in tropical areas. The oil is an almost colourless to bluish green liquid with a characteristic odour recalling that of the freshly crushed spice, although it completely lacks the pungent quality. The berries are usually crushed prior to distillation and yield between 1 and 2.6% of an essential oil, which is composed mainly of mono- and sesquiterpene hydrocarbons with minor amounts of oxygenated compounds and trace amounts of nitrogenous compounds derived from the alkaloids. The colour is due to the presence of trace amounts of azulenes and the quality of an oil has often been related to its colour. However, this can be misleading because the colour fades rapidly on exposure to light and the inclusion of pepper dust obtained from the preparation of decorticated pepper during the distillation can be used as a way of increasing the content of azulenes. Essential oil of black pepper finds use in both flavouring and perfumery.

The monoterpene hydrocarbons account for as much as 70–80% of the oil with α-pinene, β-pinene and limonene predominating, whilst the major sesquiterpene hydrocarbon is β-caryophyllene. The relative proportion of the monoterpene hydrocarbons to the other constituents may vary considerably in commercial samples as a result of the quality of the berries used for distillation and the care taken in their drying and storing.

Adulteration of pepper oil is usually confined to the addition of terpene hydrocarbons from a totally different source or by the addition of "pepper terpenes" obtained by fractionation from oleoresin production. Detection of this type of adulteration is difficult, but measurements of the physical constants and comparative examinations of fingerprint gas chromatograms can be revealing, although major changes in the terpene hydrocarbon composition may not be significant.

Typical chromatograms are shown for an English-distilled oil in Figs. 13 and 14 and the identified components and their approximate proportions in the oil are given in Tables 22 and 23; Table 24 shows the physico-chemical standards that are applied.

References

1. Analytical Methods Committee, *Analyst*, 1984, **109**, 1339.
2. Analytical Methods Committee, *Analyst*, 1980, **105**, 262.
3. Analytical Methods Committee, *Analyst*, 1981, **106**, 448.
4. Analytical Methods Committee, *Analyst*, 1973, **98**, 823.

NOTE—References 1, 2, 3, and 4 are to Parts X, VII, VIII and III of this series, respectively.

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Table 22. Essential oil of black pepper, Sarawak: methyl polysiloxane (non-polar) stationary phase

Peak identity	RRI	RPA, %
α-Thujene	916	0.2
α-Pinene	930	5.8
Camphene	944	0.1
β-Pinene	975*	10.4
Δ^3-Carene	1009	20.2
Limonene	1025	17.0
γ-Terpinene	1050	0.2
Terpinolene	1082	0.9
Terpinen-4-ol	1169	0.1
α-Terpineol	1179	0.1
δ-Elemene	1344	2.6
α-Cubebene	1359	0.2
α-Copaene	1385	2.4
β-Elemene	1394	0.5
β-Caryophyllene	1430	28.1
α-Humulene	1462	1.4
δ-Cadinene	1525	0.7

Table 23. Essential oil of black pepper, Sarawak: polyethylene glycol 20M (polar) stationary phase

Peak identity	RRI	RPA, %
α-Pinene	1027	5.8
Camphene	1068	0.1
β-Pinene	1110	10.4
Δ^3-Carene	1156	20.2
Limonene	1210	17.1
γ-Terpinene	1248	0.2
p-Cymene	1268	0.8
Terpinolene	1292	1.0
δ-Elemene	1480	2.5
α-Copaene	1510	2.3
β-Elemene	1540	0.4
β-Caryophyllene	1614	27.8
α-Humulene	1686	1.4
δ-Cadinene	1771	0.8
Caryophyllene oxide	1992	0.6

Table 24. Essential oil of black pepper: published standards

Property	ISO 3061 (1979)	BS 2999/12 (1965)	EOA No. 102	AMC/EO sample
Apparent density (mass per ml) at 20 °C: min.	0.870	0.868	0.864*	0.872
max.	0.890	0.907	0.884*	
Refractive index at 20 °C: min.	1.480	1.480	1.4795	1.4835
max.	1.492	1.492	1.4880	
Optical rotation at 20 °C: min.	−16°	−15°	−23°	−7.2°
max.	+4°	+4°	−1°	
Ester value: max.	11	11	Not given	4
Miscibility with 95% *V/V* ethanol at 20 °C	1 in 3	1 in 3	1 in 3	1 in 2.8

* Sp. gr. 25/25 °C.

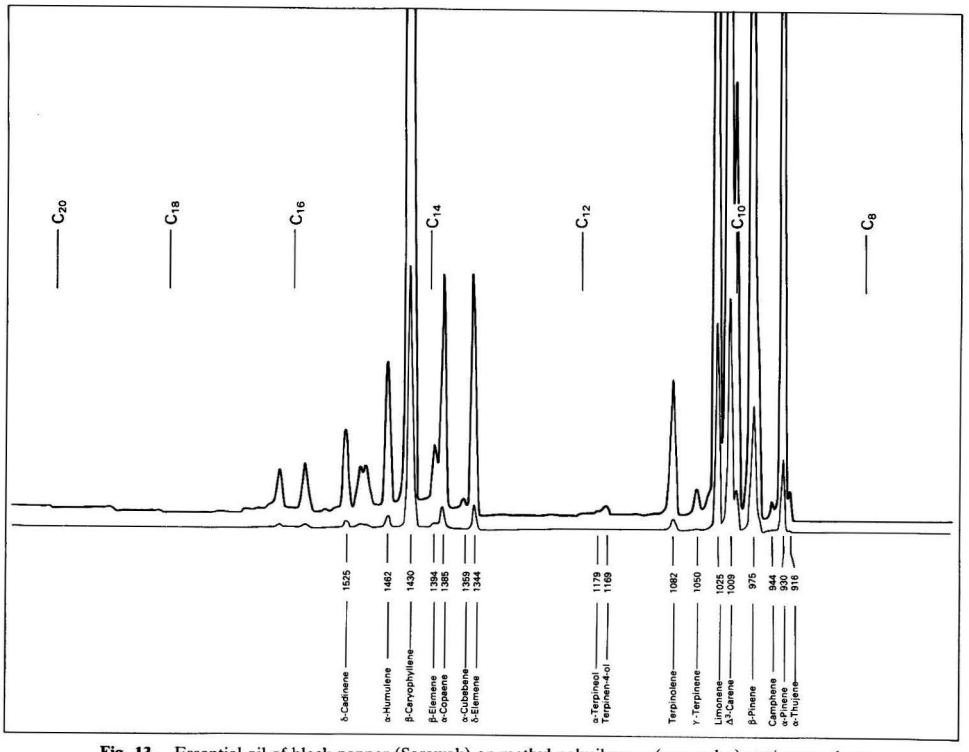

Fig. 13. Essential oil of black pepper (Sarawak) on methyl polysiloxane (non-polar) stationary phase

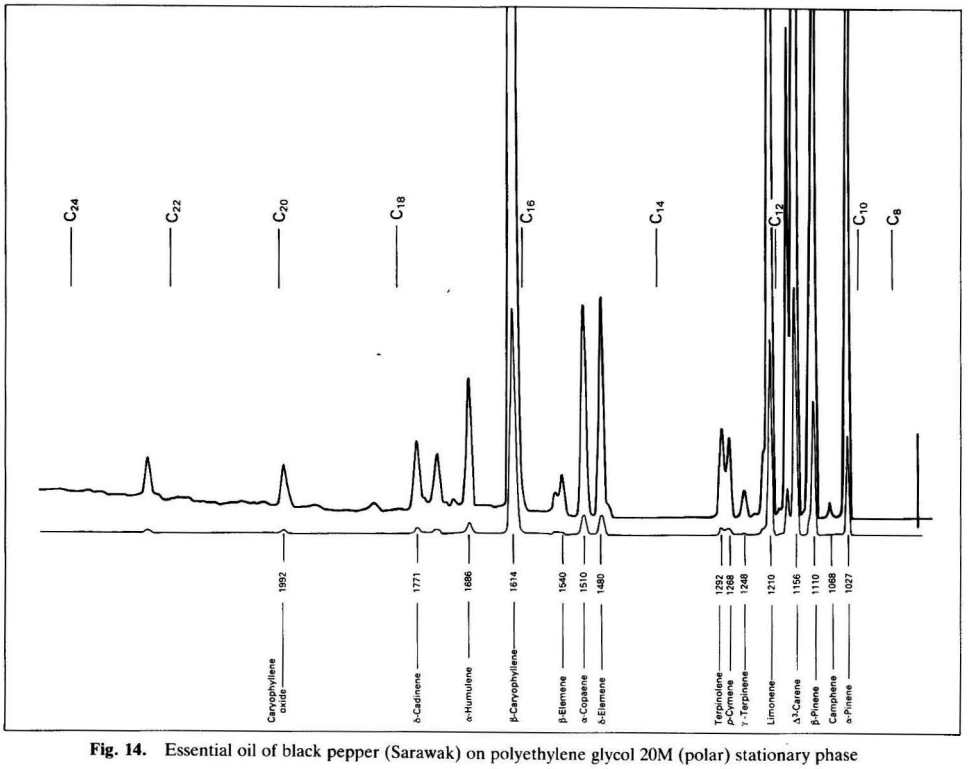

Fig. 14. Essential oil of black pepper (Sarawak) on polyethylene glycol 20M (polar) stationary phase

SHORT PAPER

Gravimetric Determination of Quinalphos by the Direct Precipitation of the 2-Hydroxyquinoxaline - Copper Chloride Complex with Copper(I) Chloride

Ramesh C. Rastogi, Nibaran C. Dey and Probin Baruah
Regional Research Laboratory, Jorhat 785 006, India

Keywords: *Quinalphos determination; copper(I) chloride; 2-hydroxyquinoxaline; gravimetry*

Quinalphos (*O,O*-diethyl *O*-2-quinoxalinyl phosphorothioate) is an important organophosphorus pesticide whose widespread use as an effective pesticide has created a demand for a quick, easy and reliable method for its determination. A few papers have recently described GLC methods[1,2] for the quantitative determination of organophosphorus pesticides. The methods are, however, inadequate for quinalphos as the compound is very sensitive to heat, and a GLC analysis leads to its decomposition on the column, which results in its inaccurate determination. Elemental analysis, *e.g.*, of nitrogen and phosphorus, is not always conclusive, because technical grade quinalphos is usually contaminated with other nitrogen base impurities such as 2-ethoxyquinoxaline and phosphorus impurities such as ethyl phosphorodithioate and *O,O*-diethyl phosphorochloridothioate. Therefore, a quick and reliable method for the determination of this pesticide is lacking. In a previous paper[3] reactions of copper(I) chloride with 2-hydroxyquinoxaline and copper(II) chloride with quinalphos have been reported to give a brick-red 2-hydroxyquinoxaline - copper chloride complex ($CuCl.C_8H_6N_2O$). The former reaction was found to be quantitative and has been reported as a method for the determination of 2-hydroxyquinoxaline,[4] but the latter did not give quantitative results, even after prolonged digestion. Recently, it was found that copper(I) chloride reacted directly with quinalphos to give the same copper complex as above. This paper describes a gravimetric method for the determination of quinalphos based on its reaction with copper(I) chloride. A direct gravimetric method for the determination of quinalphos has not previously been reported.

Experimental

Reagents

All chemicals were of analytical-reagent grade. Ethanol was distilled once before use. Acidified H_2O_2 solution was prepared by mixing 6% H_2O_2 solution with 10 N HCl.

Preparation and Purification of Quinalphos

Quinalphos was prepared by condensing 2-hydroxyquinoxaline with *O,O*-diethyl phosphorochloridothioate in the presence of anhydrous potassium carbonate and acetonitrile according to the method of Schmidt and Hammann.[5] To obtain a technical grade sample of quinalphos, the above product was diluted with xylene (2+1) and stabilised by adding epichlorohydrin. The technical quinalphos (sample A) thus obtained would be expected to contain 65–70% quinalphos, 25–30% xylene, 3% epichlorohydrin and trace amounts of other impurities. Sample A was further diluted with a different amount of xylene to give samples B, C and D. Commercially formulated samples, Ekalux and Suquin (25% *m/m*), manufactured by Sandoz India Ltd., Bombay, India and Sudarshan Chemical Ltd., Poona, India, respectively, were used as supplied.

Procedure

The stock solution of quinalphos in xylene or Ekalux or Suqin was accurately transferred into a 100-ml round-bottomed flask so as to contain approximately 50–500 mg of quinalphos. A 50–500-mg mass of finely powered anhydrous copper(I) chloride was then added followed by 10–25 ml of ethanol and the mixture was refluxed on a water-bath for 3 h using a water condenser. The mixture was cooled and the precipitate filtered through a weighed G4 sintered-glass crucible. The precipitate was first washed with benzene - hexane - diethyl ether followed by acidified H_2O_2 solution until the filtrate was free of Cu^{2+} ions. It was then washed with distilled water and rinsed with ethanol. The precipitate was dried at 120 °C for 1 h and weighed. The percentage of quinalphos in the complex is given by the following equation:

$$\% \text{ Quinalphos} = \left(\frac{m_C M_Q}{m_Q M_C}\right) \times 100$$

where m_C and m_Q are the masses of the complex and quinalphos and M_C and M_Q are the relative molecular masses of the complex and quinalphos, respectively.

Results and Discussion

As is evident from the results presented in Tables 1 and 2, the reaction of copper(I) chloride with quinalphos to produce a 2-hydroxyquinoxaline - copper chloride complex is quantitative because it can be analysed for its nitrogen content. The samples used in this study were free from 2-hydroxyquinoxaline and other nitrogenous impurities, but if present, 2-hydroxyquinoxaline can be determined in the presence of quinalphos by a previously reported method; in this procedure it is necessary to carry out a blank experiment.[4] The amount of complex thus obtained is subtracted from the amount of complex obtained by the proposed method to give the actual amount of complex formed with quinalphos. Other nitrogen impurities do not interfere with the proposed method of analysis.

It was reported earlier[3] that copper(II) chloride also reacted with quinalphos to give the brick-red complex. The reaction, however, was not quantitative even after prolonged refluxing. Copper(I) chloride reacted readily and the brick-red precipitate had good filtration properties, was stable to heating up to 250 °C without decomposition and was sparingly soluble

Table 1. Gravimetric determination of different samples of quinalphos using direct complexation with copper(I) chloride powder

Sample	Mass of sample/g	Mass of complex/g	Quinalphos, %	Mean + s.d., %	Quinalphos content from total N, %
A	1.1070	0.5949	65.37	65.38 ± 0.08	65.25
	1.0706	0.5747	65.29		
	1.2324	0.6636	65.49		
B	1.7100	0.5144	36.58	36.24 ± 0.23	36.59
	2.6737	0.8006	36.40		
	3.2230	0.9548	36.03		
C	0.9285	0.2198	28.79	28.61 ± 0.13	28.46
	0.9657	0.2262	28.49		
	0.8584	0.2015	28.55		
D	0.8979	0.1530	20.73	20.50 ± 0.18	20.49
	0.9990	0.1670	20.33		
	0.8781	0.1474	20.42		
E (Ekalux)	1.0068	0.2154	26.02	26.22 ± 0.15	25.98
	1.0366	0.2241	26.22		
	1.0074	0.2183	26.36		
F (Suquin)	1.1231	0.2378	25.75	25.61 ± 0.10	25.42
	1.0892	0.2284	25.51		
	1.0754	0.2261	25.57		

Table 2. Gravimetric determination of various amounts of quinalphos (sample B) by direct complexation with copper(I) chloride power

Sample No.	Mass of Sample/g	Mass of Complex/g	Quinalphos, %
1	0.0175	0.0324	36.60
2	0.1530	0.0460	36.57
3	0.1798	0.0533	36.03
4	0.2254	0.0681	36.73
5	0.2875	0.0867	36.66
6	0.3880	0.1010	36.35
7	0.3596	0.1069	36.17
8	0.3785	0.1134	36.43
9	0.3907	0.1200	36.51
10	0.4152	0.1236	36.22
11	0.5160	0.1559	36.75
12	0.7683	0.2323	36.77
13	1.0075	0.2993	36.13
14	1.5223	0.4566	36.48
15	1.9868	0.5933	36.52
		Mean ± s.d.:	36.45 ± 0.23

in most organic solvents. It was observed that copper(I) chloride reacted with an ethanolic solution of quinalphos even at room temperature and the reaction was completed in about 48 h. It was observed that emulsifier, stabiliser and colouring matters present in commercial samples (Ekalux or Suquin) did not interfere with the determination of the active ingredient. When emulsifier was present it was necessary to wash the precipitate thoroughly with benzene - hexane - diethyl ether, before using acidified H_2O_2 solution, otherwise the complex was partially carried by the emulsifier.

It can be concluded, therefore, that the proposed gravimetric method for the determination of quinalphos provides a unique, simple and alternative method for the determination of technical and formulated grades of quinalphos.

The authors are grateful to Dr. J. N. Baruah, Director, Regional Research Laboratory, Jorhat, for the interest he has shown in this work.

References

1. Braeqer, G., *Pflanzenschutz-Nachr.*, 1969, **22**, 308; *Chem. Abstr.*, 1971, **74**, 98301z.
2. Brun, G. L., and Mallet, V., *J. Chromatogr.*, 1973, **80**, 117.
3. Borthakur, N., Bhattacharya, A. K., Prasad, R., Garg, A., and Rastogi, R. C., *J. Heterocycl. Chem.*, 1979, **16**, 825.
4. Rastogi, R. C., Garg, A., and Baruah, P., *Analyst*, 1981, **106**, 901.
5. Schemedt, K. J., and Hammann, I., *Br. Pat.*, 1160493; *Chem. Abstr.*, 1969, **71**, 113084q.

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BOOK REVIEWS

HPLC Analysis of Biological Compounds. A Laboratory Guide.

William S. Hancock and James T. Sparrow. *Chromatographic Science Series, Volume* 26. Pp. x + 361. Marcel Dekker. 1984. Price SwFr110. ISBN 0 8247 71400.

This book is Volume 26 in the *Chromatographic Science Series*. The information given commences before opening it, as there is a diagram on the front cover that illustrates separation procedures. This leads one to expect that good practical details and applications will follow in the pages enclosed, and one is not disappointed.

There are five chapters: one introductory, then discussion of column types, mobile phases, practical details of separations and finally applications (which cover amino acids, peptides, proteins, nucleotides, carbohydrates and lipids). There is an appendix containing a list of suppliers, a good index and 200 references.

Useful tables include those giving characteristics of commercial phases of various polarities, care of columns, advantages and disadvantages of types of separations, etc. The chapter on applications includes helpful detail and is described as insights into the "minds" of chromatographers (the quotation marks being those of the authors and not the reviewer—which is puzzling).

Good details are given of those separations which are included and the text in general is clear and concise. It is a book both for libraries and the practical person inexperienced in the technique.

D. Simpson

Microcolumn High-performance Liquid Chromatography

Edited by Paul Kucera. *Journal of Chromatography Library, Volume* 28. Pp. xvi + 302. Elsevier. 1984. Price $63.50; Dfl165. ISBN 0 444 42290 0.

In recent years considerable interest has been shown in the use of narrow-bore columns for HPLC. Indeed, microbore columns and their associated equipment are now available commercially from a number of manufacturers. This book, edited by Paul Kucera, is one of the first to concern itself with a detailed study of such columns and includes both packed and open-tubular capillary varieties. Eight chapters, from a number of well known authors, cover various aspects of the subject.

The book commences with an overview by Guiochon and Colin, which details the basic properties of the different kinds of columns, their performances and limitations from a theoretical standpoint and the specifications required for the equipment associated with them. The practical advantages of using semi-microbore columns of 2 mm i.d. are also noted. The following chapter, by Kucera, deals in detail with 1 mm i.d. columns, the most usual diameter for commercial microbore columns. One disadvantage of microbore columns is their generally longer analysis times, and in Chapter 3 Hartwick and Dezaro provide a useful exploration of the theory and practice of using these columns for high-speed separations. In Chapter 4 Kucera and Manius discuss the advantages of, and practical procedures for, using column coupling and recycling techniques. Kucera is joined by Umagat in the next chapter to detail the use of chemical derivatisation techniques, and in Chapter 6, together with Hartwick, he presents various applications of microbore HPLC to "real" separation problems. Both of these chapters contain a number of interesting chromatograms that will allow the practising analyst to make a useful visual evaluation of the technique. In the penultimate chapter Novotny describes the theoretical and practical use of capillary columns, *i.e.*, those using very low flow-rates, typically around 1 $\mu l\ min^{-1}$. The unique detection opportunities offered by these columns are also discussed. Finally, Henion introduces the use of microcolumns for on-line LC - MS using both belt-transport and DLI interfaces.

In a book such as this, where each chapter has its own author, there is usually some variation in the quality of the text. In this instance each chapter is written to at least a good standard and some are particularly lucid. The various advantages and disadvantages of microcolumn HPLC are well documented throughout with many useful diagrams and photographs. The text is liberally sprinkled with numerous equations detailing the theory of the subject. The references are fairly comprehensive, although there are some omissions, with the latest being early 1983. One unfortunate aspect of this book is that each chapter is very much in the form of a monograph, and there appears to be little or no cross-reference between them. As a consequence, there is a high degree of repetition, especially concerning details of instrumental requirements. This criticism apart, however, this book provides a useful introduction to microcolumn HPLC and will answer many analysts' questions about this increasingly important technique.

C. R. Loscombe

Control of Food Quality and Food Analysis

Edited by G. G. Birch and K. J. Parker. Pp. xi + 332. Elsevier Applied Science. 1984. Price £35. ISBN 0 85334 239 3.

I am sure it has been said before that symposia do not transfer easily to the written page. Some speakers clearly are trying to instil controversy into the meeting, an admirable aim, which, however, may not come over quite so pungently on paper, lacking the speakers' personality and with no chance to question. Other speakers may have attempted to cover such a large topic that they cannot do justice to it in a few pages.

Both types are present in this book, which presents the papers read at the 14th Annual International Industry - University Symposium held at Reading in the spring of 1983. Eighteen papers are reproduced and they cover a very wide range of subjects, with food quality as the linking topic. However, food quality itself has a variable interpretation, some speakers talking of nutritional quality and others viewing quality as assessed by the analyst, the microbiologist or market research.

It would be tedious to try and comment on all eighteen papers and some are, as already mentioned, a bit thin, but other authors presented their subject in a thorough way and have added to the spoken text a list of references that make their chapters akin to a review. I shall single out eight papers that have a particular interest to me, although other readers will no doubt find that different chapters hold their attention.

Ann Walker explores the nutritional content of the national diet, its excesses and deficiencies and shows what steps are required to meet national guidelines. The story is developed concisely but clearly (62 references).

R. Sawyer discusses the problems of relating analytical information to legal standards with particular reference to

lean meat, water in meat, butter fat and dietary fibre (42 references).

Three papers cover the old and new in analytical methodology. Olga Flint describes newer developments in light microscopy, which enable it to hold its place as a valuable analysts' aid, whilst speakers from the Norwegian Food Research Institute discuss the use of NIR to predict food quality, and C. Hitchcock reviews the potential of immunology in the solution of difficult analytical problems (25, 19 and 22 references, respectively).

Microbiological quality is dealt with in two complementary papers by R. Spencer and C. S. Gutteridge, respectively. The first equates microbiological quality control with process hazard control while the second reviews rapid assay techniques (42 and 65 references, respectively).

Finally, there are a series of papers dealing with specific food products—potatoes, meat, starch, fruit and vegetables and a particularly thorough review, considering the space limitations, on dairy products by speakers from the Department of Dairy and Food Chemistry, University College of Cork (267 references).

Although the book may not be read from cover to cover, it will be an addition to the library shelf of interest to many readers.

A. G. Croft

Developments in Food Colours—2
Edited by John Walford. *Developments Series.* Pp. x + 257. Elsevier Applied Science. 1984. Price £28. ISBN 0 85334 244X.

The first volume in this series covered the history of the use of colours in foods and included chapters on synthetic colours and some of the more important natural colours that are used in the industry. The second volume represents a natural progression dealing with subjects that could not be included in the first volume.

The first chapter, by Lynne Parker, covers the important subject of worldwide legislation in the use of colours in foods, including an interesting history of the subject. It is clear from the first chapter that regulations differ widely from country to country, so that the second chapter dealing with analysis of colours is particularly welcome. G. Wadds is to be especially congratulated on the depth of knowledge that he brings. Analysts will be especially pleased that detailed analytical methods are presented to the reader.

A further chapter describes the influence of colour on customer choice and acceptance of food. The considerable influence of colour on apparent taste thresholds is particularly revealing. The fourth chapter reviews the history of synthetic food colours in North America and includes sections on specifications and certifications together with legal and toxicological aspects.

A. J. Taylor is the author of the next chapter, dealing mostly with novel sources of natural colorants. Two authors from the British Industrial Biological Research Association discuss toxicological aspects of food colours with particular reference to allergy. The book closes with a chapter on future trends in the use of food colours.

Libraries who already have a copy of the first volume will need to add the second book to their shelves. It will be in considerable demand as both a reference and source book on food colours. Many companies are likely to find a need to purchase two copies in order to provide laboratory workers with a second book for their own practical work. The publishers should be urged to consider a third volume in the future to cover some practical aspects of the subject, including the analysis of natural colours in foods.

M. J. Saxby

Treatise on Analytical Chemistry. Part 1. Theory and Practice. Second Edition. Volume 10. Section I. Magnetic Field and Related Methods of Analysis
Edited by Philip J. Elving, Maurice M. Bursey and I. M. Kolthoff. Pp. xxx + 533. Wiley-Interscience. 1983. Price £61.75. ISBN 0 471 89688 8.

This reviewer knew, and occasionally used, Kolthoff's Treatise in earlier days and he was interested to see how it had grown and expanded. He was disappointed in what he found. Clearly its expansion has caused problems so that only volumes 1, 2, 3, 5, 7, 10 and 12 of the Second Edition of Part 1, "Theory and Practice," are so far in print. This book is Volume 10, Section I. The important subject title, "Magnetic Field and Related Methods of Analysis," regrettably does not appear on the dust cover, the cloth cover, the first or second title pages or anywhere on the page relating to copyright and cataloguing; it can eventually be found on the third title page. This book (Section I) contains six chapters covering NMR of ^{1}H, NMR of ^{13}C, ESR, quadrupole resonance, secondary ion mass spectrometry (SIMS) and Mössbauer spectroscopy. SIMS is hardly a related technique, whereas NMR of other nuclei, the natural bedfellow, was not written in time and is promised for Volume 11. The probable contents of Volume 10, Section II, are not divulged.

The subjects are by now well known generally and this review is too short to comment on the individual chapters separately; each is a reasonable free-standing review in its own style. However, this book is not proffered as an "annual advances" type of series but rather as part of a coordinated, authoritative, expensive Treatise, and greater uniformity of presentation and understanding of the needs of analytical chemists could be expected. Even minor matters, such as ensuring that all spectra contained a scale, are not covered, as exemplified by Figs. 1.32, 2.8, 3.21 and 4.18, amongst others. No uniformity of units, let alone SI, is imposed and Table 3.I, giving some fundamental constants, quotes e (called the charge of the electron rather than the proton) in e.s.u., the approximate conversion factor of which to coulombs will not be known to many readers in 1984. The same table also had a value of the nuclear magneton that is too high by a factor of 10. Not all Americans or even Europeans will recognise J as the German symbol for iodine (Jod) in Fig. 4.4. Blemishes of this kind can always creep in, but the late Felix Bloch, Nobel Laureate as co-founder of NMR, surely deserves better than consistently to be called Block throughout a whole chapter and the index.

More important is the difficulty for a casual reader in finding specific items. Such a reader interested in quantitative analysis might find page 212, with its section heading "Quantitative Analysis by ^{13}C NMR." There are only three pages of text and four figures, of which the last three look very alike. They refer to different detailed pulse sequences and their relative merits are not evaluated. Indeed, they refer to a pure compound and refer to the number of ^{13}C in each site in natural abundance and not to quantitative evaluation of a mixture. Similarly, one page altogether covering fuels, lipids, other biomolecules and polymers conveys very little information. This section, and indeed the whole book, is lacking in details concerning accuracy, precision, capital costs, running costs, suitable states of matter, amount of material, concentration required, time required, suitability for industrial sites, incorporation in automated control loops, quality of staff required, merits and demerits of the method and much else that this reviewer would expect to extract conveniently from a "Treatise on Analytical Chemistry." If this series is to retain a major position in analytical chemistry it needs, in my view, a re-think of its objectives and tighter editorial control to see that they are achieved.

D. H. Whiffen

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