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THE ANALYST

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ANALYTICAL SCIENCES MONOGRAPH No. 3

Pyrolysis — Gas Chromatography

by R. W. May, E. F. Pearson
and D. Scothern

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Summaries of Papers in the Issue

Development and Evaluation of Selected Assays for Drugs and Drug Metabolites in Biological Materials

An over-all strategy for developing and evaluating assays for drugs in biological materials is described. Each stage, *i.e.*, starting points, nature of the biological sample, characterisation of the compound, choice of the analytical method, selection of an internal standard, isolation procedure, calibration, evaluation and quality control, are discussed separately. Chromatographic techniques, which are usually preferable to approaches not involving a separation step, such as competitive binding assays, are emphasised. Some controversial points concerning the use of internal standards are discussed and special approaches for calibration, including an outline of assay criteria to be evaluated, are presented. The general principles are illustrated with examples from the authors' own experience.

Keywords: *Drug assay*; *biological materials*; *assay strategy*; *chromatography*; *assay evaluation*

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Analyst, 1981, **106**, 1025–1035.

Monitoring Exposure to Toxic Gases in Workplace Atmospheres

The basis, objectives and shortcomings of personal monitoring as a means of both estimating the level of exposure and preventing the occurrence of over exposure to airborne toxic substances are discussed. Techniques that are available, and the circumstances in which they are suitable are reviewed along with recent new developments and possible future alternative approaches.

Keywords: *Toxic gas monitoring*; *workplace atmospheres*; *health and safety*

D. T. COKER

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Analyst, 1981, **106**, 1036–1041.

Analysis of Additives and Process Residues in Plastics Materials

Analytical problems, compounded by the increasing number and variety of additives used by the plastics industry and the necessity to reduce process residues and contaminants to the minimum, are described. An outline is given of general analysis schemes in use for the examination of PVC compounds, polyolefin compounds and acrylics. This is followed by some specific examples chosen particularly to illustrate the integrated approach, utilising chemical, physical and complex instrumental methods, that is required to solve some of the more difficult analytical problems. Methods used include solvent extraction, titration, ultraviolet, visible and infrared spectrophotometry, X-ray fluorescence, gas chromatography, liquid chromatography, thin-layer chromatography and mass spectrometry. The methods are often used in combination.

Keywords: *Plastics analysis*; *additives*; *process residues*

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Analyst, 1981, **106**, 1042–1056.

Various Applications of Functional Group Analysis

The importance of functional group analysis is demonstrated with a number of examples in various fields. Special attention is given to the determination of end groups in several kinds of high and low relative molecular mass materials.

Methods for the determination of the following groups are described: hydroxyl groups (polymers, esters), carboxyl groups (polymers, ester-interchange products), unsaturated groups (vinyl ester end groups in polymers), anhydride groups (polymers), amino groups (polymers, derivatised silica materials), epoxide groups (resins, derivatised silica materials) and quaternary ammonium compounds (surfactants).

The methods include derivatisation procedures and several techniques for quantitative determination (spectrophotometry, X-ray fluorescence, coulometry and potentiometric and photometric titration).

Keywords: *Functional group analysis*; *polymers*; *derivatisation*

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Analyst, 1981, **106**, 1057–1070.

Aspects of the Analysis of Drugs and Drug Metabolites by High-performance Liquid Chromatography

The biotransformation of a drug may result in a diversity of metabolites with widely differing physico-chemical properties. With the aid of suitable examples the applicability of adsorption, partition and paired ion high-performance liquid chromatography to the measurement of drugs and their metabolites is discussed in relation to their lipophilicity and polarity.

Keywords: *Drug analysis*; *drug metabolite analysis*; *high-performance liquid chromatography*

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Analyst, 1981, **106**, 1071–1075.

Determination of Mercury Vapour in Air Using a Passive Gold Wire Sampler

The determination of atomic mercury vapour in air in the range 10–120 μg m^{-3} is described. Mercury vapour was collected by exposing a 1-cm length of gold wire to the air for 5 min, then thermally desorbed from the wire for measurement by atomic-fluorescence spectrometry. The results obtained showed good agreement with those obtained from an acid permanganate wet sampling system.

Keywords: *Mercury determination*; *air analysis*; *gold wire*; *atomic-fluorescence spectrometry*

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Analyst, 1981, **106**, 1076–1081.

Field Method for the Determination of Aromatic Primary Amines in Air. Part I. Generation of Standard Atmospheres of Amines

A generator for the production of standard atmospheres of eleven aromatic amines, at very low concentrations, is described. The concentration of the amine produced is determined by gas - liquid chromatography of the heptafluorobutyrate derivative using electron-capture detection.

Keywords: *Aromatic primary amine determination*; *air*; *standard atmosphere*; *gas - liquid chromatography*

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Analyst, 1981, **106**, 1082–1087.

Field Method for the Determination of Aromatic Primary Amines in Air. Part II. Development of a Sensitive Field Test

A simple field test for primary aromatic amines in air is described in which the sample is drawn through an impregnated paper to form a coloured stain produced by the reaction of aromatic primary amine groups with 4-(dimethylamino)cinnamaldehyde. The test is sensitive to about 10 ng of amine and has been found to be applicable to eleven different amines. It does not distinguish individual compounds and can be affected by airborne alkaline or acidic compounds.

Keywords: *Aromatic primary amine determination*; *air*; *field method*

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Analyst, 1981, **106**, 1088–1095.

Determination of Gold in Tissue and Faeces by Atomic-absorption Spectrophotometry Using Carbon Rod Atomisation

A simple and accurate atomic-absorption spectrophotometric method is described for the measurement of gold in tissue and faeces following chrysotherapy. Samples and standards are disrupted with a quaternary ammonium hydroxide solubiliser in toluene, diluted with isobutyl methyl ketone, and analysed directly using a carbon rod atomiser. The detection limit for gold is 50 ± 25 pg and the calibration graph is linear up to approximately 3 ng. Concentrations of gold in tissue determined by this method correlate well with results obtained by flame atomic-absorption spectrophotometry and by neutron-activation analysis.

Keywords: *Gold determination*; *carbon rod atomic-absorption spectrophotometry*; *tissue analysis*; *faeces analysis*

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Analyst, 1981, **106**, 1096–1102.

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The Analyst

Development and Evaluation of Selected Assays for Drugs and Drug Metabolites in Biological Materials*

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An over-all strategy for developing and evaluating assays for drugs in biological materials is described. Each stage, *i.e.*, starting points, nature of the biological sample, characterisation of the compound, choice of the analytical method, selection of an internal standard, isolation procedure, calibration, evaluation and quality control, are discussed separately. Chromatographic techniques, which are usually preferable to approaches not involving a separation step, such as competitive binding assays, are emphasised. Some controversial points concerning the use of internal standards are discussed and special approaches for calibration, including an outline of assay criteria to be evaluated, are presented. The general principles are illustrated with examples from the authors' own experience.

Keywords: *Drug assay*; *biological materials*; *assay strategy*; *chromatography*; *assay evaluation*

There is a continuing increase in the number of papers being published in analytical chemistry.[1] However, analytical work, especially that of a clinical nature, is still the subject of misunderstandings and prejudices. These are attributable to a striking ignorance by many scientists of the basic difficulties involved in the analysis of drugs and endogenous compounds in biological materials, and especially the difficulties imposed by the complexity of biological matrices.

Measurements can rarely be applied directly to blood or urine and extensive sample pretreatment and/or chromatographic separation are usually required. Unlike purely "chemical assays," the drug might not be directly available but tightly bound to proteins or other macromolecules. Losses inevitably occur during isolation and require compensation by use of appropriate internal standards. In clinical assays more thorough evaluation of assay criteria is needed than with "chemical assays" because the results are crucial in the interpretation of pathological states and might be used in the diagnosis and treatment of patients. It is therefore important that each assay should be capable of measuring therapeutic levels of a drug in a realistic matrix, *e.g.*, serum or urine, and not simply for artificially high concentrations in an unreal medium such as aqueous solutions.

In this paper, we discuss our basic strategy for the development of new assays for drugs and possible metabolites in biological materials in terms of the different steps in the analytical approach, illustrated with selected examples from our experience. In this regard it should be remembered that a drug assay should always be dynamic in nature and subject to continouous modification and improvement during its application.

Development of a Drug Assay

Starting Point

A thorough definition of the need to set up a new drug assay should always precede experimental work. Requests from physicians or drug companies should be extensively docu-

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* Plenary Lecture presented at the Joint NL – UK Symposium on Quantitative Organic Analysis, Noordwijkerhout, The Netherlands, April 22–24, 1981.

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mented by an exhaustive computerised literature search. Assays for proposed new drugs are essential with a view to establishing protocols for registration. Procedures for determining levels of the compound in serum, urine and tissues are required in order to evaluate its fate in the organism, in terms of pharmacokinetics and metabolism.

Therapeutic drug monitoring is a field that is steadily gaining interest. Uncontrollable irregularities in the absorption and distribution of drugs often result in poor bioavailability and hence unsatisfactory therapeutic response. In such cases, monitoring of plasma levels in treated patients is necessary to optimise dosage schedules. Drugs that require therapeutic drug monitoring include compounds with a narrow therapeutic index, *e.g.*, cytostatics, and substances that are apt to produce serious side-effects when their plasma levels reach a toxic range, *e.g.*, antiarrhythmics. Certain pathological conditions such as renal diseases enhance the above risks because of drug accumulation and warrant close monitoring of patients. Apart from clinical considerations, the originality of the analytical problem itself should also be defined, as existing methods are often unsatisfactory and need major improvements to sample size, sensitivity, precision, specificity and analysis time.

The most logical approach in the developmental stage of an assay involves opposite chronology to the final protocol. Although isolation obviously precedes the actual measurement of a compound, it can be more convenient to develop the analytical technique first. This is followed by selection of the internal standard and development of the isolation procedure.

Nature of the Biological Sample

Several analytical problems and interferences from endogenous compounds can be anticipated and dealt with if the normal constitution and the peculiarities of the biological matrix are known and understood. However, dramatic changes in the matrix may occur in pathological conditions. As an example, samples from uraemic patients, an important group for which drug monitoring is often essential, contain numerous unidentified substances that are absent in normal humans.

In addition to potential interferences, attention should be paid to the possible binding of drugs by macromolecules. With the exception of saliva and urine, most biological materials contain at least two forms of a drug, *i.e.*, free and protein bound. Occasionally, binding to other macromolecules or other interactions, such as chelation, may occur. Concern with the phenomenon lies in the fact that only "free" drugs are pharmacologically active. However, nearly all assays determine "total" drug contents and to achieve this, special measures are often needed to release the compound quantitatively from its binding sites.

Removal of proteins and other substances such as lipids, salts and pigments (urine) is also obligatory in order to protect equipment, *e.g.*, columns, from deterioration. Finally, particular samples including tissues and faeces pose specific problems with respect to heterogeneity and interferences.

Characterisation of the Compound to be Assayed

The selection of a particular analytical technique and isolation method is dependent on the physico-chemical characteristics of the drug to be assayed. These include pK values, lipophilicity, *e.g.*, expressed as partition coefficient for octanol - water at different pHs, solubility in aqueous and organic media, polarity (presence of functional groups), and stability towards air, light, heat and pH conditions. Spectral characteristics [ultraviolet (UV) maxima, molar absorptivity], are also important.

As mentioned above, biological factors such as binding are significant for sample preparation and a knowledge of pharmacological properties can be extremely valuable. Therapeutic levels in blood and urine are also useful data for establishing the sensitivity requirements of the procedure. Finally, information about other drugs that may be co-administered will contribute to predicting expected interferences.

Choice of Analytical Method

For each drug assay, the correct choice of analytical methodology is a challenge as, in general, few methods have equivalent performance. Although many techniques are available, only a few are adequate from the standpoint of sensitivity and specificity.

Chemical techniques without a separation step

Chemical methods not involving a separation step include spectrophotometry and fluorimetry but, although convenient, both approaches have poor selectivity. Endogenous interferences are likely to occur and it is often impossible to distinguish parent compounds and metabolites. In addition, spectrophotometry, unlike fluorimetry, usually lacks the sensitivity required to determine realistic drug levels.

Competitive binding assays

The Yalow and Berson principle of competitive binding[2] was initially applied only to the determination of biopolymers, such as proteins. Organic molecules of low relative molecular mass, *e.g.*, drugs, have to be covalently linked to proteins to generate immunogenic properties. According to the nature of the protein that couples with the drug-hapten in the competitive reaction, those assays can be classified into immunoassays, using drug-specific antibodies, and competitive binding assays (CPB), using a variety of other proteins.

Radioimmunoassays (RIA) employ radioactively labelled ligands and involve separation of antibody-bound and free labelled drug-hapten. Enzyme immunoassays (EIA) are based on labelling drug-haptens with specific enzymes and can be further subdivided into so-called heterogeneous (ELISA) and homogeneous (EMIT) procedures. Other immunoassays, *e.g.*, fluorescence immunoassay (FIA), are also being developed. In other competitive protein binding assays, competition occurs between unlabelled drug and tritium-labelled tracer for drug target proteins.

More and more drugs are now analysed on a routine basis using the above techniques, partly as a result of tremendous commercial pressure and publicity. The widely available ready-to-use kits allow rapid processing, often without any sample pre-treatment, of large numbers of samples with relatively high sensitivity and can be handled by technicians without special training. However, the analytical specificity and hence accuracy of many commercially promoted assays is sometimes questionable. Structurally related and even other compounds can interfere significantly, *e.g.*, the cross-reactivity of diphenylhydantoin[3] and diazepam[4] in the protein-binding assay of thyroxine (T_4). The stereochemically structural similarity between those drugs and T_4 was discovered following X-ray crystallographic examination.

In addition, although external quality control surveys show that within-laboratory variabilities of immunoassays are controllable, between-laboratory precision is usually extremely poor. Variations are ascribed to the unpredictable quality of antisera, the properties of which are difficult to define. Combination of competitive binding assays with high-resolution separation techniques, *e.g.*, high-performance liquid chromatography (HPLC), can yield significant improvements in analytical performance. The chromatographic step enhances the specificity, while the sensitivity of the immunoassay is maintained, as seen for the RIA determination of certain peptides.[5]

Chromatographic methods

Recent developments in column chromatography have led to the present popularity of HPLC. Microparticulate packing materials and the development of stable bonded phases have improved the efficiency of column separations tremendously. Reversed-phase materials show great potential for the analysis of drugs in biological materials, as polar compounds are generally not retained on this type of stationary phase and will therefore not interfere with the analysis of more lipophilic drugs.

The sensitivity of the currently available detectors (UV absorbance, fluorescence, electrochemical) is insufficient for ultratrace analysis, but the use of new detectors (such as laser fluorescence) may eventually overcome this drawback. Detectors based upon direct coupling of liquid chromatography with mass spectrometry (LC - MS) are being tested but so far they do not show the same promise as their gas chromatography - mass spectrometry (GC - MS) counterparts.[6]

There is frequently ignorance on other ways of enhancing the intrinsic sensitivity of an HPLC system. As the detectability of a compound is a function of the peak height, which itself is directly related to the degree of dilution of the compound in the column, the use of shorter columns with smaller internal diameters than most commercial columns generally results in improved sensitivity. The theoretical concepts underlying this effect have been thoroughly described by Karger *et al.*[7] Contrary to some beliefs,[8] columns with reduced dimensions can be packed easily. In this respect, we found a 15 × 0.32 cm i.d. column to be a satisfactory compromise in terms of sensitivity, efficiency and loadability. Finally, HPLC is ideally suited for the analysis of unstable compounds: no derivatisation is necessary and separations can be carried out at room temperature, thus preventing possible thermal degradations.

Despite the growing importance of HPLC, gas chromatography (GC) continues to be of value.[9] Its inherent disadvantages, *i.e.*, obligatory derivatisation of polar compounds lacking sufficient vapour pressure and potential thermal instability of certain compounds, are largely compensated for by sensitivity and specificity.

Capillary GC offers excellent resolution, unprecedented in chromatography, and is suitable for quantitative work. However, its performance is strongly affected by technological factors, specifically the configuration of the injection device[10] and the quality of the columns used. Instruments specially designed for use with capillary columns are essential for high-performance work and have only recently appeared on the market. Nitrogen - phosphorus and electron-capture detectors provide greatly improved sensitivity and selectivity for particular compounds. The sophisticated GC - MS approach is capable of further advancing the frontiers in this respect and quantitative GC - MS has become an important tool in drug analysis.[11]

Selection of an Internal Standard

Most errors in drug analysis occur during extraction, or during subsequent evaporation of organic solvents and derivatisation.[12] Suitable internal standards should minimise these errors by compensation, provided that they are added at the earliest possible stage. A basic requirement is close structural similarity with the drug to be assayed. Too often, however, internal standards do not bear any relationship to the drug of interest, and this practice should be strongly discouraged.

In GC - MS, where selected ions of the drug and an internal standard are monitored, choice of the latter is critical. Labelled analogues are often preferable (isotope dilution MS), but there is controversy as to the superiority of this kind of internal standard.[13] Although it behaves chemically like the unlabelled compound, the position of the label may affect some of its properties, *e.g.*, p*K* values. Also, the MS fragmentation pattern may undergo changes due to an isotope effect. It is common practice to add a labelled analogue as a carrier to prevent losses of low amounts of unlabelled compound, *e.g.*, by adsorption.[13] This effect is well documented in radiochemistry,[14,15] but its relevance in isotope dilution MS is questionable at present. In no way should the use of a carrier make up for the neglect of other measures to bring an assay under control. Further, labelled analogues are usually contaminated by some unlabelled substance, which has a systematic contributory effect.

For all of these reasons, some workers claim homologues to be superior, even for GC - MS work. Frequently, a homologue will yield fragment ions with the same m/z value as the drug of interest, thus allowing single-ion monitoring. Usually, they are more readily available or cheaper to prepare than labelled analogues.

In general, the use of an internal standard belonging to the same chemical class as the drug of interest often presents more problems than is commonly assumed. Even when there are close structural similarities, such as with chemical homologues, pronounced differences in physico-chemical behaviour may arise, as exemplified later.

Isolation Procedure[16]

Prior to analysis, the drug has to be isolated from its biological matrix by methods depending on the nature of the sample and the analytical method chosen. Sample pre-treatment is also usually needed to remove interfering substances and to protect equipment from deterioration. Concentrating the extract to a small end-volume may be helpful in increasing sensitivity.

Conventional approaches for liberating a drug from binding proteins include precipitation with acids or organic solvents, dialysis and extraction of the drug in combination with salt addition and/or pH adjustment. Even a simple dilution step may be sufficient to dissociate complexes.

The commonest way of isolating drugs from a biological medium is by double-phase solvent extraction. Selection of a suitable solvent depends on extraction efficiency and selectivity, the latter with respect to co-extraction of interferents. Partition of a drug into an organic solvent is favoured by lipophilicity, which is at its maximum in the un-ionised form and is pH dependent. Compounds of high polarity are difficult to extract and require strongly polar, and hence non-selective, solvents. Sometimes, salting-out may increase the efficiency, but a special approach to extract charged species consists of adding a counter ion and extracting the lipophilic ion pair formed.

An alternative to solvent extraction for polar drugs is the use of a column of alumina or ion exchanger. Interfering materials are washed out prior to elution of the compounds preferentially with an organic solvent. The technique is usually characterised by nearly quantitative recoveries and excellent reproducibility. A less frequently used but less reliable variation to this approach is adsorption on to solids, *e.g.*, charcoal, directly suspended in the sample.

Calibration

Samples of drug-free material (serum, urine), containing known drug concentrations, should always be employed for calibration. Alternative "external" approaches based on assaying or even just injecting (in the case of chromatography) pure standard solutions are unacceptable because they ignore the specific peculiarities of the biological matrix. However, even drug-supplemented samples are subject to criticism, as an externally added drug might indeed not be present in the same physico-chemical state as an *in vivo* delivered drug.

In chromatography, peak-height or peak-area ratios (compound to internal standard) are usually plotted against concentration. The calibration graphs are calculated by linear regression analysis but in order to avoid inaccuracies near the origin weighted regression analysis should be performed. This follows because carrying out linear regression of y upon x implies, among other constraints, that there is no variance in x and a constant variance in y.[17]

In isotope dilution MS, non-linear calibration graphs are frequently obtained. Some workers propose special approaches to extend the linear range artificially,[18] but much greater accuracy is obtained by calculating the actual non-linear calibration graph by using weighted polynomial regression analysis.[19,20] A computer program calculates the different polynomials by means of matrix mathematics and the residual variances around the curves are used in an F-test to select the best fitting polynomial. This approach has been applied in our laboratories to isotope dilution MS in general and to radio-ligand assays of thyroid hormones and CPB of 25-hydroxycholecalciferol.[20]

Evaluation

Several criteria have to be evaluated in order to check the reliability and the over-all performance of an assay. Problems involved in linearity and calibration have been discussed above.

The extraction recoveries of the drug and internal standard provide useful information and can be calculated either by running radioisotopically labelled compounds through the procedure or by assaying drug-supplemented samples by an "external" calibration method.

Assessment of accuracy is a more fundamental problem because the "true" value can never be known with absolute certainty. Various parameters such as linearity and recovery indirectly provide some information about the accuracy. Comparison of the assay with other existing methods, *e.g.*, a reference method, is also very relevant.

Precision or reproducibility of an assay is defined as the coefficient of variation (relative standard deviation) of the results at a certain drug concentration. Both within-day and between-day reproducibilities can be evaluated. Sensitivity is usually expressed as the detection limit obtained under the conditions used.

The issue of analytical specificity has already been alluded to. Drugs that are therapeutically combined with the drug of interest should be checked for possible interferences or cross-reactivity. Blank samples should be included in each run in order to detect possible endogenous interfering substances. The term analytical specificity also covers more fundamental aspects. For example, in GC - MS, careful selection of ions to be monitored may affect the specificity considerably. Two comments can be made in this respect. It has been shown that the relative molecular masses of known compounds are not randomly distributed but that the frequency of occurrence is maximal between values of 200 and 350. Moreover, most compounds possess even rather than odd relative molecular masses (ratio 4 : 1). Millard[21] states that, as a result, maximum specificity will be obtained by selecting ions whose masses exceed roughly 350. Also, fragment ions with even instead of more common odd m/z values should be preferentially monitored.[21]

Quality Control

Routine drug assays should be regularly checked for reliability by using internal quality control schemes. This involves the preparation of serum pools to which known amounts of drug are added, but it is essential that the samples thus prepared can be stored for long periods under conditions of stability. With each determination on patients' samples a control sample is also analysed and the result is interpreted in terms of established limits of variation.

Inter-laboratory surveys on a similar basis are currently organised by different institutions and are often imposed by law. Control samples are distributed to the laboratories participating in such an external quality control programme and the results are statistically evaluated. The status of quality control in the UK has recently been reviewed.[22]

Applications

The above basic strategy has been used for many years in our laboratories for the development of new specific assays for drugs and metabolites in biological materials. This is illustrated below by three practical examples, *viz.*, the determination of bromhexine, doxycycline and 5-fluorouracil, 5-fluorodeoxyuridine and 5-fluorouridine. Each step is described in terms of the general principles discussed above.

Determination of Bromhexine in Plasma

Starting points

Bromhexine is a mucolytic drug that is frequently combined with antibiotics in the treatment of respiratory infections. Rapid metabolism probably accounts for the extremely low circulating levels of the drug in plasma. No pharmacokinetic parameters were available hitherto, mainly because of the lack of suitable analytical methods. Consequently, the recommended dosage schedule could be considered as unreliable. Hence, a highly sensitive technique was required to deal with this analytical challenge.

Characterisation of the drug

Chemically, bromhexine is a hydrochloride of a basic substance, containing two bromine substituents and a primary amino group in its structure. The free base is reasonably lipophilic and soluble in non-polar organic solvents.

Analytical techniques

The choice of GC as the analytical technique was straightforward for several reasons. A chromatographic method was desirable to separate the parent drug from metabolites and interfering endogenous compounds. HPLC with UV detection was dismissed in view of the relatively poor chromophore of the molecule and the extremely low concentrations to be detected. The low polarity of the molecule makes it very suitable for GC but, although it can be chromatographed without derivatisation, trifluoroacetyl derivatives were prepared for reasons of selectivity.[23] As the compound contains electronegative groups, an electron-capture detector (ECD) (nickel-63) was chosen to ensure sensitivity. Details of the procedure can be found elsewhere.[24] Concentrations below the detection limit could still be measured by GC - MS with selected ion monitoring.[25] The same technique allowed the detection of different metabolites of the drug in urine. Structures were assigned for these on the basis of the recorded mass spectra of their trifluoroacetyl derivatives.

Internal standards

A previously described GC method for determining the drug in milk and tissues of treated animals employed a structurally unrelated insecticide as an internal standard.[26] However, we synthesised various homologues of bromhexine[23] and selected the *N*-propyl derivative as an internal standard on the basis of its favourable chromatographic properties.

The GC - MS method[25] is based on the isotope dilution approach. Trideuterated bromhexine was synthesised for use as the internal standard. Ions at *m/z* 293 and 308 were chosen for monitoring the drug and the internal standard, respectively. Non-specific interferences from co-extracted endogenous substances prevented the use of ions at lower *m/z* values (112 and 115, respectively).

Isolation method

Bromhexine can be extracted from plasma with a non-polar organic solvent but alkalinisation is helpful as the free base is more lipophilic. Extraction was therefore carried out with hexane in sodium hydroxide - triethanolamine medium, the triethanolamine being necessary to avoid a low recovery of the internal standard. The same isolation method was applicable in the GC - MS technique.

Evaluation

The GC assay with electron-capture detection (ECD) was found to be linear over the two concentration intervals studied, *i.e.*, 1.6–12.7 and 7–115.8 ng cm^{-3}. These cover the therapeutic range for oral administration of the drug. An extraction recovery of 90.1 ± 5.7% ($n = 12$) was calculated for bromhexine in the 6.3–53.1 ng cm^{-3} range. The within-day coefficient of variation was 6% ($\bar{x} = 4.3$ ng cm^{-3}; $n = 8$) whereas the between-day value averaged 8.6% ($\bar{x} = 11$ ng cm^{-3}; $n = 13$). However, a substantial difference in the between-day reproducibility of both methods (GC - ECD and GC - MS) was found for levels below 10 ng cm^{-3}. The average coefficient of variation for the GC - ECD assay, as determined at five different concentrations, was 16% compared with 3.5% for the GC - MS method. The GC - MS assay was also clearly superior to the GC - ECD assay from the standpoint of sensitivity. The detection limit of the latter was 1 ng cm^{-3} but a more realistic sensitivity limit was 3 ng cm^{-3}. The GC - MS method permitted quantitation of down to 0.5 ng cm^{-3} in plasma.

Applications and conclusions

In order to obtain realistic pharmacokinetic data following the empirical dosage schedule, plasma levels as a function of time were studied in human volunteers[24] and dogs.[27] The results confirmed the low bioavailability of bromhexine. The GC - MS method was used to study the fate of the drug in horses[28] with similar conclusions on low bioavailability. As the existence of several metabolites was demonstrated, biotransformation probably causes the rapid disappearance of bromhexine from plasma. It was concluded that, provided that the metabolites are pharmacologically inactive, the recommended dosage regimen is subtherapeutic and should be readjusted.

Determination of Doxycycline in Serum, Urine, Tissues and Faeces

Starting points

Considerable doubts existed about the metabolism of doxycycline, a semi-synthetic tetracycline antibiotic. Some biological observations suggested the possibility of biotransformation but metabolites had not been isolated or analytically demonstrated. Hence, improved methodology for the analysis of this drug was desirable because existing procedures (fluorimetric, microbiological) lacked specificity.

Characterisation of the compounds

Unfavourable physico-chemical properties seriously complicate the analysis of this drug in biological materials. Tetracyclines are amphoteric, very soluble in water and almost insoluble in most organic solvents. Their polyfunctional structure accounts for a high degree of polarity, although doxycycline is more lipophilic than other members of the group. In addition, these compounds are unstable at different pHs (alkaline, weakly acidic). More promising from the analytical point of view is the presence of an extended chromophore (ϵ = 14000 l mol^{-1} cm^{-1} at 350 nm). In serum at therapeutic concentrations of a few micrograms per cubic centimetre, over 80% of the drug is reversibly bound to proteins.

Analytical technique

GC of the polysubstituted tetracyclines is difficult and inconvenient. Instability in basic media and the low chemical reactivity of some substituents prevent appropriate derivatisation. On the other hand, their favourable spectrometric characteristics led us to investigate HPLC with detection at 350 nm. Although tetracyclines display a more intense maximum at 270 nm, the former wavelength was chosen for reasons of selectivity.

Efficient liquid chromatography of doxycycline turned out to be possible only under rigorous conditions. Poor HPLC properties (tailing, low efficiency) are a direct consequence of the peculiar physico-chemical nature (amphotericity, polarity, low diffusion coefficient) of the compound. However, one particular reversed-phase material, *viz.*, an octyl-bonded phase, was superior to all other packing materials tested.[29] Classical solvent mixtures, such as water - methanol or water - acetonitrile, could not be used and the mobile phase had to be modified with acids for satisfactory efficiency. Maximum retention occurred at pH 2.1, where doxycycline exists in its mono-cationic form. Therefore, retention mechanisms can mainly be rationalised in terms of ionic, rather than hydrophobic, interactions.[30] Columns with reduced dimensions (10 × 0.2 cm i.d.) were used to optimise the sensitivity.

Internal standard

Three commercially available tetracyclines were tested for use as internal standards. They all eluted from the column more or less close to doxycycline but initially none of these derivatives proved satisfactory. Metacycline was not extractable by the adopted procedure (see below). 6-*epi*-Doxycycline, which most closely resembles doxycycline in structure, yielded approximately 20–30% lower recoveries; this was due in part to adsorption on to glass. Demeclocycline epimerised during evaporation of serum extracts in ethyl acetate. However, ascorbic acid inhibits this reaction and with this additive demeclocycline is acceptable as an internal standard.

Isolation method[31]

The polar and amphoteric nature of tetracyclines leads to substantial difficulties in their isolation by double-phase solvent extraction. Only moderately polar solvents, *e.g.*, ethyl acetate, were found to be applicable. Serum samples were buffered at pH 6 near the isoelectric point of doxycycline, where it displays minimal aqueous solubility. Only one buffer, an uncommon phosphate - sulphite system, yielded satisfactory, clean extracts. Sulphite is assumed to form conjugates with serum pigments and keep them in the aqueous phase. The high concentration of salts (2 M for both species) ensured dissociation of the drug - protein adducts and probably also contributed to the efficiency by a salting-out effect.

Evaluation

Linear relationships were observed over the therapeutic range (0–6 μg cm^{-3}). Within-day and between-day coefficients of variation were 1.8% ($\bar{x}$ = 2.5 μg cm^{-3}; n = 10) and 4.8% ($\bar{x}$ = 2.6 μg cm^{-3}; n = 9), respectively. The extraction recovery averaged 87.7 ± 4.3% (n = 12). A detection limit of 50 ng cm^{-3} was obtained, which is far beyond the range of therapeutic levels.

Application to other biological materials

In order to quantify doxycycline in heterogeneous materials such as tissues[32] and faeces,[33] a homogenisation step had to be included in the analysis. Hydrochloric acid is excellent for this purpose. Apart from giving a pH where doxycycline is stable, it has the additional advantage of dissociating chelates, especially as chelate formation between doxycycline and divalent cations may reduce the extractability of the drug from faeces. A pre-extraction stage with diethyl ether removes interfering lipids. Doxycycline was also assayed in a variety of tissues obtained from patients who had received the drug parenterally before surgery.

No modifications of the procedure were necessary to analyse urine samples for doxycycline and metabolites were not detected.[33]

"Free" doxycycline concentrations were measured in saliva. Although samples of this fluid can be injected directly on to the column, the doxycycline levels were too low to be detected this way and the normal extraction and concentration procedure was therefore followed.

A similar procedure, based on HPLC, was developed for the determination of minocycline, another semi-synthetic tetracycline.[34] Unlike doxycycline, this compound was found to be metabolised in humans, as several polar by-products were found in urine.[34]

Determination of 5-Fluorouracil, 5-Fluorodeoxyuridine and 5-Fluorouridine in Serum and Urine

Starting points

5-Fluorouracil (FU) and 5-fluorodeoxyuridine (FUdR) are useful cancer chemotherapeutic agents. Until recently, existing analytical methods lacked the sensitivity for studying the disposition of these drugs over an extended time period following administration. They are rapidly distributed and excreted, resulting in low circulating blood levels. Dosage regimens remained largely empirical and were not supported by actual pharmacokinetic data.

Characterisation of the compounds

The most striking physico-chemical property of FU and FUdR is their high polarity and the compounds act as weak acids. Protein binding is non-existent and therapeutic levels are often in the ng cm^{-3} range.

Analytical techniques

Several GC methods had been described for the determination of FU, but they all had serious disadvantages. They either lacked sensitivity, employed unsuitable internal standards or involved silylation of the compound, a reaction which is difficult to control. Few assay methods existed for FUdR. Several major improvements in the GC of these drugs were introduced. Stable butyl (FU)[35] and peralkyl (FUdR)[36] derivatives, showing excellent GC properties, were prepared. Sensitivity of the assays was greatly enhanced by use of a multiple ion detector (FU)[37] or a nitrogen - phosphorus detector (FUdR).[38] Another considerable gain in sensitivity and specificity was obtained in the FUdR analysis by replacing packed colums[38] with glass capillary systems.[39] Ultimate limits in this respect were reached in a new GC - MS method, based on chemical ionisation of the compound with ammonia gas.[40] The use of an epimeric form of FUdR as internal standard permitted single-ion monitoring, which further contributed to the excellent sensitivity of the procedure.

Internal standard

Close structural analogues of FU, *viz.*, 5-chlorouracil or ^{18}O-labelled FU (isotope dilution MS), were chosen as internal standards.[37] Similarly, 5-chlorodeoxyuridine was used for the FUdR determination,[38,39] whereas the 3′-epimer served as an internal standard in the CI - MS procedure.[40]

Isolation

Problems concerning the isolation of these compounds from aqueous media are obviously associated with their pronounced polarity. They are not readily extracted by double-phase extraction techniques unless very polar non-selective solvents are used. Column extraction overcomes these difficulties.[37,38] At pH 10, the compounds occur almost exclusively as mono-anions and are therefore retained on an anion-exchange resin. Subsequent elution was carried out with methanolic acetic acid. This approach was applied successfully to plasma samples.

The analysis of urine presented additional problems. The presence of an excess of salts was deleterious to both column performance and derivatisation and pigments also interfered with the chromatography. Two further clean-up steps were therefore introduced for the determination of FUdR in urine.[39] A barium salt precipitation served to remove inorganic sulphates and phosphates. After the anion-exchange chromatography, Sephadex LH-20 chromatography permitted further purification to remove some pigments.

A metabolite of FU and FUdR, 5-fluorouridine (FURD) (a ribonucleoside), was also relevant for the determination in urine.[41] *cis*-Diol-containing substances are known to be selectively retained on boric acid gels, and in a mildly alkaline medium a stable complex is formed between FURD and immobilised phenylboronic acid. Subsequent acidification dissociated this complex for elution of the compound to give sufficiently clean residues for use on the ion-exchange column.

Evaluation

The column extraction technique resulted in excellent recovery of the compounds, *e.g.*, 96.8 ± 2.4% (FU) and 93.2 ± 2.1% (FUdR). Even with additional clean-up steps the recoveries were still acceptable, *e.g.*, 70 ± 4% (FUdR in urine) and 68 ± 6% (FURD in urine). Within-day coefficients of variation for the FU and FUdR assays averaged 7.2% ($\bar{x}$ = 50 ng cm^{-3}; n = 5) and 4% ($\bar{x}$ = 297 ng cm^{-3}; n = 12), respectively. Corresponding between-day values were 8.4% ($\bar{x}$ = 50 ng cm^{-3}; n = 5) for FU and 6.2% ($\bar{x}$ = 295 ng cm^{-3}; n = 20) for FUdR. The detection limit of the GC - MS approach for FU was estimated to be 2 ng cm^{-3}, whereas a value of 50 ng cm^{-3} was obtained for the determination of FUdR in plasma using a nitrogen-selective detector. Serum FUdR concentrations down to 1 ng cm^{-3} could be measured by the CI - MS procedure.

Conclusion: Future Prospects

Limitations of analytical methods for the determination of drugs in biological materials mainly concern specificity, accuracy and sensitivity. Specificity can be improved either by more complex sample pre-treatment, with consequent additional sources of error, or by using more sophisticated equipment. The ultimate goal should be analysis without sample preparation.

Much progress has been made in enhancing sensitivity and detection limits of assays are steadily improving.[42] Whereas the detectability of drugs in biological media was confined to the nanogram range some years ago, current ultra-trace analysis can be applied to amounts as low as a few picograms or occasionally even femtograms (10^{-15} g). However, as pointed out elsewhere,[42] with the current rate of progress it would take at least another 20 years to reach the 10^{-23} g level. Whether or not the ideal of detecting one single molecule will be accomplished within reasonable time is still speculative.

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Monitoring Exposure to Toxic Gases in Workplace Atmospheres*

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The basis, objectives and shortcomings of personal monitoring as a means of both estimating the level of exposure and preventing the occurrence of over exposure to airborne toxic substances are discussed. Techniques that are available, and the circumstances in which they are suitable are reviewed along with recent new developments and possible future alternative approaches.

Keywords: *Toxic gas monitoring*; *workplace atmospheres*; *health and safety*

Whenever volatile substances are being manufactured, processed, transported or handled in any way in industry there is a possibility that employees may be exposed to vapours, and if this exposure becomes excessive then toxic effects may occur among those over-exposed. Most industrialised nations have safety and health legislation covering employees at work, which is designed to prevent damage to their health by imposing a statutory obligation on their employer to provide a healthy and safe workplace.

This paper will consider only how employers can fulfil this obligation with respect to preventing damage to their employees' health arising from over-exposure to toxic gases in the workplace.

Toxic Damage

It is important to note that all substances are toxic and their potential to damage the human body is a matter of their inherent toxicity and the dose the person receives. The dose is the amount of the substance that gets into the body and depends on four main factors:

(*i*) the concentration of the substance in the inhaled air;
(*ii*) the respiration rate of the person, which can vary by as much as five times between sedentary tasks and very strenuous tasks;
(*iii*) the exposure time;
(*iv*) the absorption factor, *i.e.*, the fraction of the total amount of a substance inhaled that is actually absorbed into the body. This varies considerably, water-soluble substances being more easily absorbed than insoluble ones. The pattern of exposure will also affect the absorption factor, as the amount absorbed from a continuous steady concentration will differ from that absorbed from a rapidly fluctuating concentration even though the average concentrations are the same.

Toxic damage may occur at one or more locations in the body and the degree of damage will be related to the concentration of the toxic substance or more commonly one of its toxic metabolites formed in the body, in the "at risk" or "target" organs. However, the degree of damage is dependent not only on the dose of the substance but also on individual susceptibilities, which may arise from many factors:

men and women may well be affected differently;
age may have an effect, often the young are more at risk;
the health of the person, their general fitness and such factors as fatness may have an influence;
pregnant women and the foetus are a special case;
individual variations in metabolism and body chemistry may influence the degree of toxic damage, particularly if the damage is caused by a toxic metabolite rather than the original substance;
the degree of toxic damage may be compounded by smoking, drinking alcohol, taking drugs or by exposure to other toxic substances.

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Protection of Employees

Ideally, the protection of employees' health against damage from toxic substances would best be achieved by monitoring the level of the substance or its metabolites in the target organs, but the problems of "invasive" sampling on a routine basis make this approach impractical. As a next-best option, the monitoring of body fluids, mainly urine and blood is used in certain instances, *e.g.*, blood analysis for lead and urinalysis for metabolites of trichloroethylene and benzene. There has also been a resurgence of interest in exhaled breath analysis for organic substances now that sampling and analytical techniques of sufficient sensitivity are available.

In practice, the most widely used monitoring technique is to measure the concentration of the inhaled air, sampled close to the mouth. This concentration is called the "exposure" and should not be confused with "dose." It is evident from the previous discussion that exposure will not give a very accurate assessment of the concentration of the toxic substance in the target organ as it will not account for variations in respiration volume and rate, absorption factors, exposure by other routes (dermal, oral) and non-occupational exposure. However, this is the system in widespread use and the rest of this discussion will review the principles and techniques available for personal exposure monitoring.

Air-quality Standards

Standards for acceptable levels of exposure to organic substances in the air breathed have been established and are usually referred to as Threshold Limit Values (TLVs),[1] which can be defined in several ways. All listed substances have an 8-h time-weighted average (TWA) concentration, to cover a typical workshift, but there are also 15-min averaged short-term exposure limits (STEL) and ceiling values, which should not be exceeded at any time, quoted for several substances. These different TLVs are applied to cover the different types of hazardous situation that may arise, and in general the type of TLV applied for a substance depends usually on the type of toxic effect that occurs at the lowest concentration. For instance, benzene will produce narcosis at very high concentrations, whereas at lower concentrations it does not cause narcosis but is irritating to the respiratory system; these are both acute or short-term effects. However, prolonged and repeated exposure over months or years to even lower levels can lead to damage to the blood and blood-forming organs, and in order to prevent this chronic toxic effect the TWA TLV would be applied.

Alternatively, with substances such as hydrogen sulphide there is no chronic effect from long-term exposure to low levels, only eye and throat irritation. At higher concentrations severe acute toxic effects ranging from headaches to coma and death can occur. The TWA measurement is therefore of little use and a limit on the immediate concentration should be applied.

The scope and applicability of TLVs are clearly defined but frequently exceeded. They are personal exposure limits for healthy adults in the workplace but do not apply to levels in the home or outside environment or to the very old, children, sick, etc. They refer to an airborne concentration or level and can be applied to gases, vapours, dusts, mists, noise, radiation, heat and micro-organisms. In most countries (outside the USA) they are not statutory limits except in a few specific cases. In the UK they are published by the Health and Safety Executive and are qualified by four notes:

(*i*) TLVs are not sharp dividing lines between "safe" and "dangerous" concentrations;
(*ii*) the best working practice is to reduce the concentration of all airborne contaminants as far below the TLV as is reasonably practicable, whether or not they are known to present a hazard;
(*iii*) the absence of a substance from the list does not necessarily indicate that it is safe;
(*iv*) the application of any TLV in a particular situation should be interpreted by a trained occupational hygienist.

Monitoring Objectives

The prime purpose of legislation is the "protection of employed persons at work" to ensure that their health, well-being and working efficiency are not impaired. Any monitoring programme or system should be capable of protecting the employee, and this can be achieved in several ways. For protection against substances giving acute toxic effects, the monitoring instrument should be capable of warning the wearer before a hazardous level or the dose limit is reached. For protection against substances giving chronic toxic effects, the retrospective measurement of shift average exposures offers no direct protection from over-exposure but indicates the necessity for remedial actions such as engineering control or operational procedure improvements, or the need for personal protection.

Protection of the employee as a monitoring objective is directly connected with protecting the employer, as failure to demonstrate that workplace conditions are satisfactory can result in action by the Health and Safety Inspectorate, either in improvement or closure notices or in legal prosecution. Legal action can also be taken directly by employees against the employer and in extreme cases these can be for criminal negligence. Plant control is a secondary result of monitoring as increased exposure measurements may indicate leakages, incorrect operational procedures, etc. Keeping exposure records is also very important, both as a record of working conditions should some unexpected health effect arise years after exposure and to provide data for epidemiology where medical statistics are correlated with past exposure, which is ultimately the best test of the correct setting of a TLV.

Monitoring Location

TLVs are personal exposure limits, and when a person is moving around the workplace where there are typically considerable variations in airborne contaminant levels, then measurements on the person are necessary to obtain an accurate estimate of exposure. Monitoring at fixed locations within the workplace seldom gives a good correlation with personal monitoring unless the person does not move about or when contaminant levels in the workroom are reasonably uniform and constant. Fixed location monitoring is allowable as a means of estimating exposure, but there is then an onus to show that, firstly, some correlation exists between personal and fixed location exposure estimates and, secondly, that within the limits of that correlation the personal exposure limit has not been exceeded. In practice this means that to demonstrate compliance with a TLV from fixed location exposure estimates these need to be lower than personal exposure estimates to allow for the uncertainty in the correlation.

In general, therefore, personal exposure monitoring is to be preferred and fixed location monitoring is best suited to plant leakage control or as an early warning alarm for large emissions.

Monitoring Techniques

Different techniques are necessary to account for the toxic properties of different substances and the particular objectives of the monitoring.

Biological monitoring has many advantages, as previously discussed, but unfortunately also many drawbacks. Routine blood sampling presents obvious problems and urinalysis is complicated by the need to collect the samples at set times following the end of a workshift, which can be awkward. Further, there may also be some interferences from naturally occurring metabolites. However, exhaled breath can be quickly and easily sampled at the beginning of the next workshift and, as the contaminant and not its metabolites are measured, the occurrence of interferences is much less.

Spot measurements using detector tubes or direct reading meters can be useful for preliminary assessments of the type and approximate level of any airborne substances present in the workplace, which is necessary prior to designing a comprehensive monitoring programme. Other useful devices are continuous recording meters, which can be used to identify the areas or operations in the workplace that contribute most to the over-all exposure. These include portable flame ionisation or photoionisation detectors and the paper-tape monitors that comprise a cassette of paper tape impregnated with selective reagents that is wound slowly past an air sampling port. Any contaminant in the air flowing through the port produces a stain on the paper, which is quantified using reflectance spectrometry.

For acutely toxic substances where effects are related to the immediate concentration but no chronic effects occur, then personal hazardous level warning devices offer the best protection and shift average exposure measurement is of little use. There are many such instruments available commercially, which can be worn in an overall pocket and have an audio-visual alarm that can be set to give warning when an appropriate level is reached. These usually have rapid response electrochemical cells as sensors and are used typically for substances such as hydrogen sulphide, nitrogen dioxide, ozone, oxygen deficiency, chlorine and carbonyl chloride (phosgene). A similar type is also used for substances that are acutely toxic but where the dose is more important than the immediate concentration. These integrate the concentration with time and again trigger when a pre-set "dose" is reached. They are called dosemeters, which, strictly, is a misnomer as they measure the integrated concentration with time, not the dose received by the body. Typical applications for this type are for carbon monoxide and nitrogen oxide.

Monitoring for substances with mainly chronic toxic effects, which includes most organic substances, is by measurement of the average concentration over the workshift. This can be achieved by collecting the air directly into a gas bag or evacuated container but this approach has many drawbacks and so the most widely used technique is to remove the contaminant from the air using either a liquid bubbler or a solid adsorbent.

Bubblers have many disadvantages: they are clumsy to wear; there is a large dilution factor in the absorbent solution; air sampling rates need to be high so large pumps are required; and the sampling rate must be controlled to achieve complete absorption and avoid spray and evaporative losses. Because of these disadvantages, bubblers are usually only used for very high boiling, reactive or polar substances that cannot be quantitatively recovered from solid adsorbents.

Vapour adsorption tubes

The most commonly used technique for monitoring organic vapours is the vapour adsorption (VA) tube, which is simply a bed of adsorbent in a tube or container through which air is drawn. Any contaminant in the air is trapped on the adsorbent and held for subsequent analysis. The adsorbed contaminant is removed for analysis either by solvent extraction or by heating the adsorbent and purging off the desorbed vapour in an inert gas stream. Originally these were large, clumsy, glass tubes packed with silica gel, charcoal, etc., and they also required large air sampling pumps to attain acceptable detection limits owing to the large desorption solvent volume needed. In the USA, NIOSH developed the miniature charcoal-in-glass VA tube which quickly gained acceptance as it is small and unobtrusive and needs only a small, low flow-rate personal sampling pump.

The tubes are analysed by breaking and removing the two charcoal beds (one for sampling, one as back-up) into small vials. Desorption solvent (usually carbon disulphide), is added and the extract analysed, usually by gas chromatography.

This method also has disadvantages, including: the desorption step is time consuming and uses a toxic, flammable solvent; the sensitivity is limited by a dilution of the sample with the solvent of at least 100-fold; the solvent and any impurities interfere with the analysis; desorption is often incomplete; and the sampling tube is not re-useable. These disadvantages have led to the increasing popularity of heat desorption VA tube systems over the last few years. They use a metal tube with a single adsorbent bed of either charcoal (for permanent gases) or porous polymer bead adsorbents (*e.g.*, Porapak, Tenax, Chromosorb) for vapours from liquids. These are analysed using a heat desorber, which is attached to the analyser (gas chromatograph, infrared spectrometer, atomic-absorption spectrometer, etc.). For gas-chromatographic (GC) analysis, the desorber is connected into the carrier-gas line just before the column. The desorption procedure is to clamp the VA tube into the desorber, divert the carrier gas flow through it then flash-heat the tube in a heated metal block; the trapped vapour is rapidly released on heating and carried directly on to the GC column as a discreet slug in the carrier gas. Conditions can be easily arranged so that desorption can be made on to an isothermal column giving a very rapid analysis, eliminating sample preparation, the use of and interference from desorption solvents and giving a very sensitive determination, as sample dilution is also eliminated. An alternative procedure is to desorb the vapour into a reservoir from which aliquots can be withdrawn, allowing replicate analyses or the use of more than one type of analyser. The VA tubes are clean and ready for re-use after desorption.

Passive adsorption systems

Another recent development is the so-called "dose badge" or passive sampler. This is a vapour adsorption system that does not have air pumped through an adsorbent bed but has a layer of adsorbent covered by a stagnant air layer across which contaminants diffuse and are trapped by the adsorbent at a rate proportional to their concentration in the air, eliminating the need for a pump. Analysis of these is similar to that of the pumped tubes, both solvent and heat desorption types being available. These showed promise initially in laboratory studies but field evaluations have generally been less promising, as their accuracy is rather poor in field conditions probably owing to the rapid fluctuations in concentrations that commonly occur in the workplace.

Exhaled breath analysis

As previously mentioned, this technique is attracting much interest as an easier and more logical means of assessing workplace air quality than personal (inhaled air) monitoring but at present it is mainly in the experimental stage.

Its potential advantages over personal monitoring include both technical advantages, *viz.*, (*i*) exhaled breath (EB) sampling takes only 1–2 min of the subject's time and eliminates the necessity for wearing pumps and samplers throughout the workshift with their attendant problems of encumbering the subject, intrinsic safety and reliability, (*ii*) the subject can tamper with a personal sampler but not with EB sampling and (*iii*) personal samplers can become contaminated with water or liquid sprays, etc., in the workplace; and also the opportunity for the measurement of body burden, *viz.*, the effect of a toxic substance on the body is related to the dose the body receives but personal monitoring measures only the average airborne concentration.

There is a considerable amount of literature on the subject and techniques are reasonably established but the main problem is that standards are set in terms of airborne concentrations and EB analysis has yet to prove that it is a more logical and effective means of assessing the acceptability of workplace air quality.

Future Trends

Irrespective of whatever techniques may be used in the future, it is evident that the requirement to monitor the workplace atmosphere will become a routine part of plant management. This will probably be carried out more comprehensively than at present and will be common to most industrialised countries. Although both personal and fixed location monitoring are likely to be used, it is probable that personal monitoring will be the definitive approach, with multi-point fixed location plant monitors being used for plant leakage control and as a "watchdog" on the plant atmosphere.

The ultimate personal monitor could be envisaged as a highly selective, highly sensitive and stable detector cell incorporated into a small personal monitor comprising a power supply and voltage stabiliser and circuitry to detect when a hazardous level is reached and sound a bleeper and/or a flashing light (and perhaps also to transmit this warning to the plant control room via radio). Another circuit would record the measured levels continuously on solid-state memory and also integrate the level with time; these values would be examined at the end of the shift by connection to an external read-out instrument. The level of contaminant would also be displayed on a meter built into the monitor. The electronic technology for such an instrument already exists but it is doubtful if detector cell development will reach the level of sophistication needed for monitoring one organic substance in the presence of many others.

Personal monitoring using vapour adsorption systems is still likely to be the most commonly used technique in the forseeable future. It remains to be seen how much impact passive samplers will have, but with the increasing evidence of their inaccuracy under field conditions it seems unlikely that they will displace the more reliable and accurate pumped tube method to a significant extent. It is often said that good accuracy is not a prime necessity of personal monitoring, but with the trend toward lower TLVs and a tighter control over workplace air quality the requirement for accuracy is becoming more apparent. Further, the total real cost of monitoring in terms of manpower and resources is high and the cost saving in eliminating the pump is only a small fraction of the total. It is a shortsighted policy to diminish the value of monitoring data, measured in terms of the ability of an employer to demonstrate his compliance with standards, his competence in controlling workplace conditions and their possible use as a defence against legal action by an employee claiming that a health problem arose from his employers negligence by using monitoring methods of debatable accuracy and reliability.

It is to be hoped that the advantages offered by exhaled breath sampling will be explored and adopted in the future. It is unlikely however, that this will happen very rapidly as it involves a complete change in direction that may take many years to achieve.

Reference

1. American Conference of Governmental Industrial Hygienists, "Threshhold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment," American Conference of Governmental Industrial Hygienists, Cincinnati, 1978.

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Analysis of Additives and Process Residues in Plastics Materials*

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Analytical problems, compounded by the increasing number and variety of additives used by the plastics industry and the necessity to reduce process residues and contaminants to the minimum, are described. An outline is given of general analysis schemes in use for the examination of PVC compounds, polyolefin compounds and acrylics. This is followed by some specific examples chosen particularly to illustrate the integrated approach, utilising chemical, physical and complex instrumental methods, that is required to solve some of the more difficult analytical problems. Methods used include solvent extraction, titration, ultraviolet, visible and infrared spectrophotometry, X-ray fluorescence, gas chromatography, liquid chromatography, thin-layer chromatography and mass spectrometry. The methods are often used in combination.

Keywords: *Plastics analysis; additives; process residues*

The title of this paper introduces a very large subject, even if one considers only the more common plastics. The increasing number and variety of additives used by the industry and the necessity to reduce process contaminants to the absolute minimum for quality and/or safety requirements add daily to the analytical problems. Therefore, no attempt will be made to cover the whole field, but first an outline of the general analysis schemes in current use in the author's laboratory for PVC compounds, polyolefin compounds and acrylics will be given. This is followed by some specific examples chosen particularly to show the integrated approach utilising chemical, physical and complex instrumental methods that is required to solve some of the more difficult analytical problems.

It would be wrong to say that expensive and complex instrumentation is essential for many of the analyses requested, but its availability often speeds the solution of the problems and provides additional important information.

General Schemes

In the analysis of an "unknown" plastic, characterisation into a broad group is usually relatively simple, taking into account the origin of the sample, its use, appearance, elemental composition and, most important, its infrared spectrum. Much of the detail of this and the analytical schemes given in Figs. 1–3 is outside the scope of this paper, but further information is given in reference 1.

In the schematics shown in Figs. 1–3, the major points to note are as follows. Where identification, particularly of minor organic components, is required, then some separation from the plastic compound is often necessary. Special care and specialised techniques are required when dealing with laminates and surface-coated films. For major components, the separation is made quantitatively and the analysis is completed by gravimetric, titrimetric or spectrophotometric methods. For volatile components, separation, identification and quantification can often be carried out in one analytical process. Some additives and contaminants, having strong spectral characteristics, can be determined directly in a prepared sample without any separation stage, for example, the infrared analysis of film samples.

X-ray fluorescence is of particular value and is widely used when the composition contains characteristic elements of atomic number greater than 12. Direct analysis of samples in granular form is possible and Tables I and II show that even for light elements, *e.g.*, for

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organic phosphorus compounds in nylon, precision and sensitivity are reasonable when a thin polypropylene window is used to support the granules in the presentation container. With a variation in granule size from 0.026 to 0.050 g per granule, little change in the coefficients of variation was noted.

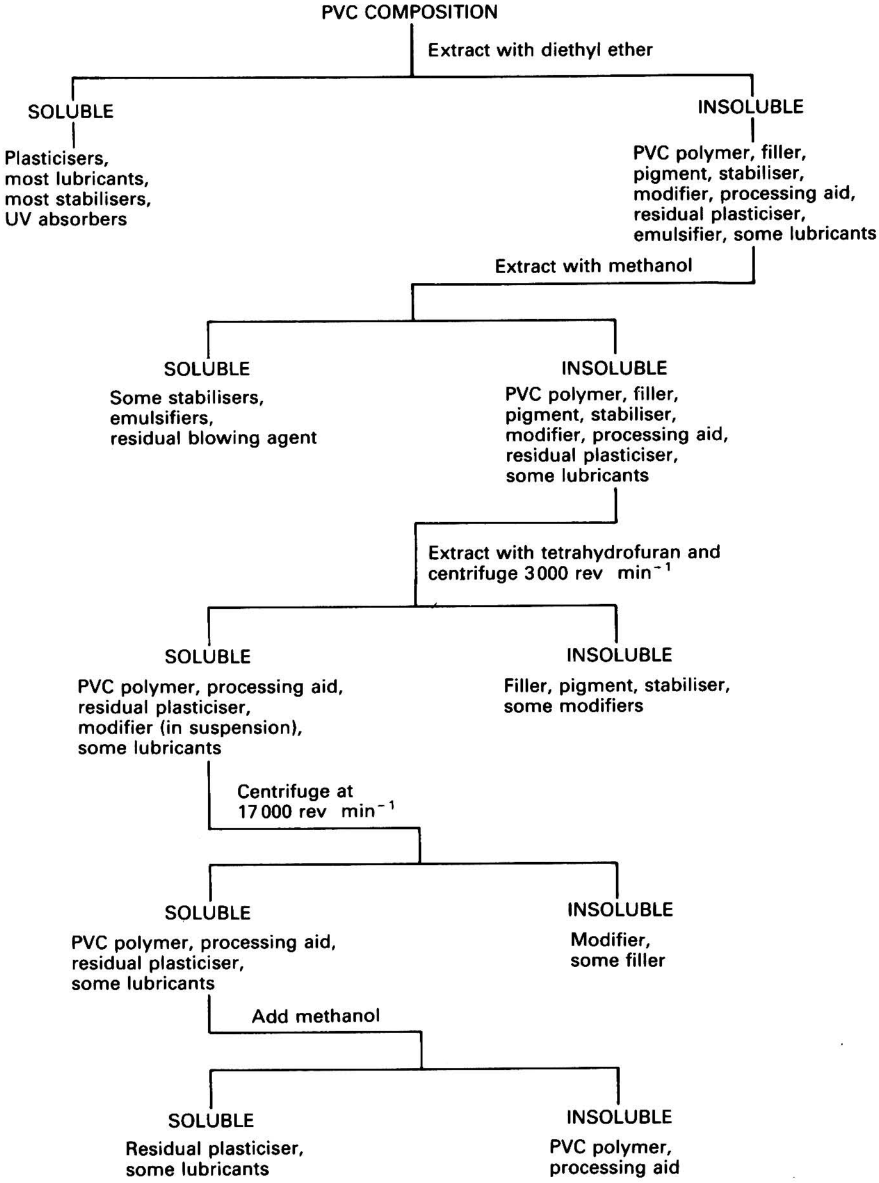


Fig. 1. Analysis of PVC compositions.

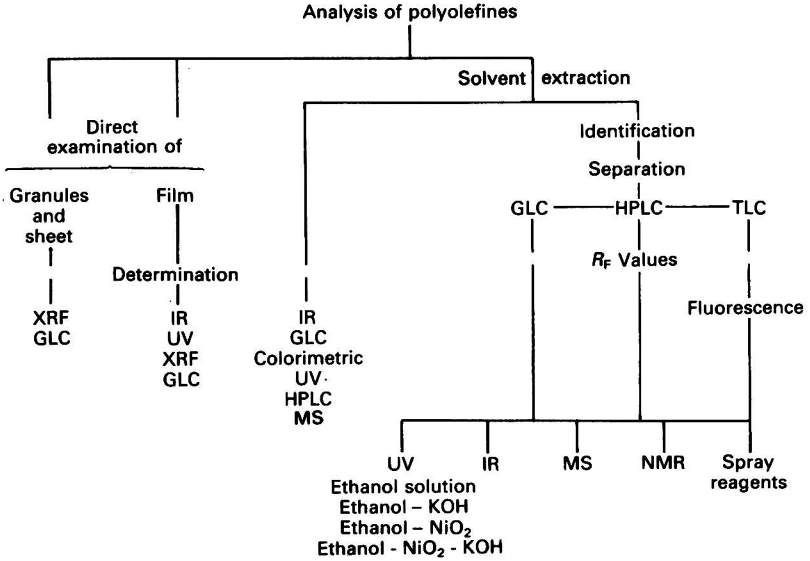


Fig. 2. Analysis of polyolefines.

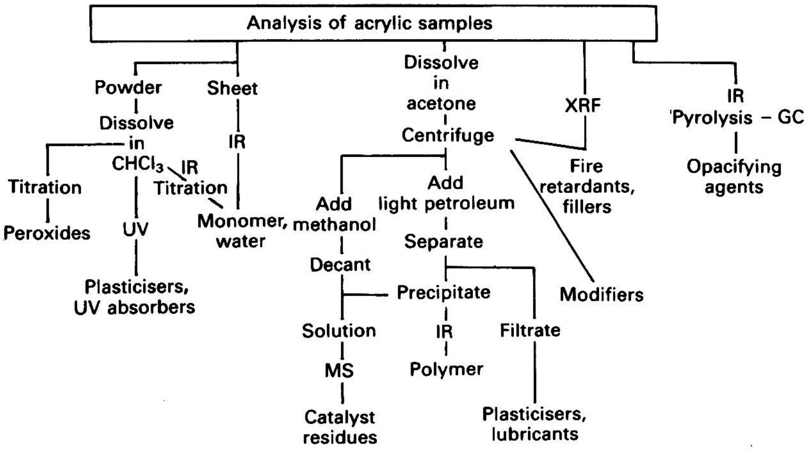


Fig. 3. Analysis of acrylic samples.

TABLE I

EFFECT OF WINDOW MATERIAL: Si Kα

	Form		
Parameter	Granules	Granules	Discs
Window material..	Polyester	Polypropylene	None
Absorption coefficient	573	345	—
Window thickness/μm	6	5	—
Counts per 1% SiO_2	44900	86300	144400
% of disc count-rate	31	60	100

TABLE II

EFFECT OF GRANULE SIZE: Zn Kα, P Kα

Parameter	Element		
	Zinc	Phosphorus	
Form	Granules	Granules	Granules
Sample cell window	Polyester (6 μm)	Polyester (6 μm)	Polypropylene (5 μm)
Readings	3 × 3 × 3	3 × 3 × 3	3 × 3 × 3
Coefficient of variation, %	1.7–2.3, depending on Zn concentration	7.8–15.9, depending on P concentration	3.5–10.2, depending on P concentration
Precision (2σ)	±0.02% at 0.4% level	±15 p.p.m. at 70 p.p.m. level	±7 p.p.m. at 70 p.p.m. level
Loss in sensitivity of disc count rate, %	10	54	33

Extraction Methods

The main extraction procedures used are summarised in Table III and, although most of them can be carried out on material cut to a particle size of less than 2 mm diameter, it is often advantageous to produce material of a smaller size and with a larger surface area to mass ratio. This is conveniently done by grinding at the temperature of liquid nitrogen using an efficient and easily cleaned cutter mill. Full details of some of these methods are provided in reference 1.

TABLE III

EXAMPLES OF EXTRACTION METHODS

Extraction method	Polymer or compound	Solvent(s)	Additives or contaminant extracted
Single solvent (Soxhlet)	PVC	Diethyl ether	Plasticisers
Single solvent (reflux)	Polyethylene	Chloroform, 1,1,1-trichloroethane	Antioxidants
Solvent + reagent (reflux)	Polypropylene	Ethylene dichloride - trichloroacetic acid	Chemisorbed amides/amines
	Polyester	Methanol - Karl Fischer reagent	Water
Solution/precipitation	Polyolefines	Toluene - methanol, xylene - methanol	UV absorbers, antioxidants, slip agents
	Acrylics	Acetone - light petroleum	Plasticiser, lubricants
Steam/solvent distillation	Packaging films	Water - diethyl ether	Odour- and taint-forming additives
Vacuum/thermal extraction	Nylon	—	Water
	Fluorocarbon polymers		Process fumes

Chemisorbed and Adsorbed Additives

The extraction of additives strongly adsorbed or chemisorbed on to the polymer - filler matrix must be carefully watched by the analyst, as a change in the method of manufacture of, for example, the filler or in the method of compounding the plastic formulation can also markedly alter the degree of adsorption bonding produced and hence invalidate an established quantitative extraction procedure. The use of "stronger" extraction reagents can cause complications at the measurement stage and hence each system must be carefully screened and frequently checked.

Solution/Precipitation Methods

This method of extraction involves dissolution of the organic phase of the plastic composition in a suitable (sometimes hot) solvent, followed by precipitation of the polymeric constituents, often in a finely divided form in suspension with the inorganic fillers, by cooling or by means of another solvent in which the analyte compound is also soluble. This method is labour intensive but very effective, and if it does not completely release any chemisorbed constituents from the polymer - filler matrix it will often leave them in a form very vulnerable to attack by the analytical reagent(s) finally used in the determination. For example, the titration of amine/amide slip additives with perchloric acid in non-aqueous media can be carried out in the presence of suspended solids.

Vacuum/Thermal/Displacement Extraction

These procedures are used extensively for the direct isolation or release of volatile components from a polymeric matrix and may involve the combined use of vacuum and heat, as for example in the mass spectrometer direct insertion probe or during dry vacuum distillation. Alternatively, the volatiles may be swept from the heated sample by a flow of inert gas for concentration by freeze trapping and/or collection on to a solid adsorbent prior to thermal or solvent desorption for gas-chromatographic or mass spectrometric examination.

A novel extension of the gas stripping technique was used when a roll of packaging film was the subject of an odour complaint. In this instance, the adsorption tube was made from a melting-point tube containing only 4–5 mg of Porapak Q, which was attached to a small low-flow pump. The reel of complaint film was slowly unrolled, moving the adsorption tube backwards and forwards like a vacuum cleaner across the two parting film surfaces (Fig. 4). The small sample of adsorbent was then inserted directly into the mass spectrometer on the end of the insertion probe and heated. In addition to small amounts of the expected solvents, acetylacetone was detected. Previous methods of direct headspace analysis, not using this concentration technique, had failed to detect this solvent. Only the thermal decomposition products, acetaldehyde and diacetyl, had been detected. The source of this odorous contamination was subsequently shown to be a printing ink solvent containing titanium acetylacetonate as an adhesion promoter.

This application is, of course, comparative rather than quantitative, but is none the less useful.

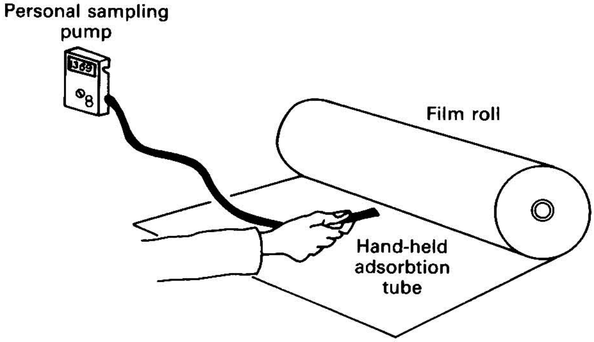


Fig. 4. Sampling volatiles from film roll.

Secondary Methods of Analytical Separation

When the above procedures for preliminary isolation of the analyte materials from the polymer matrix are complete, further separation is often required for identification and determination. Three forms of chromatography are generally used.

Gas Chromatography

Gas chromatography in all its forms with appropriate detectors and, when necessary, temperature programming, heart cutting and back-flushing techniques, is used extensively for volatile components. The details are outside the scope of this paper but mention must be made of headspace methods. These are used extensively for the determination of residual monomers and other residues in polymer compositions after dissolution or dispersion in a suitable solvent and equilibration in a sealed vial at constant temperature prior to chromatography of the headspace gas. For samples in the form of fine powders or thin films, the technique can be applied directly to the solid samples. Some examples and experimental details are given in references 2 and 3 and are summarised in Figs. 5 and 6.

Solids (direct examination)	Packaging films	Coating and printing solvent residues Residual monomers	
	Granular polymers	Polyethylene	Catalyst carrier
		Polypropylene	Process residues
		Ethylene - vinyl acetate co-polymers	Ethylene, vinyl acetate, acetic acid
	Polymer powders	PVC and co-polymers	Monomers, moisture
		Polyethylene terephthalate	Acetaldehyde
	Foodstuffs	Biscuits, cakes and crisps	Migratory trace monomers
Solids (indirect examination)	Polymer solutions (normally 10%)	PVC compositions, ABS	Residual monomers

Fig. 5. Some examples of headspace gas-chromatographic procedures applied to solids.

Liquids	Foodstuffs	Cooking oils, fruit squashes, water, wine, spirits	Migratory trace monomers
		Solution extracts of adsorption tubes (atmospheric monitoring)	Vinyl chloride in dichloromethane, acrylonitrile in benzyl alcohol, benzene in ethylene carbonate
		Latices	Butadiene, styrene, acrylate monomers, methacrylate monomers, dimers and co-dimers
	Biological samples	Whole blood	Vinyl chloride from PVC transfusion sachets, trichloroethylene, trichloroethanol
		Urine	Trichloroethanol, trichloroacetic acid

Fig. 6. Some examples of gas-chromatographic procedures applied to liquids.

Thin-layer Chromatography (TLC)

TLC separations are now usually carried out only when some preliminary separation of non-volatile materials is required prior to mass spectrometric examination or to provide confirmatory or supplementary analytical evidence. For plasticisers and extenders from PVC, 1-mm Kieselgel 60G layers on glass plates are used with 95 + 5 toluene - ethyl acetate as elution solvent. Phthalate esters, aliphatic esters, phosphate esters, epoxy esters and chlorinated hydrocarbons separate readily when 0.05–0.1 g of extract is used. The separated bands are marked under ultraviolet light, removed from the plate and extracted with diethyl ether, followed by infrared and/or mass spectrometric examination.

For additives extracted from polyolefins, usually with diethyl ether, the extract is refluxed with ethanol and the solution is decanted off the insoluble residual polymer. On cooling, additives such as dilauryl and distearyl thiodipropionate separate out and are identified by infrared examination. A 30-μl volume of the ethanolic solution is then spotted on to a thin Kieselgel 60 TLC plate and eluted with a suitable solvent, usually 98.5 + 1.5 toluene - ethyl acetate. The eluted plate is dried and sprayed with colour-developing reagents and the spots are examined. Table IV illustrates R_F values (based on Topanol OC = 1.00), colours and suitable solvent systems. If the spots are to be submitted to mass spectrometric examination, methanolic iodine is used as the colour-developing reagent as this does not over-complicate the mass spectrometry.

The normal spray procedure uses a 0.5% solution of 2,6-dibromo-*p*-benzoquinone-4-chlorimine in ethanol followed, after drying, by a 0.5% borax solution. After this spray the plate is dried at 120 °C for 5 min to develop the colours.

TABLE IV

THIN-LAYER CHROMATOGRAPHY OF COMMERCIAL ANTIOXIDANTS

	R_F value*				
Additive	Solvent 1	Solvent 2	Solvent 3	Solvent 4	Colour of spot
Topanol OC	1.00	1.00	1.00	1.00	Pale yellow centre with pink outer
DLTDP	0.40	1.00	0.05	0.30	Yellow - brown
DSTDP	0.45	1.00	0.10	0.35	Yellow - brown
Distearyl disulphide	1.10	1.05	1.20	1.20	Bright yellow
"DTB glycol ester"	0.00	0.75	0.00	0.00	Brown centre with mauve outer
Ionox 330	1.05	1.05	0.80	0.80	Pinkish brown
Irganox 259	0.30	1.05	0.00	0.65	Brown
Irganox 288	0.25	1.05	0.00	0.60	Brown
Irganox 1010	0.30	1.10	0.00	0.60	Brown
Irganox 1076	0.85	1.05	0.60	0.95	Brown
Nonox WSP	0.85	0.95	0.45	0.35	Bright yellow centre with pink outer
Polygard	0.30, 0.15	0.80, 0.70	0.10	0.05	Blue and red
Santanox R	0.35	0.80	0.10	0.00	Purple
Tinuvin 326	1.05	1.05	0.95	1.05	Yellow
Tinuvin 327	1.05	1.05	1.05	1.10	Yellow
Tinuvin 328	1.00	1.00	1.00	1.05	Yellow
Topanol CA	0.10	0.60	0.00	0.00	Brown
UV 531	0.75	1.00	0.35	0.80	Blue
Hoechst D55	0.85, 0.75, 0.55, 0.35, 0.30, 0.20	0.90, 0.85	0.55, 0.45 0.25, 0.15, 0.10, 0.00	1.10, 0.45, 0.25, 0.15	Blue and red
Oleamide	0.00	0.00	0.00	0.00	These additives give only a very faint brown coloration
Erucamide	0.00	0.00	0.00	0.00	
Ethomeen T12	0.00	0.00	0.00	0.00	
Stearic acid	0.00	0.00	0.00	0.00	

*R_F values quoted to the nearest 0.05. Solvent 1 = toluene - ethyl acetate (98.5 + 1.5); solvent 2 = toluene - isopropanol (88 + 12); solvent 3 = toluene - light petroleum (b.p. 60–80 °C) (1 + 1); and solvent 4 = cyclohexane - toluene - methanol (88 + 10 + 2).

High-performance Liquid Chromatography (HPLC)

HPLC has now become a very valuable tool for plastics analysis, particularly in the additive field. We use standard equipment with both reversed-phase and adsorption columns, isocratic and gradient elution and mainly a variable-wavelength ultraviolet detector. Two examples are appropriate.

Fig. 7 shows a chromatogram of a mixture of phthalate esters separated on an SC6 column, with a methanol - water gradient mixture as solvent. The most important region is the C_7–C_{10} fraction, which is not completely separated but which does give characteristic patterns for commercial plasticiser mixtures and hence allows identification. Of course, quantitative analysis is carried out with suitable calibration.

The second example (Fig. 8) shows a chromatogram of a mixture of antioxidants and ultraviolet absorbers. A reversed-phase system is again preferred because it can be washed clean with methanol, but this time an isocratic solvent system is used. With the variable-wavelength detector, the optimum wavelength can be set for the type of compounds being examined.

Although gradient elution is not necessary for every analysis, we find it is of great help in setting up conditions for the particular additive mixtures. Isocratic solvent mixtures are preferred for quantitative analysis.

The additives shown in Fig. 8 are almost all aromatic phenols and our remaining problem in this type of additive analysis is to detect and determine the aliphatic compounds. These are in two groups, the thioester synergistic stabilisers and the surface-active modifiers such as oleamide and glycerol monostearate. We are now looking for post-column derivatisation reactions for these compounds to give species detectable by either fluorescence or ultraviolet methods.

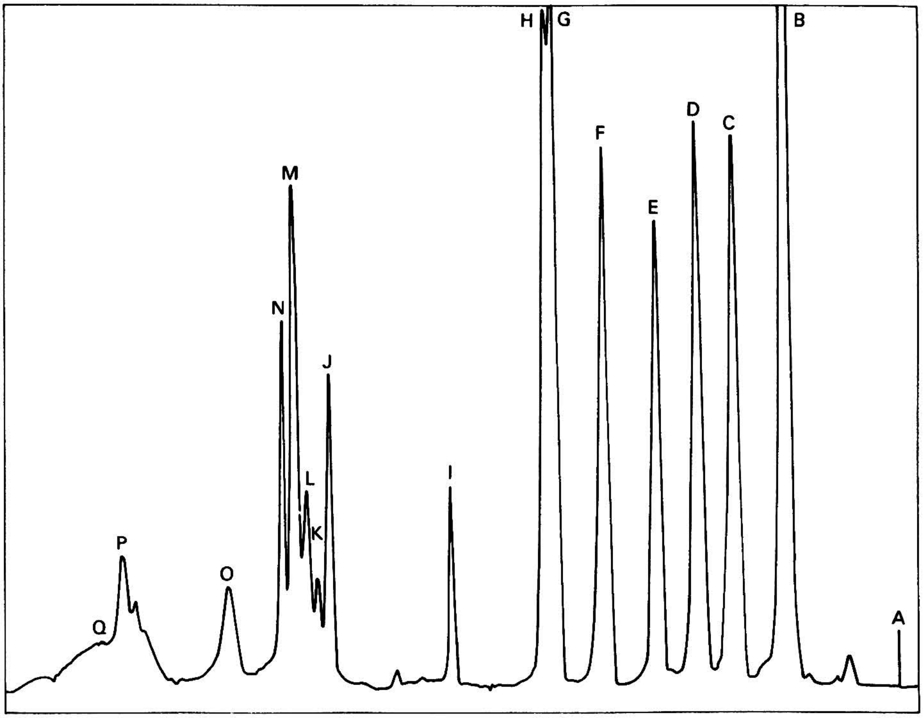


Fig. 7. Separation of phthalates by HPLC. Chromatographic conditions: column, Spherisorb SC6 (5 μm), 100 × 5 mm; solvent gradient, 60% *V*/*V* methanol in water to methanol in 20 min; flow-rate, 40 $cm^3 h^{-1}$; and detection, absorbance at 254 nm. A, Injection; B, methyl and methoxyethyl; C, ethyl; D, alkyl; E, isopropyl; F, phenyl; G, isobutyl and butylbenzyl; H, butyl; I, cyclohexyl; J, heptyl; K, impurity; L, isooctyl; M, *sec*-octyl and octyl; N, nonyl; O, isodecyl; P, undecyl; and Q, tridecyl.

Mass Spectrometry

Like other forms of molecular spectroscopy, mass spectrometry may be used as a "fingerprint" technique to identify the components of additive systems extracted from polymer compositions. The strengths of mass spectrometry are high sensitivity and the ability to distinguish between closely related compounds of differing relative molecular mass, *e.g.*, the various alkyl thiodipropionates used as synergistic stabilisers in polyolefins and the ultraviolet-absorbing benzotriazole derivatives. Often it is not necessary to separate the components before examination as some separation may be achieved by careful variation of the sample probe temperature to produce, in effect, a fractional distillation of the components. The presence, however, of large amounts of low relative molecular mass polymers from polymers such as polyethylene and polypropylene can cause interference by producing a high hydrocarbon background extending to several hundred relative atomic mass units. In such instances a thin-layer chromatographic separation can be used as a clean-up procedure, the low relative molecular mass polymer normally moving with or close to the solvent front, leaving the additive components for separate examination. Complex mixtures can be resolved and the technique then used to isolate the relevant area of coating from the plate into a small melting-point tube (Fig. 9). The additive is then eluted from the coating in the tube into the mass spectrometer sample tube or inlet probe with a suitable solvent, normally methanol.

Compounds having the same relative molecular mass and different molecular formulae may be distinguished, provided that there is sufficient material for a high-resolution examination, enabling the masses of the molecular and fragment ions to be measured to sufficient

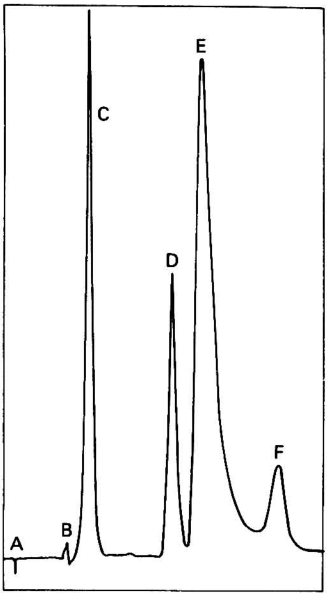


Fig. 8. Determination of additives from polypropylene by HPLC. Chromatographic conditions: column, Spherisorb ODS (5 μm), 100 × 5 mm; solvent, 90% *V*/*V* methanol; flow-rate 40 cm^3 h^{-1}; detection, absorbance at 280 nm and 0.2 a.u.f.s. A, Injection (10 μl); B, solvent front; C, Topanol CA, 10.12 mg per 50 cm^3; D, Tinuvin 326, 4.96 mg per 50 cm^3; E, UV 531, 9.82 mg per 50 cm^3; and F, Irganox 1010, 10.10 mg per 50 cm^3.

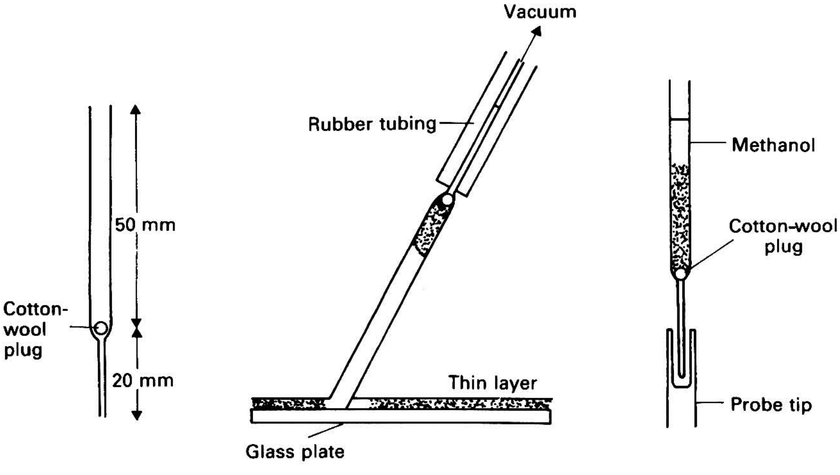
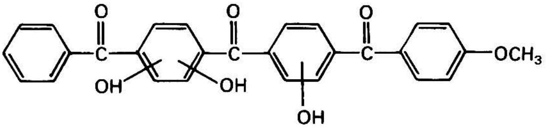


Fig. 9. Sampling of fractions from TLC plates for examination by mass spectrometry.

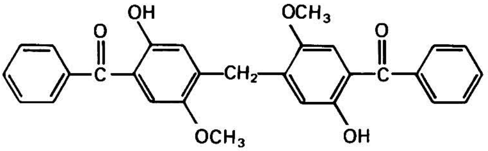
accuracy for their chemical formulae to be deduced. An example of this, also demonstrating the advantages of a multi-technique approach, is that of an ultraviolet-absorbing additive extracted from a polyethylene terephthalate composition.

The material had the same TLC R_F value as UV531, but did not exhibit the right colour or ultraviolet characteristics for this component. Infrared, ultraviolet and nuclear magnetic resonance spectroscopy indicated that it was a benzophenone derivative. The low-resolution mass spectrum showed a benzophenone of relative molecular mass 468 and from the spectrum the following structure was postulated:



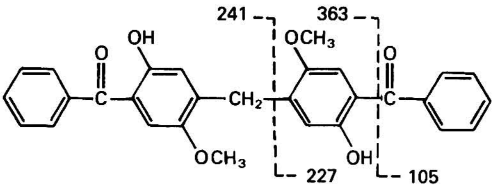
$C_{28}H_{20}O_7$ Relative molecular mass = 468.1209

Synthetic considerations, however, suggested an alternative structure:



$C_{29}H_{24}O_6$ Relative molecular mass = 468.1573

Further material was isolated and high-resolution mass spectrometry gave a relative molecular mass of 468.1544, showing $C_{29}H_{24}O_6$ to be the correct molecular formula, confirmation being obtained from the masses of the fragment ions:



Formula	Theoretical mass	Measured mass
$C_{29}H_{24}O_6$	468.1573	468.1544
$C_{22}H_{19}O_5$	363.1232	363.1244
$C_{15}H_{13}O_3$	241.0865	241.0945
$C_{14}H_{11}O_3$	227.0708	227.0703

Gas Chromatography - Mass Spectrometry (GC - MS)

Polymers contain trace amounts of residues of the catalyst used in their preparation and the identification of these is often necessary, particularly when a new catalyst is being evaluated. The use of gas chromatography in conjunction with mass spectrometry is required in order to separate the complex mixture of components that are extracted. For example, tetramethylsuccinodinitrile (TMSDN) has been detected in extracts of polymers prepared using azobisisobutyronitrile. This has been developed into a quantitative method using specific-ion GC - MS. In the development of this method it was discovered that substantial losses of TMSDN occur in the evaporation of methanolic solutions, which explained earlier difficulties in detecting residues of this catalyst. Even without concentration of the polymer extract it has been possible to achieve a lower limit of detection of 20 p.p.m. in the polymer.

A further family of catalysts often used are peroxides (*e.g.*, benzoyl or lauroyl peroxide); these produce acids as residues which may be detected by mass spectrometry or by methylation of the evaporated extract prior to GC - MS examination.

Odour and Taint

The analyst in the plastics industry may be required to trace the cause of odour and taint produced in foodstuffs packaged in plastic materials. This provides good examples of the use of high sensitivity GC - MS in the identification of such compounds. Two methods have proved useful for the concentration of these components.

(*a*) Where the sample is a sealed plastic bag, the headspace gas from the bag (or many bags) is withdrawn through an adsorption tube normally packed with Porapak Q. The trapped organic species are then thermally desorbed for GC - MS examination. This has enabled residual printing and coating solvents in the bag headspace to be identified as causes of odour and taint.

An interesting recent example was that of tainted sweets having a fatty taste. Examination of the headspace gases from the polypropylene bags containing the tainted sweets detected aldehydes (pentanal and hexanal) in addition to the flavourings (amyl acetate and limonene). Solvent washings from the internal surfaces of the bags were found to contain vegetable oil. Those from the most heavily tainted samples had a rancid odour and corresponded with the sample showing the highest concentration of aldehydes in the headspace. From these results it was deduced that the taint was not due to the film in contact with the sweets, but to the presence of rancid vegetable oil on the sweets themselves. The evolution of hexanal can apparently be used as a quantitative indicator of the rancidity of vegetable oils.

(*b*) For containers and foods, the Likens and Nickerson combined solvent extraction - steam distillation procedure[4] has proved useful. The advantage of this form of extraction is that large amounts of sample may be extracted with a small volume of organic solvent prior to further concentration by evaporation. In this procedure the evaporation of the organic solvent can concentrate trace amounts of solvent impurities to a significant proportion of the final extract and we therefore use redistilled solvents and distilled rather than deionised water, which can contain trace organics from the resin bed. A blank extraction is always carried out.

This method was used effectively in the resolution of another tainting problem. About 300–400 g of "good" and "bad" printed plastic lids were shredded and extracted. Direct gas-chromatographic analysis of the two extracts showed a large number of peaks and the two chromatograms differed in only one respect: the "bad" sample revealed one small extra peak that was not present in the "good" sample. GC - MS examination of this particular peak indicated an acetate ester of a polyol. Further extractions, followed by TLC, enabled sufficient of the component to be isolated to obtain infrared and nuclear magnetic resonance spectra, which, together with the mass spectrometric data, indicated that the contaminant was Butyl Carbitol acetate [2-(2-butoxyethoxy)ethyl acetate, $CH_3CO_2(CH_2CH_2O)_2C_4H_9$], which is used in certain ink formulations. It was subsequently proved that as little as 2 p.p.m. of this acetate could cause a detectable taint in the foodstuff in question.

In this example, the straightforward mass spectrum alone did not give a direct identification, as no molecular ion was produced. Shortly afterwards, however, this sample was used as a test of a spectrometer which could be employed in the chemical ionisation mode. Under these conditions a quasi-molecular ion of m/z 205 was formed, and had this technique been available at the time it is highly likely that the contaminant in question would have been identified without the considerable amount of extra work. We now make extensive use of chemical ionisation techniques and Fig. 10 shows electron impact and chemical ionisation mass spectra of Butyl Carbitol acetate.

Processing Volatiles

Recently there has been increased interest in the nature and amount of volatiles produced during the processing, *i.e.*, drying, extruding, moulding, etc., of plastic compositions. In our laboratories work has been carried out to identify the volatiles produced in the drying and sintering of PTFE compositions. Mass spectrometry was used, the samples being

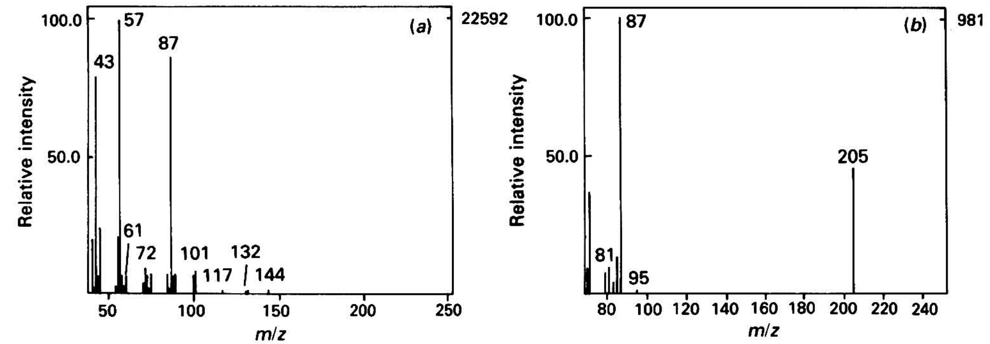
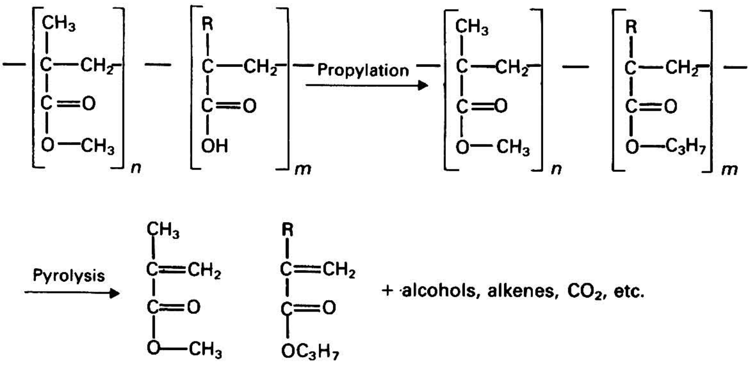


Fig. 10. Electron impact (*a*) and chemical ionisation (*b*) mass spectra of Butyl Carbitol acetate.

heated in a stainless-steel tube and the volatiles passed directly to the mass spectrometer through a heated line. Programming of the heating from 40 to 400 °C enables process conditions to be simulated. Using a data system to scan repetitively the mass spectrum and store the data from all scans, the products may be identified and specific-ion displays enable the rate of evolution of these products to be followed. For example, the evolution of perfluoroalkenes from the breakdown of a fluorinated surfactant, and alkylphenols and ethoxylated phenols from polyethylene oxide condensates used in the emulsion polymerisation have been detected.

Pyrolysis - Gas Chromatography - Mass Spectrometry

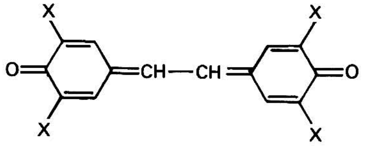
Pyrolysis - gas chromatography has been used for many years for the characterisation of plastics materials, particularly when they are of an intractable nature owing to cross-linking or are very heavily filled. The technique has now been extended to include mass spectrometry and is of particular value when minor components need to be identified. An example has been described by Sharp and Paterson[5] for the identification of small amounts (1–10%) of copolymerised unsaturated acids in acrylic polymers. The method can be summarised as follows:



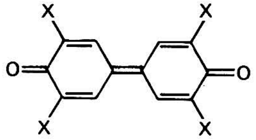
The copolymerised acid is propylated by treatment of the sample with Propyl 8 reagent, the resultant polymer is pyrolysed and the propyl ester of the acid, if present, is identified by GC - MS. By this procedure copolymerised acrylic or methacrylic acid has been identified in terpolymers with (*a*) butyl acrylate and styrene, (*b*) methyl methacrylate and ethyl acrylate and (*c*) ethylene and propylene. A methyl methacrylate - α-methylstyrene - maleic acid terpolymer, when examined by this propylation - pyrolysis procedure, yielded dipropyl fumarate and a smaller amount of dipropyl maleate. Attempts to detect copolymerised itaconic acid by this technique have failed, probably owing to the difficulty in esterifying the acid group.

Ultraviolet and Infrared Spectroscopy

The extensive use of ultraviolet and infrared spectroscopy in the analysis of antioxidants and ultraviolet-absorber systems used particularly in polyolefin products is well known[1,6] and will not be dealt with in detail here. In many instances, of course, dual wavelength measurements are required to permit the resolution of mixtures and correction for overlap absorbances. A simple example of this in the ultraviolet region concerns the resolution of two of the oxidation products of Topanol O (butylated hydroxytoluene), which in some instances can cause troublesome yellowing of the polymer and which can be evident in process solvents:



3,3′,5,5′-tetra(*tert*-butyl)stilbenequinone (SQ)



3,3′,5,5′-tetra(*tert*-butyl)diphenoquinone (DPQ)

X = *tert*-butyl

The spectrum of the extracted oxidation products is measured in hexane over the wavelength range 550–325 nm. A base line is drawn as a tangent from 480 to 350 nm. The absorbance at 419 and 444 nm is measured and referred to separate calibrations prepared with SQ and DPQ. SQ shows two absorbance maxima, at 444 and 417 nm, and DPQ a single maximum, at 419 nm. Graphs are prepared at both 419 and 444 nm, permitting algebraic calculation of the concentration of each component.

Infrared - Laser Raman Spectroscopy

Although both of the above spectroscopic methods have a wide use in their own right, the example given below demonstrates well the *complementary* value of the two methods, taking advantage of the fact that elements of high atomic number, *e.g.*, antimony and bromine, have relatively more intense Raman spectra but the lighter elements show up clearly in the infrared spectra.

Polymer granules suspected to be PVC were submitted for identification. When examined by infrared spectroscopy the strongest bands (9.8 and 14.9 μm) were due to a talc-type material and bands of medium intensity were assigned to polypropylene and possibly antimony trioxide (13.4 μm). Additional weak bands in the 7.3–7.7 μm region were possibly due to decabromodiphenyl ether. In the Raman spectrum, however, the strongest bands (250 and 185 cm^{-1} shift) confirmed the presence of antimony trioxide and some bands of medium intensity confirmed the presence of decabromodiphenyl ether (doublet at 140, triplet at 220 cm^{-1} shift) and polypropylene (800, 835, 1150, 1325, 1450 and 2900 cm^{-1} shift). The silicate bands that obscured regions of the infrared spectrum were not observed in the Raman spectrum.

Other Spectroscopic Methods and Thermal Analysis

Space does not permit a lengthy description of the other spectroscopic methods used in the analysis of plastics, but the main applications to additive and trace analysis are summarised in Table V.

TABLE V

OTHER TECHNIQUES APPLIED IN PLASTICS ANALYSIS

Technique	Principal use
X-ray diffraction	Identification of crystalline additives *in situ* (*e.g.*, hydrates) Identification of polymer phases
Thermal analysis	Detection of polymer phases with different melting-points
NMR	Identification of additives after extraction. Calibration of infrared methods (structure). Identification of "bound" additives Wide-line NMR for liquid - solid phase blends
ESCA	Surface composition studies, *e.g.*, presence of slip additive and antistatic agents at polymer surfaces
MOLE	Particulate contamination at or near film surfaces
SIMS	Ultra-surface analysis: catalyst studies
Spectrofluorescence	Degradation studies: surface oxidation; study of scintillation additives

Chemical Methods

Chemical and colorimetric methods of analysis are used, of course, in both routine and special-case analyses. The differential non-aqueous titration of slip additives extracted from polyolefin compounds is fully described in references 1 and 7 and several variations of the titrant and solvent system are used in particular circumstances. The normal slip additives encountered are mixtures of a long-chain amide with a secondary amine such as Ethomeen T12 [$R-N(CH_2CH_2OH)_2$, where R is derived from tallow], and these are differentiated by a perchloric acid titration in an organic solvent containing glacial acetic acid and acetic anhydride.

In some circumstances, particularly at the lower concentration levels and when alkali metal or calcium salts, which would interfere in the titration, are present, a colorimetric method is used for Ethomeen T12. This method relies on measurement of the chloroform-soluble complex formed by the reaction of the extracted additive with a mixed indicator solution containing bromophenol blue and bromocresol green at pH 3.5.

Interesting variations on this method are being developed by Udris, particularly for the determination of polymeric amine-type additives that present problems of extraction and that form insoluble complexes with the indicator reagents. In such instances promising results have been obtained by measuring the decrease in colour in the aqueous phase rather than the increase in the organic phase.

Instrumental methods for the determination of water in polymeric materials are numerous and many rely on the release of the water from the polymer matrix by heat. In some instances, however, particularly for polyethylene terephthalate, heating in the presence of parts per million amounts of water causes some hydrolysis of the polymer and consequent erroneous results. A simple Karl Fischer method is thus preferred and can be summarised by reference to Fig. 11. Dry methanol is placed in the sample flask, excess of dilute Karl Fischer reagent (1 ml = 1 mg of water) is added and the contents are boiled under reflux for 20 min to dry the apparatus. Slightly wet methanol is then added to the flask and the contents are immediately titrated to a visual end-point by means of the Karl Fischer reagent. The flask and contents are then refluxed for 6 h and any water picked up during this time is again titrated with the Karl Fischer reagent. This establishes that the equipment is giving a low blank value, which should be less than the equivalent of 2 ml of Karl Fischer reagent.

For the analysis of the sample the apparatus is again pre-dried and the sample introduced via the 70-ml top cup. The cup is filled to a 50-g mark with polymer granules and then the latter are allowed to drop into the flask by releasing the clip on the wide-bore rubber tubing. In this way the exposure of the sample to the atmosphere is minimal. The 6-h reflux period is then repeated and the water content of the sample is calculated from the difference between the titre for sample and blank experiments.

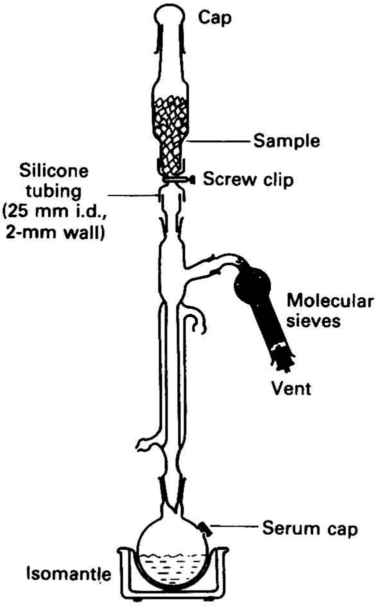


Fig. 11. Apparatus for the determination of water in polyethylene terephthalate.

Conclusion

In conclusion, I would stress that analyses for additives and trace organics in plastics materials can be made easier by a detailed understanding of the industry. Many of the modern analytical techniques are of great value in providing faster and more precise analyses, but much can also be done by the established chemical, chromatographic and spectroscopic techniques, particularly when these are supported by a degree of semi-automation for instrument control and data handling.

The procedures described in this paper are the work of a team of analysts working in close co-operation. The assistance given by Messrs. L. H. Ruddle, J. Udris, A. R. Jeffs, P. L. Warren, P. E. Arnold and J. L. Sharp is particularly acknowledged. I am also indebted to the directors of ICI Petrochemicals and Plastics Division for permission to publish this paper.

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Various Applications of Functional Group Analysis*

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The importance of functional group analysis is demonstrated with a number of examples in various fields. Special attention is given to the determination of end groups in several kinds of high and low relative molecular mass materials.

Methods for the determination of the following groups are described: hydroxyl groups (polymers, esters), carboxyl groups (polymers, ester-interchange products), unsaturated groups (vinyl ester end groups in polymers), anhydride groups (polymers), amino groups (polymers, derivatised silica materials), epoxide groups (resins, derivatised silica materials) and quaternary ammonium compounds (surfactants).

The methods include derivatisation procedures and several techniques for quantitative determination (spectrophotometry, X-ray fluorescence, coulometry and potentiometric and photometric titration).

Keywords: *Functional group analysis*; *polymers*; *derivatisation*

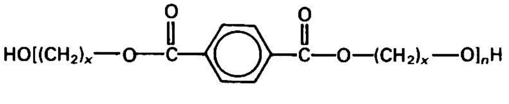
Functional groups are defined as particular reactive species present in an organic substance or mixture. They can be detected and sometimes measured directly by physical or chemical methods in a quantitative manner, but in many instances derivatisation is necessary and the substance formed or consumed is determined. Procedures for determining certain functional groups are usually not generally applicable; owing to solubility properties, steric factors and the presence of interfering reactions, the use of various reagents and special solvents is necessary.

There are about 100 possible functional groups and the determination of only a few of them will be discussed here. For a particular functional group, one or more applications for the quantitative determination of this group are given. The methods described deal mainly with procedures based on chemical derivatisation routes for the determination of hydroxyl, amino, carboxyl, anhydride, epoxide, unsaturated and quaternary ammonium groups in various products, including high relative molecular mass polymers.

Especially in the end group analysis of high relative molecular mass materials, problems due to insolubility in common analytical solvents are encountered, and finding suitable media in which derivatisation or titration procedures can be carried out is an important aspect of the development of analytical procedures. In another stage of the investigation the insolubility may be used advantageously for purification purposes. Finally, the concentration of end groups varies inversely with the relative molecular mass, which makes high demands on the analytical techniques used in this field.

Hydroxyl Groups

Procedures developed for the determination of hydroxyl groups include titrimetric, spectrophotometric, nuclear magnetic resonance spectroscopic, gas-chromatographic, electrochemical and thermometric methods. In our laboratory the quantitative determination of hydroxyl groups in low and high relative molecular mass polymers is of importance. Examples of such polymers are



with n = 10–100 and x = 2 [polyethylene terephthalate (PETP)] or 4 [polybutylene terephthalate (PBTP)], and ester-interchange products of PBTB and polypropylene glycol

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* Plenary Lecture presented at the Joint NL - UK Symposium on Quantitative Organic Analysis, Noordwijkerhout, The Netherlands, April 22–24, 1981.

(PPG) (PBTP - PPG elastomers). These products have limited solubility but are soluble in phenols, hot nitrobenzene or aniline, dichloroacetic acid and hexafluoroisopropanol.

The hydroxyl groups in these products are determined by acetylation with an excess of dichloroacetic anhydride (DCAA) in dichloroacetic acid (DCA) and measurement of the amount of acetylation by a chlorine determination. Owing to the low content of hydroxyl groups, especially in high relative molecular mass materials, the determination of the excess is inaccurate and the determination of the amount of reagent incorporated is much more attractive. This kind of procedure requires an effective elimination of the excess of reagent, which is normally carried out in dissolution and precipitation steps.

The derivatisation is carried out in a 10% *m/m* solution of DCAA in DCA at 60 °C. As can be seen in Fig. 1, a reaction time of 1 h suffices. After the reaction the solution is poured into water and the precipitated polymer is washed out. To remove the last traces of solvent and acetylation agent, re-precipitation of the derivatised polymer from a hexafluoroisopropanol (HFI) solution into water is carried out. For PETP and PBTP polymers with a low hydroxyl content (below 100 mmol kg^{-1}) the re-precipitation is carried out from a solution in nitrobenzene into cold light petroleum, in order to obtain more reproducible results; probably trace amounts of chlorine-containing impurities adhere to the polymer when using the HFI - water method.

The content of hydroxyl groups is subsequently determined by measurement of the chlorine content of the purified derivative. This is done either by potentiometric titration with silver ions after combustion or by X-ray fluorescence spectroscopy after tabletting. The latter technique is particularly suitable for rapid analysis. Details of the method have been published elsewhere.[1]

By carrying out several consecutive precipitations it could be demonstrated that only one re-precipitation from a solution in HFI (low relative molecular mass materials) or nitrobenzene (high relative molecular mass materials) into water and light petroleum, respectively, is necessary (see Fig. 2). We checked, as is necessary with this kind of procedure, that no loss of the lowest relative molecular mass fractions occurred during the precipitation step.[1]

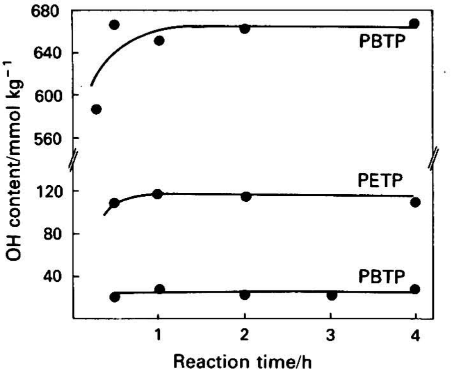


Fig. 1. Esterification of various samples with DCAA for different periods at 60 °C.

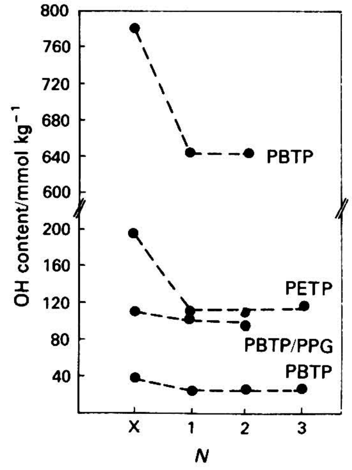
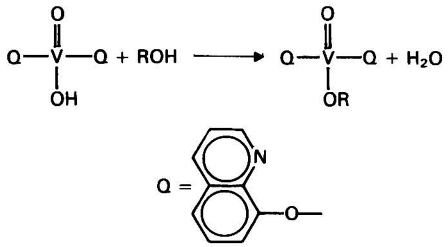


Fig. 2. OH content as a function of the number of re-precipitations (N); X = OH content of the sample after precipitation in water.

The suitability of this method for a number of samples is demonstrated in Table I.

The standard deviation of the method for high relative molecular mass PETP and PBTP materials is 0.7 mmol kg^{-1}, whereas for the low relative molecular mass materials it is about 10 mmol kg^{-1}.

For the determination of low contents of hydroxyl groups in low relative molecular mass esters we apply the frequently used method in which the sample is reacted with a solution of vanadium 8-hydroxyquinolinate (V8HQ). According to Tanaka and Kojima,[2] a coloured complex with the following structure is formed:



After removal of the excess of reagent by extraction, the complex is acidified with dichloroacetic acid and the blue colour formed is measured at 620 nm. Calibration is carried out with an alcohol as internal standard. The procedure followed is that of Harrison[3] with the following modifications: (*a*) to obtain a more stable reagent the solvent dimethylformamide - toluene is replaced with dimethylformamide - monochlorobenzene (see Table II); and (*b*) removal of the excess of reagent by extraction with 4% *V*/*V* triethylamine solution instead of sodium hydroxide solution (favourable separation of the layers).

TABLE I

HYDROXYL END GROUP CONTENT OF SEVERAL PETP, PBTP AND PBTP - PPG SAMPLES

Sample	Viscosity ratio*	Hydroxyl end groups/ mmol kg^{-1}	Remarks
PETP A	1.80	30.9–29.8 30.6–30.4	Total end groups† 76.6 mmol kg^{-1}
PETP B	1.82	14.4–14.8	Total end groups 79.1 mmol kg^{-1}
PBTP A	2.07	70.7–70.6	Total end groups 95.2 mmol kg^{-1}
PBTP B	2.08	50.6–49.3	Total end groups 92.6 mmol kg^{-1}
PBTP C	1.24	664–653	
PBTP - PPG A ..	1.25	742–729	M_N (calc.)‡ 2700; M_N (measured) 2400§
PBTP - PPG B ..	1.34	329–345	
PBTP - PPG C ..	1.50	229–212	M_N (calc.) 9000; M_N (measured) 10100
PBTP - PPG D ..	2.38	51–47	M_N (calc.) 35000; M_N (measured) 44800

* Measured for a 1% *m*/*m* solution in *m*-cresol at 25 °C (PETP and PBTP), or for a 1% *m*/*V* solution in *o*-chlorophenol at 25 °C (PBTP - PPG).

† Total end groups is sum of OH + COOH + methyl ester end groups.

‡ M_N measured by gel-permeation chromatography in *m*-cresol as a solvent.

§ Calculated from OH + COOH content.

TABLE II

STABILITY OF THE VANADIUM 8-HYDROXYQUINOLINATE REAGENT

	Decrease, %*		
Storage time/h	In benzene†	In toluene†	In monochlorobenzene
24	50	75	3
48			15
72			33

* Determined with heptan-1-ol as the alcohol.

† Values taken from reference 3.

As presented in Table III, the absorbance is only slightly dependent on the chain length for C_4–C_{18} primary alcohols.

TABLE III

ABSORBANCE OF 10 μmol OF ALCOHOL AT 620 nm (1-cm PATH LENGTH)

Alcohol	Butan-1-ol	Heptan-1-ol	Dodecan-1-ol	Octadecan-1-ol
Absorbance	0.49	0.54	0.55	0.58

The application of this method to some esters of new types of acids showed that a precise determination is possible (Table IV). The standard deviation of the V8HQ method is 1.5 mmol kg^{-1}, which is about ten times more precise than that of the classical acetylation procedure.[4]

TABLE IV

COMPARISON OF THE V8HQ METHOD WITH THE ACETYLATION METHOD

Sample	OH content/mmol kg^{-1}	
	V8HQ	Acetylation
I	47.9–47.6 43.1–46.8	50–70
II	35.5–34.9	35–50
III	130.1–126.7	170–120
IV	73.8–74.9	50–70

Carboxyl Groups

The determination of these groups in high relative molecular mass polyesters and polyamides gives, in combination with other data, important information about the degree of polymerisation, chain branching, degradation and thermal stability. The choice of the method depends on the solubility of the polymer. Most methods are based on titration in non-aqueous media with visual, potentiometric or photometric indication of the end-point.

A very convenient technique for the determination of carboxyl groups in PETP and polyamide 6 (PA6) is photometric titration. The sample is dissolved in *o*-cresol at 125 °C, cooled, diluted with chloroform and, after addition of bromophenol blue (for PETP) or bromocresol green (for PA6), the titration is carried out in a spectrophotometer with tetrabutylammonium hydroxide (TBAH). The change in absorbance is recorded continuously and the end-point is determined graphically (see Fig. 3). A detailed description was given by Van Lingen.[5]

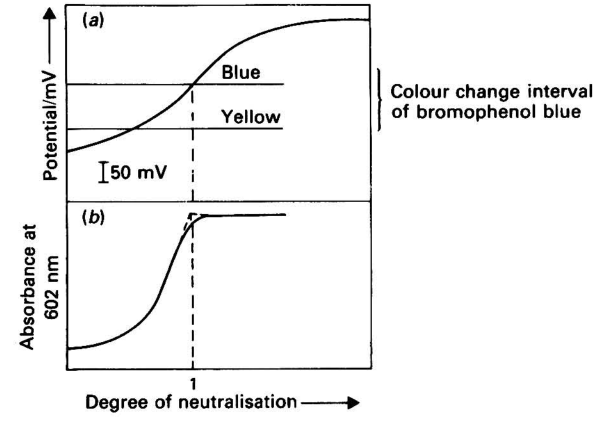


Fig. 3 (*a*) Potentiometric and (*b*) photometric titration of PETP.

Table V surveys applications of the photometric titration method, together with a comparison with a potentiometric titration method with TBAH in aniline (DMT and low relative molecular mass PETP), *o*-cresol (high relative molecular mass PETP) or benzyl alcohol - methanol - water (PA6). Generally the agreement is very satisfactory. Photometric titration, however, is much faster than potentiometric titration and has been shown to be a very convenient technique in routine analysis.

TABLE V

APPLICATION OF THE PHOTOMETRIC TITRATION METHOD TO VARIOUS SAMPLES

		Content of carboxyl groups/mmol kg^{-1}			
		Photometric titration		Potentiometric titration	
Sample		$\bar{x}$*	Standard deviation	$\bar{x}$*	Standard deviation
DMT	A	0.31	0.03	0.38	0.04
	B	0.76	0.03	0.83	0.02
PETP (M_N low)	C	4.3	0.25	4.0†	0.10
	D	12.0	0.34	12.0†	0.30
	E	17.4	0.56	17.5†	0.49
PETP (M_N high)	F	36.8	0.32	35.2	0.9
	G	68.3	0.29	68.2	1.2
	H	113.4	0.52	112.8	1.8
PA6	I	73.3	0.31	72.4‡	1.0
	J	53.2	0.22	52.5‡	0.37
	K	35.3	0.45	36.2‡	0.32

* Mean of four independent determinations.
† Titration in aniline at 40 °C.
‡ Titration in benzyl alcohol - methanol - water.

For aromatic polyamides, which are used for the spinning of high-strength and high-modulus fibres, the choice of solvents is very limited. Whereas in poly(*m*-phenylene isophthalamide) potentiometric titration in dimethylformamide is possible,[6] direct titration of poly(*p*-phenylene terephthalamide) (PPD-T) is impossible as it is soluble only in strong acids such as concentrated sulphuric acid and methanesulphonic acid.

An attractive method for determining carboxyl groups in PPD-T is based on the Schmidt reaction, in which the carboxyl group is subjected to reaction with sodium azide in sulphuric acid.[7] The acylazide formed rearranges to an amine with the liberation of nitrogen and carbon dioxide:

$$\sim\!\sim\!\sim COOH \xrightarrow[H_2SO_4]{NaN_3} \sim\!\sim\!\sim NH_2 + CO_2\uparrow + N_2\uparrow$$

The carbon dioxide evolved is measured quantitatively by an automatic non-aqueous titration. The apparatus for this determination is shown in Fig. 4.

The suitability of this method has been investigated with the model compound *NN'*-bis-(*p*-carboxybenzoyl)-*p*-phenylenediamine (BCP):

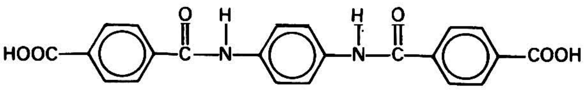


Fig. 5 shows the conversion of BCP in 100–101% sulphuric acid at 50 °C at two concentration levels. A reaction time of 2 h gives a quantitative conversion, which also suffices for polymer solutions (see Fig. 6).

As additions of known amounts of BCP to the viscous polymer solutions are recovered quantitatively,[7] the experimental conditions for complete removal of carbon dioxide are correct. The sulphuric acid concentration is very critical; as can be seen in Table VI, a concentration of 100% is essential for quantitative conversion. The standard deviation for the method is 2.6 mmol kg^{-1}.

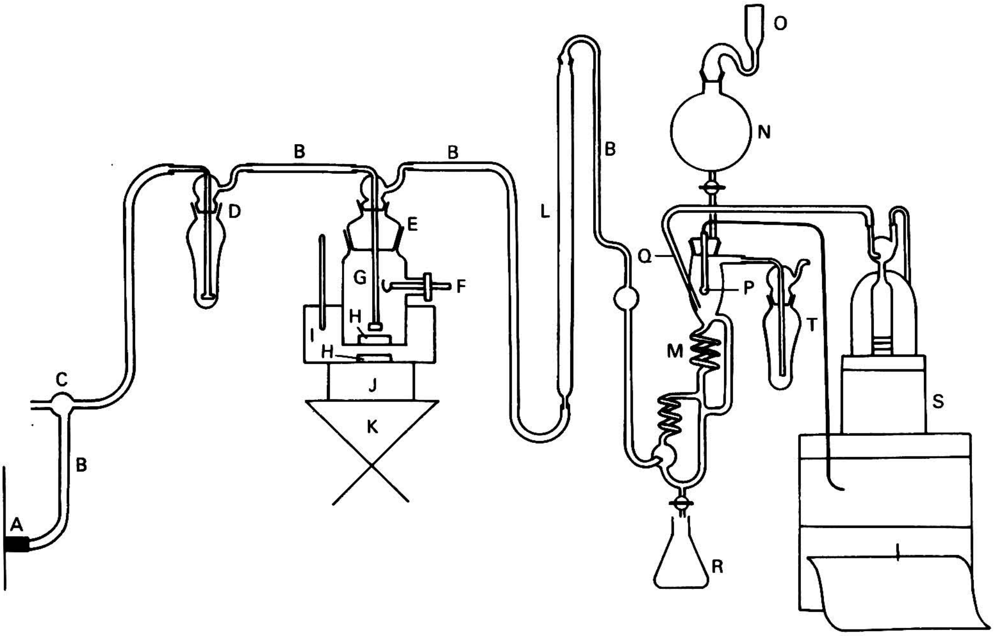


Fig. 4. Apparatus for the determination of carboxyl end groups: A, nitrogen connection; B, buty rubber tubing; C, three-way tap; D, gas absorption bottle; E, reaction vessel; F, turnable spoon; G, nitrogen inlet tube; H, magnetic stirring bars; I, oil-bath with thermometer; J, magnetic stirrer with facilities for heating; K, adjustable support; L, glass column filled with MnO_2 on asbestos (length 50 cm, diameter 2–4 cm); M, absorption and titration vessel; N, receiver; O, ascarite tube; P, combined glass - calomel electrode; Q, inlet tube burette; R, drain; S, titration equipment; and T, gas absorption bottle.

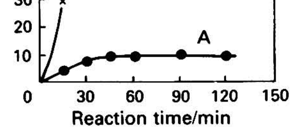


Fig. 5. Recovery of BCP after Schmidt reaction. Amount of BCP taken: A, 10.0 µmol; and B, 100.0 µmol.

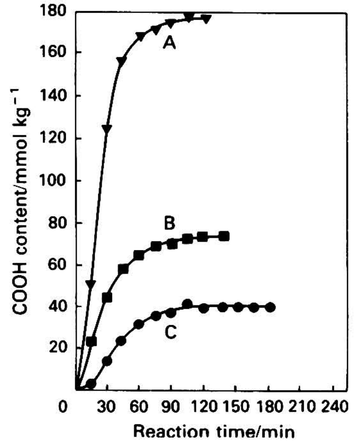


Fig. 6. Conversion of the carboxyl end groups of several samples for different periods at 50 °C. A, PPD-T (viscosity ratio = 2.09); B, yarn (viscosity ratio = 4.09); and C, PPD-T (viscosity ratio = 5.74).

Table VI

Conversion of BCP at various sulphuric acid concentrations

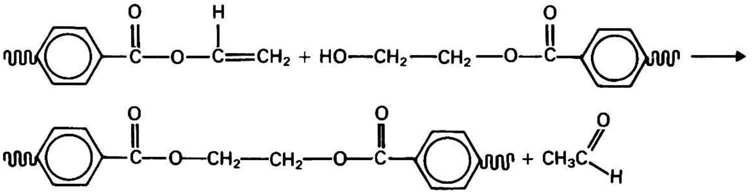
Sulphuric acid concentration, %	98.3	99.2	99.7	100.2	101.0
Conversion of BCP, %	3.8	16.2	21.9	100.1	100.5

Unsaturation

Chemical methods for the determination of unsaturation are often based on halogenation or hydrogenation. An elegant method for determining unsaturated compounds is the *in situ* generation of a halogenating agent (*e.g.*, bromine) by constant-current coulometry. The amount of reagent consumed is proportional to the amount of electricity used to generate the reagent. The reaction between the double bond and bromine is catalysed by mercury(II) chloride. The advantages of a coulometric method are that a standard reagent is not needed, minimum side-reactions (substitution) occur because the bromine concentration is kept low, and the method is precise, is suitable for low levels and can readily be automated.

A survey of applications was given by Hirozawa.[8]

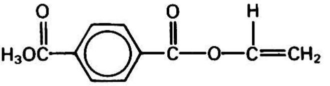
We have used coulometric bromination to determine vinyl ester end (VEE) groups in PETP formed by thermal chain scission and the source of subsequent liberation of acetaldehyde.



Only a brief description of the method is given here; more details will be published elsewhere.[9]

The constant-current generation of bromine is carried out in a medium of dichloroacetic acid (DCA), water, potassium bromide and mercury(II) chloride. To this medium an amount of the polymer, previously dissolved in HFI and diluted with anhydrous DCA, is added and bromine is generated. The end of the reaction is detected biamperometrically.

The suitability of this method was tested with the model compound methyl vinyl terephthalate (MVT):



Additions of 14.2 and 1.0 μmol of MVT (corresponding to 30 and 2 mmol of VEE group per kilogram of polymer) were recovered quantitatively (recoveries of 99.8 and 98.5%, respectively). The coulometric analysis must be completed within 30 min, because after longer times the hydrolysis of the VEE group is no longer negligible.

The VEE group is not the only reactive moiety in PETP that consumes bromine, as impurities present also do so. To determine this background, in a second sample the VEE group is previously hydrolysed at 80 °C in a dichloroacetic acid - water medium. Fig. 7(*a*) shows the relationship between the bromine consumption and the hydrolysis time for MVT and Fig. 7(*b*) represents the same relationship for two polymers containing a high and a negligible amount of VEE groups.

It can be seen that the VEE group in MVT is hydrolysed completely after 4–6 h at 80 °C. For polymer A a sharp decrease occurs, which can mainly be attributed to the hydrolysis of the VEE group in the polymer. In addition, a much smaller effect is observed due to the hydrolysis of the bromine-consuming impurities [see relationship for polymer B, Fig. 7(*b*)]. This small effect is corrected by extrapolating this relationship from time 6 h to time zero (*i.e.*, 0.7 mmol kg^{-1}).

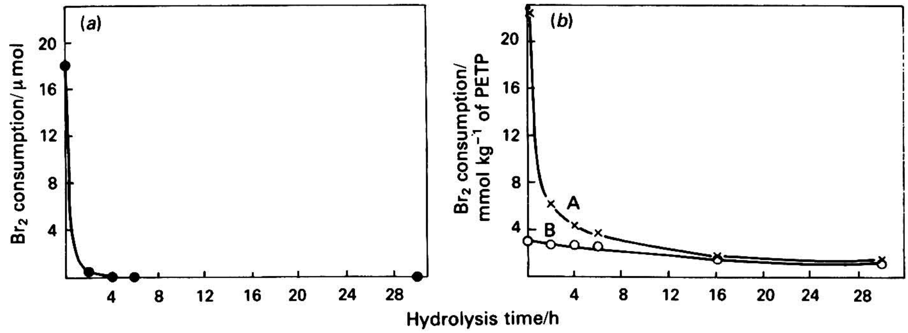


Fig. 7. (*a*) Hydrolysis of MVT. (*b*) Hydrolysis of VEE groups in PETP: A, PETP with a high VEE group level; B, PETP with a negligible VEE group level.

The VEE group content is calculated by subtracting the background (*i.e.*, the value measured after 6-h hydrolysis + 0.7 mmol kg^{-1}) from the content originally measured. The standard deviation of the method is 0.2 mmol kg^{-1}.

Application to some PETP polymers is illustrated in Table VII. It can be seen that samples with almost identical viscosity ratios (A–D) may have different levels of VEE groups, depending on the temperature at which the polycondensation is carried out. It is also apparent that, as expected, a higher level of VEE groups corresponds to a higher level of carboxyl groups. Polymerisation at higher temperatures also results in a higher background.

TABLE VII

VINYL ESTER END GROUPS IN PETP SAMPLES

Sample	Viscosity ratio*	VEE group/ mmol kg^{-1}	Background/ mmol kg^{-1}	Carboxyl groups/ mmol kg^{-1}	Polycondensation temperature/°C
A	1.59	1.1,1.0	1.5,1.4	20	280
B	1.62	2.3	2.6	33	284
C	1.62	6.1	3.5	56	294
D	1.60	18.6,17.9	4.4,4.4	80	302
E	1.89	5.5,5.6	3.3,3.3	50	280
F	1.91	1.3,1.2	2.7,2.7	22	280

* Measured for a 1% *m*/*m* solution in *m*-cresol at 25 °C.

Amino Groups

Chemical determination of amino groups is often carried out by titrimetric or spectrophotometric analysis. By proper selection of solvent and derivatisation techniques, differentiation between amines (primary, secondary, tertiary and quaternary ammonium salts) is possible.[10,11] Two examples of the determination of amino groups in insoluble or poorly soluble materials are described below.

The first example relates to the determination of this group in derivatised silica materials used for affinity chromatography. This material is illustrated schematically in Fig. 8. The amino group can be determined directly in this material by heterogeneous titration with perchloric acid and acetic acid as titration medium. It is essential to apply an equilibrium titration. This is illustrated in Fig. 9.

In Fig. 9(*a*), the first part of the titration was carried out continuously (0.2 ml min^{-1}) with a reduced titration speed on the steep part of the curve. When the titration was stopped at A, a slow shift in potential to the more negative (*i.e.*, alkaline) direction occurred. When the titration was continued at B under carefully chosen equilibrium conditions (*i.e.*, with a potential stability programmed addition) a second jump was found. The total consumption

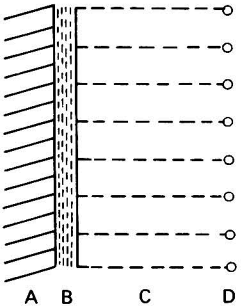


Fig. 8. Derivatised silica material: A, silica; B, coating; C, spacer group; and D, functional group.

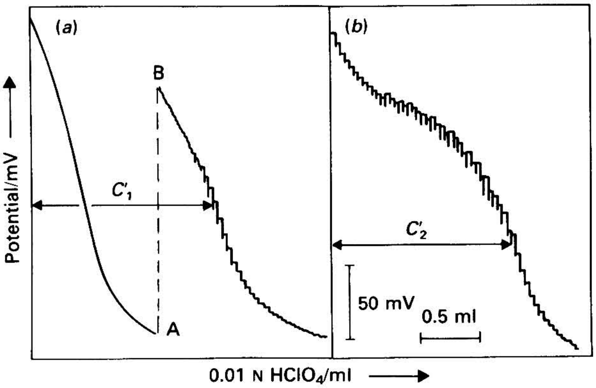
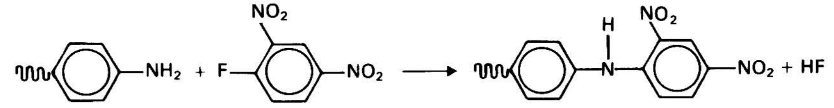


Fig. 9. Titration of silica material that contains amino groups with 0.01 N perhcloric acid under different conditions. (*a*) Continuous and equilibrium titration and (*b*) equilibrium titration. Apparatus, Mettler DK 11 and DK 15.

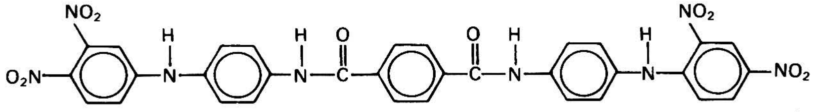
(C'_1) was equal to that of a direct equilibrium titration (C'_2). Using this technique a coefficient of variation of 2% was obtained. The accuracy of this determination was checked with results from a nitrogen analysis (Fig. 10).

In the second example, the determination of amino groups in poly(*p*-phenylene terephthalamide), titration is impossible because this polymer is soluble only in strong acids. To determine the amino groups, use is made of a heterogeneous derivatisation with 1-fluoro-2,4-dinitrobenzene (FDNB)[7]:



The polymer is treated for 4 h at 80 °C with FDNB in an ethanol - hydrogen carbonate medium. After washing out the excess of reagent, the dinitrophenyl group introduced is measured spectrophotometrically after dissolution of the derivatised polymer in methanesulphonic acid. The measurement is carried out at 430 nm against a solution of the same amount of underivatised polymer (Fig. 11). Fortunately, the molar absorptivity of unreacted FDNB at this wavelength is very low, which means that trace amounts of non-washed FDNB will contribute to only a small extent.

The spectrophotometric method was calibrated with the FDNB derivative of the model compound *NN'*-bis(*p*-aminophenyl)terephthalamide:



and the correctness of the whole procedure was confirmed by the use of carbon-14-labelled FDNB and measurement of the incorporated activity (for details, see reference 7).

The completeness of the derivatisation was checked carefully by comparing the heterogeneous derivatisation with a homogeneous derivatisation for low relative molecular mass materials (see Fig. 12) and by a re-derivatisation procedure for high relative molecular mass polymers, which showed that virtually no additional groups could be derivatised after the first derivatisation; details are given in reference 7.

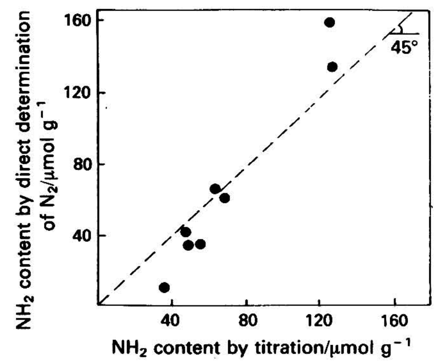


Fig. 10. Comparison of two methods for the determination of amino groups in silica.

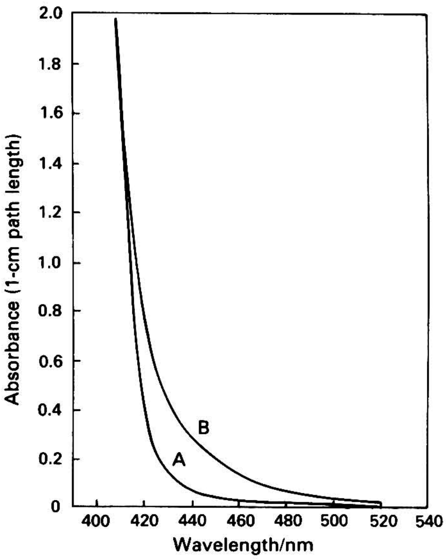


Fig. 11. Absorption spectra of underivatised (A) and FDNB-derivatised (B) PPD-T. Concentration: 100 mg per 50 ml of methanesulphonic acid.

Yarns are poorly accessible to FDNB and the derivatisation must be applied to precipitated polymer obtained from yarn dissolved in sulphuric acid (see Table VIII).

TABLE VIII

ACCESSIBILITY OF YARNS AND PPD-T TO FDNB

	NH_2/mmol kg^{-1}	
Sample	Without precipitation	After precipitation
Yarn 1	7.4	24.0
Yarn 2	25.6	48.4
Yarn 3	11.5	59.0
PPD-T 1	19.9	23.0
PPD-T 2	30.7	29.4

Quaternary Ammonium Salts

For the manufacture of galvanic metal - polytetrafluoroethane (PTFE) layers on metal, the PTFE is dispersed in galvanic baths by using two types of surfactants, *viz.*, one cationic, $[C_8F_{17}SO_2NHC_3H_6N^+(CH_3)_3]_2SO_4^{2-}$ (CAT), and the other non-ionic, $C_8F_{17}SO_2NC_2H_5$-$(CH_2CH_2O)_nH$ (NI).

Ion-selective electrodes are often used for the determination of surfactants, either via direct measurement of the potential or as an indicator electrode in a titration.[12,13] The electrodes usually have a specially prepared membrane containing the active phase. Recently Selig[14] described the use of a calcium electrode for this purpose.

A well known reagent for quaternary ammonium salts is tetraphenyl borate (TPhB), which forms insoluble complexes with cationic surfactants.[15] Applications of this reagent were described by Vytras.[16]

For the determination of CAT in baths we apply a potentiometric titration with TPhB in which a home-made electrode is used as the indicator electrode. The construction of the electrode is shown in Fig. 13.

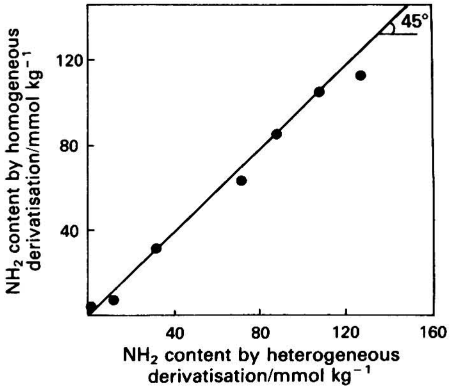


Fig. 12. Derivatisation of PPD-T under heterogeneous and homogeneous conditions.

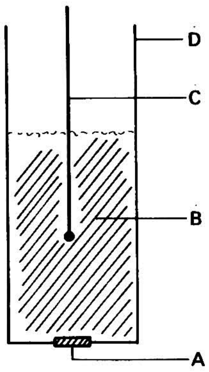


Fig. 13. Electrode for cationic surfactants: A, membrane consisting of PBTP (20%) and polytetrahydrofuran (PTHF) (80%); B, internal solution (10^{-3} M CAT+ - TPhB− complex in cyclohexanone); C, internal reference electrode (Ag - AgCl); and D, electrode housing.

Fig. 14 shows the relationship between the potential and the negative logarithm of the CAT concentration. It can be seen that the electrode follows the Nernst equation in the region p(CAT) 4–6. The slope (63 mV) is somewhat higher than the theoretical value. At concentrations exceeding 10^{-3} M, micelle formation probably occurs. The drift of the electrode is about 3 mV h^{-1}, whereas the response for a concentration change from 10^{-4} to 10^{-5} M (achievement of 95% of the maximum signal) is 8 s.

An example of a titration of an aqueous solution of CAT is presented in Fig. 15. Titrations were carried out with an equilibrium titrator (Mettler DK 15).

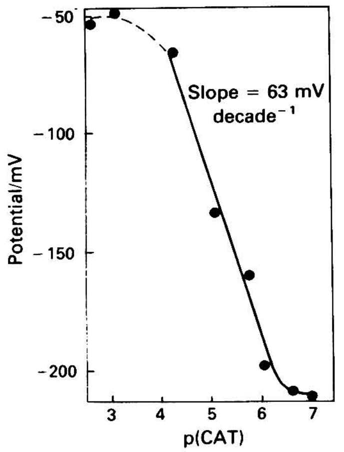


Fig. 14. Relationship between p(CAT) and potential.

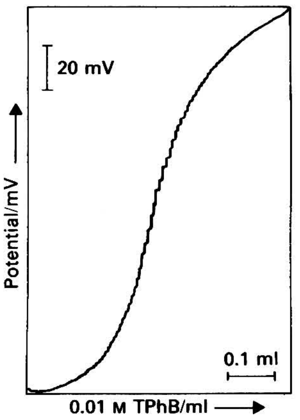
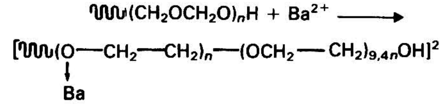


Fig. 15. Titration of about 2 mg of CAT with 0.01 M TPhB.

The non-ionic surfactant can also be determined by TPhB titration. The ethylene oxide group is charged positively by the addition of barium ions. According to Levens and Ikeda[17] the following structure is formed:



$$\sim\sim(CH_2OCH_2O)_nH + Ba^{2+} \longrightarrow$$

$$[\sim\sim(\underset{\substack{\downarrow\\ Ba}}{O}-CH_2-CH_2)_n-(OCH_2-CH_2)_{9.4n}OH]^{2+}$$

This positively charged complex also reacts with the TPhB ions to form an insoluble complex. In this way separate titrations of CAT and NI are obtained, *i.e.*, first the CAT is titrated and after the equivalence point has been passed barium chloride is added, followed by NI titration (see Fig. 16).

Ethoxylated non-ionic surfactants often contain free polyethylene oxide; this impurity is detected by the occurrence of two vague jumps in the titration (Fig. 17). As they do not correspond to the free and bound polyethylene oxide, quantitative measurement is impossible.

The surfactants are collected by extraction from the galvanic baths after removal of the PTFE. The limits of detection of CAT and NI are 2 and 5 mg l^{-1}, respectively.

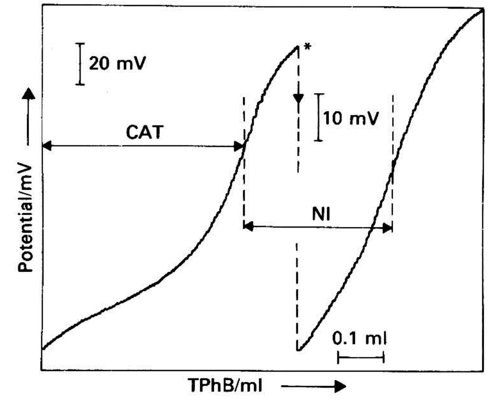


Fig. 16. Titration of a mixture of CAT and NI with TPhB; * indicates addition of $BaCl_2$.

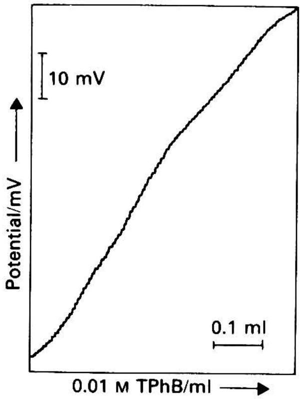
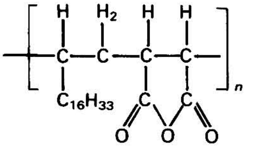


Fig. 17. Titration of NI containing free polyethylene oxide with 0.01 M TPhB.

Anhydride Groups

The example discussed here is the determination of anhydride and carboxylic groups in a commercial polyanhydride resin. The resin is derived from octadecene-1 and maleic anhydride:



The acid groups are formed by hydrolysis of the anhydride group.

The common method involving reaction with an excess of aniline and subsequent back-titration of the excess[18] is unsuitable, as the reactivity of the anhydride group is low. Even after hydrolysis with aqueous pyridine (containing 40% *V*/*V* of water) in a Parr bomb at 150 °C for 4 h, anhydride groups are still seen in the infrared spectrum.

A suitable method for determining the anhydride group is titration with aqueous potassium hydroxide in pyridine after previous esterification of the carboxyl group with diazomethane. This esterification is carried out in diethyl ether - methanol (9 + 1); after methylation, which takes about 10 min for 0.5 g of sample, the solvents are removed by evaporation and a portion of the derivatised polymer is dissolved in pyridine and titrated.

Fig. 18(*a*) and (*b*) show the infrared spectra of the resin before and after methylation, respectively. It can be seen that the absorption band of the acid group at 1710 cm^{-1} disappears and a carbonyl band of the ester at 1740 cm^{-1} is formed. The acid content of the sample is found from the difference in titres of an unmethylated and a methylated product.

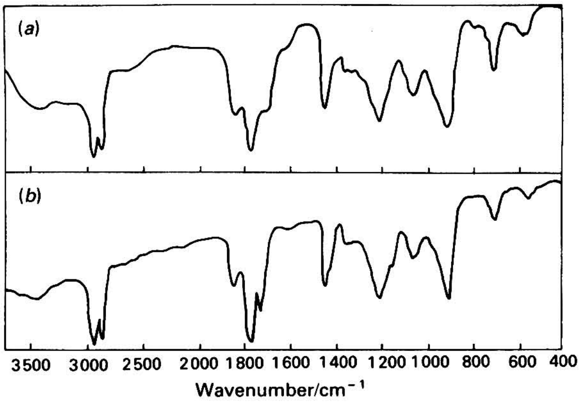


Fig. 18. Infrared spectra of polyanhydride resin: (*a*) before and (*b*) after methylation.

The suitability of this method was checked by an independent method, *viz.*, proton nuclear magnetic resonance spectroscopy. To a solution of the methylated resin in deuterochloroform a known amount of dimethyl terephthalate was added as an internal standard and the amount of methyl ester groups (corresponding to the acid content) was determined quantitatively.

The content of the alkyl part was determined by measuring the terminal methyl group content of the alkyl chain. As the resin is prepared with identical amounts of the two monomers, this amount corresponds to the sum of acid and anhydride. As can be seen in Table IX, the agreement between the methods is satisfactory.

TABLE IX

ANALYSIS OF POLYANHYDRIDE RESIN

$\bar{x}$ = Mean value; n = number of determinations; and s = standard deviation.

Method	$(C{=}O)_2O$, %	COOH, %
KOH titration	$\bar{x} = 16.0$ $n = 5$; $s = 0.29$	$\bar{x} = 5.1$ $n = 5$; $s = 0.21$
NMR	15.7	5.4

Epoxy Groups

Titrimetric methods for epoxides are mainly based on titration with a halogen acid.[19] Epoxy groups in derivatised silica materials (for composition see Fig. 8) can be titrated with perchloric acid in acetic acid containing cetyltrimethylammonium bromide. As in the determination of amino groups, in this instance also an equilibrium titration is necessary (see Fig. 19). The coefficient of variation of the titration method is 2.5% at a level of about 20–200 µmol g^{-1}.

Low contents of epoxy groups are determined in our laboratory by reaction with 2,4-dinitrobenzenesulphonic acid.[20] The reaction product is made alkaline and the resulting orange colour measured at 498 nm. Amounts down to 0.01 µmol of epoxide can easily be measured by this method. Applications to epoxide-containing polymers were described by Cramer and van Houwelingen.[21]

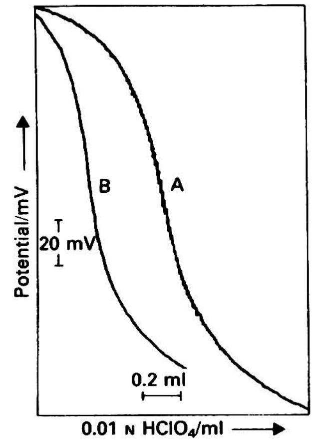


Fig. 19. Titration of equal amounts of silica material that contains epoxide groups under different conditions: A, equilibrium titration; and B, continuous titration.

Our thanks are due to Mrs. J. M. B. Pelgrim, Mrs. M. Cramer, Mr. A. H. M. Schotman and Mr. J. G. M. Aalbers for their considerable contributions to the many practical aspects of the work described.

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Aspects of the Analysis of Drugs and Drug Metabolites by High-performance Liquid Chromatography*

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The biotransformation of a drug may result in a diversity of metabolites with widely differing physico-chemical properties. With the aid of suitable examples the applicability of adsorption, partition and paired ion high-performance liquid chromatography to the measurement of drugs and their metabolites is discussed in relation to their lipophilicity and polarity.

Keywords: *Drug analysis; drug metabolite analysis; high-performance liquid chromatography*

Most drugs are by design lipophilic weak electrolytes whose relative molecular masses range from low to high. Essentially the metabolic biotransformations of a drug have been divided into two distinct types of reaction: functionalisation (phase 1) reactions involving enzymically controlled oxidations, reductions and hydrolyses, and conjugation reactions (phase 2).[1] The functionalisation reactions often produce metabolites which have physico-chemical properties similar to those of the parent drug, although there is usually an attendant increase in polarity and a decrease in lipophilicity. The metabolism of the 1,4-benzodiazepine chlordiazepoxide provides an example of a compound that is initially primarily metabolised to phase 1 metabolites (Fig. 1). However, conjugation reactions involve enzyme-catalysed reactions of the drug or its metabolite with endogenous compounds, producing polar metabolites whose physico-chemical properties are usually markedly different from those of the parent drug. As with phase 1 reactions, this often results in increased polarity and decreased lipophilicity; but the converse can be observed. *p*-Hydroxyacetanilide (paracetamol) is metabolised to a variety of polar conjugates; pK_a values range from 9.5 for the parent drug to less than 2 for its sulphate conjugate (Fig. 2).

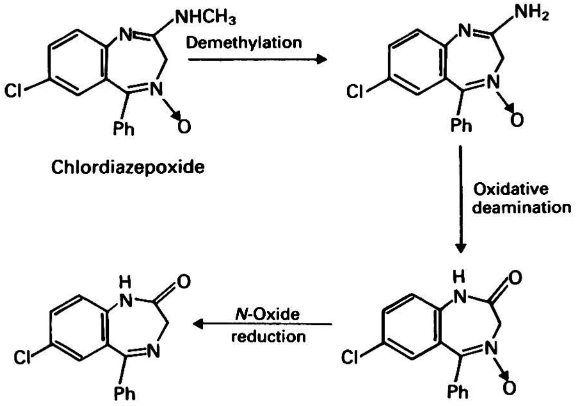

Fig. 1. Chlorodiazepoxide metabolism.

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* Keynote Lecture presented at the Joint NL - UK Symposium on Quantitative Organic Analysis, Noordwijkerhout, The Netherlands, April 22–24, 1981.

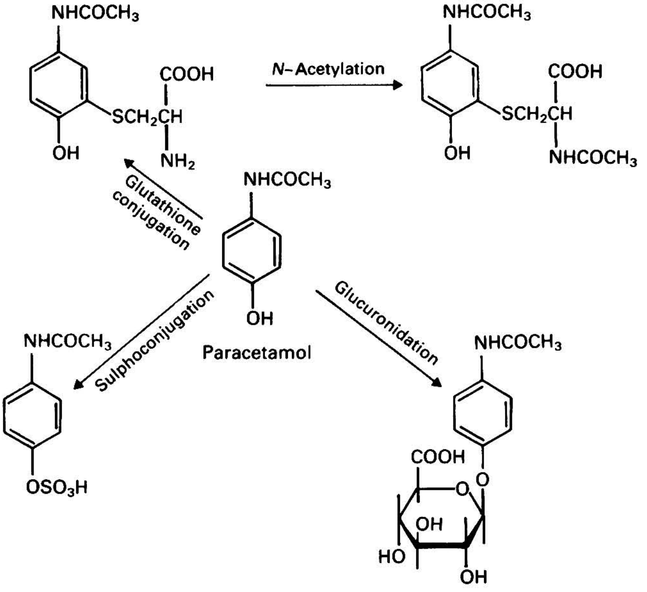

Fig. 2. Paracetamol metabolism.

Because of the aforementioned physico-chemical properties of drugs and their metabolites, high-performance liquid chromatography (HPLC) is a satisfactory method for their measurement at low concentrations in biological fluids, as it combines the advantages of other bioanalytical methods in providing rapid separation and quantitation, but at room temperature. Adsorption, partition, ion-exchange and ion-pair chromatography are all applicable in drug metabolism and pharmacokinetic studies.

Adsorption chromatography is limited to the quantitation of metabolites whose physico-chemical properties are similar to the parent drug, whereas partition chromatography (reversed-phase) using either alkyl- or phenyl-bonded supports is suitable for the measurement of compounds with wide-ranging pK_a values and lipophilicities.

A variety of methods (Table I) have been used to study in humans the elimination of the antithyroid drug methimazole (1-methylmercaptoimidazole) (Fig. 3), which is also a metabolite of the anti-thyroid drug carbimazole, its *N*-carboethoxy derivative. Those methods which do not have a chromatographic separation are non-specific, as they could include sulphur-containing metabolite concentrations in the observed drug concentrations, which would account for the longer biological half-lives of methimazole.

TABLE I

LITERATURE VALUES FOR THE BIOLOGICAL HALF-LIFE OF METHIMAZOLE AND CARBIMAZOLE

Drug	Dose administered/mg	Method of assay	Methimazole half-life/h ± S.E.M.*	Reference
[^{35}S]Methimazole ..	10	Total plasma radioactivity	13.0 (n = 1)	2
[^{35}S]Methimazole ..	10	Total plasma radioactivity	7.9 ± 0.4	3
Methimazole	10	Spectrophotometric	6.9 ± 0.6 (n = 4)	4
Methimazole	10	Gas - liquid chromatography	3.7† (n = 1)	5
Methimazole	60	HPLC	3.2 ± 0.29 (n = 5)	6
Carbimazole	60	HPLC	2.96 ± 0.34 (n = 5)	6

* S.E.M. = standard error of the mean; n = number of patients in each study.
† Calculated from information given in the paper.

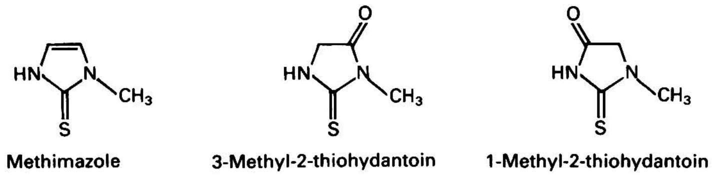

Fig. 3. Methimazole and 1- and 3-methyl-2-thiohydantoin.

In the course of our development of an HPLC method[7] to measure quantitatively methimazole in biological fluids obtained from patients receiving either methimazole or carbimazole,[6] a sulphur-containing metabolite was identified and characterised with the aid of HPLC and spectral techniques as 3-methyl-2-thiohydantoin (Fig. 3).[8] Both alumina (10 μm) and silica (10 μm) columns (10 × 0.46 cm i.d.) were examined for their suitability for the quantitative measurement of methimazole and 3-methyl-2-thiohydantoin in plasma using an internal standard at the extraction stage. The possibility that 1-methyl-2-thiohydantoin (Fig. 3) might be a metabolite was also considered. Although methimazole and the 1- and 3-methyl isomers of 2-thiohydantoin were satisfactorily resolved on a silica column (Table II) with a chloroform-based mobile phase, exogenous purines present in biological fluids, which were co-extracted, interfered with the HPLC separation. With basic alumina, 1-methyl-2-thiohydantoin has a high capacity factor, whereas methimazole, 3-methyl-2-thiohydantoin and the internal standard *p*-toluamide are satisfactorily separated from each other (Table II) in a reasonable time (10 min) with no interference from co-extracted endogenous compounds. This different behaviour of the methyl isomers of 2-thiohydantoin on silica and alumina could partly be explained by the fact that their pK_a values differ by 2 units.

TABLE II

CAPACITY FACTORS FOR METHIMAZOLE, 3-METHYL-2-THIOHYDANTOIN AND 1-METHYL-2-THIOHYDANTOIN ON SILICA AND ALUMINA HPLC COLUMNS

		Capacity factor (k')	
Compound	pK_a	Silica*	Alumina†‡
Methimazole..	11.5	2.9	3.2
3-Methyl-2-thiohydantoin	10.6	1.4	1.8
1-Methyl-2-thiohydantoin	8.6	1.9	20

* 10-μm silica with 0.75% methanol in chloroform as mobile phase.
† 10-μm alumina with 1.0% methanol in chloroform as mobile phase.
‡ k' for toluamide (internal standard) = 7.4.

For the reversed-phase systems, the capacity factor, a property of the column, is related to the partition coefficient (log P), a measure of the lipophilicity of the compound to be measured. The quantitation of chlordiazepoxide and its metabolites (Fig. 1) using an octadecylsilane (ODS) support illustrates the applicability of reversed-phase HPLC systems to the separation of a drug and its metabolites.[9] The separation, by virtue of their different lipophilicities, of chlordiazepoxide and metabolites at a particular pH, and their quantitation, without prior derivatisation or hydrolysis to their corresponding benzophenones, illustrate the advantage of reversed-phase HPLC in drug metabolism studies. Operation at room temperature is beneficial for analysing thermally labile compounds such as oxazepam,[10] another 1,4-benzodiazepine.

It is in the measurement of polar metabolites, and drugs and metabolites of high relative molecular mass that HPLC has its widest application. Paracetamol is an example of a drug that is eliminated from the body via conjugation reactions (Fig. 2), predominantly as its highly polar glucuronide and sulphate conjugates. Using silanised ODS-silica and octylamine in the mobile phase to act as a basic counter ion to form ion pairs with the weakly acidic paracetamol and strongly acidic metabolites, satisfactory separation of paracetamol from its glucuronide, its sulphate and its cysteine and mercapturic acid metabolites has been obtained.[11] With this method it is possible to measure paracetamol and its metabolites quantitatively in urine, by injecting urine samples directly on to the HPLC column.[12]

Not only can HPLC be used for the quantitation of drugs and metabolites from *in vivo* studies, but we have also been examining its applicability in enzyme kinetic studies, in particular the *in vitro* glucuronidation of paracetamol (Fig. 4) and *p*-nitrophenol by liver UDP glucuronyltransferase. The formation of the glucuronides of these substrates in incubations containing enzyme, substrate and uridine diphosphoglucuronic acid (UDPGA) was monitored by injecting samples of incubation mixture directly on to a column packed with ODS-silica (5 μm), eluted with a mobile phase containing an ion-pairing reagent, tetrabutylammonium phosphate in aqueous methanol.[13]

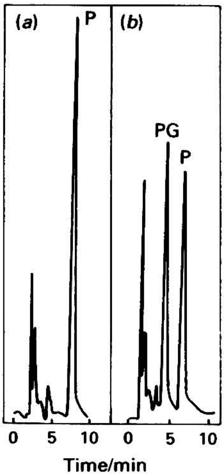

Fig. 4. Chromatograms of samples from incubation medium for studying the *in vitro* rate of glucuronidation of paracetamol. Sample taken 2 min (*a*) and 30 min (*b*) from the start of incubation. Conditions: column (10 × 0.46 cm) packed with 5-μm Spherisorb C_6; mobile phase, 5 mM tetrabutylammonium phosphate in methanol - water (12 + 88); flow-rate, 1.2 ml min^{-1}; and detection, 254 nm. Peaks: P, paracetamol; and PG, paracetamol glucuronide.

The diastereoisomeric glucuronide metabolites of the 1,4-benzodiazepine oxazepam have been quantitatively measured by HPLC[14] after their separation by preparative ion-exchange chromatography. The rates of enzyme hydrolysis of the individual diastereoisomers were studied by HPLC, subsequent to their purification.

The anthracycline cytotoxic antibiotic adriamycin, which has a high relative molecular mass (543.5), its major metabolite adriamycinol and its aglycone metabolites have been separated and quantitatively measured with the aid of an internal standard, using an ODS-silica column and a fluorescence detector.[15]

In conclusion, HPLC has been clearly shown to be a versatile technique effective in either *in vivo* or in *in vitro* drug metabolism studies. In particular it is in the measurement of polar metabolites, thermally labile drugs and metabolites and drugs and metabolites of high relative molecular mass that HPLC has its widest application.

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Determination of Mercury Vapour in Air Using a Passive Gold Wire Sampler

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The determination of atomic mercury vapour in air in the range 10–120 μg m^{-3} is described. Mercury vapour was collected by exposing a 1-cm length of gold wire to the air for 5 min, then thermally desorbed from the wire for measurement by atomic-fluorescence spectrometry. The results obtained showed good agreement with those obtained from an acid permanganate wet sampling system.

Keywords: *Mercury determination; air analysis; gold wire; atomic-fluorescence spectrometry*

There are several methods available for the sampling and determination of mercury vapour in air in the region of the Threshold Limit Value (TLV) (currently[1] 50 μg m^{-3}) and below. The mercury may be collected by drawing a measured volume of air through an absorbing solution such as iodide - iodine[2] or potassium permanganate - sulphuric acid solution,[3] or through solid adsorbents such as iodised charcoal,[4] gold-wire clippings[5] or silver foil.[6] These techniques require cumbersome apparatus incorporating a flow meter, vacuum pump and valves.

Several continuous monitors based mainly on ultraviolet atomic-absorption spectrometry are available commercially, but these instruments, although sensitive, are bulky and expensive.

Passive devices, in which a gold foil[7] or vacuum-deposited gold layer on a glass substrate[8] was exposed to the air for a fixed length of time, have been described recently. These devices are passive in that they depend upon diffusion of mercury from the air to the adsorbing surface. It is assumed that the mass of mercury deposited on to the gold surface in a given time is proportional to the concentration of mercury vapour in the air. No attempt is made to remove all of the mercury from a measured volume of air.

In the work described in this paper a 1-cm length of gold wire was exposed to the draught-free atmospheres to be measured for 5 min, then removed to the laboratory where the adsorbed mercury was thermally desorbed and determined by atomic-fluorescence spectrometry (AFS).[9] The air was simultaneously sampled with a potassium permanganate - sulphuric acid absorption system followed by analysis by AFS, and the results of the two techniques were compared.

Experimental

Reagents

All materials were of analytical-reagent grade, and all solutions were prepared using distilled water.

Potassium permanganate absorption solution, 0.01 M *in* 5% *V/V sulphuric acid.*

Hydroxylammonium chloride solution, 20% *V/V.*

Tin(II) chloride solution, 10% *V/V in hydrochloric acid.* Dissolve 20 g of tin(II) chloride in 80 ml of "concentrated" 35% hydrochloric acid. If a precipitate appears add a trace of metallic tin and warm the solution until it clears. Dilute to 200 ml with distilled water.

Sulphuric acid, 8% *V/V.*

Reduction solution. Mix 20 ml of the tin(II) chloride solution with 60 ml of 8% sulphuric acid. A continuous stream of argon should be bubbled through the reduction solution to prevent atmospheric oxidation.

Apparatus

The AFS system used was that described by Thompson and Godden.[9] Fluorescence signals were measured using a Baird Atomic, Model A 3000, spectrometer with all lenses removed. Signals were recorded on a Philips, Model 8000, flat-bed chart recorder.

Gold Wire Sampler

A 2-cm length of gold wire, diameter 0.125 mm, purity 99.99% (Goodfellow Metals Ltd.), was securely fixed vertically into a slotted rubber base in such a way that 1 cm of the wire remained exposed. A glass flask into which the base fitted protected the gold wire in transit to and from the sampling area. The exposure commenced when the wire was removed from the flask, and ended when replaced into the flask. After exposure to the draught-free air for 5 min the sampler was taken to the laboratory, where the gold wire was removed from the rubber base and thoroughly rinsed in two successive baths of chloroform. The cleaned gold wire was placed into a quartz tube (150 mm × 6 mm i.d.) through which flowed a regulated stream of argon at 1 l min^{-1}. The quartz tube was heated using a fan-tail burner, and the desorbed mercury vapour swept into the cold vapour AFS system[9] (Fig. 1).

The chloroform cleaning stage was found to be necessary to remove trace amounts of oil that had deposited from the air on to the gold wire during sampling. (This contaminant was identified as vacuum pump oil (from a nearby mercury vacuum distillation apparatus) by injecting the effluent from the heated quartz tube into a gas chromatograph. Further confirmation was obtained by injecting small amounts of the oil into the quartz tube and observing the massive fluorescence signals obtained when the quartz tube was heated.) Stainless-steel forceps were used to handle the gold wire to prevent contamination from skin oils, etc., which were found to cause positive errors during the subsequent AFS measurement,

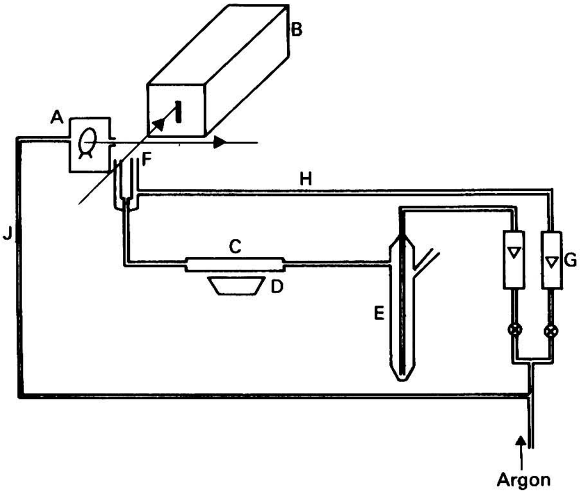

Fig. 1. The desorption and AFS system: A, lamp housing; B, spectrometer; C, quartz tube; D, fan-tail burner; E, mercury generation cell; F, fluorescence measurement head; G, flow control rotameters; H, argon shield gas; and J, lamp cooling argon.

Potassium Permanganate - Sulphuric Acid Sampler[3,10]

Five litres of air were drawn at 1 l min^{-1} through two sintered-glass absorbers[11] connected in series, each containing 20 ml of permanganate absorption solution. The second absorber served as a guard to indicate that the first absorber had not been overloaded.

After sampling, the inlet tube of each absorber was rinsed with 2 ml of water, 3 ml of hydroxylammonium chloride solution were added, the liquid level in the absorber was made up to a 25-ml calibration line if necessary and the absorber contents were mixed until a clear solution was obtained.

A 1.0-ml volume of the analyte solution was mixed with 1 ml of the reduction solution in the mercury generation cell and the mercury vapour liberated was swept into the cold vapour AFS system.

Calibration was achieved by pipetting 1.0-ml aliquots of appropriate standard mercury solutions (prepared in permanganate absorption solution and reduced with hydroxylammonium chloride solution) into the mercury generation cell and reducing with tin(II) chloride reduction solution as above.

Results and Discussion

Comparison of Gold Wire Sampler and Permanganate Absorption Solution

In order to determine the precision of the permanganate system, 20 samples were collected using in each instance five independent identical permanganate samplers operating simultaneously. During these tests the concentration of mercury in air ranged from 10 to 150 μg m^{-3} and the results showed relative standard deviations ranging from 18% at 10 μg m^{-3} to 9% at 150 μg m^{-3} (Fig. 2).

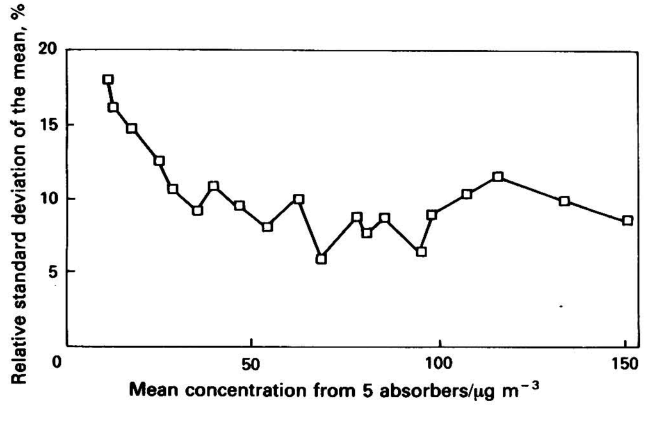

Fig. 2. Variation of precision of the permanganate absorption system with the concentration of mercury in the air.

Comparison of the results obtained from both sampling methods applied simultaneously over a series of 100 measurements made in draught-free room atmospheres showed a rectilinear relationship between the signals obtained from the gold wire system and the mass of mercury collected in the permanganate solution (and thus the concentration of mercury in the air) over the range 0–0.4 μg (0–80 μg m^{-3}), with a useful curve up to 0.6 μg (120 μg m^{-3}).

Linear regression analysis of the results from 0 to 80 μg m^{-3} gave a correlation coefficient of 0.965 at the 99.9% level, and slope of 0.693 chart divisions per μg m^{-3}, with an intercept on the y-axis (gold wire signal) of 2.17 chart divisions. This intercept, equivalent to 3.1 μg m^{-3}, was considered insignificant.

The same gold wire sampler was used for all of the tests, and showed no evidence of deterioration after 100 cycles. Delays of up to 24 h between sampling and thermal desorption produced no discernible difference in the signals obtained.

These results clearly indicated that the gold wire sampler could provide a simple means of determining the concentration of mercury in air, provided that a suitable calibration system could be devised.

Determination of Mass of Mercury Deposited on the Gold Wire

It was clear from the shapes of the signal traces that the rate of thermal desorption of mercury from the gold wire was different from the rate of gas partition desorption of mercury from the solution. Thus, although there was clear proportionality between the signals from gold wire desorption and the signals from solution desorption for given concentrations of mercury in air, it was not possible to determine the relationship between the concentration of mercury in the air to which the gold wire had been exposed and the mass of mercury deposited on the gold wire from this comparison. The solution sampling system was readily calibrated using standard mercury solutions, but calibration of the gold wire system required the additional steps of dissolving the adsorbed mercury from the gold wire in 50% nitric acid solution, followed by solution analysis with calibration against standard mercury solutions.

A series of tests was carried out in which the gold wire was exposed to draught-free atmospheres of various known mercury concentrations (as determined by the permanganate absorption method), then immersed for 15 min in 1.5 ml of 50% nitric acid solution contained in a narrow-form centrifuge tube. A 1.0-ml volume of the nitric acid solution was subsequently taken for analysis by the AFS system, using appropriate calibration standards prepared in 50% nitric acid. This method of desorption of mercury from the gold wire was found to be effective provided that it was carried out within 30 min of exposure. If the gold wire was allowed to stand for a longer period before nitric acid desorption, low recoveries of mercury were obtained, presumably owing to diffusion of mercury into the gold wire. After each nitric acid desorption, the gold wire was rinsed with distilled water and subjected to the thermal desorption process in the quartz tube. When nitric acid immersion had been delayed for longer than 30 min, residual mercury was thermally desorbed from the wire. No signals were obtained from the thermal desorption process when the nitric acid immersion had been carried out within 30 min.

A rectilinear relationship between mass of mercury in the nitric acid solution and concentration of mercury in the air during exposure was observed in the range 0–80 $\mu g\ m^{-3}$, with a useful curve up to 120 $\mu g\ m^{-3}$ (Fig. 3).

No deterioration of the gold wire sampler was observed during these tests, provided that the thermal desorption stage was carried out after each nitric acid stage.

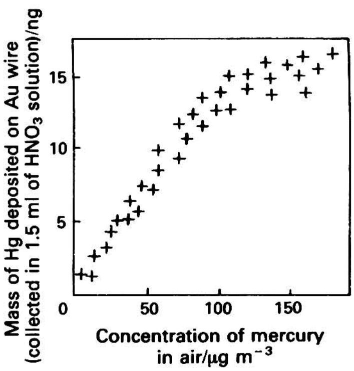

Fig. 3. Relationship between the mass of mercury deposited on the gold wire and the concentration of mercury in air.

Preparation of Standard Atmospheres

Aliquots of saturated mercury vapour in air at known temperatures were injected through a rubber septum into a sealed 5-l glass flask. The flask was shaken vigorously for 5 min to ensure homogeneity, and the gold wire sampler was introduced into the flask via a ground-glass connection.

After exposure for 5 min the wire was removed from the flask and the mercury desorbed by the thermal technique. The exposure was repeated at the same concentration (using a freshly prepared standard atmosphere) and the mercury desorbed by the nitric acid method.

The relationship between mass of mercury in the nitric acid solution and signal obtained from the thermal desorption technique was rectilinear up to 15 ng of mercury (corresponding to a concentration of mercury in air of 120 $\mu g\ m^{-3}$), although the relationship between thermal desorption signal and concentration of mercury in air was rectilinear only from 0 to 80 $\mu g\ m^{-3}$ and curved from 80 to 120 $\mu g\ m^{-3}$, as before.

Comparison of signals from both thermal and nitric acid desorption techniques, obtained from analysis of standard atmospheres of calculated concentrations, with those obtained from "real" atmospheres standardised by the permanganate absorption technique, showed good agreement in the range 0–120 μg m^{-3} of mercury.

In order to determine the precision of the gold wire system, ten standard atmospheres with mercury concentrations in the range 0–120 μg m^{-3} were prepared and the gold wire was exposed to each ten times, then thermally desorbed as previously described The gold wire was thus exposed to each standard atmosphere (freshly prepared) ten times.

The results showed a relative standard deviation of 19% at 10 μg m^{-3} of mercury, which decreased to about 6% between 80 and 120 μg m^{-3} (Fig. 4). The detection limit, corresponding to twice the standard deviation at the 10 μg m^{-3} level, was 3.6 μg m^{-3}. It should be noted, however, that the results "spread" at the 120 μg m^{-3} level showed an artificial bias as the mass of mercury deposited approached saturation.

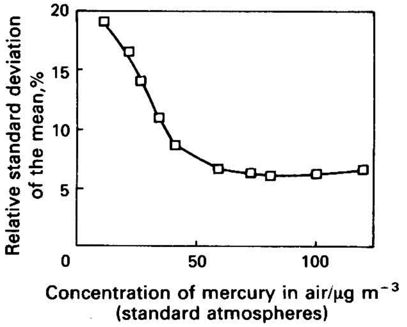

Fig. 4. Variation of the precision of the gold wire sampling system with the concentration of mercury in air.

Time of Exposure

The gold wire sampler was exposed to standard atmospheres, prepared in the 5-l flask, in the range 0–150 μg m^{-3} for varying lengths of time, then subjected to the thermal desorption procedure. In each instance the relationship between mass of mercury adsorbed by the gold wire and time of exposure was rectilinear in the range 0–12 ng of mercury (Fig. 5).

Conclusion

It has been shown that a 1-cm length of 0.125 mm diameter gold wire, exposed for 5 min to mercury vapour in air in the range 0–120 μg m^{-3}, will adsorb a mass of mercury proportional to the concentration of mercury in the air. This principle forms the basis of a simple technique for the determination of mercury vapour in air. The method is rapid and sensitive, although not particularly precise.

It has further been shown that the 1-cm length of gold wire, when exposed to a fixed concentration of mercury in air, will adsorb mercury at a constant rate until a mass of 12 ng has been adsorbed, irrespective of the time of exposure up to at least 35 min.

Further work is proposed in which a continuously moving length of gold wire will be progressively exposed to the air over an 8-h period. Subsequent progressive desorption of the mercury from this wire should thus provide a record of the variations of the mercury in air concentration over the 8-h period. It is hoped that this system will provide the basis for a personal mercury exposure monitor.

In this work mercury concentrations were determined by AFS because of the simplicity of this technique and its freedom from the use of optical cells, the windows of which could "mist up" during the thermal desorption process. It is expected, however, that atomic-absorption spectrometry would be equally effective with few modifications.

The principal function of the spectrometer used in this work was to minimise stray light entering the photomultiplier. It is expected that a simpler, non-dispersive system, such as that described by Hutton and Preston,[12] would be suitable for this purpose.

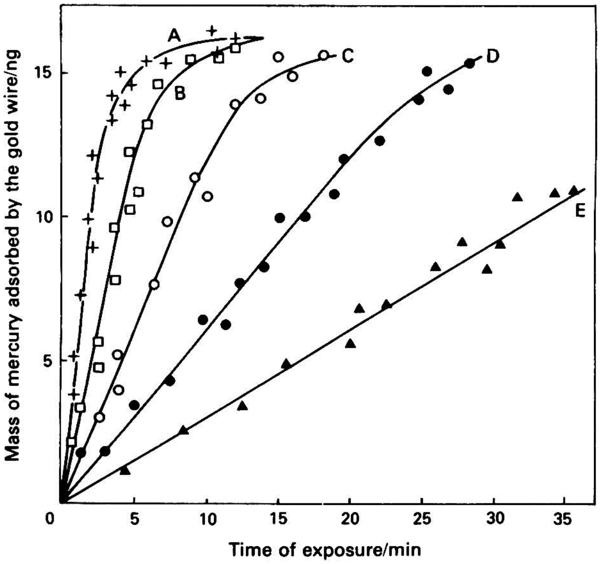

Fig. 5. Variation of the mass of mercury adsorbed by the gold wire with time of exposure for various standard mercury atmospheres. Concentration of mercury in standard atmospheres: A, 160; B, 80; C, 40; D, 20; and E, 10 μg m^{-3}.

J.S. thanks his employers, Philips Hamilton, for use of facilities, and for permission to publish this work.

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1082 *Analyst, October, 1981, Vol. 106, pp. 1082–1087*

Field Method for the Determination of Aromatic Primary Amines in Air

Part I.* Generation of Standard Atmospheres of Amines

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A generator for the production of standard atmospheres of eleven aromatic amines, at very low concentrations, is described. The concentration of the amine produced is determined by gas - liquid chromatography of the heptafluorobutyrate derivative using electron-capture detection.

Keywords: *Aromatic primary amine determination*; *air*; *standard atmosphere*; *gas - liquid chromatography*

The Carcinogenic Substances Regulations 1967[1] prohibit the manufacture or use of certain amines and also control the use of 1-naphthylamine, 4,4′-bi-*o*-toluidine, 3,3′-dimethoxybenzidine, 3,3′-dichlorobenzidine and their salts, and also auramine [4,4′-bis(dimethylamino)-benzhydrylideneamine hydrochloride] and magenta [4-(4,4′-diaminomethylbenzhydrylidene)-cyclohexa-2,5-dienylideneamine]. The Regulations require that the exposure of workers to these compounds shall be minimal and a need exists for a very sensitive test that will give a rapid indication of their presence and concentration in the air of a working environment.

A method whereby the trapped amine is coupled with a stabilised diazonium salt to give a coloured solution[2] was found to lack sensitivity. Additionally, the diazonium salt solution was insufficiently stable for use in a field test, which needs to be simple, reliable and capable of being carried out by relatively unskilled persons. Another colorimetric method involving the condensation of the amine with 4-(dimethylamino)cinnamaldehyde in a non-aqueous medium[3] was more promising. Despite the use of a variety of solvents and organic acids no satisfactory collection of amine vapour from air was achieved. However, a test has been devised in which amine vapour is collected on paper impregnated with the reagent to produce coloured stains proportional to the amount of amine present. This test will be described in Part II.

For the development of a field test it is necessary to produce a standard amine atmosphere; such a generator is the subject of this paper. As a number of primary aromatic amines have extremely low threshold limit values (TLVs), the generator must be capable of producing atmospheres of very low concentration. It was found possible to generate atmospheres of all of the amines of interest using either a vapour saturation or a diffusion technique. Most of the development work was carried out on 1-naphthylamine and it was found later that the same principle of generation could be applied to other amines. A sensitive method of analysis was required to standardise the atmosphere and this was achieved by the formation of a fluorine-containing amide derivative that was determined by gas - liquid chromatography with an electron-capture detector (EC - GLC).

Experimental

Preparation of Amine Atmospheres

Dynamic atmospheres of the amines were prepared by one of two methods.

(*i*) A vapour saturation method in which air was passed through a mixture of the amine with sand contained in a tube maintained at constant temperature. To prepare the mixture, approximately 0.5 g of the amine and 35 g of sand were intimately mixed by stirring in a glass beaker.

(*ii*) By use of a diffusion cell similar to that described by Arito and Soda[4] in which the vapour diffusing through a cylindrical tube from a reservoir containing the amine was diluted with a metered stream of air.

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* For Part II of this series, see p. 1088.

The method used for a particular amine is given in Table I. The general scheme of the generator is represented in Fig. 1, where the arrangement for controlling humidity is included.

TABLE I

METHODS USED FOR GENERATION OF AMINE ATMOSPHERES OF KNOWN CONCENTRATION

Vapour saturation at 0 °C	Diffusion cell*
2-Chloroaniline	Aniline (5)
4-Chloroaniline	*o*-Anisidine (20)
3,3′-Dimethoxybenzidine	*p*-Anisidine (29)
3,3′-Dichlorobenzidine	*o*-Toluidine (20)
2,2′-Dichloro-4,4′-methylenedianiline [4,4′-methylene bisphenyl-(2-chloraniline)][5]	
4,4′-Methylenedianiline	
1-Naphthylamine	
4-Nitroaniline (*p*-nitroaniline)[5]	
4,4′-Bi-*o*-toluidine	

* The bath temperatures (°C) are given in parentheses. The dimensions of the diffusion tubes were 250 × 1.07 mm i.d. except for *o*-anisidine for which they were 125 × 3.80 mm i.d.

The initial development work was done with dry air and the generator was operated as follows. Air supplied to the generator was cleaned by passage through a tower containing charcoal. The air passed through a metal coil in a bath containing a water - ethylene glycol mixture to establish thermal equilibrium and then either through a tube containing the sand - amine mixture or a diffusion cell as appropriate. The dilute atmosphere so produced passed through tubing having several sharp bends, which had the dual function of smoothing the flow and collecting entrained amine condensate. This tube became coated with a layer of solid and in some instances, especially for 1-naphthylamine, a crystal growth followed. If the crystallisation produced loose material the tube was cleaned thoroughly with a solvent and the generator was allowed to run for 48 h to stabilise before further samples were taken. The generated atmosphere passed into a sampling chamber with an open exit tube to ensure that samples were taken at atmospheric pressure. The atmosphere emanating from the generator was collected in an open bell and passed through a tower containing charcoal.

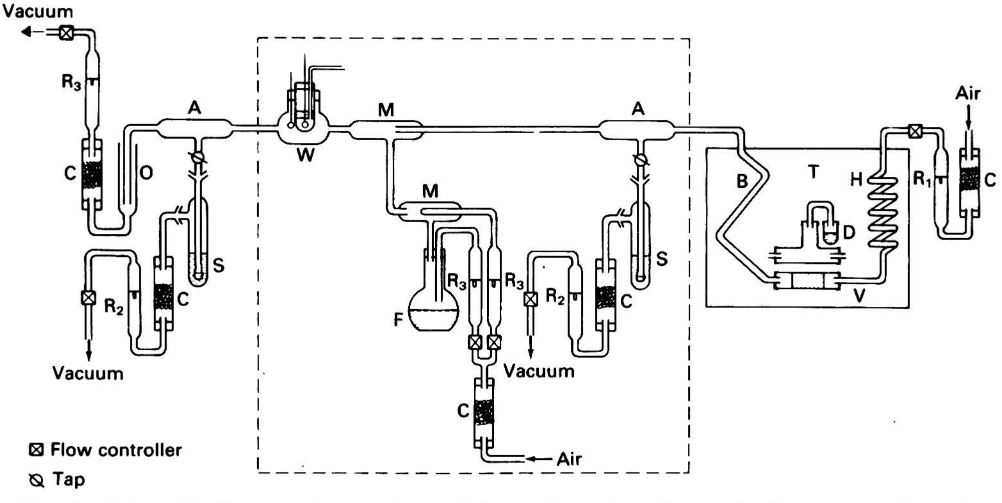

Fig. 1. Schematic diagram of generator used to produce atmospheres of primary aromatic amines. C = Charcoal tower; R = rotameters (R_1, 1 l min^{-1}; R_2, 0.5 l min^{-1}; R_3, 10 l min^{-1}); H = heating coil; T = thermostated bath; V = vapour saturation cell; D = diffusion cell; B = bent tubing; A = sampling chamber; S = sintered bubbler; F = flask containing water; M = mixing chamber; W = Regnault hygrometer; and O = open bell. The part enclosed by broken lines was omitted for atmospheres prepared using dry air.

The sampling rate, governed by a flow controller connected to a vacuum line, was measured with a rotameter protected from water vapour by a charcoal tower. As the rotameter is not open to the atmosphere it must be calibrated using an in-line soap-bubble flow meter or by disconnecting the bubbler from the generator and connecting to another rotameter, the other end of which is open to the atmosphere.

Air was removed from the bell at a flow-rate slightly greater than that through the generator in order to prevent the escape of amine vapour to the atmosphere. The amine-laden air was cleaned by passage through a tower of charcoal before being released to the atmosphere. A flow-rate of 500 ml min^{-1} was used through the generator and samples were taken at a flow-rate of 250 ml min^{-1}. The temperature of the bath when using the diffusion cell is given for individual amines in Table I.

During the course of the development of the test it was realised that humidity was a key factor (see Part II). The generator was modified to incorporate a supply of air of known humidity produced by mixing saturated and dry air streams. The humidified air, produced at 10 l min^{-1}, was added to the amine atmosphere in a mixing chamber. The humidity of the diluted amine atmosphere was determined by measurement of the dew point using a Regnault hygrometer. An additional sampling point was included in the generator before the humidified air inlet in order to estimate the concentration of the amine before dilution. Although this sampling point was not at atmospheric pressure, the back-pressure in the generator was insufficient to lead to significant error in the estimation of the amine concentration used to calculate the required flow-rate of humidified air.

Calibration of Atmosphere

Reagents

All reagents should be of recognised analytical-reagent grade where available and all solutions should be prepared with distilled or de-ionised water.

Cyclohexane. Spectrosol grade.

Hydrochloric acid, 0.1 M.

Disodium tetraborate decahydrate, 0.05 M *solution.*

Heptafluorobutyryl (HFB) chloride solution. Dissolve 0.5 ml of HFB chloride in 100 ml of cyclohexane.

HFB amide derivative. HFB amide derivatives are required as standards for gas - liquid chromatography and are prepared by the following procedure. Dissolve approximately 1 g of the amine in 20 ml of pyridine and add 1.5 ml of HFB chloride solution dropwise with shaking. Hydrogen chloride fumes and heat are generated and in some instances pyridinium chloride may be precipitated. Allow the mixture to stand for about 1 h to ensure that the reaction proceeds to completion, then pour into a large excess of water. The pyridinium chloride dissolves but the hydrophobic HFB derivative is precipitated. Filter off the precipitate and wash with copious amounts of water to remove most of the pyridine. Place derivatives of aniline, *o*-toluidine, *o*-anisidine and 4-nitroaniline, which form insoluble oils, in a refrigerator until the oil solidifies. Filter off the solid and treat in the same way as a normal precipitate. Dissolve the solids obtained in the minimum amount of ethanol. Add water until opalescence occurs and then warm the solution until it is clear again. Allow the solid to crystallise and then collect on a filter. Purify by recrystallisation until the product is white and no odour of pyridine is detectable. Usually only those derivatives that form oils need more than one recrystallisation. Dry the products at 30 °C, except for the *o*-anisidine derivative, which must be dried at 22 °C.

HFB amide derivative solutions. Prepare a solution in cyclohexane of the derivative of the amine to be determined to contain the equivalent to 50 ng ml^{-1} of amine.

Determination of concentration of amine atmosphere. Pipette 5 ml of the hydrochloric acid into the bubbler. Draw 1 l of the atmosphere through the bubbler at a flow-rate of 250 ml min^{-1}. After taking the sample, lift the inlet tube so that the sinter is clear of the liquid and by means of a blowball expel the liquid trapped in the domed sinter as completely as possible. Make any required dilution by addition of the hydrochloric acid (see Table II). To the trapping solution (or to a 5-ml aliquot if dilution was required) add, in the order given, by means of pipettes, 8 ml of disodium tetraborate solution, 1.5 ml of HFB chloride solution and 3.5 ml of cyclohexane. Stopper the tube, shake vigorously for approximately 2 min to ensure thorough mixing (see Note) and then stand to allow the two layers to separate.

Inject a 5-μl aliquot of the cyclohexane layer on to the gas - liquid chromatographic column using suitable conditions, for example, as in Table III.

NOTE—
Too vigorous shaking will result in emulsification and make separation difficult.

TABLE II

AMOUNTS OF AMINE REQUIRED FOR ANALYSIS BY EC - GLC

Amine	Threshold limit value[5]/ mg m^{-3}	Approximate amount required to give a peak height of 20 mm/pg per 5 μl	Volume of 0.1 M HCl required for dilution/ml
Aniline	(19) 10*	23	200
o-Toluidine	22	143	200
o-Anisidine	0.5	147	None
p-Anisidine	0.5	196	None
4-Nitroaniline	6	215	50
1-Naphthylamine	—†	64	None
4,4′-Methylenedianiline ..	No TLV established	18	None
4,4′-Bi-*o*-toluidine	—†	54	None
3,3′-Dimethoxybenzidine ..	—†	77	None
3,3′-Dichlorobenzidine ..	—†	34	None
2,2′-Dichloro-4,4′-methylene-dianiline	0.05	22	None

* Intended change.
† No TLV, use restricted under The Carcinogenic Substances Regulations.[1]

TABLE III

GAS-CHROMATOGRAPHIC CONDITIONS FOR PYE SERIES-104 CHROMATOGRAPH

Columns used (all glass, 1.5 m × 6 mm i.d.): A, 2% SE-52 on 80–100-mesh Chromosorb W with a nickel-63 detector; and B, 5% neopentyl glycol succinate on 80–100-mesh Chromosorb W with a tritium-foil detector. Nitrogen flow-rate: column, 60 ml min^{-1} (30 ml min^{-1} for 1-naphthylamine); and detector, 60 ml min^{-1}. Amplifer attenuation, 2000; backing-off range, × 100; and recorder, 10 mV with full-scale deflection of 200 mm.

Amine	Column	Column oven temperature/ °C	Detector oven temperature/ °C	Retention time/min	Peak height per picogram injected/ mm
Aniline	A	150	210	1.8	0.88
o-Toluidine	B	175	210	0.8	0.14
o-Anisidine	B	177	210	0.8	0.136
p-Anisidine	B	177	210	2.6	0.102
4-Nitroaniline	A	185	260	0.9	0.093
1-Naphthylamine	A	195	260	2.2	0.31
4,4 -Methylenedianiline ..	A	210	260	3.8	1.09
4,4′-Bi-*o*-toluidine	A	230	260	2.6	0.37
3,3′-Dimethoxybenzidine ..	A	210	260	6.6	0.26
3,3′-Dichlorobenzidine ..	A	230	260	2.0	0.58
2,2′-Dichloro-4,4′-methylene-dianiline	A	230	260	2.6	0.90

Results

Table IV shows results of repeated injections for solutions of the HFB amide derivative of 1-naphthylamine. It is evident that the coefficient of variation in this determination can be up to 5%. The relationship between peak height and amine concentration is not exactly linear. In Table V nine sets of results of analyses of the generated atmospheres, completed within single days, show that the concentration of 1-naphthylamine is as constant as can be determined within the precision of the analytical technique. After the initial development of the generator, samples were taken less often and there is little purpose in a statistical analysis of each daily check. However, the daily means of checks taken over a

TABLE IV

PRECISION OF THE ANALYSIS OF 5-μl ALIQUOTS OF SOLUTIONS OF THE HEPTAFLUOROBUTYRAMIDE DERIVATIVE OF 1-NAPHTHYLAMINE

Concentration in solution/mg ml^{-1}	Peak height/mm	Average/mm	Standard deviation/mm	Coefficient of variation, %
20	43, 45, 41	43	2.0	4.7
40	82, 79, 78	80	2.1	2.6
50	105, 106, 104	105	1.0	1.0
60	121, 116, 112	116	4.5	3.9
70	130, 135, 129, 130	131	2.7	2.1

period of 8 months were averaged and the mean value of 18 determinations (*i.e.*, all checks made in 8 months) was 0.20 μg l^{-1} with a standard deviation of 0.01 μg l^{-1} (coefficient of variation 5%). These results indicate that the generator is constant and reproducible, despite breaks for holiday periods and maintenance.

TABLE V

STABILITY OF 1-NAPHTHYLAMINE ATMOSPHERE WITHIN A DAY

	Concentration of 1-naphthylamine/μg l^{-1}								
	0.194	0.160	0.169	0.200	0.231	0.260	0.197	0.207	0.199
	0.196	0.163	0.160	0.200	0.237	0.249	0.197	0.205	0.197
	0.194	0.160	0.183	0.211	0.231	0.251	0.217	0.206	0.215
	0.194	0.171	0.183	0.213	0.219	0.259	0.217	0.206	0.217
	0.199	0.180	0.177	0.202	0.230	0.253	0.185	0.200	0.206
	0.199	0.185	0.172	0.205	0.228		0.191	0.200	0.206
	0.208		0.177	0.220					
			0.185	0.220					
				0.185					
				0.191					
Mean/μg l^{-1}	0.198	0.170	0.176	0.205	0.229	0.254	0.201	0.204	0.207
Standard deviation/ μg l^{-1}..	0.005	0.011	0.009	0.012	0.006	0.005	0.013	0.003	0.008
Coefficient of variation, %	2.6	6.3	4.8	5.6	2.6	1.9	6.6	1.6	3.9

The generator was used to prepare standard atmospheres of ten other aromatic amines. The method of generation is given in Table I and the results of triplicate determinations of the amine atmosphere, given in Table VI, show that a constant concentration can be established for a wide range of aromatic amines.

TABLE VI

CONCENTRATION OF STANDARD ATMOSPHERES OF VARIOUS AROMATIC AMINES

Amine	Concentration*/ μg l^{-1}	Coefficient of variation, %
Aniline	0.062	4.5
o-Toluidine	0.066	1.1
o-Anisidine	0.099	2.7
p-Anisidine	0.090	3.4
4-Nitroaniline	0.162	1.2
4,4′-Methylenedianiline	0.128	1.2
4,4′-Bi-*o*-toluidine	0.074	1.3
3,3′-Dimethoxybenzidine	0.087	3.4
3,3′-Dichlorobenzidine	0.055	4.7
2,2′-Dichloro-4,4′-methylenedianiline ..	0.004	2.3
1-Naphthylamine	0.200	5 0

* Mean of triplicate determinations.

Discussion

Aromatic amines are very toxic and a number are suspected carcinogens controlled by the Carcinogenic Substances Regulations.[1] TLVs are set at very low levels and in some instances no assignment has been made. Consequently, any field test must be as sensitive as is technically feasible and this, in turn, requires the generation of very low concentrations of these amines.

The low concentration of standard atmosphere required for these compounds makes generator design difficult. The results presented in Tables IV and VI demonstrate that the generator is applicable to a wide range of aromatic amines and, for 1-naphthylamine, it has been shown that a constant atmosphere can be maintained for several months. The main limit of precision seems to be with the analytical technique.

The emphasis in this work was to produce the lowest concentration in air that can be analysed. Where there is an assigned TLV the concentration established was much lower than this (Tables II and VI). If different concentrations of amine are required the generator is sufficiently flexible to prepare these. Higher concentrations can be achieved by reducing the air flow-rate through the generator or by increasing the bath temperature. For the diffusion technique, the range of concentration can also be varied by using capillary tubes of various lengths and diameters.

Conclusion

Atmospheres of aromatic amines can be generated in the concentration range 1–1000 ng l^{-1} either by a vapour saturation or a diffusion technique. The concentration of amine atmosphere can be kept constant for several months at least. An analysis procedure using an HFB derivative of the amine detected by EC - GLC gives the desired sensitivity.

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Field Method for the Determination of Aromatic Primary Amines in Air

Part II.* Development of a Sensitive Field Test

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A simple field test for primary aromatic amines in air is described in which the sample is drawn through an impregnated paper to form a coloured stain produced by the reaction of aromatic primary amine groups with 4-(dimethylamino)cinnamaldehyde. The test is sensitive to about 10 ng of amine and has been found to be applicable to eleven different amines. It does not distinguish individual compounds and can be affected by airborne alkaline or acidic compounds.

Keywords: *Aromatic primary amine determination*; *air*; *field method*

Part I[1] described the generation and standardisation of atmospheres of aromatic primary amines in air at very low concentrations. Part II describes a simple field test which is very sensitive to aromatic primary amines in air. There are a number of procedures for sampling and analysing amines in the workplace environment but they require laboratory facilities for the analysis. In these methods the amine is sampled by passing a metered volume of the contaminated air through a suitable sorbent, such as silica gel[2,3] or a porous polymer,[4] followed by desorption into a solvent. Analytical techniques used include gas chromatography,[2,4] high-performance liquid chromatography[3] and fluorimetry.[5] These methods can determine a few nanograms of individual amines with coefficients of variation from 1 to 14 %.

Although the methods mentioned above are sensitive and precise they cannot yield a quick result, and the object of this work was the development of a sensitive test that can be carried out in the factory by relatively unskilled persons and give a result within a few minutes. In order to achieve such a test a colour reaction is required that yields a sufficient colour change with amine concentration to permit visual comparison with colour standards. The most sensitive colour reaction found was that between aromatic primary amines and 4-(dimethylamino)cinnamaldehyde (DAC).[6] Some workers[7] have used this reaction to determine the concentration of thirteen primary amines spectrophotometrically. The limit of detection in solution ranged from 0.02 to 0.1 μg ml^{-1}, depending on the amine. When carried out in solution, the reaction was not found suitable for samples of air containing aromatic primary amines, but it was feasible to prepare a paper treated with a formulation containing DAC and which formed the basis of a very sensitive test.

The first phase of the work was to produce a test that could detect the smallest amount of 1-naphthylamine by a colour change reaction. A reaction was found that could be carried out on chemically treated paper and a set of colour standards was prepared. Similar colours were attained with other aromatic primary amines and by adjusting the volume of air sampled the same set of standards could be used, avoiding the preparation of individual standards for each amine. Not all of these amines, including 1-naphthylamine, have assigned threshold limit values (TLVs).

Development of the Paper Test

During the study of the spectrophotometric method using DAC,[6] trichloroacetic acid was replaced with toluene-4-sulphonic acid as this compound was easier to handle and could be used at much lower concentrations to achieve the same pH conditions.

The development of the paper test method was, of necessity, largely empirical because of the difficulty of predicting and controlling conditions in the treated paper after the solvent had evaporated. The initial formulation of the solution used for impregnating the paper

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* For Part I of this series, see p. 1082.

was completely speculative but a red stain was produced on the yellow paper when air from the top of a reagent bottle containing amine was drawn through the paper. During the course of the development of the impregnation solution the formulation was modified many times before the most suitable composition was found. The initial and final formulations are shown in Table I.

TABLE I

COMPOSITION OF THE IMPREGNATION SOLUTION

Component	Formulation	
	Initial	Final
4-(Dimethylamino)cinnamaldehyde	0.02 g	0.005 g
Toluene-4-sulphonic acid	0.01 g	0.11 g
Toluene-4-sulphonic acid, sodium salt	—	0.55 g
Propyl 3,4,5-trihydroxybenzoate	—	0.002 g
Glycerol	30 ml	25.2 g
Water	—	18 ml
Propan-1-ol	Variable*	—
Propan-2-ol	—	Variable*

* Sufficient solvent to give a final volume of solution of 100 ml.

The colour-forming reaction involves the condensation of the amine and the aldehyde to form an azomethine with the elimination of water. Such condensations are generally facilitated by the presence of acid and the absence of water, but it was found at a later stage in the investigation that the presence of some water in the paper was necessary to maintain an acidic pH. Glycerol was added primarily to act as a humectant, and secondly with the aim of preventing agglomerates of solutes crystallising within the paper when the solvent evaporated. Neither the initial formulation nor the paper impregnated with it were very stable, the paper darkening in colour and losing sensitivity, probably through atmospheric oxidation or by a change in pH value owing to alteration of the residual moisture content.

One of the preferred requirements of a field test is that material to be used, in this instance the impregnated paper, should have a working life of at least 24 h. Various modifications were made to the initial formulation and the effects produced on changing each component were studied.

4-(Dimethylamino)cinnamaldehyde (DAC)

The concentration of this reagent affected the intensity of the stain and its appearance. At concentrations higher than 0.005% *m/V* impregnated papers gave a more sensitive response to 1-naphthylamine but the background colour of the paper also increased, and stains tended to have a speckled appearance. The concentration of DAC was reduced from the original level of 0.02% *m/V* to the final level (0.005% *m/V*) at which prepared papers were almost white but gave a perceptible stain with about 12 ng of 1-naphthylamine.

Antioxidant

Because of the susceptibility of DAC to oxidation, an antioxidant was added to the formulation to increase its stability. Initially, hydroquinone was used but this actually increased the rate of discoloration of the impregnated papers, presumably because of compound formation between DAC and the hydroquinone or its oxidation product, *p*-benzoquinone. Hydroquinone was replaced with 2,6-di-*tert*-butyl-*p*-cresol ("butylated hydroxytoluene," BHT) which improved the sensitivity and stability of the paper. However, during later experiments, in which impregnation formulations were prepared by diluting concentrated solutions with water, BHT was found to precipitate from solution. For this reason, BHT was replaced with propyl 3,4,5-trihydroxybenzoate (propyl gallate) in all subsequent formulations. The concentration of antioxidant used in the formulation was not found to be critical.

Acidity

The apparent pH values of the solutions used for impregnating the paper were measured with a pH meter. These solutions contained varying amounts of water but were mainly organic in nature and so pH values were comparative rather than absolute but were a useful guide to the amount of acid required to produce a satisfactory test paper. It was found that formulations with a pH value below about 2 produced an almost white paper with a low but rapid response to amine. Papers produced from solutions with pH values of 2–3 gave good responses but the response again diminished above pH 3, and above pH 2.5 the papers were coloured yellow. A pH value of about 2.2 appeared to be the most promising and the concentration of toluene-4-sulphonic acid was chosen accordingly. The sodium salt of toluene-4-sulphonic acid was also added to the formulation as an aid to achieving the correct acidity in the paper by virtue of its buffering action.

Residual Solvent

A high boiling-point solvent was incorporated in the impregnating formulation in order to keep the solid reagents in solution in the prepared paper. Diethyl phthalate, dodecanol and glycerol were tried and the last proved to be best with regard to the stability and sensitivity of the paper. Over the range 5–30% *m/V* of glycerol in the impregnating solution, the sensitivity of the paper was found to decrease and the stability to increase with increasing concentration. A compromise concentration of about 25% *m/V* was adopted. Glycerol may also take part in the colour-forming reaction as it has an affinity for water, and water is produced during the condensation of the amine and the aldehyde.

Water

The effect on the prepared papers of varying the water content of the impregnating solution was similar to that of varying the acidity, and the two effects may be related. Low water contents gave yellow papers with high sensitivity to amine whereas high water contents gave white papers with reduced sensitivity. Moreover, as the water content was increased stains were found to develop more slowly and to deteriorate from an even to a speckled appearance. The investigation of the effect of water upon the colour response of the treated papers was complicated by changes in the humidity of the air. The atmosphere of 1-naphthylamine was prepared initially from a supply of compressed air which was exceptionally dry, and this meant that papers under test were being further dried during the experiment. Problems arising from this were overcome by using moist air as a diluent and raising the relative humidity of the atmosphere from the 20% found with compressed air to the range 40–90%, which resembles more closely practical atmospheric conditions.[1]

Volatile Solvent

A number of solvents for the reagents to be used for impregnating the paper were examined. These included acetone, dimethylformamide, 1,4-dioxane, propan-1-ol and propan-2-ol. The choice was governed by the completness of solution of the reagents and the absence of a speckled stain upon reaction of the amine with the treated paper. It appeared that, during the drying of the paper, the evaporation of the solvent could influence the distribution of the reagents within the paper. Propan-2-ol was finally selected as solvent.

Paper

Whatman No. 1 filter-paper was used during most of the development work but this was later changed to a silica gel loaded paper, Whatman SG 81, which improved control of the water content of the paper and helped to eliminate speckled stains. This produced a slight change in the colour of the stains in that the blue component of the predominantly pink colour was reduced.

Colour Standards

The dyestuff amaranth had been used for the visual colour standards whenpla in filter-papers were used as the basis for the test, but when silica gel loaded papers were used, Red 2G (Colour Index Number 18050) gave a better colour match. The concentrations of dye solutions required to prepare standards matching the colours of the stains produced by 25, 50 and 100 ng of 1-naphthylamine were determined by trial and error. The final choice was by consensus of a panel of observers after visual comparison. This is in keeping with the nature of a field test and also the human eye is more sensitive to small colour changes than is measurement by a reflectance spectrometer. When stored away from light, the prepared colour standards showed no sign of deterioration over a period of at least 1 year.

Reliability of Reagent

When the final conditions for the preparation of the test papers had been established, samples of DAC were purchased from all five known British sources in order to check their performance in the test. Despite obvious differences in the colour and crystalline state of the samples, they all produced test papers with similar responses to amines.

Method

Apparatus

Metal paper holder. Shown in Fig. 1.

Pump. Mains or battery operated electric pump capable of drawing air through the test paper at a flow-rate of 250 ml min^{-1}.

Flow meter. Variable-area type capable of measuring a flow-rate of 250 ml min.^{1}.

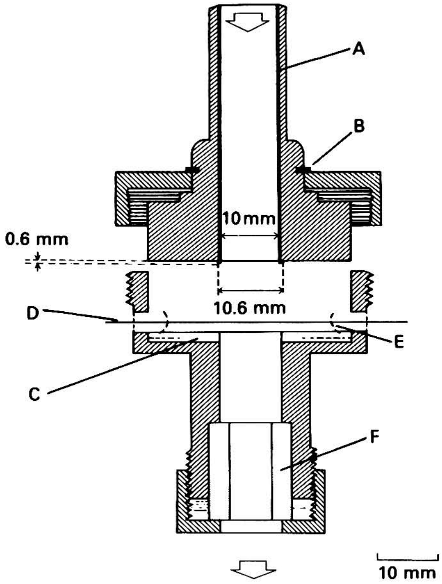

Fig. 1. Paper holder. A = Epoxide protective film; B = spring clip; C = PTFE washer; D = test paper; E = paper feed slots; and F = rubber bush.

Reagents

Reagents should be of analytical-reagent grade where possible, and water should be distilled or de-ionised.

4-(*Dimethylamino*)*cinnamaldehyde* (DAC).

Toluene-4-sulphonic acid solution, 5.5% *m/V.* Prepared in propan-2-ol.

Sodium toluene-4-sulphonate solution, 5.5% *m/V.*

Propyl 3,4,5-*trihydroxybenzoate solution* (*propyl gallate*), 0.1% *m/V.*

Glycerol.

Propan-2-ol.

Red 2G. Colour Index Number 18050.

Paper. Whatman SG 81, silica gel loaded.

Preparation of Test Papers

Prepare the impregnating solution by weighing 25.2 g of glycerol in a small beaker, then add, in the order given, 6.0 ml of water, 2.0 ml of toluene-4-sulphonic acid solution, 10.0 ml of sodium toluene-4-sulphonate solution and 2.0 ml of propyl gallate solution and mix thoroughly. Weigh 0.005 g of DAC into a second small beaker and at room temperature dissolve in successive small amounts of propan-2-ol. Transfer both solutions into a 100-ml calibrated flask and dilute to volume with propan-2-ol.

Pour the solution into a suitably sized flat-bottomed dish and immerse the paper (50 × 50 mm) for 1 min. Remove the paper, and allow surplus solution to drain off. Place the treated paper between two double sheets of plain filter-paper and roll briefly with a photographer's squeegee. Dry the treated paper for 10 min in an oven or incubator at 35 °C and store in a brown-glass screw-topped bottle overnight to stabilise the moisture content. Fresh papers should be prepared after 7 d.

Preparation of Colour Standards

Prepare three solutions of Red 2G at concentrations of 0.001, 0.002 and 0.003% *m*/*V*. Immerse one strip of Whatman SG 81 paper, 250 × 25 mm, in each of these solutions for 1 min. Remove the strips, place on a sheet of glass and remove surplus solution with a squeegee. Hang the strips in air until dry, discard the top and bottom 10 mm and punch a hole 6 mm in diameter in each to facilitate comparison with test papers. The three standards correspond to 25, 50 and 100 ng of 1-naphthylamine collected on the test paper. Store away from light and prepare fresh standards after 3 months.

Procedure

The flow meter is usually placed between the test paper and the suction pump where it will be operating under a partial vacuum. Variable-area flow meters are calibrated for use at atmospheric pressure and therefore will require re-calibration under the sampling conditions. This may be done either by connecting an additional flow meter before the test paper with its lower end open to the atmosphere or by connecting a bubble flow meter in series with the sampling flow meter. The test paper should be replaced with a fresh piece after calibration.

Clamp a strip of test paper, 50 × 25 mm, in the holder (Fig. 1) and connect to the flow meter with flexible tubing. Connect the flow meter to the pump. Draw 1 l of air through the paper at a rate of 250 ml min^{-1}. Remove the paper, allow the colour to develop for 10 min, and compare the colour with those of the standards by viewing through the holes in the paper strips. The colour standards correspond to concentrations of 1-naphthylamine in air of 25, 50 and 100 ng l^{-1}. The first perceptible colour will appear with a concentration corresponding to approximately half of the lowest standard. The limit of detection may be extended by increasing the volume of air sampled up to 10 l and applying a suitable factor to estimate the concentration.

Results and Discussion

In the development of the paper test the intensity of the colour stains was measured with a Unicam SP 500 spectrophotometer fitted with a reflectance attachment modified with a matt black top plate so that only the stain was measured. A total of eleven different compounds were examined and each yielded a stain with a maximum reflectance in the wavelength range 520–540 nm. The results in Table II demonstrate that the test is sensitive and yields reproducible results with all of the amines investigated. Sampling times could be varied from 0.5 to 100 min in order to collect the amine in the mass range 25–100 ng. The absence of any stain on the downstream side of the paper indicated efficient collection. This was verified by the failure to detect amine in a back-up bubbler or on a second test paper.

The results are presented in a different form in Table III. If the mass of amine collected is converted into moles, it is found that the reflectance per mole divided by the number of amine groups in the molecule is constant. The average value of this amount has a coefficient of variation of 5% for the amines at all levels tested. This is well within the precision of

TABLE II

REFLECTANCE VALUES OF STAINS FROM VARIOUS AROMATIC PRIMARY AMINES

	Concentration in atmosphere/ ng l^{-1}	*ca.* 25 ng level			
		Sampling volume/ ml	Mass collected/ ng	Reflectance	Average reflectance
Aniline	62.4	375	23.4	0.018, 0.021, 0.018, 0.021, 0.020, 0.021, 0.025, 0.020, 0.021, 0.025	0.021
o-Toluidine	66	375	24.7	0.020, 0.018, 0.020, 0.019	0.019
o-Anisidine	98.6	250	24.6	0.018, 0.018, 0.018, 0.016, 0.020	0.018
p-Anisidine	90.4	250	22.6	0.018, 0.016, 0.016, 0.015	0.016
4-Nitroaniline	162	125	20.2	0.013, 0.012, 0.015, 0.013, 0.013, 0.014	0.013
1-Naphthylamine	22.5	1250	28.1	0.016, 0.017, 0.017, 0.017	0.017
4,4′-Methylenedianiline	128	190	24	0.019, 0.019, 0.020, 0.021, 0.021	0.020
4,4′-Bi-*o*-toluidine	74	310	23.1	0.018, 0.021, 0.019, 0.018, 0.020	0.019
3,3′-Dimethoxybenzidine	87	250	21.8	0.015, 0.016, 0.016, 0.016, 0.016	0.016
3,3′-Dichlorobenzidine	55	440	24	0.016, 0.017, 0.016	0.016
2,2′-Dichloro-4,4′-methylenedianiline*	4.04	6250	25.2	0.017, 0.017, 0.018, 0.016, 0.017	0.017

	Concentration in atmosphere/ ng l^{-1}	*ca.* 50 ng level			
		Sampling volume/ ml	Mass collected/ ng	Reflectance	Average reflectance
Aniline	62.4	750	46.8	0.038, 0.038, 0.040, 0.040, 0.040, 0.039, 0.038	0.039
o-Toluidine	66	750	49.5	0.036, 0.036, 0.037, 0.036, 0.038	0.037
o-Anisidine	98.6	500	49.3	0.034, 0.035, 0.035, 0.037	0.035
p-Anisidine	90.4	500	45.2	0.032, 0.033, 0.032, 0.033	0.032
4-Nitroaniline	162	310	50.6	0.028, 0.029, 0.030, 0.033	0.030
1-Naphthylamine	22.5	2250	50.6	0.032, 0.029, 0.030, 0.029	0.030
4,4′-Methylenedianiline	128	375	48	0.038, 0.040, 0.036, 0.038, 0.037	0.038
4,4′-Bi-*o*-toluidine	74	625	46.2	0.036, 0.034, 0.035, 0.038	0.036
3,3′-Dimethoxybenzidine	87	500	43.5	0.032, 0.028, 0.032, 0.029, 0.029	0.030
3,3′-Dichlorobenzidine	55	875	48	0.031, 0.033, 0.031, 0.031	0.031
2,2′-Dichloro-4,4′-methylenedianiline*	4.04	1250	50.5	0.032, 0.032, 0.033, 0.032	0.032

TABLE II continued

	Concentration in atmosphere/ ng l^{-1}	*ca.* 100 ng level			
		Sampling volume/ ml	Mass collected/ ng	Reflectance	Average reflectance
Aniline	62.4	1500	93.7	0.079, 0.069, 0.078, 0.088, 0.080, 0.079, 0.080	0.079
o-Toluidine	66	1500	99	0.076, 0.073, 0.074, 0.076	0.075
o-Anisidine	98.6	1000	98.7	0.070, 0.071, 0.074, 0.072	0.072
p-Anisidine	90.4	1000	90.4	0.065, 0.065, 0.065, 0.068, 0.066	0.066
4-Nitroaniline	162	750	101.25	0.065, 0.066, 0.065, 0.065	0.065
1-Naphthylamine	22.5	4500	101.25	0.063, 0.064, 0.065, 0.067	0.065
4,4′-Methylenedianiline	128	750	96	0.073, 0.076, 0.074, 0.076, 0.075	0.075
4,4′-Bi-*o*-toluidine	74	750	92.5	0.069, 0.070, 0.068, 0.072	0.070
3,3′-Dimethoxybenzidine	87	1000	87	0.063, 0.062, 0.061, 0.057, 0.062	0.061
3,3′-Dichlorobenzidine	55	1750	96	0.061, 0.065, 0.065, 0.066, 0.067	0.065
2,2′-Dichloro-4,4′-methylenedianiline*	4.04	2500	101	0.064, 0.064, 0.066	0.065

* 4,4′-Methylene bis(2-chloraniline).

the reflectance measurement and the values have a Gaussian distribution. The implications of this observation have important practical results. The constancy of the colour intensity of these amine groups presumably arises from the fact that the colour-forming centre is not affected by the nature of the aromatic structure of the amine. Consequently, a single set of colour standards can be used as the mass of any other amine can be estimated from, say, a set of standards based on 1-naphthylamine. Alternatively the volume of air sampled can be selected so that the colour standards correspond to masses of 25, 50 and 100 ng. It seems reasonable to assume that aromatic amines, other than those studied in this work, will yield a predictable colour stain. The only instance envisaged where this may not apply is if steric restrictions inhibit the formation of the azomethine.

Assessment of the proposed method was carried out under field conditions at an industrial site where 4,4′-methylenedianiline was being used. Samples of the atmosphere were taken by the field method and were collected simultaneously in dilute hydrochloric acid for subsequent analysis in the laboratory by gas - liquid chromatography.[1] Good agreement was obtained between the two methods.

The test cannot distinguish individual amines and if mixtures are present the result will relate to the total concentration of amines. By considering the extremes of the composition of the mixture, a range of concentrations can be estimated. The colour-forming reaction may be adversely affected by the presence of alkaline or acidic compounds in the atmosphere if these are present in sufficient amount to change the pH of the paper.

Conclusions

The reaction of aromatic primary amines and 4-(dimethylamino)cinnamaldehyde provides the basis of a sensitive field test for airborne amines. The sensitivity of the method is about 10 ng and the method can be quantified either by comparison with colour standards or by measuring colour intensity by reflectance with a spectrophotometer and then relating this to a calibration graph. The intensity of the colour stain is proportional to the molar amount of amine collected and the number of primary amine groups in the molecule. The test is specific for aromatic primary amines but does not distinguish individual compounds. The test may not operate reliably in the presence of airborne alkaline or acidic compounds.

TABLE III

RELATIONSHIP BETWEEN COLOUR INTENSITY OF STAIN AND MASS OF AMINE COLLECTED

Amine	Relative molecular mass	Mass collected on paper*/ng	Average reflectance*†	Reflectance × 10^7 / No. of moles	Reflectance × 10^7 / No. of moles × No. of amine groups
Aniline ..	93	23.4	0.021	8.3	8.3
		46.8	0.039	7.8	7.8
		93.7	0.079	7.8	7.8
o-Toluidine ..	107	24.7	0.019	8.2	8.2
		49.5	0.037	8.0	8.0
		99	0.075	8.1	8.1
o-Anisidine ..	123	24.7	0.018	9.0	9.0
		49.3	0.035	8.7	8.7
		98.7	0.072	9.0	9.0
p-Anisidine ..	123	22.6	0.016	8.7	8.7
		45.2	0.032	8.7	8.7
		90.4	0.066	9.0	9.0
4-Nitroaniline ..	138	20.2	0.013	8.9	8.9
		50.6	0.030	8.2	8.2
		101.2	0.065	8.9	8.9
1-Naphthylamine..	143	28.1	0.017	8.7	8.7
		50.6	0.030	8.5	8.5
		101.2	0.065	9.2	9.2
Methylene-dianiline ..	198	24.0	0.020	16.5	8.3
		48.0	0.038	15.7	7.8
		96.0	0.075	15.5	7.7
4,4′-Bi-*o*-toluidine	212	23.1	0.019	17.4	8.7
		46.2	0.036	16.5	8.3
		92.5	0.070	16.0	8.0
3,3′-Dimethoxy-benzidine ..	244	21.8	0.016	18.0	9.0
		43.5	0.030	16.9	8.4
		87	0.061	17.1	8.6
3,3′-Dichloro-benzidine ..	253	24.0	0.016	16.9	8.4
		48.0	0.031	16.3	8.2
		96.0	0.065	17.5	8.6
2,2′-Dichloro-4,4′-methylene-dianiline ..	268	25.2	0.017	18.1	9.0
		50.5	0.032	17.0	8.5
		101	0.065	17.3	8.6

* See Table I.

† Average of at least three determinations.

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NOTE—Reference 1 is to Part I of this series.

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Determination of Gold in Tissue and Faeces by Atomic-absorption Spectrophotometry Using Carbon Rod Atomisation

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A simple and accurate atomic-absorption spectrophotometric method is described for the measurement of gold in tissue and faeces following chrysotherapy. Samples and standards are disrupted with a quaternary ammonium hydroxide solubiliser in toluene, diluted with isobutyl methyl ketone, and analysed directly using a carbon rod atomiser. The detection limit for gold is 50 ± 25 pg and the calibration graph is linear up to approximately 3 ng. Concentrations of gold in tissue determined by this method correlate well with results obtained by flame atomic-absorption spectrophotometry and by neutron-activation analysis.

Keywords: *Gold determination*; *carbon rod atomic-absorption spectrophotometry*; *tissue analysis*; *faeces analysis*

Numerous analytical techniques have been utilised to measure gold in tissue following chrysotherapy. Colorimetric,[1–3] polarographic[4] and voltammetric[5] methods are relatively insensitive (and, in some instances, non-selective) and generally require extensive sample digestion, which leads to inaccuracies. Tracer studies with radioactively labelled gold have been performed,[6,7] but the technique is not suitable for a multiple dosing regimen over a prolonged period of time. Neutron-activation analysis[8,9] is free of these difficulties, but it is costly and neither generally available nor suitable for routine analysis.

Recently, atomic-absorption spectrophotometry has become the method of choice for gold measurement. In addition to speed of analysis and general availability of instrumentation, small amounts of sample are required and results compare favourably with those obtained by neutron-activation analysis.[10,11] Biological applications have been extensive in the areas of fluid[12–15] and blood cell[16–19] analysis, but the measurement of gold in tissue has received comparatively little attention.[20–22]

This paper describes a method for the determination of gold in tissue by atomic-absorption spectrophotometry. Tissue samples were disrupted with Soluene 100 and diluted with isobutyl methyl ketone (IBMK) prior to analysis by carbon rod techniques. Results of this method are compared with flame atomisation atomic-absorption spectrophotometry and neutron-activation analysis over a wide concentration range in several types of tissues.

Experimental

Reagents

Doubly distilled, de-ionised water was used for dilutions where indicated.

Gold sodium thiomalate, 10, 50 *and* 100 mg ml^{-1}. Sterile aqueous solutions of gold sodium thiomalate (Merck, Sharp and Dohme) containing 0.5% benzyl alcohol as a preservative were used.

Gold(III) chloride stock solution, 1000 μg ml^{-1}. A solution of gold(III) chloride (Varian Techtron) in 1% *V/V* aqua regia was used.

Soluene 100, 0.5 N. A solution of Soluene 100 (Packard Instrument Company) in toluene was used.

Animal Experiments

Groups of 3–4 male Sprague - Dawley rats weighing 200–225 g were administered subcutaneous injections of 12.5, 50.0 or 100.0 mg kg^{-1} of gold sodium thiomalate weekly for 8 weeks. Control animals received an equal volume of 0.5% benzyl alcohol. Animals were

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killed by exsanguination through the heart at various times during the treatment and blood was collected in heparinised tubes for the separation of plasma. Tissue samples were promptly frozen at −20 °C until analysis.

Sample Preparation

Where necessary, blood clots were removed and any excess of liquid was blotted gently from the tissue. Skin was frozen in liquid nitrogen for 1 min then hair was removed by scraping with a scalpel blade against the nap. Approximately 100 mg of tissue was weighed accurately into 12 × 75 mm polypropylene test-tubes. The volume of plasma or fluid samples used was 100 μl. Following the addition of 1 ml of the Soluene 100 solution to the samples, tubes were capped and heated at 50 °C in a water bath until the tissue had dissolved. Samples were allowed to cool and 2 ml of IBMK were added immediately prior to the determination of the gold content. Care was taken to re-cap tubes to prevent solvent loss.

Preparation of Calibration Standards

Gold standards (100.0 and 10.0 μg ml^{-1}) were prepared by dilution of the gold(III) stock solution with water. Standards were not stable and were freshly prepared for each analysis. Appropriate volumes of standards were added by Hamilton syringe to tissue from control animals at the outset of sample preparation. Final concentrations of 50 to 2000 μg ml^{-1} were prepared. Calibration graphs were prepared using control tissue for each tissue group at each analysis.

Apparatus

A Varian, Model AA6, atomic-absorption spectrophotometer with a Jarrell Ash gold hollow-cathode lamp was employed. The instrument was equipped with a BC-6 background corrector with a hydrogen continuum hollow-cathode lamp, a Model 63 carbon rod atomiser and an air - acetylene burner. Operating conditions were as follows: wavelength, 242.8 nm; lamp current, 5 mA; slit width, 10 mm; and band pass, 1 nm. Settings for carbon rod atomisation were as follows: drying conditions, 15 s at 0.8 relative volts; ashing conditions, 10 s at 6.5 relative volts; and atomise conditions, 2 s at 7.0 relative volts. The atomiser was cooled continuously both with nitrogen maintained at approximately 10 lb in^{-2} and with tap water at a flow-rate of 1–2 l min^{-1}. Flame settings were 7.75 units of air + 1 unit of acetylene with a flow-rate of 3 ml min^{-1}.

A 10-kW nuclear reactor of the swimming-pool type (Ohio State University, Columbus, Ohio) was used for all irradiations. Samples were counted on a lithium-drifted germanium detector (Princeton Gamma Tech, Model 301) and analysis of gold-198 was based on the principal energy peak at 412 keV and a half-life of 2.7 d.

Procedure

Carbon rod analysis

A 10-μl Hamilton syringe with a Chaney adapter was used to apply 2-μl samples lengthwise to the carbon rod. A disposable PTFE tip (1.125 in long) designed for use with the syringe was used to take up each sample, thus avoiding contamination among samples. The highest absorbance obtained during the atomisation stage was electronically monitored and was employed for all absorbance readings. In general, samples and standards were analysed in triplicate. If the absorbance value of the gold in a sample exceeded the linear portion of the standard graph, then a smaller amount of treated and untreated tissue was used for analysis and generation of the standard graph, respectively. Alternatively, samples were diluted with control tissue prepared in an identical manner.

Flame analysis

Samples and standards prepared for carbon rod analysis were subsequently analysed using a flame without further manipulation. The average absorbance for a 3-s aspiration period was electronically determined and was employed for all readings. Samples and standards were analysed in duplicate.

Neutron-activation analysis

Duplicate samples of tissue (50 mg) or plasma (0.1 ml) were placed in polyethylene snap-cap vials of 1.3-ml capacity and dissolved in 0.5 ml of the Soluene 100 solution overnight at room temperature. Standard graphs were constructed to cover the range of gold concentrations to be analysed by adding appropriate amounts of gold chloride stock solution by syringe to control samples of the same tissue. Vials were friction-sealed prior to activation. Samples and standards were irradiated for 1.5 h in the central facility of the reactor at a thermal neutron flux of 2.0×10^{11} neutrons $cm^{-2}\,s^{-1}$. Radioactivity was determined 1.5 h after removal of the samples from the reactor to allow for decay of the short-lived isotopes that are predominant components of the sample, *e.g.*, ^{24}Na and ^{38}Cl. In general, samples and standards were counted for 10 min. The gold content of the samples was calculated, based on regression analysis of the standard graph data of radioactivity generated *versus* amount of gold added.

Results

Sample Preparation

Tissue disruption using Soluene 100 was satisfactory for faeces and all tissues considered with the exception of bone, whole blood and red blood cells. Bone did not dissolve in Soluene 100 even with prolonged heating. However, matrix effects of solubilised whole blood or red blood cells effectively masked the absorbance of gold concentrations near the limit of detection when analysed by atomic-absorption spectrophotometry.

Standardisation

Tissues from control animals exhibited zero absorbance when analysed for gold by atomic-absorption spectrophotometry. This permitted standardisation to be accomplished directly by the addition of known amounts of gold stock standard solution to control tissue. Daily variations resulting from the preparation of standard graphs were minimised by the generation of an over-all average standard graph for each tissue.

Carbon Rod Atomic-absorption Spectrophotometry

Instrument parameters

Settings for the carbon rod atomiser that gave the optimum absorbance values were determined. For a 2-µl sample, the drying stage allowed for evaporation of liquid without loss of sample by spluttering or by seepage outside the light path. The ashing stage was adjusted to destroy remaining organic material without premature volatilisation of gold, and the atomisation stage was selected to give the maximum absorbance reading for gold with no residual gold remaining on the carbon rod.

Linearity

Calibration graphs generated for tissues were linear up to absorbance values of 1.0 corresponding to concentrations of 3–4 ng for 2-µl samples.

Precision

Precision is reported as the relative standard deviation (RSD, %) of ten replicate determinations. Typical RSD values for gold concentrations of 0.1, 0.5, 1.0 and 1.5 µg ml^{-1} in spleen tissue were 6.3, 8.2, 3.9 and 4.9%, respectively.

Sensitivity

The mass of gold that produced an absorbance reading of 0.0044 was determined for 2 µl of sample applied. The average and standard error of the mean (SEM) for all of the tissues and faeces was 18 ± 4 pg.

Detection limits

Detection limits were determined as the mass of gold that produced an average absorbance peak signal corresponding to twice the peak to peak noise of the base line. The average and SEM was 53 ± 25 pg where the absorbances of corresponding control tissues and faeces were used as the respective base lines.

Recovery studies

The recovery of varying amounts of gold standard added to spleen samples of constant gold content ranged from 96 to 107% (average = 101%) as indicated in Table I. The recovery of a constant amount of standard added to samples of varying gold content ranged from 86 to 106% (average = 94%) (Table I).

TABLE I

RECOVERY OF GOLD ADDED TO SPLEEN SAMPLES FROM GOLD-TREATED RATS

Amount of gold/μg			
Present in sample	Added	Recovered*	Recovery, %
0.068	0.05	0.049	98
0.068	0.10	0.107	107
0.068	1.00	0.990	99
0.068	1.50	1.513	101
0.310	0.50	0.470	94
0.534	0.50	0.530	106
0.740	0.50	0.450	91
0.880	0.50	0.430	86

* Mean of three determinations.

Sample dilution considerations

Spleen samples whose gold absorbance exceeded the linear portion of the standard graph were diluted and re-analysed using methods of standard additions to establish if the manner of diluting samples affected the accuracy of the proposed procedure. The results are shown in Fig. 1. The slope of the absorbance *versus* concentration graph for each set of samples compared with that of control tissue containing the same amounts of added gold (average standard graph) (line A) indicated that the samples diluted with control spleen dissolved in

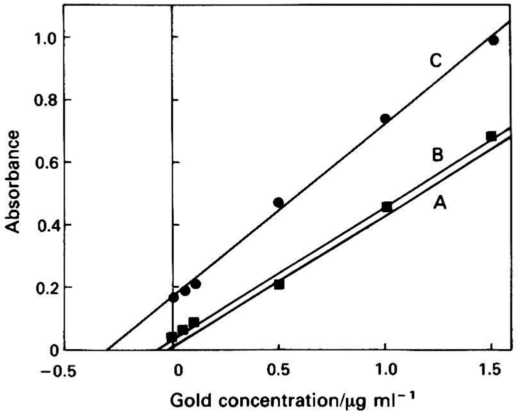

Fig. 1. Method of standard additions applied to control spleen samples (A) and to spleen from rats treated with gold sodium thiomalate following dilution with control spleen dissolved in a 1 + 2 mixture of Soluene 100 + IBMK (B) or with a 1 + 2 mixture of Soluene 100 + IBMK alone (C). Slope B/slope A = 1.0 and slope C/slope A = 1.2 where slope A = 0.429 ml μg^{-1}.

a 1 + 2 mixture of Soluene 100 + IBMK (line B) produced results identical with those of the average calibration graph. However, samples diluted with Soluene 100 + IBMK alone (line C) produced 20% higher results. Similar results were obtained when IBMK alone was used for dilution.

Comparison of Carbon Rod Atomic-absorption Spectrophotometry with Flame Atomic-absorption and Neutron-activation Analysis

In determining the gold concentrations of plasma and spleen samples, excellent correlation (correlation coefficient = 0.99) was found between the results obtained by carbon rod and flame atomic-absorption techniques over the range 20–80 µg of gold per gram wet mass of tissue (Fig. 2). The results of these two techniques were nearly identical, as shown by a slope of 1.03 for the line of best fit *versus* a slope of 1.0 for the line of equality. Similarly, results obtained by carbon rod atomic-absorption spectrophotometry and neutron-activation analysis correlated well for plasma and thymus samples over the concentration range 1–125 µg of gold per gram wet mass of tissue (correlation coefficients = 0.99 and 0.97, respectively) as shown in Fig. 3. However, it was apparent that neutron-activation analysis gave results 7% higher than those obtained by atomic-absorption spectrophotometry.

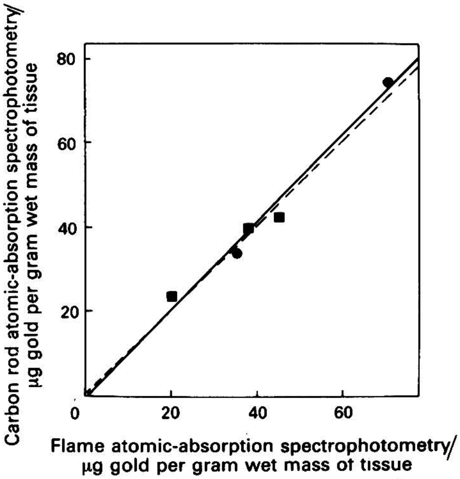

Fig. 2. Correlation between the results of carbon rod and flame atomic-absorption spectrophotometry in the determination of the gold concentration of plasma (●) and spleen (■) samples from rats treated with gold sodium thiomalate. Line of equality: ----. Line of best fit: ———.

Discussion

The disruption of samples with Soluene 100 followed by destruction of the organic matrix during the ashing stage of the atomisation process simplified the determination of gold in tissue. Ashing procedures to destroy organic matter prior to the gold determination[20] were eliminated. The use of the same tube for weighing, disruption and dilution of the sample minimised sample preparation and improved upon the drying, grinding, mixing and transfer manipulations of similar methods.[21] Addition of IBMK allowed for uniform sample distribution and reproducible sampling. Further, disrupted samples are also suitable for liquid scintillation counting.[23]

A detection limit of 10 pg for gold in serum or plasma using carbon furnace atomisation has been reported[18] and the detection limit of 53 pg and sensitivity of 18 pg (for an absorbance of 0.0044) determined by this method for a 2-µl sample compare favourably. These values are lower when individual tissues are considered.

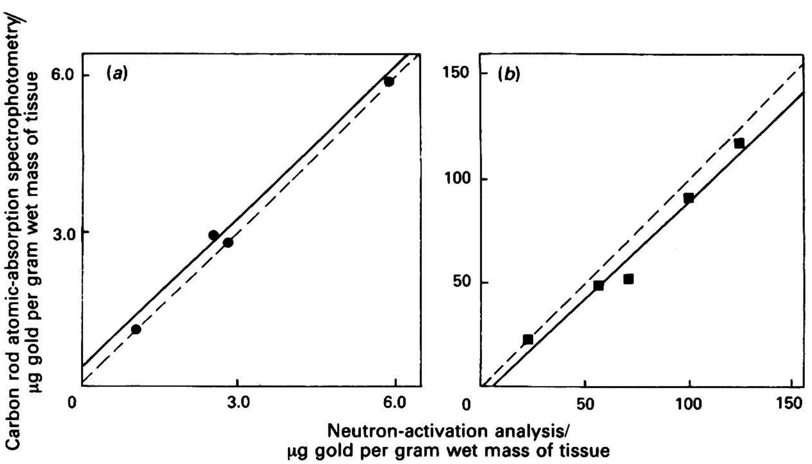

Fig. 3. Correlation between the results of carbon rod atomic-absorption spectrophotometry and neutron-activation analysis in the determination of (*a*) the gold concentration of plasma (●) and (*b*) thymus (■) samples from rats treated with gold sodium thiomalate. Line of equality: – – –. Line of best fit: ———.

The accuracy of the procedure was established by the recovery of 98% (86–107%) of various amounts of gold standard added to tissues containing various amounts of gold. Further, the results obtained by carbon rod atomisation were in close agreement with those obtained by flame atomic-absorption spectrophotometry and neutron-activation analysis over the range 1–125 μg of gold per gram wet mass of tissue. It is apparent that carbon rod atomic-absorption results are slightly lower than those obtained by neutron-activation analysis. Similar results were seen by Ward *et al.*[11] in their measurement of gold in plasma and plasma fractions. The reason for this difference in gold values is not yet understood.

Enhanced absorption values for samples diluted with Soluene 100 + IBMK or IBMK alone *versus* samples diluted with control tissue dissolved in Soluene 100 + IBMK suggested that, in addition to diluting the gold concentrations, the procedure also diluted matrix effects present in the tissue. Such effects correspond to physical, chemical or ionisation interferences,[24] which probably vary from tissue to tissue. Preparation and dilution of samples to maintain a matrix identical to the standards avoids this inaccuracy.

As little as 0.7 ± 0.4 μg of gold per gram of tissue can be determined at the limit of detection of this method, assuming 100 mg of tissue were to be analysed in a final volume of 3 ml. This value can be improved by decreasing the final volume of samples and standards. Comparison with electrothermal atomic-absorption techniques[11,12,15,16,18] indicates that the method described is equally able to detect gold in small volumes of serum and plasma of patients receiving chrysotherapy. Moreover, neutron-activation analysis results[8,9] indicate that the concentration of gold in various tissues of these patients is suitable for analysis by the method described.

The small amount of sample preparation and manipulation required and the speed of analysis permit the processing of large numbers of samples. This, along with the nearly universal application of this technique to various tissues, makes a systematic study of gold accumulation in the body possible. Further, unlike neutron activation, atomic-absorption instrumentation is readily available and costs are low.

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Determination of Riboflavin and Flavin Mononucleotide in Foodstuffs Using High-performance Liquid Chromatography and a Column-enrichment Technique

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A high-performance liquid chromatographic method has been developed for the determination of riboflavin and flavin mononucleotide (FMN) using a C_{22} reversed-phase column packing material. Mean recoveries of 100% (±5%) for riboflavin and 102% (±2%) for FMN were obtained. Multiple determinations of the vitamin in two samples indicated a precision of ±5%. The results agree well with those obtained by microbiological assay. Riboflavin and FMN are eluted within 9 min, and the total time for the analysis of most samples is 3.5 h. The lifetime of the reversed-phase column is far greater than that of silica columns. After 500 sample loadings no deterioration of column efficiency occurred. The method is suitable for the determination of riboflavin down to the 0.01 mg per 100 g level. For samples with a lower vitamin content trace enrichment can be used, which involves only a few minutes' extra analysis time.

Keywords: *Riboflavin determination*; *flavin mononucleotide determination*; *high-performance liquid chromatography*; *microbiological assay*; *trace enrichment*

Microbiological assay and fluorimetry are the two most widely used techniques for the determination of riboflavin in foods. Microbiological assay is time consuming, subject to interferences and requires specialised facilities. Fluorimetric procedures have been automated, but they are also susceptible to interferences, and complex clean-up procedures may have to be used. The use of high-performance liquid chromatography (HPLC) was therefore investigated as it offers the advantages of speed and increased specificity, using equipment that is versatile in its application.

HPLC has been applied to the determination of riboflavin in foods, although few papers have appeared on the subject. Van de Weerdhof *et al.*[1] used a column of 20–30 µm diameter silica with a mobile phase of 0.1 M acetate buffer solution at pH 4.8 and fluorescence detection; however, a problem encountered was the contamination of the silica by food extracts. This resulted in a change in column permeability and gave rise to non-reproducible peak heights, which necessitated a change of column after only a few days of operation. Richardson *et al.*[2] used a modified procedure in which 10 µm diameter silica was used with a mobile phase of 0.1 M sodium acetate solution, and found that the silica column lasted for about 150 injections before needing replacement. In general, values for the riboflavin content of foods using this system were found to agree well with results obtained by microbiological assay. Reversed-phase packings have also been used for the chromatography of riboflavin in food extracts. Rhys Williams and Slavin[3] determined riboflavin in milk, and Toma and Tabekhia[4] determined this vitamin in rice. Neither paper gave any indication of column life. Because of the contamination problem associated with silica columns it was decided to investigate the use of reversed-phase materials.

Natural riboflavin usually occurs in foods at concentrations ranging from 0.01 to 0.5 mg per 100 g, either in the free form or bound as flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD). The first step in the extraction of riboflavin from foods is acid hydrolysis, which causes the FAD to be hydrolysed to FMN. Determination of riboflavin activity at this stage would therefore entail a measurement of FMN and free riboflavin. However, in most non-biological procedures for the determination of riboflavin a further enzymic hydrolysis is used to convert FMN into free riboflavin, so that total activity is measured as riboflavin. In order to study the efficiencies of the extraction procedures, in particular the enzymic hydrolysis, an HPLC method for the separation of riboflavin and FMN was developed.

Experimental

Chromatographic System

This consisted of the following: a Waters M 6000A solvent delivery system; a Perkin-Elmer LC1000 fluorescence detector, with excitation at 449 nm and emission at 520 nm; a 150 × 4 mm i.d. stainless-steel analytical column packed with Magnusil C_{22} Supergrade (Magnus Scientific Instrumentation Ltd.) protected by a 40 × 4 mm i.d. stainless-steel guard column packed with Spherisorb ODS (Phase Separations Ltd.), which was attached to the top of the analytical column with a low dead-volume coupling; and a Rheodyne, Model 70-100, loop injector.

Reagents

Aqueous methanol mobile phase, pH 5.8. A mixture of 0.1 M trisodium citrate solution (382 ml), 0.1 M citric acid solution (118 ml), water (100 ml) and methanol (400 ml). The mixture was passed through a 0.4-μm Millipore filter under vacuum before use.

Takadiastase. Serva (UK suppliers: Uniscience, Cambridge).

Sodium acetate buffer solution, 1.6 M. A mixture of sodium acetate trihydrate (217.6 g) and glacial acetic acid (60 ml), diluted to 1 l with distilled water.

Riboflavin standard. Koch-Light Laboratories.

Riboflavin-5'-monophosphate (*FMN*). Koch-Light Laboratories.

Sample Extraction and Chromatography

Prepare a stock riboflavin standard solution (40 μg ml^{-1}) by dissolving 20 mg of the vitamin in 50 ml of 0.1 M hydrochloric acid and diluting to 500 ml with water. This stock solution is further diluted with water to produce standards covering the range 0–0.6 μg ml^{-1} of riboflavin.

Load 100-μl aliquots of these standards on to the HPLC guard column via the loop injector, and construct a calibration graph by plotting riboflavin peak height *versus* mass (nanograms of riboflavin loaded). Weigh up to 4 g of the sample into a screw-top conical flask and add 25 ml of 0.1 M hydrochloric acid (swirl the flask to prevent aggregation of the sample). Autoclave the mixture at 121 °C for 30 min, cool, then add 5 ml of acetate buffer solution and 0.3 g of takadiastase. Incubate this mixture for 2 h at 40 °C (4 h for samples with a high fat content). If necessary, dilute the extract after incubation so that the riboflavin concentration is within the calibration range. Filter the extract through a No. 42 Whatman filter-paper and load 100-μl aliquots on to the HPLC guard column via the loop injector. Set the mobile phase flow-rate at 0.8 ml min^{-1}. Measure the riboflavin peak heights obtained, and compare with the calibration graph for quantification.

For foods containing very low levels of the vitamin (0.01 mg per 100 g or less) it may be necessary to use the trace-enrichment process (see under Results and Discussion).

Results and Discussion

Separation of Riboflavin and Flavin Mononucleotide

The separation of riboflavin and FMN was investigated on three types of 5-μm reversed phase packing, *viz.*, C_8, C_{18} and C_{22}. For each packing, methanol - water mixtures were used as mobile phases and fluorescence measurement for detection. All three materials separated riboflavin from FMN, the C_{22} column (150 × 4 mm i.d.) giving the best separation using a mobile phase of 40 + 60 methanol - water, with a flow-rate of 0.8 ml min^{-1}. It was found that riboflavin and FMN gave maximum fluorescent emission at pH 5.8; the mobile phase was therefore buffered to this pH with 0.05 M trisodium citrate and citric acid solutions.

In order to achieve the sensitivity required for analysis of most foodstuffs, 100-μl sample injections were found to be necessary. This was achieved using a loop injector. A 40-mm guard column packed with C_{18} material was placed between the injector and the C_{22} analytical column. The reproducibility of injection was investigated by repeat injections of a riboflavin standard. The peak-height measurements had a coefficient of variation of ±1.4%. Standard calibration graphs for riboflavin and FMN were constructed, and were shown to be linear up to a loading of 60 ng.

Extraction Techniques

An acid hydrolysate of foodstuffs was prepared by autoclaving samples with 0.1 M hydrochloric acid for 30 min at 120 °C. The same extraction procedure is used in this laboratory for microbiological assay of riboflavin. It was found that if strengths of hydrochloric acid of greater than 0.1 M were used, interfering peaks occurred in resulting chromatograms. Enzymic hydrolysis with takadiastase caused some problems. It was found that samples of takadiastase varied considerably in their phosphatase activity. Also, acid extracts of samples with a high fat content required a longer incubation period to achieve quantitative conversion of FMN into riboflavin. The enzyme preparations supplied by several manufacturers were compared, using an acid extract of ham paste as a substrate and incubation at 40 °C for 2 h. After chromatography, the efficiency of conversion was calculated. Acid phosphatase was tried as a possible alternative, but poor results were obtained. The enzyme preparation supplied by Serva was found to be most efficient for the conversion of FMN into riboflavin; this was also found by Richardson *et al.*[2] Fig. 1 shows chromatograms obtained after incubation of the ham paste extract with Serva takadiastase, and takadiastase produced by another manufacturer. Ham paste extract was chosen as a substrate because quantitative conversion of FMN into riboflavin in this sample had caused problems during this investigation. Extended incubation of this sample with Serva takadiastase (4 h) gave quantitative conversion of FMN into riboflavin.

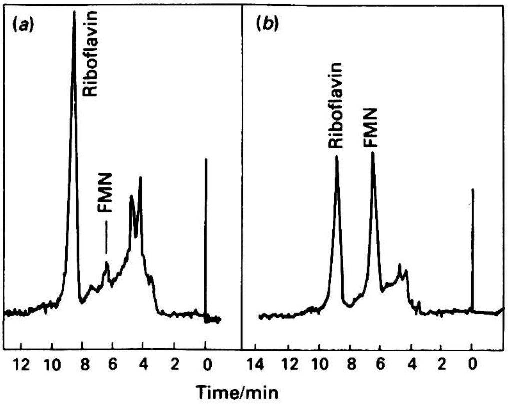

Fig. 1. Chromatograms obtained from ham paste extract after incubation with (*a*) Serva takadiastase and (*b*) takadiastase from an alternative source. Column: 150 × 4 mm i.d. Magnusil C_{22} Supergrade. Mobile phase: methanol - water (2 + 3), pH 5.8, flow-rate 0.8 ml min^{-1}. Fluorescence detection, excitation at 449 nm, emission at 520 nm. Chart speed: 2 min cm^{-1}.

Trace Enrichment

For foods with riboflavin levels greater than 0.01 mg per 100 g, injection of 100 μl of extract is sufficient for quantitative analysis. If analysis of foods with levels of 0.01 mg per 100 g or less is required, concentration of the extracts is necessary. Several attempts using conventional techniques, *e.g.*, evaporation under vacuum and solvent extraction, resulted in poor recoveries of the vitamin. However, it was found that riboflavin had an extremely long retention time on the reversed-phase columns when only water was used as a mobile phase. This made it possible to concentrate sample extracts directly on the HPLC column. Concentration was achieved by loading successive 100-μl volumes of sample extract on to the guard column whilst eluting with water. This had the effect of concentrating the riboflavin on the top of the column. The vitamin could then be eluted as a sharp band by changing to the methanol - water mobile phase. Trace enrichment also enables lower detector sensitivities to be used, thus reducing background noise. Fig. 2 shows chromatograms of an extract of shortcrust pastry (0.009 mg of riboflavin per 100 g of freeze-dried sample) before and after trace enrichment. It can be seen that a measurable riboflavin peak is obtained after loading four 100-μl aliquots of the extract on to the column. Resolution and peak symmetry are not lost using this technique. Up to 2 ml of sample extract has been loaded on to the column in this way and chromatographed successfully.

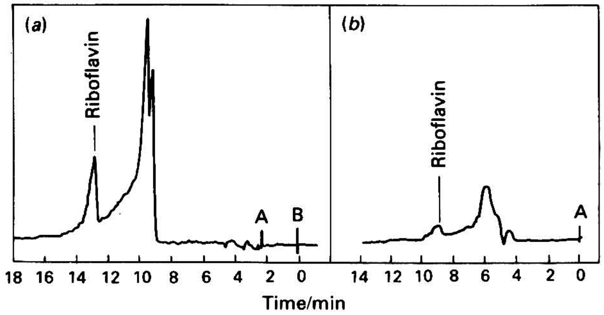

Fig. 2. Trace enrichment of a sample extract: (*a*) chromatogram after trace enrichment; and (*b*) before trace enrichment; 4 × 100 μl loaded. Sample: shortcrust pastry (9 μg of riboflavin per 100 g). Mobile phase: A, methanol - water (2 + 3), pH 5.8, flow-rate 0.8 ml min^{-1}; and B, water, flow-rate 0.8 ml min^{-1}.

Recovery Experiments

Riboflavin standard was added to several samples, and then the acid and enzyme extraction procedure was carried out. After chromatography the recovery of riboflavin was calculated. The same procedure was carried out using the FMN standard. The recovery results are shown in Tables I and II, respectively.

TABLE I

RIBOFLAVIN RECOVERY EXPERIMENTS

	Riboflavin/mg per 100 g				
Sample	Found	Added	Theoretical total	Experimental total	Recovery,* %
Ham paste	0.21	0.06	0.28	0.29	104
	0.21	0.16	0.37	0.37	100
Wholemeal flour	0.07	0.05	0.12	0.12	100
	0.07	0.13	0.20	0.19	95
Cows' milk (old sample)	0.09	0.03	0.12	0.13	108
	0.09	0.05	0.14	0.15	107
	0.09	0.08	0.17	0.17	100
Cows' milk (fresh)	0.15	0.08	0.23	0.22	96
	0.15	0.16	0.31	0.29	94
Hen egg†	1.4	0.53	1.93	1.9	98
	1.4	1.33	2.73	2.6	95

* Mean recovery: 100 ± 5%.
† Freeze-dried.

TABLE II

FLAVIN MONONUCLEOTIDE RECOVERY EXPERIMENTS

Sample	Flavin mononucleotide/mg per 100 g Found	Added	Theoretical total	Experimental total	Recovery,* %
Hen egg†	1.4	0.24	1.64	1.7	104
	1.4	0.37	1.77	1.8	102
	1.4	0.49	1.89	1.9	101
	1.4	0.33	1.73	1.7	98
	1.4	0.83	2.23	2.3	103

* Mean recovery: 102 ± 2%.
† Freeze-dried.

Comparison of HPLC with Microbiological Assay

The riboflavin content of 19 food samples was determined using the described HPLC method and the results compared with those obtained by microbiological assay using the method of Bell.[5] *Streptococcus zymogenes* was used as the test organism. The results obtained are shown in Table III. It can be seen that the HPLC results compare well with those obtained by microbiological assay. A typical sample chromatogram is shown in Fig. 3.

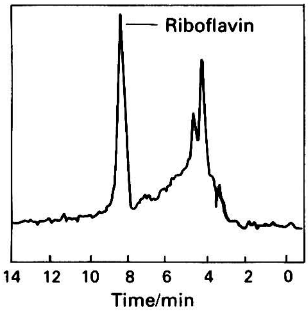

Fig. 3. Chromatogram obtained from wholemeal bread extract; 100 μl loaded. Column: 150 × 4 mm i.d. Magnusil C_{22} Supergrade. Mobile phase: methanol - water (2 + 3), pH 5.8, flow-rate 0.8 ml min^{-1}. Fluorescence detection, excitation at 449 nm, emission at 520 nm. Chart speed: 2 min cm^{-1}.

Repeat riboflavin determinations on wholemeal flour and cows' milk were carried out over a period of 1 week. The coefficients of variation of the mean results were found to be ±4 and ±5%, respectively, indicating the precision of the method.

The method is suitable for the determination of riboflavin down to the 0.01 mg per 100 g level. For samples with a lower content of the vitamin, trace enrichment can be used. The column life far exceeds that of silica columns; after 500 injections no deterioration of column efficiency was observed. The total analysis time for most samples is 3.5 h, so the method has a distinct advantage over microbiological procedures in which the analysis time is 2.5 d.

TABLE III

COMPARISON OF HPLC AND MICROBIOLOGICAL ASSAY FOR THE DETERMINATION OF RIBOFLAVIN

Sample	Riboflavin/mg per 100 g HPLC	Riboflavin/mg per 100 g MBA	Sample	Riboflavin/mg per 100 g HPLC	Riboflavin/mg per 100 g MBA
Wholemeal flour	0.08	0.09	Instant dessert	0.04	0.02
Cows' milk	0.15	0.16	Chicken in sauce*†	0.25	0.27
Wholemeal bread	0.07	0.09	Beefburger*†	0.31	0.34
Hovis bread	0.08	0.09	TVP mince	2.8	2.8
Sliced white bread	0.03	0.03	Oatmeal	0.07	0.09
Brown bread	0.06	0.07	Savoury rice	0.08	0.09
Brown flour	0.06	0.06	Shreddies	1.7	1.7
Household flour	0.02	0.03	Mousse*	0.33	0.38
Spaghetti	0.03	0.02	Hen egg*	1.4	1.3
Egg noodles	0.05	0.07			

* Freeze-dried.

† Denotes 4-h incubation with takadiastase.

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Automatic Titration by Stepwise Addition of Equal Volumes of Titrant

Part VII.* Potentiometric Precipitation Titrations

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The method used throughout this series to add the titrant stepwise with equal volumes each time is well suited to precipitation titrations. The advantage that one has to wait for equilibrium to be reached at only a few points is of special importance.

Two methods for calculation of the equivalence volume in precipitation titrations have been evaluated. One is based on the solution of a set of linear equations and the other is an extended version of the Gran I method. The methods are characterised by the following facts: (1) side-reactions can be accounted for; (2) the values of the solubility product and the stability constants of complexes may be unknown; (3) the solutions may be so dilute that a complete precipitation is not obtained; and (4) no accurate calibration of the electrode couple is required.

The calculation methods have been tested on the determination of chloride by titration with silver nitrate solution. The two methods agree closely and the errors in the calculations are negligible. The over-all errors in the determinations are 0.1–0.2% at chloride concentrations down to 10^{-4} M. At lower concentrations the relative errors are greater.

The methods have also been tested on the determination of sodium fluoride with lanthanum nitrate in (*a*) neutral and unbuffered solution, (*b*) acidic solution, (*c*) solution buffered with acetic acid - acetate and (*d*) solution buffered with formic acid - formate. The calculations were performed on experimental curves according to Lingane. For (*a*) and (*b*) good agreement between calculated and expected values were obtained. In the acetate-buffered solution too high a value was obtained in spite of the fact that acetate and mixed acetate - fluoride complexes were considered. For (*d*) the buffer capacity was too small to give satisfactory results.

Keywords: *Gran I method; potentiometric precipitation titrations; sodium fluoride determination; chloride determination*

Previous parts of this series have covered calculations of the equivalence volume in acid - base titrations. In Part III[1] a program, EKVOL, was presented, which utilises a system of linear equations with a maximum of three unknown variables. In Parts V[2] and VI[3] a method was described based on an extended Gran I method.[4] In order to make it easy to refer to the new method we have named it QUOTEQ.

These two methods can be used also for the calculation of the equivalence volume in a precipitation titration. A program that could be used for all types of precipitation titrations would be difficult to develop. It is therefore more practical to adapt a program to the actual problem. In order to illustrate the method of evaluation of a precipitation titration, we have chosen a special example, namely the titration of a chloride ion solution with a standard silver nitrate solution in which the course of the titration is followed with a silver - silver chloride electrode.

The titrant is added stepwise with equal volumes each time (a principle used throughout this series). This method is also well suited for precipitation titrations. The advantage that it is necessary to wait for equilibrium to be reached at only a few points is of special importance in this type of titration.

If the reaction were complete and as simple as $Ag^+ + Cl^- \rightarrow AgCl(s)$, the calculation of the equivalence volume would be very simple. Before the equivalence point, the chloride ion concentration would decrease linearly with the addition of titrant and after the equivalence point the silver ion concentration would increase linearly with the addition of titrant.

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* For details of Part VI of this series, see reference list, p. 1118.

A graph of $(V_0 + V)[\mathrm{Cl}^-]$ *versus* V would be a straight line, intersecting the volume axis at $V = V_e$, where V_0 is the volume of the sample solution at the start of the titration, V is the volume of the titrant added and V_e is the equivalence volume. After the equivalence point a graph of $(V_0 + V)[\mathrm{Ag}^+]$ would be a straight line intersecting the volume axis and the graph of $(V_0 + V)[\mathrm{Cl}^-]$ *versus* V at $V = V_e$.

This method of determining the equivalence volume has been described by Gran.[5] It has to be assumed that the precipitation is complete and that no side-reactions take place. Such an assumption is virtually true if the initial concentration of chloride ions in the titrand is 10^{-3} M or higher. This is illustrated in Fig. 1 for the titration of 100 ml of 0.001 M chloride solution with 0.01 M silver nitrate solution. The S-shaped graph shows e.m.f. values *versus* titrant volumes. The corresponding Gran II plot is a straight line intersecting the volume axis at $V = 10.07$ ml close to the correct equivalence volume of 10.04 ml.

This method of evaluation gives less accurate results when a 10^{-4} M chloride solution is titrated in the same manner with 0.001 M silver nitrate solution. It is difficult to observe a potential jump at the equivalence point and the Gran II plot gives an erroneous result (Fig. 2). This is due to the fact that precipitation at the equivalence point is not complete. The solubility product of silver chloride is about $10^{-9.4}$ and therefore about 20% of the chloride ions are not precipitated at the equivalence point if the initial chloride ion concentration is 10^{-4} M. A correct evaluation method has to consider this fact and also take into account the side-reactions that may occur.

The evaluation methods derived were also tested on the titration of sodium fluoride with lanthanum nitrate using experimental curves according to Lingane.[6]

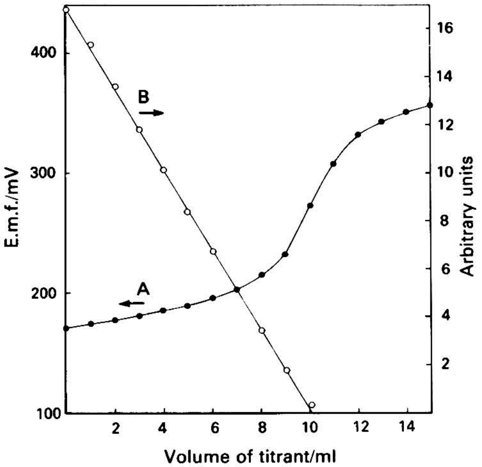

Fig. 1. Titration of 100 ml of 1.005×10^{-3} M chloride ion solution with 1.000×10^{-2} M silver nitrate solution. Graph A is e.m.f. in mV (left-hand scale) *versus* added volume of titrant; graph B is the Gran II plot in arbitrary units (right-hand scale).

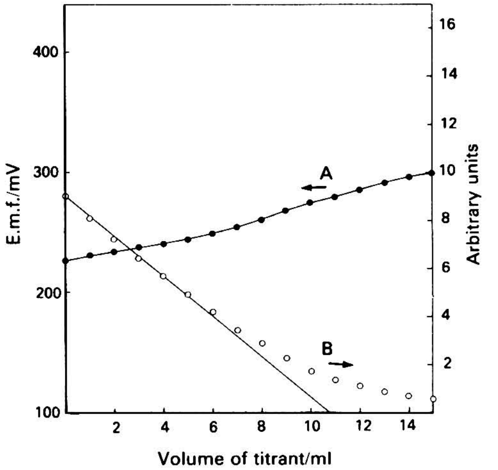

Fig. 2. Titration of 100 ml of 1.005×10^{-4} M chloride ion solution with 1.000×10^{-3} M silver nitrate solution. Graph A is e.m.f. in mV (left-hand scale) *versus* added volume of titrant; graph B is the Gran II plot in arbitrary units (right-hand scale). The straight line through the first few points gives a very imprecise value of V_e.

Theoretical

The Titration Curve

Assume that V_0 ml of a C_0 M chloride solution is titrated with C_B M silver nitrate solution. After addition of V ml of titrant, P mmol of silver chloride have been precipitated and therefore

$$(V_0 + V)[\mathrm{Cl}^-]_{\mathrm{tot}} = V_0 C_0 - P \tag{1}$$

$$(V_0 + V)[\mathrm{Ag}^+]_{\mathrm{tot}} = V C_B - P \tag{2}$$

where $[\mathrm{Cl}^-]_{\mathrm{tot}}$ and $[\mathrm{Ag}^+]_{\mathrm{tot}}$ are the total concentrations of chloride and silver ions in the solution. If V_e is the equivalence volume, then by definition

$$V_0 C_0 = V_e C_B \tag{3}$$

Combination of equations (1), (2) and (3) gives

$$V - \frac{V_0 + V}{C_B}([\mathrm{Ag}^+]_{\mathrm{tot}} - [\mathrm{Cl}^-]_{\mathrm{tot}}) - V_e = 0 \tag{4}$$

The following equilibrium conditions are valid[7]:

$$\begin{array}{ll}
\mathrm{AgCl(s)} \rightleftharpoons \mathrm{Ag}^+ + \mathrm{Cl}^- & \log K_{s0} = -9.4 \\
\mathrm{AgCl(s)} \rightleftharpoons \mathrm{AgCl} & \log K_{s1} = -6.6 \\
\mathrm{AgCl(s)} + \mathrm{Ag}^+ \rightleftharpoons \mathrm{Ag_2Cl}^+ & \log K_{s12} = -4.6 \\
\mathrm{AgCl(s)} + \mathrm{Cl}^- \rightleftharpoons \mathrm{AgCl_2}^- & \log K_{s2} = -4.7 \\
\mathrm{AgCl(s)} + 2\mathrm{Cl}^- \rightleftharpoons \mathrm{AgCl_3}^{2-} & \log K_{s3} = -4.4 \\
\mathrm{AgCl(s)} + 3\mathrm{Cl}^- \rightleftharpoons \mathrm{AgCl_4}^{3-} & \log K_{s4} = -3.5
\end{array}$$

By combining these equilibrium conditions with equation (4) the following expression is obtained (for convenience, charge signs have been omitted):

$$V - \frac{V_0 + V}{C_B} \cdot [\mathrm{Ag}]\,(1 + K_{s12}) + \frac{V_0 + V}{C_B} \cdot [\mathrm{Cl}](1 + K_{s2} + 2K_{s3}\,[\mathrm{Cl}] + 3K_{s4}\,[\mathrm{Cl}]^2) - V_e = 0 \tag{5}$$

The values of the parentheses are close to unity. In other types of titrations where the constants are unknown, it may be safe to consider the constants for the first complexes, *i.e.*, K_{s12} and K_{s2}. The concentrations of the higher complexes usually are too low to interfere in any practical titration.

If the concentration of free silver ions is measured, $K_{s0}/[\mathrm{Ag}]$ is substituted for [Cl] and equation (5) becomes

$$V - \frac{V_0 + V}{C_B} \cdot K_1\,[\mathrm{Ag}] + \frac{V_0 + V}{C_B} \cdot \frac{K_2}{[\mathrm{Ag}]} - V_e = 0 \tag{6}$$

where $K_1 = (1 + K_{s12}) \approx 1$ and $K_2 = (1 + K_{s2})K_{s0} \approx K_{s0}$. In equation (6) K_1, K_2 and V_e are unknown, V_0, V and C_B are known and [Ag] is measured.

If a silver electrode combined with a reference electrode is used for following the titration, the e.m.f. (E) between the electrodes will depend on the concentration of silver ions in the solution:

$$E = E'_o + Q \log\,[\mathrm{Ag}] \tag{7}$$

where E'_o is the conditional standard potential of the electrode pair, including the reference electrode potential and the activity coefficient, Q is the Nernst factor $= (RT\ln 10)/F = 59.158$ at 25 °C and [Ag] is the silver ion concentration (not the activity), as the electrode pair is calibrated using solutions with known silver ion concentrations. This means that the activity factors must be kept constant, *i.e.*, it is necessary to carry out the titration in a solution of constant ionic strength. From equation (7) we obtain

$$[\mathrm{Ag}] = 10^{(E-E'_o)/Q} \tag{8}$$

As an actual value of E'_o may be difficult to determine, it is practical to assume that the values of the silver ion concentrations calculated from equation (8) may contain a constant but unknown error, so that

$$[\mathrm{Ag}] = f[\mathrm{Ag}'] \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \qquad (9)$$

where [Ag] is the correct silver ion concentration and [Ag′] is the silver ion concentration obtained by using an incorrect value of E'_0. If this expression is inserted in equation (6), the following equation is obtained:

$$\frac{(V_0 + V)[\mathrm{Ag}']}{C_\mathrm{B}} \cdot K'_1 - \frac{V_0 + V}{[\mathrm{Ag}']C_\mathrm{B}} \cdot K'_2 + V_\mathrm{e} - V = 0 \qquad \ldots \quad \ldots \qquad (10)$$

where $K'_1 = K_1 f$ and $K'_2 = K_2/f$.

Calculation of the Equivalence Volume

Equation (10) may be used for calculating the equivalence volume. The quantities V_0, V and C_B are known and [Ag′] is measured. Two different calculation procedures, EKVOL and QUOTEQ, have been used for the evaluation. The procedures require pairs of measurements (volumes of titrant *versus* e.m.f.) plus initial volume, titrant concentration, temperature and a rough value of the conditional standard potential.

Calculation with the aid of EKVOL

No preliminary estimates of equivalence volume or of the constants K'_1 and K'_2 are required in this procedure. Equation (10) is a linear equation with three unknowns, K'_1, K'_2 and V_e. To determine the values of these unknowns a set of at least three equations is required, *i.e.*, at least three data pairs (V; [Ag′]) are needed. With more than three measurement points, a least-squares treatment should be used.

For a certain data pair (V_i; $[\mathrm{Ag}']_i$) equation (10) may be written as

$$A(1,i)\, K'_1 + A(2,i)\, K'_2 + A(3,i)\, V_\mathrm{e} + A(4,i) = 0 \qquad \ldots \quad \ldots \qquad (11)$$

where

$$\begin{aligned} A(1,i) &= (V_0 + V_i)[\mathrm{Ag}']_i/C_\mathrm{B} \\ A(2,i) &= -\,(V_0 + V_i)/([\mathrm{Ag}']_i C_\mathrm{B}) \\ A(3,i) &= 1 \\ A(4,i) &= -V_i \end{aligned}$$

The computer program EKVOL[1] can carry out these calculations. Initially it calculates the different $[\mathrm{Ag}']_i$ values using a rough value of E'_0. The values of V_0, V_i, C_B and the calculated $[\mathrm{Ag}']_i$ are then used to calculate the coefficients in the set of linear equations (11). A least-squares procedure is then used to calculate the coefficients in the normal equations and these equations are solved.

The resulting values of K'_1, K'_2 and V_e include random errors and some systematic errors derived from errors in V_0, V, E'_0 and E. Accurate pipetting will make volume errors practically negligible. A constant error in E'_0 will influence the values of K'_1 and K'_2 but not the value of the equivalence volume, V_e. As $K'_1K'_2 = K_1fK_2/f$, it follows that $K'_1K'_2 = K_1K_2$, which means that the solubility product, K_{S0}, is roughly equal to K_1K_2.

Calculation with the aid of QUOTEQ

The titration data are divided into two groups: (1) before the equivalence point where [Ag] is small and thus the term $(V_0 + V)K'_1[\mathrm{Ag}']/C_\mathrm{B}$ in equation (10) is small, and (2) after the equivalence point where $[\mathrm{Cl}] = K'_2/[\mathrm{Ag}']$ and thus the term $(V_0 + V)K'_2/([\mathrm{Ag}']C_\mathrm{B})$ in equation (10) is small. Before the equivalence point has been reached, the following equation can be applied:

$$(V_\mathrm{e} - V)C_\mathrm{B} = \frac{K'_2}{[\mathrm{Ag}']}(V_0 + V) \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \qquad (12)$$

For two additions of titrant, V_i and V_j, the following expressions are obtained:

$$(V_{\mathrm{e}} - V_i)\, C_{\mathrm{B}} = \frac{K'_2}{[\mathrm{Ag}']_i}\,(V_0 + V_i) \qquad \ldots \quad \ldots \quad \ldots \quad (13)$$

and

$$(V_{\mathrm{e}} - V_j)\, C_{\mathrm{B}} = \frac{K'_2}{[\mathrm{Ag}']_j}\,(V_0 + V_j) \qquad \ldots \quad \ldots \quad \ldots \quad (14)$$

Divisions of equation (13) by equation (14) and rearrangement as in Part V[2] gives

$$V_{\mathrm{e}} = V_j + \frac{V_j - V_i}{\dfrac{V_0 + V_i}{V_0 + V_j} \cdot \dfrac{[\mathrm{Ag}']_j}{[\mathrm{Ag}']_i} - 1} \qquad \ldots \quad \ldots \quad \ldots \quad (15)$$

After the equivalence point the following expression is obtained in a similar way:

$$V_{\mathrm{e}} = V_j - \frac{V_i - V_j}{\dfrac{V_0 + V_i}{V_0 + V_j} \cdot \dfrac{[\mathrm{Ag}]_i}{[\mathrm{Ag}]_j} - 1} \qquad \ldots \quad \ldots \quad \ldots \quad (16)$$

In neither of the instances is it necessary to know the value of the solubility product. All values, except V_{e}, are known or may be measured. Note also that only ratios of silver ion concentrations are used, which means that the calculations are independent of the value of f.

In dilute solutions equations (15) and (16) will not give sufficiently accurate results. In such instances it is necessary to use the complete expression (10). The following equation is obtained:

$$V_{\mathrm{e}} = V_j + \frac{V_j - V_i}{\dfrac{V_0 + V_i}{V_0 + V_j} \cdot \dfrac{K'_2/[\mathrm{Ag}']_i - K'_1[\mathrm{Ag}']_i}{K'_2/[\mathrm{Ag}']_j - K'_1[\mathrm{Ag}]_j} - 1} \qquad \ldots \quad \ldots \quad (17)$$

In this equation K'_1, K'_2 and V_{e} are unknown. However, if the calibration of the electrode couple is not too bad, the factor f has a value close to unity and it is possible use $K'_1 \approx K_1 \approx 1$ and $K'_2 \approx K_2 \approx 10^{-9.4}$ as a first approximation. In fact, it is even possible to start by setting $K'_1 = K'_2 = 0$. The program will still calculate the values for the three unknowns.

As in the similar method described in Parts V[2] and VI,[3] V_j should be as close as possible to the expected V_{e} value. As the equivalence potential is known to be approximately 270 mV, it is fairly easy to choose a value for V_j (*cf.*, reference 8). Other titration points are used as V_i.

In order to refine the value of V_{e} and to calculate values for K'_1 and K'_2 an iterative procedure is used, in which K'_1 and K'_2 are calculated from

$$K'_1 = \frac{C_{\mathrm{B}}}{[\mathrm{Ag}']_\omega} \cdot \frac{V_\omega - V_{\mathrm{e}}}{V_0 + V_\omega} + \frac{K'_2}{[\mathrm{Ag}']^2_\omega} \qquad \ldots \quad \ldots \quad \ldots \quad (18)$$

and

$$K'_2 = C_{\mathrm{B}}\,[\mathrm{Ag}']_\alpha \cdot \frac{V_{\mathrm{e}} - V_\alpha}{V_0 + V_\alpha} + K'_1\,[\mathrm{Ag}']^2_\alpha \qquad \ldots \quad \ldots \quad \ldots \quad (19)$$

In these two equations (V_α; $[\mathrm{Ag}]_\alpha$) is chosen from one of the very first points and (V_ω; $[\mathrm{Ag}]_\omega$) from one of the last points in the titration. By doing so, the value of K_1 is least influenced by the value of K'_2, and *vice versa* for the calculation of K'_2.

The calculation program is thus built up around three fixed data pairs (V_α; $[\mathrm{Ag}]_\alpha$), (V_j; $[\mathrm{Ag}]_j$ and (V_ω; $[\mathrm{Ag}_\omega]$). For each data pair (V_i; $[\mathrm{Ag}]_i$) a $V_{\mathrm{e}(i)}$ is calculated, where the iteration is carried on until two consecutive values for $V_{\mathrm{e}(i)}$ do not deviate by more than, *e.g.*, 10^{-5} ml. The first data pair (V_i; $[\mathrm{Ag}]_i$) used normally requires several iterations. The subsequent data pairs usually require only a few, as all values of $V_{\mathrm{e}(i)}$, K'_1 and K'_2 are very close to the values to be found in the next iteration.

The Quotec program also includes the possibility of making a linear regression of the various $V_{e(i)} = a + bV_i$. This line is then intersected by the line $V_{e(i)} = V_i$ to give a final value for V_e, *i.e.*, $V_e = a/(1 - b)$, see Table I.

TABLE I

TITRATION OF 10^{-4} M CHLORIDE SOLUTION

99.88 ml of a 1.005×10^{-4} M hydrochloric acid solution were titrated with a 1.000×10^{-3} M silver nitrate solution. The supporting electrolyte was 0.1 M in potassium nitrate and 0.1 M in nitric acid. The temperature was 25 °C. E_0' was assumed to be 555 mV. Pipette volume, 1.0021 ml.

	Experimental values*		V_e/ml		
Value No.	V/ml	E/mV	Calculated from equation (10)	Calculated from equations (17), (18) and (19)	
0	0	228.6		10.0754	10.0833
1	1.002	231.5	10.108	10.0759	10.0731
2	2.004	234.6	10.072	10.0763	10.0660
3	3.006	237.9	10.071	10.0764	10.0657
4	4.008	241.6	10.055	10.0766	10.0614
5	5.010	245.6	10.072	10.0764	10.0659
6	6.013	250.2	10.062	10.0767	10.0622
7	7.015	255.2	10.087	10.0760	10.0728
8	8.017	260.9	10.091	10.0757	10.0770
9	9.019	267.2	10.086	10.0757	10.0766
10	10.021	273.8	10.081		10.0758
11	11.023	280.3	10.078	10.0758	
12	12.025	286.3	10.082	10.0761	10.0649
13	13.027	291.7	10.077	10.0759	10.0718
14	14.029	296.4	10.079	10.0760	10.0709
15	15.031	300.5	10.081	10.0761	10.0704
16	16.034	304.1	10.081	10.0760	10.0708
17	17.036	307.3	10.073	10.0759	10.0731

* Using the experimental data in columns 2 and 3 the following values were obtained with the aid of Ekvol: $V_e = 10.079$ ml, standard deviation = 0.011; $\log K_1' = 0.004$; $\log K_2' = -9.497$. These values for the constants were used to calculate the individual V_e values in column 4 using equation (10).

From the same experimental data values for V_e, K_1' and K_2' were calculated by iteration of equations (17), (18) and (19). In columns 5 and 6 individual V_e values are given for $j = 10$ ($V_j = 10.206$ ml) and $j = 11$ ($V_j = 11.227$ ml), respectively. Linear regression on column 5 gives $V_e = 10.0761 - 9.068 \times 10^{-6}\ V_i$, which, with $V_i = V_e$, gives $V_e = 10.076$ ml, standard deviation = 0.0003 ml; $\log K_1' = -0.004$; $\log K_2' = 9.498$, in good agreement with the corresponding values obtained by the Ekvol program.

Evaluation of the Programs

Synthetic Titration Data

The programs were first evaluated using synthetic data, which were calculated from the titration equation (10). The sample volume was 100 ml, $\log K_1' = 0.2$, $\log K_2' = -9.6$ and $V_e = 10$ ml. C_0 was varied from 10^{-2} to 10^{-6}. In all instances correct values were obtained.

Experimental Data

The e.m.f. measurements were carried out with a silver - silver chloride electrode (according to Brown[9]) combined with a Radiometer K701 reference electrode and a digital millivoltmeter. Titrant aliquots of 1.0021 ml were added with a pneumatically driven pipette. The titration vessel was immersed in a bath maintained at 25.0 ± 0.1 °C.

The chloride solutions were prepared from a 0.1005 M stock solution of hydrochloric acid. The titrant solutions were prepared from a 0.1000 M solution of silver nitrate. The concentrations of the two stock solutions were determined by precipitation and weighing as silver chloride. Five different concentrations of hydrochloric acid were used:

(1) 100 ml 10^{-2} M chloride titrated with 0.1 M silver nitrate solution;
(2) 100 ml 10^{-3} M chloride titrated with 0.01 M silver nitrate solution;

(3) 100 ml 10^{-4} M chloride titrated with 0.001 M silver nitrate solution;
(4) 100 ml 5×10^{-5} M chloride titrated with 0.001 M silver nitrate solution;
(5) 100 ml 10^{-5} M chloride titrated with 0.001 M silver nitrate solution.

All solutions were 0.1 M in potassium nitrate and 0.1 M in nitric acid.

An approximate value of E'_0 was determined by measuring the e.m.f. of the electrode pair in the 10^{-3} M silver nitrate solution. The value found was $E'_0 = 555$ mV and this value was used throughout all calculations.

Example 1

Three titrations of a 0.01 M chloride solution were performed. The results were V_e = 10.118, 10.060 and 10.040 ml using EKVOL and V_e = 10.053, 10.053 and 10.057 ml using QUOTEQ. The values for $\log K_{s0} = \log K'_1 + \log K'_2$ were −9.41 to −9.43.

Example 2

A titration of a 0.001 M chloride solution is shown in Fig. 1. A Gran II plot gives V_e = 10.07 ml. A numerical evaluation of this titration and a duplicate one gave V_e = 10.023 and 10.024 ml (EKVOL) and 10.038 and 10.037 ml (QUOTEQ). All four evaluations gave $\log K_{s0} = -9.49$.

Example 3

A Gran II plot of a titration of a 10^{-4} M chloride solution gives completely wrong results (Fig. 2). However, a numerical evaluation gives correct results. Duplicate titrations gave V_e = 10.08 and 10.03 ml (EKVOL) and V_e = 10.07 and 10.03 ml (QUOTEQ). Impurities in the supporting electrolyte could be calculated to consume about 0.03 ml of 0.001 M silver nitrate solution. The V_e values obtained thus agree well with the expected value of 10.04 ml. The four evaluations gave $\log K_{s0} = -9.48$ to -9.50. See Table I for further details.

Example 4

Even a chloride solution as dilute as 5×10^{-5} M can be evaluated with good results. EKVOL gave V_e = 5.18 and 5.18 ml and QUOTEQ gave V_e = 5.20 and 5.20 ml.

Example 5

The results of titrations of 10^{-5} M chloride solutions with 10^{-3} M silver nitrate solution were 1.1, 1.4, 1.5 and 1.6 ml (all obtained with the EKVOL program). Log K_{s0} varied between −9.40 and −9.45. The QUOTEQ program did not work, as too narrow an e.m.f. range was obtained. However, if average values for K'_1 and K'_2, taken from examples 2 and 3, were used in the QUOTEQ program, the results were V_e = 1.47, 1.40, 1.38 and 1.59 ml. It is obvious that at this very low concentration the results are scattered.

Discussion

In general, evaluations by the EKVOL and the QUOTEQ programs give results that agree fairly well; only for the first titration in example 1 a small difference is found: QUOTEQ gave V_e = 10.053 ml, close to the expected value of 10.04 ml, whereas EKVOL gave V_e = 10.118 ml. This discrepancy can be explained as follows. EKVOL is based on the solution of a system of linear equations. All data therefore have the same weight, and if some data are bad this shows up in the result. In the first titration some data are bad because the solution was not stirred effectively enough to prevent some precipitate from adhering to the electrodes. In QUOTEQ $V_e = V_j +$ quotient; with $j = 10$, *i.e.*, V_j = 10.021 ml, the quotient is only 0.055 ml, and for $j = 11$, *i.e.*, V_j = 11.023 ml, V_e was calculated to be 10.071 ml and the quotient was −0.952 ml. In QUOTEQ the V_j term normally dominates and the value of the quotient should not exceed the volume of the titrant pipette, V_p. It is suitable to use two V_j values, one on each side of V_e, in the calculations.

The over-all errors in the determinations are 0.1–0.2% down to chloride concentrations of 10^{-4} M. The errors due to the evaluation are negligible.

Other Titrations

In order to illustrate the evaluation of more complicated precipitation titrations we have chosen the titration of sodium fluoride with lanthanum nitrate. These titrations have been studied by Lingane,[6] who used a lanthanum fluoride membrane electrode to follow the change in the fluoride ion activity during the titration. The main reaction is

$$La^{3+} + 3\,F^- = LaF_3(s) \quad .. \quad .. \quad .. \quad .. \quad (20)$$

However, the fluoride and lanthanum ions may also take part in a number of side-reactions. In acidic solutions HF may be formed, as hydrofluoric acid is a weak acid. Further, HF_2^- may be formed. At the start of the titration, F^-, HF and HF_2^- may be present in the sample solution. The total concentration of fluoride ions in the solution is thus

$$[F]\,(1 + K_{HF}[H] + 2K_{HF_2}[H][F]) \quad .. \quad .. \quad .. \quad (21)$$

where K_{HF} is the stability constant for the reaction $H^+ + F^- = HF$ and K_{HF_2} is the stability constant for the reaction $H^+ + 2F^- = HF_2^-$. Log K_{HF} is about 3.5 and log K_{HF_2} is about 4.0.

In dilute fluoride solutions, the term $2K_{HF_2}[H][F]$ can be neglected and thus equation (21) is reduced to

$$[F](1 + K_{HF}[H]) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (22)$$

The factor $(1 + K_{HF}[H])$ can be replaced with a constant K_1 if the hydrogen ion concentration is kept constant or if it is low, *e.g.*, pH > 5. In the titration of unbuffered fluoride solutions the pH is usually 5–6 and then K_1 is very close to unity.

In the titration of pure fluoride solutions, only LaF_3 and LaF^{2+} have to be considered, but in buffered solutions many different complexes can be formed in addition to these two. If an acetate buffer is chosen, the pH is so high that the formation of HF and HF_2^- can be neglected. The addition of acetate ions leads to the formation of acetate complexes in addition to the fluoride complexes and further mixed complexes may be formed.

During the titration in an acetate-buffered solution, varying amounts of F^-, La^{3+}, $LaFAc^+$, $LaAc_2^+$, LaF^{2+}, $LaAc_3$, LaF_2^+ and LaF_3 are present. Anfält *et al.*[10] used the program HALTAFALL[11] to calculate the equilibrium concentrations of the various species formed during the titrations. They used the experimental values from Lingane's paper[6] and equilibrium constants from "Stability Constants"[12] and other sources in their calculations. In this way it is possible to decide which species will dominate and which can be neglected.

In the determination of the equivalence point the method of maximum slope of the titration curve cannot be used. In these titrations,[13] the equivalence point and the point of maximum slope do not coincide. Anfält and co-workers[10,14] and Eriksson[15] determined the equivalence volume by the Gran II method. By applying the same principles as in the chloride titration above, the following equation, corresponding to equation (5), can be deduced:

$$\frac{V_0 + V}{3C_B}[F](1 + K_{HF}[H]) - \frac{V_0 + V}{3C_B}\cdot\frac{3K_{s0}}{[F]^3} - \frac{V_0 + V}{3C_B}\cdot\frac{2[Ac]^2}{[F]^2}\cdot K_{s0}K_{112} -$$

$$\frac{V_0 + V}{3C_B}\cdot\frac{2}{[F]^2}\cdot K_{s0}K_{LaF} - \frac{V_0 + V}{3C_B}\cdot\frac{[Ac]}{[F]}\cdot K_{s0}K_{121} + V - V_e = 0 \quad .. \quad (23)$$

where K_{LaF}, K_{112} and K_{121} are the stability constants for the formation of the complexes LaF^{2+}, $LaFAc_2$ and LaF_2Ac, respectively.

If the hydrogen and acetate ion concentrations can be considered to be constant, the following titration equation, corresponding to equation (10), is obtained:

$$\frac{V_0 + V}{3C_B}.[F'] \,.\, K'_1 - \frac{V_0 + V}{3C_B}\cdot\frac{K'_2}{[F']^3} - \frac{V_0 + V}{3C_B}\cdot\frac{K'_3}{[F']^2} -$$

$$\frac{V_0 + V}{3C_B}\cdot\frac{K'_4}{[F']} + V - V_e = 0 \quad .. \quad .. \quad .. \quad (24)$$

The constants K'_1, K'_2, K'_3 and K'_4 are thus conditional constants in which both the error factor f and stoicheiometric coefficients are included. Equation (24) is linear with five unknowns and can in principle be solved, *e.g.*, by the program EKVOL, if sufficiently many accurate measurement data are available. However, in practice, a few terms dominate and the contribution from the other terms is so slight that they do not influence the data. In the choice among the terms a knowledge of the approximate values of the stability constants is a good help; otherwise, one has to resort to the trial and error method.

This technique has been tested on the evaluation of four titrations shown in Fig. 6 in Lingane's paper.[6] In all four titrations 100 ml of 0.004 M sodium fluoride solution were titrated with 0.03189 M lanthanum nitrate solution, *i.e.*, $V_e = 4.18$ ml. The experimental data were read from the figure, which of course involved a high risk of inaccuracy. $E'_0 = -170$ mV was chosen for the calculation of the fluoride concentrations.

Titration 1: *unbuffered neutral solution*

It can be assumed that $[F^-]$ dominates before the equivalence point and $[LaF_3]$ after the equivalence point. Some LaF^{2+} may be formed.

Result: $V_e = 4.15 \pm 0.05$ ml, $K'_1 = 0.93$, $\log K'_2 = -17.70$ and $\log K'_3 = -13.2$. Nearly the same results were obtained even if the K'_3 term was omitted: $V_e = 4.19 \pm 0.07$ ml, $K'_1 = 1.04$ and $\log K'_2 = -17.53$.

A calculation on the same titration using a QUOTEQ program gave $V_e = 4.20 \pm 0.04$ ml, $K'_1 = 1.04$ and $\log K'_2 = -17.52$.

The QUOTEQ program in this instance iterates the three equations

$$V_e = V_j + \frac{V_j - V_i}{\dfrac{V_0 + V_i}{V_0 + V_j} \cdot \dfrac{K'_1[F']_i - K'_2/[F']_i^3}{K'_1[F']_j - K'_2/[F']_j^3} - 1} \quad \cdots \quad (25)$$

$$K'_1 = \frac{3C_B}{[F']_\alpha} \cdot \frac{V_e - V_\alpha}{V_0 + V_\alpha} + \frac{K'_2}{[F']_\alpha^4} \quad \cdots \quad (26)$$

and

$$K'_2 = 3C_B[F']_\omega^3 \cdot \frac{V_\omega - V_e}{V_0 + V_\omega} + K'_1[F']_\omega^4 \quad \cdots \quad (27)$$

Titration 2: *acidified solution*

This titration was performed in the same way as titration 1, except that 10 ml of 0.100 M hydrochloric acid solution was added to the 100 ml of neutral sample solution before the start of the titration. In this instance the formation of hydrofluoric acid cannot be disregarded.

Equation (23) then becomes

$$\frac{V_0 + V}{3C_B} \cdot [F']K'_1 + \frac{V_0 - V}{3C_B} \cdot [F'][H]K'_{HF} - \frac{V_0 + V}{3C_B} \cdot \frac{K'_2}{[F']^3} - V_e + V = 0 \quad (28)$$

where K'_1 is the error factor for [F], *i.e.*, about 1.0 if the electrode couple is fairly well calibrated. As the K'_1 term is small in comparison with the other terms it is better not to calculate K'_1 but to assume that $K'_1 = 1.0$.

The hydrogen ion concentration is not constant, but it can of course be measured during the titration. However, it is also possible to calculate the hydrogen ion concentration if the total number of moles, V_0[Cl], of hydrochloric acid added is known. In this instance the titration equation is changed to

$$\frac{V_0[Cl]}{3C_B} \cdot \frac{[F']}{1 + K'_{HF}[F']} \cdot K'_{HF} - \frac{V_0 + V}{3C_B} \cdot \frac{K'_2}{[F']^3} - V_e + V + \frac{V_0 + V}{3C_B}[F'] = 0 \quad \cdots \quad (29)$$

As in principle, K'_{HF} is unknown the calculations must be iterated. In the first cycle of the iteration K'_{HF} in the denominator can be given an arbitrary value, *e.g.*, 1000.

Result: $V_e = 4.20 \pm 0.06$ ml, $\log K'_{HF} = 3.04$ and $\log K'_2 = -17.3$.

A calculation of the same titration using a QUOTEQ program gave $V_e = 4.18 \pm 0.02$ ml, $\log K'_{HF} = 3.01$ and $\log K'_2 = -17.1$. In this instance, the QUOTEQ equations, of course, were derived from equation (29).

Titration 3: *acetate-buffered solution*

This titration was performed as titration 2 except that the hydrochloric acid addition was replaced with 10 ml of 1 M acetate buffer solution of pH 4.88. The hydrogen ion concentration can be assumed to remain constant during the whole titration. The best precision in the results of the calculations of V_e was obtained when the K'_3 term in equation (24) was excluded, in which event the following result was obtained: $V_e = 4.52 \pm 0.06$ ml, $K'_1 = 1.08$, $\log K'_2 = -15.6$ and $\log K'_4 = -7.0$.

The equivalence volume obtained is much too high and the value for the solubility product which can be calculated from K'_2, $\log K_{s0} = -15.8$, is not in accordance with accepted values. Titration of fluoride in acetate-buffered solution is therefore not suitable. Certainly, too far-reaching conclusions should not be drawn from these results, as the data obtained from Lingane's diagrams are rather uncertain. However, our results agree reasonably well with those obtained by Anfält and co-workers[10,14,16] who studied the problem in more detail using Lingane's data and their own data. They found that acetate ions enter the solid phase during the titration. The precipitate is therefore not pure LaF_3, which is a requirement for the calculation of a correct fluoride concentration.

Titration 4: *formate-buffered solution*

This titration resembled titration 3 except that the acetate buffer was replaced with 10 ml of 0.2 M formate buffer solution of pH 3.00. The result, $V_e = 4.40$ ml, is too high and the precision is too low, which may be due to too low a buffer capacity and thus to a changing hydrogen ion concentration. $K'_1 = 2.11$, which is in fair agreement with the expected value $K'_1 = (1 + K_{FH}[H]) = (1 + 10^{3.1} \times 10^{-3}) = 2.25$.

The authors are indebted to Professor Folke Ingman for stimulating discussions and to Mrs. Karin Lindgren for skilful experimental assistance.

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NOTE—References 1, 2 and 3 are to Parts III, V and VI of this series, respectively.

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SHORT PAPERS

High-performance Liquid Chromatographic Determination of Chlorpromazine and Thioridazine Hydrochlorides in Pharmaceutical Formulations

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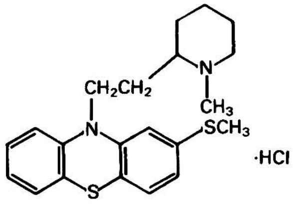
Keywords: *Chlorpromazine hydrochloride determination*; *thioridazine hydrochloride determination*; *pharmaceutical formulations*; *high-performance liquid chromatography*

Chlorpromazine and thioridazine are major tranquillisers and are widely used in the treatment of mental illness. In addition, chlorpromazine is a valuable adjunct for the relief of pain and vomiting. They belong to the phenothiazine class of drugs and are used in a variety of dosage forms mainly as their hydrochloride salts.

Chlorpromazine hydrochloride



Thioridazine hydrochloride

The widespread use of these drugs has necessitated the development of rapid, simple and precise methods for their quality control. High-performance liquid chromatography (HPLC) is ideal for this purpose as it satisfies the above criteria by minimising sample preparation. Methods currently in use for the analysis of these and other phenothiazines have been reviewed by Fairbrother.[1] Recently, Ramappa *et al.*[2] have described the use of arsenomolybdic acid as a reagent for the spectrophotometric determination of a number of phenothiazines including chlorpromazine hydrochloride, and Takahashi[3] has published an HPLC method for determining chlorpromazine hydrochloride and its oxidation products using an amino-bonded column in a normal-phase mode and fluorescence detection.

Experimental

Materials and Reagents

Chlorpromazine and thioridazine drug substances (hydrochlorides and free bases) were received from May and Baker Ltd. and R.P. Drugs Ltd., respectively. BP-grade cinchocaine hydrochloride, analytical-reagent grade methanol, hydrochloric acid and ethanolamine and distilled water were used when required.

Apparatus

A reversed-phase column (250 × 5 mm) containing 10-μm Hypersyl ODS (HPLC Technology Ltd.), equipped with a septumless injector was used in conjunction with a variable-wavelength spectrophotometric detector (Varian Variscan, Model 635), a pump (Altex, Model 110A) and a recorder (Smiths Servoscribe, Model RE 511–20).

Procedure

Samples were diluted with appropriate solvents to give an expected concentration of 0.05% of the drug. Cinchocaine hydrochloride (0.2% *m/V*) was used as an internal standard (IS). All solutions were protected from the light and kept in well closed containers.

Chlorpromazine

Syrup. Dilute 1.0 ml to 10.0 ml with a solution of IS in water.

Injections. Dilute 1.0 ml to 50.0 ml as above.

Tablets. Weigh 20 tablets and crush to a powder. Weigh an amount of the powder containing about 25 mg of the drug and dissolve it in 50.0 ml of a solution of IS in 0.1 N hydrochloric acid. Centrifuge and use the supernatant liquid for HPLC.

Suppositories. Weigh an amount containing 0.2 g of chlorpromazine hydrochloride and dissolve in 200 ml of warm methanol containing IS. Cool and dilute 5.0 ml of the clear solution to 10.0 ml with methanol containing IS.

Thioridazine

Syrup. Dilute 1.0 ml to 10.0 ml with a solution of IS in 0.1 N hydrochloric acid.

Tablets. Weigh 20 tablets and crush to a powder. Weigh an amount of the powder containing about 25 mg of the drug and dissolve it in methanol (50 ml) containing IS. Centrifuge and use the supernatant liquid for HPLC.

HPLC conditions

The following conditions were used: column, 250 × 5 mm, 10-μm Hypersyl ODS; mobile phase, methanol - water (90 + 10 *V/V*) containing 0.2% *V/V* of ethanolamine; flow-rate, 2.0 ml min^{-1}; injection volume, 15 μl; chart speed, 120 mm h^{-1}; wavelength, 257 nm (chlorpromazine) and 265 nm (thioridazine); and detector range, 1.0 a.u.f.s.

Standard solutions of drugs were prepared by dissolving 50.0 mg of drug substance in 100.0 ml of the same solvent (containing IS) as used for the corresponding sample. In preparing the standard solutions for liquid dosage forms, 1.0 ml of the sample was replaced with 1.0 ml of water. Standards were analysed in a similar manner to the samples. A standard was injected together with each sample and the drug peak height was compared with that of the IS. Two injections were made for each sample. The results were calculated as follows:

$$\%\ \text{Drug} = \frac{\begin{array}{c}\text{Average peak-height ratio}\\ \text{of drug to IS in sample}\end{array}}{\begin{array}{c}\text{Average peak-height ratio}\\ \text{of drug to IS in standard}\end{array}} \times \text{Concentration of standard} \times \text{Dilution factor}$$

From this the amount of drug per tablet or suppository was calculated, the sample mass and the average mass of a tablet or a suppository being taken into consideration. The samples were also analysed by the BP[4] or BPC[5] methods for comparison. The combined results are shown in Table I.

Results and Discussion

The conditions used gave well resolved peaks for each component (Fig. 1). The retention times for cinchocaine, chlorpromazine and thioridazine hydrochlorides were 182, 305 and 540 s, respectively. Each drug was analysed at the wavelength of maximum absorption, thereby imparting maximum sensitivity and precision to the assay. The change in the concentration of ethanolamine in the mobile phase had a significant effect on the elution of chlorpromazine and thioridazine hydrochlorides but not on cinchocaine hydrochloride (Fig. 2). Retention times and volumes were calculated from the point of injection. A concentration of ethanolamine of 0.2% was found to be the optimum under the other chromatographic conditions employed.

Although the comparison of peak heights was found suitable for the analysis, calibration graphs were drawn to check the linearity of response. For both drugs, in their respective formulations, the sample to IS peak-height ratios were found linear within the concentration

TABLE I

DETERMINATION OF CHLORPROMAZINE AND THIORIDAZINE HYDROCHLORIDES IN PHARMACEUTICALS

Sample	Drug content, %*	
	Proposed method	BP/BPC method
Chlorpromazine—		
Syrup, 0.5%	96.0	103.0
Injection, 2.5%	103.0	103.0
Tablets—		
10-mg	95.1	98.5
25-mg	104.0	103.0
50-mg	105.0	104.0
100-mg	100.0	103.0
Suppository, 10-mg†	101.0	101.0
Thioridazine—		
Syrup, 0.5%	97.5	—‡
Tablets—		
10-mg	105.0	104.0
25-mg	100.0	99.6
50-mg	98.5	102.0
100-mg	101.0	101.0

* With respect to the content claimed; average of two determinations.
† Contains chlorpromazine, determined as the free base.
‡ No BP/BPC method available for this product.

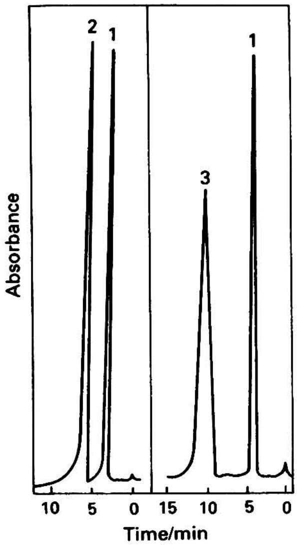


Fig. 1. Chromatograms of tablet sample extracts: 1, cinchocaine hydrochloride; 2, chlorpromazine hydrochloride; and 3, thioridazine hydrochloride.

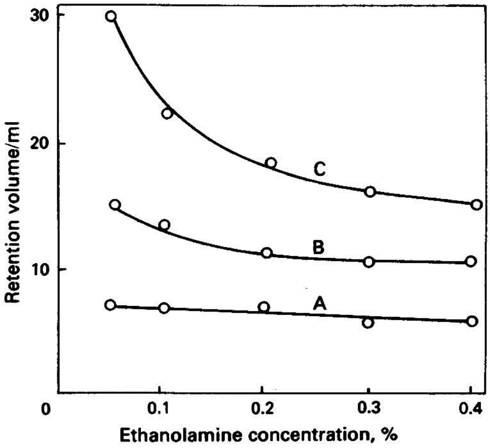


Fig. 2. Effect of ethanolamine concentration on elution volumes of A, cinchocaine hydrochloride; B, chlorpromazine hydrochloride; and C, thioridazine hydrochloride.

range 0.025–0.1% m/V. For example, the regression equations for the calibration graphs for chlorpromazine and thioridazine hydrochlorides in 0.1 N hydrochloric acid are given in equations 1 and 2, respectively:

$$y = 16.38x + 0.024, \text{ correlation coefficient } (r) = 0.99 \qquad (1)$$

$$y = 18.99x + 0.019, \text{ correlation coefficient } (r) = 0.99 \qquad (2)$$

Good reproducibility of the method was indicated when ten separate determinations of chlorpromazine hydrochloride and thioridazine hydrochloride in 50-mg tablets gave coefficients of variation of 1.4 and 1.9%, respectively. In each formulation the sample chromatogram compared very well with the standard chromatogram. No extra peaks were obtained even when sample chromatograms were run for longer periods. These observations suggest the absence of non-active ingredients or degradation products. No attempt was made to investigate the stability-indicating potential of the method by chromatographing degradation products or by actually degrading the drugs. (It is worth mentioning that, for chlorpromazine, previous work[3] has shown that, except for the concentrate, no degradation was detected from 2-year-old samples of commonly available preparations.) The time required for the chromatography is 10 and 15 min for chlorpromazine hydrochloride and thioridazine hydrochloride, respectively. No lengthy sample preparation is required and the results show no significant difference between the proposed and the official methods at the 95% confidence level. The method demonstrates its suitability for the routine quality control of these drugs in a variety of formulations.

The author thanks May and Baker Ltd. and R.P. Drugs Ltd. for providing chlorpromazine and thioridazine drug substances, and Mr. B. Midcalf for helpful discussions.

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Study of 3-Propyl-5-hydroxy-5-D-arabinotetrahydroxybutyl-3-thiazolidine-2-thione as a Reagent for the Spectrophotometric Determination of Thallium(I)

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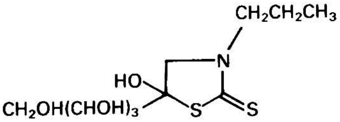
Keywords: *3-Propyl-5-hydroxy-5-*D*-arabinotetrahydroxybutyl-3-thiazolidine-2-thione reagent*; *spectrophotometry*; *thallium*(I) *determination*

The thiazolidine-2-thiones (3-alkyl-5-hydroxy-5-D-arabinotetrahydroxybutyl-3-thiazolidine-2-thiones), which have ethyl, methyl, butyl or dodecyl substituents in the 3-position, have some similarities to diethyldithiocarbamate and it was considered probable that they would form complexes with some metal ions.

Until now, only the copper(II) complex with 3-propyl-5-hydroxy-5-D-arabinotetrahydroxybutyl-3-thiazolidine-2-thione (PHTTT, shown below) has been described by Chow[1]



and Stiff,[2] who reported a specific method for the determination of copper in natural waters with PHTTT. Corbett,[3] Jochims[4] and Jochims and Abu-Taha[5] described the application of 3-methyl-5-hydroxy-5-D-arabinotetrahydroxybutyl-3-thiazolidine-2-thione (MHTTT) as a spectrophotometric reagent for copper(II), mentioning the nature and structures of the metal complexes and the application[3] to the determination of copper in steels, aluminium alloys, lead, zinc and water.

In this paper, the reactivity of PHTTT and the spectrophotometric characteristics of the complexes formed between PHTTT and nickel(II), beryllium(II), silver(I), bismuth(III) and thallium(I) are described. The spectrophotometric method has been applied to the determination of thallium(I) in solutions containing other ions.

Experimental

Apparatus

A Pye-Unicam SP 8-100 spectrophotometer was used for recording spectra in the ultraviolet and visible regions of the spectrum. A Beckmann DU spectrophotometer was also used for absorbance measurements at fixed wavelengths in the ultraviolet region.

An Orion Research Ionanalyzer 901 pH meter with glass and saturated calomel electrodes was used for pH measurements.

Reagents

All solutions were prepared with analytical-reagent grade chemicals and diluted using doubly distilled water.

PHTTT was prepared by Chow's method[1] and analytically pure material was obtained on recrystallisation from ethanol - water (7 + 3). Found: C, 40.3; N, 4.9; H, 6.5; and S, 21.8%. Calculated for PHTTT: C, 40.3; N, 4.7; H, 6.4; and S, 21.6%.

The PHTTT solutions were prepared by dissolving 0.1 g in 10 ml of ethanol - water (1 + 1) and diluting to 100 ml with water.

Solutions of various ions (10^{-3} M) were prepared from high-purity metals or analytical-reagent grade chemicals and were diluted immediately prior to use.

Procedures

General procedure for spectrophotometric studies

Aliquots of the ion solutions (10^{-3} M) to be studied were placed in 100-ml calibrated flasks, the required amount of the ligand (PHTTT solution, 0.1% *m/V*) and 5 ml of the appropriate Clark and Lub's buffer solution were added, and the mixtures were diluted to 100 ml with water. The spectra were recorded or the absorbances measured using 10-mm cells and a reagent blank as the reference.

Procedure for spectrophotometric determination of thallium(I)

To 10 ml of sample solution [containing 2–11 μg ml^{-1} of thallium(I)] in a 50-ml calibrated flask were added 5 ml of buffer solution (pH 2.2) and 2 ml of PHTTT solution (0.1% *m/V*), and the solution was diluted to 50 ml with doubly distilled water. The absorbance of the solution was measured at 210 nm against 0.1% *m/V* PHTTT solution and the appropriate buffer solution, using 10-mm cells. The calibration graph was prepared with standard solutions of thallium(I) treated in the same way.

For the preparation of the calibrations graphs, the ionic strength of the solutions was kept constant.

Results and Discussion

The reactivity of PHTTT with silver(I), copper(I) and -(II), cadmium(II), lead(II), arsenic(III) and -(V), tungsten(VI), mercury(I) and -(II), thallium(I) and -(III), niobium(V), tantalum(V), bismuth(III), palladium(II), iron(II) and -(III), antimony(III) and -(V), germanium(IV), chromium(III) and -(VI), aluminium(III), molybdenum(VI), tin(II) and -(IV), selenium(IV) and -(VI), tellurium(IV) and -(VI), platinum(IV), gold(I) and -(III), beryllium(II), uranium(IV), thorium(IV), titanium(IV), zirconium(IV), lanthanum(III), cerium(III) and -(IV), vanadium(V), nickel(II), cobalt(II), manganese(II), zinc(II), calcium(II), strontium(II), barium(II), magnesium(II), boron(III), lithium(I), sodium(I), potassium(I) and caesium(I) was studied between pH 1.0 and 12.5. The PHTTT formed coloured complexes with nickel(II) (green), copper(II) (yellow), cadmium(II) (yellow) and cobalt(II) (yellow - green) only, but the reactions with cadmium and cobalt had sensitivities too low to be applicable to spectrophotometric methods of determination, and the reaction with copper had been studied[1,2] previously.

The spectrophotometric characteristics and chemical conditions for the nickel(II) - PHTTT complex are shown in Table I. A green complex was formed in solution at pH values higher than 3.6 with the optimum pH value lying between 5.5 and 7.5. Complex formation was complete after 2 h at 20 °C, but heating to 70 °C reduced this time to 5 min. The green colour was stable for more than 10 h over the pH range 5.5–7.5. Only cobalt and copper at concentrations higher than 25 μg ml^{-1} interfered under the conditions used.

TABLE I

DATA FOR THE COMPLEX FORMED BETWEEN PHTTT AND VARIOUS METAL IONS

Cation	pH range	Cation - PHTTT stoicheiometry	$\lambda_{max.}$/nm	$\epsilon_{max.}$/l mol^{-1} cm^{-1}	Sandell's sensitivity/μg cm^{-2}	Maximum concentration at which Beer's law is obeyed, p.p.m.
Ni^{2+}	5.5–7.5	1:4	395	8100	0.0072	14
Be^{2+} (BeO_2^{2-})	>10.0	1:2	279	8700	0.0010	8
Ag^+	4.0–12.0	1:2	308	12500	0.0086	15
Bi^{3+}	1.0–4.0	1:4	305	8700	0.0240	12
Tl^+	1.0–12.0	1:2	210	14800	0.0138	12

PHTTT also forms colourless complexes with beryllium(II), silver(I), bismuth(III) and thallium(I), which absorb in ultraviolet region. The characteristics of these metal complexes are given in Table I. For concentrations of silver(I) higher than 10^{-2} M, the silver cation is reduced to elemental silver to produce a yellow colloidal suspension, the colour of which is dependent on the concentrations of silver(I) and PHTTT.

The most important reaction of PHTTT with those metals that form ultraviolet-absorbing complexes is that with thallium(I), because there are few good spectrophotometric reagents available for the determination of thallium. The Rhodamine B method[6,7] is subject to interference from gold(III), iron(III), antimony(V), mercury(II) and gallium, and often a previous separation of thallium by extraction with dithizone is needed. The Brillant Green method[8,9] is subject to interference from cations that form chloro complexes, and for the starch - iodine method oxidation to thallium(III) is necessary. Other less well known methods use xylenol orange,[10] 4-(2-pyridylazo)resorcinol and 4-(2-thiazolylazo)resorcinol,[11] 5-(2-pyridylazo)-5-diethyl-*m*-aminophenol[12] and 1-(2-pyridylazo)-2-naphthol.[13] Each method presents some difficulties.

Thallium(I) and PHTTT react to form a colourless complex at pH 1.0–12.0. The pH range is large and it is possible to choose the working pH to eliminate the interferences. At 20 °C complex formation is complete in 5 min. The ratio of thallium(I) to PHTTT in the complex has been determined by the continuous variation and the molar ratio methods. At 210 nm the absorbance is stable for more than 10 h within the optimum pH range.

With the choice of a suitable pH range, the formation of the complex $Tl(PHTTT)_2$ is very specific, except in the presence of copper and bismuth, between pH 1.0 and 3.0, when the elimination of these interferences is not possible and separation of thallium is necessary. By working at pH values higher than 4.5, the interference of bismuth is eliminated. The optimum concentration range of the method [2–11 μg ml^{-1} of thallium(I)] has been evaluated by Ringbom's method.

The PHTTT - spectrophotometric method has been applied to the determination of thallium(I) in solutions containing other ions, as artificial samples. The results listed in Table II were obtained by the following method. To 10 ml of sample solution, containing 2–11 μg ml^{-1} of thallium(I) and the final concentration of foreign ions given in Table II were added 5 ml of buffer solution (pH 2.27) and 2 ml of 0.1% *m*/*V* PHTTT solution and the solution was diluted to 50 ml with doubly distilled water. The absorbance was measured at 210 nm, against 0.1% *m*/*V* PHTTT solution and the same buffer solution, using 10-mm cells. The average relative error was 2.22%.

TABLE II

DETERMINATION OF VARYING AMOUNTS OF THALLIUM(I) IN A MIXTURE OF OTHER IONS

Composition of mixture, p.p.m.: Ni^{2+}, 10.0; Be^{2+} and Ag^{+}, 5.0; As^{3+}, 2.0; H_3BO_3, 0.25; Se^{4+}, 0.2; and VO_3^-, 0.06.

Thallium, p.p.m.			Thallium, p.p.m.		
Present in mixture	Found	Relative error, %	Present in mixture	Found	Relative error, %
2.0	2.01	+0.5	7.0	6.83	−2.4
3.0	3.12	+4.0	8.0	8.21	+2.6
4.0	3.97	−0.75	9.0	9.19	+2.1
5.0	4.82	−3.6	10.0	9.87	−1.3
6.0	6.17	+2.8			

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Communication

Material for publication as a Communication must be on an urgent matter and be of obvious scientific importance. Rapidity of publication is enhanced if diagrams are omitted, but tables and formulae can be included. Communications should not be simple claims for priority: this facility for rapid publication is intended for brief descriptions of work that has progressed to a stage at which it is likely to be valuable to workers faced with similar problems. A fuller paper may be offered subsequently, if justified by later work.

Manuscripts are not subjected to the usual examination by referees and inclusion of a Communication is at the Editor's discretion.

Flow Injection Sample Introduction for Atomic-absorption Spectrometry: Applications of a Simplified Model for Dispersion

Keywords: *Atomic-absorption spectrometry*; *flow injection*; *sample introduction*; *dispersion model*

In this paper a simple model is proposed to account for the absorbance - time curves obtained when a flow injection analysis (FIA) system is used for introducing samples into a flame atomic-absorption spectrometer. Three different uses of the FI system are described, based on limited dispersion (the analogue of discrete nebulisation), medium dispersion (standard addition method) and high dispersion (production of concentration - time profiles for calibration purposes). It is suggested that the way of looking at a flow injection system described here with particular reference to atomic-absorption spectrometry could be applied to a whole range of other FIA systems and, furthermore, FI sample introduction has more to offer than just reproducible passage of sample to instrument.

It is well known that the dispersion of a sample plug in a continuously flowing carrier stream depends on (1) the carrier tube dimensions (diameter and length), (2) the flow-rate and (3) the volume of the sample plug; also, as the quantitative parameter used is nearly always peak height, considerable efforts have been devoted to obtaining equations relating C_p/C_m, the ratio of the concentration at the peak maximum to that injected, to the above parameters.[1,2] The value of the equations seems to be limited, as most publications in which FIA is used appear to adopt an approach to achieving the appropriate dispersion, residence time, etc., based on the guidelines set out in reference 1.

Basic Model

The dispersion effects due to injection, carrier tubing, etc., and detector are considered to be due to a single well stirred mixing chamber of volume V, *i.e.*, this hypothetical tank is the only part of the system that produces a concentration gradient. If a volume V_i of concentration C_2 is injected into a carrier stream of concentration C_1 flowing with a velocity u then the peak maximum, C_p, occurs after time $t_m = V_i/u$ and the appropriate equations to describe the resulting concentration - time behaviour are

$$C = C_2 - (C_2 - C_1)\exp(-ut/V) \text{ for } t < t_m \quad \ldots \quad (1)$$

$$C_p = C_2 - (C_2 - C_1)\exp(-V_i/V) \text{ for } t = t_m \quad \ldots \quad (2)$$

$$C = C_1 - (C_1 - C_p)\exp[-u(t - t_m)/V] \text{ for } t > t_m \quad \ldots \quad (3)$$

where C is the concentration at time t.

If C_1 is zero and $C_2 = C_m$, *i.e.*, the normal flow injection condition, then equation (2) reduces to

$$C_p = C_m\,[1 - \exp(-V_i/V)] \quad \ldots \quad (4)$$

Dispersion, D, is conveniently quantified[1] as C_m/C_p and thus in this model the dispersion is given by the reciprocal of $1 - \exp(-V_i/V)$. The value of V has to be determined experimentally (as described below) for any particular set-up, but once this has been done, dispersion as a function of volume injected can be easily calculated, as can the time taken to reach the peak maximum and the time for the concentration to fall to a given fraction of the maximum value. With an atomic-absorption spectrometer as detector, it was found best to operate at a flow-rate compatible with maximum sensitivity of the nebuliser response (about 4–5 ml min^{-1}) and to vary the dimensions of the tubing between injector and nebuliser in order to change the value of V.

If standards are injected into the continuously flowing sample stream then this system provides the analogue of the standard addition method. At the "peak" maximum, equation (2) applies, where C_1 is the unknown concentration of the sample and C_2 is the concentration of the standard. The maximum change in concentration, $\Delta C = C_p - C_1$, is thus given by

$$\Delta C = (C_2 - C_1)[1 - \exp(-V_i/V)] \quad \ldots \quad (5)$$

and thus a plot of ΔC *versus* C_2 would intercept the C_2 axis at C_1 (*i.e.*, when $\Delta C = 0$, $C_2 = C_1$). If it is assumed that an interference in the sample acts on the standards in the same way, then a plot of ΔA (maximum change in absorbance) *versus* C_2 will intercept the C_2 axis at C_1. If the further, but not necessary, assumption is made that absorbance is a linear function of concentration, then the plot of ΔA *versus* C_2 would be a straight line.

Experimental and Results

Apparatus

Injection valves. (1) Six-port Rheodyne Type 5020; (2) eight-port Altex Type 201–25. A home-made flap valve[3] was used in initial work but was found to give significantly poorer precision.

Pump. Gilson Minipuls 2 peristaltic pump. Use of the suction of the nebuliser alone, as has been reported recently,[4] was found to be unsatisfactory, but the use of constant-head vessels provide a satisfactory, inexpensive alternative to peristaltic pumps.

Tubing and connectors. Anachem PTFE tubing, 0.58 mm i.d., and couplings.

Atomic-absorption spectrometers. Pye Unicam SP9, SP90A Series 2, Shandon Southern A3300 and Perkin-Elmer 290B.

Determination of Volume, V, of Hypothetical Mixing Chamber

The flow-rate was first determined either by timing the delivery of a fixed volume or by injection of a known volume and measuring, using a chart recorder, the time taken to reach the peak maximum from when the trace first deviated from the base line. This method is not particularly suitable with low values of V as the time involved may be less than 1 s. A step change in concentration from zero is then made so that the steady-state signal, A_m, is reached and the rising part of the curve is analysed according to the absorbance form of equation (1) with $C_1 = 0$, namely

$$A = A_m[1 - \exp(-ut/V)] \quad \ldots \quad (6)$$

This equation can be rearranged to give

$$V = ut/\{\ln[A_m/(A_m - A)]\} \quad \ldots \quad (7)$$

where A is the absorbance at time t. Thus, substitution of pairs of A, t values into equation (7) yields values of V; suitable values of t may be chosen so that $A_m/(A_m - A)$ has convenient values or alternatively a plot of t *versus* $\ln[A_m/(A_m - A)]$ has a slope of V/u.

Limited Dispersion ($1 < D < 3$)

With the injection valve located close to the nebuliser and a flow-rate of 82 μl s^{-1}, a value of 42 μl was found for V with the Shandon Southern instrument. Thus, injection of 100 μl of solution is expected to give a signal 0.908 of the maximum value (*i.e.*, the dispersion is 1.10). The value found for a series of calcium solutions was 1.09 ± 0.03. It is expected that the peak maximum is achieved in 1.22 s and that the signal will have decreased to 1% of the peak value after a further 2.3 s and to 0.1% after 3.5 s.

The use of limited dispersion FI sample introduction provides the same benefits as discrete nebulisation with the additional features that effects due to sample viscosity are reduced and that the nebuliser is continuously washed with carrier stream. A precision of about 1% relative standard deviation was obtained.

Medium Dispersion ($3 < D < 10$)

The interference effects of phosphate and aluminium on calcium are well known and were used to test the applicability of the FI analogue of the standard addition method. With phosphate, which exerts a constant depressive effect above a certain phosphate to calcium ratio, the method works provided that the dispersion is greater than a certain critical value depending on the amount of phosphate present in the sample. The FI system must be designed so that when the standard is dispersed in the flowing sample stream the phosphate to calcium ratio never falls below the value required to give the constant depression. This is illustrated in Fig. 1. The system does

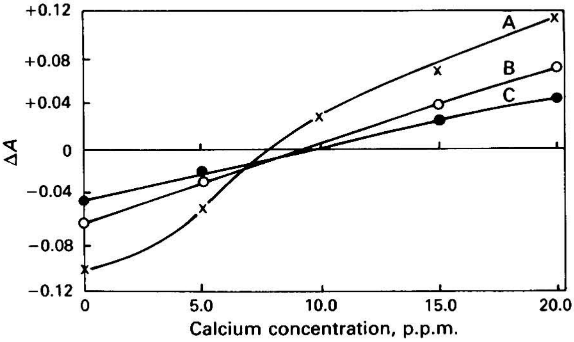

Fig. 1. Effect of dispersion on flow injection standard addition calibration for calcium in the presence of phosphate. Dispersion: A, 1.7; B, 2.6; and C, 3.4. Carrier stream, 10 p.p.m. of calcium plus 500 p.p.m. of phosphate.

not work with aluminium as interferent as these is no constant depression plateau. As the aluminium to calcium ratio is increased the calcium signal eventually drops to zero. The interference was overcome by the addition of lanthanum to the sample stream. As well as acting as a releasing agent, the lanthanum enhances the signal due to its ionisation-suppressant action. This is illustrated in Fig. 2. The results for the analyses of some BCS iron ores are given in Table I. In all instances an air - acetylene flame was used.

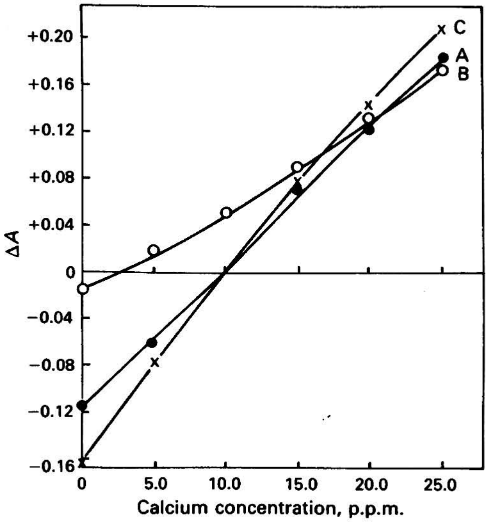

Fig. 2 Flow injection standard addition calibrations for calcium in the presence of aluminium. A, 10 p.p.m. of calcium; B, 10 p.p.m. of calcium plus 20 p.p.m. of aluminium; and C, 10 p.p.m. of calcium plus 20 p.p.m. of aluminium plus 0.25% *m*/*V* of lanthanum. Dispersion 4.5.

The FI standard addition method has the advantages over the conventional method of (a) using the same standard solutions for a number of samples, thus reducing considerably the amount of volumetric manipulation necessary, and (b) being an interpolative method, thus increasing the accuracy over the normal extrapolative method.

TABLE I

DETERMINATION OF CALCIUM IN SOME BCS IRON ORES BY THE FLOW INJECTION STANDARD ADDITION METHOD

Sample	Calcium present, %	Calcium found, %
BCS 303	14.0	14.2*
BCS 378	4.70	4.67
BCS 302/1	2.89	2.67

* Sulphosalicylic acid and strontium were used instead of lanthanum.

In recent work on the determination of chromium in steel, problems have been encountered with burner blockage on prolonged nebulisation of a sample stream containing a high salt concentration. It has been found that the method works in the alternative configuration in which a discrete volume of the sample solution is injected into the standards, used in turn as the carrier stream.

High Dispersion ($D > 10$)

A high dispersion was achieved by incorporating a small (approximately 10 ml) glass mixing chamber into the FI system. Passage of a step concentration change from zero to C_m produces the concentration - time profile given by equation (1) with the appropriate substitutions, namely

$$C = C_m[1 - \exp(-ut/V_m)] \quad \ldots \quad (8)$$

where V_m is the volume of the mixing chamber.

If the corresponding absorbance - time response of the instrument is recorded, this curve may be used as a calibration as the time axis may be replaced by concentration according to equation (8). In practice, the absorbance of the sample is observed at the same flow-rate at which the calibration graph was generated, the time to which this corresponds found by interpolation and finally the concentration by calculation using equation (8) (this can be achieved rapidly with a programmable calculator). Preliminary studies have shown the method to be accurate to about 2%. The method has the advantages over the conventional working curve method of (a) generating a continuous response curve over the desired concentration range, (b) using only one standard solution, (c) allowing rapid re-calibration over a different concentration range and (d) producing a calibration amenable to handling directly by a bench-top computer.

One of us (A.B.I.) gratefully acknowledges financial support from the National University of Malaysia.

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Book Reviews

HAZARDS IN THE CHEMICAL LABORATORY. Third Edition. Edited by L. BRETHERICK. Pp. xxii + 567. The Royal Society of Chemistry. 1981. Price £15. ISBN 0 85186 419 8.

This is the sort of book that grows in value with each edition as the contents are refined, amended or extended in the light of experience gained over the period intervening between editions. For example, the 4 years in the current instance have seen considerable progress in the consolidation of UK legislation, and the stress put on the implications of the Health and Safety at Work Act some 7 years after its enactment reflects the continuing concern and debate on its provisions. I suppose in the circumstances there is a certain inevitability that a full chapter be devoted to the Act and that the book as a whole be very firmly centred around UK regulations and controls, with a nod or two towards EEC regulations (which seem to be permanently imminent). However, this does not detract too much from the over-all value of the book to the international reader, and probably adds to it from the point of view of the UK reader. The role of good management is stressed in the chapter on safety planning, which has been completely recast by the Editor himself and is mercifully free from both jargon and platitudes—well, almost free (I am still not certain precisely what an organigram is). The chapter on fire protection has been rewritten by a new author, and the chapter on reactive chemical hazards written by the Editor continues to be one of the most useful in the book despite (or maybe because of) its brevity.

The chapter on chemical hazards and toxicology has been extended to include some detail of the toxicity and carcinogicity testing required for commercial chemicals together with comments on the predictive value of such testing. The chapter on medical services and first aid now has the more fashionable title of Health Care and First Aid and now deals with mutagens and carcinogens and also provides sources of information on toxicity and medical emergencies both in the UK and in Europe. The chapter on precautions against radiations has also been updated, but the chapter on safety in hospital biochemical laboratories has been dropped on the reasonable grounds that the specific aspect of infectious biological hazards was too important, both technically and legally, to be dealt with adequately in a brief chapter in a volume devoted to chemical and toxic hazards.

However, the most substantial and valuable part of the text remains the famous "yellow pages," which include monographs describing briefly the hazardous properties of over 480 substances or groups of substances, recommending first aid and fire-fighting procedures, and suggesting methods of dealing with spillages. It also incorporates references to dangerous reactions or preparations, and several monographs have been added covering a selection of solvents and research reagents which it is now known show unusually hazardous properties. Information on proven and suspected human carcinogenic substances is included, and to assist in the general recognition of potential carcinogens a brief chemical classification of all these types has been attempted and references to more detailed sources are given.

Over all the book is well edited and I believe that the information contained is so useful that the majority of chemists will want access to it.

G. E. PENKETH

INORGANIC REACTION CHEMISTRY. VOLUME 1. SYSTEMATIC CHEMICAL SEPARATION. By D. T. BURNS, A. TOWNSHEND and A. G. CATCHPOLE. Pp. 248. Ellis Horwood. 1980. Price £18.50. ISBN 0 85312 118 4; 0 470 268956.

Senior chemists still recall the joys of six-radical "mixtures," the basis of qualitative analysis for undergraduates two generations ago. After 1955 advances in instrumental analysis and chromatography and the "renaissance," which often stemmed from inorganic reactions in non-aqueous systems, made the present pedagogic approach inevitable. The educational debates, fierce then, continue as we prepare for the 1980s. Professor Belcher's introductory remark that "We seem to be breeding a generation of chemists who have to go to a reference book to find a reaction that at one time most schoolboys would have known" summarises one view of the matter.

This book is timely from that viewpoint, and encourages those of us who want to keep a sense of "green fingers" in chemical education. It presents revised practical schemes for the systematic separation of inorganic mixtures by chemical means, carefully tested by the Midland Association for Qualitative Analysis (MAQA), with the emphasis on teaching.

Chapters One and Two, after a brief historical introduction, are concerned with the theory based on the thermodynamic treatment of ionic equilibria and kinetic effects, such as coprecipitation, at about the first-year undergraduate level. Chapter Three records MAQA's reasons for incorporating different improvements in their schemes. Thus, "calcium group" cations are precipitated early as insoluble sulphates immediately after the "silver group," in order to avoid coprecipitation, and a reagent of purified hydrogen sulphide in acetone improves the "copper - tin group" separation. There is elegance also in the division into elements within the groups; thus, EDTA makes the separation of calcium, strontium and barium more quantitative and alkaline chloroacetate gives smooth dissolution of the "tin group" precipitates. The removal of anions that interfere, such as phosphate with zirconium prior to the "iron and zinc group" separations, and a more systematised separation for the anions are improvements also. The practical techniques, explained in detail, involve manipulation of 15–30-mg amounts in 3–5 ml of solution based on centrifugation using the semi-micro methods of 1945–55. Chapters Four and Five are set out as a laboratory handbook, with the procedures and tests tabulated, and include the preparation of reagents. There are sections on treatments of insolubles and mixtures containing less common elements. The final chapter on the ring-oven technique probably marks the extent to which this kind of qualitative analysis was applied. Here, and at the end of the other chapters, there are short lists of references for further reading.

In its own way this is a highly recommendable book, and together with the second volume, entitled "Reactions of the Elements and Their Compounds," will help lecturers in Universities and Polytechnics to devise courses that demonstrate the chemical processes that depend on reactions in aqueous media. Perhaps they would need to consider more chromatographic separations and increase the emphasis on solvent extraction techniques. More imponderable is the value of them returning to this type of course in the 1980s. On the one hand, when new graduates find employment they should be able to automate chemical tests for "pattern recognition" of the cations and anions in mixtures, including setting-up a microcomputer programmed for this purpose, and on the other hand they should also be able to develop and prove better tests such as these—can they be expected to do both? J. A. W. DALZIEL

PROGRESS IN DRUG METABOLISM. Volume 4. Edited by J. W. BRIDGES and L. F. CHASSEAUD. Pp. xii + 335. John Wiley. 1980. Price £25. ISBN 0 471 27702 9.

This volume is probably the best of the four so far produced. The authors include experts from the pharmaceutical industry, academic pharmacy and consultant research who present well written and up-to-date reviews on drug absorption and drug measurement. There are five chapters; the first three deal with pharmacokinetics in drug development, gastrointestinal absorption and drug bioavailability and the last two are concerned with the analysis of drugs in biological fluids by gas chromatography (GC) and high-performance liquid chromatography (HPLC).

The chapter on pharmacokinetics is not a treatise or an introduction to the mathematical side, but a description of the important factors affecting bioavailability and how they may be described by pharmacokinetic parameters. This information is then put into context of the development of a new drug. It is not for the novice wishing to learn about pharmacokinetics, but the large number of tables, figures and references make it a good explanatory source for those wishing to know how the study of pharmacokinetics can be used in the pharmaceutical industry.

The second chapter on the gastrointestinal absorption of drugs and other xenobiotics reviews the literature from 1970. It is a pity that the review concentrates on absorption in the rat rather than in humans. However, it has the advantage that studies on the mechanisms of absorption and methods for obtaining quantitative data are dealt with in some detail because of this. The chapter is complementary to the first in that why and how drugs are absorbed are described rather than what you do with the information. An interesting part of the chapter is a discussion of the absorption of ionised drug molecules.

Drug bioavailability and its clinical significance is the third of the three chapters on absorption. It is written in a clear, easy style and concentrates on orally administered drugs intended for systemic activity. Because it describes oral dosage forms and the factors affecting absorption from the standpoint of bioavailability, it forms a good companion to the first two chapters.

Between them they cover a large amount of ground with the citing of a large number of modern references. At the end, the reader is left wanting more, with the relationship between pharmacokinetics and pharmacodynamics in clinical practice being left almost untouched. Perhaps the Editors will include this in one of their future volumes.

The review on GC covers a large amount of modern literature with references up to December 1978 being included. After an introduction concerning the manipulation of biological samples prior to GC a lengthy discussion on derivatisation techniques leads to a description of the gas chromatograph. Some of the description of the equipment is too general for this type of review and not enough space is devoted to the choice of columns, although the use of general and specific detectors is covered at some length. An extremely useful table describing GC analyses of 294 individual drugs or groups of drugs (*e.g.*, barbiturates and anticonvulsants) from a total of 631 references is given. Details such as the biological fluid analysed, internal standard, derivative formed, stationary phase used and detection system are all included.

The last chapter on HPLC is divided in two parts, *viz.*, general principles and specific analyses. The first contains many references from 1977–78, contains a useful section on sample preparation and provides an introduction to the use of the technique. Details of specific analyses are given in tabular form for 150 drugs or groups of drugs arranged in pharmacological groups. Information concerning choice of column, detectors, sensitivity and specificity is given in the table. In addition, a description of the best way to analyse groups of drugs in relation to their functional chemical groups is given in the text to complement the data in the table. Both this review and that on GC analyses are timely and should prove useful to analysts who have need to analyse biological fluids for drugs.

This is a valuable book, well worth the price, and the Editors are to be congratulated on putting together such a worthwhile collection of reviews.

A. C. MOFFAT

SIZE EXCLUSION CHROMATOGRAPHY (GPC). Edited by THEODORE PROVDER. *ACS Symposium Series*, 138. Pp. viii + 312. American Chemical Society. 1980. Price $30.75 (available in the UK and Eire from the RSC, price £16). ISBN 0 8412 0586 8.

This volume is based on a symposium held in September, 1979, and has 17 contributions.

Size exclusion chromatography has been called variously gel permeation chromatography, gel filtration chromatography and molecular sieve chromatography, among others, and the book is concerned mainly with the fractionation and subsequent determination of macromolecules or their relative molecular mass distribution. The use of the technique as a means of determining the particle sizes of polymeric latices is included, as also is the determination of polymer intrinsic viscosity values. Other contributions include the characterisation of branched polymers, determination of the purity of hydrophilic polymeric dyes containing hydrophobic impurities, polymerisation and dilute solution characterisation of poly(dichlorophosphazene) and the characterisation of oligomers used in coatings. Detector systems mentioned include light-scattering (including low-angle laser), ultraviolet, differential refractometric and mass detectors.

Abbreviations are used throughout and it is necessary to be aware of their meaning: for example, GPC is used for size exclusion chromatography and HPGPC is self-explanatory from that (SEC is also used as an abbreviation for size exclusion chromatography.); SEC/LALLS is employed, the latter part referring to low-angle laser light scattering; HDC is used for hydrodynamic chromatography, etc.

Many of the chapters contain a considerable amount of theoretical matter, which is inescapable in a book devoted to such a technique, but more practical detail would have been an advantage on occasion. The words "latices" and "lattices" to refer to latex products were observed, but this may have been merely an original error (of which there are several), the papers having been reproduced by a photographic method.

This book is an account of papers given at a specialised symposium and will doubtless appeal to specialists in the field or to libraries of plastics companies or polymer chemistry/physics departments of colleges and universities.

D. SIMPSON

METAL CONTAMINATION OF FOOD. By CONOR REILLY. Pp. xvi + 235. Applied Science. 1980. Price £17. ISBN 0 85334 905 3.

The importance of metallic elements in human nutrition and health has been recognised for many years. Although the title suggests that only contaminant metals are reviewed, in fact this book gives a comprehensive account of both beneficial and toxic elements, metallic and metalloidal, which are found in foods. Part I consists of a survey of the role of metals in the environment and the various pathways by which food becomes contaminated as a result of agricultural and industrial processes, including packaging. Methods of control are covered in a general way in a chapter on international legislation. Outline methods for the analysis of foods for trace elements are included. Particular attention is given to sampling, destruction of organic matter and end-determination techniques. The discussion concentrates on the principles of alternative procedures rather than on recipes, which are included in the comprehensive bibliography. Instrumental techniques are emphasised and their strengths and weaknesses discussed. Part II discusses individual elements in more detail. There are separate chapters on lead, mercury, cadmium, arsenic, antimony, selenium, aluminium, tin, the transition metals, zinc and some other elements in lesser detail. Each discussion covers sources, production and uses, biological effects in the human system and levels found in foods. Again, analytical methods are described only in general terms.

This book is essentially a much needed update of the classical work of G. W. Monier-Williams, "Trace Elements in Food," Chapman and Hall, London, first published in 1949. For the analyst it gives a well balanced introduction to a complex and difficult subject. It is therefore a companion and guide to what is now a vast literature. As a consequence, it is perhaps less useful to the practising and more experienced specialist analyst but it does provide a valuable background from which new data can be evaluated. The work is supported by extensive references to the scientific literature collected together by chapter at the end of the book. The volume is well produced with very few errors. Part I contains informative tables but surprisingly no diagrams or figures. Part II contains only two tables, but the text is broken up by suitable sub-headings. The index is comprehensive but some headings, such as Africa, Additives, Elements, Toxic metals and Valence Electrons are, perhaps, of limited usefulness. As this is the first major review of an important subject in 30 years, most libraries and chemists will be able to justify its purchase.

N. T. CROSBY

RADIOISOTOPE LABORATORY TECHNIQUES. Fourth Edition. By R. A. FAIRES and G. G. J. BOSWELL. Pp. xvi + 335. Butterworths. 1981. Price £15. ISBN 0 408 70940 5.

Most practising radiochemists will be familiar with earlier editions of this useful book, which have been translated into German, Japanese, Polish and Spanish. As one of the earlier authors died in 1974, his place has been taken by Dr. Boswell. Extensive revisions have been made, notably in the chapters on counting devices and techniques, and both units and references have been updated to 1979. The text reads well, and the extensive practical experience of the authors is displayed in many places.

The content of the book can be summarised briefly as 42 pages on Introductory Nuclear Physics, 61 pages on Health Physics, 154 pages on Counting Techniques and 30 pages on Applications of Radioisotopes. The chapters give practical guidance on handling, using and counting radioactive materials in ordinary laboratories, with emphasis on economy and feasibility. The applications have only been outlined in the space available. Among the revised sections, those on autoradiography, laboratory neutron sources, quenching corrections in liquid scintillation counting and gamma spectrometry with Ge - Li detectors are particularly useful and informative, but the discussion of Mössbauer spectrometry is not. The list of suppliers of equipment given in one of the appendices is a novel and valuable feature.

The photographs are of dubious value. On p. 60 we learn that "moveable cylinder stands are not good," but Plate 1A shows one prominently exposed in a Class A laboratory. Specific activity and molar activity are confused on pp. 255–256, cross-section is poorly defined on p. 33 and there are a number of printing errors. However, these are minor blemishes in a book which should be consulted by all those in charge of radiochemical work, and for which no alternative exists.

H. J. M. BOWEN

ANALYTICAL TECHNIQUES IN ENVIRONMENTAL CHEMISTRY. PROCEEDINGS OF THE INTERNATIONAL CONGRESS, BARCELONA, SPAIN, NOVEMBER 1978. Edited by J. ALBAIGES. *Pergamon Series on Environmental Science, Volume 3.* Pp. xii + 646. Pergamon Press. 1980. Price $85. ISBN 0 08 023809 2.

The book is a collection of about 60 papers covering almost all aspects of environmental analytical chemistry. To make detailed comments upon the papers would be most inappropriate because presumably their contents would have been vetted by the Organising Committee.

The first 40 or so papers are concerned with organic compound recognition and determinations covering the normal range of pollutants including PNAs, petroleum hydrocarbons, halocarbons (PCBs and alkyl types), among others. Frankly it would be intriguing to consider where the subject of organic trace analysis would be without the techniques of gas chromatography, particularly with high-efficiency capillary columns, and mass spectrometry. The interfacing of the two techniques brings symbiotic benefits.

All of the recognition techniques are instrumental ones using the conventional sampling approaches, that is, taking discrete samples and bringing them back to the laboratory for examination. An intriguing alternative is the "mussel watch" where bivalve organisms, *e.g.*, oysters or mussels, are used as sentinel organisms to monitor heavy metals, petroleum hydrocarbons and synthetic organic compounds as well as artificial radionuclides at over 100 sites in US coastal waters. Perhaps that is how a great deal of monitoring work, judging long-term exposures, will develop.

The papers describing the inorganic methods make up the final third of the book and are concerned with radionuclides; the "black-list" elements, mercury, arsenic and selenium receive due attention. Neutron-activation analysis, PIXE, ion exchange, ion-selective electrodes, atomic-absorption spectrometry, electron microprobe X-ray spectrometry, in fact all the panoply of surface analysis, as well as novel electrochemical techniques, are employed to elicit data on inorganic pollutants in natural substances.

This is an impressive book, with a continuity missing from similar reports on other environmental conferences. However, in view of the price and the relatively specialised nature of the subject, it is a book for the specialist laboratory or reference library. G. NICKLESS

IMMOBILIZED ENZYMES IN ANALYTICAL AND CLINICAL CHEMISTRY. FUNDAMENTALS AND APPLICATIONS. By PETER W. CARR and LARRY D. BOWERS. *Chemical Analysis. A Series of Monographs on Analytical Chemistry and its Applications, Volume* 56. Pp. x + 460. John Wiley. 1980. Price £24. ISBN 0 471 04919 0.

This book is intended as a guide and introduction to the use of immobilised enzymes for analytical purposes. It does not attempt to cover the use of immobilised enzymes as models of membrane-bound enzymes, for pharmaceutical use or in manufacturing processes. It is always difficult for authors of books that straddle conventional disciplinary boundaries to decide how much introductory material should be included. On the one hand, one is beckoned by the Scylla of scantiness in order to keep the price competitive. On the other, the Charybdis of comprehensiveness tempts the author to write *the* definitive text regardless of price. In my view, the authors of this book have sailed a hesitant passage between the two. The first two chapters on basic enzymology and enzyme kinetics are so elementary that any reader who needed instruction at this level would almost certainly sink without trace in the deeper and more exciting waters that follow. In economic terms, one eighth of the book and hence of the price could have been saved.

Chapter 3 provides a fundamental examination of the principles of equilibrium and kinetic methods of analysis of enzyme-catalysed processes. The theoretical aspects of methodological precision are nicely related to various practical techniques and their limitations. The following chapter describes the various methods available for immobilising enzymes on various supports. There are plenty of references to the original literature for those requiring experimental detail. Examples of the properties of some enzymes immobilised on different supports are given. Chapter 5 is devoted to the theory and application of enzyme electrodes. Important practical considerations such as sensitivity, response time and stability of enzyme electrodes are clearly discussed. Chapters

6 and 7 deal with chemical kinetics in flow systems and the problems of mass transfer and diffusional processes in heterogeneous systems involving immobilised enzymes. Although the reader is gently guided through the requisite calculus and algebra, avoidance of clumsy abbreviations such as CSTR (continuously stirred tank reactor), PFR (plug flow reactor), HETP (height equivalent to a theoretical plate) and CFA (continuous-flow analysis) could have made the passage easier. The final chapter brings together the foregoing concepts and there are useful tables of applications with references.

Overall, this is a useful book for the analytical chemist and biochemist, especially for those who have to teach the subject at postgraduate level. There are places in the text where the style is irritatingly obtuse. Apart from the abbreviations mentioned above, the reader is sometimes assailed by a volley of adjectival nouns. Thus, we are told (p. 89), "In Pardue's classification scheme, this amounts to a two-point, fixed-time variable sensor signal kinetic assay." There must be a better way of writing that!

D. T. ELMORE

CHROMATOGRAPHIC METHODS IN INORGANIC ANALYSIS. By G. SCHWEDT. Pp. viii + 226. Dr. Alfred Hüthig Verlag. 1981. Price DM75; $38. ISBN 3 7785 0690 0.

While the central place of chromatographic separation in organic analysis is self-evident, the over-all emphasis in inorganic analysis has often been on elemental determinations rather than molecular separation and characterisation. Inorganic separation problems have therefore attracted less over-all attention but with the rapidly developing emphasis on speciation of metal and metalloid compounds, the call for a review text such as that of Schwedt has become very strong.

This book is one of the initial volumes in a series *Chromatographic Methods* in which the publisher is clearly addressing the need for up-to-date material and practical applications to aid the working analytical chemist. Other volumes have ranged in concept from collections of journal article reprints on selected topics, through symposium collections to theoretical treatments of good pedagogic value; however, this volume is purely a review text and indeed follows journal critical review articles in style. Such is the range of chromatographic methods available for inorganic samples that this review fulfils a very important need and although necessarily requiring future updating to maintain current coverage, it offers an excellent present-day overview.

It is refreshing to see a review text which, while seeking to be comprehensive in both techniques and applications (as shown by the listing of 814 references, mostly from 1970 onwards), is nevertheless organised with exceptional clarity for rapid access of information. The author has wisely emphasised that in inorganic chromatography the detection mode chosen is often of equal importance to the separation procedure and element-selective detection holds a major place in developing methodology. His organisation thus follows the form: (1) Separation Methods and Systems, (a) Liquid Chromatography and (b) Gas Chromatography; (2) Detection Methods, (a) Layer Chromatography, (b) Column Chromatography and (c) Gas Chromatography; (3) Applications, (a) Inorganic Materials, (b) Organic, Biological and Environmental Materials, (c) Chromatographic Enrichment, (d) Chromatographic Analysis of Element Species and (e) Comparison with Alternative Methods.

The greatest strength of this work is the exhaustive and sophisticated use of tables (62) to gather great amounts of data together. The researcher can readily refer to many different aspects of an analytical problem and find access to relevant materials. Reference to inorganic and other specific reviews is very well organised and examples (with figures—not always well reproduced, unfortunately) and a sufficient amount of editorial comment, help to make a useful introduction to the primary literature.

The indexing and cross-referencing is very well accomplished and apparently error free. An over-all element documentation index is included, as is a keyword method index. References are made through the 1979 literature and include full article titles.

Review articles and primary monographs frequently gather dust on office shelves as the "real world" of analytical chemistry proceeds on its way. That is not the fate of Schwedt's text. It is pre-eminently a working handbook, to be referenced in the laboratory over and over again. It will save both the researcher and the practising analyst a great amount of time and will become an essential desk volume for anyone involved in the expanding field of inorganic chromatography.

P. C. UDEN

NICKEL TOXICOLOGY. PROCEEDINGS OF THE SECOND INTERNATIONAL CONFERENCE ON NICKEL TOXICOLOGY, 3–5 SEPTEMBER 1980, SWANSEA, WALES. Edited by STANLEY S. BROWN and F. WILLIAM SUNDERMAN, JR. Pp. xx + 193. Academic Press. 1980. Price £15; $36. ISBN 0 12 137680 X.

The three main sections of this book are (1) Studies in Carcinogenesis, both epidemiological (6 papers) and experimental (7 papers); (2) Actions *in Vivo* and *in Vitro*, with 12 papers on the uptake, distribution and excretion of nickel and 10 papers on pharmacological studies, and (3) Analytical Aspects, with only 8 papers.

The analytical studies form only a minor part of this book and contain little new information on the analysis of blood and urine. There is a report of an inter-laboratory study of serum and urine analysis for nickel by a provisional IUPAC reference method, which showed an improvement over previous studies. Stoeppler and Bagschik extended their earlier investigations using ^{63}Ni to study solvent extraction and electrothermal atomisation for atomic absorption and showed some unusual matrix effects. Other analytical techniques discussed were polarography and energy-dispersive X-ray emission spectroscopy. This latter technique was used to show high concentrations, up to 116 p.p.m. of nickel, in particulate material from lung tissues of exposed workers, and in a separate study, the presence of high levels of nickel in chromosomes of fresh water and marine species cultured in the laboratory.

Some interesting analytical data are presented in other sections of the book. Cronin *et al.* used syringes fitted with platinum capillaries and gold-plated Luer hubs to collect blood samples without contamination with nickel. In this study, volunteer women were given up to 2.5 mg of nickel orally as nickel sulphate to test for skin sensitivity. No toxicity was observed but blood levels increased from less than 4 to 24 μg l^{-1}. The interesting observation that about 63% of blood nickel may be in white blood cells (Barton *et al.*) requires further investigation.

The use of computer models to simulate chelation therapy to remove nickel(II) indicated synergism between NaDDC and DTPA to be a promising line of investigation (Jones *et al.*) and partial support for this was provided by Reiss *et al.*, who found that the latter reagent removed 94% of the nickel bound to albumin.

The main theme of this book is the carcinogenicity of nickel and its compounds. Morbidity and mortality studies showed an increased incidence of sinonasal and lung cancers among workers employed in nickel smelters prior to 1950–60. Studies on those employed after 1960 do not show clearly such effects and there is evidence that nickel exposure has been significantly reduced.

There are many studies on the carcinogenicity of Ni_3S_2 administered to animals. Although it was shown that phagocytosis was a primary stage in this process (Costa and Mollenhower), it was also reported that, *in vitro*, this compound as well as particulate nickel and nickel matter were very soluble in whole blood (Andersen *et al.*).

Those concerned with industrial hygiene control and with nickel toxicology will find this book of immense value.

H. T. DELVES

Erratum

JULY (1981) ISSUE, p. 784, Table I and p. 785, Table II: *for* Standard deviation†, % *read* Standard deviation†/ mg kg^{-1}.

Determination of Riboflavin and Flavin Mononucleotide in Foodstuffs Using High-performance Liquid Chromatography and a Column-enrichment Technique

A high-performance liquid chromatographic method has been developed for the determination of riboflavin and flavin mononucleotide (FMN) using a C_{22} reversed-phase column packing material. Mean recoveries of 100% (±5%) for riboflavin and 102% (±2%) for FMN were obtained. Multiple determinations of the vitamin in two samples indicated a precision of ±5%. The results agree well with those obtained by microbiological assay. Riboflavin and FMN are eluted within 9 min, and the total time for the analysis of most samples is 3.5 h. The lifetime of the reversed-phase column is far greater than that of silica columns. After 500 sample loadings no deterioration of column efficiency occurred. The method is suitable for the determination of riboflavin down to the 0.01 mg per 100 g level. For samples with a lower vitamin content trace enrichment can be used, which involves only a few minutes' extra analysis time.

Keywords: *Riboflavin determination*; *flavin mononucleotide determination*; *high-performance liquid chromatography*; *microbiological assay*; *trace enrichment*

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Analyst, 1981, **106**, 1103–1108.

Automatic Titration by Stepwise Addition of Equal Volumes of Titrant. Part VII. Potentiometric Precipitation Titrations

The method used throughout this series to add the titrant stepwise with equal volumes each time is well suited to precipitation titrations. The advantage that one has to wait for equilibrium to be reached at only a few points is of special importance.

Two methods for calculation of the equivalence volume in precipitation titrations have been evaluated. One is based on the solution of a set of linear equations and the other is an extended version of the Gran I method. The methods are characterised by the following facts: (1) side-reactions can be accounted for; (2) the values of the solubility product and the stability constants of complexes may be unknown; (3) the solutions may be so dilute that a complete precipitation is not obtained; and (4) no accurate calibration of the electrode couple is required.

The calculation methods have been tested on the determination of chloride by titration with silver nitrate solution. The two methods agree closely and the errors in the calculations are negligible. The over-all errors in the determinations are 0.1–0.2% at chloride concentrations down to 10^{-4} M. At lower concentrations the relative errors are greater.

The methods have also been tested on the determination of sodium fluoride with lanthanum nitrate in (*a*) neutral and unbuffered solution, (*b*) acidic solution, (*c*) solution buffered with acetic acid - acetate and (*d*) solution buffered with formic acid - formate. The calculations were performed on experimental curves according to Lingane. For (*a*) and (*b*) good agreement between calculated and expected values were obtained. In the acetate-buffered solution too high a value was obtained in spite of the fact that acetate and mixed acetate - fluoride complexes were considered. For (*d*) the buffer capacity was too small to give satisfactory results.

Keywords: *Gran I method*; *potentiometric precipitation titrations*; *sodium fluoride determination*; *chloride determination*

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Analyst, 1981, **106**, 1109–1118.

High-performance Liquid Chromatographic Determination of Chlorpromazine and Thioridazine Hydrochlorides in Pharmaceutical Formulations

Short Paper

Keywords: *Chlorpromazine hydrochloride determination*; *thioridazine hydrochloride determination*; *pharmaceutical formulations*; *high-performance liquid chromatography*

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Analyst, 1981, **106**, 1119–1122.

Study of 3-Propyl-5-hydroxy-5-D-arabinotetrahydroxybutyl-3-thiazolidine-2-thione as a Reagent for the Spectrophotometric Determination of Thallium(I)

Short Paper

Keywords: 3-*Propyl*-5-*hydroxy*-5-D-*arabinotetrahydroxybutyl*-3-*thiazolidine*-2-*thione reagent*; *spectrophotometry*; *thallium*(I) *determination*

J. AZNÁREZ, J. R. CASTILLO and C. LUESMA

Department of Analytical Chemistry, Science Faculty, University of Zaragoza, Zaragoza, Spain.

Analyst, 1981, **106**, 1122–1125.

Flow Injection Sample Introduction for Atomic-absorption Spectrometry: Applications of a Simplified Model for Dispersion

Communication

Keywords: *Atomic-absorption spectrometry*; *flow injection*; *sample introduction*; *dispersion model*

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Analyst, 1981, **106**, 1125–1129.

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