The ANALYST

The Journal of the Society for Analytical Chemistry

A monthly International Publication
dealing with all branches of
Analytical Chemistry

Published by the
SOCIETY FOR ANALYTICAL CHEMISTRY

Volume 96
No. 1147, Pages 681-752 **October, 1971**

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

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Published by The Society for Analytical Chemistry
Distributed by The Chemical Society, Publications Sales Office,
Blackhorse Road, Letchworth, Herts., SG6 1HN

Volume 96, No. 1147

October, 1971

THE ANALYST

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Liquid Scintillation Counting as an Analytical Tool
A Review

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J. A. B. GIBSON and A. E. LALLY
Health Physics and Medical Division, A.E.R.E., Harwell, Didcot, Berkshire.

Analyst, 1971, **96**, 681–688.

A Method for the Determination of Disodium Cromoglycate and Other Chromones

The hydrolysis of disodium cromoglycate in alkaline solution has been studied and a method for its determination is presented. The method is based on spectrophotometric measurement of the change in absorption at 310 nm, which takes place on opening the γ-pyrone ring of the chromone nucleus. The technique is applicable, with suitable minor modifications, to the determination of other compounds containing the chromone nucleus, such as flavones.

J. TILLMAN and D. W. WHYMARK
Fisons Limited, Pharmaceutical Division, Research and Development Laboratories, Bakewell Road, Loughborough, Leics.

Analyst, 1971, **96**, 689–698.

The Flame-photometric Determination of Alkalis in Ceramic Materials

The flame-photometric method in general use for the determination of alkalis in the ceramic industries was originally devised for the EEL, Model 100, flame photometer, with a coal gas flame, which is not now generally available. Current supplies of town gas, propane and methane (natural gas) flames are compared and interferences evaluated. Perchloric and hydrochloric acids are found to have a depressant effect; sodium is enhanced by potassium and is subject to spectral interference by calcium.

Propane is chosen as the preferred fuel. The effect of chlorine-containing acids is eliminated by the use of sulphuric and nitric acids for the initial decomposition and the spectral interference from calcium by the addition of aluminium sulphate; sodium - potassium inter-element effects are eliminated by the use of a caesium buffer. Although the procedure is principally devised to give optimum results with aluminosilicates, its extension to high-lime materials is also considered.

R. P. EARDLEY and R. A. REED
British Ceramic Research Association, Queens Road, Penkhull, Stoke-on-Trent, ST4 7LQ.

Analyst, 1971, **96**, 699–711.

A Potentiometric Procedure for the Assay of Isonicotinic Acid Hydrazide (Isoniazid)

A potentiometric procedure for the assay of isonicotinic acid hydrazide (isoniazid) with vanadium(V) at room temperature is described. The reduction of vanadium(V) to vanadium(IV) by isoniazid in an acidic medium is catalysed by osmium tetroxide, and the application of this method to the assay of isoniazid in pharmaceutical preparations is considered. Oxalic acid interferes in the determination, although commonly used excipients such as starch, dextrin, sucrose, glucose, lactose and gum acacia do not interfere.

P. V. KRISHNA RAO and G. BALA BHASKARA RAO
Chemistry Department, Andhra University, Waltair, India.

Analyst, 1971, **96**, 712–715.

The Determination of Nikethamide and Other Compounds in Pharmaceutical Dosage Forms by Thin-layer Chromatography

The qualitative examination and quantitative assay of pharmaceutical dosage forms containing nikethamide by thin-layer chromatography is described. When applicable, the determination of accompanying active compounds such as adenosine, caffeine, strychnine and theophylline by the same method is also described. Individual quantitative determinations of the eluted drugs are performed by ultraviolet spectrophotometry.

W. M. CARMICHAEL
Analytical Division, Ciba-Geigy Limited, 4000 Basel 2, Switzerland.

Analyst, 1971, **96**, 716–720.

The Gas-chromatographic Determination of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in 2,4,5-Trichlorophenoxyacetic Acid ("2,4,5-T"), 2,4,5-T Ethylhexyl Ester, Formulations of 2,4,5-T Esters and 2,4,5-Trichlorophenol

A gas-chromatographic method for the determination of trace amounts of a toxic impurity, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, is described. A purified extract of the sample was subjected to gas chromatography on a column containing either 2 per cent. of OV-17 on Diatomite CQ or 1 per cent. of Hi-Eff 8 BP on Gas-Chrom Z with electron-capture detection. 2,4,5-Trichlorophenoxyacetic acid and 2,4,5-trichlorophenol were purified by chromatography of an ether extract of the sample on a column of alumina, followed by shaking with sulphuric acid. For 2,4,5-trichlorophenoxyacetic acid esters and formulations, saponification and chromatography on a Celite - sulphuric acid column, followed by chromatography on a column of alumina, were necessary.

Recoveries of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin ranged from 89 to 98 per cent., and the standard deviation of the method at a level of 0·3 p.p.m. was 0·03 p.p.m. The limit of detection was about 0·05 p.p.m.

D. A. ELVIDGE
Quality Control, Analytical Research, Boots Pure Drug Co. Ltd., Pennyfoot Street, Nottingham.

Analyst, 1971, **96**, 721–727.

Determination of Total Hydrolysable Nitrogen in Acidic Aqueous Solutions of Nitriles Containing Cyanide

A method is described for the determination of total hydrolysable nitrogen in acidic aqueous solutions containing acrylonitrile, acetonitrile, hydrogen cyanide and ammonia. A recovery of about 97 per cent. is obtained for all the components examined. The method is based on the classical Radziszewski reaction, and involves reaction with 30 per cent. w/v hydrogen peroxide followed by alkaline hydrolysis to ammonia. The important feature of the method is that it enables hydrogen cyanide to be determined together with the other components.

D. C. WHITE
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Analyst, 1971, **96**, 728–733.

OCTOBER, 1971 Vol. 96, No. 1147

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Liquid Scintillation Counting as an Analytical Tool

A Review*

By J. A. B. GIBSON and A. E. LALLY
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Introduction

Liquid scintillation counting is commonly used for counting a wide range of β- and α-emitting radioisotopes in many chemical forms. The technique provides for a good detection efficiency (up to about 100 per cent.), and normally involves a minimum of chemical preparation. The relatively high backgrounds obtained in this method compared with other detection systems, *e.g.*, proportional counters, limit the sensitivity for some isotopes, particularly α-emitters. A second, more serious, limitation is the variable efficiency caused by a reduction in the light output (quenching) in the presence of certain chemical impurities. Other impurities may introduce chemiluminescence, which gives an unknown and variable background.

These effects are most important for low-energy β-emitters such as ^{3}H and ^{14}C. Reduction of quenching and chemiluminescence have been widely discussed throughout the literature, and many methods of measuring the efficiency have been devised.

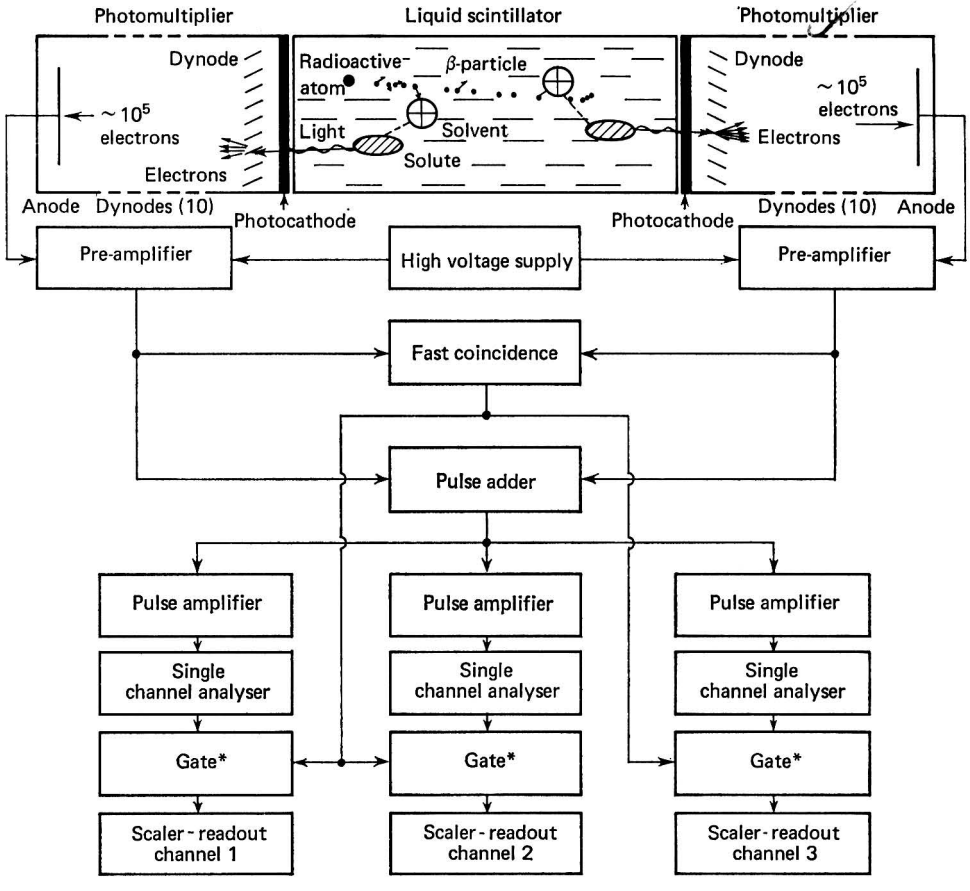
There already exist several reviews dating from the proceedings of a symposium in 1958, edited by Bell and Hayes,[1] and culminating in a second symposium (edited by Branksome[2]) in 1970. The proceedings of the latter will provide much up-to-date information for the experienced user of the technique. This more limited review cannot compete with the depth and coverage of such a symposium. We therefore aim to provide a critical introduction to the methods and materials used in liquid scintillation counting.

The review is divided into four general sections, the basic concepts of the technique, instrumentation, the choice of scintillators and additives, and the techniques used in sample preparation and standardisation and analysis of the subsequent data. The information is finally summarised as a table in the Appendix, which gives an indication of the range of isotopes that can be counted with a liquid scintillation counter. The techniques that will not be discussed include the use of liquid scintillators for neutron measurements and as anti-coincidence shields. These and other techniques are discussed in detail by Birks.[3]

Basic concepts

The basic organic liquid scintillator consists of a solution of one or more fluorescent aromatic solutes dissolved in an aromatic solvent (usually toluene or dioxan). Other compounds (additives) can be added to this basic solution so as to incorporate the various radioactive samples into the scintillator.

* Reprints of this paper will be available shortly. For details see summaries in advertisement pages.



* Gates only open when pulses are observed from both photomultiplier tubes.

Fig. 1. Schematic diagram of the scintillation process and electronics

Alpha and beta radiations from radioisotopes in the scintillator deposit energy in the solvent. This energy is transferred through the solvent until it reaches a solute molecule which converts the energy to a light quantum (Fig. 1). Light quanta from several solute molecules are detected by the photomultiplier tubes (usually two), which convert about 25 per cent. of the light quanta into electrons. The over-all quantum efficiency from the original particle to the production of electrons is about 0·2 per cent., and the deposition of 5 keV of electron energy (approximate mean energy for ^3H β-particles) results in only one or two electrons being produced in the photocathode of the photomultiplier tube. Because of the statistical nature of the process,[4] some events will not be observed at all, and therefore the maximum theoretical counting efficiency is less than 100 per cent.[5] (typically 60 per cent. for ^3H with two tubes in coincidence). The electrons are accelerated through a series of dynode stages that produce between two and six electrons for each electron input. This multiplication may amount to a 10^5-fold increase over eleven stages.

Spontaneous emission from the photocathode results in a background noise output from the photomultiplier tube. By using two photomultipliers in coincidence, the single-noise event in one tube only can be rejected, but excessive noise will result in accidental coincidences that can be reduced by cooling to below ambient temperature. The external background of gamma and cosmic radiation will produce events in the scintillator that can be reduced

by using an anti-coincidence shield. Added materials may be chemiluminescent and produce single photons, but this effect is largely eliminated by using the coincidence system.

Chemical impurities may interfere with the transfer of energy from solvent to solute to produce "chemical quenching," or they may absorb the light emitted from the solute molecules to produce "colour quenching." Both effects will reduce the light output of the scintillator[6] and thus reduce the counting efficiency for the sample. The two types of quenching have different effects on the output spectrum of the counter, and chemiluminescence will give yet a third change in the spectrum. It is these spectrum changes and shifts that form the basis of most techniques for the determination of counting efficiency and the detection of chemiluminescence. These techniques are discussed later.

Chemical quenching can be avoided with high-energy β-emitters ($\beta_{max.} > 0{\cdot}15$ MeV) by the use of Cerenkov counting. The sample is counted directly without a scintillator and the light output is produced when the speed of the β-particles exceeds the speed of light in the medium.[7] This technique, although simpler, is still subject to colour quenching and chemiluminescence.

Instrumentation

A large number of complete instrument systems are commercially available for use with liquid scintillators. They vary in complexity from single-sample instruments to systems handling hundreds of samples with an output of efficiencies, corrections and the disintegration rates for up to three isotopes. The choice depends upon the application and the variety of isotopes encountered in a particular laboratory.

The basic components of a typical coincidence system are shown in Fig. 1. The photomultiplier tubes are normally contained in a temperature-controlled box, which also contains the sample changer. The choice of the temperature is a compromise between the lower backgrounds obtainable at 0 °C and the miscibility of some samples at reduced temperatures. At present, the majority of new systems operate at temperatures slightly below ambient to ensure that the sample is homogeneous. The system may contain a number of independent channels (usually three), each with a separate amplifier and channel-width controls. This enables two or perhaps three isotopes to be counted simultaneously. Some systems incorporate an automatic external source of γ-radioactivity. Following an initial sample count, the source is automatically transferred from a shielded container to a position close to the sample and a second count in the three channels is obtained. This second count with the external source enables an estimate to be made of any quenching present; details of the method are discussed later.

The output data are normally printed on to a paper tape and, if necessary, can be punched on to tape for computer analysis. In some machines the computer is built into the system and the processed results are produced directly.

Solvents, scintillators and additives

The purpose of the solvent is (*a*) to provide a medium for containing the sample, (*b*) to transport the energy from the source of radiation and (*c*) to contain the solutes and allow for the emission of light. The scintillation solute transforms the energy into light, but if the wavelength is unsuitable for the photomultiplier tube then a secondary solute can be used as a wavelength shifter.

The two basic solvents used are alkylbenzenes, such as toluene and xylene, and aliphatic ethers, the most common being 1 + 4 dioxan - water. Although the dioxan base produces a scintillator with a lower scintillation efficiency than that of the alkylbenzene bases, it has the advantage of being miscible with water. All solvents must be of the highest purity to avoid quenching effects which can be caused by trace amounts of impurities.

Typical primary solutes are 2,5-diphenyloxazole (PPO) and 2-(4′-t-butylphenyl)-5-(4″-biphenyl)-1,3,4-oxadiazole (Butyl-PBD). The two common secondary solutes (wavelength shifters) are 1,4-bis-(5-phenyloxazol-2-yl)benzene (POPOP) and 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene (DM-POPOP). The solutes must be soluble at the operating temperature of the system and also must not be precipitated by the addition of the sample, *e.g.*, water.

Radioisotopes are most commonly prepared for counting in aqueous solutions, so that water miscibility is an essential requirement in many scintillators. Dioxan-based systems are therefore most suitable for this purpose, but naphthalene is usually added to increase

the energy transfer from the solvent to solute and hence to increase the counting efficiency. Many popular dioxan-based scintillators originate from the Bray[8] solution, comprising naphthalene, PPO, POPOP, methanol and ethylene glycol in dioxan. Ethanol and monomethyl and monoethyl ethers of ethylene glycol can be used to reduce the freezing-point of dioxan scintillators.[9,10]

Toluene-based scintillators can be diluted with various polar solvents, such as ethanol and methanol, to increase water miscibility. This system was designed mainly for organic solvents, but now that solubilisers are freely available, a wide variety of sample materials, *e.g.*, blood, urine and biological tissue, can be counted in toluene-based systems. Typical solubilisers are hyamine hydroxide, NCS (Nuclear Chicago) and the "Bio-Solv" range (Beckman Instruments). Other additives such as Triton X-100 enable emulsion counting to be performed in toluene systems.[11]

Gel counting is a technique to be used when the sample is insoluble in the scintillator or in any convenient solvent. Normal dioxan-based scintillators can be converted into gels by stirring in finely divided silica to provide a thixotropic phase in which the insoluble material is suspended.

The choice between buying ready-made scintillators and preparing one's own depends upon the number and variety of samples to be analysed.[12,13] Shelf-life is an important consideration. Dioxan-based systems should be kept under nitrogen to prevent oxygen absorption and in tightly stoppered vessels to prevent loss of solvent and the subsequent crystallision of naphthalene, both of which reduce the counting efficiency. Toluene-based scintillators are not affected by either of these phenomena.

Techniques

This section covers the factors involved in selecting the technique, sample preparation (homogeneous, heterogeneous or for Cerenkov counting), standardisation and data handling. Initially, when a new isotope or material is to be counted, various factors must be considered:

(*a*) the energies and types of radiation emitted by the sample, *e.g.*, α-, β- or γ-radiation;

(*b*) the chemical form of the sample and whether chemical preparation could improve the effectiveness of the method;

(*c*) the choice of the scintillator system and the possible inclusion of additives to obtain miscibility, reduce quenching and improve the counting efficiency;

(*d*) instrumental conditions to ensure adequate sensitivity and an accurate assessment of the efficiency and background; and

(*e*) the method of data analysis should be included in the selection of the technique.

After choosing the technique, it is finally necessary to ensure that conditions remain constant by regular checks with calibrated standards.

Sample preparation for homogeneous systems—

Sample preparation should normally be kept to a minimum so as to avoid losses from incomplete chemical recovery and isotope effects caused by the different properties of the natural and radioactive isotopes. Chemical impurities introduced by processing may also introduce unknown quenching effects.

Aqueous systems can be added directly to dioxan-based scintillators and to toluene-based scintillators when a solubiliser is added.[14,15,16]

Biological materials such as blood, urine, salts, sugars and the alkali digests of plasma and tissue samples can be incorporated into toluene-based scintillators with Bio-Solv (BBS2 and BBS3, Beckman Instruments Ltd.). BBS2 is an acid solubiliser for alkaline tissue digests and aqueous solutions, and it must be neutralised before counting. BBS3 is a general-purpose solubiliser for all types of aqueous samples and for blood and plasma.[16] NCS (Nuclear Chicago Corporation) and Hyamine 10X can also be used for tissues and purified biological material.[17] However, the quaternary ammonium base of hyamine hydroxide is strongly chemiluminescent[18] and should be used with caution. It is necessary always to use some method to check for chemical and colour quenching and chemiluminescence.

Materials that are strongly luminescent or produce severe quenching require further treatment. Colour quenching can often be eliminated, or at least reduced, by digestion with

hydrogen peroxide and perchloric acid. This method has been used successfully for soft tissues, solid biological materials[19] (*e.g.*, teeth and bones) and for the determination of radioactivity on filter-papers.[20] Isotopes investigated include ^{32}P, ^{35}S, ^{45}Ca, ^{55}Fe and ^{57}Co.

Alternatively for ^{3}H and ^{14}C, complete combustion of the sample to water or to a soluble carbonate produces a simple counting method. The Schöniger oxygen-flask method[21] was the forerunner of this technique and many modifications have been made.[22] An automatic version of the instrument is now available commercially as a "Tritium Oxidiser" (Packard Instruments Ltd.). Recovery experiments to investigate the chemical yield and any isotope effect are an essential part of any combustion experiment.

SAMPLE PREPARATION FOR HETEROGENEOUS SYSTEMS—

Insoluble materials and other samples that cannot be processed chemically can be measured as suspensions in gels or as emulsions. If necessary, a solid support such as a filter-paper can be used. A reduction in counting efficiency may occur through self-absorption in particles, supports, etc., and standardisation may be difficult.

Suspension counting in gels is useful for incorporating reasonable amounts of precipitates that are otherwise insoluble in liquid scintillators. A transparent gelling agent such as Cab-o-Sil, a finely divided silica powder, is mixed to give a thixotropic gel that is fluid when shaken but firm when at rest. This technique has been used for barium and strontium carbonates, perchlorates, etc., and for an iron ferriphosphate complex for the determination of ^{55}Fe and ^{59}Fe (Eakins and Brown[23]) and ^{239}Pu and ^{241}Pu (Eakins and Lally[24]).

Emulsion counting can be used for incorporating large volumes of aqueous samples into scintillators. An example of this technique is the use of Triton X-100 detergent with a toluene-based scintillator.[12] This technique is very sensitive to pH, temperature and salt concentrations.

SAMPLE PREPARATION FOR CERENKOV COUNTING—

In the simplest form no sample preparation is required and the solution is placed directly into a counting phial. The efficiency is improved by effecting an increase in the refractive index (to reduce the energy threshold) and this may be necessary for β-emitters of lower energy. The introduction of a wavelength shifter will further increase the efficiency.[7] Chemical quenching is eliminated in this method, but colour quenching and chemiluminescence are still important. Quenching can be reduced by using the decolorising techniques discussed above. Standardisation is carried out either by adding an internal standard or by using a high-energy γ-emitter to produce photoelectrons in the solution. The major advantage of this technique is the high sensitivity with large volumes, or for flow monitoring without changing the liquid passing through the detector.

STANDARDISATION—

The counting efficiency for a high-energy β-emitter can be nearly 100 per cent., and the effects of quenching are then small. If all samples in a particular experiment have the same composition, then a simple standardisation technique is all that is necessary. However, for low-energy β-emitters in a wide range of materials, either chemical or colour quenching, or both, will normally be present. The three methods most commonly used are the use of an added internal standard, an automatic external standard[25] (usually a long-lived γ-ray emitter), and a channels ratio method.[26] The advantages and disadvantages are summarised in Table I.

The normal procedure is to choose the method, *e.g.*, channels ratio, in which the efficiency is plotted against the ratio for a series of standards with different amounts of a quenching material. The ratio obtained during an experiment can then be converted into an efficiency by the use of this graph. Such a calibration curve is necessary for each isotope, scintillator and instrument setting. A similar technique is used with the external standard method, but the internal standard will give the efficiency directly for each sample.

The background of the counter is also affected by quenching, and for low-level counting it is necessary to know the background for various values of the channels ratio. The presence of chemiluminescence introduces an increased variable background and can lead to erroneous results if it is significant compared with the radioactivity of the sample.

DATA HANDLING—

The output from manual instruments can normally be analysed with a desk calculator. Automatic systems that process hundreds of samples per day can produce five or more items of information per sample, and manual processing becomes tedious. The simplest technique normally involves the use of a small desk-top computer,[27] which can be programmed to take information about the sample (perhaps counts in three channels), the external standard (three more counts) and the time of the measurement, and produce the mean disintegration rate for that sample. The operator of the computer will have prepared a suitable polynomial fit for the efficiency *versus* ratio curve, and the data can be transferred by hand or by paper tape. The use of more sophisticated computers may be necessary for larger outputs or for a wide variety of samples.[28] If each sample is different in radioisotope, scintillator or type of quenching, then manual methods are usually the most efficient. The use of both the external standard and the channels ratio methods for each sample will normally reveal the type of quenching or the presence of chemiluminescence, and it is good practice to compare the efficiencies determined by these methods by using statistical tests.

TABLE I

METHODS OF DETERMINING COUNTING EFFICIENCY

Method	Advantages	Disadvantages
Internal standard	Gives individual results Best method for highly quenched samples Only reliable method when solid support material is present Corrects for both colour and chemical quenching	Possible errors when measuring small amounts of standard solution with a pipette Second count needed, *i.e.*, time consuming with large numbers of samples Sample cannot be re-counted Not suitable for dual-label samples
Automatic external standard	Automatic with no handling problems Only short repeat count needed Composition of sample unchanged	Dependent upon sample volume and the accurate positioning of the source Sample must be homogeneous Poor accuracy with highly quenched samples Instrumental costs and maintenance
Channels ratio	Only one count needed No handling of the sample required Composition of the sample unchanged Independent of sample volume Independent of inhomogeneity in the sample	Long counting time needed for accuracy with low-activity samples Poor accuracy with highly quenched samples Needs at least two channels

CONCLUSIONS

Liquid scintillation counting is the accepted technique for many radioisotopes in a wide range of chemical forms. It lacks sensitivity for very low levels of α-activity and cannot compete with internal gas counters used for natural tritium levels. This leaves a wide field of analytical application in chemistry, biochemistry and medicine. The presence of quenching agents in most samples can normally be detected, and their effect either reduced or corrected for by the choice of suitable standardisation methods. Similarly, chemiluminescence can be detected and reduced by using a different technique.

Improvements in the future may come from increased photocathode efficiency and from improved chemical techniques, with the best use of scintillators and solubilisers to reduce unwanted effects. Use of the technique is essentially a practical problem, which presents new facets with each new type of sample.

Appendix

ISOTOPES MEASURED IN A LIQUID SCINTILLATION COUNTER

This appendix is intended as a preliminary guide to the versatility of the technique and gives some idea of the sensitivity of the method. The information in Table II includes a range of isotopes and the matrix from which they were extracted. The preparation technique can be obtained from the references in the final column, but brief details of the additives are given, together with the scintillator used. The efficiency of counting is given only as a

guide, but it can be used to give an approximate indication of sensitivity if the background is taken as typically 10 to 25 counts per minute for most systems.

The reference list is fairly limited considering the vast literature on this subject, and represents methods, techniques and theoretical information that we have found to be useful in the theory and application of liquid scintillation counting.

Table II

Isotopes measured in a liquid scintillation counter

Isotope	Matrix	Counting form	Scintillator	Counter temperature/°C	Efficiency, per cent.	Reference
^{3}H	Water	Water	PPO - *p*-bis(*o*-methylstyryl)benzene in dioxan	0 to 25	23	14
^{3}H	Water	Water emulsion	Triton N-101 in *p*-xylene	17 to 25	24	15
^{3}H	Water	Water emulsion	Triton X-100 in toluene	4	27	11
^{3}H	Blood, plasma, urine	Blood, plasma, urine	BBS3 solubiliser in toluene	2	37 30	16
^{3}H	Plasma, urine	Plasma, urine, emulsion	Triton X-100 Hyamine 10X in toluene	0	20	29
^{14}C	^{14}C-toluene ^{14}C-fructose	^{14}C-toluene ^{14}C-fructose	BBS1 solubiliser in toluene	N.S.	88	16
^{14}C	Organic compounds	Aqueous solutions	Triton X-100 in toluene	0	75	11
^{32}P	Various	Aqueous solutions	None + wavelength shifter	N.S. N.S.	25 50	7 7
^{35}S	Vegetation	$BaSO_4$ precipitate in a gel	Dioxan	N.S.	65	30
^{35}S	Various	H_2SO_4 on glass-fibre disc	3 g l^{-1} of *p*-terphenyl in toluene	12	78	31
^{36}Cl	Various	Aqueous solution of NaCl	Dioxan	N.S.	85	32
^{45}Ca	Various	$CaCl_2$ in dibutyl phosphate	Toluene	−5	85	33
^{55}Fe ^{59}Fe	Blood	Ferriphosphate complex in gel	Dioxan	4	19·4 33·4	23
^{63}Ni	Aqueous solution	Tetrapyridine-nickel dithiocyanate	Dioxan	0	65	34
^{90}Sr ^{90}Y	Various	2-Ethylhexanoic acid solution of $SrCO_3$	Toluene	−4	83 95	35
^{131}I	Plasma	Plasma in gel	Toluene	−2	85	36
^{147}Pm	Urine	Di-2-ethylhexyl phosphate complex	Toluene	N.S.	95	37
^{210}Pb	Aqueous solutions	Aqueous concentrate	Dioxan	N.S.	97	38
Pu (α) ^{241}Pu	Urine, faeces, blood	Ferriphosphate complex in gel	Dioxan	4	86 21	24
^{239}Pu	Bone, liver, spleen, urine	Acidic solution after in-vial oxidation	0·4 g l^{-1} of PPO in ethanol Toluene	N.S.	85	39

N.S. Not stated.

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Received *April 8th*, 1971
Accepted *June 25th*, 1971

Analyst, October, 1971, Vol. 96, *pp.* 689–698

A Method for the Determination of Disodium Cromoglycate and Other Chromones

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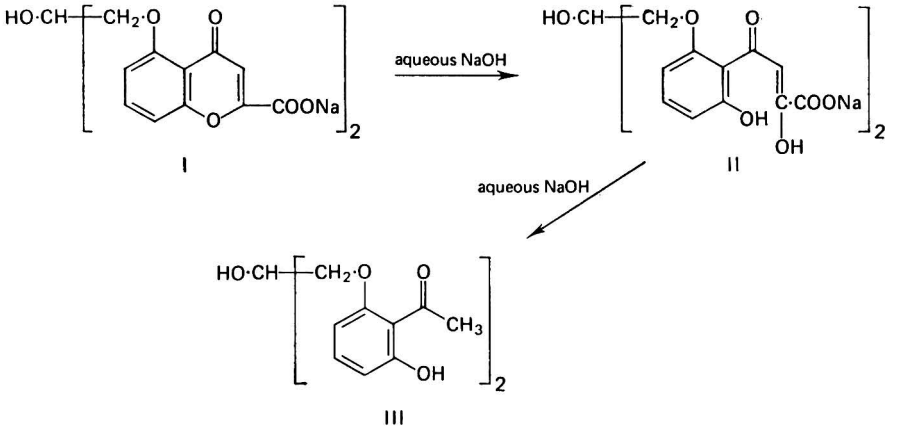
The hydrolysis of disodium cromoglycate in alkaline solution has been studied and a method for its determination is presented. The method is based on spectrophotometric measurement of the change in absorption at 310 nm, which takes place on opening the γ-pyrone ring of the chromone nucleus. The technique is applicable, with suitable minor modifications, to the determination of other compounds containing the chromone nucleus, such as flavones.

DISODIUM cromoglycate (DSCG), the disodium salt of 1,3-bis(2-carboxychromone-5-yloxy)-2-hydroxypropane, is a new therapeutic substance[1] of value in the treatment of allergic asthma. In the course of the commercial development of disodium cromoglycate (I) it became necessary to devise a method of analysis suitable for the examination of aged material. The most likely type of degradation was considered to be hydrolytic, analogous to the reaction of flavones in alkaline solution.[2] Such a degradation would involve the opening of the γ-pyrone ring of the chromone nucleus, followed by the loss of a two-carbon fragment as oxalate, and the formation of a bisacetophenone derivative (III). The open-ring intermediate (II) had not previously been isolated and was thought to be unstable.

The bisacetophenone derivative has phenolic properties and can be determined spectrophotometrically by coupling with diazotised amine (*e.g.*, *p*-nitroaniline).[3] It was considered that the determination of this compound before and after quantitative hydrolysis should



give an accurate value for the amount of unchanged chromone present in a formulation. The hydrolysis of disodium cromoglycate in alkaline solution was therefore studied in detail. It was soon shown that the open-ring intermediate (II) was reasonably stable in strongly alkaline solution and that in this enol form its ultraviolet absorption spectrum was different from those of DSCG and the bisacetophenone. This difference was such that it was feasible to base a method of analysis on the formation of the intermediate. As it is possible that the open-ring compound (II) might be present in aged material it is essential that any method

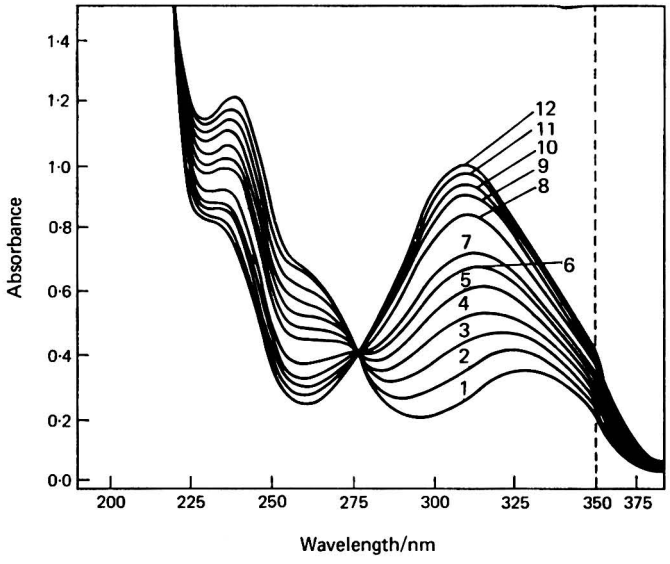


Fig. 1. Ultraviolet spectra recorded during the hydrolysis of DSCG in 0·1 M sodium hydroxide at 37 °C. DSCG concentration 1·8 mg per 100 ml in 10-mm cell. Time after preparation of solution: 1, 2 minutes; 2, 19 minutes; 3, 36 minutes; 4, 53 minutes; 5, 70 minutes; 6, 86 minutes; 7, 100 minutes; 8, 141 minutes; 9, 170 minutes; 10, 189 minutes; 11, 222 minutes; and 12, 253 minutes

of analysis used in stability testing should permit discrimination between this compound and DSCG. A method based on the formation of this intermediate is therefore potentially very specific for the chromone nucleus.

THE HYDROLYSIS OF DSCG IN ALKALINE SOLUTION AND THE NATURE OF THE HYDROLYSIS PRODUCT—

The hydrolysis of disodium cromoglycate in 0·1 M sodium hydroxide solution at 95 °C was followed by a thin-layer chromatographic determination. The bisacetophenone derivative was shown to be the ultimate product of the reaction, but the presence of at least one inter-

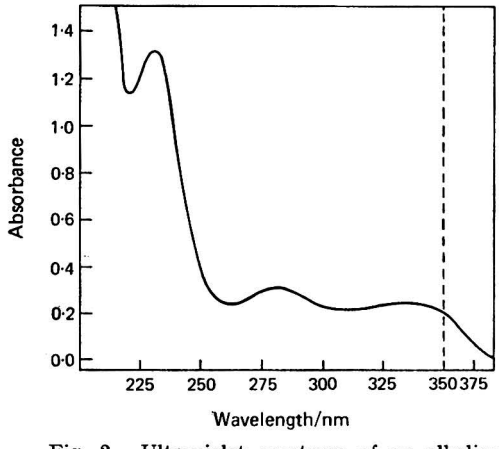
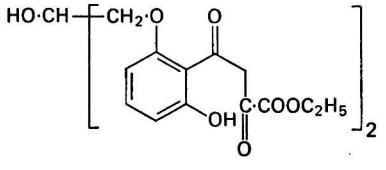


Fig. 2. Ultraviolet spectrum of an alkaline solution of the bisacetophenone derivative of DSCG at a concentration of 2·2 mg per 100 ml in 10-mm cell

mediate was indicated. The course of the hydrolysis of a more dilute solution at 37 °C was followed spectrophotometrically (Fig. 1). Graph 1 in this figure corresponds to DSCG itself, with an absorption maximum at a wavelength of 326 nm. The course of the reaction is indicated by a hypsochromic shift to 310 nm, accompanied by an increase in absorbance (graph 12). A maximum is reached after 4 hours and the system remains stable for about 30 minutes before further changes occur which eventually result in the spectrum of the bisacetophenone derivative (Fig. 2). It can be inferred from the isosbestic point at 277 nm (Fig. 1) that under the given conditions there are only two species present during the initial stages of the hydrolysis. These experiments, therefore, confirmed the formation of an intermediate during the hydrolysis of DSCG to its bisacetophenone derivative. At 25 °C in 0·1 M sodium hydroxide solution complete production of the intermediate takes 16 hours and further hydrolysis to the bisacetophenone is slow.

A 1 per cent. solution of DSCG that had been hydrolysed under controlled conditions produced on acidification a yellow precipitate that was extracted into solvent ether. Separation and evaporation of the ether layer yielded a yellow solid. The parent acid of DSCG is white and completely insoluble in solvent ether, and the infrared spectrum of the yellow solid differs from that of cromoglycic acid although it does indicate that the compound is a carboxylic acid. The yellow solid was soluble in dilute alkali and gave an ultraviolet spectrum identical with that of the intermediate formed in the hydrolysis of DSCG. It was also readily soluble in ethanol from which it deposited, either on standing or, more rapidly, on acidification and heating, a white precipitate with an infrared spectrum identical with that of cromoglycic acid. It was apparent that no carbon atoms had been lost during the hydrolysis and subsequent reactions and it was therefore concluded that the intermediate in the hydrolysis reaction was the acyclic sodium salt (II), the parent acid of which is yellow and soluble in ether and which readily cyclises in acid - ethanol solution.

The ester (IV) is known.[4]



IV

A solution of (IV) in chloroform was extracted with aqueous alkali and the ultraviolet absorption spectrum of this extract was found to be identical with that of the intermediate in the hydrolysis of DSCG. This is further evidence for the acyclic nature of the intermediate.

An attempt was made to isolate the acyclic sodium salt by freeze-drying a hydrolysate. Two experiments were performed. In one the hydrolysate was freeze-dried directly and in the other the hydrolysate was carefully neutralised to pH 7·5 with dilute hydrochloric acid before drying. These procedures produced two apparently different residues, that obtained from the alkaline solution being bright yellow and that from the neutral solution being white. Their infrared spectra were different and also different from that of DSCG. Both salts redissolved in water, but on acidification the salt from the alkaline solution gave a yellow precipitate while that from the neutral solution gave a white precipitate. The infrared spectra of these two precipitates were different from each other and from that of cromoglycic acid (V, Fig. 3). However, both could be converted into cromoglycic acid by digestion in acidified ethanol. This evidence confirms that the two sodium salts were the keto and enol forms of the acyclic compound, from which the chromone structure could readily be regenerated. Their proton nuclear magnetic resonance spectra also support this in that the band at about 3·5 τ, which is caused by the proton in the 3-position in the chromone ring, is not observed.

Further evidence that supports the tautomeric structures theory was obtained by examination of the spectral changes that occurred when the pH of the hydrolysate was adjusted from 13 to 6·5 (Fig. 4). The strong band at 310 nm disappears and is replaced by two weaker bands at 268 nm and 333 nm. On making the solution alkaline again the original spectrum can be regenerated. This is consistent with a tautomeric system involving labile

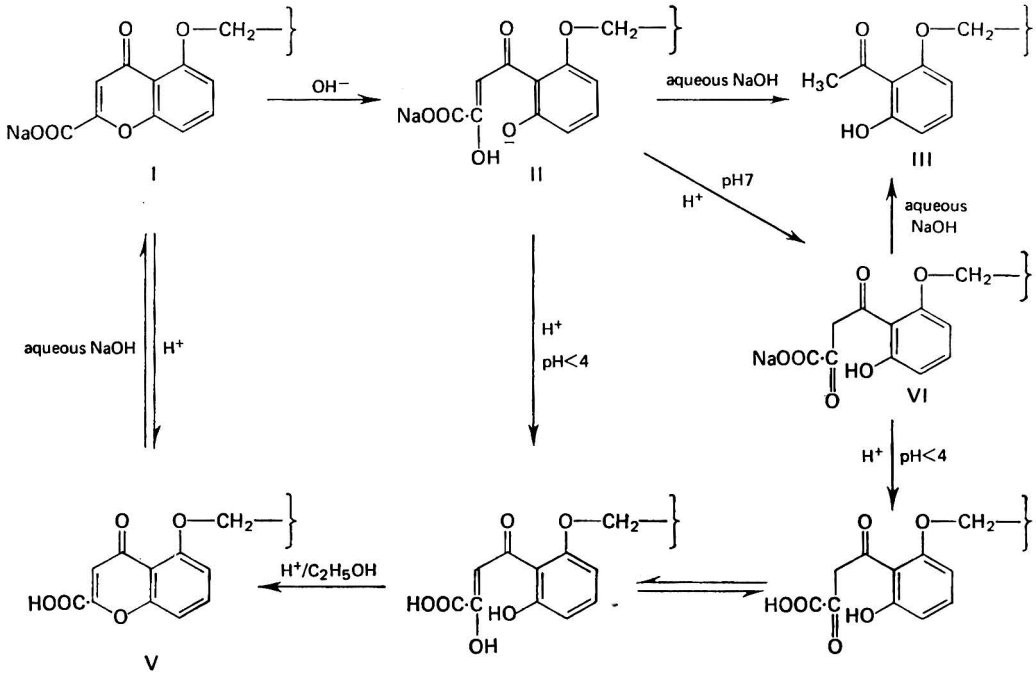


Fig. 3. Summary of the relationships between DSCG and its hydrolysis products

protons, and is explained by shifts in the equilibrium between II and VI (Fig. 3). The foregoing evidence is summarised in the reaction plan (Fig. 3). The alkaline hydrolysis of DSCG proceeds via ring opening to form a β-diketo compound, which is accompanied by a change in the ultraviolet absorption spectrum. The proposed method makes use of this reaction and the associated increase in absorbance at 310 nm.

The β-diketo compound exists in the diketo form in neutral and acidic solutions, and in the enol form in alkaline solution. Further hydrolysis results in the formation of a bis-acetophenone derivative by loss of a two-carbon atom fragment as oxalate.

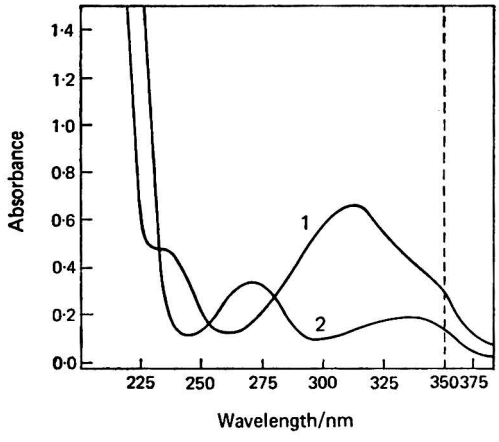


Fig. 4. Ultraviolet spectra of the initial hydrolysis product of DSCG at: 1, pH 13; and 2, pH 6·5. Concentration of 1·2 mg per 100 ml in 10-mm cell

Experimental

Reagents—

Sodium hydroxide solutions, 0·10 and 0·50 M.

Procedure—

Weigh accurately an amount of sample containing about 250 mg of DSCG and transfer it to a 500-ml calibrated flask. Add about 200 ml of distilled water, shake to dissolve the sample and dilute to the mark with distilled water. Mix thoroughly. Transfer a 2-ml aliquot with a bulb pipette to a 100-ml calibrated flask containing 10 ml of 0·1 M sodium hydroxide solution and 70 ml of distilled water. Dilute to the mark with distilled water and mix thoroughly. (This is solution A.) Measure the absorbance of solution A at 310 nm in 1-cm silica cells, with distilled water in the reference cell.

Also prepare a reagent blank consisting of 10 ml of 0·1 M sodium hydroxide diluted to 100 ml with distilled water. Read the absorbance of this solution against the distilled water reference at 310 nm. Subtract the reading from the sample reading, $(A_{310})_A$. All sample measurements must be completed within 10 minutes of adding the sample aliquot to the dilute sodium hydroxide solution.

To a second 100-ml calibrated flask transfer by pipette 20·0 ml of 0·5 M sodium hydroxide solution and approximately 60 ml of distilled water. Swirl to mix. To this flask transfer 2 ml of the sample solution by using a bulb pipette, again swirl to mix and immediately dilute to the mark with distilled water. Mix thoroughly. (This is solution B.) Store solution B at 25 $\pm$ 0·5 °C in a thermostatically controlled water-bath for at least 16 hours and not longer than 19 hours. Measure the absorbance of the solution at 310 nm in a 1-cm silica cell, with distilled water in the reference cell. Prepare a reagent blank by diluting 20 ml of 0·5 M sodium hydroxide solution to 100 ml with distilled water. Read the absorbance of this solution against the distilled water reference at 310 nm. Subtract this reading from the sample reading, $(A_{310})_B$. It is important to adhere to the order of addition of reagents and sample in the preparation of solution B, and to the storage temperature and time of 16 to 19 hours before measurement of absorbance.

Calibration graph—

Weigh accurately about 1·5 g (equivalent of dry powder) of pure DSCG into a 500-ml calibrated flask. Dissolve, make up to volume with distilled water and mix (solution C). Transfer aliquots of 5, 10, 15, 20 and 25 ml by pipette into 100-ml calibrated flasks, dilute them to the mark with distilled water and mix thoroughly (solutions D). Carry out the procedure for each of the solutions D and plot a graph of ΔA against the concentration of anhydrous DSCG (in mg per 100 ml) in the final 100 ml of solution. The latter is calculated from the expression—

$$\text{DSCG (mg per 100 ml)} = 0{\cdot}04\ VW_3\ \frac{(100 - M)}{100}$$

where W_3 is the weight (in g) of pure DSCG taken for the stock solution C, M the moisture content of the pure DSCG determined by drying under vacuum over phosphorus pentoxide at 105 °C, and V the volume (in ml) of the aliquot taken to prepare solution D. The points obtained should form a straight line passing through the origin.

Calculation—

The increase in absorbance caused by hydrolysis $= \Delta A = (A_{310})_B - (A_{310})_A$. Read from the calibration graph the weight of DSCG in mg per 100 ml of solution corresponding to the experimental value of ΔA, say W_2 mg.

Then the percentage of DSCG in the sample $= \frac{W_2}{W_1} \times 25\,000$

where W_1 is the weight of sample in mg.

Results and discussion

Relationship between DSCG concentration and ΔA—

It was confirmed that the increase in absorbance at 310 nm, ΔA, was proportional to the DSCG concentration in the range from 0·1 to 1·5 mg per 100 ml when the procedure was applied to samples of the pure compound. The calibration graph was found to be reproducible and to pass through the origin.

Effect of sodium hydroxide concentration on the recovery of DSCG—

The procedure was applied to identical aliquots of the stock solution. The amount of sodium hydroxide used to effect the hydrolysis was varied to give different final concentrations of alkali but all other conditions were as stated under Procedure. The results are shown in Table I. It is evident from these results that a 5 per cent. variation in the concentration of alkali has no effect on the result but that insufficient alkali leads to low recoveries caused by incomplete reaction.

Table I

Effect of variation of sodium hydroxide concentration on the recovery of DSCG

Sodium hydroxide concentration/M	ΔA	Recovery of DSCG, per cent.
0·105	0·395	100·0
0·105	0·395	100·0
0·100	0·396	100·3
0·100	0·394	99·7
0·095	0·395	100·0
0·095	0·398	100·8
0·090	0·385	97·5
0·080	0·368	93·2
0·070	0·347	87·8
0·060	0·325	82·3
0·050	0·302	76·5

Effect of temperature of hydrolysis on the recovery of DSCG—

The procedure was applied to identical aliquots of the stock solution but the temperature at which hydrolysis was carried out was varied. The results are shown in Table II. Variation of the temperature has a marked effect as low results are obtained at temperatures both higher and lower than 25 °C. A temperature of 25 $\pm$ 0·5 °C was therefore adopted for the routine operation of the method. At lower temperatures the hydrolysis is slow, and incomplete reaction leads to low results. At higher temperatures the decomposition of the open-ring compound, II, to the bisacetophenone derivative, III, occurs at a significant rate and also leads to low results.

Table II

Effect of temperature on the recovery of DSCG

Temperature/°C	21	21	25	25	30	30
Recovery of DSCG, per cent.	94·8	93·4	100·2	99·8	96·6	96·4

Effect of time of hydrolysis on the recovery of DSCG—

An alkaline solution of DSCG was prepared according to the procedure and its absorbance at a wavelength of 310 nm measured as a function of time. The results are shown in Table III. A maximum is reached and maintained for about 3 hours. On the basis of these results a reaction time of 17·5 $\pm$ 1·5 hours was adopted for the routine operation of the method.

Table III

Effect of time of hydrolysis on the recovery of DSCG

Time/hours	2	4	7	11	16·5	17·5	18·5	19·5	21·8
Recovery of DSCG, per cent.	38·0	62·0	82·3	94·2	99·5	100·0	99·5	98·7	94·9

INTERFERENCES—

Lactose—DSCG is normally formulated for clinical use as a blend with lactose; therefore the effect of lactose on the recovery of DSCG by the proposed procedure was investigated. Two solutions containing identical concentrations of DSCG (approximately 50 mg per 100 ml) were prepared. One solution also contained lactose at a concentration of 50 mg per 100 ml. Aliquots (2 ml) of each of the solutions were treated as under Procedure. The results are shown in Table IV and confirm that lactose has no effect on the determination of DSCG by the proposed method.

TABLE IV

EFFECT OF 1 mg PER 100 ml OF LACTOSE ON THE RECOVERY OF DSCG

Lactose absent	..	..	100·3	100·5	99·5	100·0	100·0	Mean 100·1
Lactose present	..	..	100·5	99·7	100·5	100·5	100·0	Mean 100·2

Bisacetophenone derivative—The bisacetophenone derivative, III, is a possible degradation product of DSCG, and it was considered necessary to show that its presence would not interfere in the proposed method. It was shown that no change in the ultraviolet spectrum of a solution of the bisacetophenone derivative in 0·1 M sodium hydroxide occurred during a period of 16 hours at a temperature of 25 °C. In particular, the absorbance at a wavelength of 310 nm of an alkaline solution containing 2 mg per 100 ml of the bisacetophenone derivative was unchanged over this period. The presence of this compound in the sample will not, therefore, interfere in the determination of DSCG by the procedure outlined in this paper.

Open-ring compound, II—If the degradation of DSCG in a storage sample occurs by a hydrolytic process, the open-ring compound, II, may also be present. This compound does not contribute to the increase in absorbance at 310 nm and therefore it will not interfere in the determination of DSCG. However, it can be seen from Fig. 3 that its absorption spectrum is pH-dependent. It is essential that the initial absorbance be measured in an alkaline solution with a pH greater than 11 so that II is in its enol form, which has an

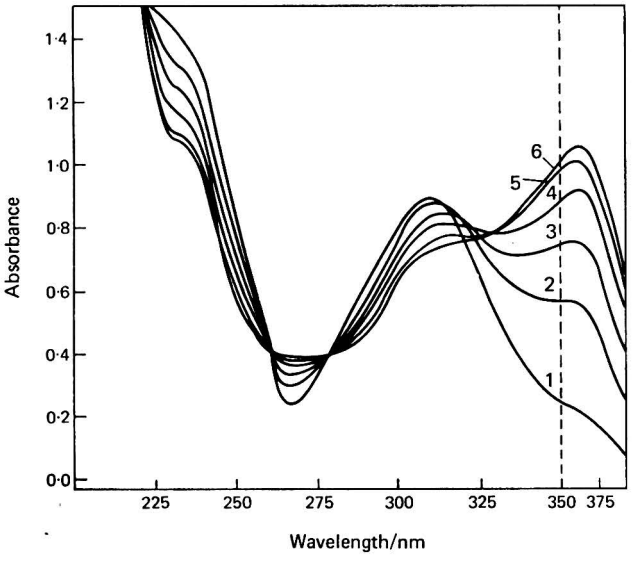


Fig. 5. Ultraviolet spectra recorded during the hydrolysis of compound VII in 0·3 M sodium hydroxide at 25 °C. Concentration 2·0 mg per 100 ml in 10-mm cell. Time after preparation of solution: 1, 6 minutes; 2, 18 minutes; 3, 29 minutes; 4, 43 minutes; 5, 60 minutes; and 6, 77 minutes

absorption maximum at 310 nm. If the initial measurement is made while using 0·1 M sodium hydroxide solution, then the rate of hydrolysis is such that the absorbance increases too rapidly for an accurate initial reading to be made. At a sodium hydroxide concentration of 0·01 M, II is in its desired form and the rate of hydrolysis is such that there is no measurable change in absorbance for up to 10 minutes after preparing the solution.

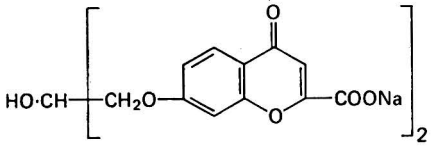
ACCURACY AND PRECISION—

The DSCG content of a fresh 50 per cent. blend with lactose was determined by ultraviolet spectrophotometry at a wavelength of 326 nm, at which the absorption maximum occurs. The mean of twenty determinations was 51·4 per cent. w/w and this value was taken to be the true DSCG content of the blend. The standard deviation was 1·00 per cent., which includes assay variation and slight blend heterogeneity.

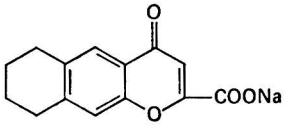
The DSCG content of the blend was then determined twenty times by using the procedure given above. The mean of the twenty results was 52·0 per cent. w/w and the standard deviation was 1·23 per cent. The mean recovery obtained with the proposed method, with respect to the direct spectrophotometric method, was 101·2 per cent. The accuracy and precision of the proposed procedure are therefore comparable with those of the direct method.

APPLICATION OF THE HYDROLYSIS APPROACH TO OTHER CHROMONES—

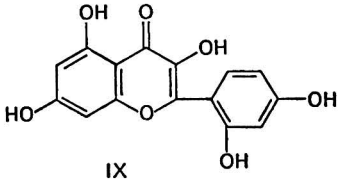
The reaction upon which the procedure is based is general for compounds with the chromone structure. With suitable modifications it was possible to devise methods of analysis for other compounds with a basic chromone structure. Three further compounds were examined: 1,3-bis(2-carboxychromone-7-yloxy)-2-hydroxypropane disodium salt (VII), which is the 7,7′ isomer of DSCG; cyclohexano[*g*]chromone-2-carboxylic acid, sodium salt (VIII); and morin (3,5,7,2′,4′-pentahydroxyflavone) (IX).



VII



VIII



IX

It was found that by using suitable conditions of alkalinity and temperature, slow changes in the ultraviolet spectra of solutions of these compounds could be effected. In each case a steady state was reached. The changes in the spectra are shown in Figs. 5, 6 and 7. The

TABLE V

SUMMARY OF CONDITIONS SUITABLE FOR THE DETERMINATION OF VARIOUS COMPOUNDS CONTAINING THE CHROMONE NUCLEUS

Compound	Sodium hydroxide concentration/M	Temperature/°C	Optimum hydrolysis time	Wavelength/nm	Change in absorbance for 1 mg per 100 ml
I	0·1	25	17 ± 1·5 hours	310	+0·42
VII	0·3	25	110 ± 10 minutes	354	+0·57
VIII	0·2	10	4 ± 0·5 hours	354	+0·31
IX	0·1	37	3·5 ± 0·5 hours	415	−0·68
				318	+0·61

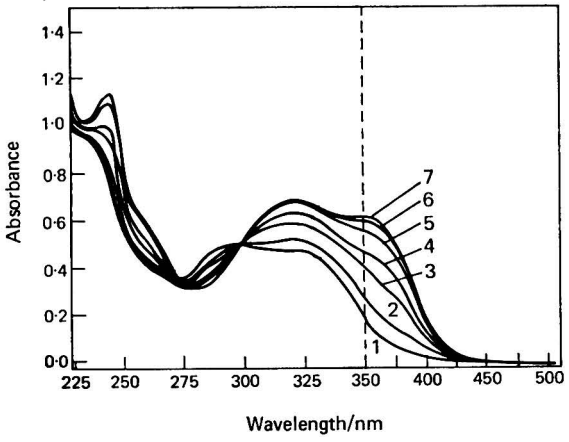


Fig. 6. Ultraviolet spectra recorded during the hydrolysis of compound VIII in 0·2 M sodium hydroxide at 10 °C. Concentration 1·9 mg per 100 ml in 10-mm cell. Time after preparation of solution: 1, 1 minute; 2, 6 minutes; 3, 43 minutes; 4, 69 minutes; 5, 120 minutes; 6, 177 minutes; 7, 240 minutes.

increase (or decrease) in absorbance at a suitable wavelength was shown to be directly proportional to the concentration of the individual compound. The conditions appropriate for the determination of the four compounds are summarised in Table V. The existence of isosbestic points in the spectra in Figs. 1, 5, 6 and 7 supports the contention that after a steady state has been achieved there exists in solution an equilibrium between the chromone

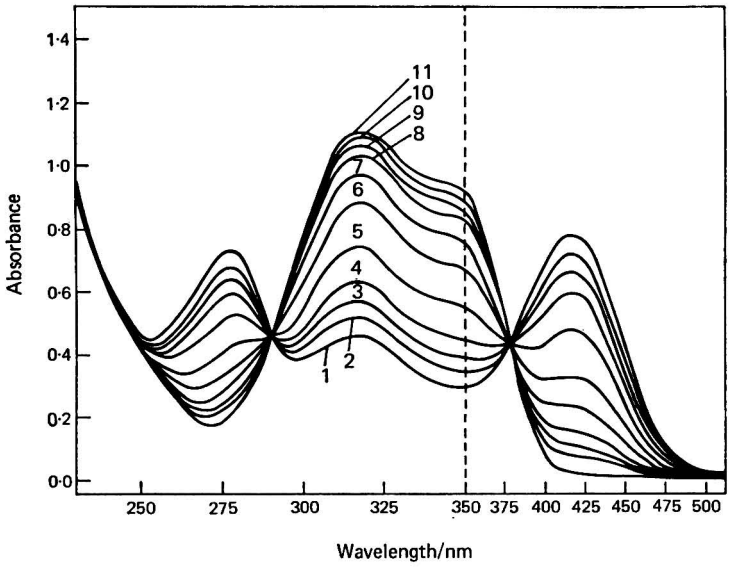


Fig. 7. Absorption spectra recorded during the hydrolysis of morin, compound IX, in 0·1 M sodium hydroxide at 37 °C. Concentration 1·1 mg per 100 ml in 10-mm cell. Time after preparation of solution: 1, 2 minutes; 2, 10 minutes; 3, 20 minutes; 4, 35 minutes; 5, 55 minutes; 6, 85 minutes; 7, 105 minutes; 8, 120 minutes; 9, 135 minutes; 10, 150 minutes; and 11, 180 minutes

structure and the open-ring compound, probably in its enol form. It may be that the equilibrium is such that all or nearly all of the original chromone has been converted into the open-ring species and that the spectrum of a pure compound is observed.

Experiments carried out during these investigations showed that the two most important parameters were the alkalinity and the temperature at which hydrolysis was carried out. In the reaction sequence—

Chromone → open-ring compound → acetophenone derivative

the first reaction is favoured by high alkali concentrations and the second by higher temperatures. In order to obtain maximum effect, the extent of the second reaction should be minimised, while allowing the first reaction to proceed more rapidly. A compromise must be made between alkali concentration and temperature so that a steady state is maintained for a sufficient period to enable accurate final measurements to be made.

Conclusions

This approach to the determination of DSCG provides the high degree of specificity required in testing the stability of formulations. It is sufficiently accurate and the precision is of the order that would be expected from a spectrophotometric method. Its successful application to three other chromones, including a flavone, suggests that it may have wide applicability for the assay and determination of purity of such compounds.

We thank Mr. H. Cairns for helpful discussions and Mr. M. L. Ray-Johnson for technical assistance. We also thank Dr. J. S. G. Cox and Fisons Limited, Pharmaceutical Division, for permission to publish this paper.

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Received *March 16th*, 1971
Accepted *May 24th*, 1971

Analyst, October, 1971, Vol. 96, *pp.* 699–711

The Flame-photometric Determination of Alkalis in Ceramic Materials

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The flame-photometric method in general use for the determination of alkalis in the ceramic industries was originally devised for the EEL, Model 100, flame photometer, with a coal gas flame, which is not now generally available. Current supplies of town gas, propane and methane (natural gas) flames are compared and interferences evaluated. Perchloric and hydrochloric acids are found to have a depressant effect; sodium is enhanced by potassium and is subject to spectral interference by calcium.

Propane is chosen as the preferred fuel. The effect of chlorine-containing acids is eliminated by the use of sulphuric and nitric acids for the initial decomposition and the spectral interference from calcium by the addition of aluminium sulphate; sodium - potassium inter-element effects are eliminated by the use of a caesium buffer. Although the procedure is principally devised to give optimum results with aluminosilicates, its extension to high-lime materials is also considered.

The method for the determination of alkalis in ceramic materials is based on work carried out 14 years ago[1] with the Evans Electroselenium, Model 100, flame photometer. This work showed that it was legitimate to compare simple solutions of the alkali sulphates with sample solutions (prepared by decomposition with hydrofluoric acid) in which the acid used for dissolution was hydrochloric acid. In the intervening years small changes in the apparatus, *e.g.*, in the construction of nebulisers, and considerable changes in the composition of town gas have occurred, and during the past year or so occasional discordant results obtained have suggested that these changes might have altered interferences both qualitatively and quantitatively.

At the time of the original work, town gas consisted mainly of coal gas. At the present time town gas may take many forms ranging from natural gas containing 84 to 90 per cent. of methane to a mixture of this with low pressure gas and high pressure gas. This composition may vary from day to day and even from hour to hour.

Flame temperature is therefore likely to vary (Table I) and this variation, together with the impending introduction of North Sea gas, created interest in the effect of the fuel gas on the determination of alkalis, particularly from the point of view of the possible interference effects. The ultimate objective was to ensure that the method for their determination

TABLE I

COMPARISON OF THE COMPOSITION OF COAL GAS, LOW AND HIGH PRESSURE GAS AND TWO SAMPLES OF TOWN GAS

Constituent	Composition, per cent. v/v				
	Coal gas[3]	Low pressure gas	High pressure gas	Town gas[2]	Town gas[2]
Hydrogen	49·8	40·6	47·1	50	44·9
Methane	25·8	29·8	34·3	23·5	19·3
Ethane	1·5	—	—		
Carbon monoxide	11·8	8·4	1·8	2·7	9·1
Carbon dioxide	2·4	7·6	16·8	12·3	2·7
Nitrogen	4·2	11·2	—	8·0	18·2
Unsaturated hydrocarbons	4·5	1·4	—	3·0	5·4
Calculated flame temperature/°C	1916	1810	1970	1870	1880

The calculated flame temperature for propane is 1925 °C and for methane 1880 °C.

yielded correct results by evaluating the respective interference effects for various gases, selecting the best of the gases for further experiments and attempting to overcome the interferences with this gas. Three gases were chosen for study, town gas, propane, which is available commercially in a reasonable degree of purity and is already used in several other commercial instruments, and methane (the major constituent of North Sea and Saharan gases). Andrew and Nichols[2] drew attention to the variability of town gas and of commercial butane, and for this reason butane was not included in our experiments.

It should be made clear that during the period of experimental work the town gas used was from the normal supply. The results presented for use of town gas may therefore have been subject to any variation that could arise from fluctuations in gas composition such as those illustrated in Table I.

Experimental

Modifications to the instrument—

Preliminary experiments indicated that neither propane nor methane could be burnt satisfactorily on the normal EEL burner head. As soon as a satisfactory flame had been produced, as judged by the appearance of separate blue cones, the flame "lifted off." This was caused by the low burning velocity of the two gases. A satisfactorily stable flame could be obtained by the use of a Méker-type head but a serious loss of sensitivity occurred, probably resulting from the obstruction of the aerosol flow. At the suggestion of Mr. V. R. Williamson of Evans Electroselenium Ltd., the thickness of the standard burner head was increased to about half an inch and a stable flame of good sensitivity was obtained. The dimensions of the burner head are shown in Fig. 1. The head used in these studies was

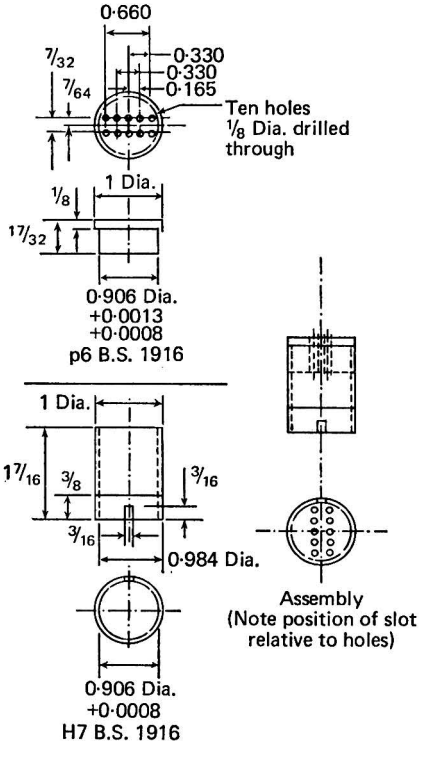

Fig. 1. Design of burner for EEL, Model 100, flame photometer to burn propane and natural gas. Material is of 18/8 Cr - Ni steel (all measurements are in inches)

constructed of brass and performed satisfactorily during the 4 months in which it was in use. However, for prolonged use its construction in stainless steel would seem preferable.

During operation of the instrument, difficulty was often found in setting the full-scale reading, which was caused by the coarseness of the sensitivity control. This problem was overcome by replacing the carbon potentiometer with a wire-wound multi-turn helical potentiometer (20 kΩ) and the sensitivity knob with a geared multi-turn control knob. Even with the new arrangement the second-to-second stability of the readings was still not satisfactory. By trial and error it was found that optimum damping was obtained by attaching a 250-μF electrolytic capacitor across the terminals of the photocell.

With the bottled gases the pressure of the gas supplied to the instrument was controlled at 0·432 p.s.i. (12 inches w.g.) by the use of a Calor propane regulator. For propane this can be attached directly to the cylinder; for methane, because of the higher cylinder pressure, an adaptor to a British Oxygen Co. hydrogen regulator was made to take the Calor regulator.

STANDARDISATION—

The procedure for the determination of alkalis commonly used in the ceramic industries is described in several standard methods,[4] and is based on decomposition of the sample with hydrofluoric acid. Sulphuric acid was originally chosen to eliminate the fluorides, so that it was logical to standardise by using solutions of the alkali sulphates. In later years it was found that nitric and perchloric acids could be used to remove fluorides and that hydrochloric acid could be used for the dissolution of the residue without altering the validity of sulphate standards.

To test the current validity of this basis of standardisation, solutions containing 10 and 5 p.p.m. of K_2O, 5 and 2·5 p.p.m. of Na_2O and 20 and 10 p.p.m. of Li_2O were prepared from their sulphates and chlorides. The instrument was adjusted in each instance to give full-scale deflection on the highest sulphate standard and zero on distilled water, and the other solutions were sprayed. The results observed for town gas, propane and methane are shown in Table II.

TABLE II

COMPARISON OF SULPHATE AND CHLORIDE STANDARDS

Solution concentration, p.p.m.	Mean reading		
	Town gas	Propane	Methane
Sodium—			
Na_2O (sulphate) 5	100	100	100
(chloride) 5	100	100	99·5
(sulphate) 2·5	54	54	54·5
(chloride) 2·5	53·5	54	54·5
Potassium—			
K_2O (sulphate) 10	100	100	100
(chloride) 10	100	100	100
(sulphate) 5	52	54	54
(chloride) 5	52	54	54
Lithium—			
Li_2O (sulphate) 20	100	—	—
(chloride) 20	98	—	—
(sulphate) 10	51	—	—
(chloride) 10	49·5	—	—

From the results in Table II it seems that the temperatures of the flames are adequate to overcome the differences between the boiling-points of the alkali sulphates and chlorides. With lithium the slight difference may arise from uncertainty in the assay of the lithium carbonate used for preparation of the chloride standard. It was decided at this stage to restrict attention to effects on sodium and potassium only, as they are the most important industrially, and in any event the usual problem with the determination of lithium in ceramic materials lies in determining the significance of a deflection of one or two divisions.

EFFECT OF ACIDS ON THE DISSOLUTION OF THE SAMPLE—

Although it is usual to use hydrochloric acid for the final dissolution of the residue, depending on the nature of the material the fluorides can be removed with sulphuric or perchloric acid. It was, therefore, necessary to investigate the effect of each of the three

acids. Hydrochloric acid will be present in fixed amounts, *i.e.*, 20 ml of the dilute acid (1 + 19) used to dissolve the residue. On the other hand, sulphate and perchlorate concentrations will depend on the composition of the residue, most of each anion being present as a salt, and possibly a little free acid remaining trapped in the residue. From calculations based on the possible composition of the residue, 3 ml of 1 + 4 perchloric acid (sp.gr. 1·54) and 5 ml of sulphuric acid (1 + 9) would yield equivalent amounts of perchlorate and sulphate.

To test the effect of these anions at these concentrations, standard solutions prepared from alkali sulphates and chlorides to yield combined solutions equivalent to (*i*) 10 p.p.m. of K_2O and 5 p.p.m. of Na_2O and (*ii*) 5 p.p.m. of K_2O and 2·5 p.p.m. of Na_2O with appropriate additions of acid were compared with similar solutions without addition of acid, setting full scale on the latter and the zero on distilled water. The comparisons were carried out by using town gas, propane and methane (Table III).

TABLE III

EFFECTS OF ADDITIONS OF ACID ON SULPHATE AND CHLORIDE STANDARDS WITH THE DIFFERENT FUELS

Solution concentration, p.p.m.	Acid added	Mean readings *less* blanks: Sulphate standards			Chloride standards		
		Town gas	Propane	Methane	Town gas	Propane	Methane
Potassium—							
10	Nil	100	100	100	100	100	100
10	HCl	96·7	96·3	93·5	95·0	95·5	95·5
10	H_2SO_4	99·0	99·5	99·5	98·0	100·0	99·5
10	$HClO_4$	97·3	97·0	95·8	95·8	97·0	97·0
5	Nil	51·5	55·0	55·0	51·0	54·5	55·3
5	HCl	48·7	53·8	53·3	48·0	53·0	52·8
5	H_2SO_4	50·0	55·5	55·3	50·0	55·0	55·5
5	$HClO_4$	49·0	54·0	54·0	49·0	53·5	54·0
Sodium—							
5	Nil	100	100	100	100	100	100
5	HCl	97·0	98·5	96·0	96·0	98·3	97·8
5	H_2SO_4	97·5	99·5	99·3	97·0	99·5	99·8
5	$HClO_4$	98·0	98·5	97·8	97·2	97·8	98·3
2·5	Nil	52·0	54·0	54·5	51·3	54·5	55·0
2·5	HCl	50·3	53·0	53·0	49·3	53·5	54·0
2·5	H_2SO_4	51·3	54·0	54·0	51·5	55·5	56·0
2·5	$HClO_4$	51·0	54·0	53·5	50·8	54·5	54·8

The fact that there was little difference between the behaviour of sodium and potassium from either sulphate or chloride standards indicated that there was little merit in altering the basis of calibration. The investigation of chloride standards was therefore discontinued at this juncture.

It can be seen that both perchloric and hydrochloric acids produce significant negative errors with all gases. The depression with sulphuric acid is smaller and, with the exception of town gas, of negligible proportion.

The effect of perchlorate ion is particularly disturbing in that the concentration in the solution can be so variable. It was decided to check if the hydrochloric acid addition could be increased to reach a saturation point beyond which the perchloric acid might have a negligible effect. The results of these experiments are shown in Table IV.

Plateaux were not attained in the range of 10 to 50 ml of dilute hydrochloric acid (1 + 19) per 250 ml of solution. An increase to the equivalent of 20 ml of hydrochloric acid (1 + 1) led to a depression more or less proportional to the acid content.

Experiments were also carried out to ascertain if the depression noted for perchloric acid was at saturation level, by increasing the addition of dilute acid (1 + 4) from 3 to 10 ml, and also if the depressions for the two acids were additive. The results are also shown in Table IV. For town gas and methane, the perchloric acid depressions were not saturation values and the effects of the two acids were in fact additive. It was decided to return to the use of sulphuric and hydrofluoric acids as the decomposition medium, but to use nitric acid for the dissolution so as to avoid depressing the solubility of calcium sulphate (such as would result from the decomposition of a bone ash or a building clay) by the presence of a large excess of sulphuric acid.

TABLE IV

EFFECTS OF VARIOUS ADDITIONS OF ACID ON THE INTENSITIES OBSERVED FOR 5 P.P.M. OF Na_2O AND 10 P.P.M. OF K_2O

Addition/ml	Mean readings for 5 p.p.m. of Na_2O			Mean readings for 10 p.p.m. of K_2O		
	Town gas	Propane	Methane	Town gas	Propane	Methane
Control	100	100	100	100	100	100
HCl (1 + 19) 10	98·5	99·5	99·5	97·5	97·5	97·0
(1 + 19) 20	97·0	99·0	97·8	95·3	95·3	93·5
(1 + 19) 30	96·0	98·0	97·0	93·3	93·3	91·5
(1 + 19) 40	96·0	97·5	97·8	91·8	91·3	89·8
(1 + 19) 50	94·0	96·5	95·8	89·3	90·0	88·3
HCl (1 + 1) 20	83·5	86·0	84·8	68·5	70·0	67·5
$HClO_4$ (1 + 4) 3	98·0	98·5	97·8	97·3	97·0	95·8
(1 + 4) 10	95·5	98·5	95·8	91·8	93·8	90·3
$HClO_4$ (1 + 4) 10 *plus* HCl (1+19) 20	94·0	98·3	93·8	89·5	91·5	87·8
HNO_3 (1 + 19) 20	100	100	100	99·5	100	99·5

Tests also showed (Table IV) that the effect of nitric acid is negligible, thus permitting the addition of nitric acid to the decomposition mixture. This addition assists the complete destruction of organic matter and the removal of excess of sulphuric acid as nitrosulphonic acid. In samples with relatively high calcium contents it is desirable to reduce the sulphate-ion concentration. Tests on decomposition with sulphuric acid - nitric acid and hydrofluoric acid revealed no interferences in the complexometric determination of calcium or magnesium.

Table IV also shows that the effects of addition of acid were most serious with methane as fuel, only a little less serious with town gas and relatively less important with propane.

EFFECTS OF ONE ALKALI ON THE INTENSITY OF ANOTHER—

In the original work on the determination of alkalis, inter-element effects between sodium and potassium were found to be negligible, but because of the changes in interference caused by anions the validity of this finding was checked with the three fuels.

Solutions containing 4 p.p.m. of Na_2O were sprayed alone and with additions of 8 and 100 p.p.m. of K_2O and compared with the usual 5 p.p.m. of Na_2O - 10 p.p.m. of K_2O standard. At the same time blanks were carried out on 8 and 100 p.p.m. of K_2O. These solutions would represent, respectively, samples of high sodium-to-potassium ratio such as borax frit, soda feldspar and alumina, the ratio normally used as a combined standard solution and low sodium-to-potassium ratios, such as in potash feldspar. Similarly, solutions containing 8 p.p.m. of K_2O were prepared with additions of 0, 4 and 80 p.p.m. of Na_2O and compared with the normal 10 p.p.m. of K_2O - 5 p.p.m. of Na_2O standard, and blanks were measured. The results are shown in Table V.

TABLE V

INTER-ELEMENT EFFECTS BETWEEN SODIUM AND POTASSIUM FOR VARIOUS RATIOS

Solution concentration, p.p.m.	Mean readings *less* blanks		
	Town gas	Propane	Methane
Sodium filter—			
Na_2O 4 K_2O 0 ..	79·2	79·3	79·0
4 8 ..	81·8	82·5	82·6
4 100 ..	81·5	82·5	82·7
Potassium filter—			
K_2O 8 Na_2O 0 ..	81·7	81·3	81·5
8 4 ..	81·7	81·5	81·5
8 80 ..	82·5	82·3	82·0

The effect of potassium was most marked, and appeared to reach a maximum with 8 p.p.m., or with a ratio of K_2O to Na_2O of 2:1. Further experiments with smaller increments of potassium were carried out at the 4 p.p.m. and 2 p.p.m. levels of Na_2O (Table VI), by using propane alone. At this stage of the investigation it was decided to use propane as fuel gas in the remaining work. This gas had the advantage of relatively constant composition (at least it is known when variation can occur, *e.g.*, when a new cylinder is used) and it also yielded lower levels of interference from additions of acid.

TABLE VI

EFFECT OF INCREASING AMOUNTS OF POTASSIUM ON THE LIGHT EMITTED BY Na_2O

K_2O added, p.p.m.	(Full scale set on 5 p.p.m. of Na_2O in the presence of 10 p.p.m. of K_2O) Test solution 4 p.p.m. of Na_2O Mean readings *less* blank			(Full-scale set on 2·5 p.p.m. of Na_2O in the presence of 5 p.p.m. of K_2O) Test solution 2 p.p.m. of Na_2O Mean readings *less* blank
	Town gas	Propane	Methane	Propane
0	79·2	79·3	79·0	77·8
0·5	79·8	78·8	78·8	—
1	80·5	79·3	79·3	78·4
2	81·3	79·8	79·8	78·6
4	80·7	80·5	81·2	80·0
8	81·8	82·5	82·0	80·8
100	81·5	82·5	82·7	80·5

Although the results for the lower sodium concentration were less reproducible, because of increased "scale expansion," both experiments indicated that a steady figure was reached when the K_2O content reached 8 p.p.m.

EFFECT OF OTHER ELEMENTS—

In normal aluminosilicates, the maximum concentrations of other elements that would be expected to be present are: calcium oxide 50 p.p.m. (5 per cent.), aluminium oxide 400 p.p.m. (40 per cent.), magnesium oxide 50 p.p.m. (5 per cent.) and iron(III) oxide 100 p.p.m. (10 per cent.). The same levels apply to aluminous materials (containing more than 45 per cent. of Al_2O_3) as these are not completely dissolved, so that only the soluble alumina needs to be considered. To determine the possible effects of these constituents, solutions of 4 p.p.m. of Na_2O and 8 p.p.m. of K_2O were sprayed in the presence and absence of calcium oxide 50 p.p.m., aluminium oxide 400 p.p.m., magnesium oxide 50 p.p.m. and iron(III) oxide 100 p.p.m. The magnesium and calcium solutions were prepared from Specpure materials and the iron and aluminium solutions from analytical-reagent grade metals. Blanks were also sprayed to evaluate either impurity levels or the possibility of spectral interference. The results obtained are shown in Table VII.

TABLE VII

EFFECTS OF CALCIUM, MAGNESIUM, ALUMINIUM AND IRON ON THE DETERMINATION OF SODIUM AND POTASSIUM

Full-scale deflection set on 5 p.p.m. of Na_2O or 10 p.p.m. of K_2O

Solution concentration, p.p.m.	Sodium		Potassium	
	Mean	Mean *less* blank	Mean	Mean *less* blank
Na_2O 4 + K_2O 8	—	82·0	—	82·0
Na_2O 4 + K_2O 8 + CaO 50	85·5	(81·5)	82·3	82·3
Na_2O 4 + K_2O 8 + MgO 50	83·0	81·0	82·0	82·0
Na_2O 4 + K_2O 8 + Al_2O_3 400	82·8	82·0	81·6	81·6
Na_2O 4 + K_2O 8 + Fe_2O_3 100	81·5	81·5	82·0	82·0
Blank on CaO (50)	4	—	0	—
Blank on MgO (50)	2	—	0	—
Blank on Al_2O_3 (400)	0·8	—	0	—
Blank on Fe_2O_3 (100)	0	—	0	—

All of the interferences are within experimental error; with calcium, however, the blank reading is not wholly due to the presence of Na_2O, as spectral interference also occurs. In this case, therefore, it is not legitimate merely to deduct the blank.

ELIMINATION OF INTERFERENCES—

Three forms of interference had been found; that arising from the use of perchloric and hydrochloric acids in the dissolution of the sample had already been eliminated by modification of the decomposition procedure. The spectral interference of calcium oxide band spectra on sodium can be suppressed by the addition of aluminium, preferably as the sulphate, so that the calcium is complexed by both aluminium and sulphate ions. It was necessary that

the addition should increase neither the blank errors of the determination nor the salt content of the solution to an undesirable extent and must therefore be kept to a minimum. (The purity of AnalaR aluminium sulphate is not adequate for this purpose.) The effect of potassium on sodium probably results from the ionisation equilibria in the flame. Once the temperature of the flame is adequate to cause the dissociation of the molecules of alkali salts into atoms (from which the characteristic radiation is emitted), an equilibrium exists between atoms, ions and electrons, *i.e.*,

$$Na\ (atom) \rightleftharpoons Na^+\ (ion) + e$$
$$K\ (atom) \rightleftharpoons K^+\ (ion) + e$$

As the ease of ionisation increases in the order of lithium, sodium, potassium, rubidium and caesium, it follows that potassium will ionise to a greater extent than sodium and hence produce a greater concentration of electrons. If sodium is also present the effect would be to displace the sodium equilibrium to the left, increase the concentration of sodium atoms and thus the amount of light passing through the sodium filter. For the same reasons it is unlikely that sodium could affect the potassium intensity, and these predictions are borne out by the facts observed.

It is clear that this effect could be overcome either by matching the Na_2O - K_2O of the standards to that of the sample or by adding an excess of potassium to ensure maximum light emission. Neither of these solutions was attractive as both would complicate the determination of the two main alkalis.

Caesium had already been used as an ionisation buffer by Sanui and Pace.[5] They used a concentration of 550 p.p.m. of caesium, but as their work was carried out with an air - acetylene flame in which the ionisation could be expected to be greater as a result of the higher temperature, it seemed reasonable to expect that less caesium would be required for the lower temperature of the air - propane flame.

The addition of both aluminium sulphate and an ionisation buffer would need to be made on an aliquot of the main alkali solution, as EDTA end-points for lime and magnesia, which were also determined on this solution, would deteriorate in the presence of a large excess of aluminium. A 1 + 1 dilution would appear to be convenient and it would then follow that by using a standard at 10 p.p.m. of K_2O and 5 p.p.m. of Na_2O, full-scale deflection would be equivalent to 2 per cent. of K_2O and 1 per cent. of Na_2O (0·25 g of sample in 250 ml of stock solution). As this would obviate the need for dilution in most sodium determinations, it was decided to propose the use of standards containing 20 p.p.m. of K_2O, thus eliminating the need for any dilution with most samples. Calibration graphs indicated that the degree of curvature at the higher concentration was acceptable.

To determine the amount of aluminium required to overcome the effect of up to 10 per cent. of calcium oxide in the sample (50 p.p.m. as presented to the instrument), a solution of this concentration was sprayed with the addition of 0, 100, 200, 300, 400 and 500 p.p.m. of Al_2O_3.

Blanks were also measured. The mean readings are shown in Table VIII. None of the normal sources of aluminium sulphate was found to be of sufficient purity; ultimately, a satisfactory solution was prepared from aluminium metal. The metal is dissolved in nitric and sulphuric acids, and the solution is evaporated until all nitrous fumes have been removed and the sulphuric acid fumes strongly; the residue is then dissolved in water.

TABLE VIII

EFFECT OF ALUMINIUM ADDED AS SULPHATE ON THE SPECTRAL INTERFERENCE OF LIME IN THE SODIUM DETERMINATION

Full scale set on 5 p.p.m. of Na_2O - 10 p.p.m. of K_2O; 50 p.p.m. of CaO

Al_2O_3 added, p.p.m.	0	100	200	300	400	500
Mean reading *less* blank	10·75	1	0·25	0·25	0·25	<0·25

The presence of 200 p.p.m. of Al_2O_3 appears to be adequate to suppress the calcium emission. This concentration of alumina had no interfering effect on the intensity of the alkali emission.

After establishing the desired Al_2O_3 concentration it was now necessary to determine the amount of caesium required to overcome ionisation effects. Additions of 10, 20 and 30 p.p.m. of caesium were made to solutions containing 200 p.p.m. of Al_2O_3 and various amounts of Na_2O and K_2O (Table IX).

TABLE IX

EFFECT OF INCREASING CAESIUM ADDITION ON THE INTENSITY OBSERVED FROM 4 P.P.M. OF Na_2O AND 16 P.P.M. OF K_2O IN THE PRESENCE OF 200 P.P.M. OF Al_2O_3

Full scale set on 5 p.p.m. of Na_2O - 20 p.p.m. of K_2O

Solution concentration, p.p.m.				Sodium reading		Potassium reading	
Na_2O	K_2O	Al_2O_3	Cs	Mean	Mean *less* blank	Mean	Mean *less* blank
4	16	200	10	82·3	81·5	87·8	85·9
4	16	200	20	83·3	81·6	91·3	87·0
4	16	200	30	83·4	81·4	94·0	87·0
4	0	200	10	81·8	81·0	—	—
4	0	200	20	82·8	81·1	—	—
4	0	200	30	83·2	81·2	—	—
0	16	200	10	—	—	87·8	85·9
0	16	200	20	—	—	91·0	86·7
0	16	200	30	—	—	94·4	87·4
0	0	200	10	0·8	—	1·9	—
0	0	200	20	1·7	—	4·3	—
0	0	200	30	2·0	—	7·0	—

The results indicate that 30 p.p.m. of caesium are required to ensure freedom from ionisation effects for sodium and potassium. The efficiency of the addition in the presence of various alumina contents up to a maximum of 450 p.p.m. of Al_2O_3 was examined (*i.e.*, addition of 200 p.p.m. to overcome interference by lime *plus* 250 p.p.m., for example, from the 50 per cent. contributed by raw bauxite). Although the effect of alumina variation was negligible there seemed to be a slight indication of an effect on the potassium intensity (see Table X). It therefore seemed advisable to calibrate the instrument in the presence of the median alumina content of, say, 300 p.p.m. of Al_2O_3.

TABLE X

EFFECTS OF VARIOUS ALUMINA CONTENTS ON THE INTENSITY FROM 4 P.P.M. OF Na_2O AND 16 P.P.M. OF K_2O IN THE PRESENCE OF 30 P.P.M. OF Cs

Full scale set on 5 p.p.m. of Na_2O - 20 p.p.m. of K_2O

Solution concentration, p.p.m.				Mean reading *less* blank	
Na_2O	K_2O	Al_2O_3	Cs	Sodium	Potassium
4	16	200	30	81·8	86·7
4	16	320	30	81·8	86·4
4	16	450	30	81·6	85·9

CALIBRATION FOR SODIUM, POTASSIUM AND LITHIUM—

A propane flow-rate of 400 ml minute^{-1} was reached as the mean figure obtained when several analysts set the flame to give "well defined blue cones." The variation in gas setting was considerable, but although the sensitivity varied the shape of the calibration graph was unchanged.

Lithium—The sensitivity of the instrument was inadequate to permit calibration with less than 20 p.p.m. of Li_2O standard and for this reason it was decided to continue the practice of determining this element on the undiluted stock alkali solution. As potassium is known to interfere spectrally with lithium a graph was constructed of this interference (Fig. 2) to enable it to be corrected for.

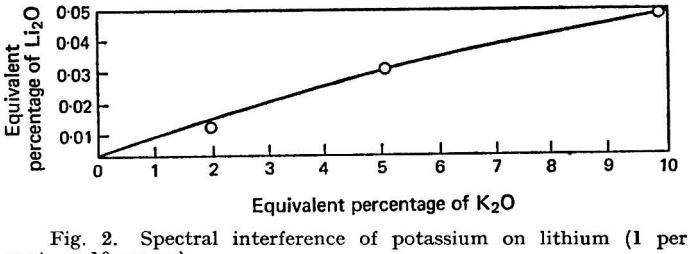

Fig. 2. Spectral interference of potassium on lithium (1 per cent. ≡ 10 p.p.m.)

Sodium and potassium—Sodium was then calibrated for up to 1 per cent. (5 p.p.m.) of Na_2O and potassium for 4 per cent. (20 p.p.m.) of K_2O on the assumption that the stock solution would be at a concentration of 1 g l^{-1} of sample and that this solution would be diluted 1 + 1. Calibration solutions, in addition to their appropriate alkali contents, also contained 30 p.p.m. of caesium, 300 p.p.m. of Al_2O_3 and nitric acid so as to be as nearly as possible equivalent to the diluted sample solution.

The curvature of the potassium calibration graph is slightly greater at 20 p.p.m. than at 10 p.p.m. full-scale deflection, but is still acceptable. Each scale division of the top 10 per cent. of the graph is now equal to 0·055 per cent. of K_2O whereas, on the previous basis (10 p.p.m. of K_2O) and allowing for the 4-fold dilution needed, it was 0·044 per cent.

TRIALS ON SYNTHETIC SAMPLES—

As most of the available standards have been analysed by the old procedure the accuracy of the alkali determinations must be in doubt. Synthetic sample solutions were therefore prepared covering a wide range of composition, with and without the appropriate alkali content, the latter solutions acting as blanks. The composition of these standards and the results for alkalis are shown in Table XI. The solutions were made at a sample concentration of 1 g l^{-1} except for the high-silica materials, which were made at 4 g l^{-1} as required by the British Standard method.

TABLE XI

COMPOSITION OF SYNTHETIC STANDARDS AND RESULTS FOR ALKALIS

	Oxide content, per cent.									
Synthetic samples	Al_2O_3	Fe_2O_3	CaO	MgO	K_2O added	K_2O found	Na_2O added	Na_2O found	Li_2O added	Li_2O found
Building clay CAS 3	16	6	2·4	2	2·50	2·46	0·40	0·49	0·02	0·03
Clay CAS 5	30	1	0·2	0·4	2·50	2·46	0·40	0·49	0·04	0·04
Earthenware body CAS 7	18	0·4	0·8	0·4	1·25	1·27	0·50	0·49	0·01	0·01
Firebrick B.C.S. 269	32	3·2	0·2	1	2·50	2·49	0·50	0·48	0·13	0·13
Firebrick B.C.S. 315	40	3·2	0·4	0·4	0·50	0·51	0·10	0·10	0·09	0·09
Sillimanite B.C.S. 309	40*	1·6	0·4	0·2	0·50	0·48	0·30	0·29	0·01	0·01
High purity silica B.C.S. 313	0·2	0·04	0·02	0	0·025	0·023	0·013	0·013	0	0
Silica brick B.C.S. 314	0·8	0·5	1·8	0·05	0·063	0·063	0·050	0·049	0·01	0·015
Silica brick B.C.S. 267	0·8	0·8	1·8	0·05	0·125	0·128	0·075	0·073	0	0·006
Silica brick US 102	2	0·65	2·3	0·15	0·313	0·313	0·025	0·025	0	0·005
Potash feldspar 2 CAS 11	18	0·4	0·4	0·1	10·0	10·0	3·00	2·95	0	0
Soda feldspar 2 CAS 13	16	0·1	0·25	0	0·75	0·77	8·00	7·95	0	0·01
Potash feldspar US 70a	18	0·1	0·1	0	12·5	12·5	2·50	2·48	0	0
Feldspar US 99a	20	0·1	0·5	0	5·00	4·98	6·00	6·00	0	0
Borax frit AN 30	8	0·2	14	0·2	1·25	1·27	9·00	9·00	0·10	0·13

CAS, 2 CAS and AN samples are analysed samples used by the British Ceramic Research Association for co-operative work.

* To represent the proportion of alumina dissolved during decomposition.

The blank solutions were made for a dual purpose, firstly to evaluate the sodium and potassium blanks arising from the additions of other constituents and also to evaluate the potential interferences in the determination of lithium. Positive blank errors for lithium were found in samples equivalent to B.C.S. 314, B.C.S. 267, US 102 and AN 30. All of these samples had a low Al_2O_3-to-CaO ratio. Although the errors are small their occurrence would suggest that caution must be applied in the interpretation of results for lithium if the Al_2O_3-to-CaO ratio in the stock solution is less than that required to overcome the calcium interference, *i.e.*, 4:1. The remainder of the results were satisfactory and so several standard samples were re-analysed to determine the alkali content.

ANALYSIS OF ACTUAL SAMPLES—

The results (Table XII) indicate, as might be expected, little difference between the old and new figures when the alkali content is low. With the feldspars the results show the expected movements in that the potassium results are generally higher following removal of the depressant effect of chlorine-containing acids, and the sodium results are higher because of removal of ionisation errors.

TABLE XII

ALKALI DETERMINATIONS ON ANALYSED AND STANDARD SAMPLES

		K_2O, per cent.		Na_2O, per cent.		Li_2O, per cent.	
Sample type	Code	Found	Previous value	Found	Previous value	Found	Previous value
Building clay ..	CAS 3	2·44	2·41	0·58	0·63	0·02	0·02
Clay	CAS 5	2·57	2·65	0·37	0·40	0·02	0·04
Earthenware body	CAS 7	1·51	1·45	0·72	0·74	0·03	0·01
Firebrick	B.C.S. 269	2·63	2·62	0·38	0·37	0·13	0·13
Firebrick	B.C.S. 315	0·56	0·52	0·12	0·13	0·10	0·09
Sillimanite	B.C.S. 309	0·48	0·46	0·31	0·34	0·02	0·01
High purity silica ..	B.C.S. 313	0·04	0·04	0·007	<0·01	<0·01	<0·01
Silica brick ..	B.C.S. 314	0·09	0·09	0·04	0·05	<0·01	0·01
Silica brick ..	B.C.S. 267	0·13	0·14	0·05	0·06	<0·01	—
Silica brick ..	US 102	0·33	0·32	0·015	0·015	<0·01	<0·01
Feldspar	2 CAS 11	10·61	10·35	2·91	2·88	<0·01	—
Feldspar	2 CAS 13	0·65	0·62	8·28	8·02	0·01	—
Feldspar	US 70a	11·78	11·8*	2·48	2·55*	0·01	—
Feldspar	US 99a	5·34	5·2*	6·26	6·2*	0·01	—
Borax frit	AN 30	1·14	1·13	8·49	8·2† to 8·8‡	0·05	0·10

* United States Bureau of Standards (provisional figures).
† Determined by the old flame-photometric method; closer simulation of standards gave 8·44.
‡ Gravimetric method, with the zinc uranyl acetate precipitate dried at room temperature.
Comparison with the borax frit is difficult because of the wide range of results previously obtained. The original flame-photometric results were low, as might be expected from the ionisation effects, and most errors in the gravimetric method (giving sodium zinc uranyl acetate) used tend to be positive.

ALKALIS IN HIGH-LIME MATERIALS—

For samples of high-lime content such as bone ash, dolomites, cements and calcium aluminates, the addition of 200 p.p.m. of Al_2O_3 was not expected to remove the interference in the determination of sodium. To allow for the effects of 60 per cent. of calcium oxide, it was found that the addition of alumina needed to be increased to 1500 p.p.m.

To test the accuracy of the modification, standards were synthesised representing the top, bottom and mid-points of the calibration graph for sodium and potassium. The degree of bowing was identical with that with the lower Al_2O_3 content. Two high-lime samples were synthesised representing a bone ash and an ignited dolomite in both a "test" and blank form. The results for the determination of alkalis in these samples are shown in Table XIII, together with the composition of the synthetic solutions.

"Blanks" of 0·03 and 0·07 per cent. of Li_2O were found for the bone ash and dolomite, respectively. In view of the low alkali contents of this type of sample, it may be better to treat the whole of the sample solution with caesium and Al_2O_3 and then determine Na_2O, K_2O and Li_2O without dilution. It is not usual to determine calcium and magnesium oxides on the solution prepared for the alkali determination with these types of material. However, it would seem that atomic-absorption spectroscopy offers a better solution to the problem of the analysis of these materials for alkalis.

TABLE XIII

COMPOSITION AND RESULTS FOR SYNTHETIC SAMPLES WITH HIGH LIME CONTENT

	Oxide content, per cent.										
Sample	Al_2O_3	Fe_2O_3	CaO	MgO	P_2O_5	K_2O added	K_2O found	Na_2O added	Na_2O found	Li_2O added	Li_2O found
Bone ash	0·1	0·1	56	1	40	0·05	0·04	0·30	0·30	0·05	0·08
Dolomite	0·2	0·2	60	40	—	0·05	0·05	0·10	0·10	0·05	0·12

CONCLUSIONS

Town gas is now too variable in composition to form the basis of an instrumental method for the flame-photometric determination of alkalis, and methane has been found to be less satisfactory than propane with regard to depressive effects and to flame stability. This would suggest that North Sea gas would be equally unsuitable. Bottled propane would seem to offer the best source gas of constant composition and is less subject to depressive effects.

The flame-photometric method, although free from interference from anions as originally given, has now been found to be subject to depressive effects because of the chlorine-containing acids used for the decomposition, to give low results for some sodium determinations because of ionisation effects and to give some high results because of spectral interference of calcium on sodium and lithium.

The depressive effects caused by the presence of hydrochloric and perchloric acids can be removed by the use of sulphuric and nitric acids for the decomposition of the sample.

The original method showed no discernible interference of one alkali on another, but it was now found that potassium interfered in the determination of sodium. The tendency to obtain low results for sodium when the K_2O-to-Na_2O ratio is less than 2:1 can be overcome by the addition of a caesium sulphate ionisation buffer to give a final concentration of 30 p.p.m. of caesium.

The spectral interference of calcium on the sodium determination can be excluded by the addition of aluminium sulphate solution (containing 200 p.p.m. of Al_2O_3) to an aliquot of the main alkali stock solution. The interference on lithium is not serious unless the alumina-to-lime ratio in the sample is less than 4:1. When this is so, more accurate results would be obtained either by measurement on the aliquot diluted for the sodium and potassium determination, with a 2-fold loss in sensitivity, or by addition of caesium and aluminium to the whole of the stock alkali solution. If accurate results for lithium oxide are required the additions of caesium and an increased amount of aluminium must be made to the whole of the 250 ml of stock alkali solution.

METHOD

APPARATUS—

The modification essential to the use of propane gas is shown in Fig. 1. Optional modifications that aid the reproducible operation of the instrument are replacement of the carbon potentiometer used in the sensitivity control by a 20-kΩ wire-wound helical potentiometer and the addition of a 250-μF electrolytic condenser to improve the damping of the galvanometer.

REAGENTS—

Unless otherwise stated, all reagents should be of analytical-reagent grade when available and distilled water should be used throughout the analysis.

Aluminium sulphate solution equivalent to approximately 10 *mg ml*$^{-1}$ *of* Al_2O_3—Clean analytical-reagent grade aluminium metal by washing it with hydrochloric acid, ethanol and ether. Weigh 10·58 g of the clean, dry metal, add 40 ml of sulphuric acid (sp.gr. 1·84), 120 ml of nitric acid (sp.gr. 1·42) and about 50 ml of water. Allow to react in the cold, then raise the temperature gradually until dissolution of the metal is complete and heat the solution until all of the nitric oxides are expelled and the sulphuric acid fumes strongly. Cool, dissolve the crystalline melt in distilled water and dilute to 2 litres.

Aluminium sulphate solution equivalent to approximately 2000 *p.p.m. of* Al_2O_3—Dilute 400 ml of the above aluminium sulphate solution to 2 litres.

Caesium sulphate solution equivalent to approximately 300 *p.p.m. of Cs*—Dissolve 0·41 g of Cs_2SO_4 (Johnson Matthey Specpure) in 1 litre of water.

Hydrofluoric acid, 40 *per cent.*

Dilute nitric acid (1 + 19).

Sulphuric acid - nitric acid mixture—To 650 ml of water, add 100 ml of dilute sulphuric acid (1 + 1) and 250 ml of nitric acid (sp.gr. 1·42).

STANDARD SOLUTIONS—

Lithium solution A equivalent to 400 *p.p.m. of* Li_2O—Dehydrate lithium sulphate monohydrate by heating it at 150 °C for 24 hours. Dissolve 1·4719 g of the anhydrous lithium sulphate in 1 litre of water.

Lithium top standard equivalent to 20 *p.p.m. of* Li_2O—Dilute 25·0 ml of the lithium solution A *plus* 40 ml of dilute nitric acid (1 + 19) to 500 ml.

Potassium - sodium solution A equivalent to 400 *p.p.m. of* K_2O *and* 100 *p.p.m. of* Na_2O—Dissolve 0·7400 g of anhydrous potassium sulphate (dried at 150 °C) and 0·2292 g of anhydrous sodium sulphate (dried at 150 °C) in 1 litre of water.

Potassium - sodium solution B equivalent to 40 *p.p.m. of* K_2O *and* 10 *p.p.m. of* Na_2O—Dilute 50·0 ml of the potassium - sodium solution A to 500 ml.

Potassium - sodium top standard equivalent to 20 *p.p.m. of* K_2O *and* 5 *p.p.m. of* Na_2O—To a 1-litre calibrated flask, add 40 ml of dilute nitric acid (1 + 19), 100 ml of the caesium sulphate solution, 30 ml of aluminium sulphate solution (equivalent to approximately 10 mg ml^{-1} of Al_2O_3) and 50·0 ml of the potassium - sodium solution A; dilute to 1 litre.

Potassium - sodium zero standard—This solution is used as a diluent for alkalis in excess of 4 per cent. of K_2O and 1 per cent. of Na_2O. To a 1-litre calibrated flask, add 40 ml of dilute nitric acid (1 + 19), 100 ml of the caesium sulphate solution and 30 ml of aluminium sulphate solution (equivalent to approximately 10 mg ml^{-1} of Al_2O_3); dilute to 1 litre. Check the blanks for Na_2O and K_2O by spraying with the zero set on distilled water and the full scale set on the top standard. Typical values are deflections of two divisions for Na_2O and seven for K_2O. Readings greatly in excess of these invalidate the use of the buffer, and the origin of the blank must be investigated and rectified.

INTERMEDIATE CALIBRATION SOLUTIONS—

Lithium—To four 50-ml calibrated flasks, add 4 ml of dilute nitric acid (1 + 19) and 10, 20, 30 and 40 ml of the lithium top standard; dilute to 50 ml. These concentrations are equivalent to 4, 8, 12 and 16 p.p.m. of Li_2O or 0·4, 0·8, 1·2 and 1·6 per cent. of Li_2O.

Potassium - sodium—To seven 200-ml calibrated flasks, add 8 ml of dilute nitric acid (1 + 19), 20 ml of the caesium sulphate solution, 6 ml of aluminium sulphate solution (equivalent to 10 mg ml^{-1} of Al_2O_3), and 5, 10, 20, 40, 50, 60 and 80 ml of the potassium - sodium solution B to give 1, 2, 4, 8, 10, 12 and 16 p.p.m. of K_2O and 0·25, 0·5, 1, 2, 2·5, 3 and 4 p.p.m. of Na_2O, respectively, equivalent at the dilution used to 0·2, 0·4, 0·8, 1·6, 2·0, 2·4 and 3·2 per cent. of K_2O and 0·05, 0·1, 0·2, 0·4, 0·5, 0·6 and 0·8 per cent. of Na_2O, respectively.

CALIBRATION—

Construct calibration graphs by setting the zero of the instrument on the zero standard and full-scale deflection on the appropriate top standard.

DECOMPOSITION OF THE SAMPLE—

Weigh 0·250 g (1 g for high-silica materials) of the sample dried at 110 °C into a platinum dish and ignite gently to remove organic matter. To the cool dish, add 10 ml of sulphuric acid - nitric acid mixture and 10 ml of hydrofluoric acid, and evaporate to dryness on a sand-bath in a fume cupboard, taking care to prevent spurting. Cool the dish, add 10 ml of the sulphuric acid - nitric acid mixture and rinse down the sides of the dish with water. Evaporate carefully to dryness.

To the cool dry residue, add 20 ml of dilute nitric acid (1 + 19) and warm to dissolve. Cool, filter the solution if necessary through a No. 42 Whatman filter-paper and wash the dish and filter-paper with cold water. Dilute the filtrate and washings with water to 250 ml to give stock solution A.

DETERMINATION OF ALKALIS—

Potassium and sodium—Transfer 25·0 ml of the stock solution A to a 50-ml calibrated flask containing 5 ml each of the caesium sulphate solution and aluminium sulphate solution (equivalent to 2000 p.p.m. of Al_2O_3) (Note). Dilute to 50 ml to give solution B.

Set up the instrument, insert the appropriate filter and spray the sample solution B, setting full scale on the potassium - sodium top standard and the zero with the potassium - sodium zero standard. If the alkali content is in excess of the normal range, readings can be brought on to scale, either by dilution of solution B with zero standard or by appropriate dilution of an aliquot of solution A before addition of caesium and aluminium sulphates. It is essential to maintain, in the solutions presented to the instrument, concentrations equivalent to additions of 5 ml of the caesium sulphate solution and 5 ml of aluminium sulphate solution (equivalent to 2000 p.p.m. of Al_2O_3) in 50 ml of solution.

NOTE—

Attempts to simplify the procedure by making a mixed solution of caesium and aluminium sulphates are not advised, as serious loss of caesium will occur because of the slow precipitation of sparingly soluble caesium aluminium sulphate.

Lithium—Insert the lithium filter and spray the stock solution A, setting full scale on 20 p.p.m. of Li_2O and zero on distilled water.

Lithium is subject to positive errors caused by potassium light passing through the filter. The magnitude of the error must be ascertained by setting up the instrument as for the lithium calibration and spraying solutions of 20, 50 and 100 p.p.m. of K_2O, equivalent to sample contents of 2, 5 and 10 per cent. of K_2O, and a correction graph prepared. Note the reading and apply the appropriate correction for the K_2O content determined, by reference to the correction graph.

EXTENSION OF THE GENERAL METHOD TO THE ANALYSIS OF HIGH-LIME MATERIALS

Only the modification for sodium and potassium will be described. The determination of lithium is rarely required in these materials and, because of the low sensitivity and susceptibility to interference, it is of doubtful accuracy.

SPECIAL SOLUTIONS REQUIRED—

High-lime potassium - sodium zero standard—To a 1-litre calibrated flask, add 40 ml of dilute nitric acid (1 + 19), 100 ml of the caesium sulphate solution and 150 ml of aluminium sulphate solution (equivalent to approximately 10 mg ml^{-1} of Al_2O_3); dilute to 1 litre.

High-lime potassium - sodium top standard equivalent to 20 p.p.m of K_2O and 5 p.p.m. of Na_2O—To a 1-litre calibrated flask, add 40 ml of dilute nitric acid (1 + 19), 100 ml of the caesium sulphate solution, 150 ml of aluminium sulphate solution (equivalent to approximately 10 mg ml^{-1} of Al_2O_3) and 50·0 ml of the potassium - sodium solution A; dilute to 1 litre.

PROCEDURE FOR THE DETERMINATION OF SODIUM AND POTASSIUM—

Transfer 25·0 ml of the stock solution A to a 50-ml calibrated flask containing 5 ml of the caesium sulphate solution and 7·5 ml of aluminium sulphate solution (equivalent to about 10 mg ml^{-1} of Al_2O_3). Dilute to 50 ml. Spray the solution against the top and zero standards synthesised above.

We thank Dr. N. F. Astbury, Director of Research of the British Ceramic Research Association, for permission to publish this paper.

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Received *July 29th*, 1970
Accepted *March 22nd*, 1971

A Potentiometric Procedure for the Assay of Isonicotinic Acid Hydrazide (Isoniazid)

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A potentiometric procedure for the assay of isonicotinic acid hydrazide (isoniazid) with vanadium(V) at room temperature is described. The reduction of vanadium(V) to vanadium(IV) by isoniazid in an acidic medium is catalysed by osmium tetroxide, and the application of this method to the assay of isoniazid in pharmaceutical preparations is considered. Oxalic acid interferes in the determination, although commonly used excipients such as starch, dextrin, sucrose, glucose, lactose and gum acacia do not interfere.

Several colorimetric[1,2,3,4] and titrimetric[5 to 17] procedures have been reported for the assay of isoniazid in pharmaceutical preparations. The hydrazino group in the compound is susceptible to oxidation and many of the cited titrimetric procedures depend on this property. The potentiometric titration of isoniazid with potassium bromate[7] in an 8 to 12 per cent. hydrochloric acid medium and in the presence of potassium bromide is considered to be the best method, although Kühni, Jacob and Grossglauser[13] state that the titration of isoniazid with 0·05 N potassium bromate gave results that were 0·5 per cent. too high.

The redox reaction between isoniazid and quinquivalent vanadium does not appear to have been considered as the basis of a quantitative titrimetric method. Recently Krych and Lipiec[18] have reported a spectrophotometric method for the determination of vanadium(V) with isoniazid that involves measuring at a wavelength of 420 nm the absorbance of the orange - red complex formed between the reactants at a pH of 1·98. These authors state that the complex is not stable for longer than 10 to 15 minutes and that the results are reproducible only to within 4 per cent. Gowda and Gopala Rao[19] proposed a method for the assay of isoniazid that involves treating an aliquot of an aqueous solution of the compound with an excess of 0·05 M sodium vanadate solution in a medium of 4 M sulphuric acid, allowing the reaction mixture to stand for one minute and titrating the unreacted vanadate with a standard solution of ammonium iron(II) sulphate. *N*-Phenylanthranilic acid is the redox indicator used in this case. These authors claimed definite advantages for their method over iodimetric and bromate titration procedures because the common excipients such as lactose, glucose and starch did not interfere in the determination. We have undertaken a detailed study of the reaction and have succeeded in developing an accurate potentiometric method for the assay of the compound in pharmaceutical preparations.

Experimental

Reagents—

Sodium vanadate solution, 0·1 N—A standard vanadium(V) solution was prepared (by dissolving sodium orthovanadate in water) and standardised against a standard solution of potassium dichromate, which was in turn standardised against a solution of ammonium iron(II) sulphate, the end-points in both the titrations being located potentiometrically.

Isonicotinic acid hydrazide—The isoniazid used in this investigation was of U.S.P. grade. A 0·05 M solution in water was prepared from a sample that had been twice recrystallised from aqueous ethanol and dried at 110 °C for 2 hours. The aqueous solution thus prepared was standardised against a standard solution of potassium bromate following the potentiometric method described by Vulterin and Zyka.[7]

Osmium tetroxide—A 0·1 per cent. solution in 0·1 N sulphuric acid was prepared and stored in an amber glass bottle (sample supplied by Johnson Matthey Chemicals Ltd., London).

This reagent (in 0·2-ml amounts) was used as a catalyst in all the experiments described in this paper.

Orthophosphoric acid—E. Merck's "Pro Analysi" grade orthophosphoric acid (85 per cent.) was used throughout this investigation (any analytical-reagent grade acid can be used).

Sulphuric acid—Analytical-reagent grade nitrogen-free sulphuric acid was used without further purification.

All other reagents and chemicals used were of analytical-reagent grade.

Nydrazid and Isonex tablets, manufactured by Squibb Pharmaceuticals and Dumex Pharmaceuticals (India), respectively, were extracted with water and the aqueous extracts analysed to determine the active constituent (isoniazid).

APPARATUS—

The potentiometric titration assembly used consists of a Pye potentiometer graduated in millivolts, a galvanometer (Cambridge Instrument Co., London), a saturated calomel reference electrode, a bright-platinum rod (0·2 mm in diameter) as indicator electrode and a porous-plate salt-bridge filled with a saturated solution of potassium chloride. The titration mixture is stirred with an electromagnetic stirrer.

Preliminary experiments showed that a direct titration of isoniazid with sodium vanadate in an acidic medium containing any mineral acid would not be possible because the red complex formed on the addition of sodium vanadate decomposed slowly and the potentials were not stable. The use of several catalysts such as orthophosphoric acid, iodine monochloride, osmium tetroxide and copper(II) sulphate did not improve the situation. The reverse titration, *i.e.*, that of vanadate with isoniazid, is also slow at both room and elevated temperatures in media containing various concentrations of sulphuric, perchloric and phosphoric acids (ranging from 0·25 to 4·0 M). However, the reaction was markedly catalysed by osmium tetroxide, although in a 0·5 to 2·0 M sulphuric acid medium the potentials did not attain stable values. Nevertheless, further experiments showed that the addition of 1·0 ml of orthophosphoric acid gave stable potentials. We have therefore carried out experiments to ascertain the optimum concentrations of the catalyst and phosphoric or sulphuric acid that will give a satisfactory potentiometric titration, each time keeping one of the parameters constant.

Effect of varying the catalyst concentration—Experiments on the variation of the osmium tetroxide catalyst concentration in the range 0·05 to 2·0 ml of a 0·1 per cent. solution in 50 ml of the titration mixture (containing the optimum concentration of either phosphoric acid or sulphuric acid for this reaction) have shown no significant deviations in the accuracy of the method. However, we noticed that when the catalyst solution was present in volumes below 0·2 ml, the potentials were not stable near the equivalence point, thus causing considerable delays with each determination.

Effect of varying phosphoric or sulphuric acid concentration—In these experiments 0·2 ml of osmium tetroxide was added to the titration mixture and the acid concentration was varied. Experiments showed that variation of the phosphoric acid volume from 0·5 ml to 20·0 ml (in a total volume of 50 ml of titration mixture) did not affect either the accuracy or the speed of the titration, while greater concentrations of phosphoric acid resulted in a higher consumption of isoniazid and an abnormal drift in the potentials near the equivalence point. Moreover, the potential break near the equivalence point was not sharp but evenly distributed between successive additions of the titrant, thus leading to substantial errors.

In the case of titrations in media 0·25 to 4·0 M in sulphuric acid, we found that without the addition of at least 1·0 ml of phosphoric acid the potentials were not stable and that the break in potential at the equivalence point could not be located accurately. At higher concentrations of sulphuric acid the results were always several per cent. lower, even if phosphoric acid and osmium tetroxide were added. Because of these findings, we recommend the following procedure for the potentiometric assay of isoniazid with sodium vanadate at room temperature.

RECOMMENDED PROCEDURE—

A suitable volume of the standard vanadium(V) solution is transferred to a 150-ml titration vessel and 1 to 20 ml of 85 per cent. orthophosphoric acid (or a mixture of 2·5 to 10·0 ml of sulphuric acid (1 + 1) and 1·0 ml of 85 per cent. orthophosphoric acid) *plus* 0·2 ml

of 0·1 per cent. osmium tetroxide solution are added. The resulting mixture is diluted to 50 ml and titrated potentiometrically with 0·05 M isoniazid solution, the potentials being noted 1 minute after the addition of each portion of the titrant. A typical potential *versus* volume graph is given in Fig. 1.

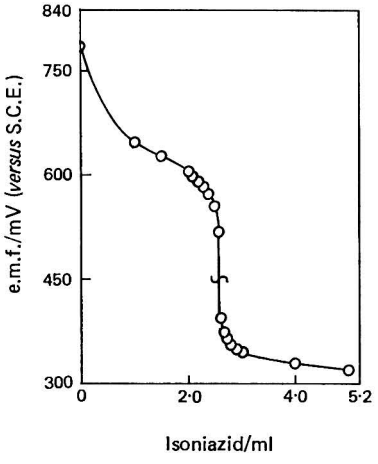

Fig. 1. Potentiometric titration of vanadium(V) with isoniazid

The potential break at the equivalence point is about 100 to 120 mV per drop (approximately 0·04 ml) of 0·05 M isoniazid. A large number of determinations of isoniazid have been carried out according to the recommended procedure and the results compared with those obtained by the B.P. method.[20] Some typical results are given in Table I and show that the proposed method yields results that agree with those by the standard B.P. method with an average deviation of 0·45 per cent.

TABLE I

COMPARATIVE ASSAYS OF ISONIAZID

	Isoniazid/mg							
By B.P. method ..	1·04	3·19	6·04	9·34	12·2	16·3	22·4	25·9
By proposed method ..	1·05	3·21	6·03	9·29	12·1	16·4	22·4	26·1
Deviation, per cent. ..	0·96	0·63	0·17	0·54	0·82	0·61	0·00	0·71

Mean deviation = 0·56 per cent.

APPLICATION OF THE PROPOSED METHOD TO THE ASSAY OF ISONIAZID IN PHARMACEUTICAL PREPARATIONS—

Two tablets are dissolved in 50 ml of water, the resulting mixture is filtered through an IG4 sintered-glass crucible and the filtrate is made up to 100 ml. This solution is then transferred to a microburette and the isoniazid content ascertained by potentiometric titration against a standard solution of vanadium(V) according to the recommended procedure. The results thus obtained were compared with those obtained by using the standard B.P. method,[20] and are given in Table II. The relative deviation for each method is also shown.

Interferences—Sucrose, glucose, lactose, starch, dextrin and gum acacia, which are usually used as excipients in pharmaceutical preparations, do not interfere in this determination. However, oxalic acid interferes at all concentrations and uranium(VI) and chromium(III) also interfere. In all the interference studies the excipients were added in amounts up to a 50-fold excess relative to the calculated amount of isoniazid at the end of each titration.

TABLE II

ASSAY OF ISONIAZID IN TABLETS

Tablet assayed	Amount of isoniazid found with the B.P. method/mg	Deviations from average	Amount of isoniazid found with the proposed method/mg	Deviations from average
Nydrazid	94·9	−0·20	95·4	+0·20
	94·7	−0·40	94·9	−0·30
	94·9	−0·20	95·4	+0·20
	95·3	+0·20	94·9	−0·30
	95·4	+0·30	95·0	−0·20
	95·4	+0·30	95·5	+0·30
	Mean 95·1	s.d. 0·30	Mean 95·2	s.d 0·28
Isonex	101·2	+0·10	101·4	+0·30
	101·1	0·00	101·0	−0·10
	101·1	0·00	100·9	−0·20
	101·2	+0·10	101·0	−0·10
	100·9	−0·20	101·1	+0·00
	101·0	−0·10	101·3	+0·20
	Mean 101·1	s.d. 0·12	Mean 101·1	s.d. 0·20

DISCUSSION

Under the experimental conditions prescribed, 4 moles of vanadium(V) are reduced per mole of isoniazid oxidised, according to the reaction—

$$C_5H_4NCONH.NH_2 + 4\ V(V) + H_2O \rightarrow C_5H_4NCOOH + 4\ V(IV) + N_2 + 4H^+$$

Whereas an orange-red 1:1 complex formed between vanadium(V) and isoniazid at a pH of 1·98 has been reported by Krych and Lipiec,[18] under our experimental conditions isoniazid is quantitatively oxidised to nitrogen and isonicotinic acid. (When a drop of osmium tetroxide solution is added to the vanadium(V) - isoniazid complex at pH 2, copious evolution of nitrogen occurs and the solution turns blue, thus showing that the complex undergoes internal oxidation - reduction under the catalytic influence of octavalent osmium.) Further work on the elucidation of the reaction mechanism is in progress and will be reported separately.

One of us (G.B.B.R.) thanks the Council of Scientific and Industrial Research, India, for the award of a Junior Research Fellowship. We also thank Albert David (Private) Ltd., Calcutta, India, for the generous gift of a sample of isoniazid, and Professor G. Gopala Rao for his helpful suggestions and keen interest in this investigation.

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Received *July 27th*, 1970
Accepted *January 12th*, 1971

The Determination of Nikethamide and Other Compounds in Pharmaceutical Dosage Forms by Thin-layer Chromatography

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The qualitative examination and quantitative assay of pharmaceutical dosage forms containing nikethamide by thin-layer chromatography is described. When applicable, the determination of accompanying active compounds such as adenosine, caffeine, strychnine and theophylline by the same method is also described. Individual quantitative determinations of the eluted drugs are performed by ultraviolet spectrophotometry.

NN-DIETHYLNICOTINAMIDE (Coramine, Ciba; generic name nikethamide) is a widely used respiratory stimulant for oral and parenteral use, which is formulated alone and in multi-component pharmaceutical dosage forms. It can be synthesised by treating nicotinic acid with thionyl chloride and allowing the resultant acid chloride to react with diethylamine hydrochloride.[1] The liquid amide is purified by vacuum distillation, during which a very minor fraction consisting of *N*-ethylnicotinamide is occasionally obtained (Candolfi, E., and Hürzeler, H., personal communication) as a result of the presence of monoethylamine impurity in the diethylamine used for the synthesis. The two solvent systems used in the quantitative thin-layer chromatographic determination of nikethamide (see Table II) enable the two compounds to be adequately separated.

Nikethamide is a markedly stable compound, and although the amide linkage can be hydrolysed by refluxing it with acid or, more readily, with a base, hydrolytic or oxidative decomposition has not been observed in accelerated stability studies on dosage forms containing the compound.[2] In the course of the present work, no evidence could be found *in vitro* of oxidative *N*-oxide formation or ring cleavage, although *N*-oxide formation is a known metabolic pathway for nikethamide in mammalian tissues.[3]

Copious analytical literature exists on the assay of nicotinic acid amides, including nikethamide. Dosage control of nikethamide has been traditionally carried out by non-aqueous titrimetry with perchloric and acetic acids,[4] the method having been applied to both the free base and its calcium chloride and thiocyanate salts. Colorimetric methods have involved the use of the hydroxamic acid reaction for amides and subsequent complexation with iron(III),[5] ion-pair formation with bromophenol blue[6] and the Zincke - König reaction,[7,8] in which cleavage of the pyridine ring is followed by condensation of the resulting aldehyde with a suitable amine.[9,10,11] The last method suffers from the disadvantage that it requires the use of the highly toxic reagent cyanogen bromide. Gas - liquid chromatographic assay with a range of stationary phases has been described for nikethamide alone and in the presence of nicotinic acid.[12,13]

Several paper and thin-layer chromatographic methods for detecting nikethamide have been published.[14,15,16] Spectrophotometric determination of the drug following its isolation by thin-layer chromatography has also been described[17] in connection with the control of doping of horses. The present paper describes the identification and quantitative determination of nikethamide in solid and liquid pharmaceutical dosage forms by thin-layer chromatography on silica gel. In the quantitative assays, the evaluation, with the exception of ephedrine (see below), is performed by ultraviolet spectrophotometry ($\lambda_{max.}$ for nikethamide in ethanol = 261 nm; ϵ = approximately 3400 at a concentration of 0·02 mg ml^{-1} and A = about 0·3). Absorption maxima and end dilution concentrations for the other four active compounds determined by this method are given in Table I.

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Experimental

Reagents—

All reagents used were of analytical-reagent grade.

Standard substances, which were obtained commercially, were of pharmaceutical purity [*e.g.*, B.P., U.S.P. or DAB (Deutsche Arzneibuch) grades]. The nikethamide used was a typical production batch of Coramine.* The calcium chloride and thiocyanate salts of nikethamide were obtained from the same source.

For thin-layer chromatography, plates pre-coated with a 0·25-mm layer of silica gel HF_{254}† were used without further treatment.

Developing solvent systems—

1. Chloroform - ethanol (95 per cent.) (10 + 1 v/v).
2. Ethyl methyl ketone - ammonia solution (sp.gr. 0·9) (10 + 1 v/v) or isopropyl alcohol - ammonia solution (sp.gr. 0·9) (10 + 1 v/v).
3. Chloroform - ethanol (95 per cent.) - ammonia solution (sp.gr. 0·9) (100 + 20 + 1 v/v).
4. Chloroform - methanol - glacial acetic acid (25 + 65 + 10 v/v).
5. Isopropyl alcohol - formic acid - water (70 + 20 + 10 v/v).
6. Ethyl acetate - ethanol (95 per cent.) (90 + 10 v/v).
7. Cyclohexane - chloroform - diethylamine (5 + 4 + 1 v/v).
8. n-Butyl alcohol - glacial acetic acid - diisopropyl ether - water (9 + 6 + 3 + 1 v/v), without chamber saturation.
9. Chloroform - ethanol (95 per cent.) - diethyl ether (5 + 5 + 2 v/v), plates developed three times with intermediate drying in cool air stream.

Chromatographic procedure—

The standard techniques of ascending thin-layer chromatography were used[18]; when not otherwise indicated, plates were developed over a distance of 10 to 15 cm in a saturated chamber, saturation being achieved by lining the tank with strips of chromatographic paper soaked in developing solvent. At least half an hour was allowed for the tank atmosphere to reach equilibrium before plates were developed at room temperature.

Viewing of spots—

Nikethamide was made visible by its fluorescence-quenching effect when developed plates were viewed under an ultraviolet lamp (254 nm) or by the Munier and Macheboeuf variation of the Dragendorff reagent (iodobismuthate, reagent No. 92 in reference 19). *N*-Ethylnicotinamide was made visible by fluorescence quenching or with the Reindel-Hoppe reagent (chlorine followed by *o*-tolidine, reagent No. 45 in reference 19), which also gave a faint coloration with nikethamide. Other active compounds accompanying nikethamide in dosage forms were also made visible by one of these methods or by charring with methanolic sulphuric acid (1 + 1 v/v) at 120 °C.

Qualitative examination—

For the identity test, active compounds were extracted from powdered solid dosage forms with water, ethanol or ethanol - water (1 + 1 v/v). Dilutions were normally adjusted to give a nikethamide concentration of 20 mg ml^{-1}. Standard solutions of each active compound were prepared at the same concentration as for the test solution and with the same solvent; 5 μl of standard and test solutions were chromatographed. Developed plates were dried in a stream of warm air, and the compounds were made visible. The identity of each active compound was confirmed when corresponding standard and test spots behaved in a similar way and exhibited the same R_F value after being made visible.

Quantitative assay—

Dosage forms were extracted (by using an ultrasonic bath and temperatures of up to 50 to 60 °C, if necessary) or diluted with water or ethanol - water (1 + 1 v/v) to give a nikethamide concentration of 2 mg ml^{-1}. A standard solution at the same concentration

* Obtained from the Pharmaceutical Chemical Production Division, Ciba-Geigy (Klybeck) Limited, Basel.

† Merck, Darmstadt.

was prepared with the same solvent. By using a 100-μl microsyringe (*e.g.*, Terumo, Japan), 100 μl of standard and test solutions were applied as separate 5 to 7-cm long narrow bands on the starting line of a 20 × 20-cm pre-coated thin-layer chromatographic plate. Experience has shown that manual sample application can be carried out with a reproducibility of better than ± 2 per cent. Before and after development of the plates, excess of solvent was removed with a stream of cool air. Standard and test zones of nikethamide were identified while viewing the developed plates under ultraviolet light (254 nm). After marking them, the relevant zones of adsorbent were scraped off the plate and each was placed in a 25-ml conical flask fitted with a ground-glass stopper. Standard and test blanks were prepared from a clean area of adsorbent of equal size. With a pipette, 10·0 ml of ethanol - water (1 + 1 v/v) were introduced into each flask and the suspensions were thoroughly shaken for about 5 minutes, either by hand or by mechanical shaker, before being centrifuged for 5 minutes at about 3000 r.p.m. The clear solutions were evaluated by ultraviolet spectrophotometry in 1-cm quartz cuvettes, the absorbance of each standard and test solution being measured at 261 nm against the corresponding blank.

In the multi-component dosage forms listed in Table II, caffeine, theophylline, adenosine and strychnine were determined in an analogous way. The caffeine and theophylline determinations were performed by using the plate used for the nikethamide assay, to which corresponding mixed standards were applied with the appropriate test solution. Higher relative concentrations were required for the adenosine and strychnine determinations, which were performed with separate plates. Each of these four compounds was determined at a wavelength and dilution normally adopted for its determination by ultraviolet spectrophotometry.[20] These parameters for all five compounds determined in this way are presented in Table I.

TABLE I

END DILUTION CONCENTRATIONS AND ABSORPTION MAXIMA FOR ACTIVE COMPOUNDS DETERMINED BY THIN-LAYER CHROMATOGRAPHY, ELUTION AND ULTRAVIOLET SPECTROPHOTOMETRY

Compound	$\lambda_{max.}$/nm	Concentration at final dilution/mg ml^{-1}
Nikethamide	261	0·02
Adenosine	260	0·005
Caffeine	272	0·005
Strychnine	254	0·02
Theophylline	270	0·008

Solvent: ethanol - water (1 + 1 v/v)

RESULTS AND DISCUSSION

The calcium chloride and thiocyanate salts of nikethamide are frequently incorporated in solid dosage forms, rather than the free amide, which is a liquid. As a result of the weak ion-exchange effect of the adsorbent, these salts behave like nikethamide itself when chromatographed on silica gel, even when neutral developing solvents are used. Details of the dosage forms investigated and the thin-layer chromatographic results accumulated are presented in Table II. All nine solvent systems are suitable for the identification of nikethamide. Quantitative determinations have been performed by using the solvent systems 1 and 7, system 7 being particularly adaptable to nikethamide - caffeine combinations. For the quantitative determination of strychnine in the latter combinations, continuous development with methanol for 1 to 1½ hours was found to be the best method of separation. While the strychnine travelled 2 to 3 cm from the starting line, nikethamide and caffeine migrated to the top of the plate. System 1 was adopted for the determination of each active compound in the nikethamide - adenosine combinations. Two developments were required to ensure that adenosine travelled sufficiently far from the point of application and was adequately separated from adenine, a potential contaminant arising from the acid-catalysed hydrolysis of adenosine. Ephedrine in nikethamide - ephedrine combinations was chromatographed with solvent systems 3 and 4 consecutively. After elution with methanol, ephedrine was determined by a previously published method involving colorimetry of the copper(II) complex of the dithiocarbamate formed by reacting ephedrine with carbon disulphide in alkaline solution.[21]

Quantitative thin-layer chromatography with solvent system 1 or 7, and for ephedrine systems 3 *plus* 4, serves as both a dosage control and stability assay procedure for nikethamide, adenosine, caffeine, ephedrine, theophylline and strychnine. Each compound is separated from adjuvants and from known decomposition products, should degradation occur. Adsorption effects on adjuvants or on the chromatographic adsorbent were overcome by the high polarity of the extraction solvents used, and by the use of ultrasonics and elevated temperatures for extracting the active compounds from solid dosage forms. The latter were found to be most efficiently pulverised by using a domestic electric coffee grinder prior to extraction. The absorbance of the blanks was found in every case to be below 0·05 absorbance units, unless a faint turbidity was present arising from inadequate centrifugation. This turbidity was readily removed from the standard, test and blank solutions by adding Celite analytical filter aid (Johns-Manville) and re-centrifuging the mixtures after briefly shaking them. In all instances, recoveries of active compounds from the adsorbent were found to exceed 95 per cent. when compared with parallel, unchromatographed solutions. In the absence of detectable decomposition, results obtained with the quantitative thin-layer chromatographic methods normally fell within ± 4 per cent. of the declared dosages. A spread of this order is to be expected for determinations of this type.[22]

TABLE II

THIN-LAYER CHROMATOGRAPHIC RESULTS FOR NIKETHAMIDE DOSAGE FORMS

Combination	Dosage form*	Dosage ratio (mg ml^{-1} or mg per unit)	Solvent system	Ratio of 100 R_F values (approximate)
Nikethamide	i, ii, iii	100 to 250	1	50[a]
Nikethamide - glucose..	iii, iv, v	125:1500	1	50:0
			8	60:50
Nikethamide - glucose - ascorbic acid ..	iv	125:1500:550	1	50:0:0
			8	60:50:80[b]
Nikethamide - theophylline - adenosine ..	i, ii	200:68:1	1	55:40:07[c,d]
		100:34:0·5	—	—
Nikethamide - caffeine - strychnine sulphate[f]	iii	65:50:0·25	7	70:40:45[e]
Nikethamide - caffeine - strychnine sulphate[f] - sodium salicylate	i	100:50:0·25:50	7	60:70:45:0
			9	90:80:30:50 to 70
Nikethamide : ephedrine hydrochloride[g] ..	iii	130:15	1	50:0
			2	50:20
			3	67:17
			4	80:70
			5	50:65
			6	55:30

[a] *N*-Ethylnicotinamide, 100 $R_F = 35$; [b] dehydroascorbic acid, 100 $R_F = 90$; [c] for adenosine determination, develop twice; [d] adenine, 100 $R_F = 15$; [e] *N*-ethylnicotinamide, 100 $R_F = 55$; [f] quantitative determination of strychnine by continuous development (1½ hours) with methanol; and [g] quantitative determination of ephedrine by development with systems 3 *plus* 4.

* Dosage forms: (*i*) aqueous solution capable of injection; (*ii*) gelatine capsule; (*iii*) tablet (may contain nikethamide as its calcium chloride or thiocyanate salt); (*iv*) effervescent tablet; and (*v*) caramel sweet.

Solvent systems that were found to be suitable for the qualitative examination of particular dosage form combinations are given in Table II. For nikethamide, system 1 was most widely applicable. The chromatographic behaviour of the compounds was good, with the exception of ephedrine, which produced tailing effects with systems other than 3 and 4. Slight aerial oxidation of ascorbic acid occurred during chromatography and gave rise to a satellite spot (dehydroascorbic acid).

In most instances, quantitative assay of each component in nikethamide combination preparations was possible by thin-layer chromatography. Two important exceptions were glucose and ascorbic acid, which were more conveniently determined by established titrimetric methods[23] involving oxidation with hexacyanoferrate(III) or iodine reagents for glucose, and with iodine for ascorbic acid. For the determination of glucose in combinations containing ascorbic acid, interference by the latter was eliminated by retaining it on Amberlite IRA-410 (OH^- form) anion-exchange resin (Rohm & Haas), the glucose being eluted with warm water.

Conclusions

Thin-layer chromatography is a rapid, sensitive and selective method for the identification and quantitative assay of preparations of nikethamide combinations that compares favourably with other methods currently available. While the quantitative determinations described above involve the elution of the active compounds from the adsorbent, preliminary studies have shown that with the possible exception of ephedrine, the ideal behaviour of these compounds during thin-layer chromatography as regards spot symmetry and the absence of tailing effects should permit the application of direct densitometric evaluation of chromatograms (fluorescence-quenching mode) as a routine procedure.

The author thanks Mrs. P. Brunschwiler and Miss R. Matthies for experimental assistance, and Mr. E. Candolfi for the sample of *N*-ethylnicotinamide. The management of Ciba-Geigy is thanked for permission to publish this paper.

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Received *January* 15*th*, 1971
Accepted *April* 26*th*, 1971

Analyst, October, 1971, Vol. 96, *pp.* 721–727

The Gas-chromatographic Determination of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in 2,4,5-Trichlorophenoxyacetic Acid ("2,4,5-T"), 2,4,5-T Ethylhexyl Ester, Formulations of 2,4,5-T Esters and 2,4,5-Trichlorophenol

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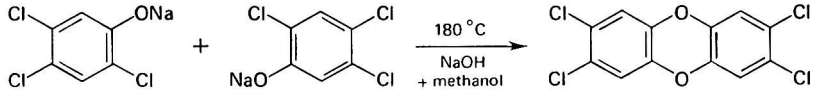
A gas-chromatographic method for the determination of trace amounts of a toxic impurity, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, is described. A purified extract of the sample was subjected to gas chromatography on a column containing either 2 per cent. of OV-17 on Diatomite CQ or 1 per cent. of Hi-Eff 8 BP on Gas-Chrom Z with electron-capture detection. 2,4,5-Trichlorophenoxyacetic acid and 2,4,5-trichlorophenol were purified by chromatography of an ether extract of the sample on a column of alumina, followed by shaking with sulphuric acid. For 2,4,5-trichlorophenoxyacetic acid esters and formulations, saponification and chromatography on a Celite - sulphuric acid column, followed by chromatography on a column of alumina, were necessary.

Recoveries of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin ranged from 89 to 98 per cent., and the standard deviation of the method at a level of 0·3 p.p.m. was 0·03 p.p.m. The limit of detection was about 0·05 p.p.m.

2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN is known to cause chloracne and liver damage at very low levels of concentration in rabbits,[1] and is believed to possess teratogenic properties. Under certain conditions it is formed during the manufacture of 2,4,5-trichlorophenol and of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) from 2,4,5-trichlorophenol.



One method of producing 2,4,5-trichlorophenol commercially is by hydrolysis of 1,2,4,5-tetrachlorobenzene with methanolic sodium hydroxide solution. If other chlorinated benzenes are present, then other polychlorinated phenols could be formed, which would in turn give rise to chlorinated dioxins, the level of which would be expected to be far lower than that of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin itself.

In the United States, work at the Bionetics Research Laboratories[2] has shown that one sample of 2,4,5-T examined contained about 27 p.p.m. of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, whereas the normal level is about 1 p.p.m. or less.

During the last few years it has been shown that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin is a component of the chick oedema factor that causes hydropericardium in chickens.[3] The more highly chlorinated dioxin 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin has also been isolated from toxic fats and identified by gas chromatography[3] and X-ray crystallography.[4] Qualitative identification of the chick oedema factor in oils and fats by gas chromatography after suitable clean-up has been reported,[5,6,7] and bio-assay methods have also been used.[8,9] However, no methods for the quantitative determination of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or any of the other individual components of chick oedema factor have been published.

This paper describes a gas-chromatographic procedure for the quantitative determination of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in 2,4,5-T, 2,4,5-T ethylhexyl ester, formulations of 2,4,5-T esters and 2,4,5-trichlorophenol. Confirmation of the presence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin is obtained by gas chromatography on a second stationary phase.

Experimental

CAUTION—Because of the highly toxic nature of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin extreme care must be taken when handling solutions and materials containing this compound.

Reagents—

Hexane, distilled—Two litres of hexane were distilled through a column, 8 inches × 1 inch, of Fenske helices, 4 mm in diameter, and the first 1800 ml of distillate were collected. The suitability of the distilled material for use in the subsequent procedures was checked as follows: 50 ml were evaporated to 1 ml and 20-μl aliquots were subjected to gas chromatography on columns containing the stationary phases OV-17 and Hi-Eff 8 BP, under the conditions described later. Hexane that showed peaks with the same retention times as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin was rejected. The Puriss grade supplied by Koch-Light was found to be satisfactory after distillation.

Benzene—Analytical-reagent grade.

Diethyl ether—Anaesthetic grade B.P., distilled. Two litres of diethyl ether were distilled through a column, 8 inches × 1 inch, containing Fenske helices, 4 mm in diameter, and the first 1800 ml of distillate were collected.

Ethanol, 95 per cent.—B.P. grade.

Methanol—Analytical-reagent grade.

Diethyl ether - hexane (1 + 9 v/v) and (1 + 4 v/v).

Methylene chloride—Analytical-reagent grade.

Sulphuric acid, concentrated—Analytical-reagent grade.

Sodium sulphate, anhydrous—Analytical-reagent grade.

Potassium hydroxide, M.

Hydrochloric acid, concentrated—Analytical-reagent grade.

Alumina—Chromatographic grade, neutral, Brockmann activity I, 100 to 240 mesh. Camag material (Hopkin and Williams Ltd.) was found to be suitable.

Celite 535, acid-washed—Celite 535 (Johns-Manville) was soaked in concentrated hydrochloric acid overnight, washed with water until neutral and the "fines" were removed by decantation. The Celite was then filtered off, washed with methanol, then with methylene chloride and finally dried overnight at 50 °C.

2,3,7,8-*Tetrachlorodibenzo*-p-*dioxin, reference material*—This was prepared by the following method: 98·75 g (0·5 mole) of 2,4,5-trichlorophenol were dissolved in 250 ml of toluene, the solution being stirred and heated on a steam-bath; 33 g (0·5 mole) of potassium hydroxide pellets were added, which dissolved with precipitation of the potassium phenoxide. Water was removed azeotropically and 10 g of copper powder (activated by iodine[10]) and 1 g of copper(II) acetate were added. About 150 ml of toluene were distilled off, 500 ml of dry nitrobenzene were added and distillation was continued until an internal temperature exceeding 210 °C was reached. The mixture was gently refluxed in an atmosphere of nitrogen for 18 hours, cooled below 5 °C and filtered under suction. The solid on the filter was washed with nitrobenzene (50 ml) and then with about 75 ml of methanol. After sucking it as dry as possible the solid (80 g) was slurried with 400 ml of N sodium hydroxide solution, filtered, washed with water and air dried. The resultant olive green - brown coloured solid (28 g) was recrystallised from anisole to give 12·5 g of a pinkish white crystalline powder with melting-point 302 to 304 °C (literature value 295 °C[11]). The infrared spectrum, as a paraffin mull, was identical with the published spectrum.[11] Gas chromatography of the compound on OV-17 under the conditions described gave one major well defined peak and a small amount of impurity with a relative retention time of 0·55.

2,3,7,8-*Tetrachlorodibenzo*-p-*dioxin standard solution*—Ten milligrams of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, accurately weighed, were dissolved, with warming, in 5 ml of benzene contained in a 100-ml calibrated flask and the solution was adjusted to volume with hexane; 5·0 ml of this solution were diluted to 50 ml with hexane and a further dilution of 5·0 ml of this solution to 50 ml was made with hexane (1·0 ml of the final solution contained 1·0 μg of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin).

Apparatus—

All glassware was cleaned in chromic - sulphuric acid mixture, washed thoroughly with water, dried and rinsed with hexane.

A Perkin-Elmer F11 gas chromatograph, equipped with an electron-capture detector (with a 500-mCi tritium source) and a 1-mV Leeds and Northrup Speedomax W recorder, was used.

Columns—(*a*) A 1·8-m glass column of 3-mm i.d. was packed with 2 per cent. of OV-17 (Supelco Inc.) on 80 to 100-mesh Diatomite CQ (Pye Unicam Ltd.) and conditioned at 250 °C for 48 hours without the detector attached.

(*b*) A 1·8-m glass column of 3-mm i.d. was packed with 1 per cent. of cyclohexane dimethanol succinate (Hi-Eff 8 BP) (Applied Science Laboratories Inc.) on 100 to 120-mesh Gas-Chrom Z (Applied Science Laboratories Inc.) and conditioned at 230 °C for 48 hours without the detector attached.

The following operating conditions were used: detector voltage 2 V, detector temperature 205 °C, recorder chart speed 12 inches hour^{-1} and carrier gas nitrogen (oxygen-free); for the OV-17 column: column temperature 200 °C, injection port temperature 210 °C and carrier gas flow-rate 60 ml min^{-1}; and for the Hi-Eff 8 BP column: column temperature 200 °C, injection port temperature 210 °C and carrier gas flow-rate 50 ml min^{-1}.

Attenuation settings were selected so that an injection of 20 ng of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin produced a peak height of at least 30 per cent. of the full-scale deflection.

PROCEDURE FOR PRELIMINARY TREATMENT OF THE SAMPLE FOR 2,4,5-T AND 2,4,5-TRICHLOROPHENOL—

Extraction—About 1·0 g of sample, accurately weighed, was transferred to a dry 500-ml separating funnel and dissolved in 50 ml of ethanol; 100 ml of water were added, followed by 10 ml of M potassium hydroxide. The alkaline solution was extracted with three 50-ml portions of diethyl ether, the ether extracts were combined and the separating funnel was rinsed with 10 ml of diethyl ether, which were added to the main ether extracts. The combined ether extract was washed with two 20-ml portions of M potassium hydroxide and then with three 25-ml portions of water, after which it was dried by shaking it with 10 g of anhydrous sodium sulphate and filtered through a cotton-wool plug into a 500-ml round-bottomed flask; the separating funnel and cotton-wool were rinsed with two 15-ml portions of diethyl ether and the rinsings added to the flask. The ether was removed in a rotary evaporator at 40 °C, 5 ml of methanol were added to the residue and the solution was again evaporated to dryness. The residue was then dissolved in 2 ml of hexane.

Alumina column chromatography—A column was prepared in a glass tube, 150 × 10 mm, which was fitted with a tap and plugged with a small wad of cotton-wool, by filling the tube with hexane, adding 5 g of alumina and allowing the alumina to settle while gently tapping the tube to release any air bubbles.

The hexane solution from the extraction step was transferred with a Pasteur pipette to the top of the alumina column. The 500-ml flask was rinsed with three successive 2-ml portions of hexane, and the washings were added to the alumina column. The column was washed with 50 ml of hexane and then with 50 ml of diethyl ether - hexane (1 + 9 v/v).

The 2,3,7,8-tetrachlorodibenzo-*p*-dioxin was then eluted with 50 ml of diethyl ether - hexane (1 + 4 v/v), the eluate being collected in a 100-ml flask and the solvent removed in a rotary evaporator at 40 °C. The residue was dissolved in 1 ml of hexane, which was subjected to treatment with sulphuric acid as follows.

Sulphuric acid treatment—The hexane solution was transferred with a Pasteur pipette to a 10-ml stoppered cylinder and the flask was rinsed with two 1-ml portions of hexane, the washings being added to the cylinder; 3 ml of sulphuric acid were added and the cylinder was shaken rapidly for 3 minutes on a mechanical shaker. After allowing the layers to separate the hexane layer was transferred to a second cylinder containing 3 ml of sulphuric acid, the first cylinder being washed with two 0·5-ml portions of hexane and the washings added to the second cylinder. After shaking the contents of this cylinder for 3 minutes the layers were allowed to separate and the hexane layer was transferred to a 100-ml flask via a funnel containing 2 g of sodium hydrogen carbonate supported on a plug of cotton-wool. The sulphuric acid layer in the cylinder was washed with three 2-ml portions of hexane, each washing being transferred via the sodium hydrogen carbonate layer to the 100-ml flask. The solvent was removed in a rotary evaporator at 40 °C and the final residue dissolved in 1·0 ml of hexane.

PROCEDURE FOR PRELIMINARY TREATMENT OF THE SAMPLE FOR 2,4,5-T ESTERS AND 2,4,5-T ESTER FORMULATIONS—

Saponification and extraction—A sufficient amount of 2,4,5-T ester or ester formulation to contain the equivalent of about 1·0 g of 2,4,5-T was accurately weighed and transferred to a 250-ml flask; it was then dissolved in 50 ml of ethanol, 5 ml of M potassium hydroxide (or sufficient to provide a 1-ml excess over the amount required for saponification) and 2 ml of water were added, and the mixture was refluxed on a steam-bath for 10 minutes. After cooling, the solution was transferred with 50 ml of water to a separating funnel containing 50 ml of water. The extraction procedure described above was then followed, commencing at "The alkaline solution was extracted . . .," except that the final residue was dissolved in 10 ml of hexane.

Celite - sulphuric acid column chromatography—A Celite - sulphuric acid column was prepared by intimately mixing 6 g of Celite with 4 ml of concentrated sulphuric acid and packing this mixture, in portions, into a glass column, 200 × 18 mm, fitted with a tap and plugged with a small wad of cotton-wool, tamping each portion firmly with a glass rod.

The hexane solution from the saponification and extraction step was transferred to the Celite column and the flask was rinsed with three 5-ml portions of hexane, which were added to the column. A further 50 ml of hexane were added to the column and the total eluate was collected in a 250-ml flask and evaporated to dryness in a rotary evaporator at 40 °C. The residue was dissolved in 2 ml of hexane and treated as described below.

Alumina column chromatography—The hexane solution was chromatographed on a column of alumina as described for 2,4,5-T and 2,4,5-trichlorophenol. The residue from the diethyl ether - hexane (1 + 4 v/v) fraction was dissolved in 1·0 ml of hexane.

PROCEDURE FOR THE GAS-CHROMATOGRAPHIC DETERMINATION OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN—

Aliquots of 20 μl of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin standard solution and the sample solution from the above final preliminary treatments were chromatographed on the OV-17 and Hi-Eff 8 BP columns under the conditions already described. On either column, the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin was eluted after about 15 minutes; its concentration in the samples was calculated from the following formula—

$$\text{2,3,7,8-Tetrachlorodibenzo-}p\text{-dioxin in sample, p.p.m.} = \frac{h_E}{h_S} \times \frac{1{\cdot}0}{W}$$

where h_E is the height of its peak in the sample chromatogram, h_S is the height of its peak in the standard chromatogram and W is the weight, in grams, of the sample taken.

RESULTS AND DISCUSSION

The use of the electron-capture detector had the advantage of high sensitivity, and thus avoided the necessity of handling relatively large amounts of sample. However, such a choice required a vigorous clean-up procedure to prevent gross contamination of the detector by other chlorinated compounds present and to remove interfering substances with the same retention times as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. No single clean-up procedure was found to be adequate, but while a combination of extraction, alumina column chromatography and sulphuric acid treatment reduced the background to a satisfactory level for 2,4,5-T and 2,4,5-trichlorophenol, for 2,4,5-T esters and their formulations a preliminary saponification, extraction, Celite - sulphuric acid column chromatography and alumina column chromatography were required. It was necessary to carry out the Celite - sulphuric acid column treatment of the unsaponifiable fraction from the esters and their formulations before chromatography on alumina to remove compounds that otherwise would have been detrimental to the latter procedure. The same clean-up technique could probably have been applied to 2,4,5-T and 2,4,5-trichlorophenol with equal success, but as this had no apparent advantage the simpler procedure of shaking with sulphuric acid after alumina column chromatography was adopted for these substances. Similar clean-up procedures have been used for the detection of chick oedema factor.[5,6,7]

Hexane of satisfactory purity proved difficult to obtain. Most commercial material contained a significant amount of an impurity that had the same retention time as that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and efforts to remove it were mainly unsuccessful.

Treatment with fuming sulphuric acid or potassium permanganate, or refluxing with sodium or alkaline silver nitrate showed little improvement. Eventually distillation of carefully selected hexane, initially of low impurity content, provided a satisfactory reagent.

RECOVERY OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN—

Amounts of 0·5 and 1·0 ml of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin standard solution were added to 1·0-g portions of 2,4,5-T that were free from the impurity (less than 0·05 p.p.m.), dissolved in 50 ml of ethanol, and the samples were analysed by the method described. Recoveries of 98 and 95 per cent., respectively, were obtained. Also, 1·0 ml of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin standard solution was added to 1·5 g of 2,4,5-T ethylhexyl ester dissolved in 50 ml of ethanol and the mixture was analysed by the method described. A recovery of 89 per cent. was obtained.

PRECISION—

The precision of the gas-chromatographic procedure was determined by replicate injections of 20 μg of the standard 2,3,7,8-tetrachlorodibenzo-*p*-dioxin solution. The standard deviation of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin injected was 0·6 ng. The precision of the whole procedure was determined by analysing five 1·0-g portions of one of the 2,4,5-T samples, and the results ranged from 0·25 to 0·31 p.p.m., with a mean value of 0·27 p.p.m. and a standard deviation of 0·03 p.p.m.

LINEARITY OF RESPONSE OF THE ELECTRON-CAPTURE DETECTOR—

Portions of 5·0, 10·0, 15·0 and 20·0 μl of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin standard solution were injected on to the OV-17 column. The graph of peak height *versus* nanograms of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin was rectilinear over the range examined and passed through the origin.

LIMITS OF DETECTION—

Under the conditions of the method the lowest level of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin detectable (twice the noise level) was found to be 0·05 ng per μl of final solution. For a sample concentration equivalent to 1·0 g ml^{-1}, the limit of detection was, therefore, 0·05 p.p.m.

Table I shows the results obtained on several samples of 2,4,5-T from four manufacturers. The determinations were carried out in duplicate on separately weighed amounts of sample.

TABLE I

2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN CONTENT OF 2,4,5-T

	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, p.p.m.	
Sample	OV-17	Hi-Eff 8 BP
A_1	0·30, 0·27	0·27
A_2	0·17, 0·12	0·16
A_3	0·50, 0·42	0·50
B	0·29, 0·31	—
C	0·27, 0·28	0·33
D	<0·05, <0·05	<0·05

TABLE II

2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN CONTENT OF 2,4,5-T ESTER AND FORMULATIONS

	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, p.p.m.	
Sample	OV-17	Hi-Eff 8 BP
2,4,5-T ethylhexyl ester—		
E	0·26, 0·28	0·20
F	0·27, 0·24	0·22
2,4,5-T ester formulation—		
G	0·19, 0·17	0·16
H	0·09, 0·13	0·09

All of the samples contained less than 0·5 p.p.m. of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Sample B contained an impurity that interfered in the determination with Hi-Eff 8 BP as the stationary phase. This impurity was not present in any other sample.

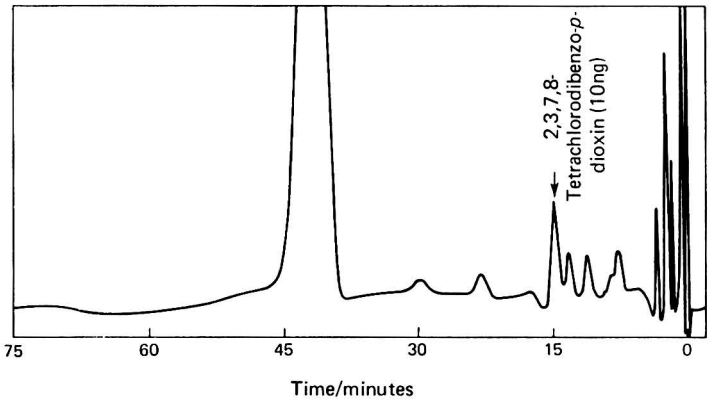


Fig. 1. Chromatogram of an extract of 2,4,5-T on 2 per cent. OV-17 on Diatomite CQ

A typical chromatogram of an extract from 2,4,5-T containing about 0·5 p.p.m. of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, with OV-17 as the stationary phase, is shown in Fig. 1.

Table II shows the results on samples of 2,4,5-T ethylhexyl ester and ester formulations. Again the contents of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin were less than 0·5 p.p.m. A typical chromatogram of an extract from 2,4,5-T ethylhexyl ester containing about 0·2 p.p.m. of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, with Hi-Eff 8 BP as the stationary phase, is shown in Fig. 2.

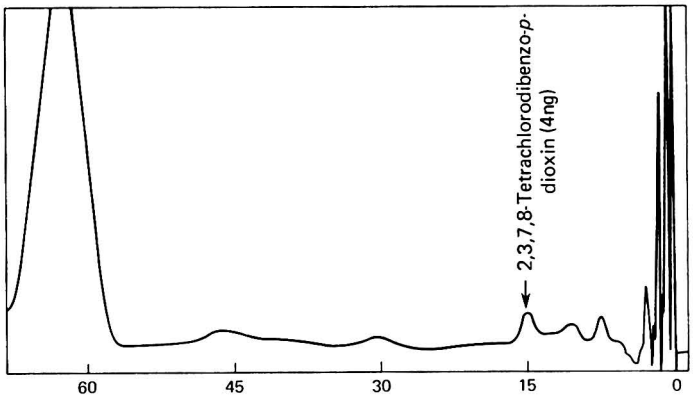


Fig. 2. Chromatogram of an extract of 2,4,5-T ethylhexyl ester on 1 per cent. Hi-Eff 8 BP on Gas-Chrom Z

Finally the results on two samples of 2,4,5-trichlorophenol are shown in Table III. Neither sample contained appreciable amounts of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

In nearly all of the samples of 2,4,5-T and 2,4,5-T esters a large impurity peak was observed with a relative retention time of about 3·5 on OV-17 and 4·2 on Hi-Eff 8 BP (2,3,7,8-tetrachlorodibenzo-*p*-dioxin = 1·00). This impurity was not removed even by further

treatment with sulphuric acid, but as it did not interfere in the determination its elimination was of no consequence. Evidence from its mass spectrum suggests that the impurity is not a polychlorinated dioxin but possibly bis-(2,4,5-trichlorophenoxy)methane.

All chromatograms were run for 1½ to 2 hours before injection of further samples to clear the columns of long-running impurities.

TABLE III

2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN CONTENT OF 2,4,5-TRICHLOROPHENOL

Sample	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, p.p.m.	
	OV-17	Hi-Eff 8 BP
J	0·32, 0·32	0·25
K	<0·05, <0·05	<0·05

CONCLUSIONS

The highly toxic impurity 2,3,7,8-tetrachlorodibenzo-*p*-dioxin can be detected, and its concentration determined, in 2,4,5-T, 2,4,5-T esters, formulations and 2,4,5-trichlorophenol by means of gas chromatography with either OV-17 or Hi-Eff 8 BP as stationary phase after suitable clean-up procedures have been applied. The lower limit of detection, taking the equivalent of 1 g of 2,4,5-T in the initial sample, is about 0·05 p.p.m. The standard deviation of the procedure at a level of 0·3 p.p.m. is 0·03 p.p.m.

The author thanks Mr. W. H. Stephenson for his interest and encouragement, and Mr. R. Banks for preparing the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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Received *December 11th*, 1970
Accepted *April 15th*, 1971

Determination of Total Hydrolysable Nitrogen in Acidic Aqueous Solutions of Nitriles Containing Cyanide

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A method is described for the determination of total hydrolysable nitrogen in acidic aqueous solutions containing acrylonitrile, acetonitrile, hydrogen cyanide and ammonia. A recovery of about 97 per cent. is obtained for all the components examined. The method is based on the classical Radziszewski reaction, and involves reaction with 30 per cent. w/v hydrogen peroxide followed by alkaline hydrolysis to ammonia. The important feature of the method is that it enables hydrogen cyanide to be determined together with the other components.

THE method of Peterson and Radke[1] for the determination of acrylonitrile in air, which involved absorption of the vapour in sulphuric acid followed by alkaline hydrolysis to ammonia in the presence of hydrogen peroxide, was considered for the determination of total hydrolysable nitrogen in aqueous solutions of acrylonitrile, acetonitrile, hydrogen cyanide and ammonia. Such solutions were usually decinormal with respect to sulphuric acid. We had inadequate information as to the actual recovery of acrylonitrile and acetonitrile and also assumed that hydrogen cyanide would not be determined. Experiments showed, however, that hydrogen cyanide was partially determined by this method; because of the difficulty in removing the latter from solution without loss of the other components, it was decided to develop a method in which all of the hydrogen cyanide would be determined, together with the other components.

DEVELOPMENT OF THE METHOD

METHOD OF PETERSON AND RADKE[1]—

In this method the sample solution was treated with 0·2 g of copper(II) acetate, to prevent the polymerisation of acrylonitrile, followed by 50 ml of 50 per cent. w/w sodium hydroxide solution. After dropwise addition of 20 ml of 100-volume hydrogen peroxide the solution was heated slowly to boiling and then under reflux for 30 minutes, after which the ammonia was distilled over and titrated with 0·05 N hydrochloric acid.

A set of experiments carried out with this method gave the results shown in Table I. Aliquots of a solution containing about 0·7 milli-equivalent of nitrogen were taken for each experiment. Further experiments were carried out at the same time omitting the copper acetate and then omitting both copper acetate and hydrogen peroxide, the sample being refluxed for 30 minutes before distillation in each case. These results are also shown in Table I. It was noticed that when copper was present the hydrogen peroxide decomposed

TABLE I

RESULTS OBTAINED BY USE OF THE METHOD OF PETERSEN AND RADKE WITH VARIATIONS

	Recovery, per cent.		
Solution	Sodium hydroxide *plus* hydrogen peroxide *plus* copper acetate	Sodium hydroxide *plus* hydrogen peroxide only	Sodium hydroxide only
Acetonitrile (neutral solution)	87·3	94·4	92·2
Acrylonitrile (neutral solution)	88·3	97·6	93·7
Potassium cyanide (neutral solution)	8·7	33·2	10·6

immediately it was added to the solution in the cold, so that its existence in the reaction mixture was of negligible duration. There is no evidence to show that polymerisation of acrylonitrile takes place to any significant extent, so it was judged that the copper acetate fulfils no useful purpose.

It can be seen that alkaline hydrolysis alone gives quite high recoveries of acetonitrile and acrylonitrile, although only of about 10 per cent. in the case of potassium cyanide. When hydrogen peroxide is also present all of the recoveries are increased. The effect of hydrogen peroxide on the course of the hydrolysis reactions will be discussed later.

METHOD OF WHITEHURST AND JOHNSON[2]—

These workers describe a method for the determination of simple aliphatic nitriles by reaction with alkaline peroxide solution (50 ml of N potassium hydroxide *plus* 10 ml of 30 per cent. hydrogen peroxide *plus* 90 ml of water). They state that the conversion of nitrile into amide is completed in 5 minutes at room temperature, although complete hydrolysis to the acid salt takes place only after the alkali concentration has been raised to at least 2 N by removal of water by boiling.

The following experiments, with more alkali, were therefore carried out. Volumes (5 ml) of acetonitrile, acrylonitrile and potassium cyanide solutions (approximately 0·15 g of nitrogen per 100 ml) were mixed with 40 ml of 5 N sodium hydroxide solution, 10 ml of 30 per cent. hydrogen peroxide and 25 ml of water, then stood at room temperature for 5 minutes before heating and distilling. The results are shown in Table II. The extent of conversion of cyanide into ammonia is still very small (about 14 per cent.).

TABLE II

RESULTS OBTAINED BY USE OF THE METHOD OF WHITEHURST AND JOHNSON AS MODIFIED BY US

Solution	Recovery, per cent.
Acetonitrile (neutral solution)	99
	100·4
Acrylonitrile (neutral solution)	93·7
	94·0
Potassium cyanide (neutral solution)	14

USE OF ACETALDEHYDE—

The recovery of potassium cyanide was increased to 88 per cent. by reaction of the sample solution (total volume 30 ml) with 1 ml of acetaldehyde at about pH 4 before the addition of 10 ml of 30 per cent. hydrogen peroxide and 1 ml of 5 N sodium hydroxide. The mixture was allowed to react at room temperature for 10 minutes before the addition of a further 40 ml of 5 N sodium hydroxide and subsequent distillation. The increased recovery is probably the result of conversion of the cyanide into cyanhydrin before reaction with the alkaline peroxide.

USE OF STRONGLY OXIDISING CONDITIONS—

It was finally discovered that reaction of the sample solution with a high concentration of hydrogen peroxide (*e.g.*, 30 ml of 30 per cent. peroxide in a total volume of 40 ml made alkaline with 1 ml of 5 N sodium hydroxide) at room temperature followed by distillation led to about 95 per cent. recoveries of cyanide without the need for prior reaction with acetaldehyde. The recoveries of acetonitrile and acrylonitrile were also very good. This last observation provided the basis for the recommended method.

CONCLUSIONS—

Summarising the above experimental work it appears that there are two main courses of reaction in the case of nitriles, namely simple alkaline hydrolysis via the amide to the ammonium salt, or epoxidation followed by further re-arrangement or oxidation to the amide, which is then converted by alkaline hydrolysis into the ammonium salt. In the recommended method it seems likely that the latter course of reaction predominates.

In the case of hydrogen cyanide, alkaline hydrolysis to ammonia takes place to only a small extent, even in the presence of a moderate amount (10 ml) of 30 per cent. hydrogen

peroxide (Tables I and II). The improvement in recovery after reaction with acetaldehyde is probably caused by oxidation of the acetaldehyde cyanhydrin so formed. When a large amount of hydrogen peroxide is used, as in the final recommended method, almost complete oxidation of hydrogen cyanide to oxamide or ammonia takes place.

Reactions

The following reactions, as proposed in literature references 3 to 5, are those thought to take place in the recommended method.

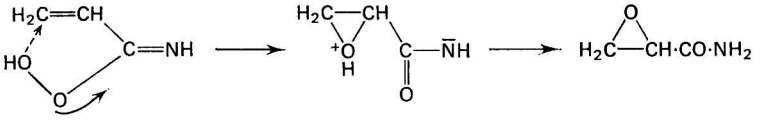
Acetonitrile and acrylonitrile—

Payne and co-workers describe the reactions of alkaline hydrogen peroxide with acrylonitrile and other nitriles.[3,4]

Acrylonitrile and hydrogen peroxide react in aqueous solution to form glycidamide. This takes place via peroxyacrylamidic acid

$$H_2C{=}CH.CN + H_2O_2 \xrightarrow{OH^-} H_2C{=}CH.\underset{\underset{NH}{\|}}{C}OOH$$

which is then thought to undergo an intramolecular re-arrangement to the amide—



Hydrogen peroxide reacts with nitriles in general in slightly alkaline solution to form a peroxycarboximidic acid intermediate. In the absence of any other reducing agent or olefin this then reacts with hydrogen peroxide to give an amide and oxygen.

$$RCN + H_2O_2 \xrightarrow{OH^-} R\underset{\underset{NH}{\|}}{C}OOH + H_2O_2 \longrightarrow RCO.NH_2 + O_2 + H_2O$$

(This over-all reaction is the classical Radziszewski reaction.)

Hydrogen cyanide—

Sheridan and Brown describe the reaction of hydrogen cyanide and hydrogen peroxide to form oxamide.[5] The reaction apparently proceeds in at least two stages such as

$$2HCN + H_2O_2 \longrightarrow (CN)_2 + 2H_2O$$
$$(CN)_2 + 2H_2O_2 \longrightarrow (CONH_2)_2 + O_2$$

or over-all

$$2HCN + 3H_2O_2 \longrightarrow (CONH_2)_2 + 2H_2O + O_2$$

Ammonia and carbon dioxide are also produced indicating the side reaction

$$HCN + H_2O_2 = CO_2 + NH_3$$

Effect of phosphate—

Ogata and Sawaki[6] state that, in a 75 per cent. methanolic solution, such peroxycarboximidic acids are rapidly stabilised by the phosphate dianion, HPO_4^{2-}, which prevents their reaction with hydrogen peroxide to form an amide. If this were true in the present instance, low recoveries of nitrogen would result if phosphate were present. Ogata and Sawaki's experiments were carried out with acetonitrile and substituted benzonitriles. However, they further state that when boric acid is added to a reaction mixture containing phosphate, the oxidation of peroxycarboximidic acids to amides by hydrogen peroxide does take place even in the presence of an excess of phosphate, the rate increasing with increasing addition of boric acid. We carried out experiments on solutions of acetonitrile and acrylonitrile to which was added 0·5 ml of M potassium dihydrogen phosphate per 40 ml of reaction mixture. Any interference that takes place under these conditions does not result in recoveries that are more than 1 per cent. low. This effect was not further investigated.

Recommended method

Reagents—

Hydrogen peroxide, 30 *per cent. w/v*—General-purpose reagent.
Sodium hydroxide solution, 5 N.
Boric acid solution, about 4 *per cent.*—Boil 4 g of boric acid with 100 ml of distilled water.
Hydrochloric acid, 0·05 N.
Mixed indicator—(*a*) Dissolve 0·166 g of methylene blue in ethanol, then (*b*), dissolve 0·250 g of methyl red in ethanol and make up to 100 ml in each instance. Mix equal volumes of (*a*) and (*b*). The exact proportions are important.

Apparatus—

This is shown in Fig. 1. The quartz-wool plug in the side-arm from the flask is essential to prevent the carry-over of alkaline spray formed as a result of the decomposition of the hydrogen peroxide during the initial heating of the reaction mixture.

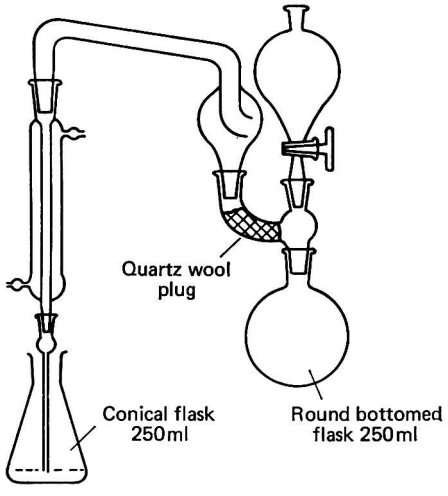


Fig. 1. Apparatus for the determination of total hydrolysable nitrogen

Procedure—

Measure 30 ml of hydrogen peroxide into the reaction flask and add sufficient sodium hydroxide solution to neutralise any acidity in the sample *plus* 1 ml in excess. (Should the acidity of the sample be unknown, titrate a separate aliquot with N sodium hydroxide, with phenolphthalein as indicator.) Transfer accurately by pipette into the flask a volume of sample containing about 0·7 milli-equivalents of nitrogen (but not more than 10 ml). Then add water to make the volume up to 10 ml if necessary. Attach the flask to the rest of the apparatus, and allow to stand for 10 minutes. Place a 250-ml conical flask containing 10 ml of boric acid solution and 3 drops of indicator below the condenser, the end of the condenser dipping below the surface of the solution. Add 40 ml of sodium hydroxide *plus* 10 ml of water through the tap funnel, mix by swirling, and then heat very gently until the effervescence ceases. Remove the source of heat if at any time the effervescence becomes vigorous. Then, increase the heat and distil off the ammonia until about 30 ml of liquid remains in the reaction flask. Titrate the ammonia with 0·05 N hydrochloric acid to the neutral grey end-point. It is advisable at this juncture to add a further 20 ml of water to the distillation flask, replace the titration flask below the condenser and distil over a further 10 ml. A small additional titration is usually obtained. Carry out a blank on all the reagents used.

An alternative way of decomposing the hydrogen peroxide prior to distillation is to add 0·2 ml of 1 per cent. cobalt sulphate solution. If necessary, this last decomposition can be initiated by very careful warming, but as the decomposition is exothermic there is some risk of loss of control. Decomposition by heating alone is probably safer.

CALCULATION—

$$\text{Nitrogen/g per 100 ml} = \frac{(T - B) \times F \times 14{\cdot}008 \times 1{\cdot}03}{2 \times 100 \times \text{sample volume}}$$

where T is the sample titration in ml, B is the blank titration in ml, F is the factor for the 0·05 N hydrochloric acid, and 1·03 is a factor assuming a 97 per cent. recovery of ammonia from all the components (see below).

RESULTS

TESTING THE METHOD—

The recommended method was tested by analysing separate solutions of acetonitrile, acrylonitrile, potassium cyanide and hydrogen cyanide made 0·2 N in sulphuric acid because acidic solutions of this strength were normally encountered in practice. (A neutral solution of potassium cyanide was also analysed.) Solutions of the first three were made up by weight; the hydrogen cyanide solutions were assayed during use by titration with silver nitrate (Liebig - Dénigès method). The results are shown in Table III. The mean recovery was 97·0 per cent., with a standard deviation of 1·0 per cent. It was therefore decided to assume an average recovery of 97 per cent. for all the above components in the analysis of unknown mixtures.

TABLE III

ANALYSIS OF INDIVIDUAL COMPONENTS BY THE RECOMMENDED METHOD

Solution	Nitrogen in solution/ g per 100 ml	Nitrogen taken/mg	Nitrogen found/mg	Recovery, per cent.
Acetonitrile in 0·2 N sulphuric acid ..	0·1521	7·61	7·36	96·7
		15·21	14·89	97·9
Acrylonitrile in 0·2 N sulphuric acid ..	0·1058	5·29	5·09	96·2
		5·29	5·07	95·8
		10·58	10·09	95·4
	0·2398	11·99	11·63	97·0
			11·53	96·2
	0·1930	19·20	18·63	97·0
			18·45	96·1
	0·1953	19·43	19·05	98·0
Potassium cyanide in 0·2 N sulphuric acid	0·2076	10·38	10·05	96·8
			10·14	97·7
Potassium cyanide in neutral solution ..	0·2145	10·73	10·33	96·3
	0·2103	10·52	10·12	96·2
Hydrogen cyanide in 0·2 N sulphuric acid	0·1450	7·25	7·17	98·9
			7·12	98·2
		14·50	14·09	97·2
			14·17	97·7
			14·16	97·7

Mixtures of acrylonitrile and hydrogen cyanide, and of acetonitrile, acrylonitrile and hydrogen cyanide, both in 0·2 N sulphuric acid, were then made up and analysed. The recovery was calculated on the basis of a 97 per cent. recovery of ammonia from all the components. The results are shown in Table IV. Ammonium sulphate was not included in these mixtures as it was assumed, and then confirmed experimentally, that ammonia was recovered quantitatively.

POSSIBLE SOURCES OF ERROR—

It was found to be difficult to obtain consistent results on neutral solutions of acrylonitrile, for which there was no explanation, and work was restricted to acidic solutions. Some later check experiments on neutral solutions of acetonitrile gave recoveries of 100·0, 99·7 and 100·0 per cent. as compared with the value of 97 to 98 per cent. shown in Table III for acidic solutions.

It is possible that the method of making up synthetic solutions also allowed for variation. These solutions were made up by weighing a stoppered 100-ml calibrated flask containing

TABLE IV

ANALYSIS OF MIXTURES BY THE RECOMMENDED METHOD

Solution	Nitrogen in solution/ g per 100 ml	Nitrogen taken/mg	Nitrogen found × 1·03/mg	Recovery, per cent.
Acrylonitrile *plus* hydrogen cyanide in 0·2 N sulphuric acid, solution (*a*)	0·770	7·70	7·75	100·6
			7·66	99·5
Acrylonitrile *plus* hydrogen cyanide in 0·2 N sulphuric acid, solution (*b*)	0·1165	11·65	11·65	100·0
			11·59	99·5
Acetonitrile *plus* acrylonitrile *plus* hydrogen cyanide in 0·2 N sulphuric acid, solution (*c*)	0·1241	6·20	6·21	100·2
			6·18	99·7
		12·41	12·40	99·9
			12·47	100·5

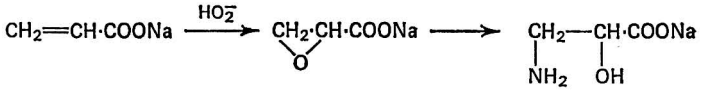
Solution (*a*)—
Acrylonitrile nitrogen = 0·0525 g per 100 ml
Hydrogen cyanide nitrogen = 0·0245 g per 100 ml

Solution (*b*)—
Acrylonitrile nitrogen = 0·0677 g per 100 ml
Hydrogen cyanide nitrogen = 0·0488 g per 100 ml

Solution (*c*)—
Acetonitrile nitrogen = 0·0154 g per 100 ml
Acrylonitrile nitrogen = 0·0811 g per 100 ml
Hydrogen cyanide nitrogen = 0·0276 g per 100 ml

about 60 ml of water *plus* sulphuric acid, rapidly adding the correct amount of nitrile, restoppering the flask and reweighing it. Hydrogen cyanide solution, if required, was added at this juncture (*i.e.*, before making up to volume).

It seemed possible that the recovery of less than 100 per cent. with acrylonitrile could be caused by epoxidation of sodium acrylate (from hydrolysis of the acrylamide) and subsequent addition of ammonia to form sodium 3-amino-2-hydroxypropionate during the heating stage when ammonia and peroxide are present together.



Experiments in which equivalent amounts of ammonium sulphate and acrylic acid were taken through the procedure yielded recoveries of 98·9 and 99·7 per cent., which indicated that this epoxidation does not take place to any appreciable extent.

Permission to publish this paper has been given by the British Petroleum Company Limited.

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Received *October 9th*, 1970
Accepted *May 27th*, 1971

Analyst, October, 1971, Vol. 96, *pp.* 734–738

The Theoretical and Practical Aspects of Electronic Timing as One Method of Increasing the Analysis Rate on the Technicon AutoAnalyzer I

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The need for an increase in the speed of analysis of soil and plant samples is discussed with reference to the use of the Technicon AutoAnalyzer I. A reliable electronic timer that controls the operation of the Technicon Sampler is described. A circuit diagram of the timer is shown, with a list of components used.

THE use of the Technicon AutoAnalyzer I as an analytical tool for the analysis of biological samples is well established. In the original concept, the AutoAnalyzer I system was used for the determination of parameters in vital body fluids. Such analyses were performed at rates of forty to seventy tests per hour on samples the variability of which about a so-called normal value rarely exceeded twice this value, and in many instances was considerably less.[1,2,3,4]

Botanical samples such as leaf material originating from a single-species source show, on analysis, more variability than is found in body fluids. In one such instance, the highest expected value for a single element may be up to five times the lowest expected value.[5] When drawn from different parts of the world, the expected variation from low to high for a single element may be as much as a factor of thirty.

Soil samples can be expected to give an even wider range of values. For a single element the high value can be as much as one hundred times the low value. Even samples collected from a relatively small area of land often show a very wide range of values for a single element, and during a series of analyses a high value can be immediately followed by a low value. It is essential, therefore, that in any scheme in which the Technicon AutoAnalyzer is used the wash-out between consecutive samples is as complete as possible while maintaining a high rate of analysis.

These two factors, effective wash-out and a high rate of analysis, directly oppose each other. The faster the rate of throughput the less time is available for wash-out. On the other hand, if a good wash-out characteristic is required, then a low rate of throughput of samples usually results.

In this laboratory we have used the AutoAnalyzer I system for several years for the analysis of soil and plant materials. For this work we have adopted as a specification for a single determination a minimum of sixty tests per hour, with a wash-out of at least 90 per cent. between tests. This has been achieved by the critical examination of each manifold, and modifications were made until this specification was met.

As more samples for analysis are expected and more tests are automated, it becomes necessary to examine the possibility of increasing the rate of analysis to speeds of about eighty to one hundred tests per hour. It would also be an advantage if these increased rates of analysis could be achieved by using manifolds that are unmodified, or only slightly modified, from those in current use.

At first sight a simple reduction in the time taken for analysis of each sample, with a subsequent increase in flow-rate through the colorimeter flow cell to compensate for the shortened time, would appear to solve the problem.

Unfortunately, within the range of analyses being considered (eighty to one hundred tests per hour) the form of the trace on the chart recorder passes from "steady-state analysis" to "non-steady-state analysis," and the method of timing becomes most important. An examination of the forms of traces obtained under different conditions will illustrate this aspect.

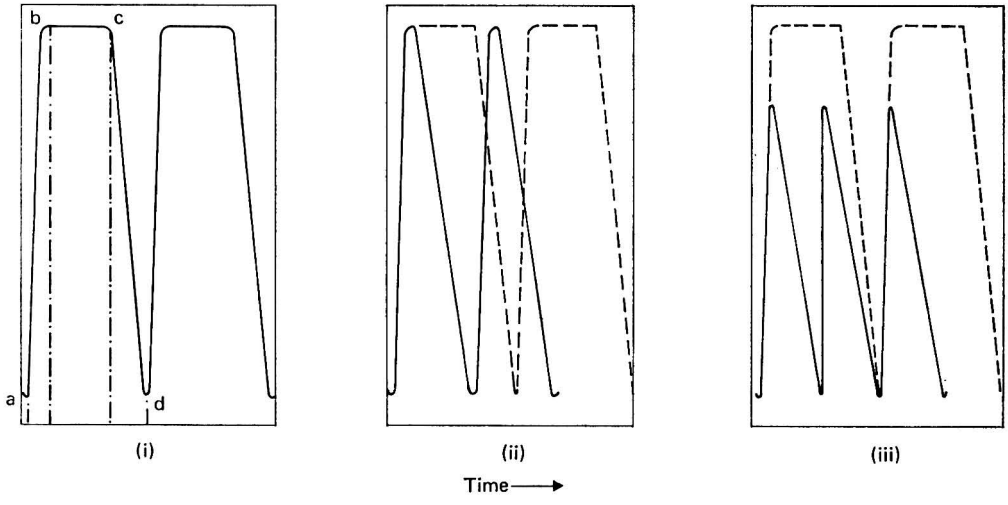

Fig. 1. Diagrammatic representation of trace characteristics: solid lines (*i*), forty tests per hour; (*ii*), sixty tests per hour; and (*iii*), eighty tests per hour

TRACE CHARACTERISTICS—

A typical trace is shown in Fig. 1 (*i*), which consists of a rise time, ab, followed by a plateau, bc, at steady state, and finally a period of wash-out, cd, before the next sample enters at point d. In this form of "steady-state analysis" a plateau region produced over a time interval of, say, up to 30 s removes the need for accurately machined timing cams. In practice, the length of castellation on a timing cam as used on the AutoAnalyzer I may vary by 10 per cent. On a cam with six castellations good reproducibility would be obtained only on every sixth sample unless a system of "steady-state analysis" was used.

All the useful information in this form of trace is contained in the leading edge point, b, and no additional information is obtained from the plateau region, bc. The plateau time, bc, may be reduced to zero so that b = c, thus providing a greater output in terms of number of tests per hour. This type of trace is illustrated in Fig. 1 (*ii*) and may be described as "critical steady-state analysis."

The slope of the trace between a and b will vary, depending on the analysis being performed. Therefore, the time taken to reach b from a is variable and dependent on the method. Similarly, the wash-out characteristic, cd, also varies between different determinations.

If a sample-to-wash ratio of 2:1 is used it might be possible to reach point b but fall short of a 90 per cent. wash-out. On the other hand a sample-to-wash ratio of 1:1 may give the required 90 per cent. wash-out, but fall short of point b. This assumes, of course, that the same number of tests per hour is performed. A sample-to-wash ratio of 1:1 at a slower rate of tests per hour would overcome this difficulty.

When high-speed working of about eighty to one hundred tests per hour is examined it is found that point b cannot be reached in the time available. This is illustrated in Fig. 1(*iii*) and may be termed "non-steady-state analysis." In this case, because of the steep slope of ab with respect to time, both sample and wash time must be constant if reproducibility is to be achieved. In addition, the wash-out characteristic in this type of analysis should approach 100 per cent. to ensure absence of carry-over between samples.

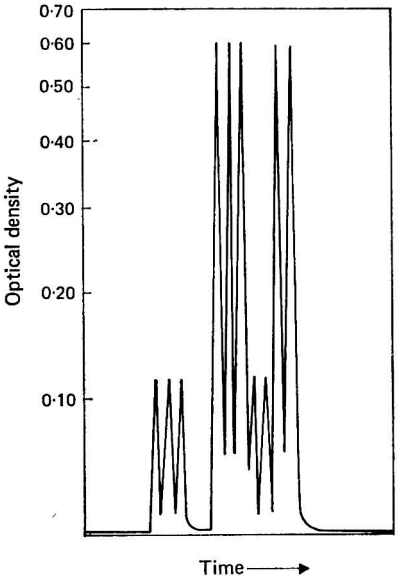

Fig. 2. Repeatability of peaks obtained during the determination of ammonium-nitrogen by using the indophenol blue method. Analysis rate ninety tests per hour.

Fig. 2 illustrates the repeatability of peaks obtained in the determination of ammonium-nitrogen by using a modification of the indophenol blue method described by Van Slyke and Hiller.[6] The solutions used are potassium chloride extracts of soil (for cation-exchange capacity according to the method of Metson[7]). Two extracts, one of low and the other of high concentration, have been sampled in the order shown, with two blank samples following the first three low peaks. The rate of analysis was ninety tests per hour, with a sample time of 21 s and a wash time of 19 s. The total flow-rate of sample or wash *plus* reagents was 9·3 ml min^{-1} *plus* 2·5 ml min^{-1} of air. After de-bubbling, the flow-rate through the colorimeter flow cell was 3·9 ml min^{-1}.

The use of "non-steady-state analysis" could perhaps be further extended as a means of reducing the sensitivity of a method in which an unmodified manifold is used. This is useful when a set of samples with values beyond the expected range has to be analysed. Standard solutions of higher concentration are used, and the higher peaks that would normally have been obtained are cut short by reducing the time of sampling. Such a method usually exhibits good wash-out, for if high-speed working is not required, the extra time available can be used to extend the wash-out period. However, success with this method is entirely dependent on accurate timing.

The cam system of timing as used in the AutoAnalyzer I system provides speeds in multiples of ten tests per hour with fixed sample-to-wash ratios and has limited flexibility. It is possible to obtain greater flexibility by using variable-ratio cams. However, on such cams increasing the time of sampling reduces the wash time and *vice versa*.

It is obvious from the previous discussion that there is a definite need for a more flexible timing system, preferably one in which the sample and wash times are independent of each other, accurately reproducible and extremely variable.

Experimental

Apparatus—

Earlier work with a simple resistor - capacitor circuit for controlling the operation of the Technicon AutoAnalyzer Sampler was unsatisfactory because of poor repeatability of the time intervals. An alternative circuit was constructed incorporating unijunction transis-

tors, which has proved to be stable for long periods with good repeatability. The circuit diagram of this unit can best be described by dividing it into three sections, as shown in Fig. 3.

Section (*i*) is the basic timing circuit in which the time interval starts when power (—12 V d.c.) is applied via S_1 and terminates when the voltage is applied to the load, RLY_1. The unijunction transistor, TR_2, is used in the oscillator, which pulses base 2 of the complementary unijunction, TR_1. This reduces the effective peak-point current of TR_1 and allows the use of a much smaller timing capacitor, $C_1 + C_2$, and larger timing resistors, R_{14} to R_{32} or R_{33} to R_{51}, than would otherwise be possible. At the expiration of the interval, a pulse of approximately —3·5-V amplitude is produced at the base 1 of TR_1. This pulse, which is of short duration, is led via D_1 to the base of TR_3 in section (*ii*). Section (*ii*) is a pulse-lengthening circuit, the length of the pulse being determined by the resistor - capacitor network of R_{12} and C_4 on the base of TR_4.

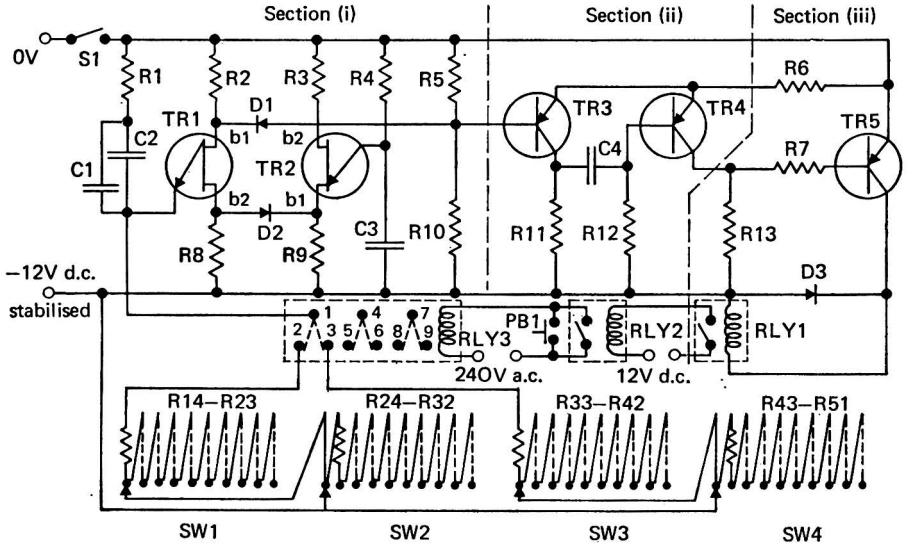

Fig. 3. Circuit diagram for electronic timer

Section (*iii*) comprises the reed relay, RLY_1, and the switching transistor, TR_5. The reed switch closes momentarily at the expiration of the timed period as governed by the basic timing circuit in section (*i*). This momentarily closes the contacts of the relay, RLY_2, which in turn energises the bistable relay, RLY_3, which changes state. This has the effect of switching from one pair of resistors, as set by SW_1 and SW_2, to the other pair, SW_3 and SW_4, via the first change-over contacts on pins 1, 2 and 3 of the bistable relay. The process is repeated and continues to alternate from one timed period to the other while power is being applied.

The second change-over contacts on the bistable relay, pins 4, 5 and 6, are connected to the Technicon Sampler in such a way as to take over the function of the cam microswitch. The state of the bistable, therefore, also governs the position of the sample tube, either in the "wash" or "sample" position.

The third change-over contacts on the bistable, pins 7, 8 and 9, are wired to two neon lamps on the front of the unit. These lamps give a visual indication of the state of the bistable, and the position of the sample tube.

CALIBRATION—

Calibration of the timer* is made by adjustment of the value of the capacitors, C_1 and C_2, while the unit is in operation before connection to the sampler, but connected to a suitable calibrated timing device, such as a chart recorder. A voltage source is connected to the

* Patent applied for.

recorder via pins 4 and 5 of the bistable relay. In this way a record of the timing intervals is shown on the chart paper as a series of square waves, and the necessary "padding" of C_1 with various values of C_2 can be made until the required calibration is achieved (1 MΩ is equivalent to 1 s).

The value of $C_1 + C_2$ will be approximately 1 μF, and a high stability polycarbonate capacitor of this value can be used for C_1 as a starting point.

OPERATION—

Selection of the time intervals for "sample" and "wash" are made by operation of SW_1 *plus* SW_2, and SW_3 *plus* SW_4, which are marked in seconds and seconds × 10. Time intervals are therefore variable from 1 to 100 s in 1-s steps for both "sample" and "wash" positions of the sample tube on the Technicon Sampler.

A push-button switch, PB_1, as shown in Fig. 3, enables the operator to change manually the position of the sample tube on the Sampler while setting up a programme of analyses.

CONCLUSION

The timer described has been in continuous use for 12 months with a Technicon Auto-Analyzer I, and has resulted in a general increase of about 30 per cent. in the analysis rate for all the automated determinations carried out in this laboratory. It has proved to be reliable in use, with no malfunctions. The cost of the timer was approximately £30. A list of components used in the construction is given below.

The authors thank Mr. R. M. Baker of this unit for his helpful suggestions, and Mr. P. C. Chambers, Deputy Director, Land Resources Directorate of Overseas Surveys, for permission to publish this paper.

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Received *September 30th*, 1970
Accepted *January 19th*, 1971

Appendix

LIST OF COMPONENTS

R_1, R_2, R_9	= 47-Ω, ½-W resistors	Metal oxide, 2 per cent.
R_3	= 330-Ω, ½-W resistor	
R_4	= 33-kΩ, ½-W resistor	
R_5, R_{13}	= 10-kΩ, ½-W resistors	
R_6	= 1-kΩ, ½-W resistor	
R_7	= 330-kΩ, ½-W resistor	
R_8	= 820-Ω, ½-W resistor	
R_{10}	= 220-kΩ, ½-W resistor	
R_{11}	= 27-kΩ, ½-W resistor	
R_{12}	= 500-kΩ, ½-W resistor	
R_{14} to R_{23}, R_{33} to R_{42}	= 1-MΩ, 1-W resistors	Carbon, 1 per cent. selected
R_{24} to R_{32}, R_{43} to R_{51}	= 10-MΩ, 1-W resistors	
C_1	= 1-μF capacitor, 63-V d.c. working, polycarbonate	
C_2	= Polyester capacitor selected, as described in text	
C_3, C_4	= 0·22-μF capacitors, 250-V d.c. working, polyester	
TR_1	= D5K transistor	
TR_2	= 2N2646 transistor	
TR_3, TR_4	= NKT213 transistors	
TR_5	= 2N1305 transistor	
D_1, D_2, D_3	= OA202 diodes	
RLY_1	= 12-V reed relay, 1-kΩ coil	
RLY_2	= 12-V relay, 600-Ω coil, 240-V, 3-A a.c. contacts	
RLY_3	= 240-V a.c. bistable relay, type 521 (Magnetic Devices Ltd., Newmarket, Suffolk)	
SW_1, SW_2, SW_3, SW_4	= 1-pole, 10-way rotary switches	
S_1	= Light-duty single-pole, single-toggle switch	
PB_1	= Push-button switch	

Analyst, October, 1971, Vol. 96, *pp.* 739–740

The pH Meter as a Hydrogen-ion Concentration Probe: A Postscript

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Measurements of the ratio between the quantity H ($= 10^{-pH}$) and the true hydrogen-ion concentration in solutions of fixed ionic composition have been made in alkaline solutions, and are in agreement with values previously determined in acidic solutions.

Although the e.m.f. of suitable chemical cells can be empirically related to the logarithm of the hydrogen-ion concentration[1] within the range $10^{-2} > [H^+] > 10^{-12}$ M, the practice followed in most laboratories when the acidity of solutions is being studied is to measure pH with a combination of glass and calomel electrodes. When it is necessary to derive values of the hydrogen-ion concentration from such measurements, as might be required for a mass balance, it has been commonly recommended[2] that the pH value be interpreted as $-\log a_{H^+}$, and that a suitable estimate of the activity coefficient y_{H^+} be used to convert a_{H^+} to $[H^+]$. This practice does not solve the problems of (*i*) specific ionic interactions with background electrolyte and the not uncommon use of moderately concentrated supporting electrolytes to secure a medium of essentially constant ionic strength; and (*ii*) the liquid-junction potential. In 1969 the author described[3] direct measurement of the factor Γ relating H ($= 10^{-pH}$) and $[H^+]$, and listed values of this factor found at various concentrations of three background electrolytes. It is clear from the response to that paper and from other allusions in the literature that increased interest is being taken in this relationship between H and $[H^+]$.

Irving, Miles and Pettit,[4] of whose work we were unaware at the time, have dealt with direct calibrations in a fashion similar to our own. Perrin and Childs[5] treated the quantity F (identical with our Γ) as an adjustable parameter in applying a least-squares computer programme to the results of pH titrations for the determination of acidity constants or metal-complex stability constants; they sought the value of F corresponding to a minimum in the standard deviation of titre values. Subsequently Childs[6] has shown that the two approaches lead to essentially the same result. Powell and Hedwig (University of Canterbury, N.Z., private communication) calibrated the cell

glass electrode$|H^+_{(aq)}$, $NaCl_{(aq)}$, buffer|calomel electrode

against acetate or ethylenediamine solutions of known $[H^+]$ over the pH range 4·0 to 10·3 and at various ionic strengths. The relationship between pH and p[H] found by them closely matched that observed in this laboratory.

In the work previously reported the ratio Γ ($= H/[H^+]$) was determined over the range from approximately 10^{-2} to 10^{-4} M with respect to hydrogen ion, but the question remained as to whether this ratio could be assumed to be constant at higher pH values, so that, for instance, it could be used to convert mixed acidity constants into concentration quotients at any pH value, or to calculate $[OH^-]$ at higher pH values in order to complete a mass or charge balance. To some extent Powell and Hedwig's observations justify extension of the range of application of Γ.

Experimental

We have extended the calibration of the pH meter assembly into the alkaline region and have observed a linear relationship between H^{-1} ($= 10^{pH}$) and $[OH^-]$ in the approximate range 10^{-2} to 10^{-4} M with respect to hydroxide ion. The slope or proportionality constant for this relationship was found graphically and by regression analysis. If we call this constant Γ_{OH} and that obtained in acidic solutions Γ_H, it follows that $(\Gamma_H\Gamma_{OH})^{-1}$ must be equal to K_{cw}, the ionic concentration product for water for the salt medium and temperature in question.

Such an approach to the measurement of K_{cw} has been described previously.[7,8,9] Alternatively, if K_{cw} is known or can be estimated, the value of Γ_H in alkaline solution can be calculated and compared with the value observed under the same conditions in acidic solution—

$$\Gamma_H = (K_{cw}\Gamma_{OH})^{-1} \text{ in alkaline solution.}$$

TABLE I

COMPARISON OF Γ_H MEASURED IN ALKALINE AND ACIDIC SOLUTIONS

	Example 1	Example 2
Ionic medium	0·5 M KNO_3	0·15 M KNO_3
Temperature	25·0 °C	37·0 °C
pH meter	EIL Vibron	Orion
Electrodes	EIL	Beckman
Observed Γ_{OH}	$0{\cdot}680 \times 10^{14}$	$0{\cdot}296 \times 10^{14}$
Number of observations ..	60	12
Standard deviation of Γ_{OH} ..	$0{\cdot}013 \times 10^{14}$	$0{\cdot}002 \times 10^{14}$
$\gamma_H\gamma_{OH}/a_{H_2O}$*	0·535	0·593
K_{cw}	$1{\cdot}887 \times 10^{-14}$	$4{\cdot}077 \times 10^{-14}$
Γ_H (calculated)	0·782	0·826
Γ_H (observed)	0·801	0·804

* See Harned and Owen.[10]

Table I shows some typical results from measurements in potassium nitrate solutions at two molarities and at two temperatures. The values of K_{cw} were estimated[10] by using interpolated values of the factor $\gamma_H\gamma_{OH}/a_{H_2O}$ appropriate to potassium chloride solutions (for lack of corresponding values for potassium nitrate). The values of Γ_H measured in acidic solutions and those derived from measurements in alkaline solutions agree to within 3 per cent. This agreement appears to be satisfactory; it corresponds to a misfit of slightly more than 0·01 pH units in a graph of pH *versus* –log [H^+] between the two experimentally contrived regions for which comparisons were made. With commercial electrodes it is not uncommon to encounter discrepancies of this order in aligning pH-meter readings on three buffers of widely spaced pH values.

This evidence offers further justification for the use of empirical values of Γ to convert pH readings into concentrations of hydrogen or hydroxyl ion over the useful range of the pH scale.

The author gratefully acknowledges financial support for this work from the National Research Council of Canada, and the technical assistance of Miss Janet Rohr.

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NOTE—Reference 3 is to the paper to which the present paper constitutes a postscript.

Received *March 12th*, 1971
Accepted *April 27th*, 1971

Analyst, October, 1971, Vol. 96, *pp.* 741–743

Analytical Methods Committee

REPORT PREPARED BY THE METALLIC IMPURITIES IN ORGANIC MATTER SUB-COMMITTEE

The Determination of Small Amounts of Copper in Organic Matter by Atomic-absorption Spectroscopy

THE Analytical Methods Committee has received the following Report from its Metallic Impurities in Organic Matter Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been approved by the Council.

REPORT

The constitution of the Metallic Impurities in Organic Matter Sub-Committee responsible for the preparation of this Report was: Dr. L. E. Coles (Chairman), Mr. W. Cassidy, Dr. J. C. Gage, Dr. R. A. Hoodless, Miss E. M. Johnson, Mr. D. A. Lambie, Dr. H. Liebmann, Dr. R. F. Milton, Mr. W. L. Sheppard, Mr. G. B. Thackray and Mr. C. A. Watson, with Mr. P. W. Shallis as Secretary.

INTRODUCTION

In 1963 the Sub-Committee recommended a procedure involving the use of diethylammonium diethyldithiocarbamate for the determination of small amounts of copper in organic matter.[1] This method is still considered to be satisfactory, but advances in instrumentation and technique have, in the opinion of members of the Sub-Committee, made atomic-absorption spectroscopy equally satisfactory for the determination of copper and, in general, the latter method is more convenient and rapid to apply. This Report gives details of conditions that have been found to be satisfactory and the Sub-Committee recommends that either the diethylammonium diethyldithiocarbamate procedure[1] or atomic-absorption spectroscopy can be used for the determination of copper in organic matter; the method selected will depend on the material to be analysed.

SAMPLE PREPARATION

The determination of copper in organic materials by atomic-absorption spectroscopy is less affected by the use of different acids than are the determinations of other trace metals, and hence it is the least affected by the type of sample preparation adopted. However, it is still advisable to use the same acid composition and concentration for standards and blanks as are used for the samples. In general, the acid concentration should not exceed about 2 N in the aspirated solution, although in certain circumstances the use of considerably higher acid concentrations may be satisfactory.

Dry or wet ashing is suitable for the destruction of organic matter before the determination of copper. For certain types of liquid samples, *e.g.*, ready-to-drink beverages, and for semi-solid foodstuffs alternative satisfactory procedures are described.

DRY ASHING—

Even if the organic matter can be readily dry ashed it is still necessary to check the recovery of copper, which may not be complete.[2] Porcelain crucibles should not be used, as copper can be extracted from the glaze.[3] The residue from a dry-ashing procedure is best dissolved in a mixture of hydrochloric acid and water (1 + 1 v/v). If the copper in the sample does not dissolve completely in this mixture, a mixture of hydrochloric acid, nitric acid and water (2 + 1 + 3 v/v) can be used, provided that the containing vessel is not made of platinum.

WET ASHING—

Any of the usual techniques[4] can be used, but the easiest method of digesting many organic materials is with a mixture of 50 per cent. hydrogen peroxide and sulphuric acid.[5]

For both wet and dry ashing sample weights and final volumes can be chosen to give the optimum sensitivity required.

READY-TO-DRINK BEVERAGES—

These can be aspirated without destruction of organic matter. A suitable dilution with water should be prepared to minimise the effect of the presence of soluble solids in the beverage. Soluble solids are troublesome in the aspirated sample only at concentrations above about 3 per cent.

SEMI-SOLID FOODSTUFFS—

A quick method for the analysis of foodstuffs is based on that described by Simpson and Blay.[6] To 10 g of the homogenised sample, 40 ml of water and 10 ml of concentrated hydrochloric acid are added. The mixture is heated to boiling and then simmered gently for not more than 5 minutes. The solution is next cooled, transferred to a 100-ml calibrated flask, diluted to volume, and mixed. A sufficient volume of solution (normally 10 to 20 ml) for the analysis is removed and filtered.

It is essential not to prolong the boiling, as charring may occur, which could hold back some trace metal at the filtration stage. Similarly, a higher acid concentration can also increase charring and for this reason the mixture recommended here is less concentrated than that used by Simpson and Blay.[6]

OILS, FATS AND FATTY FOODS—

It may be difficult, and sometimes impossible, to wet-oxidise these materials to give an aqueous solution. At copper contents below 0·1 p.p.m., contamination from reagents and vessels used for preparing aqueous solutions may be greater than the actual copper contents. It is then simpler to aspirate solutions of the fatty matter in an organic solvent directly into the flame.

When this is done, care must be taken that the spraying characteristics of the reference sample and the unknown are identical. Oils and fats that are from different sources and have different physical properties (*e.g.*, density and viscosity) will give different background responses and also modify the atom-producing capacity of the flame. It is, therefore, preferable to use the maximum possible dilution of the fatty matter in an appropriate solvent. When dilution causes too great a loss in sensitivity it is possible to aspirate a 50 per cent. w/w solution of fatty matter in the solvent, but the aspirating air must be pre-heated and solutions should be incubated to the same temperature (30 to 40 °C). Fats can then be aspirated without precipitation in the nebuliser and all oils will tend towards common spraying characteristics at the higher temperature. A calibration curve is prepared by supplementing a similar metal-free oil with an organic copper compound, such as oleate or naphthenate.

Pentyl acetate is a good general solvent for this type of work.

Suitable adjustments to the fuel flow will be necessary to compensate for the burning capacity of the fat and solvent.

It may be necessary to separate fatty matter from formulations, *e.g.*, from carbohydrates by solvent extraction, or from the aqueous phase by stirring at 80 °C in the case of fat - water emulsions such as margarines.

THE USE OF AMMONIUM PYRROLIDINEDITHIOCARBAMATE (APDC)

This reagent, which was introduced by Malisia and Schoffmann,[7] can be used for the extraction of copper from a wide range of aqueous solutions into an organic solvent suitable for direct atomic-absorption spectrophotometry.[8] Extraction of the copper into an organic phase for atomic-absorption spectrophotometry affords several advantages: the concentration of the metal in the organic phase may be 100 times that in the aqueous phase, which enables smaller amounts to be determined, the atomic-absorption signal for copper in an organic solvent is considerably enhanced in comparison with that obtained from a similar concentration in aqueous solution, and the copper is separated from high concentrations of salts, which inevitably arise when certain organic materials are wet-digested, ashed or diluted.

The sensitivity of copper determinations (A = 0·004) by atomic absorption varies with the equipment being used, but in organic solvents such as 4-methylpentan-2-one it is usually between 0·02 and 0·1 p.p.m. and a linear calibration graph is usually obtained up to at least 100

times this figure, so that if the copper is extracted into 10 ml of solvent the method can be used for amounts of between 0·2 and 1 μg of copper up to amounts between 20 and 100 μg of copper, according to the equipment available.

The copper - APDC complex is readily extracted from aqueous solution, within the range strongly acidic (5 N) to pH 10, into polar organic solvents such as chloroform, 4-methylpentan-2-one and heptan-2-one. Chloroform is useful if very high concentration factors are required but leads to flame instability and attack on the nebuliser with some equipment when sprayed directly. For most purposes 4-methylpentan-2-one or heptan-2-one is more satisfactory, the latter having a lower solubility in acidic aqueous solutions.

Owing to the solubility of the solvents in acidic aqueous solutions, the solution should be saturated with the solvent before extraction of the APDC - copper complex, which is formed immediately upon addition of the APDC. For amounts of copper below 100 μg two extractions with 4 ml of solvent, each after the addition of 1 ml of 1 per cent. aqueous APDC, will remove all detectable amounts of copper from 100 ml of solution; the two extracts are then combined and diluted to 10 ml in a calibrated flask and sprayed into the flame of the atomic-absorption spectrophotometer. The calibration graph must be prepared by extraction of standard amounts of copper under conditions similar to those used for the samples, as the sensitivity may vary according to the solvent composition.

Extraction of copper with APDC can be applied directly to the aqueous solutions obtained by the above general methods of sample preparation providing they are diluted, when necessary, so that the acidity is not greater than 5 N.

Operating conditions

The optimum operating conditions recommended by the instrument manufacturer should be used. The zero should be set by using an appropriate blank, and standard solutions for the calibration should be prepared so that they have the same acid composition.

The instrument should be set up with a copper hollow-cathode lamp, operated at the recommended current, with the monochromator set at 324·8 nm, and the slit-width adjusted to give an acceptable signal-to-noise ratio. Instruments are available that are capable of detecting as little as 0·01 p.p.m. of copper.

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Analyst, October, 1971, Vol. 96, *pp.* 744–745

Analytical Methods Committee

REPORT PREPARED BY THE FISH PRODUCTS SUB-COMMITTEE

Nitrogen Factor for Coal Fish

THE Analytical Methods Committee has received the following Report from its Fish Products Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The constitution of the Fish Products Sub-Committee responsible for the preparation of this Report was: Dr. S. M. Herschdoerfer (Chairman), Dr. G. H. O. Burgess (resigned October, 1968), Dr. J. H. Bushill (resigned October, 1969), Mr. C. B. Casson (resigned February, 1970), Dr. W. T. Little (deputy—Mr. W. P. Cowie), Dr. J. A. Lovern (resigned March, 1969), Mr. T. McLachlan, Mr. R. McLay, Mr. D. J. Ward (deputy—Dr. M. F. Gould), Mr. R. E. Weston and Dr. M. L. Windsor (deputy—Mr. I. N. Tatterson), with Mr. P. W. Shallis as Secretary.

In 1966 the Sub-Committee issued its report on the nitrogen factor for cod flesh,[1] and a similar approach to the determination of a nitrogen factor to be used in the analysis of products made from coal fish (saithe; coley) has since been applied. Results for the nitrogen content of coal fish were collected over the period May, 1967, to March, 1969, and the results are shown in Fig. 1. It can be seen from Fig. 1 that the collaborating laboratories were not able to obtain any fish for analysis during some months of any of the years covered.

A wide spread of nitrogen contents was observed, similar to that previously found for cod, and this serves to illustrate the inevitable degree of possible error attached to any individual analysis of such fish products. The over-all range of the ninety-five results was 2·52 and 3·27 per cent., the mean value for the nitrogen content of coal fish flesh being 2·937 per cent., with a standard deviation of $\pm 0{\cdot}165$.

The Sub-Committee has examined the possibility of using other analytical values in the analysis of fish products, *e.g.*, the non-protein nitrogen content. It has been found, however, that other factors are not less variable than is the total nitrogen content and therefore do not reduce the range of possible error. A method based on the physical determination of fish fibre has been investigated by some members of the Sub-Committee.[2] This method suffers from certain disadvantages and cannot at present be recommended by the Sub-Committee, but further investigations are to be carried out. The Sub-Committee cannot therefore at present recommend any more accurate method for the determination of the fish content of fish products than that based on a determination of total nitrogen content.

RECOMMENDATION

After due consideration of all the relevant data the Sub-Committee recommends that an average nitrogen factor of 2·90 should be used in the analysis of coal fish (saithe; coley) products.

ACKNOWLEDGMENT

The Sub-Committee thanks those listed below for their help and communications—

Department of Trade and Industry, Humber Laboratory.
Department of Trade and Industry, Torry Research Station.
Unilever Ltd.

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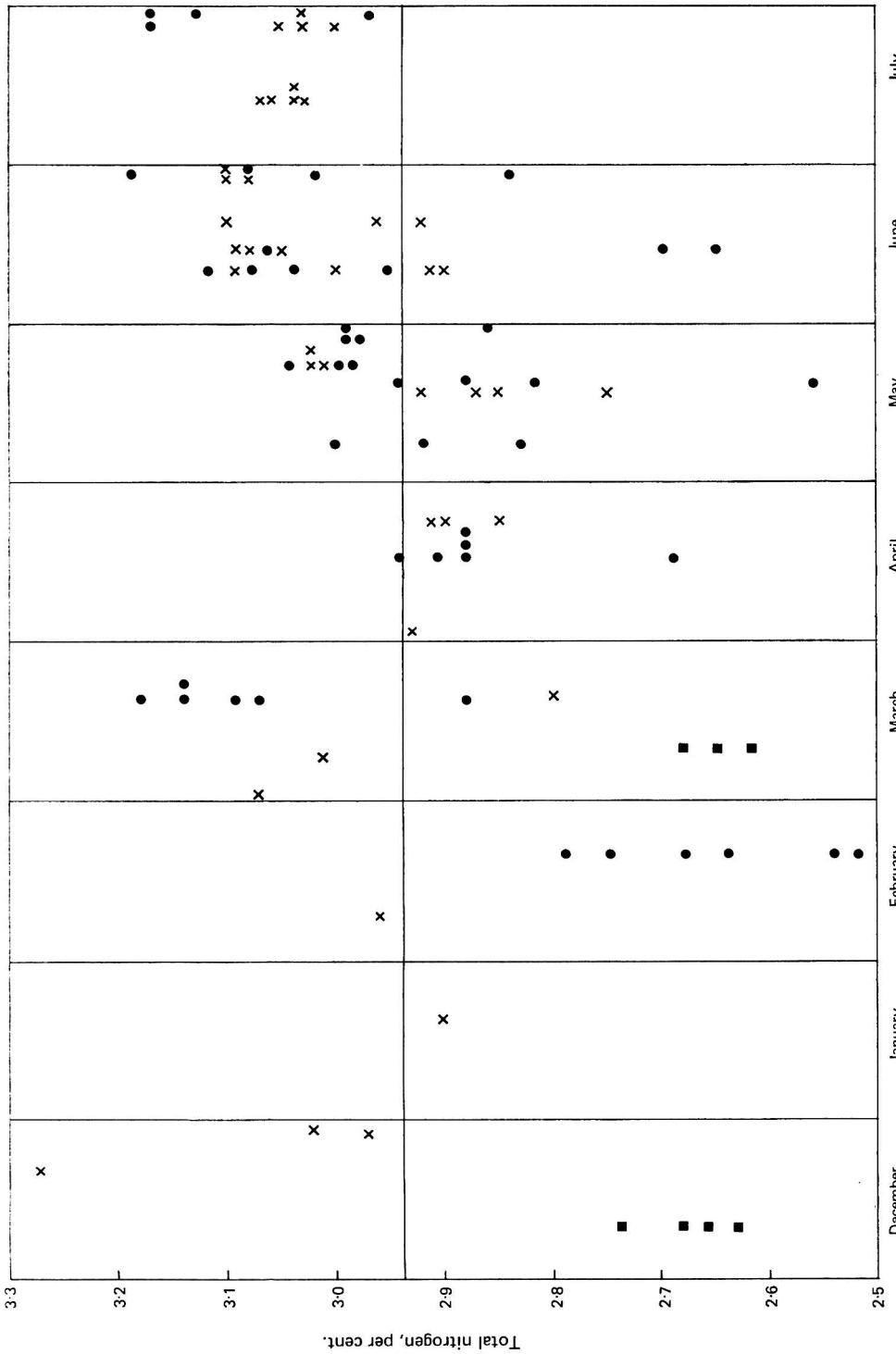

Fig. 1. Nitrogen content of coal fish determined during the period from May, 1967, to March, 1969. ●, Laboratory A; ×, laboratory B; ■, laboratory C

Analyst, October, 1971, Vol. 96, *pp.* 746–749

Analytical Methods Committee

REPORT PREPARED BY THE PROPHYLACTICS IN ANIMAL FEEDS SUB-COMMITTEE

The Determination of Dimetridazole in Animal Feeds: Revised Method

THE Analytical Methods Committee has received the following Report from its Prophylactics in Animal Feeds Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The constitution of the Prophylactics in Animal Feeds Sub-Committee responsible for the preparation of this Report was: Mr. S. G. E. Stevens (Chairman), Mr. R. J. Anderson, Mr. A. G. Croft, Mr. C. E. Dodd, Mr. G. Drewery, Mr. J. Hartley, Mr. R. S. Hatfull, Mr. S. P. Hayes, Mr. J. S. Leahy, Mr. I. McLachlan, Mr. J. Markland, Mr. D. H. Mitchell, Mr. R. C. Spalding, Mr. J. A. Stubbles, Mr. R. E. Weston and Dr. D. R. Williams, with Mr. P. W. Shallis as Secretary.

INTRODUCTION

In October, 1969, the Sub-Committee published its recommended polarographic method[1] for determining dimetridazole (1,2-dimethyl-5-nitroimidazole) in poultry feeds. Shortly after this method had been published one member of the Sub-Committee received for analysis several samples of feedingstuff from a single manufacturer that were stated to have been medicated with dimetridazole at the usual level, *viz.*, 125 p.p.m. Application of the recommended method to these samples gave very low results; in all instances less than 10 p.p.m. of the drug was indicated and on one of the samples no polarographic wave for dimetridazole was found. The presence of dimetridazole in this sample was, however, indicated by a thin-layer chromatographic technique[2] and, although this method is not claimed to be quantitative, a matching procedure against standards gave an estimate of the dimetridazole content of about 100 p.p.m.

The Sub-Committee's attention was also drawn to the fact that an official agricultural analyst had received for examination a sample of turkey pellets stated to contain 125 p.p.m. of dimetridazole. By the recommended polarographic procedure this analyst had found a negligible content of the drug. A member of the Sub-Committee analysed a portion of the same sample by a spectrophotometric method[3] that had previously been investigated by the Sub-Committee and reported a result of 128 p.p.m. of dimetridazole.

An unsatisfactory position had therefore arisen in which the recommended method, although obviously satisfactory in the collaborative tests carried out originally by the Sub-Committee, was now known not to be generally applicable. Consideration was given to abandoning the polarographic method and re-investigating the available spectrophotometric procedures that in the earlier work had been found to be not entirely satisfactory, and to attempting to find the reason for the failure of the polarographic method in certain instances. It had for some time been realised that changes in the composition of feedingstuffs could occasionally introduce some interfering species that would render a method of analysis inapplicable. Enquiries elicited the fact that the essential difference between the feedingstuffs used by the Sub-Committee in its original work and those from which extremely poor recoveries of dimetridazole were obtained was that the latter contained a bentonite-based hardening ingredient at a concentration of 56 lb per ton. As it was considered that this could affect the extraction of the drug from the feed, it was decided to extend the investigation of the polarographic method to see if some simple modification could overcome the difficulties that had been encountered. A cautionary note on the use of the recommended method was published in April, 1970.[4]

Experimental

The laboratory that first noted the low recoveries of dimetridazole when analysing a batch of samples obtained some further samples from the same manufacturer that were of the same composition except that the bentonite-based hardening ingredient was absent. On all these samples satisfactory recoveries of dimetridazole were obtained by the recommended method.[1] It was thought, therefore, that because of its high absorptive capacity the bentonite was retaining the drug and that the comparatively mild extraction with dilute acid used in the recommended method was not sufficient to recover it for analysis. All previous methods recommended by the Sub-Committee for the determination of prophylactic drugs in feedingstuffs involved initial extraction with an organic solvent, and it was decided to investigate the application of a preliminary solvent extraction followed by transfer of the extracted drug to dilute acid solution for the polarographic determination of dimetridazole.

One laboratory investigated the use of methanol, hexane, carbon tetrachloride, toluene, ether and light petroleum for the preliminary extraction of dimetridazole and of these found only methanol to be wholly satisfactory, as with all the others either low recoveries were obtained or there was a pronounced shift in the voltage at which the polarographic peak occurred. By using the procedure of pre-treatment with methanol this laboratory reported results of 122, 125 and 123 p.p.m. of dimetridazole in a portion of the sample of turkey pellets referred to earlier in this Report: a further test at the same time by the unmodified procedure gave a result of 22 p.p.m. of the drug.

A collaborative test of the modified procedure was arranged in which three samples of different feedingstuffs medicated with dimetridazole and Pancoxin and containing also a bentonite-based hardening ingredient at a concentration equivalent to 28 lb per ton were circulated to four laboratories. Each laboratory determined the dimetridazole contents of these samples by both the original and the modified procedures.

The results of these tests were inconclusive as, although in almost every instance the recoveries by the modified procedure were low (mean recovery 72 per cent.), the inhibitive effect of the presence of bentonite was far less pronounced in the original method (mean recovery 62 per cent.) than it had been with the earlier samples. The Sub-Committee considered that the low recoveries by the modified procedure may have been a reflection on the adequacy of mixing and sampling, as a departure from normal procedure had been followed in that the samples had been medicated before distribution. In nearly all its previous work the Sub-Committee has avoided confusing the testing of an analytical method with the testing of the efficiency of mixing dry ingredients of different particle size by carrying out medication to the required level within each laboratory by adding the drug directly to the portion for analysis.

Two possible reasons were advanced for the more efficient recovery of the drug by the original method, *viz.*, the proprietary bentonite-based hardener used was known not to be the same as that incorporated in the commercial samples that had caused difficulties, and in these tests the level of incorporation of the hardener was only half that contained in the commercial samples. As the absorptive capacity of bentonite from different sources is known to vary greatly, the Sub-Committee decided to carry out a further collaborative test in which bentonite from the same source as that incorporated in the commercial samples was to be used.

A single sample of unmedicated feed was circulated to four laboratories, together with common samples of dimetridazole and bentonite hardener. Medication was carried out in each laboratory and the hardener was incorporated at a level equivalent to 56 lb per ton. Triplicate tests by the modified procedure were carried out in each laboratory and the results are shown in Table I. Laboratory A also carried out a determination by the original method and obtained a recovery of only 17 per cent.

Conclusions

The Sub-Committee is satisfied that by carrying out a preliminary extraction with methanol the difficulties encountered in the determination of dimetridazole by the previously recommended method[1] are overcome. It is recommended that the method given in the Appendix to this Report should be used for the determination of dimetridazole in animal feedingstuffs.

TABLE I

RECOVERY OF DIMETRIDAZOLE BY MODIFIED PROCEDURE

Laboratory	Dimetridazole added, p.p.m.	Dimetridazole found, p.p.m.	Recovery, per cent.
A	117	91	77·8
	133	123	92·5
	162	130	80·2
B	125	131	104·8
	125	109	87·3
	125	104	83·2
C	125	125	100·0
	125	121	97·0
	125	122	97·6
D	126	129	102·1
	126	114	90·5
	126	122	96·8

Appendix

RECOMMENDED METHOD FOR THE DETERMINATION OF DIMETRIDAZOLE IN FEEDS

PRINCIPLE OF THE METHOD—

The method is based on that recommended by Cooper and Hoodless.[5] The sample is extracted with methanol and the extracted drug is transferred to 0·65 per cent. hydrochloric acid solution. The acidic solution is treated with sodium tetraborate and then with potassium cyanide to complex any copper present. The solution is polarographed and the peak height at about −0·35 V is measured. The dimetridazole content is calculated from the difference between this peak height and that for a similarly treated solution containing a known amount of added dimetridazole. This technique of standard addition to the feed extract is necessary as co-extractives from the feed depress the slope of the standard curve.

APPLICABILITY—

The method is suitable for determining the usual concentrations of dimetridazole in poultry feeds of the types currently marketed. There is not likely to be any interference from copper, iron, nickel, zinc and manganese present in the feed up to the levels indicated in the earlier Report.[1]

REAGENTS—

Methanol—Analytical-reagent grade.

Potassium cyanide solution, 5 per cent. w/v.

Sodium tetraborate.

Dimetridazole standard solution—Dissolve 0·1 g of pure dimetridazole in 200 ml of water containing 5 ml of dilute hydrochloric acid.

Hydrochloric acid, 0·65 per cent. w/v—Dilute 17·6 ml of concentrated hydrochloric acid (sp.gr. 1·18) to 1 litre with water.

PROCEDURE—

Weigh 10·0 g of feed sample and transfer it to a Soxhlet thimble. Place the thimble in a continuous extractor and extract with methanol for 2 hours. Reduce the volume of methanol extract to between 3 and 4 ml either under reduced pressure or on a low-temperature hot-plate or water-bath (dimetridazole tends to sublime at temperatures above about 70 °C). Remove the last traces of methanol under reduced pressure, applying heat only from the palm of the hand. Shake the oily residue with 80 ml of warm 0·65 per cent. hydrochloric acid and then filter the solution.

By pipette place 1 ml of water into a calibrated 25-ml flask and 1 ml of dimetridazole standard solution into another similar flask. Make both up to volume with the acidic feed extract solution. Transfer the solutions to separate dry centrifuge tubes, add 3 g of sodium tetraborate to the contents of each tube and shake them for 1 minute. Add 1 ml of 5 per cent. w/v potassium cyanide solution to each, and shake the tubes to mix. Spin them in a centrifuge at 3000 to 4000 r.p.m. for about 3 minutes.

Transfer sufficient solution from the two tubes to separate dry polarographic cells and de-oxygenate the solutions with oxygen-free nitrogen. Record the polarograms of the solutions over a suitable potential range to allow measurement of the peak height at about −0·35 V. Calculate the dimetridazole content of the sample solution from the difference between the peak heights of the two solutions.

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5. Cooper, P. J. and Hoodless, R. A., *Analyst*, **1967**, **92**, 520.

Communication

Manuscripts are *not* submitted to the usual examination by referees. Inclusion of a Communication is at the Editor's discretion; a manuscript not accepted as a Communication may, if the author wishes, be re-submitted as a possible paper and subjected to the usual scrutiny by referees.

CRACKING OF COMBUSTION TUBES WHEN USING A SEMI-AUTOMATIC FLASH-COMBUSTION APPARATUS

We have observed a peculiar phenomenon that occurs periodically with our Thomas semi-automatic flash-combustion apparatus (Francis, H. J., and Minnick, E. J., *Microchem. J.*, 1964, **8**, 245) for the determination of carbon and hydrogen in organic compounds. Samples of 3 mg are weighed into small aluminium tubes, which are crimped and then dropped into the vertical combustion tube. Normally a 2 to 3-second burn is observed and this leads to good results, but occasionally, after a combustion tube has been in use for some time, a prolonged, brilliant and rather awe-inspiring burn takes place, lasting sometimes for up to 3 minutes. The flow-rate for oxygen observed during the dynamic phase at the combustion tube exit drops from 80 to 10 ml min^{-1}, but gradually recovers as the burn dies away. Following this manifestation the weight of water produced is about right, but that of carbon dioxide is negligible. The phenomenon was initially ascribed by us to a "thermit" type of reaction between metallic residues from organometallic samples and the aluminium capsules, but on the last occasion a prolonged bright burn occurred after the tube had received fifty non-metallic samples and only one inadvertent organometallic sample.

The only combustible material present in the tube in an amount sufficient to give the reaction described is aluminium. This element must accumulate in the tube over a period of time and then have its oxidation initiated by an as yet unknown mechanism. Aluminium oxide is found inside broken tubes. While our apparatus normally gives satisfactory results (provided that the humidity is carefully controlled), these vigorous combustions are annoying in that they usually crack the very expensive ceramic combustion tube.

If any readers have had similar experiences, or can offer any explanation or advice, we should be grateful for their comments.

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M. A. Leonard
W. J. Swindall

Book Reviews

SECHS- UND ACHTGLIEDRIGE RINGSYSTEME IN DER PHOSPHOR-STICKSTOFF-CHEMIE. By S. PANTEL and M. BECKE-GOEHRING. Pp. x + 301. Berlin, Heidelberg and New York: Springer-Verlag. 1969. Price $14.90.

This book is a well presented, comprehensive review of the literature of the title subject. It is most welcome because of the massive volume of literature published on phosphorus - nitrogen compounds in the last decade.

A clear explanation of the nomenclature of phosphorus - nitrogen compounds is set out at the beginning of the book.

The major portion of the book is devoted to a review of cyclic phosphazenes. In addition to referring to methods of synthesis and reactions of the various compounds, physico-chemical data and i.r. and n.m.r. spectra are presented where these have been published in the literature. The extensive patent literature, on phosphorus - nitrogen ring compounds, is also adequately covered. The structure and formation of inorganic rubbers and oil-like polymers are discussed in a section devoted to cyclic phosphonitrilic chlorides. Analytical methods are briefly reviewed.

A relatively small section is devoted to ring systems containing carbon, sulphur and oxygen in addition to phosphorus and nitrogen. The small size of this section reflects the gaps in our knowledge rather than a lack of interesting chemistry in these particular systems.

One minor criticism of the book is that the references should also have listed the corresponding Chemical Abstracts location. This would have been particularly useful in following up the references to patents and the more obscure literature.

The authors have succeeded in producing a coherent and interesting book which is highly recommended, both as an account of recent chemistry in this field, and as a source of literature references.

A. F. CHILDS

FLAME PHOTOMETRY: LABORATORY PRACTICE. By JOSEF DVORAK, IVAN RUBESKA and ZDENEK REZAC. Translated by R. E. HESTER. Pp. 325. London: Butterworth Group (Iliffe Books). 1971. Price £4·50.

Flame-photometric methods, both emission and absorption, have been in use for a considerable time, and this volume aims to give an up-to-date account of both techniques, with particular emphasis on practical applications.

The book is in three sections: Part I—General, Part II—Theoretical and Part III—Practical. The theoretical section describes the emission and absorption of radiation, various properties of flames, and problems associated with introducing sample solutions into a flame. Part III, which accounts for about two-thirds of the book, deals with instruments and equipment, interferences and practical applications; there are also more than 800 references.

Flame-emission spectroscopy, having been eclipsed for several years by atomic-absorption spectroscopy, partly due to the use of inferior instrumentation, is now returning to favour, but it is doubtful if this volume will encourage the current resurgence. For example, there is no account of the nitrous oxide - acetylene flame for flame-emission work; this is disappointing, because its application is capable of providing superior results to those obtained by using total-consumption burners and air - acetylene type flames.

One of the instruments described in detail, and also used for several of the practical applications, is a filter-type photometer, whereas modern instruments incorporate a scanning monochromator to give much improved resolution and better interpretation of background effects. The practical applications described are largely confined to the alkali and alkaline earth metals.

The book deals almost entirely with flame-emission spectroscopy, although atomic-absorption spectroscopy is discussed very briefly, but it is doubtful if it will appeal to many analysts working in the field of flame photometry.

H. PUGH

ANALYTICAL FLAME SPECTROSCOPY. SELECTED TOPICS. Edited by R. MAVRODINEANU. Pp. xxii + 772. London: Macmillan and Co. 1970. Price £14.

As a comprehensive compilation of any aspect of analytical chemistry, involving (in the widest sense) the use of flames to emit, absorb or re-emit specific radiations in conjunction with the material under investigation, this book stands out as giving an authoritative coverage of the whole field.

Each of the book's thirteen chapters bears the hallmark of one or more of its nineteen authors, whose names in their specialised fields are known throughout the world.

The majority of the contributions are from the U.S.A.; two chapters originate from the Netherlands, one from Germany, one from France and one from Australia.

To attempt to review a publication of this magnitude, recognising its wide and detailed coverage, is virtually impossible within the customary length of these reviews, especially if justice is to be done to all the individual chapters, because each is a competent exposition in itself, and where any overlapping does occur, this is understandable and defensible.

Perhaps the best way to reinforce the reviewer's favourable impression of the book is to list its chapter numbers and headings, name the author(s), and include a random selection of main sub-headings.

1. "From Sample to Signal in Emission Flame Photometry: An Experimental Discussion," by C. Th. J. Alkemade (1.2. Brief outline of the basis of emission flame photometry; 1.7. Excitation of metal vapor to light emission).

2. "Sensitivity, Detection Limit, Precision and Accuracy in Flame Emission and Atomic Absorption Spectrometry," by O. Menis and T. C. Rains (2.3. The optimization of components; 2.5. Criteria for improvement of precision and accuracy).

3. "Some Considerations on Optical Design and Selection of Spectroscopic Instruments," by W. Müller-Herget (3.2. The compensation principle; 3.6. Illumination of spectroscopic instruments. Luminosity and astigmatism).

4. "Principles of Electronic Instrumentation," by P. G. Cath (4.2. Analog circuits; 4.3. Digital circuit techniques).

5. "Nonmetals," by P. T. Gilbert (5.2. Hydrogen; 5.17. The noble gases).

6. "Atomic Emission and Absorption Spectrometry of the Rare Earth Elements," by R. N. Kniseley, V. A. Fassel and C. C. Butler (6.2. Atomic and molecular emission spectra; 6.4. Analytical applications).

7. "Some Recent Spectroscopic Investigations of Low-Pressure Oxyacetylene Flames," by R. Bleekrode (7.3. Emission; 7.4. Absorption).

8. "Agricultural Applications of Flame Photometry," by M. Pinta (8.2. Analysis of rocks and soils; 8.5. Analysis of fertilizers).

9. "The Applications of Flame Photometry in Biology and Medicine," by R. Herrmann (9.4. Properties of the starting material in medical and biological investigations; 9.6. Interference and ways to reduce it).

10. "Atomic Absorption Spectrometry," by J. B. Willis (10.5. The technique of atomic absorption spectrometry; 10.6. Application to (72) individual elements).

11. "Atomic Fluorescence Flame Spectrometry," by J. D. Winefordner and R. Smith (11.1. Theoretical considerations; 11.5. Comparison with other flame spectrometric methods).

12. "Hollow Cathode Discharge Devices," by J. C. Burger, W. Gillies and G. K. Yamasaki (12.3. Development of sealed-off devices; 12.7. Current status of hollow cathode devices).

13. "Bibliography on Flame Spectroscopy—Analytical Applications," by R. Mavrodineanu (13.1. Emission; 13.2. Atomic absorption spectroscopy; 13.3. Electrical discharges having the aspect of combustion flames). This collection of over 2000 references, from about 1966 to 1968, is a continuation of an earlier bibliography containing over 5000 references (R. Mavrodineanu, "Bibliography on Flame Spectroscopy, Analytical Applications, 1800–1966," National Bureau of Standards Miscellaneous Publications, 281, 1967).

This excellent volume has been commendably co-ordinated for unified presentation by R. Mavrodineanu. It is reasonable to speculate that the majority of analysts, having once seen the book, will feel that they could be missing something vital in being without it.

W. T. ELWELL

ANALYTICAL CHEMISTRY OF NITROGEN AND ITS COMPOUNDS. Edited by CARL A. STREULI and PHILIP R. AVERELL. Part I. Pp. viii + 429. Part II. Pp. viii + 431–763. New York, London, Sydney and Toronto: Wiley-Interscience. 1970. Price £16·50.

This book is Volume No. 28 of "Chemical Analysis: A series of Monographs on Analytical Chemistry and its Applications," edited by P. J. Elving and I. M. Kolthoff, and published by Wiley-Interscience. It consists of two parts. Part I deals with inorganic and simple organic compounds while Part II deals with complex organic compounds. In such a book, reference to the nuclear magnetic resonance of nitrogen is obviously necessary; in some ways it is a pity that a chapter has been included in Part I rather than Part II but no doubt it was a difficult choice to make. The book is unusual in that it really is in two parts and that the pagination runs on from one part to the next. This is perhaps not a bad idea because it results in two volumes of reasonable size and there is an index at the end of Part I for that part only and a cumulative index for both parts at the end of Part II. The latest list price for both parts of the book is £16·50; presumably the parts may be available separately but this is not indicated in the price lists to which we have access.

Chapters 1 to 9 (Part I) deal with: (1) Total nitrogen; (2) Nuclear magnetic resonance of nitrogen; (3) Nitrogen - hydrogen compounds; (4) Nitrogen - oxygen compounds; (5) Nitrogen compounds of carbon, silicon and boron; (6) Nitrogen compounds of the halogens; (7) Nitrogen compounds of sulphur and phosphorus; (8) Amines and related compounds; and (9) Amides and related compounds.

Chapters 10 to 16 (Part II) deal with the analysis of various groups of organic nitrogen compounds comprising: (10) Nitro and nitroso compounds; (11) Urea and its derivatives; (12) Functional groups with the –C≡N linkage; (13) Functional groups with N–N links; (14) Heterocyclics; (15) Amino acids, polypeptides and proteins; and (16) Synthetic polymers.

Each chapter is a separate entity compiled by experts. It is therefore to be expected that variations in style and quality of the compilation occur, and in these circumstances criticisms made of the whole volume may not be strictly fair to individual parts.

There are, however, some general comments that emerge as one reads through; one is that this is a collection of reviews, some of which are less critical than one might wish and lack the authoritative comment that is so valuable to someone wishing to get a feel for a subject.

In the chapters on total nitrogen, nitrogen - hydrogen compounds and nitrogen - oxygen compounds, with which one reviewer is familiar, the treatment is comprehensive and the trend towards automatic methods is clearly indicated.

In the second volume there is a surprising preponderance of volumetric and colorimetric analysis in the text; admittedly these methods are still widely used in research and control analysis, but in an era when so many analytical problems are solved either by chromatography or by spectroscopy, or by a combination of the two, one might have expected greater emphasis on these methods in a new book on analysis.

On the credit side, there is a very large amount of clearly referenced information in each chapter and for anyone who is faced with a new field of nitrogen chemistry to study analytically, the book would provide a valuable and readable lead into the subject.

A. C. DOCHERTY
H. E. STAGG

APPOINTMENTS VACANT

FOR SALE

REPORTS OF THE ANALYTICAL METHODS COMMITTEE

The Reports of the Analytical Methods Committee listed below may be obtained direct from The Society for Analytical Chemistry, Book Department, 9/10 Savile Row, London, W1X 1AF (not through Trade Agents), at the price of 10p. to members of the Society and 15p. to non-members. Remittances must accompany orders and be made payable to "Society for Analytical Chemistry."

Certain Reports published before 1946 have been omitted from this list, but are still available.

Additives in Animal Feeding Stuffs Sub-Committee:

Report of the Antibiotics Panel: The Determination of Penicillin, Chlortetracycline and Oxytetracycline in Diet Supplements and Compound Feeding Stuffs.
Report of the Hormones Panel: The Determination of Stilboestrol and Hexoestrol in Compound Feeding Stuffs.
Report of the Prophylactics Panel: The Determination of Nitrofurazone in Compound Feeding Stuffs.
Report of the Vitamins (Water-soluble) Panel: The Determination of Water-soluble Vitamins in Compound Feeding Stuffs.
Report of the Vitamins (Fat-soluble) Panel: The Determination of Fat-soluble Vitamins in Diet Supplements and Compound Feeding Stuffs.
Report of the Prophylactics in Animal Feeds Sub-Committee: The Determination of Amprolium in Animal Feeding Stuffs.
Report of the Prophylactics in Animal Feeds Sub-Committee: The Determination of Sulphaquinoxaline.
Report of the Prophylactics in Animal Feeds Sub-Committee: The Determination of Acinitrazole.
Report of the Prophylactics in Animal Feeds Sub-Committee: The Determination of Ethopabate in Feeds.
Report of the Prophylactics in Animal Feeds Sub-Committee: The Determination of Furazolidone in Feeds.
Report of the Prophylactics in Animal Feeds Sub-Committee: The Determination of Dimetridazole in Animal Feeds.
Report of the Prophylactics in Animal Feeds Sub-Committee: The Determination of Dinitolmide (Zoalene) in Animal Feeds.
Report of the Prophylactics in Animal Feeds Sub-Committee: The Determination of Amprolium, Sulphaquinoxaline and Ethopabate when Present Together in Animal Feeds.

Analytical Standards Sub-Committee:

Sodium Carbonate as a Primary Standard in Acid - Base Titrimetry.
Sulphamic Acid as a Primary Standard in Acid - Base Titrimetry.

Essential Oils Sub-Committee:

Report No. 15. Determination of Linalol in Essential Oils.
Application of Gas - Liquid Chromatography to Essential-oil Analysis: Interim Report on the Determination of Citronellol in Admixture with Geraniol.
Spectral Characteristics of Eugenol.

Fish Products Sub-Committee:

Nitrogen Factor for Cod Flesh.

Meat Products Sub-Committee (formerly Meat Extract Sub-Committee):

Analysis of Meat Extract.
Determination of Gelatin in Meat Extract and Meat Stocks: Interim Report.
Nitrogen Factors for Pork *and* Nitrogen Content of Rusk Filler (as one reprint).
Nitrogen Factors for Beef.
Nitrogen Factors for Chicken.
Nitrogen Factors for Liver.
Nitrogen Factor for Veal.
Nitrogen Factors for Turkey.
Nitrogen Content of Rusk Filler.
Nitrogen Factor for Kidney.
Nitrogen Factor for Tongue.
Nitrogen Factor for Barley.
Nitrogen Factor for Blood.

Metallic Impurities in Foodstuffs Sub-Committee:

Determination of Lead in Foodstuffs: Tentative Method.

Metallic Impurities in Organic Matter Sub-Committee:

Methods for the Destruction of Organic Matter.
Notes on Perchloric Acid and its Handling in Analytical Work.
The Determination of Lead.
The Determination of Small Amounts of Arsenic in Organic Matter.
The Determination of Small Amounts of Copper in Organic Matter.
The Determination of Small Amounts of Mercury in Organic Matter.
The Determination of Small Amounts of Tin in Organic Matter. Part 1. Amounts of Tin up to 30 μg
The Determination of Small Amounts of Zinc in Organic Matter.
The Use of 50 per cent. Hydrogen Peroxide for the Destruction of Organic Matter.
The Determination of Small Amounts of Tin in Organic Matter. Part 2. Amounts of Tin from 30 to 150 μg.
The Determination of Small Amounts of Cadmium in Organic Matter.

The Theoretical and Practical Aspects of Electronic Timing as One Method of Increasing the Analysis Rate on the Technicon AutoAnalyzer I

The need for an increase in the speed of analysis of soil and plant samples is discussed with reference to the use of the Technicon AutoAnalyzer I. A reliable electronic timer that controls the operation of the Technicon Sampler is described. A circuit diagram of the timer is shown, with a list of components used.

J. A. VARLEY and K. F. BAKER

Tropical Soils Analysis Unit, Land Resources Division, Overseas Development Administration, Coley Park, Reading, Berkshire.

Analyst, 1971, **96**, 734–738.

The pH Meter as a Hydrogen-ion Concentration Probe: A Postscript

Measurements of the ratio between the quantity H ($= 10^{-pH}$) and the true hydrogen-ion concentration in solutions of fixed ionic composition have been made in alkaline solutions, and are in agreement with values previously determined in acidic solutions.

W. A. E. McBRYDE

Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada.

Analyst, 1971, **96**, 739–740.

The Determination of Small Amounts of Copper in Organic Matter by Atomic-absorption Spectroscopy

Report prepared by the Metallic Impurities in Organic Matter Sub-Committee.

ANALYTICAL METHODS COMMITTEE

9/10 Savile Row, London, W1X 1AF.

Analyst, 1971, **96**, 741–743.

Nitrogen Factor for Coal Fish

Report prepared by the Fish Products Sub-Committee.

ANALYTICAL METHODS COMMITTEE

9/10 Savile Row, London, W1X 1AF.

Analyst, 1971, **96**, 744–745.

The Determination of Dimetridazole in Animal Feeds: Revised Method

Report prepared by the Prophylactics in Animal Feeds Sub-Committee.

ANALYTICAL METHODS COMMITTEE

9/10 Savile Row, London, W1X 1AF.

Analyst, 1971, **96**, 746–749.

Cracking of Combustion Tubes when using a Semi-automatic Flash-combustion Apparatus

Communication

M. A. LEONARD and W. J. SWINDALL

Department of Analytical Chemistry, David Keir Building, The Queen's University of Belfast, Belfast, BT9 5AG, Northern Ireland.

Analyst, 1971, **96**, 749.

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Potassium Dichromate N/10
Potassium Hydroxide N/1
Potassium Hydroxide N/2
Potassium Hydroxide N/10
Potassium Iodate N/10
Potassium Iodate N/100
Potassium permanganate N/10
Potassium permanganate N/100
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Silver Nitrate N/100
Sodium Arsenite N/10
Sodium Carbonate N/10
Sodium Chloride N/10
Sodium hydroxide N/1
Sodium hydroxide N/2
Sodium hydroxide N/10
Sodium hydroxide N/100
Sodium Thiosulphate N/10
Sodium Thiosulphate N/100
Sulphuric acid N/1
Sulphuric acid N/2
Sulphuric acid N/10
Sulphuric acid N/100

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Volume 96 No. 1147, Pages 681-752 October, 1971

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

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Printed for the Society for Analytical Chemistry by W. Heffer & Sons Ltd., Cambridge, England
Communications to be addressed to the Editor, J. B. Attrill, 9/10 Savile Row, London, W1X 1AF
Enquiries about advertisements should be addressed to J. Arthur Cook, 9 Lloyd Square, London WC1 9BA

Entered as Second Class at New York, U.S.A., Post Office.