Analysis of Surfactants in Waters, Wastewaters and Sludges, 1981

Methods for the Examination of Waters and Associated Materials

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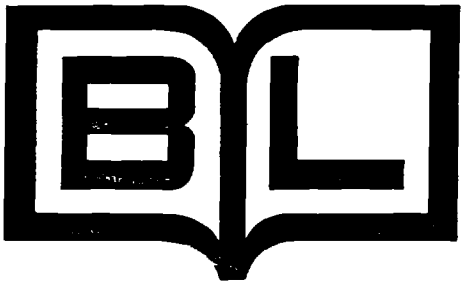

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This booklet contains a selection of methods and reviews for the analysis of surfactants* of various types. Some of the methods have been fully tested, others only have tentative status. This is indicated in the titles of the individual methods. To help the user compare results mention is made of proprietary brands used in developing and testing these methods. Equivalent materials may be substituted if the analytical accuracy and precision are comparable.

Contents

*Surfactants is the preferred term for synthetic detergents or surface active agents.

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Warning to users

The analytical procedures given in this booklet should only be carried out by competent trained persons, with adequate supervision when necessary. Local Safety Regulations must be observed. Laboratory procedures should be carried out only in properly equipped laboratories. Field operations should be conducted with due regard to possible local hazards, and portable safety equipment should be carried. Care should be taken against creating hazards. Lone working, whether in the laboratory or field, should be discouraged. Reagents of adequate purity must be used, along with properly maintained apparatus and equipment of correct specification. Specifications for reagents, apparatus and equipment are given in manufacturers' catalogues and various published standards. If contamination is suspected, reagent purity should be checked before use.

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Code of Practice for Chemical Laboratories' and 'Hazards in the Chemical Laboratory' issued by the Royal Society of Chemistry, London; 'Safety in Biological Laboratories' (Editors Hartree and Booth), Biochemical Society Special Publication No 5, The Biochemical Society, London, which includes biological hazards; and 'The Prevention of Laboratory Acquired Infection' Public Health Laboratory Service Monograph 6, HMSO, London.

Where the Committee have considered that a special unusual hazard exists, attention has been drawn to this in the text so that additional care might be taken beyond that which should be exercised at all times when carrying out analytical procedures. It cannot be too strongly emphasised that prompt first aid, decontamination, or administration of the correct antidote can save life; but that incorrect treatment can make matters worse. It is suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after any accident involving chemical contamination, ingestion, or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries require specialist treatment not normally encountered by most doctors. Similar warning should be given if a biological or radio chemical injury is suspected. Some very unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and when sampling in the field. In the latter case, all equipment including footwear should be disinfected by appropriate methods if contamination is suspected.

The best safeguard is a thorough consideration of hazards and the consequent safety precautions and remedies well in advance. Without intending to give a complete checklist, points that experience has shown are often forgotten include: laboratory tidiness, stray radiation leaks (including ultra violet), use of correct protective clothing and goggles, removal of toxic fumes and wastes, containment in the event of breakage, access to taps, escape routes, and the accessibility of the correct and properly maintained first-aid, fire fighting, and rescue equipment. If in doubt, it is safer to assume that the hazard may exist and take reasonable precautions, rather than to assume that no hazard exists until proved otherwise.

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About this series

This booklet is part of a series intended to provide recommended methods for the determination of water quality. In addition, the series contains short reviews of the more important analytical techniques of interest to the water and sewage industries. This booklet is one such review. In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, has issued volumes of methods for the analysis of water and sewage culminating in 'Analysis of Raw, Potable and Waste Waters'. These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print. The present series will be published as individual methods, thus allowing for the replacement or addition of methods as quickly as possible without need of waiting for the next edition. The rate of publication will also be related to the urgency of requirement for that particular method, tentative methods being issued when necessary. The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users—the senior analytical chemist, biologist, bacteriologist etc, to decide which of these methods to use for the determination in hand. Whilst attention of the user is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user.

The preparation of this series and its continuous revision is the responsibility of the Standing Committee of Analysts (to review Standard Methods for Quality Control of the Water Cycle). The Standing Committee of Analysts is one of the joint technical committees of the Department of the Environment and the National Water Council. It has nine Working Groups, each responsible for one section or aspect of water cycle quality analysis. They are as follows:

1.0 General principles of sampling and accuracy of results
*2.0 Instrumentation and on-line analysis
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General nonmetallic substances
6.0 Organic impurities
7.0 Biological methods
*8.0 Sludge and other solids analysis
9.0 Radiochemical methods

The actual methods etc are produced by smaller panels of experts in the appropriate field, under the overall supervision of the appropriate working group and the main committee. The names of those associated with this method are listed inside the back cover.

Publication of new or revised methods will be notified to the technical press, whilst a list of Methods in Print is given in the current HMSO Sectional Publication List No 5, and the current status of publication and revision will be given in the biennial reports of the Standing Committee of Analysts.

Whilst an effort is made to prevent errors from occurring in the published text, a few errors have been found in booklets in this series. Correction notes for booklets in this series are given in the Reports of The Standing Committee of Analysts, published by the Department of the Environment but sold by the National Water Council, 1 Queen Anne's Gate, London SW1H 9BT. Should an error be found affecting the operation of a method, the true sense not being obvious, or an error in the printed text be discovered prior to sale, a separate correction note will be issued for inclusion in the booklet.

T A DICK
Chairman

L R PITTWELL
Secretary

25 September 1981

*These two Working Groups are in process of being wound up. Their tasks are being redistributed among the other working groups.

Analysis of Surfactants in Waters, Wastewaters and Sludges, 1980

Introduction

This booklet gives methods for the analysis of the major surfactant (surface active agent) types when present at low concentrations in waters and wastewaters. The three major categories dealt with are anionic, nonionic and cationic surfactants. The most heavily used of these are the anionic surfactants, these being salts whose detergency property is essentially vested in the anion. Amphoteric compounds, containing both cationic and anionic surfactant groupings in the same molecule, are not considered in this booklet. Although they have value for specialized applications they are at present of minor overall importance.

The main groups of synthetic anionic compounds in widespread use as surfactants* are alkyl aryl sulphonates, long chain fatty alcohol sulphates and alcohol ethoxysulphates; various other chemical types are used as anionic surfactants to a lesser extent. In addition to there being a wide range of anionic surfactant classes, the individual commercial products themselves are generally complex isomeric mixtures. Consequently manual and automatic colorimetric procedures that are generally applicable for the determination of all anionic surfactants containing a single sulphate or sulphonate grouping are described in this booklet (see Part A), and estimations are made relative to the response of an arbitrary standard anionic surfactant, namely 'Manoxol OT'. In these methods anionic surfactants are determined following chloroform extraction of the ion-association complex formed with methylene blue. Various other compounds present in environmental samples can also form chloroform extractable methylene blue complexes, and at low surfactant concentrations such compounds may have a significant effect. It is therefore rather more realistic to refer to the material determined in such samples as 'methylene blue active substance' (MBAS) rather than anionic surfactant. A procedure is also given in this booklet to allow anionic surfactant adsorbed on sewage sludge to be recovered by solvent extraction (see Method A3). By determining the amount of MBAS in the resulting extract the level of anionic surfactant on the sludge can be assessed.

The second most heavily used group of surfactants are the nonionic type and in recent years their relative significance has increased. The most important surfactants of this type in use are those produced by the condensation of ethylene oxide with a hydrophobe such as an alkyl phenol or fatty alcohol. Various analytical methods are given in this booklet for these alkoxylated nonionic surfactants and the reader may well find it helpful initially to read the review of these methods (see Method B1) which gives guidance on the relative scope and characteristics of the various methods. In most of the nonionic methods analysis is carried out relative to an arbitrary standard nonionic surfactant product such as 'Synperonic NP8' for essentially similar reactions to those outlined above for the analysis of anionic surfactants, and results may be expressed as reagent active substance due to possible positive interferences in environmental samples.

At present cationic surfactants, generally of the long chain alkyl and dialkyl quaternary ammonium salt type, have a lower usage than both anionic and nonionic surfactants. Although relatively expensive and poor in detergency action their properties make them of value as fabric conditioners and germicidal detergents where their use is increasing. Because of their more recent introduction in significant amounts to the environment, methods for their determination at low levels in water and wastewaters are not as well established as the other two major surfactant classes. Consequently, only a tentative spectrophotometric method for their determination is put forward in this booklet, ie, the Disulphine Blue method (see Method C1), for the determination of cationic surfactant in biodegradation test liquor and effluent samples. However, the procedure has the advantage that it incorporates an anion-exchange separation step which allows cationic surfactant to be determined in the presence of anionic surfactant and other anionic components that interfere in a direct colorimetric estimation. Determinations on environmental samples are made relative to an arbitrary standard such as cetyl trimethyl ammonium chloride, and the results are often quoted as 'disulphine blue active substance' (DSBAS), 'disulphine blue' being an anionic dyestuff which forms a chloroform soluble complex with a cationic surfactant in the same way that 'methylene blue' forms such a complex with an anionic surfactant.

* Soaps, which may also be considered as anionic surfactants, are salts of long chain fatty acids; they do not respond to the 'methylene blue' analytical procedures described herein.

A Anionic Surfactants

Note, various standard substances are used in different countries. British preference at the present time is for sodium dioctyl sulphosuccinate Na ($C_8H_{17}O_2C.CH(SO_3)CH_2.CO_2C_8H_{17}$) also known as Manoxol OT. The French standard referred to in some directives on water quality as lauryl sulphate is sodium lauryl sulphate $NaSO_4C_{12}H_{25}$. Conversion factors are used based on molecular weight per active group. Thus as both of the above substances contain one active (SO_3 or SO_4) group and the molecular weights are 444 and 288 respectively the factor for converting Manoxol OT units to lauryl sulphate units is $282/444 = 0.635$. For alkylbenzene sulphonates and the like the factor will depend on the alkyl and other groups substituted onto the benzene ring and may, for mixtures, be empirical.

A1 Manual Determination of Anionic Surface Active Materials by Abbott's Modification of the Methylene Blue Spectrophotometric Method

A1.1 Performance Characteristics of the Method (39)

A1.1.1	Substance determined	Synthetic anionic surfactants and other anionic surface active material of natural origin.
A1.1.2	Type of sample	Settled sewage, sewage effluents, waters and certain trade effluents.
A1.1.3	Basis of method	Spectrophotometric measurement of the chloroform extracted methylene blue/anionic surfactant complex (1, 2, 3, 4).
A1.1.4	Range of application (a)	0.1–1.8 mg/l anionic surfactant as Manoxol OT for 100 ml sample.
A1.1.5	Calibration curve (b)	Linear $C = K(AT-AB)$ where C = concentration of surfactant (as mg/l Manoxol CT) K = constant AB = blank absorbance AT = absorbance for concentration C

A1.1.6 Standard deviation (a)

Concentration of Manoxol OT in distilled water (mg/l)	Total Standard Deviation (mg/l)	Degrees of Freedom
0.16 (a)	0.017	6
0.40 (b)	0.046	41
1.6 (c)	0.045	6
7.45 (d)	0.31	42

A1.1.7	Limit of detection (a)	0.02 mg/l. Calculated from the standard deviation of the blanks for 100 ml samples (6 degrees of freedom).
A1.1.8	Sensitivity (a)	0.04 absorbance units for 0.1 mg/l Manoxol OT with 100 ml samples and 10 mm cuvettes.
A1.1.9	Bias	Has not been detected see Section A1.3.
A1.1.10	Interference	See Section A1.3.
A1.1.11	Time required for analysis	Approximate analytical and operator time for a batch of five samples or standards = 2 hours (excluding initial reagent preparation). Approximate stock reagent preparation time = 1 hour (required only periodically).

(a) and (c) Data obtained at the Laboratory of the Government Chemist on standard distilled water solutions of Manoxol OT (100 ml samples).

(b) Data obtained in 42 laboratories on a standard distilled water solution of alkyl benzene sulphonate. (Reported by Finney, EE and Nicholson, NJ in Proc. Soc. for Wat. Treat. and Exam. 17, No 8 p 25 1968).

(d) Data obtained in 43 laboratories on filtered domestic sewage containing 7.45 mg/l of alkyl benzene sulphonate. (Reported by Finney, EE and Nicholson, NJ in Proc. Soc. for Wat. Treat. and Exam. 17, No 8 p 25 1968).

A1.2 Principle

A1.2.1 The Abbott method (3) is generally considered to be an improved version of the Longwell and Maniece procedure (2). The latter was accepted by the UK Standing Technical Committee on Synthetic Detergents (1956) as the official methylene blue method for determining anionic surface active material. However, improvements in the former method, ie use of pre-extracted reagents, etc have resulted in greater sensitivity and reproducibility for methylene blue estimations.

A1.2.2 Anionic surfactant is associated with the intensely coloured methylene blue cation to form a chloroform extractable ion-association complex; the unassociated dye has an extremely small solubility in chloroform. The methylene blue-anionic complex is partitioned into chloroform from an alkaline methylene blue solution to avoid the negative interference of proteinaceous material present in environmental samples. The chloroform phase is then back-extracted with an acidified methylene blue solution in order to remove the interference of those materials such as inorganic anions eg nitrate, chloride etc, that form methylene blue complexes of low chloroform extractability. The absorbance of the final chloroform phase is determined at 650 nm.

A1.3 Scope and Limitations (including Interferences)

For distilled water solutions of synthetic anionic surfactant the method will give an accurate determination of the surfactant present. However, for unknown water samples, ie sewage, effluent and surface waters, the results obtained represent anionic surface active material rather than synthetic anionic surfactant. In theory, any compound containing a single strong anionic grouping (eg sulphate or sulphonate) and a hydrophobic moiety is capable of forming an extractable ion-association compound with the methylene blue cation and can therefore positively respond in a determination for synthetic anionics. It has been shown or predicted that organic sulphates, sulphonates, carboxylates and phenols and even simple inorganic anions such as cyanate, nitrate, thiocyanate and sulphide can be methylene blue active (5) (7). Although negative interferences can also occur as a result of the direct competition of other 'cationic' materials for example proteins and quaternary ammonium compounds, for the anionic surfactant, they are generally considered to be less important than positive interferences (8). However, the increased usage of materials such as cationic surfactant may alter this situation. The reaction conditions in the Abbott method have been selected, as far as possible, to eliminate or minimise some of the above interferants, in particular proteinaceous material and inorganic anions which result in negative and positive interferences respectively. Common constituents of sewage and effluents including urea, ammonia and nitrate, as well as the preservatives formaldehyde and mercuric chloride have been shown to give no interference at 100 mg/l (6). Sodergren (4) showed that sodium chloride (100–30,000 mg/l), sodium sulphide (100 mg/l), sodium sulphate (400 mg/l) and sea water do not interfere. Nevertheless, not all natural interferants can be eliminated to allow a direct methylene blue determination of the synthetic anionic surfactant in environmental samples. For this reason, the entities detected by the method are more correctly referred to as anionic surface active material or methylene blue active substances (MBAS). For domestic waste waters the MBAS response can generally be taken as an acceptable over-estimate of the synthetic anionics present, but synthetic anionic surfactants may contribute only a small proportion to the total MBAS in some surface waters (5) (9) (10) (11).

A1.4 Hazards

Sulphuric acid, chloroform and mercuric chloride are potentially harmful reagents.

A1.5 Reagents

Reagents should be analytical reagent quality unless otherwise specified.

A1.5.1 Water—distilled or deionized.

A1.5.2 Sodium tetraborate, approx 0.05M

Dissolve 19.0 ± 0.1 g of sodium tetraborate decahydrate $Na_2B_4O_7.10H_2O$ (laboratory reagent) in 1.00 ± 0.01 litre of water. Store in a glass stoppered glass bottle. This solution is stable for at least two weeks.

A1.5.3 Sodium hydroxide, approx 0.1M

Dissolve 4.00 ± 0.05 g of sodium hydroxide pellets in 1.00 ± 0.01 litre of water. Store in a polythene stoppered glass bottle. This solution is stable for at least two weeks.

A1.5.4 Alkaline Borate solution

Mix equal volumes of 0.05M sodium tetraborate solution (A1.5.2) and 0.1M sodium hydroxide solution (A1.5.3). Store in a polythene stoppered glass bottle. This solution is stable for at least one week.

A1.5.5 Sulphuric acid, approx 0.5M

Carefully add 17 ± 1 ml of concentrated sulphuric acid (d_{20} 1.840) to approximately 500 ml of water. Cool and make up to 1.00 ± 0.01 litre with water. Store in a glass stoppered bottle.

A1.5.6 Chloroform

It is most important that a suitable grade of chloroform is used. The quality of commercial chloroform is variable: when inferior grades are used, prolonged preliminary extraction of reagents is required. To achieve the required degree of purity, all chloroform to be used should be treated as follows:

A1.5.6.1 Commercial chloroform

Before use, all commercial chloroform is passed through a glass column containing self-indicating silica gel, 6–20 mesh, on top of neutral alumina (Al_2O_3). The length and diameter of the glass column, the height of silica gel and alumina in the column, and the rate of flow of eluting chloroform do not appear to be critical. The approximate dimensions are shown in Figure 1a.

A1.5.6.2 All used chloroform is dried over self-indicating silica gel (6–20 mesh) for a minimum of two hours and then distilled in the apparatus shown in Figure 1b. The first 200 ml of distillate are discarded, and the remainder is passed through a glass column containing self-indicating silica gel on top of neutral alumina (as shown in Figure 1(a)). The alumina removes the more polar constituents, such as surfactants, that might be present in the chloroform.

A1.5.6.3 Alternative method of Preparation of Chloroform for Re-Use

Distil chloroform from anhydrous calcium chloride, discard the first 30–50 ml of distillate, collect the distillate following this and discard the final 50 ml of distillate. Wash three times with distilled water.

The chloroform is then ready for use without drying. This guarantees there will not be any turbidity problems. The used chloroform is stored in a dark bottle and recovered immediately prior to use.

A1.5.7 Methylene blue solution (250 mg/l)

Dissolve 0.250 ± 0.005 g of methylene blue (BP grade) in water and dilute to 1.00 ± 0.01 litre with water. Store in a glass stoppered glass bottle in the dark. This solution is stable for at least two weeks.

A1.5.8 Manoxol OT solution (100 mg/l)

Dissolve 0.1000 ± 0.0005 g of Manoxol OT (sodium dioctyl sulphosuccinate) in water and make up to 1 litre in a volumetric flask. Store in a glass stoppered glass bottle in a refrigerator below 5°C. This solution is stable for at least two months.

A1.5.9 Manoxol OT solution (10 mg/l) (1 ml = 10 μg)

Pipette 10 ml of the 100 mg/l Manoxol OT solution (A1.5.8) into a 100 ml volumetric flask and make up to the mark with water. This solution is stable for at least one week.

A1.6 Apparatus

Unless otherwise specified all glassware used for quantitative volumetric measurements should be accurate to a Class B tolerance or better as defined in the relevant British Standards.

A1.6.1 Common laboratory glassware including pipettes, volumetric flasks, burettes, measuring cylinders, separating funnels fitted with PTFE stopcocks, and beakers.

A1.6.2 Glassware should not be washed in detergents but should be cleaned using 10% v/v hydrochloric acid, water and deionised water.

A1.7 Sample Collection and Preservation

Because of their nature surfactants will be adsorbed on to any suspended solids as well as on the walls of the containing vessel. It must therefore be clearly stated to the analyst as to what is required, ie the total surfactant or only that in solution. If the former is required it must be first ascertained whether the level of solids in the sample will permit a satisfactory estimation of the total surfactant. When the latter is needed, centrifugation rather than filtration of the sample should be employed to remove the suspended solids.

Sampling bottles should be completely filled with sample and if they are not to be analysed within a few hours of collection the samples should be stored in a refrigerator and sterilized, for example, by addition of 1% v/v of a 40% formaldehyde solution. To obtain a representative sample either stir the whole sample with a magnetic stirrer or slowly invert the sample bottle twice before taking aliquots. As far as practical avoid the formation of foam during the stirring or inversion but in any case do not withdraw an aliquot while the bulk sample is foaming.

A1.8 Analytical Procedure

Step	Procedure	Notes
	Preparation of Calibration Graph	
A1.8.1	Using a 25 ml burette measure out 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 ml volumes of the 10 mg/l Manoxol OT solution. Make up in each case to approximately 100 ml, then carry out the procedure below (Steps A1.8.2–8.9 and 8.11) (note a). Deduct the absorbance of the blank from those of the standard solutions and prepare graphs relating net absorbance to μg of Manoxol OT for 10 and 40 mm cells.	(a) It is only necessary occasionally to carry out this procedure.
	Analysis of Sample	
A1.8.2	Measure into a 250 ml glass-stoppered separating funnel 50 ml of water, 10 ml of alkaline borate solution and 5 ml of methylene blue solution. Add 10 ml of chloroform, shake for 30 seconds, and allow to seperate.	
A1.8.3	Run off the chloroform layer as completely as possible (note b) and rinse the aqueous layer without shaking with 2 to 3 ml of chloroform. Repeat the extraction with a further 10 ml of chloroform and rinse as before. Discard the chloroform extracts (note c).	(b) Wherever running off a chloroform layer from the separating funnel take care that none of the aqueous phase enters the tap of the funnel.
A1.8.4	Into a second 250 ml glass-stoppered separating funnel measure 100 ml of water 10 ml of alkaline borate solution and 5 ml of methylene blue solution. Extract with chloroform as in steps A1.8.2 and 8.3 (note c).	(c) The alkaline methylene blue may be cleaned up in one large separating funnel by taking suitably increased volumes of alkaline borate and methylene blue solutions and extracting with appropriate volumes of chloroform.
A1.8.5	To the extracted aqueous layer in the second separating funnel add 3 ml of sulphuric acid, mix well, and ensure that the tap and stem of the funnel are dry.	
A1.8.6	To the first separating funnel add a suitable volume of sample, up to 100 ml containing 20 to 160 μg of anionic surfactent (note d). Add 15 ml of chloroform, shake evenly and gently twice a second for 1 minute, preferably in a horizontal plane. Allow the layers to separate as completely as possible and swirl the funnel to dislodge droplets from the sides of the funnel (note e).	(d) Generally take up to 10 ml of settled sewage, 50 ml of sewage effluent, and 100 ml of river water. (e) It is frequently advantageous to incline the funnel and rotate it slowly about an almost horizontal axis in order to brake up the fine emulsion coating the walls.

Step	Procedure	Notes
A1.8.7	Allow to settle for two minutes then run as much as possible of the chloroform layer into the second separating funnel containing the extracted and acidified methylene blue solution. Shake the contents of this funnel and allow then to separate as described in step A1.8.6.	
A1.8.8	Allow to settle for two minutes. Run the chloroform solution through a small filter funnel plugged with cotton wool, which has been freshly washed with chloroform, into a clean dry 50 ml volumetric flask (note b).	
A1.8.9	Repeat steps A1.8.6 to 8.8 from the addition of 15 ml of chloroform onwards a further twice. Dilute the combined extracts in the volumetric flask to the mark with chloroform.	
A1.8.10	For each batch of samples for which steps A1.8.2–8.9 are carried out also complete these steps for a blank determination on 100 ml of deionised water and one standard from the range given in step A1.8.1.	
A1.8.11	Measure the absorbances of chloroform extracts for standards, samples and blank in 10 mm cells against a chloroform reference at 650 nm. The volumetric flasks should be shaken just before filling the cells and the cells rinsed out three times with the solutions before taking the reading. Wash out the cells with chloroform after each reading (notes f and g).	(f) Check the cell error frequently by measuring the absorbance difference when chloroform is used in both cells and correct for this error. If the cell error increases clean the cells by immersion in dilute nitric acid, rinse with deionised water and dry with acetone and chloroform. Mark one cell and reserve for the reference chloroform. (g) If the absorbance of the test solution of the sample when read in a 10 mm cell is less than 0.1 repeat readings of standards, blank and sample in 40 mm cells using the technique described in step 8.11.
A1.8.12	Deduct the absorbance of the blank from that of the sample and from the calibration graph corresponding to the size of cell used read the number of micrograms of Manoxol OT equivalent to the corrected absorbance (note h).	(h) If the standard run with the sample batch differs significantly from the calibration graph value, repeat the method with all samples and a full range of standards.

A1.9 Calculation of Results

Let V ml = volume of sample extracted
a μg = equivalent weight of Manoxol OT as read off the calibration graph
Then the equivalent concentration of anionic surfactant in the sample

$$= \frac{a}{V} \text{ mg/l (expressed as Manoxol OT).}$$

A2 Determination of Anionic Surface Active Material by an Automated Version of the Abbott Methylene Blue Method (Tentative Method)

A2.1 Performance Characteristics of the Method (39)

A2.1.1	Substance determined	Synthetic anionic surfactants and other anionic surface active material of natural origin.
A2.1.2	Type of sample	Settled sewage, sewage effluents and waters and certain trade effluents.
A2.1.3	Basis of method	Automated solvent extraction (water/chloroform) and colorimetric measurement of the anionic surfactant as its methylene blue complex (1) (2) (3) (4).
A2.1.4	Range of application (a)	Up to 20 mg anionic surfactant per litre as Manoxol OT.
A2.1.5	Calibration curve (a)	Logarithmic; $C = K (\log B - \log T)$ where C = concentration as mg Manoxol OT per litre K = constant B = baseline transmission (per cent) T = transmission (per cent) for concentration C

A2.1.6 Standard deviation (a)

Concentration of Manoxol OT in distilled water (mg/l)	Total Standard Deviation (mg/l)	Degrees of Freedom
0.2	0.07	9
1.0	0.03	9
10.0	0.06	9
20.0	0.11	9

A2.1.7	Limit of detection (a)	0.2 mg Manoxol OT per litre.
A2.1.8	Sensitivity (a)	$\frac{dT}{dC} = 0.07T$
A2.1.9	Bias	Not known.
A2.1.10	Interference	See section 3 for substances tested.
A2.1.11	Time required for analysis	Approx analytical time (80 + 4.3N) min; approx operator time (15 + 0.5N) min; where N = number of samples.

(a) These data were obtained at the Water Research Centre (Stevenage Laboratory) (6) using a procedure exactly the same as this method with a basic Technicon Auto Analyser AA1 system.

A2.2 Principle

A2.2.1 The Abbott method (3) is generally considered to be an improved version of the Longwell and Maniece procedure (2). The latter was accepted by the UK Standing Technical Committee on Synthetic Detergents (1956) as the official methylene blue method for determining anionic surface active material. However, improvements in the former method, ie use of pre-extracted reagents, etc have resulted in greater sensitivity and reproducibility for methylene blue estimations.

A2.2.2 Anionic surfactant is associated with the intensely coloured methylene blue cation to form a chloroform extractable ion-association complex; the unassociated dye has an extremely small solubility in chloroform. The methylene blue-anionic complex is partitioned into chloroform fron an alkaline methylene blue solution to avoid the negative interference of proteinaceous material present in environmental samples. The chloroform phase is then back extracted with an acidified methylene blue solution in order to remove the interference of those materials such as inorganic anions eg nitrate, chloride etc, that form methylene blue complexes of low chloroform extractability. The absorbance of the final chloroform phase is determined at 650 mm.

A2.3 Scope and Limitations (including Interferences)

A2.3.1 For distilled water solutions of synthetic anionic surfactant, the method will give an accurate determination of the surfactant present. However, for unknown water samples, ie sewage, effluent and surface waters, the results obtained represent anionic surface active material rather than synthetic anionic surfactant. In theory, any compound containing a single strong anionic grouping (eg sulphate or sulphonate) and a hydrophobic moiety is capable of forming an extractable ion-association compound with the methylene blue cation and can therefore positively respond in a determination for synthetic anionics. It has been shown or predicted that organic sulphates, sulphonates, carboxylates and phenols and even simple inorganic anions such as cyanate, nitrate, thiocyanate and sulphide can be methylene blue active (5) (6). Although negative interferences can also occur as a result of the direct competition of other 'cationic' materials, for example proteins and quaternary ammonium compounds, for the anionic surfactant, they are generally considered to be less important than positive interferences (8). However, the increased usage of materials such as cationic surfactant may alter this situation. The reaction conditions in the Abbott method have been selected, as far as possible, to eliminate or minimize some of the above interferants, in particular proteinaceous material and inorganic anions which result in negative and positive interferences respectively. Common constituents of sewage and effluents including urea, ammonia and nitrate, as well as the preservatives formaldehyde and mercuric chloride have been shown to give no interference at 100 mg/l. Sodergren (4) showed that sodium chloride (300–30,000 mg/l), sodium sulphide (100 mg/l), sodium sulphate (400 mg/l) and sea water do not interfere. Nevertheless, not all natural interferants can be eliminated to allow a direct methylene blue determination of the synthetic anionic surfactant in environmental samples. For this reason, the entities detected by the method are more correctly referred to as anionic surface active material or methylene blue active substances (MBAS). For domestic waste waters the MBAS response can generally be taken as an acceptable over-estimate of the synthetic anionic surfactant present. However, there are good indications that synthetic anionics may contribute only a small proportion to the total MBAS in some surface waters (5) (9) (10) (11).

A2.3.2 Sodergren (4) established that there was more than a 90% probability of obtaining the same value by the automated and manual procedures for homogeneous aqueous samples containing Manoxol OT (0.02 to 2.7 mg/l). It has been observed, however, that the automated procedure can give significantly lower MBAS values than the manual method when applied to samples containing suspended solids (on which anionic surfactant is concentrated). Two major factors probably account for the lower recoveries of anionic material in the automated method, (i) suspended solids tend to settle out in the sampling cups of the analyser while awaiting analysis and the adsorbed surfactant is not quantitatively analysed and (ii) in the less vigorous extraction system of the analyser (ie mixing coils) a less complete recovery of surfactant from the sampled solids is obtained compared with the manual procedure. The analyst should therefore determine how satisfactory the automatic analysis is for samples containing high levels of suspended solids by correlating its performance with that of the manual method.

A2.4 Hazards

Besides sulphuric acid, other potentially harmful reagents used in this method are chloroform, petroleum ether and mercuric chloride.

Chloroform is not expected to be a hazard during its use in the analysis but an efficient distillation unit, housed in a fume cupboard, should be employed for its recovery.

Petroleum ether bp 40° to 60° is a highly flammable liquid and must not be used near any naked light or flame. Smoking while using this material must be prohibited.

The eyes should be protected by wearing suitable glasses in the event of the bursting of the plastic tubing on the Auto Analyser.

A2.5 Reagents

A2.5.1 Water

Use distilled or deionized water for wash water, blank determinations, and for preparing standard and reagent solutions. This water should have the equivalent of anionic-surfactant content which is negligible compared with the smallest concentration to be determined in samples by this method.

A2.5.2 Sodium tetraborate, 0.05M

Dissolve 19.05 g of sodium tetraborate in 1.0 litre of distilled water. Store in a glass-stoppered glass bottle. This solution is stable for at least two weeks.

A2.5.3 Sodium hydroxide, approx 0.1M

Dissolve 4.0 ± 0.2 g of sodium hydroxide pellets in 1.0 litre water. Store in a polythene-stoppered glass bottle. This solution is stable for at least two weeks.

A2.5.4 Alkaline borate solution

Mix equal volumes of 0.05M sodium tetraborate solution and 0.1M sodium hydroxide solution. Store in a polythene-stoppered glass bottle. This solution is stable for at least one week.

A2.5.5 Sulphuric acid, approx 0.5M

Carefully add 27 ± 0.5 ml of concentrated sulphuric acid (d_{20} 1.840) to approx 500 ml water, cool and make up to 1.0 litre with water in a volumetric flask. Store in a glass-stoppered glass bottle.

A2.5.6 Chloroform

It is most important that a suitable grade of chloroform is used. The quality of commercial chloroform is variable: when inferior grades are used, prolonged preliminary extraction of reagents is required, and a high and continuous baseline drift results. To achieve the required degree of purity, all chloroform to be used should be treated as follows:

A2.5.6.1 Commercial chloroform

Before use, all commercial chloroform is passed through a glass column containing self-indicating silica gel, 6–20 mesh, on top of neutral alumina (Al_2O_3 grade W200). The length and diameter of the glass column, the height of silica gel and alumina in the column, and the rate of flow of eluting chloroform do not appear to be critical. The approximate dimensions used at present are shown in Figure 1a.

A2.5.6.2 Used chloroform

All used chloroform is dried over self-indicating silica gel (6–20 mesh) for a minimum of two hours and then distilled in the apparatus shown in Figure 1b. The first 200 ml of distillate are discarded, and the remainder is passed through a glass column containing self-indicating silica gel on top of neutral alumina (as shown in Figure 1a). The alumina removes the more polar constituents, such as surfactants, that might be present in the chloroform.

A2.5.6.3 Alternative Method of Preparation of Chloroform for Re-Use

Distil chloroform from anhydrous calcium chloride, discarding the first 30–50 ml of distillate. Collect the distillate following this and discard the final 50 ml of distillate. Wash three times with distilled water.

The chloroform is then ready for use without drying. This guarantees there will not be any turbidity problems. The used chloroform is stored in a dark bottle and recovered immediately prior to use.

A2.5.7 Stock methylene-blue solution

Dissolve 0.25 ± 0.005 g of methylene-blue in water, transfer quantitatively to a 1 litre volumetric flask and make up to the mark with water. Store in a glass-stoppered glass bottle in the dark. The solution is stable for at least two weeks.

A2.5.8 Alkaline methylene-blue solution

Measure 60 ± 0.5 ml of the stock methylene-blue solution into a 250 ml separating funnel and add 100 ml of alkaline borate solution. Extract this mixture with four 20 ml portions of chloroform. After the fourth extraction the chloroform layer should be colourless.

Wash the methylene-blue layer with 25 ml of petroleum ether (bp 40° to 60°C) (HIGHLY FLAMMABLE LIQUID), run the lower layer into a 1 litre measuring cylinder containing 200 ml of alkaline borate solution, and make up to 1 litre with water. Store in a polythene-stoppered glass bottle in the dark. This solution is stable for up to five days.

A2.5.9 Acid methylene-blue solution

Measure 80 ml of the stock methylene-blue solution into a 500 ml separating funnel and add 40 ml of alkaline borate solution and 160 ml of distilled water. Extract this mixture with five 25 ml portions of chloroform. After the fifth extraction the chloroform layer should be colourless.

Wash the methylene-blue layer with 25 ml of petroleum ether (bp 40° to 60°C) (HIGHLY FLAMMABLE LIQUID), run the lower layer into a 2 litre measuring cylinder containing 20 ml of 0.5M sulphuric acid and make up to the mark with water. Store in a glass-stoppered glass bottle in the dark. This solution is stable for up to five days.

A2.5.10 Standard Manoxol OT solutions

A2.5.10.1 Stock solution A, 1 ml $\equiv$ 1 mg Manoxol OT

Dissolve 1.00 ± 0.05 g of Manoxol OT (sodium dioctylsulphosuccinate) in water. Transfer quantitatively to a 1-litre volumetric flask and make up to 1 litre with water. Store in a glass-stoppered glass bottle in a refrigerator below 5°C. This solution is stable for at least three months.

A2.5.10.2 Stock solution B, 1 ml $\equiv$ 0.1 mg. Manoxol OT

Add 50.0 ml of solution A to a 500 ml volumetric flask and make up to 500 ml with water. Store in a glass-stoppered glass bottle in a refrigerator below 5°C. This solution is stable for at least two months.

A2.5.10.3 Prepare a series of standard solutions containing 1, 2, 5, 10, 15, and 20 mg Manoxol OT per litre by adding 1.0 ml, 2.0 ml, 5.0 ml, 10.0 ml, 15.0 ml, and 20.0 ml respectively, of solution B to 100 ml volumetric flasks and making up to the mark with water. These solutions are stable for at least one week.

A2.6 Apparatus

The apparatus consists basically of a sampler, proportioning pump, colorimeter, and strip chart recorder, assembled as shown in Figure 2.

A2.7 Sample Collection and Preservation

Because of their nature surfactants will be adsorbed on to any suspended solids as well as on the walls of the containing vessel. It must therefore be clearly stated to the analyst as to what is required, ie the total surfactant or only that in solution. If the former is required it must be first ascertained whether the level of solids in the sample will permit a satisfactory estimation of the total surfactant. When the latter is needed, centrifugation rather than filtration of the sample should be employed to remove the suspended solids.

Sampling bottles should be completely filled with sample and if they are not to be analysed within a few hours of collection the samples should be stored in a refrigerator and sterilized, for example, with 1% of a 40% formaldehyde solution. To obtain a representative sample either stir the whole sample with a magnetic stirrer or slowly invert the sample bottle twice before taking aliquots. As far as practical avoid the formation of foam during the stirring or inversion but in any case do not withdraw an aliquot while the bulk sample is foaming. Samples expected to contain more than 20 mg anionic surfactant as Manoxol OT per litre should be first diluted with water.

A2.8 Analytical Procedure

Step	Procedure	Notes
	Starting Operation	
A2.8.1	Connect the system as shown in flow diagram and switch on photometer (note a) and select the appropriate wave-length. (650 nm).	(a) Allow photometer lamp to warm up for at least 15 min.
A2.8.2	Place the reagent lines into their respective reagents, start the pump and allow the reagents to pump through the system for 15 min (note b).	(b) Straighten out any 'snaked' pump tubes and check that the bubble pattern and hydraulic behaviour of the system are satisfactory. If not, eliminate difficulties before proceeding to stage 3.
A2.8.3	Connect the second phase splitter to the flow cell (note c).	(c) Pinch the acid-resistant tubing at the bottom of the phase splitter with the fingers and disconnect the screw-clip attachment. Gently ease the finger pressure to allow chloroform to flow out slowly until it is seen to be free of methylene-blue solution then re-pinch the tubing and connect to the flow cell inlet line. Ensure that the chloroform layer in the phase splitter does not drop so low that methylene-blue solution enters the flow-cell line. If this happens reconnect screw-clip attachment, wash out the flow-cell with hot water followed by acetone, air dry, and repeat the connection procedure.
A2.8.4	Switch on the drive for the recorder chart, and set the pen to 95–100 per cent T.	
	Sample Analysis	
A2.8.5	For a typical 40-positioned sample tray* load in the following order (note d):	(d) The tray can be loaded during the stabilization period.
	Position Nos 37 diluted sewage sample prelim. 38 sewage sample check 39, 40 distilled water samples 1–6 standard solutions in ascending order 7–9 distilled water 10–21 samples (note e) 22 Control Standard (note f) 23 Intermittent Standard (note g)	(e) A second portion of the first sample in each block of samples is analysed as the last sample in each block, for quality-control purposes. (f) A standard solution of known concentration corresponding to 0.5 C is analysed at the end of each block of samples for quality-control purposes; C is the highest concentration that the calibration is intended to cover. (g) One of the calibrating standards, in ascending order, is repeated for calibration check purposes.
	24–25 distilled water Repeat the sequence 10–25 inclusive until all the samples have been analysed (note h).	(h) Standards not used intermittently are to be grouped in ascending order at the end of the run.

*applicable to the type indicated in the flow diagram.

Step	Procedure	Notes
A2.8.6	Start the sampler (note i) with a sample rate of 20/h and a sample: wash ratio 1:2 (note j).	(i) The first peak will appear after approximately 7 min. (j) When a sample having a low concentration of anionic surfactant immediately follows a sample with a high concentration, the test will be repeated on the low sample at the end of the run, separated by two distilled water samples. Samples whose concentration is found to be $>$ 20 mg/l are repeated after suitable dilution with distilled water. End a run of samples by repeating the calibration curve, if considered necessary.
A2.8.7	When all the peaks have appeared and a final base line has been obtained, switch off the drive for the recorder chart.	
	Calculation of Results	
A2.8.8	Plot a calibration curve of per cent T against concentration of standard solution (notes k and l).	(k) Take the mean percentage reading for T of two aliquots of each standard. The calibration is linear if plotted on semi-log paper. (l) All peaks heights of standards and samples are first corrected for any base-line drift.
A2.8.9	Calculate the equivalent concentration of Manoxol OT in the samples.	
A2.8.10	Shut-down procedure. (Required at the end of each working day). Transfer both the acid and alkaline methylene-blue lines to water and pump water for 10 min while continuing to pump chloroform through the chloroform lines. Then pinch the flow cell inlet line, disconnect, and reconnect the screw-clip attachment. When the flow cell is free of chloroform, pump air through the chloroform reagent lines until the first phase splitter is free of chloroform. Place the chloroform reagent lines into a solution of methanol containing approximately 10 per cent v/v of concentrated hydrochloric acid, and place the other reagent lines in water; wash the system through for ten minutes, then place the chloroform reagent lines in water and wash through for fifteen minutes. The methanol/hydrochloric acid solution effectively removes methylene-blue which adheres to the glass surfaces; it also removes any adsorbed surfactant. Switch off the pumps, release the pump tubes, and switch off the colorimeter. Wash the sample cups (note m).	(m) If cups are to be re-used wash well with **hot** water.

A2.9 Extension of the Concentration Range of the Method

The method has been tested in the concentration range 0 to 20 mg Manoxol OT per litre. The response is not linear at higher concentrations when employing a semi-logarithmic plot. However, by using range expansion, $\times 10$, a much lower working range of 0 to 2.0 mg Manoxol OT per litre can be accommodated, but each sample should be separated by a solution of 0.02M KH_2PO_4 potassium dihydrogen phosphate on the sample tray for improved precision.

A3 Extraction of Anionic Surface Active Material from Sewage Sludges (Tentative Method)

A3.1 Performance Characteristics of the Method (39)

This method has not been tested to the normal tentative method standard; nevertheless the Committee think that it should be published.

A3.1.1	Substance determined	Synthetic anionic surfactants and other anionic surface active material of natural origin.
A3.1.2	Type of sample	Wet or dry, primary, secondary and digested sludges.
A3.1.3	Basis of method	The concentration of the anionic surfactant extracted from the sewage sludge by alkaline methanol is determined by the standard methylene blue procedure (Method A1).
A3.1.4	Range of application	0.1–5.0% m/m as Manoxol OT on dry solids.
A3.1.5	Standard deviation	—
A3.1.6	Limit of detection	0.005% m/m*.
A3.1.7	Interferences	Anything extracted which interferes with methylene blue active substance determination. (See Section A3.3).
A3.1.8	Time required for analysis	Total time: 8 h for preparation of 12 aqueous samples for MBAS analysis.

* Based on extraction of ½ g solids.

A3.2 Principle

Anionic surfactant present in sludge is first extracted into alkaline methanol. After evaporation of the methanol, the residue is dissolved in water and analysed by the standard methylene blue procedure. (Method A1.) (12).

A3.3 Interferences

The methylene blue analysis is not specific for anionic surfactant alone; many other anion active materials (Synthetic and natural) have been shown to be responsive (see Section A1.3). Therefore the analysis of the residue extracted from a sludge will give a total estimate of the anionic surface active material present (ie methylene blue active substances, MBAS). In addition, negative interferences can also occur in the methylene blue analysis as a result of the direct competition of other 'cationic' material, for example, proteins and quaternary ammonium compounds, for the anionic surfactant. These have generally been considered to be less important than positive interferences although the increased usage of cationic surfactant may have altered this situation. Detergent-free sludge has been found to have the equivalent of 0.2% Manoxol OT m/m equivalent on dried solids.

Sludges heavily contaminated with mineral oils might give rise to difficulties through the precipitation of oil in the cold methanol extracts and its subsequent interference with the methylene blue analysis.

A3.4 Hazards

A high standard of hygiene should be maintained since primary and secondary sludges may contain pathogenic organisms. Some of the reagents used, including methanol, chloroform (for MBAS) and sodium hydroxide, are potentially harmful.

A3.5 Reagents

A3.5.1 **Methanol,** analytical reagent grade

A3.5.2 **Sodium hydroxide,** analytical reagent grade **0.1M methanolic solution.**

Dissolve 10 ± 0.1 g sodium hydroxide in 250 ± 5 ml methanol (caution).

A3.6 Apparatus

A3.6.1 **250 ml round bottomed flask fitted with a reflux condenser.**

A3.6.2 **Steam bath or heating mantle.**

A3.6.3 **Buchner funnel and filter flask.**

A3.6.4 **Whatman GF/C glass fibre filter papers of diameter 50 mm,** or equivalent.

A3.6.5 **Filter pump.**

A3.6.6 **Porous porcelain chips.**

A3.7 Sample Preservation

Sludge samples should be analysed immediately. If this is not possible, samples should be stored at 1–4°C but for not more than 48 hours.

A3.8 Analytical Procedure (See also note at the end of this procedure.)

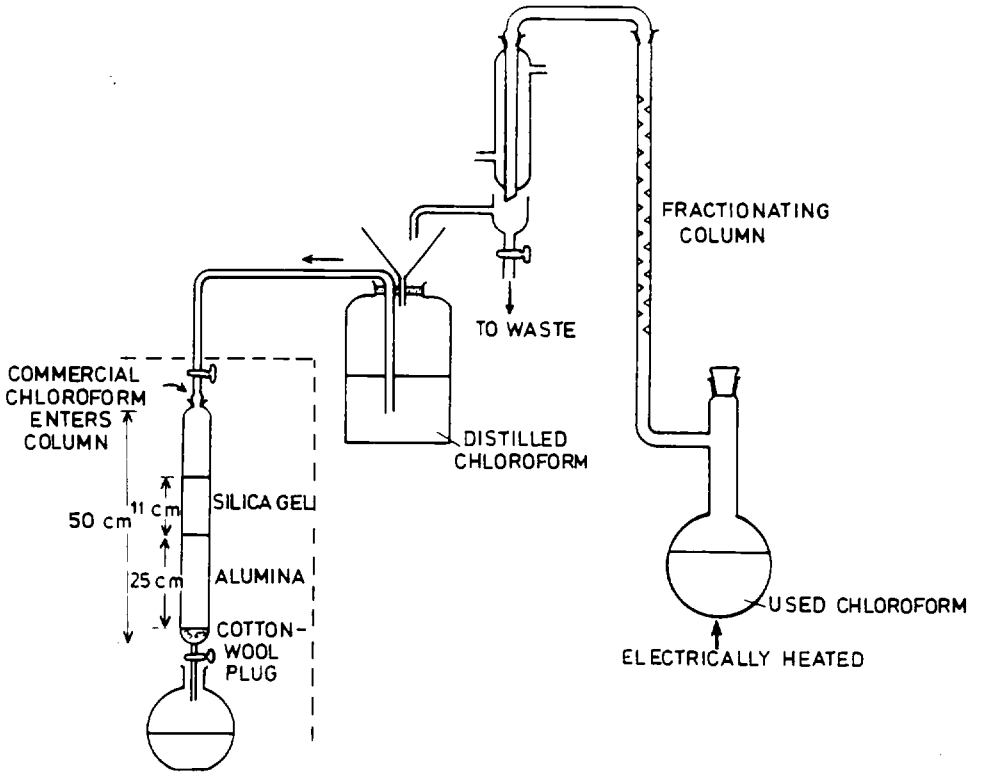
Step	Procedure	Notes
A3.8.1	Weigh out accurately about 10 ml sieved wet sludge or about 0.5 g dried sludge (note a). Transfer with the aid of a little methanol to a 250-ml round bottomed flask (note b).	(a) If necessary, coarse solids are removed by sieving (about 5 mm). The dry weight of the sludge must be determined (36). Dried sludge is ground and sieved before use (37). (b) Glassware should be rinsed with methanol before use to avoid contamination.
A3.8.2	Add 150 ± 5 ml methanol and 10 ± 1 ml of a 0.1M methanolic solution of sodium hydroxide.	
A3.8.3	Fit a reflux condenser to the flask and reflux vigorously for about 30 mins over a steam bath or heating mantle.	
A3.8.4	After refluxing allow the sludge to settle in the flask (note c) and decant the methanolic extract through a glass-fibre filter paper under suction (note d) on a Buchner funnel into a 500 ml filter flask. Transfer the whole contents of the flask to the filter funnel.	(c) Initial settling facilitates filtering. (d) Use a filter pump.
A3.8.5	Rinse out the flask into the filter with 10–20 ml methanol making sure all the solids are transferred.	
A3.8.6	Wash the solids on the filter paper with three separate 20 ml portions of methanol.	
A3.8.7	Return the filtrate to the round bottomed flask and distil off (note e) the methanol until about 10 ml remains.	(e) Porous porcelain chips should be used to prevent bumping.
A3.8.8	Disconnect the flask and allow to cool for 10 min. (note f).	(f) This allows the remaining methanol to evaporate.

Step	Procedure	Notes
A3.8.9	Rinse down the sides of the flask and dilute the residue to not less than 250 ml with distilled water (note g).	(g) Dilution is normally to 250 ml up to 1000 ml depending upon the concentration of anionic surfactant in the sample.
A3.8.10	Analyse this solution by the standard methylene blue procedure (Method A1).	

Note: A soxhlet extraction may be used instead of the refluxing in steps A3.8.1–A3.8.4 using methanol only (13).

A3.9 Calculation

Let the volume of final extract be V ml and the concentrations of MBAS be x mg/l. If the weight of dry sludge used is w mg, the percentage MBAS in dry solids $= \frac{xV}{10w}\%$



(a) Commercial chloroform (b) Used chloroform

Fig. 1. Distillation of chloroform

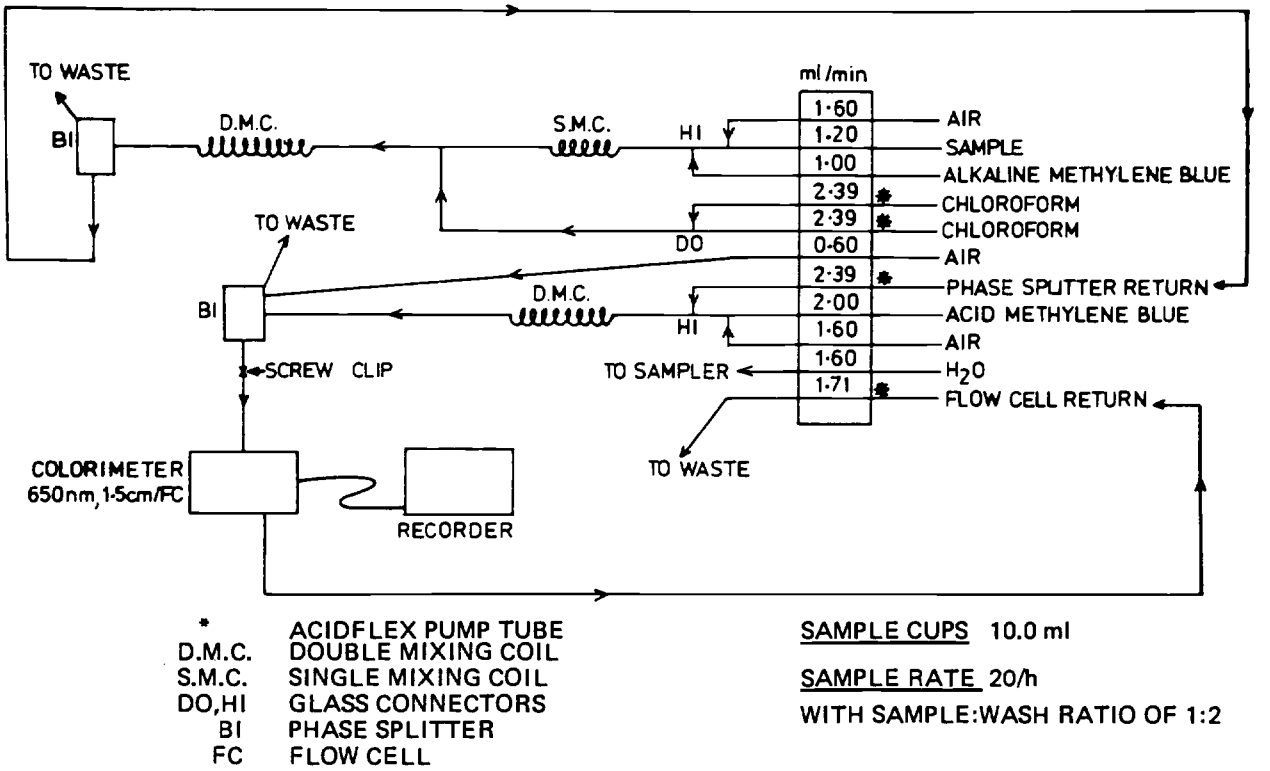


Fig. 2. Flow diagram of analytical system for anionic surfactants

B Nonionic Surfactants

B1 A Review of Analytical Methods for the Determination of Low Levels of Alkoxylated Nonionic Surfactants in Aqueous Samples

Introduction

The two most widely used groups of nonionic surfactants, accounting for about 80% of domestic and industrial use in Europe (14), are the alcohol ethoxylates (AE) and the alkylphenol ethoxylates (APE); these are prepared by the reaction of ethylene oxide (EO) with fatty alcohols and alkylphenols respectively:

$$ROH + n\ H_2C\text{–}CH_2 \rightarrow R(OCH_2CH_2)_n\ OH$$
$$\qquad\qquad\quad \backslash\ /$$
$$\qquad\qquad\quad\ O$$

where R is an alkyl (C_8–C_{18}) or alkylphenyl group (octyl/nonyl phenol)

n = an average of 4–20 (EO) units.

Other less important classes of alkoxylated nonionic surfactants include the polyoxyalkylene oxide block copolymers (containing ethylene oxide and propylene oxide (PO)), polyoxyethylene esters and polyoxyethylene alkylamides, etc (15).

The use of substantial quantities of alkoxylated nonionic surfactant of the types indicated above has resulted in a need for analytical methods that can be used to determine them in a number of sample matrices. To date, only a small number of procedures have gained any general acceptance for determining low levels of these materials (0–20 mg/litre) in a range of aqueous samples which include biodegradation test liquors, sewage, effluents and surface waters. Four of the most applicable methods* for use in nonionic surfactant biodegradability studies and environmental monitoring are considered. These are:

1. the Wickbold titrimetric method (and modifications) (16, 17), (Method B2),
2. the American Soap and Detergent Association's (SDA) cobaltothiocyanate method (18), (Method B5).
3. the Patterson TLC method (19), (Method B3), and
4. a chemical fission—GLC procedure (tentative method) (20, 21), (Method B4).

An important first step in all these procedures in the concentration and separation of the intact alkoxylated nonionic surfactant free from interfering material in the aqueous sample prior to its analytical estimation. Concentration is generally necessary since the analytical estimation steps lack sufficient sensitivity to enable a direct analysis of samples (c. 1 mg required for accurate estimation) even if they contain no interfering components. In the Patterson TLC method a series of solvent extraction steps (water/chloroform) is used to concentrate and separate nonionic surfactant from some of the interfering material in aqueous samples. The technique results in the extraction of nonionic surfactant degradation products and polyoxyethylene glycols (ie non-surface active components). These materials do not generally interfere in the TLC estimation of intact nonionic surfactant since they have significantly different Rf's (and if required can be separately assessed). For the other three procedures the intact nonionic surfactant is concentrated and separated by the Wickbold solvent sublation technique free (22) from non-surfactant material that could interfere in its subsequent estimation. With this technique all surface active materials including the nonionics are transported from aqueous samples into an overlying layer of organic solvent (ethyl acetate) by bubbling gas (nitrogen or air) through it. The phenomenon is due to the adsorption of the surfactant on the surface of the bubbles and its subsequent transfer to the liquid-liquid interface where the isolated substances partition into the organic solvent. On increasing the ethylene oxide content of a surfactant structure the recoverability of a material in this sublation step can be significantly reduced due to its increasing hydrophilic character. Nevertheless, acceptable recoveries have been obtained for materials containing up to an average of 50 (EO) units. It has been indicated that for samples containing high levels of solids eg raw and settled sewage, additional sublations (to the two recommended by Wickbold) are often needed to ensure acceptable recoveries of nonionic surfactant (18).

The resulting sublation extracts are generally ion-exchanged under non-aqueous conditions to remove ionic-surface active materials, that can interfere in the subsequent estimation of the nonionic surfactant in these methods, eg cationic surfactants, alcohol ethoxylate sulphates, etc. However, the ion-exchange step in these procedures can be left out provided that interfering materials are known to be absent from a sample, eg usually the case for biodegradation test liquors.

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*Other minor classes of non-alkoxylated nonionic surfactants such as the anhydrohexitol derivatives, sugar esters, fatty alkanolamides and fatty amine oxides are unlikely to be responsive to these methods.

The solvent sublation step is equally applicable for preparing a nonionic surfactant extract suitable for a Patterson TLC analysis (providing ion-exchange has been carried out for those samples requiring it).

The chemical basis of the analytical estimation step in three of the methods ie Wickbold, SDA cobaltothiocyanate and Patterson TLC procedures is essentially similar and depends upon the formation of heavy metal—alkoxylated nonionic surfactant complexes. The stoichiometry of complex formation is dependent on the length of the polyoxyalkylene chain and little or no reaction is obtained for nonionic surfactants containing less than an average of 4 (EO) units. Therefore, wherever possible results should be expressed with the aid of standards for the surfactant under investigation eg in biodegradation test studies, etc. For samples in which the nature of the nonionic surfactant is unknown, particularly true of environmental samples, results have to be expressed arbitrarily in terms of a suitable reference surfactant. A nonyl-phenol ethoxylate with an average of eight to ten ethoxylate units (such as 'Synperonic NP8' manufactured by ICI Ltd and 'Marlophen 810' manufactured by Hüls AG) may be used for this purpose. The fourth method, however, is based on the chemical degradation of the nonionic surfactant to give specific products that can be analysed by GLC. The response of this method, unlike the other three, is dependent only on the polyoxyalkylene content of the extracted nonionic surfactant; both polyoxyethylene and polyoxypropylene units are assessed. A brief description of each method is given below.

B1.1 Wickbold titrimetric procedure and modifications

This procedure has been officially adopted for the determination of nonionic surfactants in biodegradation test liquors in German, French and UK methods and has also been incorporated into the draft EEC directive for determining the biodegradability of nonionic surfactants. A simple solvent sublation technique is used to concentrate selectively intact nonionic surfactants free from non-surface active materials including polyoxyethylene glycols (PEG's) and nonionic surfactant degradation intermediates. The residues isolated from environmental samples are cation-exchanged to remove other interfering surface active materials, such as cationic surfactants, that are concentrated in the solvent sublation. The nonionic surfactant is precipitated from aqueous solution with a modified Dragendorff reagent (barium chloride—potassium tetraiodobismuthate (III)); the resulting nonionic surfactant—complex precipitate is dissolved and the liberated bismuth ion is titrated potentiometrically with pyrrolidine dithiocarbamate complexone as a measure of the nonionic surfactant.

In other modifications the bismuth is estimated (17 and Method B2), probably with greater ease, by atomic absorption or UV colorimetry. Since it is recognised that materials (synthetic and natural) other than nonionic surfactant can positively respond to this method, the entities detected are commonly referred to as either Dragendorff active substances (DAS) or bismuth active substances (BiAS).

The Wickbold procedure has been found to be applicable to a wide range of alkoxylated nonionic surfactants. Although Wickbold indicated that alcohol ethoxylates and alkylphenol ethoxylates with an average of 6–30 (EO) units could be assessed it has been found that compounds with as many as 50 (EO) units and those containing (EO) and (PO) units can also be estimated. The response of a nonionic surfactant is dependent upon both its molecular weight and polyoxyalkylene chain length; short chain nonionic surfactants containing an average of 4 or less (EO) units have little or no response. The method has a limit of detection for alkoxylated nonionic surfactant of about 0.05 mg/l (expressed as 'Synperonic NP8').

B1.2 SDA Cobaltothiocyanate Procedure

The American SDA has claimed that its cobaltothiocyanate method (CT) is simpler and faster to operate than the Wickbold titrimetric procedure in biodegradation and environmental studies. A modified Wickbold solvent sublation step is used to concentrate selectively intact nonionic surfactant; for environment samples the resulting nonionic surfactant residue is both anion and cation-exchanged to remove ionic surface active materials that might subsequently interfere in the nonionic surfactant estimation. The nonionic surfactant is reacted with the ammonium cobaltothiocyanate in aqueous solution to form a coloured complex which is readily extractable into dichloromethane. The intensity of the colour of the complex in the organic phase is a measure of the nonionic surfactant present. The CT method, like the Wickbold, is not completely specific for nonionic surfactant and the entities detected are therefore correctly referred to as cobaltothiocyanate active substances (CTAS). (See also Ref 38.)

The applicability of the method has been established for alcohol and alkylphenol ethoxylates with linear or branched alkyl chain lengths of C_8 to C_{18} and ethoxylate chain lengths of 4 to 20 (EO) units although other longer (EO) chain length nonionic surfactants are expected to be responsive. The response of a nonionic surfactant is highly dependent upon its polyoxylalkylene chain length and molecular weight (probably more so than the Wickbold procedure). It has a limit of detection of about 0.1 mg/l.

Work has been carried out at the Water Research Centre on the automation of this procedure (23) allowing the analysis of three samples an hour. Although limitations were experienced in this approach it could form the basis for further investigation.

B1.3 Patterson TLC Method

This procedure has found considerable application in the monitoring of nonionic surfactant in sewage, effluent and surface waters particularly in the UK. A liquid/liquid solvent extraction technique is used to concentrate and separate the nonionic surfactant from some of the interfering material in aqueous samples. The resulting nonionic surfactant residue, made up to small volume with chloroform, is spotted alongside standards on a TLC plate and run in a suitable solvent system. The sample spots are visually quantified with the aid of the standard spots after the plate is sprayed to produce the coloured Dragendorff—nonionic surfactant complex (see Wickbold method). It is the subjective comparative estimation of the nonionic surfactant in this procedure that has been seen as one of its major disadvantages for use in statutory biodegradation tests.

The Patterson TLC method is applicable to commercial alkoxylated nonionic surfactants with ethoxylated chain lengths of at least 4 (EO) units. Compounds containing both (EO) and (PO) units are also estimatable. However, streaks rather than compact spots are formed on TLC plates with compounds that have more than about 20 (EO) units. The response of a nonionic surfactant in the TLC estimation is dependent upon its molecular weight and polyoxyalkylene chain length. The sensitivity of the method is significantly better (limit of detection about 0.01 mg/l 'Synperonic NP8') than that of the Wickbold and CT methods.

B1.4 Chemical Fission—GLC Method (Tentative)

The chemical fission--GLC technique has been well established for the macrodetermination of the polyoxyethylene and/or polyoxypropylene content of a range of alkoxylated compounds. Methods using this principle are now available for the microdetermination of alkoxylated nonionic surfactant and degradation intermediates in aqueous samples. However, for the specific determination of intact nonionic surfactant preliminary solvent sublation and ion exchange steps have to be incorporated into the method; if an estimate of the total alkoxylated material in a sample is required an appropriate alternative concentration technique can be used (ie solvent extraction, simple evaporation). The intact nonionic surfactant, isolated by solvent sublation, is reacted with hydrogen bromide to break the ether linkages between adjacent oxyalkylene units to form the corresponding dibromoalkanes which are subsequently quantitatively estimated by GLC. The amount of nonionic surfactant is calculated from the levels of dibromoalkanes produced and with the aid of suitable reference standards.

$$RO(CH_2CH_2O)_n\,H + HBr \rightarrow R\,Br + n\,BrCH_2CH_2Br$$

$$RO(CH_2CH_2O)_x\,(CH_2.\underset{\displaystyle CH_3}{\underset{|}{CHO}})_y\,H + HBr \rightarrow R\,Br + x\,BrCH_2CH_2Br + y\,BrCH_2\underset{\displaystyle CH_3}{\underset{|}{CH}}.Br$$

Unlike the other three methods, the response of nonionic surfactant in the chemical fission—GLC procedure is dependent only upon the polyoxyalkylene content of the nonionic extract; the method is therefore applicable to all alkoxylated nonionic surfactants irrespective of their (EO) chain length provided that they are solvent sublatable. The limit of detection for the method is claimed to be 0.005 mg/l (as 'Synperonic NP8').

B1.5 The Applicability of the Methods to Aqueous Samples

B1.5.1 Biodegradation Test Liquors

All four methods can be used to determine low levels of a wide range of commercial alkoxylated nonionic surfactants in biodegradation test liquors with acceptable accuracy and precision. However, the Wickbold and CT procedures are likely to find the greatest usage since they are simpler and quicker to operate. The use of the Wickbold solvent sublation technique in these methods provides a convenient means of selectively concentrating small amounts of intact nonionic surfactant. The two methods are particularly suited to the range of nonionic surfactant concentrations found in biodegradation test liquors (0.1–20 mg/l) and give an unequivocal estimate (via a direct titration or absorbance reading) of the nonionic present (unlike the Patterson TLC method which relies upon a subjective comparative estimation). Although the Wickbold and CT methods are probably more susceptible to positive interference than the other two methods this is unlikely to be important for biodegradation test liquors (ie where appropriate control liquors are generally available to correct for any interfering material).

The Patterson TLC and chemical fission—GLC methods are very much less convenient but can offer some distinct advantages over the simpler methods on occasions. For example, both methods are able to determine much lower levels of nonionic surfactant (down to 0.005 mg/l), the TLC method can give additional information on the nature of the nonionic and its degradation intermediates and the chemical fission—GLC procedure can estimate a wider range of materials including the short (EO) chain nonionic surfactants.

B1.5.2 Sewage, Effluent and Surface Water Samples

In environmental samples there are many more materials that can interfere with the estimation of alkoxylated nonionic surfactants. Of the four methods, the Patterson TLC and chemical fission—GLC procedure are less likely to be

susceptible to the possible interference of naturally occurring material since they utilise chromatographic techniques that result in an additional separation of the nonionic surfactant from interferants. Since these two methods are also the most sensitive they are more applicable for monitoring the whole range of nonionic levels found in environmental samples. Additional useful information is obtainable from the Patterson TLC method on the nature of the nonionic surfactant, for example, an indication of the length of the ethoxylate chain and type of nonionic surfactant can be given.

Although the Wickbold and CT methods can be applied to environmental samples (to determine BiAS and CTAS levels respectively) they are not particularly suited (ie detection limit of 0.05–0.1 mg/l) for the estimation of the low levels of nonionic found in most UK sewage effluents and surface waters (<0.5 mg/l).

A summary of the main features of the four nonionic surfactant methods considered in this review is given in Table 1.

Table 1 Main Features of the Four Nonionic Methods

Analytical Method	Concentration and Separation steps (max sample size (litre))	Analytical Estimation Step	Recommended Sample Requirements (μg)	Detection Limit (mg/l Synperonic NP8	Ease of Operation	Application	Additional Comments
Wickbold titrimetric method (and modifications)	(i) Solvent sublation (1 litre) (ii) Cation-exchange of extract	Complexometric titration. Other modifications use atomic absorption and UV colorimetric estimations	250–800	0.05	Relatively simple and quick to operate on a routine basis (particularly the modifications).	Highly applicable to biodegradation test liquor samples. Less suitable for environmental monitoring (particularly at low levels).	Response of nonionic dependent upon its M.Wt. and polyoxyalkylene chain length. Estimations for mixed nonionics must be expressed arbitrarily in terms of a suitable reference std. Nonionics containing 4 or less (EO) units are not readily detected.
SDA cobaltothiocyanate method	(i) Modified Wickbold solvent sublation (0.5 litre) (ii) Cation- and anion exchange of extract	Colorimetric estimation	200–2000	0.1	Relatively simple and quick to operate on a routine basis.	Highly applicable to biodegradation test liquor samples. Less suitable for environmental monitoring (particularly at low levels).	
Patterson TLC method	(i) Liquid/liquid solvent extraction (0.5 litre) (ii) Cation exchange of extract	Comparative TLC estimation	10–750	0.01	Generally less convenient than Wickbold and CT methods.	More suited to environmental monitoring but can be used for a range of samples.	
Chemical fission—GLC method (tentative)	(i) Wickbold solvent sublation (1 litre) (ii) Cation- and anion exchange of extract	GLC estimation of the dibromoalkanes derived from the chemical fission of nonionic	50–500	0.005	The most complex and time consuming of the methods—requires some relatively sophisticated equipment.	More suited to environmental monitoring but can be used for a range of samples.	Response dependent only on polyoxyalkylene content—applicable to all solvent sublatable nonionics.

B2 Determination of Ethoxylated Nonionic Surfactants by the Wickbold Method (Tentative Method)

B2.1 Performance Characteristics of the Method (39)

B2.1.1	Substance determined	Ethoxylated nonionic surfactants with 6–30 ethylene oxide groups. Propoxylated surfactants also react.
B2.1.2	Type of sample	Settled sewage (a), sewage works effluents, waters and certain trade effluents.
B2.1.3	Basis of method	Concentration of the nonionic surfactant by sublation followed by precipitation of the concentrated extract with barium tetraiodobismuthate (modified Dragendorff reagent). The precipitate is dissolved and the liberated bismuth is determined by titration (16). Alternatively atomic absorption or ultra violet spectrophotometry may be used to determine the liberated bismuth in the sample aliquot.
B2.1.4	Range of application (optimum)	250–800 µg. Corresponding to 0.25–0.8 mg/l.
B2.1.5	Calibration curve	Linear.
B2.1.6	Standard deviation (b)	A. Standard solutions of Marlophen 810. 1 litre samples:

Concentration mg/l		Standard Deviation	Degrees of
Nominal	Found	mg/l	Freedom
0.2	0.194	0.031	4
0.25	0.258	0.034	4
0.3	0.324	0.027	4
0.4	0.373	0.028	4
0.5	0.504	0.011	4
0.6	0.557	0.054	4
0.7	0.699	0.030	4
0.8	0.858	0.030	4
0.9	1.005	0.070	4

B. Crude Sewage 50 ml samples:

Found mg/l	Standard Deviation mg/l	Degrees of Freedom
5.97	0.39	4

C. Sewage Effluent 1 litre samples:

0.138	0.020	4

B2.1.7	Limit of detection (c)	(1) Five determinations simultaneously: $2 \times 2.t \times SD = 0.039$ mg/l (2) Seventeen single determinations: $2 \times 2.t \times SD = 0.086$ mg/l t = tabulated value of Student's–t (single-sided) SD = within batch standard deviation.

B2.1.8	Sensitivity	(1) Titration method: 1 ml titrant = 0.050 mg/l. (2) Atomic Absorption Spectrophotometry Depends on the equipment characteristics. (3) Ultra Violet Spectrophotometry (2 cm cell) 0.685 Absorbance at 263.5 nm per mg as Marlophen 810.
B2.1.9	Bias	Not known.
B2.1.10	Interferences	The known interferences are anionic surfactants in concentrations greater than 15 times the nonionic; cationic surfactants and heavy metals except when bismuth is determined by Atomic Absorption Spectrophotometry.
B2.1.11	Time required for analysis	10 samples: total analytical times: approximately 8 hours, total operator times: approximately 8 hours.

(a) The recovery of nonionic surfactant from settled sewage may not be quantitative.
(b) Results obtained by Water Research Centre (Stevenage Laboratory).
(c) Limit of detection for titrimetric method only.

B2.2 Principle

B2.2.1 The method described is based on experimental evaluatory work carried out in the United Kingdom, on the Wickbold recommended method for the determination of nonionic surfactants (16, 17).

B2.2.2 Air or nitrogen is bubbled through the sample and the nonionic surfactants are extracted from the aqueous phase into a less dense organic phase by physical sorption at the liquid/bubble interface. The surfactants are carried upwards by the continuous flow of gas bubbles and dissolve in the organic solvent layer above. This process is termed sublation.

The organic layer is separated from the aqueous phase and is concentrated by evaporation. The residue is reacted with barium tetraiodobismuthate (modified Dragendorff reagent) to form an orange precipitate. This precipitate is filtered off, dissolved in ammonium tartrate solution and the bismuth determined by titration. Alternatively atomic absorption or ultra violet spectrophotometry methods may be used (see Sections B2.9.3 and B2.9.4 respectively).

B2.3 Interferences

There is no detailed information concerning interfering substances and their effect on this method. The extraction stage excludes polyethylene glycols and the majority of non-surface active substances which might otherwise interfere in the complex formation stage of the analysis. Interferences due to other materials have not been evaluated. Cationic surfactants interfere by reacting with the modified Dragendorff reagent and must be removed if necessary by a cation exchange procedure (see Section B2.10).

Anionic surfactants do not interfere provided their concentration is less than 15 times that of the nonionic surfactants.

Heavy metals will interfere in the titrimetric and ultra violet methods for the determination of bismuth.

B2.4 Hazards

The following potentially hazardous reagents are used in this method. Glacial acetic acid, sodium hydroxide, hydrogen peroxide, concentrated ammonia, nitric acid, barium chloride.

B2.5 Reagents

All reagents are stored in glass bottles. 'Analytical Reagent' grade chemicals are suitable except where otherwise indicated.

B2.5.1 Deionized water or water distilled from an all glass apparatus is suitable.

B2.5.2 Ethyl Acetate

Freshly re-distilled taking the fraction boiling between 76–78°C.

B2.5.3 5% w/v Sodium Bicarbonate

Dissolve 50±1 g sodium bicarbonate in 1 litre of distilled water. Stir whilst adding the solid.

B2.5.4 Sodium Chloride

B2.5.5 Glacial Acetic Acid

Acid of purity 99–100% only should be used.

B2.5.6 Methanol

B2.5.7 0.1% w/v Bromocresol Purple

Dissolve 0.1±0.005 g bromocresol purple in 100 ml methanol.

B2.5.8 Precipitating—Complexing Agent

This is the modified Dragendorff reagent and is a mixture of 2 volumes of solution A and 1 volume of solution B described in Sections 5.8.1 and 5.8.2. The mixture is prepared freshly each day and stored in a brown glass stoppered bottle.

B2.5.8.1 Partial Solution A

Dissolve 1.7±0.01 g basic bismuth (III) nitrate ($BiONO_3.3H_2O$) in 20 ml (±1 ml) glacial acetic acid and make up to 100±5 ml with water. Dissolve 65.0±0.5 g potassium iodide in 200±5 ml water.

Mix these two solutions in a 1 litre volumetric flask, add 200±5 ml glacial acetic acid and make up to 1 litre with water. After ensuring complete mixing, store the solution in a brown glass stoppered bottle. The solution is stable for at least 10 days.

B2.5.8.2 Partial Solution B

Dissolve 290±0.5 g barium chloride ($BaCl_2 2H_2O$) in 1 litre of water in a volumetric flask.

B2.5.9 Ammonium Tartrate Solution

Dissolve 15.20±0.01 g ammonium tartrate and make up to 1 litre water in a volumetric flask.

Alternatively 12.40±0.01 g tartaric acid and 12.40±0.01 ml of ammonia solution (d_{20} 0.910) are mixed in a 1 litre volumetric flask and made up to 1 litre with water.

Store the solution of ammonium tartrate in a refrigerator.

B2.5.10 Dilute Ammonia Solution

20±1 ml of ammonia (d_{20} 0.910) are added carefully to 80±5 ml water in a conical flask.

B2.5.11 Acetate Buffer Solution

Dissolve 40±0.5 g solid sodium hydroxide in 500±5 ml water in a beaker and allow to cool. Add 120±5 ml glacial acetic acid and allow to cool. After thorough mixing and cooling, make up to 1 litre with water in a volumetric flask.

B2.5.12 0.0005M Sodium Pyrrolidinedithiocarbamate ('Carbate' solution)

Dissolve 0.103±0.005 g sodium pyrrolidinedithiocarbamate ($C_4H_8N.CS_2Na.2H_2O$) in approximately 500 ml water in a 1 litre volumetric flask. Add 10±0.5 ml n-amyl alcohol (pentan-1-ol) and 0.5±0.0005 g sodium bicarbonate. Make up to 1 litre with water in a volumetric flask. Store both the solution and the solid chemical in a refrigerator. The ammonium salt may be used as an alternative.

B2.5.13 Copper Sulphate Solution

This is used to standardize the 'carbate' solution.

B2.5.13.1 Copper Sulphate Stock Solution (approx 0.005M)

Accurately weigh approximately 1.249 g copper sulphate ($CuSO_45H_2O$) add 50 ml of 0.5M sulphuric acid and make up to 1 litre with water in a volumetric flask. Broken and soft crystals of copper sulphate should be rejected when making up this solution. The actual molarity of this solution is calculated from the weight of copper sulphate actually taken.

B2.5.13.2 Copper Sulphate Standard Solution (approx 0.00025M)

Pipette 50 ml of the stock solution and 10 ml of 0.5M sulphuric acid into a 1 litre volumetric flask and make up to 1 litre with water.

B2.5.14 Potassium Permanganate Solution (approx 1% w/v)

Dissolve 10±1 g potassium permanganate in 1 litre of water with a few drops of concentrated sulphuric acid (d_{20} 1.840) (see Section 8).

B2.5.15 Hydrogen Peroxide (10 Volume)

Acidify with a few drops of sulphuric acid (d_{20} 1.840) before use. Extreme caution must be observed when adding the acid.

B2.5.16 Additional Reagents for the determination of bismuth by Atomic Absorption Spectrophotometry

B2.5.16.1 Nitric acid (Aristar Grade is recommended) d_{20} 1.420

B2.5.16.2 Bismuth, pure powder

B2.5.17 Additional Reagents for the Determination of Bismuth by Ultra Violet Spectrophotometry

B2.5.17.1 Disodium Ethylenediaminetetracetic Acid EDTA (0.02M)

Dissolve 7.5±0.5 g. EDTA in water and make up to 1 litre in a volumetric flask.

B2.6 Apparatus

B2.6.1 Extraction Apparatus and Sublator

The general layout of the gas stripping and sublation apparatus is shown in Figure 3.

B2.6.2 Apparatus for Precipitation, Solution and Standardization

B2.6.2.1 1 vacuum pump

B2.6.2.2 4 Buchner filtration flasks. (250 ml and 1000 ml), fitted with suitable adaptors

B2.6.2.3 Sintered glass crucibles (porosity 4 and capacity 40 ml)

Alternatively Gooch crucibles (diameter 21 mm) and glass fibre filter papers (Whatman GF/A or GF/C or equivalent) may be used. The use of glass fibre filter papers with the sintered glass crucibles prolongs the useful life of the crucible.

B2.6.2.4 Magnetic stirrers

B2.6.2.5 Electric hot plate

B2.6.2.6 Hot water bath

B2.6.2.7 10 ml pipette of Grade A standard and other normal laboratory glassware.

B2.6.3 Automatic Titration Apparatus

An automatic titrator is the least time-consuming method for measuring the bismuth in the final solution. A recording potentiometer using a bright platinum electrode and a calomel (or a silver/silver chloride) reference electrode with a 250 mV range in conjunction with an automatic burette, 20–25 ml capacity, are required. The electrodes must be checked regularly. The platinum electrode may be cleaned with abrasive paper when it loses its brightness and the saturated potassium chloride in the calomel electrode should be replaced at approximately fortnightly intervals, depending on the number of samples titrated.

B2.7 Sample Collection and Preservation

Because of their nature, surfactants will be adsorbed on to any suspended solids as well as on the walls of the containing vessel. It must therefore be clearly stated to the analyst as to what is required, ie the total surfactant or only that in solution. If the former is required it must be first ascertained whether the level of solids in the sample will permit a satisfactory estimation of the total surfactant. When the latter is needed centrifugation rather than filtration of the sample should be employed to remove the suspended solids.

Sampling bottles should be completely filled with sample and if they are not to be analysed within a few hours of collection the samples should be stored in a refrigerator and sterilized for example with formaldehyde solution, a 1% v/v addition of a 40% formaldehyde solution should be adequate. Before taking aliquots from the sample for analysis, the whole should be stirred with a magnetic stirrer. Do not withdraw an aliquot while the bulk sample is foaming.

B2.8 Cleaning of Apparatus

Detergents containing nonionic and/or cationic surfactants must not be used. All glassware must be cleaned by using 10% v/v HCl, water and distilled water except for the filter crucibles.

The passage of water through the sinter of the crucibles should be reasonably fast (30 secs for a sample), otherwise high blanks will be recorded as the crucibles become blocked. They should be cleaned by storing them in acidified 1% w/v potassium permanganate and then rinsing in acidified hydrogen peroxide (10 vols) followed by a thorough rinse with distilled water. Backwashing is also recommended.

Do not wash any glassware in chromic acid as this interferes with the titration stage of the procedure.

B2.9 Analytical Procedure

Step	Procedure	Notes
B2.9.1	Sublation	
B2.9.1.1	Add a known volume of sample to the Wickbold tube such that it contains between 250 and 800 μg nonionic surfactant.	
B2.9.1.2	Add 100±1 ml sodium bicarbonate solution and 100±0.5 g sodium chloride. If necessary add water up to the level of the stopcock. Dissolve the sodium chloride by inverting the Wickbold tube several times or by bubbling air or nitrogen through the liquid.	
B2.9.1.3	Add 100±1 ml ethyl acetate carefully on top of the aqueous phase.	
B2.9.1.4	Fill the Dreschel bottle in the gasline two-thirds full with ethyl acetate.	

Step	Procedure	Notes
B2.9.1.5	Pass a stream of air or nitrogen at the rate of 0.6 l/min through the apparatus for 5 min (note a). Discharge the organic phase into a 500 ml separating funnel and return any water (from the aqueous phase) to the Wickbold tube. Run the organic phase from the separating funnel into a 250 ml beaker.	(a) There should be no violent turbulence between the 2 phases, otherwise the ethyl acetate will dissolve in the aqueous layer and negate the sublation. If as a result, there is a reduction of more than 20% of the volume of the organic phase the sublation must be discarded and a fresh sample aliquot taken for a new test.
B2.9.1.6	Repeat steps 3 and 5, combining the 100 ml aliquots in the beaker. Rinse the separating funnel with a few mls of ethyl acetate and add the washings to the beaker.	
B2.9.1.7	In a fume cupboard, evaporate the ethyl acetate extract to dryness on a steam bath with a gentle stream of air directed onto the surface of the solution to accelerate the evaporation process (note b).	(b) The batch of samples may all be processed to this step. Any cationic surfactants present must be removed before proceeding to step B2.9.1.8 (see Section B2.10).
	Precipitation and Filtration	
B2.9.1.8	Dissolve the residue from step B2.9.1.7 in 5 ml$\pm$0.1 ml methanol and add 40$\pm$1 ml water.	
B2.9.1.9	Using a magnetic stirrer stir the solution and add 3–5 drops of bromocresol purple. If necessary add glacial acetic acid dropwise until the solution turns yellow.	
B2.9.1.10	Add 30$\pm$1 ml of precipitating agent and stir for 10 mins. Leave to stand for at least 5 mins.	
B2.9.1.11	Place a sintered glass crucible (see Section B2.6.2.3) into a suitable adaptor attached to a 500 ml filter flask (notes c and d). Apply suction to the flask using a vacuum pump, filter 2 $\times$ 40 ml aliquots of distilled water through the crucible.	(c) If used, the rubber collars must not come into contact with any of the reagents. (d) The life of glass sintered crucibles may be prolonged by inserting a glass fibre filter paper (GF/A or C is suitable).
B2.9.1.12	Filter the precipitate through the crucible, carefully transferring the plastic covered magnet of the stirrer from the beaker to the crucible (note e).	(e) At this stage the transfer of the precipitate into the crucible need not be quantitative (see step B2.9.2.3).
B2.9.1.13	Take 100$\pm$1 ml of glacial acetic acid and pour about 30–40 ml into the beaker, swirling it around so as to wash any precipitate left. Transfer the acid from the beaker to the crucible and filter through the precipitate (note f).	(f) Fill the crucible to the top each time to wash any precipitate on the sides of the crucible.
B2.9.1.14	Repeat step B2.9.1.13 with further aliquots of the acid until all the 100 ml have been used to wash the precipitate.	
B2.9.2	Determination of Bismuth by Titration Solution of Precipitate	
B2.9.2.1	Heat to about 80°C, 50$\pm$1 ml ammonium tartrate in a 150 ml conical flask on a hot plate.	
B2.9.2.2	Transfer the crucible to a 250 ml filter flask set up as in step B2.9.1.11.	

Step	Procedure	Notes
B2.9.2.3	Add the hot ammonium tartrate to the crucible until it is totally full. Add the excess to the original 250 ml beaker and swirl around to dissolve any remaining precipitate.	
B2.9.2.4	Apply suction to the crucible when the precipitate has dissolved and add the ammonium tartrate from the beaker as the crucible empties.	
B2.9.2.5	Wash the crucible with 2 × 40 ml aliquots of water.	
B2.9.2.6	Remove the suction and wash the base of the crucible, and adaptor with a jet of water adding the washings to the filter flask. Transfer the stirrer magnet and the flask contents back into the 250 ml beaker and rinse the flask twice with water adding the washings to the beaker.	
B2.9.2.7	Stir the solution with the magnetic stirrer, add a few drops of bromocresol purple and then add ammonia solution dropwise until the colour turns violet. Add 10±0.5 ml of acetate buffer (note a).	(a) See Section B2.9.3 and B2.9.4 for alternative methods for the determination of bismuth.
B2.9.2.8	Pre-set the automatic titrator according to the manufacturers instructions ready to carry out the titration.	
B2.9.2.9	Immerse the electrodes and burette tip in the sample solution and titrate potentiometrically with 0.0005M 'carbate' solution until the jump in potential. The end point is the intersection of the potential curve. Let the volume at the end point be y ml.	
	Standardization of 'carbate' solution	
B2.9.2.10	Add 100 ml±1 ml of water and 10 ml±0.5 ml of acetate buffer to 10 ml (measured with a Grade 'A' pipette) of copper sulphate standard solution in a 250 ml beaker (note b). Titrate as in step B2.8.2.3 and let the average volume at the end point be x ml. Then f = 10/x (note c).	(b) Standardization is carried out in duplicate. (c) f is called the carbate factor (see step B2.9.2.12).
	Blank Determinations	
B2.9.2.11	Two blanks must be run through the whole procedure with each batch of samples. Add 5±0.5 ml methanol and 40±1 ml water to a 250 ml beaker and treat as a sample from step B2.9.2.9. Let the average volume at the end point of their titration be z ml.	
	Calculation of Results	
B2.9.2.12	The amount of nonionic surfactant in the sample = 54(y − z).f. μg = 0.054(y − z).f. mg	
	Expression of Results	
B2.9.2.13	Results are usually expressed in mg/l in the following manner: Less than 1 mg/l to 2 decimal places more than 1 mg/l to 1 decimal place	

Step	Procedure	Notes
	Control of the factor of the 'carbate' solution	
B2.9.2.14	This should be determined before a series of determinations (or when a new 'carbate' solution is made) as described in step B2.9.2.10. If the volume at the end point is x ml, the factor, f, is calculated: $f = 10/x$	
	Conversion Factor for each Nonionic Surfactant	
B2.9.2.15	Every nonionic surfactant has a conversion factor, depending on the length of the ethylene oxide chain. As the type of nonionic surfactant is usually unknown when analysing sewages and effluents, calculations are related to a standard substance. A nonyl phenol ethoxylate with an average of 10 ethylene oxide units has been chosen as the standard substance and has a conversion factor of 54. From it, the amount of detergent present in the quantity of sample taken is found and expressed as of the nonyl phenol ethoxylate. (A suitable standard is Marlophen 810 or Synperonic NP8.)	
B2.9.3	Bismuth Determination by Atomic Absorption Spectrophotometry	
	Solution of Precipitate	
B2.9.3.1	The precipitate is prepared as shown in Section B2.9.1 steps 8 to 14.	
B2.9.3.2	Add 2 ml concentrated nitric acid (d_{20} 1.420) to the washed precipitate in the crucible. Impart a swirling movement to the crucible but do not apply vacuum. Add 2 to 3 ml water with swirling and apply vacuum. Repeat the process twice (note a) until the precipitate is dissolved and removed. Rinse the crucible and adaptor with water to ensure complete transfer into the Buchner flask. An alternative method of dissolution is indicated in note b.	(a) Three 2 ml portions of nitric acid are usually sufficient to dissolve the precipitate. If more is required the corresponding extra quantity must be added to the standard bismuth solutions. (b) Alternatively the precipitate may be dissolved using an appropriate volume of ammonium tartrate solution (see Section B2.9.2). Standard bismuth solutions should contain the same concentration of ammonium tartrate solution.
B2.9.3.3	Transfer the solution in the Buchner flask to a 100 ml volumetric flask and make up to the 100 ml mark with water.	
B2.9.3.4	Set up the absorption spectrophotometer and operate it in accordance with the manufacturers' instructions, measuring absorbances of sample and standard (B2.9.3.5) solutions. Hence calculate the concentrating of bismuth in the sample solutions and thus the corresponding concentrations of nonionic surfactant in the original samples.	

Step	Procedure	Notes
	Preparation of Standard Bismuth Solution	
B2.9.3.5	Accurately weigh out 0.500 ± 0.005 g of pure bismuth powder and dissolve in a few drops of concentrated nitric acid and then dilute with water to 500 ml in a volumetric flask. (1 ml of this solution contains approximately 1 mg of bismuth.) Prepare standard solutions from this solution which will contain 1, 2, 3, 4 and 5 mg/l of bismuth. Ensure that each standard solution contains approximately the same concentration of nitric acid as the sample (ie 6 mls/100 ml solution) (note a).	
B2.9.4	Bismuth Determination by Ultra-Violet Spectrophotometry(2)	
B2.9.4.1	Isolate the nonionic surfactant as described in Section B2.9.1 steps 1 to 14.	
B2.9.4.2	Heat to about 80°C on a hot plate, 50 ± 1 ml of ammonium tartrate contained in a 150 ml conical flask.	
B2.9.4.3	Transfer the crucible to a suitably sized filter flask fitted with a suitable adaptor.	
B2.9.4.4	Dissolve the precipitated complex in 15 to 20 ml of the hot ammonium tartrate solution.	
B2.9.4.5	Use a further 15 ml of the tartrate solution to rinse the sides of the beaker (see step B2.9.2.9) to dissolve any remaining precipitate.	
B2.9.4.6	Filter the contents of the beaker through the sintered glass crucible (see step B2.9.1.11).	
B2.9.4.7	Wash the crucible with the remainder of the tartrate solution followed by 10 ml of water.	
B2.9.4.8	Transfer the contents of the filter flask to the beaker.	
B2.9.4.9	Wash out the filter flask with two portions of water (10 and 5 ml) and add the washings to the beaker (note a).	(a) The total volume of solution should be approximately 75 ml.
B2.9.4.10	Add 4 ml of 0.02M EDTA solution to the bismuth solution obtained in step B2.9.4.9 mix the solution and transfer it to a 100 ml volumetric flask.	
B2.9.4.11	Wash the beaker with water and use the washings to make up the solution in the flask to 100 ml (note b).	(b) The final pH values of the samples are generally in the range 4–5. Since the adsorptivity of the bismuth—EDTA complex at 263.5 nm remains constant over the pH range 2–9, no pH control in the colorimetric estimation is required.
B2.9.4.12	Set up the UV spectrophotometer in accordance with the manufacturers instructions.	
B2.9.4.13	Using a 2 cm cell read the absorbance of the solution obtained in step B2.9.4.11 against a water reference at a wavelength of 263.5 nm.	

Step	Procedure	Notes
	Preparation of Calibration Curve	
B2.9.4.14	Prepare solutions containing 0, 200, 400, 600 and 1000 µg of the standard nonionic surfactant (note c) by dissolving it in 5 ml of methanol and 40 ml of water. Prepare the complex compound as described in section B2.9.1 and dissolve the precipitate as described in steps 2 to 11 above.	(c) Suitable reference nonionic surfactants are Marlophen 810 or Synperonic NP8.
B2.9.4.15	Read the absorbance of these standard solutions with the cells used in step B2.9.4.13. Plot a calibration curve correlating absorbance at a wavelength of 263.5 nm against the concentration of nonionic surfactant (note d).	(d) Both the tartrate ion and EDTA absorb slightly at 263.5 nm and contribute to the absorbance reading for the reagent blank (typical reagent blanks are 0.03–0.04 absorbance).
B2.9.4.16	Calculate the weight of nonionic surfactant in the sample by reference to the standard calibration curve.	

B2.10 Removal of Interfering Cationic Surfactants

Cationic surfactants also react with tetraiodobismuthate to form an insoluble orange precipitate and therefore must be removed if their presence is suspected.

The residue from step B2.9.1.7 should be dissolved in 20 ± 1 ml methanol. Run this solution through an ion-exchange column, filled with 10 ml macroporous cation-exchange resin (Dowex 50WX2-H form is suitable). Adjust the rate of flow to a series of fast drops. Wash the column with 50–60 ml methanol and add the washings to the first 20 ml methanol. If the nonionic surfactant is known to contain 25 or more ethylene oxide groups the methanol should be replaced by a mixture containing 80% methanol and 20% methylene dichloride.

Evaporate the methanol solution on a water bath and treat the residue as in step B2.9.1.8 and onwards.

Regenerate the cation-exchange resin with 5% v/v hydrochloric acid in methanol before use. Then wash with methanol until the effluent is no longer acid to methyl red. Keep the resin under methanol.

B3 Determination of Alkoxylated Nonionic Surfactants by Thin Layer Chromatography

B3.1 Performance Characteristics of the Method (39)

B3.1.1	Substance determined	Alkoxylate nonionic surfactants (see Section B3.2.3).
B3.1.2	Type of sample	Settled sewage, sewage works effluents, waters and certain trade effluents.
B3.1.3	Basis of method	Extraction of surfactant into chloroform with subsequent clean-up, evaporation to small bulk and final quantification by thin layer chromatography.
B3.1.4	Range of application	0.05–2.5 mg/l for 250 ml sample.
B3.1.5	Calibration curve	Not applicable. Quantification by visual comparison with 1–5 μg quantities of standard material.
B3.1.6	Standard deviation	

Concentration (mg/l)	Total Standard Deviation (mg/l)	Degrees of Freedom
0.4(a)	0.06	7
0.8(b)	0.14	6
1.2(b)	0.21	11

B3.1.7	Limit of detection	0.5 μg on plate spot corresponding to 0.025 mg/l for a 500 ml sample.
B3.1.8	Sensitivity	The spot on the TLC plate can be determined to ± 0.5 μg.
B3.1.9	Bias	Not known.
B3.1.10	Interferences	Anionic surfactants are partially coextracted and can affect the accuracy of the determination by changing the shape of the nonionic surfactant sample spot on the thin layer plate. Cationic surfactants can similarly interfere and may also affect the apparent nonionic surfactant concentration since they are also often Dragendorff reactive.
B3.1.11	Time required for analysis	For six samples, total analysis and operator times are approximately 8 hours including preparation of materials and calculation of results.

(a) Results obtained on 250 ml samples in 8 laboratories on a detergent-free effluent with added Lissapol NX.
(b) Results obtained on 250 ml samples in 7 laboratories on a detergent-free effluent with added Lissapol NX.

B3.2 Principle

B3.2.1 The method described is based on the thin-layer chromatographic method for the determination of nonionic surfactants developed in the Laboratory of the Government Chemist by S J Patterson et al (24) and evaluated in various OECD and other inter-laboratory studies.

B3.2.2 Nonionic surfactant present in a strong magnesium sulphate solution of the water sample is extracted into chloroform. Co-extracted impurities in the chloroform are removed by saline acid and alkaline wash solutions. The cleaned-up chloroform extract is evaporated to dryness and made up to small volume in chloroform. It is then spotted alongside standards on TLC plates coated with silica gel and run in suitable solvents. Nonionic surfactant is finally characterised and visually quantified on the plates by formation of a coloured derivative with a modified Dragendorff spray reagent.

B3.2.3 It is applicable to the commercial nonionic surfactants of the alkyl phenol ethoxylate and alcohol ethoxylate types with chain lengths of at least 4 ethylene oxide groups. Alkyl phenol ethoxylates with greater than about 20 ethylene oxide groups in their chain lengths form a streak rather than a spot on the TLC plates. In some cases they can be conveniently estimated against standards of a suitable molecular weight polyethylene glycol. Compounds containing polypropoxylene units will react similarly.

B3.3 Interferences

Solvent extraction and clean-up removes many potentially interfering substances. Others surviving in the chloroform solution are not generally Dragendorff active substances with similar Rf characteristics to nonionic surfactants.

Anionic and cationic surfactants are partially co-extracted and if present in high concentrations in the original water, may affect the accuracy of the estimation by changing the shape and colour of the sample spot on the TLC single spot plate. If the ratio of anionic to nonionic surfactant in the sample is less than 10:1 the accuracy of the result will not be significantly affected. In the rare cases where this interference is sufficient to affect the nonionic surfactant determination consideration may have to be given to removal of ionic surfactant species by passing the extract in a suitable solvent through ion exchange resins (Ref 18, Method B2).

Fats and greases may interfere with the thin layer chromatography. They can be separated in a preliminary clean-up on the TLC plate as described in B3.8.2.4.

Rarely an organic dyestuff may be present which interferes with the estimation. A preliminary clean-up on the TLC plate which is helpful in some cases is described in B3.8.2.5.

B3.4 Hazards

Potentially harmful reagents used in this method include chloroform, diethyl ether, sodium hydroxide, hydrochloric acid and barium chloride.

B3.5 Reagents

Chemicals should be AR grade unless otherwise specified.

B3.5.1 Distilled or deionized water must be used to prepare reagent solutions.

B3.5.2 Extraction Reagents

B3.5.2.1 Chloroform

B3.5.2.2 Magnesium sulphate ($MgSO_4.7H_2O$)

B3.5.2.3 Acid wash solution

Dissolve 1000 ± 10 g of sodium chloride in 3650 ± 20 ml of water in a 5 litre beaker. Heating may be used to increase the rate of solution. Allow to cool, then slowly add 330 ± 10 ml of hydrochloric acid (d_{20} 1.18) stirring well. Transfer from the 5 litre beaker to a suitable polythene bottle.

B3.5.2.4 Alkaline wash solution

Dissolve 1000±10 g of sodium chloride in 4000±20 ml of water in a 5 litre beaker. Heating may be used to increase the rate of solution. Allow to cool then add 320 g of sodium hydroxide pellets (care, heat evolved). Stir well until the sodium hydroxide dissolves. After cooling transfer from the 5 litre beaker to a suitable polythene bottle.

B3.5.3 Thin Layer Chromatography Reagents

B3.5.3.1 Powdered silica gel with gypsum binder of a suitable type.
(Kieselgel G nach Stahl Type 60 manufactured by Merck is suitable).

B3.5.3.2 Nonionic surfactant standard solution (1 mg/ml)

Accurately weigh out 0.100±0.001 g of an alkyl phenol nonionic surfactant condensed with an average of 8 ethylene oxide units (Synperonic NP8 manufactured by ICI Ltd is a suitable material). Make up to 100 ml as a chloroform solution in a volumetric flask.

B3.5.3.3 Diethyl ether

B3.5.3.4 Acetone

B3.5.3.5 Modified Dragendorff reagent

Suspend 1.7 g bismuth oxynitrate GPR in 220 ml glacial acetic acid. Add 40 g potassium iodide dissolved in 100 ml of water. Mix well. (A clear solution is obtained without heating.) Dilute the solution to 1 litre with water. Transfer to a brown glass bottle and store in a dark place.

B3.5.3.6 Spray reagent

Mix in the order given 10 ml modified Dragendorff reagent (B3.5.3.5), 1 ml orthophosphoric acid, 10 ml ethanol and 5 ml of a 20% w/w aqueous solution of barium chloride ($BaCl_2.2H_2O$). The spray reagent is usually satisfactory for 2 to 3 days.

B3.5.3.7 Solvent System A

Mix 40 ml of ethyl acetate, 30 ml of water and 30 ml of glacial acetic acid and use the same day.

B3.5.3.8 Solvent System B

Mix 70 ml of ethyl acetate, 15 ml of water and 16 ml of glacial acetic acid and use the same day.

B3.6 Apparatus

B3.6.1 Normal laboratory apparatus for the extraction and concentration stage, including separating funnels of 2 litre and 500 ml capacities.

B3.6.2 Normal thin layer chromatography equipment including:

Thin layer glass plates 20 cm × 20 cm
Chromatographic tanks approximately 10 cm wide, 30 cm long and 20 cm high.

B3.6.3 Dessicator or air drying cabinet containing silica gel (for the storage of activated plates).

B3.6.4 Glassware should not be washed in detergents BUT SHOULD BE CLEANED USING 10% V/V HYDROCHLORIC ACID, WATER AND DISTILLED WATER.

B3.7 Sample Collection and Preservation

Because of their nature surfactants will be adsorbed on to any suspended solids as well as on the walls of the containing vessel. It must therefore be clearly stated to the analyst as to what is required, ie the total surfactant or only that in solution. If the former is required it must be first ascertained whether the level of solids in the sample will permit a satisfactory estimation of the total surfactant. When the latter is needed, centrifugation rather than filtration of the sample should be employed to remove the suspended solids.

Sampling bottles should be completely filled with sample and if they are not to be analysed within a few hours of collection the samples should be stored in a refrigerator and sterilized, for example, by a 1% v/v addition of a 40% formaldehyde solution. To obtain a representative sample either stir the whole sample with a magnetic stirrer or slowly invert the sample bottle twice before taking aliquots. As far as practical avoid the formation of foam during the stirring or inversion but in any case do not withdraw an aliquot while the bulk sample is foaming.

B3.8 Analytical Procedure

Read Section B3.4 on Hazards before starting this Procedure.

Step	Procedure	Notes
B3.8.1	Extraction	
B3.8.1.1	Add 150±2 g magnesium sulphate to a 2 litre separating funnel fitted with a PTFE tap. Add 250±5 ml of deionised or distilled water, shake until solution is complete (note a).	(a) For a sample of river water, use 300±3 g magnesium sulphate and 500±5 ml demineralised or distilled water.
B3.8.1.2	Measure 250±5 ml of the acid wash solution into a second separating funnel of 500 ml capacity, fitted with a PTFE tap.	
B3.8.1.3	Measure 250±5 ml of the alkaline wash solution into a third separating funnel of 500 ml capacity, fitted with a PTFE tap.	
B3.8.1.4	Add 50±1 ml chloroform to all three funnels, shake each for 2 minutes and then allow to settle. Reject the chloroform washings (note b).	(b) For a sample of river water, use 100±1 ml chloroform in the 2 litre separating funnel.
B3.8.1.5	Add 250±2 ml (note c) unfiltered sample to the washed magnesium sulphate solution in the first funnel (note d). Add 50±1 ml chloroform (note e), shake gently (note f) for 2 minutes and allow to stand until the emulsion formed shows no further sign of settling. Rotate the funnel back and forth to aid demulsification (note g).	(c) If it is suspected that the sample will contain more than 2.5 mg/l of nonionic surfactant a smaller volume may be taken and the balance made up to 250 ml with deionized or distilled water. (d) For a river water take a sample of 500±5 ml. (e) Use 4 × 100±1 ml portions of chloroform to extract a sample of river water. (f) Vigorous shaking of the first extract may lead to the formation of an indefinitely stable emulsion. (g) If there is very little separation of a lower emulsified chloroform layer, reduce 250±2 ml of effluent (diluted or otherwise) to about 100 ml by boiling it in a beaker. Cool and transfer to a 250 ml graduated cylinder. Wash the walls of the beaker with 25 ml 1M hydrochloric acid and transfer the washings to the graduated cylinder. Dilute the solution to 250 ml and then treat as a normal sample.

Step	Procedure	Notes
B3.8.1.6	Transfer the chloroform layer directly to the funnel containing the acid wash if the emulsion formed in step B3.8.1.5 is negligible. If the emulsion layer has persisted, run it through a conical pad made from approximately 0.8 g of chloroform-washed white cotton wool placed in a filter funnel.	
B3.8.1.7	Extract the effluent for a further 3 times with 50 ± 1 ml portions of chloroform, shaking vigorously (note e). Run each extract through the cotton wool pad, if used, into the acid wash funnel (note h). Wash the pad with about 10 ml chloroform.	(h) When a river water is analysed, the first two combined extracts are put through the acid and alkaline wash stage and then transferred to a 500 ml round bottom flask. The last two extracts are then put through the acid and alkaline wash stage. It is not necessary to rinse the funnels with chloroform for the first two extracts.
B3.8.1.8	Shake the acid wash solution and combined chloroform extracts for 2 minutes. Allow to stand and allow the layers to separate; emulsification should not give rise to any difficulties at this stage. Transfer the chloroform layer to the alkaline wash funnel. Wash the second funnel by shaking briefly with 10 ml chloroform; transfer the washings to the alkaline wash.	
B3.8.1.9	Shake the alkaline wash solution and chloroform extract for 2 minutes; allow to stand until the layers have separated. Run off the washed chloroform extract through a dry filter paper (previously washed with chloroform; an 11 cm diameter Whatman No 1 paper is suitable) into a 250 ml round bottom flask (note h). Rinse the aqueous solution by shaking briefly with 10 ml chloroform and use this to wash the filter paper.	
B3.8.1.10	Distil off the chloroform in a fume cupboard until about 2 ml remains. Transfer this residue to a test tube of the type which takes a ground glass stopper.	
B3.8.1.11	Wash out the flask with 1–2 ml of chloroform adding the washings to the contents of the test tube. Evaporate the chloroform carefully on a water bath.	
B3.8.1.12	Repeat the washing and evaporation step B3.8.1.11 a further three times.	
B3.8.2	Thin Layer Chromatography	
B3.8.2.1	Shake 30 ± 1 g Kieselgel G with 60 ± 1 ml water for 2 minutes in a 150 ml capacity stoppered conical flask. Immediately after shaking, spread the mixture on to five plates to a thickness of 250 μm. Allow the plates to dry overnight (note a). Activate them the following morning by heating them in an oven for 60 mins at 110 ± 5°C.	(a) The prepared plates may be stored until required over silica gel in a desiccator or other air tight container. The plates should be re-activated on the morning of the day they are required. High ambient temperatures adversely affect the 'compactness' of the spots on the chromatograph. Under such conditions the spots may be kept compact by spreading the plate with the Kieselgel G to a thickness of about 350 μm. The amount of gel and water must be scaled up accordingly.

Step	Procedure	Notes
B3.8.2.2	Prepare two chromatography tanks, each of sufficient capacity to hold a single plate, about 30 minutes before the plates are inserted. Line one tank with a strip of filter paper running around the internal walls of the tank. Place the prepared volume of Solvent A in this tank and allow the solvent to ascend the paper lining. Into the second unlined tank, place the prepared volume of Solvent B.	
B3.8.2.3	Dissolve the residue of the extract in the test tube (step B3.8.1.12) by adding accurately 0.5 ± 0.01 ml chloroform washing down the sides of the tube. Apply accurately spots of 2, 5, 10 and 20 µl of this chloroform solution and 1, 2, 3, 4 and 5 µl (ie 1 to 5 µg) of the standard solution of nonionic surfactant at suitable intervals along one plate (Plate A), 15 to 20 mm above the bottom edge. When 'overspotting' is required, allow each spot to dry before applying the next. On another plate (Plate B) apply 20 µl of the standard of nonionic surfactant, and 5, 10 and 20 µl of the extract.	
	If appreciable fat or grease are not present proceed to B3.8.2.5.	
B3.8.2.4	If the extract contains appreciable amounts of fat or grease the thin layer plates should now be run in a tank containing 100 ml of diethyl ether. The ether should be allowed to rise about 150 mm above the base line before the plate is removed and air dried until there is no odour of ether. It is then further dried for 10 minutes at 110°C in an oven and allowed to cool.	
	If organic dyestuffs are not present in the extract proceed to B3.8.2.6.	
B3.8.2.5	If the extract contains an organic dyestuff which may interfere with the subsequent chromatographic estimation, a procedure similar to that used for the removal of fat and grease may be used. Acetone is substituted for diethyl ether although success cannot be guaranteed.	
B3.8.2.6	Mark a line on the surface of the plates 100 mm above the line containing the spotted material. As soon as the spots have dried insert Plate A (B3.8.2.3) into the lined tank containing solvent A and Plate B (B3.8.2.3) into the tank containing solvent B. Allow the solvents to rise up the plate to the lines marked on their surfaces.	
B3.8.2.7	Remove the plates from the tanks, allow them to dry in air and then heat them in an oven set at 110°C for 10 minutes. Allow the plates to cool, then spray evenly with the spray reagent taking care not to over wet the chromatogram. Cover the chromatogram with a clean glass plate when spraying is finished (note b).	(b) The nonionic surfactant has a typical RF value of about 0.75. If the average ethylene oxide chain length of the surfactant differs appreciably from 10 units there will be a corresponding shift in the RF value.

Step	Procedure	Notes
B3.9 Determination of Nonionic Surfactants		
B3.9.1	Between 5 and 30 minutes after spraying determine the amount of nonionic material present by visually matching sample and standard spots on Plate A. If the colour density of the sample spots is greater than the upper limit of the standards, the sample extract should be diluted to a larger known volume in chloroform and further plates run (note a). The plate run in solvent B is examined to confirm that the material tested resolves into a streak or series of spots characteristic of polyalkoxylated nonionic surfactants.	(a) If a large amount of anionic surfactant is present; this will run just above (ca Rf 0.80) the nonionic surfactant, appearing as a slightly darker yellow spot against the yellow background and causing distortion of the nonionic surfactant spot. This is unlikely to occur unless certain primary effluents are being examined. Certain cationic surfactants interfere with the estimation of the nonionic surfactant spot. If such interferences are present consideration may need to be given to removal of ionic surfactants from the sample extract using ion exchange resins (see Section B3.3).

B3.10 Calculation of Results

Let m = µgs of standard surfactant matching V_1 µl spot of sample on Plate A.
Let V_2 ml be the original sample volume which was extracted to a 0.5 ml final chloroform extract.

Then concentration of nonionic surfactant in the original sample

$$= \frac{m \times 500}{V_1 \times V_2} \text{ mg/l}$$

B3.11 Estimation of Polyglycols and Other Related Materials

Polyglycols and their derivatives are Dragendorff active and form streaks in other positions on plate A (the single spot plate used for estimating nonionic surfactant in the above procedure). By preparing suitable comparison standards a useful estimate can be obtained of the concentration of such material in the original sample, especially if the nature of the original sample material is known. The main applications of this extension of the method are:

Sample Material	Suggested Comparison Standards
1. Polyglycols.	Polyglycols of similar type and molecular weight range.
2. Very long ethylene oxide chain length nonionic surfactants.	Nonionic surfactant of comparable ethylene oxide chain length.
3. Initial degradation material from rapidly degrading nonionic surfactant substantially of a polyglycol nature.	Polyglycol of a molecular weight range which gives a comparable Rf range on the plate as the sample material.

B4 Determination of the Oxyalkylene Content of Nonionic Surfactants by Chemical Fission and Gas Chromatography (Tentative Method)

B4.1 Performance Characteristics of the Method (39)

B4.1.1	Substance determined	Alkoxylate nonionic surfactants.
B4.1.2	Type of sample	Sewage (a), sewage effluents, biodegradation test solutions and river waters.
B4.1.3	Basis of the method	Nonionic surfactants are concentrated by the Wickbold method (Method B2 of this booklet) from the sample and then subjected to ether fission with hydrogen bromide. The bromoalkanes produced are determined by gas chromatography.
B4.1.4	Range of application	35–350 µg of polyoxyethylene or polyoxypropylene (equivalent to about 50–500 µg of nonylphenol-9-alkoxylate) in the extract.
B4.1.5	Calibration curve	Linear over the range of application.
B4.1.6	Standard deviation (b) (total)	Nonylphenol 9-ethoxylate µg: 50; 250; 500 — Standard Deviation µg: 4.7; 10.1; 26.4 — Degrees of Freedom: 5; 5; 5
B4.1.7	Limit of detection (b) (estimated)	7.5 µg of nonylphenol-9-ethoxylate.
B4.1.8	Bias	Not Known.
B4.1.9	Interferences	Compounds which are foam sublatable and which after treatment with the reagent give rise to gas chromatographic peaks having similar retention times as 1,2-dibromoethane or 1,2-dibromopropane under the chromatographic conditions employed (see Section B4.3).
B4.1.10	Time required for analysis	10 hours total time for 2 or 3 samples and the necessary standards and blanks. Operator time approximately 7 hours.

(a) The recovery of nonionic surfactants from settled sewage may not be quantitative but may be maximized by increasing the number of solvent sublations.
(b) Data supplied by Lankro Chemicals Ltd obtained on biodegradation test liquors.

B4.2 Principle

The procedure described determines small amounts of alkoxylated nonionic surfactants extracted from aqueous media such as effluents, biodegradation test solutions etc. by solvent sublation (see Method B2 of this booklet).

The method (20, 25, 26) depends on the chemical fission of the ether linkage between adjacent oxyalkylene units by means of hydrogen bromide in acetic acid under controlled conditions to form dibromoalkanes which are determined quantitatively by gas liquid chromatography. A known amount of polytetrahydrofuran [polyoxy-(1,4-butylene)] is incorporated into the reaction mixture to provide a measurable internal fissionable standard. Under the conditions of reaction this generates 1,4-dibromobutane. The ratio of peak areas of dibromoethane or dibromopropane to that of the dibromobutane is determined. A range of standards of a nonionic surfactant is similarly analysed, and a calibration graph of peak area ratios against quantity of surfactant drawn up. The corresponding quantity of surfactant in the sample can then be found from this graph.

The method is effective for the determination of nonionic surfactants containing the oxyethylene, and/or oxypropylene moeity and is independent of the molecular weight or oxyalkylene distribution. The method is not applicable to those molecules in which the oxyalkylene units are joined directly to a nitrogen atom eg alkanolamide; although it does determine other oxyalkylene units coupled to them through oxygen.

B4.3 Interferences

The same considerations apply to the extraction stage as indicated in the Wickbold method (see Method B2). Apart from these the only predictable interferences arise from materials giving chromatographic peaks with similar retention times to the dibromoalkanes under the conditions used. Overlapping interfering peaks may be separated by changing the gas chromatographic conditions, but any material containing the 1,4-dioxybutane group [-O-(CH_2)4-O-] will produce 1,4-dibromobutane and thus interfere by increasing the peak area of the internal standard. If anionic or cationic surfactants which contain alkoxy groups are present, they may be removed using an ion-exchange technique similar to that described in the cobaltothiocyanate method for the determination of nonionic surfactants (see Method B5 of this booklet).

B4.4 Hazards

Ethyl acetate, dichloromethane, carbon disulphide and hydrogen bromide are all potentially hazardous materials. Carbon disulphide and ethyl acetate are easily flammable and it is recommended that a 'NO SMOKING', 'NO NAKED LIGHTS' rule be operated. Hydrogen bromide gives off irritating fumes and should be handled in a fume cupboard.

THE SEALED GLASS REACTION TUBES USED IN THE FISSION STAGE SHOULD BE PLACED INSIDE A SUITABLE CONTAINER CAPABLE OF RETAINING FRAGMENTS OF GLASS WHICH MAY RESULT FROM AN EXPLOSION.

B4.5 Reagents

Analytical reagent grade chemicals should be used except where otherwise specified.

B4.5.1 Hydrogen bromide 45% w/v in glacial acetic acid

This reagent is obtainable in 100 ml sealed ampoules and should be transferred to separate 5 ml ampoules which are then sealed. One of these 5 ml ampoules is used for each set of fission experiments on a given day. The remaining contents of an open ampoule should be discarded.

B4.5.2 Polytetrahydrofuran

The technical product 'Polymeg' supplied by the Quaker Oats Co Ltd, is suitable.

B4.5.2.1 Polytetrahydrofuran 0.02% w/v in ethyl acetate

Weigh out 0.1000 ± 0.001 g of polytetrahydrofuran and make up to 500 ml with ethyl acetate in a volumetric flask.

B4.5.3 Nonionic surfactant

The material for standards may be varied depending on the material being analysed. For general application a technical product of average structure nonylphenol-8-ethoxylate is suitable, such as 'Synperonic NP8' obtainable from the Petrochemical Division of Imperial Chemical Industries Ltd.

B4.5.3.1 Nonionic Surfactant 0.02% w/v in ethyl acetate

Weigh out 0.1000±0.001 g of Synperonic NP8, or other standard material if appropriate, and make up to 500 ml with ethyl acetate in a volumetric flask.

B5.4.4 Ethyl acetate

B4.5.5 Dichloromethane

B4.5.6 Carbon disulphide

B4.5.7 Methanol

B4.6 Apparatus

B4.6.1 Gas Chromatograph

Any gas chromatographic system capable of producing well separated and symmetrical peaks for 1,2-dibromoethane, 1,2-dibromopropane and 1,4-dibromobutane is adequate.

Instrument:	*Dual flame ionization chromatograph*
Column:	*2 m × 0.6 cm (approximately 7 ft × 0.25 in)*
Stationary phase:	*15% Apiezon L on Universal B support*
Temperature:	*125°C, isothermal*
Carrier gas:	*Nitrogen, rate 40 ml/min*

B4.6.2 Glassware

B4.6.2.1 Normal laboratory glassware is suitable.

B4.6.2.2 Thick walled glass tubes (8 mm internal diameter, 2 mm wall thickness). Pyrex glass is suitable.

B4.6.2.3 It is vital that all glassware be thoroughly cleaned to remove all traces of detergents etc. CLEANING AGENTS INCORPORATING DETERGENTS MUST NOT BE USED. The following procedure has been found satisfactory:

B4.6.2.3.1 Wash 3 times with hot distilled water.
B4.6.2.3.2 Wash 3 times with methanol.
B4.6.2.3.3 Rinse with distilled water and finally dry in an oven at a temperature of 110±5°C.

B4.6.3 Variable pipettor capable of dispensing 0–2 mls.

B4.6.4 Ancillary Equipment

B4.6.4.1 Electric hot plate.

B4.6.4.2 Forced draught oven, controllable to a temperature of 150±2°C.

B4.6.4.3 Drying oven controllable to a temperature of 110±5°C.

B4.6.4.4 Stainless steel tubing, 4 mm internal diameter.

B4.6.4.5 Gas chromatograph injection syringe, capacity 1 µl.

B4.6.4.6 Natural gas-oxygen torch (for sealing glass tubes).

B4.7 Sample Collection and Preservation

Because of their nature surfactants will be adsorbed on to any suspended solids as well as on the walls of the containing vessel. It must therefore be clearly stated to the analyst as to what is required, ie the total surfactant or only that in solution. If the former is required it must be first ascertained whether the level of solids in the sample will permit a satisfactory estimation of the total surfactant. When the latter is needed, centrifugation rather than filtration of the sample should be employed to remove the suspended solids.

Sampling bottles should be completely filled with sample and if they are not to be analysed within a few hours of collection the samples should be stored in a refrigerator and sterilised, for example, with 10 ml of 40% m/v formaldehyde solution per litre. To obtain a representative sample either stir the whole sample with a magnetic stirrer or slowly invert the sample bottle twice before taking aliquots. As far as practical avoid the formation of foam during the stirring or inversion but in any case do not withdraw an aliquot while the bulk sample is foaming.

B4.8 Analytical Procedure

Step	Procedure	Notes
B4.8.1	Sample and Standard Preparation	
B4.8.1.1	Extract surfactant from aqueous samples by the Wickbold sublation procedure (note a) thus obtaining an ethyl acetate solution containing the equivalent of 50–500 μg of nonylphenol-8-ethoxylate.	(a) See Method B2 of this booklet.
B4.8.1.2	Add 1±0.01 ml of the 0.02% w/v ethyl acetate solution of polytetrahydrofuran to the ethyl acetate solution of the sample contained in a 250 ml beaker.	
B4.8.1.3	Add 1±0.01 ml of the 0.02% w/v ethyl acetate solution of polytetrahydrofuran to a series of four 250 ml beakers each containing approximately 100 ml of ethyl acetate. Add in turn 0.5, 1.0, 1.5 and 2.0 ml of the 0.02% w/v ethyl acetate solution of nonionic surfactant standard into the beakers.	
B4.8.1.4	Add 1±0.01 ml of the 0.02% w/v ethyl acetate solution of polytetrahydrofuran to a further 250 ml beaker containing 100 ml of ethyl acetate (note b).	(b) This solution provides a method blank. It also establishes the position of the 1,4-dibromobutane peak for each batch of samples.
B4.8.2	Evaporation and Transfer to Fission Tubes	
B4.8.2.1	Transfer the beakers prepared in steps B4.8.1.2 to 8.1.4 to a hot plate (placed in a fume cupboard) and evaporate the contents of each beaker to a volume of approximately 5 ml.	
B4.8.2.2	Transfer this volume from each of the beakers to separate 10 ml beakers. Rinse each large beaker with small volumes of ethyl acetate and transfer the washings to the respective 10 ml beakers. Continue evaporating the contents of the small beakers almost, but not completely, to dryness.	
B4.8.2.3	Transfer the contents of the small beakers into separate glass tubes previously sealed at one end, using 2 ml of dichloromethane to effect the transfer (note c).	(c) This operation concentrates the sample into a small area of the base of the tube. It is recommended that the volume in the tube does not exceed 2.5 ml at any time to avoid the possibility of frothing.

Step	Procedure	Notes
B4.8.2.4	Direct a gentle current of air through 4 mm internal diameter stainless steel tubes inserted into the open end of the glass tubes onto the surface of the liquid to evaporate the dichloromethane. The ends of the steel tubes should be maintained at about 20 mm above the surface of the liquid. The bases of the glass tubes should be in contact with the hot plate.	
B4.8.2.5	The small beakers (step B4.8.2.3) should each be rinsed with 2 × 2 ml volumes of dichloromethane and the rinsing transferred to the respective glass tubes during the evaporation process and the evaporation process continued to complete dryness.	
B4.8.3	Ether Fission Procedure	
B4.8.3.1	To each of the glass tubes containing the dried residue add 0.4±0.05 ml of the hydrogen bromide-acetic acid reagent and seal the open ends with an oxy-acetylene flame (note d).	(d) This seal is the weak point of the fission tube and unless it is sound it can give way under the pressure generated by the fission reaction. It is recommended that the operator prepares each tube in duplicate until a reliable sealing technique has been developed.
B4.8.3.2	Place the sealed tubes in a forced draught oven at 150±2°C and leave for 3 hours (note e). Remove and allow to cool to room temperature.	(e) The oven must be connected to a fume ducting system and for safety reasons the tubes must be enclosed in a metal container.
B4.8.3.3	Cut open the tubes and transfer the contents to separate screw capped glass bottles (10 ml capacity) using in each case 4 ml of water to complete the transfer. Add 0.5±0.05 ml carbon disulphide to each bottle. Replace the screw cap, shake the bottles vigorously for 1 minute and allow the layers to settle.	
B4.8.4	Gas Chromatography	
B4.8.4.1	Set up the gas chromatograph in accordance with the manufacturer's instructions.	
B4.8.4.2	Using the syringe withdraw a 0.5 µl aliquot from the lower (organic) layer in one of the bottles; inject this volume into the gas chromatograph. Carry out each injection in duplicate. Repeat for all sample, standard and blank solutions.	
B4.8.5	Calculation of Results	
B4.8.5.1	Determine the gas chromatogram peak areas of 1,2-dibromoethane and 1,4-dibromobutane by triangulation or integration.	
B4.8.5.2	Calculate the ratios of the peak areas of dibromoethane to dibromobutane for each individual chromatogram.	
B4.8.5.3	Average duplicate ratio values in all cases.	

Step	Procedure	Notes
B4.8.5.4	Prepare a standard graph plotting dibromoethane/dibromobutane peak area ratios against weight of nonionic surfactant standard (note f).	(f) The 0.5, 1.0, 1.5 and 2.0 ml volumes of standard nonionic surfactant solution pipetted out in step B4.8.1.3 correspond to 100, 200, 300 and 400 µg of nonionic surfactant respectively.
B4.8.5.5	Read off weights of nonionic surfactant in sample aliquots by finding the weights corresponding to sample dibromoethane/dibromobutane peak area ratios on the standard graph prepared in step B4.8.5.4.	

B4.9 Nonionic Surfactants Containing Polyoxypropylene Units

If any of these compounds are present, they will be indicated by the presence of 1,2-dibromopropane in the gas chromatographic determination. If required, quantitative measurement may be obtained using a suitable oxypropylene containing standard (eg polypropylene glycol). A similar calculation, but suitably modified, to that in Section B4.8 may be made.

B5 Determination of Ethoxylated Nonionic Surfactants by the Cobaltothiocyanate Method

B5.1 Performance Characteristics of the Method (39)

This method has not been tested by the committee to its normal standards for tentative methods. Some data is available in the original paper on which this method is based (Ref 18).

B5.1.1	Substance determined	Ethoxylated nonionic surfactants with 6–30 ethylene oxide units. Propoxylated surfactants also react.
B5.1.2	Type of sample	Settled sewage, sewage effluents, raw and potable waters and biodegradation test liquors.
B5.1.3	Basis of method	Concentration of the nonionic surfactant by sublation, removal of ionic material from the concentrate with ion exchange resins, determination of nonionic surfactant by measurement of the absorbance of a methylene chloride solution of the cobaltothiocyanate complex at 620 nm.
B5.1.4	Range of application	0.2 mg to 2 mg in sample aliquot.
B5.1.5	Calibration curve	Linear over the range of application.
B5.1.6	Standard deviation	See reference 18 for a range of data.
B5.1.7	Limit of detection	0.1 mg/l for 500 ml environmental sample (18).
B5.1.8	Sensitivity	Absorbance of 0.2–0.36 (2 cm cell) for 1 mg of surfactant, the absorbance varying in this way dependent on the relative amount of ethylene oxide chain material present in the molecule.
B5.1.9	Bias	Not known.
B5.1.10	Interferences	See Section B5.3.
B5.1.11	Time required for analysis	10 samples: total analytical and operator time: 8 hours.

B5.2 Principle

The procedure is based on the reaction of alkoxylated nonionic surfactant with ammonium cobaltothiocyanate to form a highly coloured solvent extractable ion-association complex. This particular method, published on behalf of the American Soap and Detergent Association by S L Boyer et al (18), is a modification of the Brown and Hayes procedure (27), and has an improved sensitivity and specificity for nonionic surfactant. It uses a preliminary solvent sublation step, essentially as described by Wickbold (16) for the separation of low concentrations (0.1–20 mg/l) of nonionic surfactants in aqueous samples. A method, similar in principle, has been published by Hey and Jenkins (38). Ionic surface active materials concentrated in the sublation are subsequently removed by an ion-exchange technique.

The response of nonionic surfactant in this method is highly dependent upon its polyoxyalkene chain length in addition to its molecular weight. Therefore, whenever possible, results should be expressed with the aid of standards for the surfactant under investigation. For samples in which the nature of the nonionic is unknown, particularly true of environmental samples, results have to be expressed arbitrarily in terms of a suitable reference surfactant. A nonyl phenol ethoxylate with an average of eight ethoxylate units (such as Synperonic NP8 manufactured by ICI Ltd) is generally considered acceptable for this purpose.

B5.3 Interferences

There is no detailed information concerning interfering substances and their effect on this method. The extraction stage excludes polyethylene glycols and the majority of non-surface active substances which might otherwise interfere in the colorimetric stage. Since it is acknowledged that materials other than nonionic surfactant can positively respond to this method, particularly for environmental samples, the entities detected are commonly referred to as cobaltothiocyanate active substances (CTAS). In some samples, for example settled sewages, multiple sublations are required or low recoveries will be obtained. For samples containing high levels of suspended solids, for example sewage treatment liquors, four separate sublation steps have been found to be necessary to ensure acceptable recoveries of nonionic surfactant (90% recovery). Ionic surface active agents may be removed by the use of ion exchange resins.

B5.4 Hazards

Potentially harmful reagents used in this method include methanol, dichloromethane and sodium hydroxide.

B5.5 Reagents

Use Analytical Reagent grade chemicals except where otherwise identified.

B5.5.1 Deionized water or distilled water from an all glass apparatus is suitable.

B5.5.2 Sodium Chloride

B5.5.3 Sodium Bicarbonate

B5.5.4 Ethyl Acetate

B5.5.5 Sodium Sulphate (anhydrous)

B5.5.6 Methanol

B5.5.7 Anion-exchange resin, hydroxide form, 50–100 mesh (for example 'Bio-Rad AG1-X2' converted from chloride to hydroxide by eluting with 1 N sodium hydroxide followed by methanol until water is displaced).

B5.5.8 Cation-exchange resin, acid form, 50–100 mesh (for example Bio-Rad 50W-8).

B5.5.9 Dichloromethane

B5.5.10 Ammonium Cobaltothiocyanate Reagent

Dissolve 30 ± 0.1 g of cobalt nitrate hexahydrate and 200 ± 0.1 g of ammonium thiocyanate in water and dilute to 1000 ml with water in a volumetric flask. This reagent is stable for at least 1 month at 25°C.

B5.5.11 Nonionic Surfactant Standard Solution (0.1 g/l)

Weigh out 0.1000 ± 0.0005 g of an alkyl phenyl ethoxylate of average ethylene oxide chain length about eight units (Synperonic NP8 manufactured by ICI Ltd is suitable). Make up to 1000 ml with water in a volumetric flask.

B5.6 Apparatus

B5.6.1 Extraction Apparatus and Sublator

The general layout of the gas stripping and sublation apparatus is shown in Figure 3.

B5.6.2 A spectrophotometer equipped with 2 cm cells suitable for measuring absorbances at 620 nm.

B5.6.3 A centrifuge equipped to hold tubes of approximately 12 ml capacity and operating at up to 4000 rpm.

B5.6.4 Steam bath

B5.6.5 Glass chromatographic column approximately 30 cm long and 1 cm diameter equipped with a tap to control the rate of elution.

B5.7 Sample Collection and Preservation

Because of their nature surfactants will be adsorbed on to any suspended solids as well as on the walls of the containing vessel. It must therefore be clearly stated to the analyst as to what is required, ie the total surfactant or only that in solution. If the former is required it must be first ascertained whether the level of solids in the sample will permit a satisfactory estimation of the total surfactant. When the latter is needed, centrifugation rather than filtration of the sample should be employed to remove the suspended solids.

Sampling bottles should be completely filled with sample and if they are not to be analysed within a few hours of collection the samples should be stored in a refrigerator and sterilized, for example, with 10 ml of 40% m/v formaldehyde solution per litre. To obtain a representative sample either stir the whole sample with a magnetic stirrer or slowly invert the sample bottle twice before taking aliquots. As far as practical avoid the formation of foam during the stirring or inversion but in any case do not withdraw an aliquot while the bulk sample is foaming.

B5.8 Cleaning Apparatus

Detergents containing nonionic and/or cationic surfactants must not be used. All glassware must be cleaned by using 10% v/v HCl, water and distilled water.

B5.9 Analytical Procedure

Step	Procedure	Notes
B5.9.1	Concentration by Sublation	
B5.9.1.1	Measure out a known volume of sample containing up to 2 mg of nonionic surfactant into the sublator.	
B5.9.1.2	Add 100 ± 1 g sodium chloride and 5 ± 0.05 g sodium bicarbonate to the sublator (note a).	(a) The sodium chloride and bicarbonate may be added as solids or in solution. In the former case agitation or bubbling with air or nitrogen to effect solution is necessary.
B5.9.1.3	Dilute with water until the level is brought up to the upper stopcock.	
B5.9.1.4	Add 100 ± 1 ml ethyl acetate carefully on top of the aqueous phase.	

Step	Procedure	Notes
B5.9.1.5	Pass a steam of air or nitrogen at a rate of about 30 l/hour until 4 litres of gas have bubbled through the liquid (note b) the gas having been passed through a Dreschel bottle containing ethyl acetate.	(b) There should be no violent turbulence between the two phases, otherwise the ethyl acetate will dissolve in the aqueous layer and negate the sublation. If as a result there is a reduction of more than 20% of the volume of the organic phase the sublation must be discarded and a fresh sample aliquot taken for a new test.
B5.9.1.6	Drain the ethyl acetate layer into a 500 ml separating funnel and return any aqueous phase to sublator.	
B5.9.1.7	Repeat steps B5.9.1.4 to B5.9.1.6 a further three times (note c).	(c) Two of these further sublations may be omitted for samples low in suspended solids, for example, many river water and biodegradation test samples, but not sewage samples.
B5.9.1.8	Add 10–15 g anhydrous sodium sulphate to the extracts in the separating funnel and shake. Decant the liquor into a 600 ml beaker. Wash the sodium sulphate with a further 20 ml of ethyl acetate; decant into the beaker. Evaporate the extract to dryness on a steam bath directing a gentle stream of air or nitrogen onto the surface of the solution to accelerate the evaporation process. The sample should be removed from the steam bath as soon as the solvent has evaporated.	
B5.9.2	Removal of Ionic Material (note d)	
B5.9.2.1	Insert a small glass wool plug at the base of the chromatographic column and pour in sufficient anionic resin in a methanol slurry to fill the bottom 10 cm of the column.	(d) If ionic materials are known to be absent this stage may be omitted.
B5.9.2.2	Cover with a plug of glass wool and add a similar quantity of cationic resin in a methanol slurry (note e).	(e) The column thus prepared may be used for the treatment of up to six samples before repacking.
B5.9.2.3	Dissolve the residue from step B5.9.1.8 in methanol and quantitatively transfer to the chromatographic column in 5–10 ml of methanol.	
B5.9.2.4	Elute with methanol at a rate of 1 drop per second into a clean dry 150 ml beaker until a volume of 125 ml has been collected.	
B5.9.2.5	Evaporate the eluate to dryness on a steam bath, directing a gentle stream of air or nitrogen onto the surface of the solution to accelerate the evaporation process. The sample should be removed from the steam bath as soon as the solvent has evaporated.	
B5.9.3	Colorimetric Estimation (CTAS)	
B5.9.3.1	Dissolve the residue from step B5.9.2.5 or step B5.9.1.8 as appropriate in 15.0 ± 0.05 ml of dichloromethane. Pipette 10.0 ± 0.05 ml of this solution into a 125 ml separating funnel (note f).	(f) During this and subsequent steps minimize evaporation of the dichloromethane solution to avoid volume changes.

Step	Procedure	Notes
B5.9.3.2	Add 5 ml of the cobaltothiocyanate reagent and shake vigorously for 60 ± 5 seconds.	
B5.9.3.3	After the layers have separated, drain the dichloromethane layer into a centrifuge tube and centrifuge at about 4000 rpm for 3 minutes.	
B5.9.3.4	Transfer the dichloromethane layer into a 2 cm cell with the aid of a Pasteur pipette and measure the absorbance of the solution against dichloromethane at 620 nm in the spectrophotometer.	
B5.9.4	Preparation of Calibration Graph	
B5.9.4.1	Pipette out 0, 2, 5, 10 and 20 ml aliquots of the nonionic surfactant standard solution (B5.5.11) and make up to approximately 500 ml with distilled water in each case (note g).	(g) Where a specific nonionic surfactant is known to be present in the samples use this surfactant to prepare standards.
B5.9.4.2	Carry out steps B5.9.1 and B5.9.3 above.	
B5.9.4.3	Draw a graph of absorbance readings against weight of surfactant (note h).	(h) The full range of standards need only be run periodically. At least one standard and a blank should be analysed with each batch of samples.
B5.9.4.4	Using the calibration curve produced in step B5.9.4.3, convert absorbance values produced by sample solutions into weights of surfactant.	
B5.9.5	Calculation of Results Let the initial volume of sample taken = V ml and the weight of CTAS found = a mg. Then concentration of nonionic surfactant in the samples as CTAS $= \frac{(a \times 1000)\ \text{mg/l}}{V}$	

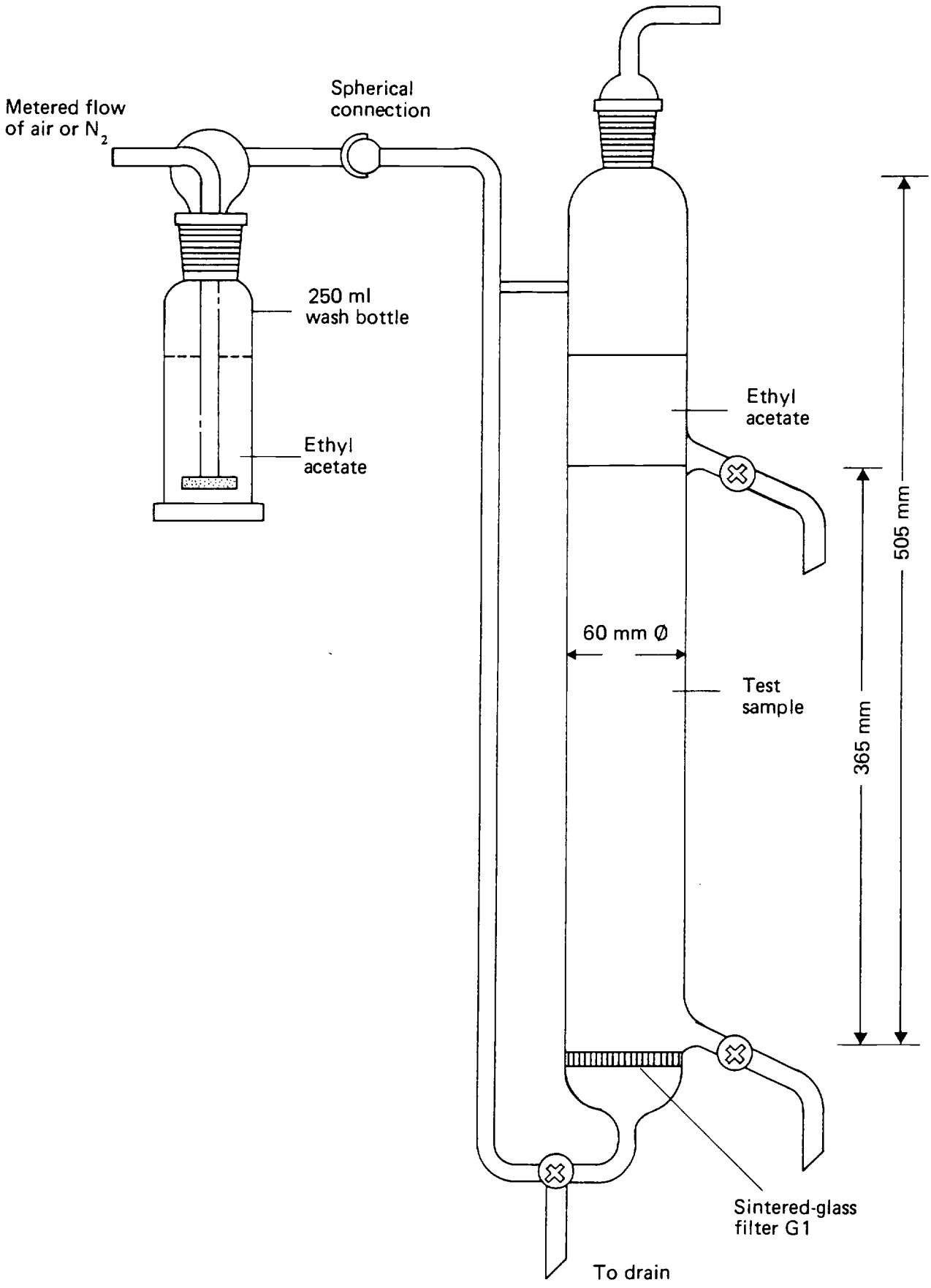

Fig. 3. Detail of gas stripping and sublation tube apparatus (OECD version)

Cationic Surfactants

C1 The Disulphine Blue Spectrophotometric Method for the Determination of Cationic Surfactants

The complete procedure described (incorporating concentration, separation and colorimetric estimation stages) can determine cationic surfactant in biodegradation test liquors and effluents in the presence of anionic surfactant and other anionic components ie substances which cause negative interferences in existing direct colorimetric procedures. However, for samples known to contain no interfering anionic components a direct disulphine blue determination on the aqueous sample can be made to estimate the cationic surfactant present.

C1.1 Performance Characteristics of the Method

C1.1.1	Substance determined	Long chain synthetic cationic surfactants and other cationic material of natural origin (see Section C1.3).
C1.1.2	Type of sample	Biodegradation test liquors such as those recommended for use in the OECD Screen and Confirmatory tests (28).
C1.1.3	Basis of method	Extraction of the cationic surfactant from the residue of an evaporated sample, its separation from anionic material on an ion-exchange resin and colorimetric estimation as its disulphine blue complex in chloroform (A max 628 nm).
C1.1.4	Range of application	0.1–10.0 mg/l cationic surfactant as cetyltrimethylammonium chloride for a maximum sample size of 200 ml.
C1.1.5	Calibration curve	Linear over the range of application.
C1.1.6	Standard deviation	

Concentration of cationic* surfactant (mg/l)	Total Standard Deviation (mg/l)	Degrees of Freedom
5 (a)	0.13	7
5 (b)	0.06	6
1 (c)	0.03	5
0.65 (d)	0.03	5

C1.1.7	Limit of detection	0.05 mg/l as cetyltrimethylammonium chloride for 100 ml sample volumes calculated as a between batch standard deviation with 10 degrees of freedom.
C1.1.8	Sensitivity	0.075 absorbance units for 0.1 mg/l cetyltrimethyl ammonium chloride for 100 ml samples and 10 mm optical cells.

*Data supplied by Unilever Research, Port Sunlight Laboratory.

C1.1.9	Bias	Unknown.
C1.1.10	Interferences	See Section C1.3.
C1.1.11	Time required for analysis	12 hours total time for 10 samples and the necessary standards and blanks. Operator time approximately 8 hours.

(a) Data for distearyldimethylammonium chloride in OECD Confirmatory inlet liquor on 50 ml sample volumes.
(b) Data for cetyltrimethylammonium chloride in OECD Confirmatory inlet liquor on 50 ml sample volumes.
(c) Data for distearyldimethylammonium chloride in OECD Screen Liquor on 100 ml sample volumes.
(d) Data for cetyltrimethylammonium chloride in OECD Screen Liquor on 100 ml sample volumes.

C1.2 Principle

The method described (29) can determine cationic surfactant in biodegradation test liquors and effluents in the presence of anionic surfactant and other anionic components which normally negatively interfere with existing direct colorimetric procedures (30–33) ie these fail because the anionic surfactant interacts more strongly with cationic than does the anionic dye reagent.

The cationic surfactant is concentrated by evaporation of the aqueous sample and separated from the resulting solid residues by a methanol extraction step. The resulting extract is treated under non-aqueous conditions (methanol) on an ion-exchange resin in the chloride form to remove interfering anionic components. The isolated cationic surfactant is reacted with the intensely coloured disulphine blue anion in aqueous solution to form a chloroform extractable ion-association complex. The absorbance of the final chloroform phase is determined at 628 nm.

However, for test liquors that contain no interfering anionic components a direct disulphine blue determination on the aqueous sample can be made to estimate the cationic surfactant present.

C1.3 Scope and Limitations, including Interferences

Only the long alkyl chain quaternary ammonium compounds will react to form stable chloroform-extractable disulphine blue complexes in the colorimetric estimation step. Alkyltrimethylammonium and dialkyldimethylammonium compounds with more than ten and eight (or more) carbons respectively in the alkyl groups have been found to form completely extractable complexes with disulphine blue. On this basis, the commercially important cationic surfactants should be responsive to the disulphine blue reaction.

To date, the complete procedure has only been validated for a comparatively small number of cationic surfactants ie distearyldimethyl ammonium chloride, cetyltrimethylammonium chloride, octadecyltrimethylammonium chloride, didecyldimethylammonium chloride, didodecyldimethylammonium chloride and dodecylbenzyldimethylammonium chloride. The recovery of standard additions (5 mg/l) of these surfactants from biodegradation test liquors containing an excess of anionic surfactant has generally been better than 95%. The applicability of the procedure for any new surfactant should be checked out in advance by determining its recoverability from control test liquor samples. It may be found to be necessary to adapt the analytical conditions somewhat (ie number of extractions, extractant, etc) in order to obtain an acceptable recovery for other materials.

As a class of surfactants, in aqueous solution they are highly substantive to negatively charged surfaces (including glass). It is for this reason that the cationic extracts, following the initial evaporation step, are kept in organic solvents to minimise losses of cationic by adsorption. The pre-condition of the equipment to cationic surfactant will also help to reduce such losses (see C1, Sections 5.5, 8.2, 8.3, 9.2.2 and C1.9.4.6 note k). The removal of anionic surfactant in the ion-exchange step must be consistently high if it is not to interfere negatively in the estimation of the cationic. The efficiency of each batch of new resin should therefore be checked using standard cationic-anionic solutions.

The disulphine blue reaction is not specific for synthetic cationic surfactant in biodegradation test liquors. Any long-chain amines (34) and other compounds that can be protonated in weakly acidic conditions, or which contain a quaternary nitrogen group, may also form extractable disulphine blue compounds that can positively interfere. For example, the OECD synthetic sewage, which contains no added cationic surfactant gives a measurable disulphine blue active substance (DSBAS) level (0.1–0.3 mg/l). Therefore, control liquors are generally required to correct for low levels of other materials that are reactive to disulphine blue before the actual cationic surfactant content of the test liquor can be determined. For waste and surface water samples, the levels of natural substances that react with disulphine blue are likely to be so high that the procedure cannot be used for monitoring their cationic surfactant contents; for example, surface waters are found to have Disulphine Blue Active Substance (DSBAS) levels in the range 0.1–0.3 mg/l expressed as distearyldimethyl ammonium chloride.

C1.4 Hazards

Chloroform, glacial acetic acid and methanol are potentially harmful reagents.

C1.5 Reagents

Reagents should be analytical reagent quality except where otherwise identified.

C1.5.1 Water—distilled

C1.5.2 Disulphine blue solution (Acid Blue 1; CI 42045; 1.3×10^{-3}M)

Dissolve 0.16 ± 0.005 g of disulphine blue in 20 ml of 10% (v/v) ethanol in water and dilute to 250 ± 2.5 ml with water. Store in a glass stoppered glass bottle. This solution is stable for at least three months.

C1.5.3 Acetate buffer solution, 2M, pH5

Dissolve 114.8 ± 0.1 gm of anhydrous sodium acetate in water, add 34.8 ± 0.05 ml of glacial acetic acid and make up to 1.00 ± 0.01 litre with water. Store in a glass stoppered glass bottle. This solution is stable for at least three months.

C1.5.4 Standard cationic surfactant solutions (10 and 100 mg/l)

For biodegradation studies the surfactant under test will generally be used to prepare the calibration curve. However, a useful reference surfactant is cetyltrimethylammonium chloride.

C1.5.4.1 Cationic surfactant solution (100 mg/l)

Dissolve 0.1000 ± 0.0005 g of active cationic surfactant in water and make up to litre in a volumetric flask. Store in a glass stoppered glass bottle in a refrigerator below 5°C. This solution is stable for at least two months.

C1.5.4.2 Cationic surfactant solution (10 mg/l)

Add 10 ± 0.01 ml of the 100 mg/l cationic surfactant solution (C1.5.4.1) into a 100 ml volumetric flask and make up to the mark with water.

C1.5.5 Methanol solution of cetyltrimethylammonium chloride (approximately 100 mg/l)

For use in pre-conditioning the resin material.

C1.5.6 Alkylbenzene sulphonate (ABS) solution (approximately 1000 mg/l)

Use an ABS such as Marlon A350 (manufactured by Hüls AG). This solution is used to make up the anionic surfactant concentration to about 10 mg/l in those samples containing low levels of anionic components eg biodegradation test effluents. This has been found to aid the recovery of cationic surfactant (as its neutral ion-association complex) from the dry residue of an evaporated sample.

C1.5.7 Chloroform

C1.5.8 Methanol

C1.5.9 Anion-exchange resin, chloride form, 50–100 mesh such as Biorad AG1-X2.

C1.6 Apparatus

C1.6.1 Unless otherwise specified all glassware used for quantitative volumetric measurements should be accurate to a Class B tolerance or better.

C1.6.2 Standard laboratory glassware including pipettes, volumetric flasks, burettes, measuring cylinders, separating funnels fitted with PTFE stopcocks and beakers.

C1.6.3 The extraction device described by Taylor and Fryer (35) is used to transfer the chloroform extracts from centrifuge tubes to optical cells (see Figure 4).

C1.6.4 A spectrophotometer equipped with matched 10 mm glass optical cells.

C1.6.5 Glass centrifuge tubes with conical bases—nominal volume of 40 ml.

C1.6.6 Centrifuge equipped to take the above tubes.

C1.6.7 Steam bath

C1.6.8 Ion-exchange columns of conventional design having a 19/24 joint to take a 250 ml separating funnel as a solvent reservoir (see Figure 5).

C1.6.9 Variable high speed electrical stirrer—fitted with a small glass spiral stirring rod.

C1.7 Sample Collection and Preservation

Because of their nature surfactants will be adsorbed on to any suspended solids as well as on the walls of the containing vessel. It must therefore be clearly stated to the analyst as to what is required, ie the total surfactant or only that in solution. If the former is required it must be first ascertained whether the level of solids in the sample will permit a satisfactory estimation of the total surfactant. When the latter is needed, centrifugation rather than filtration of the sample should be employed to remove the suspended solids.

Sampling bottles should be completely filled with sample and if they are not to be analysed within a few hours of collection the samples should be stored in a refrigerator and sterilized, for example, with 10 ml of 40% m/v formaldehyde solution per litre. To obtain a representative sample either stir the whole sample with a magnetic stirrer or slowly invert the sample bottle twice before taking aliquots. As far as practical avoid the formation of foam during the stirring or inversion but in any case do not withdraw an aliquot while the bulk sample is foaming.

C1.8 Cleaning of Apparatus

C1.8.1 The beakers used in the sample concentration and ion-exchange steps must be cleaned by successively washing them in hot tap water (twice), deionized water (twice) and finally methanol (twice). Allow the beakers to air dry before use.

C1.8.2 The centrifuge tubes used in the colorimetric estimation step must be washed with tap water (twice) and deionized water (twice) only. In this way the tubes remain suitably 'conditioned' to reagent and samples. Occasionally, a more rigorous methanol wash may be required but the tubes will then need to be re-conditioned.

C1.8.3 Pre-condition all new glassware to cationic surfactant by soaking in a 100 mg/l aqueous cationic surfactant solution (see C1.5.4.1). Wash free of any excess soluble cationic by washing with water and methanol before using.

Step	Procedure	Notes
C1.9.1	Separation and Concentration of Cationic Surfactant	
C1.9.1.1	Proceed directly to Section C1.9.4.7 if the sample contains no anionic surfactant or other anionic interferences and carry out a direct colorimetric estimation. For samples containing anionic surfactant take a volume of unfiltered test sample (note a) containing 25–1000 μg of cationic surfactant and evaporate to dryness in a 250 ml beaker. A volume of standard control solution equivalent to that taken for the test determination is also evaporated to dryness on a steam bath. Make up the anionic surfactant concentration to about 10 mg/l, using the ABS reagent, (C1.5.6), in those samples containing low levels of anionic components eg biodegradation test effluents (note b).	(a) The volume of sample should preferably not exceed 200 ml. (b) This aids the recovery of cationic surfactant (as its neutral ion-association compound) from the dry residue of an evaporated sample.
C1.9.1.2	Break-up the sample residue thoroughly with a glass rod and extract it with 20 ml of boiling methanol. Filter the cationic extract through a small cotton wool pad plugged into the neck of a filter funnel and collect the filtrate in a 250 ml beaker. Wash the pad with 10 ml of cold methanol.	
C1.9.1.3	Repeat the extraction with two further 20 ml portions of boiling methanol. Finally wash the extraction beaker, pad and filter funnel with 20 ml of cold methanol. Evaporate the combined extracts to dryness on a steam bath.	
C1.9.2	Preparation of Anion-Exchange Columns	
C1.9.2.1	Plug the lower constriction above the tap of the column with a small wad of cotton wool and fill one third full with methanol. Slurry 12 ml of wet resin into the column with methanol (note c). If necessary stir the resin bed with a glass rod to remove any gas bubbles and plug the top with a small pad of cotton wool.	(c) Soak the resin in methanol for ½–1 hr prior to preparing columns to allow for shrinkage.
C1.9.2.2	Pre-condition the resin bed to cationic surfactant by passing 10 ml of cetyltrimethylammonium chloride solution in methanol at a rate of 2 ml min^{-1}. Wash the column by passing 600 ml of methanol at a rate of 2–3 ml min^{-1}. Check that all free cationic has been removed from the resin by carrying out a control blank determination (note d).	(d) Evaporate a 100 ml of the final eluate and proceed as indicated in Section C1.9.4.1–9.4.6. The column blank should be similar to the reagent blank for the colorimetric step (0.02–0.03 absorbance).
C1.9.2.3	Ensure that the resin bed is free from gas bubbles before use.	
C1.9.3	Ion-Exchange Removal of Anionic Interferences	
C1.9.3.1	Dissolve the cationic residue in 10 ml of methanol, using a glass rod to break-up any residue, and transfer the whole extract to the top of a prepared ion-exchange column (C1.9.2).	

Step	Procedure	Notes
C1.9.3.2	Pass the sample through the resin bed at a rate of less than 1 ml min^{-1}, and collect the eluate in a 250 ml beaker. Use a further two 10 ml portions of methanol to ensure quantitative transfer of the sample to the column. Wash the sample through the column with 100 ml of methanol at a rate of 2–3 ml min^{-1} (note e). Evaporate the column eluate to dryness carefully on a steam bath.	(e) Wash the columns with a further 150 ml of methanol after each separation. The capacity of the resin bed is such that a column can be used 5–10 times.
C1.9.4	Colorimetric Determination of Cationic Surfactant	
	Ion-exchanged Samples (note f)	
C1.9.4.1	Redissolve the cationic surfactant residue (from C1.9.3.2) in 20 ml of boiling water-saturated chloroform (note g and h). Allow the boiling solvent to be in contact with the residue before using a glass rod to break it up.	(f) An alternative colorimetric extraction technique, which uses separating funnels, is given in C1.11. (g) Prepared by partitioning chloroform with water for 1 min in a separating funnel. (h) For residues that are very sticky and insoluble in chloroform a small measured volume of methanol (eg 2.5 ml) is used initially to dissolve the extract before the further addition of 15 ml of cold water saturated chloroform (which generally results in precipitation of extraneous material). A similar washing procedure to that used in C1.9.4.2 results in quantitative recovery of cationic surfactant. The final chloroform solution should contain no more than 2.5% (v/v) of methanol.
C1.9.4.2	Transfer the cationic extract to a suitably sized graduated flask (50/100/200 ml). The volume of the flask is chosen so that a 10 ml aliquot of the final solution contains 5–50 μg of cationic. Use a further portion of hot solvent and several portions of cold solvent to wash the beaker and make the solution up to the appropriate volume.	
C1.9.4.3	Add 2.5 ± 0.5 ml of 2M acetate buffer and 1 ml of disulphine blue reagent into a 40 ml conical centrifuge tube (note i) and add 15 ± 0.5 ml of distilled water. Pipette 10 ± 0.05 ml of the chloroform solution into the tube.	(i) Previously conditioned to reagents and sample.
C1.9.4.4	Stir the contents of the tube vigorously for 90 ± 5 secs with the high speed stirrer, so that the two layers are completely mixed (note j). Centrifuge for about 30 sec to separate the layers.	(j) The speed setting and the length of stirring should be closely controlled.
C1.9.4.5	Attach a rubber teat to the short arm of the extraction device (see figure 4). Introduce the long arm through the aqueous layer into the chloroform layer maintaining a slight positive pressure on the teat to produce a slow stream of air bubbles. Secure the bung into the mouth of the centrifuge tube and then remove the teat.	
C1.9.4.6	Transfer the chloroform extract directly to a glass 10 mm optical cell (note k) by applying pressure with the syringe via the needle. Wash the cell once with the extract and then immediately measure the absorbance of the extract at 628 nm against a chloroform reference.	(k) Previously conditioned with a disulphine blue-cationic chloroform standard solution. Quartz optical cells should not be used because of their high substantivity for the disulphine blue complex.

Step	Procedure	Notes
	Aqueous Samples that Contain no Anionic	
C1.9.4.7	Place a measured volume of aqueous sample, up to 15 ml, into a centrifuge tube. Make up the volume of the aqueous phase to 15 ± 0.5 ml (if required) and add 2.5 ± 0.5 ml of 2M acetate buffer and 1 ± 0.5 ml of disulphine blue reagent. Pipette 10 ± 0.05 ml of chloroform into the tube. Proceed from C1.9.4.4 to C1.9.4.6.	
C1.9.5	Preparation of Calibration Graph	
C1.9.5.1	Add by burette 0, 1, 2, 3, 4 and 5 ml (0–50 µg) of a 10 mg l^{-1} cationic surfactant solution (note l) into a series of 40 ml centrifuge tubes and make up the volume to 15 ml with distilled water. Add 2.5 ml of acetate buffer, 1 ml of disulphine blue reagent and 10 ± 0.05 ml of chloroform (note m) and proceed from C1.9.4.4 to C1.9.4.6.	(l) This is generally a known sample of the surfactant under test. (m) For sample residues re-dissolved in methanol (see note h) use an equivalent volume of an appropriate methanol chloroform mixture (no more than 2.5% (v/v) methanol) to perform the standard determinations.
C1.9.5.2	Construct a calibration graph of absorbance against the concentration (µg) of cationic surfactant (note n).	(n) Identical calibrations were obtained regardless of whether the cationic standards were prepared in water or chloroform. Calibrations showing a tendency to intersect the concentration axis rather than pass through the origin may be due to equipment not being sufficiently condition to reagents and samples. The full range of standards need only be run periodically. The top 50 µg standard should be analysed with each batch of samples.

C1.10 Calculation of Results

The following expression can be used to calculate the amount (mg/l) of cationic in the aqueous samples (put through the complete procedure)

$$C \text{ mg/l} = \frac{a \times V_c}{10 \times V_s}$$

where a = number of µg cationic found in a 10 ml aliquot (corrected for control liquor)
V_c = volume of the final chloroform extract
V_s = volume of aqueous sample taken.

C1.11 Alternative Technique for the Colorimetric Estimation (which is based on the use of separating funnels)

Re-dissolve the cationic residue obtained from the ion-exchange separation (ie steps 9.1–9.4) in 20 ml of warm, water-saturated chloroform and add to a 100 ml separating funnel containing 2.5 ml acetate buffer, 15 ml water and 1 ml of disulphine blue. Shake the separating funnel on a flask shaker at a fixed rate for 5 mins. Allow the phases to separate and run the chloroform phase into a 50 ml graduated flask. Repeat the transference and extraction steps with a further 20 ml portion of warm, water-saturated chloroform. Combine the chloroform extracts and make up to the mark with chloroform. Read the absorbance of the solution at 628 nm in a 10 mm cell. Further dilution of extracts may be necessary to bring them into the range of a 0–250 µg calibration curve.

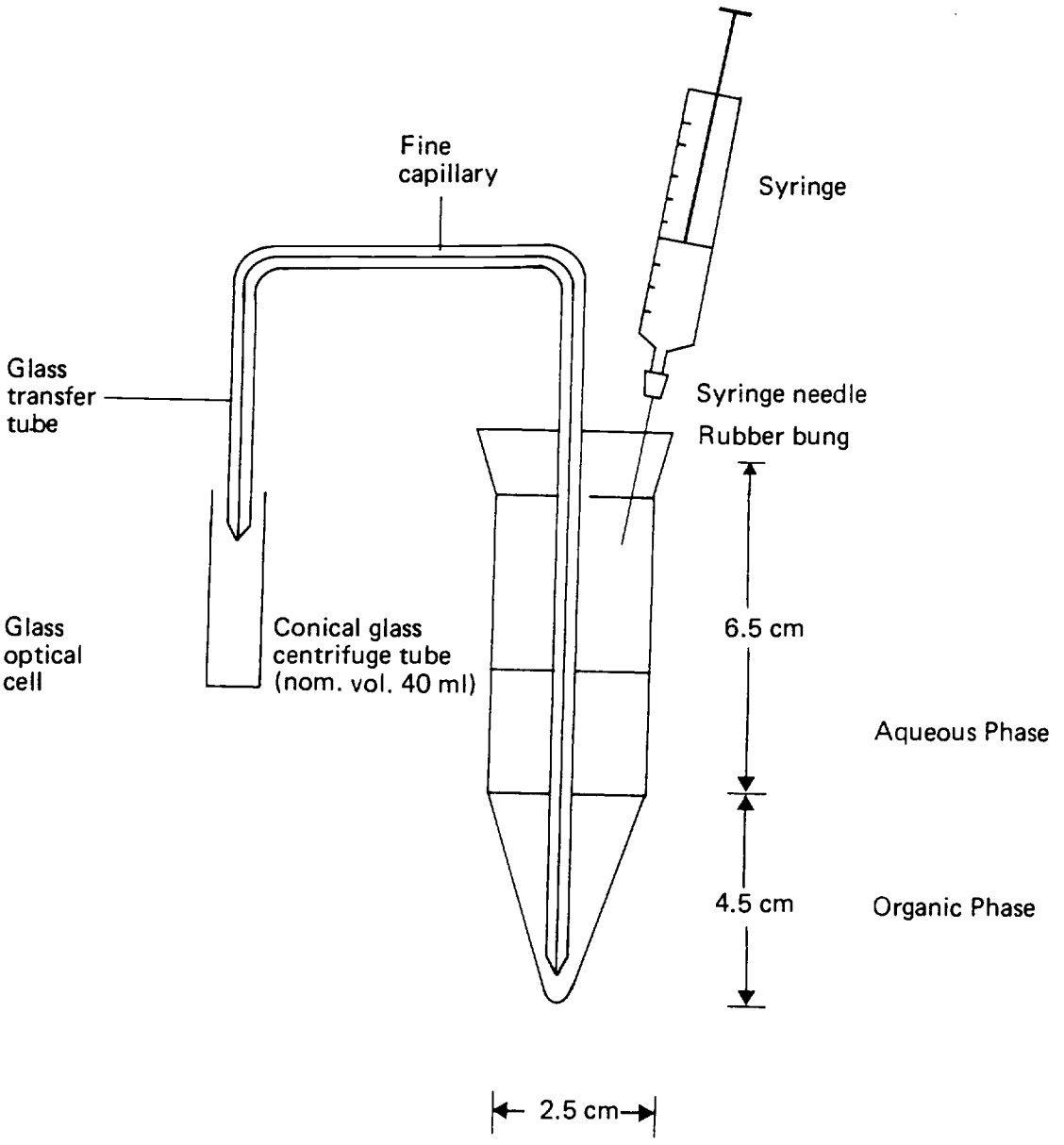

Fig. 4. Extraction device

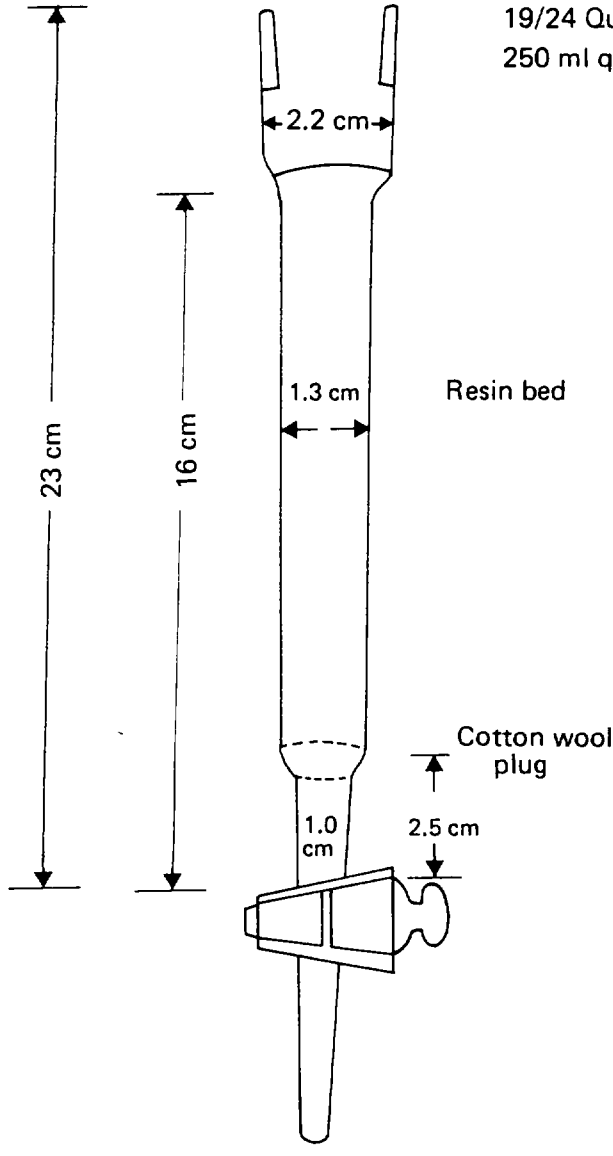

Fig. 5. Ion exchange column

D Checking the Accuracy of the Analytical Results

In normal routine operation many factors may subsequently affect the accuracy of the results. Therefore it is recommended that the accuracy should be tested initially and then at regular intervals thereafter. This may be done by analysing a standard solution of known concentration in the same manner as normal samples; that is by carrying out the whole procedure.

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However thoroughly a method may be tested, there is always the possibility of a user discovering a hitherto unknown problem. Users with information on this method are requested to write to:

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The Standing Committee of Analysts
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