DRAFT AFRICAN STANDARD DARS 1462: 2026

Second Edition 2026

Determination of biodegradability of surfactants - Test Method

Reference No. DARS 1462:2026(E)
ICS 71.100.40

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This African Standard was prepared by the ARSO Technical Harmonization Committee Number 5 on Chemistry and Chemical Engineering (ARSO/THC 05).

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Introduction

The methods described in this standard can be successfully applied in the field by water and sewerage authorities to determine the biodegradability of surfactants used in the manufacture of detergents.

Determination of biodegradability of surfactants – Test Method

1 Scope

This Draft African Standard describes a procedure for the biological degradation of surfactants and a method for assessing results, which is valid for both anionic and non-ionic surfactants.

2 Normative references

There is no normative reference

3 Principle

Stock solutions of predetermined concentration of the candidate sample, and the hard and soft reference surfactants shall be diluted with biochemical oxygen demand (BOD) dilution water. The diluted solutions shall be inoculated with air-dried activated sewage sludge and maintained at 20 °C while being agitated with air for a period of between 7 days and 21 days. Duplicate runs are made.

Samples are withdrawn periodically for the determination of the concentration of residual active material and a graph is prepared showing the relationship between time and degradation of surfactant.

The candidate sample is assessed by comparing its biodegradability behaviour with that of the reference samples.

4 Reagents and materials

4.1 Biochemical oxygen demand (BOD) dilution water

4.1.1 Prepare the following solutions:

a) *ferric chloride* ($FeCl_3.6H_2O$) — Dissolve 0.1 g of ferric chloride hexahydrate in 1 litre of distilled water;

b) *calcium chloride* ($CaCl_2$) — Dissolve 27.0 g of anhydrous calcium chloride (or the appropriate mass of the hydrated salt) in 1 litre of distilled water;

c) *magnesium sulphate* ($MgSO_4.7H_2O$) — Dissolve 25.0 g of magnesium sulphate heptahydrate in 1 litre of distilled water;

d) *ammonium sulphate* [$(NH_4)_2SO_4$] — Dissolve 2.0 g of anhydrous ammonium sulphate in 1 litre of distilled water;

e) *phosphate buffer (stock solution)* — Dissolve 42.5 g of anhydrous monobasic potassium phosphate (KH_2PO_4) in 700 mL of distilled water. Add 8.8 g of sodium hydroxide and make up to 1 litre with distilled water. The buffer solution should have a pH of 7.2 ± 0.1.

4.1.2 Prepare the BOD dilution water by adding 1.0 mL of each of the above solutions to 1 litre of distilled water. Aerate the dilution water overnight at ambient temperature to remove carbon dioxide and saturate with oxygen.

NOTE 1 All solutions are made using high purity distilled water (e.g. copper content less than 0.01 mgL) as heavy metals are generally toxic to bacteria.

NOTE 2 Unused dilution water shall be discarded. The container shall then be cleaned, preferably by sterilizing with a sodium hypochlorite solution (300 ppm available chlorine), rinsed with distilled water to remove any residues, and stored out of direct sunlight.

NOTE 3 Freshly prepared dilution water shall be not used to “top up” old stock solution.

4.2 Reference standard surfactants

To allow for the inherent variability of biological processes, the biodegradability of the candidate sample shall be assessed against that of two reference standards, one of which shall be a biologically hard surfactant and the other a biologically soft surfactant.

Two pairs of reference standards for anionic and non-ionic surfactants shall be described as follows:

a) for testing anionic surfactants:

i) soft standard — A linear alkyl benzene sulphonate materialasprepared at the time (see 6.1).

ii) hard standard — A tetrapropylene benzene sulphonate material as prepared at the time (see 6.1).

b) for testing non-ionic surfactants:

i) Soft standard— A synthetic lauryl alcohol ethoxylate material as prepared at the time (see 6.2).

ii) Hard standard — An alkyl phenol ethoxylate type material as prepared at the time (see 6.2).

NOTE Typical non-ionic surfactants are subject to limited shelf life and therefore the reference standards shall not be older than 12 months. The standards held shall carry a termination date.

4.3 Inoculum

Air-dried activated sewage sludge shalbe used as the inoculum to found the bacteria colony. The sewage sludge shall be collected from an identifiable source and be typical of the locality. The suspended solids are best separated by centrifugation, dried in a thin layer, at room temperature, shielded from light.

The separation and drying of the sludge shall be carried out immediately after receiving the sample. Prior to use, the dried sludge shall be powdered and used between 1 week and 15 weeks after collection.

The sewage sludge shall not be freeze-dried nor shall the sludge, as obtained by the prescribed drying procedure, be stored in a refrigerator. The inoculum shall be stored in an airtight brown glass container and maintained at room temperature.

NOTE 1 If the test does not meet the time criteria laid down then the sludge should be rejected and another sludge obtained.

NOTE 2 Sludges collected from different sources, and from the same source at different times, display variations in biological activity. The method minimizes the influence of biological variability as far as possible by internal standardization of each set of tests against reference standards. An extensive program of test work has shown that similar results are obtained with inoculum from any of the common sources.

5 Apparatus

5.1 General

Suitable apparatus for the biodegradation of the candidate and reference samples is shown in Figure 1 and the apparatus for the determination of residual surfactant are described in Annexes A, B and C.

5.2 Cleaning of glassware

Glassware used for biodegradation shall be kept separate from all other glassware and cleaned

internally with a nitric acid/sulphuric acid mixture (1:1 by volume of the concentrated acids).

The cleaned glassware shall be thoroughly rinsed with distilled water just before using. On no account shall washing with soap or detergent be permitted as residues may subsequently interfere with the test.

Chromic acid cleaning mixtures shall not be used to clean biodegradation glassware as traces of the chromic ion may discourage the growth of bacteria.

5.3 Test vessel

A Drechsel type bottle of 2.5 litres to 3 litres capacity, fitted with a centre tube reaching to within 2 cm of the bottom of the vessel and terminated with a hemispherical sintered glass frit of porosity 1 or 0.

If the method described in Annex A or B is adopted, then a bottle of 1.5 litres to 2 litres capacity may be used, but the biodegradation procedure is written for the 2.5 litres to 3 litres capacity vessel as required for analysis by the method described in Annex C.

A suitable biodegradation vessel is shown in Figure 2.

NOTE A hemispherical-shaped frit is specified as this will deliver a constant stream of small air bubbles giving better aeration and mixing.

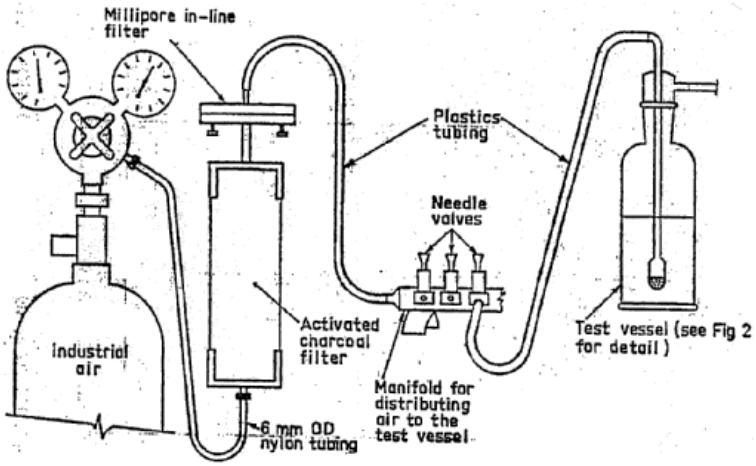


Figure 1— Suggested apparatus for biodegradation

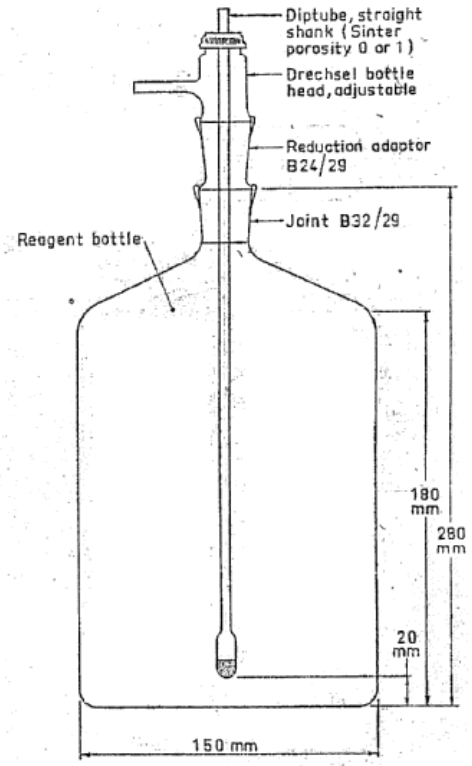


Figure 2 — Diagram of a suitable Biodegration vessel

5.4 Air supply

Clean air shall be used to aerate and mix the contents of the test vessel. The flow of air shall be adjustable to a rate of 10 mL/min. to 15 mL/min. and care shall be exercised to ensure that the air does not carry contaminants into the test solution. If 1.5 litres to 2 litres capacity bottles are used, the airflow shall be adjustable to 5 mL/min. to 10 mL/min.

All air, irrespective of source of supply, shall be passed through a column of activated carbon in order to avoid contamination of the system by volatile organic contaminants e.g. phenols, benzene, chlorinated solvents.

To prevent extraneous bacteria from entering the biodegradation vessel via the air supply, the air shall be sterilized by means of an in-line filter of suitable pore size. An in-line millipore filter holder fitted with 0.45µm and 0.22 µm filters has been found suitable. The recommended instructions of the manufacturer of the equipment shall be observed, whichever filter system is used.

A suitable glass or metal manifold shall be employed for distribution and adjustment of cleaned air to the test vessel.

NOTE 1 A variable airflow rate is necessary to avoid losing solution through the vent tubes when high foaming surfactants are being tested.

NOTE 2 Industrial compressed air is suitable.

5.5 Means of temperature control

The biodegradation is carried out at a temperature of 20 ± 1 °C. A means, such as an incubator, constant temperature room or water bath, for maintaining this temperature is required.

NOTE The test vessel shall be screened from direct sunlight to prevent algal growth.

6 Preparation of stock test solutions

6.1 Anionic surfactants

Stock solutions that will yield, after appropriate dilution, a concentration of 30 micro equivalents of methylene blue active substance (MBAS) per litre at the beginning of the biodegradation test, shall be prepared as follows:

a) candidate surfactant:

i) prepare a solution containing 2 g to 3 g of the candidate surfactant per litre using distilled water for the diluent. Dilute 10.0 mL of this solution to 1 litre and determine the concentration of MBAS in accordance with the method described in Annex A;

ii) calculate the mass of candidate surfactant required to prepare a stock solution containing 3 000 microequivalents of linear alkyl benzene sulphonate (LAS) per litre using the following formula:

$$m_1 = \frac{m_2 \times 30}{E}$$

where

m_1 = the required mass of candidate surfactant, in grams;

m_2 = the 2 g to 3 g mass of the candidate surfactant used in the determination of MBAS, in grams;

E = MBAS concentration of the diluted solution (0.02 g/L to 0.03 g/L) of the candidate surfactant, in microequivalents per litre.

iii) weigh out the mass of candidate surfactant calculated in step ii);

iv) dissolve the mass of candidate surfactant in distilled water and dilute to 1 litre.

b) soft and hard reference standard surfactants:

i) for both soft and hard reference surfactants, weigh out the mass required to make a solution containing 3 000 microequivalents of LAS per litre;

ii) separately dissolve the mass of each reference surfactant in distilled water and dilute each to 1 litre.

NOTE The reference solutions should be discarded after a week.

6.2 Non-ionic surfactants

Stock solutions containing 1 g of active matter[1)] per litre shall be prepared as follows:

a) For the candidate surfactant and each of the reference surfactants, transfer approximately 1 g, weighed to the nearest 10 mg, to a tarred petri dish of approximately 80 mm diameter and heat in an oven at 105 ± 2 °C for 40 minutes. Cool, reweigh and calculate the percentage (m/m) of non-volatile matter;

b) Calculate the mass of each surfactant required to prepare stock solutions containing 1 g of active matter per litre using the following formula:

$$m = \frac{100}{M} \times 100$$

where
m = the required mass of surfactant, in grams;
M = the calculated percentage (m/m) of non-volatile matter.

c) For each of the candidate and reference surfactants, weigh out the mass calculated in step b).

d) Separately dissolve the mass of each surfactant in distilled water and dilute to 1 litre.

7 Procedure

a) Transfer 20.0 mL of each prepared stock solution of the reference surfactants and the candidate samples into individual test vessels. Add 60 mg of air-dried activated sewage sludge to each test vessel and dilute to 2 litres with standard BOD dilution water (if 1.5 litres to 2 litres capacity bottles are being used, halve all these quantities). Duplicate biodegradations on all samples are required and the biodegradation shall be started on the same day for the group of samples being tested.

b) Determine the initial surfactant concentration on all samples (including reference standards). The anionic surfactants shall be determined as MBAS (methylene blue active substance) in accordance with the procedure described in Annex A. The mean value of the duplicate is the initial concentration C_o; this concentration must be between 29 and 31 microequivalent of MBAS per litre and should be reported to the nearest 0.3 microequivalent of MBAS per litre. The non-ionic surfactants shall be determined as bismuth active substance (BiAS) in accordance with the procedure described in either Annex B or C. The mean value of the duplicates is the initial concentration C_0 which must be between 9.5 mg and 10.5 mg of BiAS per litre and should be reported to the nearest 0.1 mg of BiAS per litre.

c) Use a flow meter to adjust the flow of air through the sintered glass frit to a rate of approximately 10 mL/min. to 15 mL/min. and allow the test to proceed. If 1.5 litres to 2 litres capacity bottles are being used, adjust the flow of air to 5 mL/min to 10 mL/min.

NOTE If the recommended flow rate causes loss of foam through the vent tube, reduce the flow of air to a minimum rate of 1 mL/min. When the foam abates, restore the flow of air to the recommended rate.

[1)] For the purposes of this test, “active matter” is taken to mean “non-volatile mater” (NVM).

d) Collect samples at intervals during the biodegradation and determine the concentration of residual active material using the appropriate method described in Annex A, B or C. Should the method of Wickbold be preferred for non-ionics, follow the procedure described in Annex C.

NOTE 1 Carry out the first analysis for surfactant concentration after 1 day and subsequent analyses at 1 day to 2 days intervals until the test is completed.

NOTE 2 Shut off the air supply about 1 h before samples are collected and allow the foam to collapse. If necessary, the test solution may be swirled in the bottle to collapse the foam. This procedure is necessary as the foam contains a higher concentration of surfactant than the solution.

NOTE 3 The volume of sample required will increase as the biodegradation proceeds. The relationships between the expected concentration of any of the anionic or non-ionic surfactants being tested (including the respective reference standard surfactants) and the recommended sample sizes are given in Tables 1, 2 and 3.

NOTE 4 Commence the analyses of the test solution aliquots within a period not exceeding 4 hours after sampling. Wherever this period is exceeded the test solution aliquots must then be preserved by the addition of mercuric chloride. A final concentration of 50 mg $HgCl_2$/L has been found adequate to destroy the bacteria present to prevent any further degradation.

Table 1 — Recommended sample sizes for anionic surfactant (see Annex A)

Expected MBAS concentration microequivalents/L	Test solution sample aliquot mL
21 to 30	10
9 to 21	20
2.0 to 9	50
1.0 to 2.0	100
0.3 to 1.0	250

NOTE All the above aliquots should be made up to 250 mL with BOD water.

Table 2 — Recommended sample sizes for non-ionic surfactants — TLC procedure (see Annex B)

Expected BiAS concentration mg/L	Test solution sample aliquot mL
5.0 to 10.0	50
2.5 to 5.0	50
1.0 to 2.5	50
0.5 to 1.0	50
0.25 to 0.5	100

Table 3 — Recommended sample sizes for non-ionic surfactants — Wickbold procedure (see Annex C)

Expected concentration mg BiAS/L	Test solution sample aliquot mL	BiAS content of test solution aliquot µg
5.0 – 10.0	50	250 — 500
2.5 – 5.0	50	125 — 250
1.0 – 2.5	100	100 — 250
0.25 – 1.0	500	125 — 500

e) Continue the test until an apparent end-point is reached (see 7.1).

NOTE For the biodegradation test to be valid, an end-point must be reached within 7 days to 21 days.

f) Continue monitoring the biodegradation for 4 days after the end-point to prevent invalid extrapolation through the end-point.

g) Express the residual concentration of the surfactant at time *t* (sampling day) as the average for each duplicate set of results. Calculate the % degradation of a sample and the appropriate standards using the following formula:

$$A_t = \frac{C_o - C_t}{C_o}$$

where

A_t = % degradation at time *t* (days);
C_o = mean initial concentration (at day 0) of the test solution, in microequivalents of MBAS per litre or milligrams of BiAS per litre;

C_t = mean concentration of the test solution at time *t*, in microequivalents of MBAS per litre or milligrams of BiAS per litre.

h) Prepare a biodegradation curve by plotting, on linear graph paper, the percentage degradation (at time *t*) versus time *t*, allowing a margin of ± 5 percentile units for each point and draw a curve of best fit through the plotted points.

8 Assessment and expression of results

8.1 Determination of end-point

Find the point at which a line whose slope represents a 1 % degradation per day is tangential to the degradation curve for the soft standard. Read off the time t_d at this tangent point. The end-point of the test is taken at time 1.2 t_d days (to the nearest day) provided that this lies between 7 days and 21 days.

NOTE An example of the graphical construction for end-point determination is shown in Figure 3.

8.2 Criteria for validity of a set of tests

The criteria for the validity of a set of tests are as follows:

a) the end-point time shall be between 7 days and 21 days;

b) at the end-point, the soft standard shall have degraded by at least 90 %;

c) at the end-point, the hard anionic standard shall not have degraded by more than 50 %, and the hard non-ionic standard shall not have degraded by more than 60 %.

If these criteria are not met, the set of tests shall be rejected and repeated except that if a candidate material degrades by 80 % minimum at the end-point, but criterion b) has not been met, the test may be deemed valid and the candidate reported as being biologically soft.

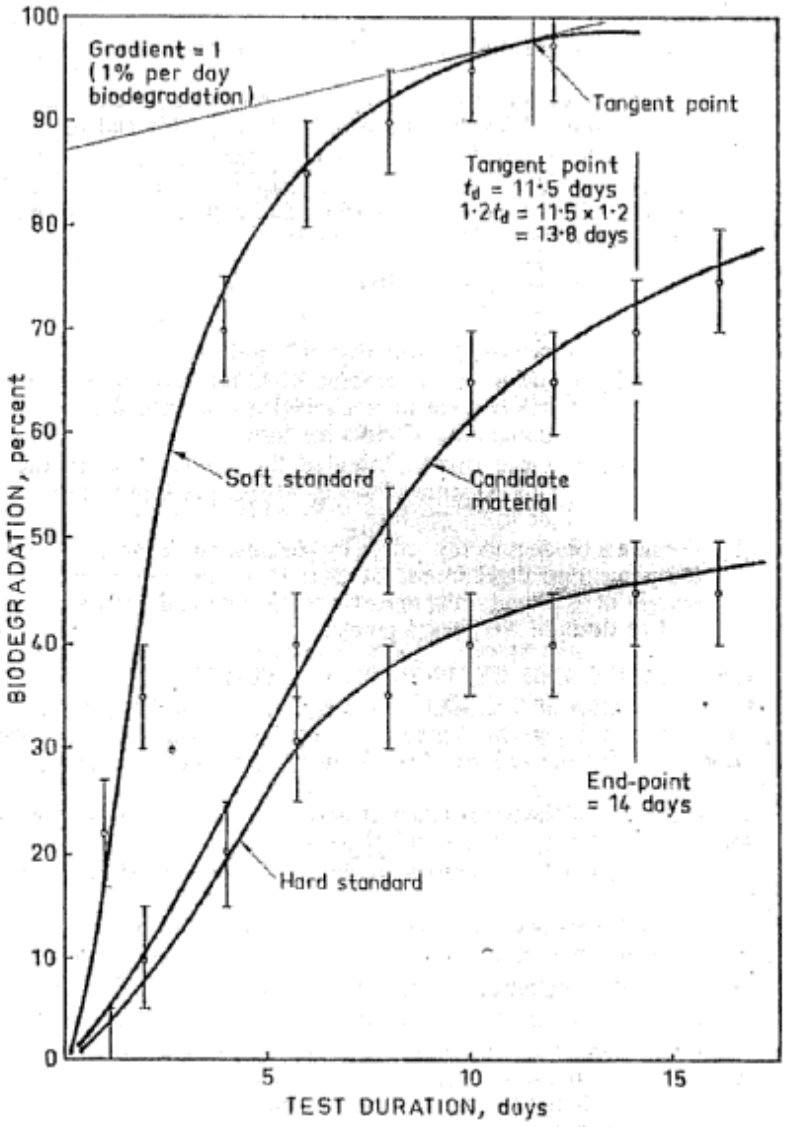


Figure 3 — Graphical construction for end-point determination

9 Test report

The following shall be reported:

a) materials which have degraded by 80 % or more at the end-point, as being biologically soft;

b) materials which have degraded by less than 80 % at the end-point, as being biologically hard.

If it is desired to give quantitative expression to the result, this may be done, but only if criteria a), b) and c) in 8.2 are complied with.

Annex A
(normative)

Method of analysis for anionic surfactants

A.1 The Annex describes a procedure for the determination of anionic surfactants, measured as methylene blue active substance (MBAS) in aqueous solutions, for following the progress of biodegradation of the stated surfactant type. The method is applicable over the range 0.00 microequivalents of MBAS to 0.45 microequivalents of MBAS.

A.2 Principle

Anionic surfactant shall be separated from an aqueous sample solution by direct extraction with chloroform in the presence of an alkaline methylene blue/borate solution at pH 10 to remove a number of potentially interfering substances. Subsequent treatment of the chloroform extracts with an acid methylene blue solution at pH 2 shall further ensure the removal of interfering substances so that only the anionic surfactant is retained in the chloroform layer.

The methylene blue, initially present as the chloride or sulphate salt, reacts with anionic surfactant to form a blue-coloured, chloroform-soluble complex, the absorbance of the colour being proportional to the concentration. The absorbance of the chloroform solution shall be measured by means of photo-electric colorimeter at a wavelength of approximately 655 nm and shall be compared with that produced under the same conditions by known concentrations of a standard anionic surfactant.

A.3 Reagents

Unless otherwise indicated, all reagents shall be of analytical grade.

- *sodium tetraborate (TBS), 0.05M solution* — Dissolve 19.07 g of borax ($Na_2B_4O_7.10H_2O$) in distilled water and make up to 1 litre in a volumetric flask;
- *sodium hydroxide*, *0.10M solution* — Dissolve 4.00 g of NaOH (pellets) in distilled water and make up to 1 litre in a volumetric flask;
- *sulphuric acid*, *0.5M* — Add 50.00 g of 98 % H_2SO_4 (ρ = 1.84) to approximately 500 mL of distilled water, cool to room temperature and make up to 1 litre;
- *methylene blue, 0.025 % m/V solution* — Dissolve 0.250 of methylene blue in 1 litre of distilled water.
- *chloroform* — AR grade;
- *sulphuric acid/nitric acid cleaning mixture* — Add 25 mL concentrated H_2SO_4 (ρ = 1.84) to 900 mL of distilled water, mix, cool to 20 — 30°C and add 75 mL — concentrated HNO_3 (ρ = 1.42).
- *linear alkyl benzene sulphonate (LAS), stock solution* — Weigh an amount of the reference material equal to 3 000 microequivalents LAS. Dissolve in distilled water and dilute to 1 litre. Prepare fresh each week.

- **BOD dilution water (see 3.1).**

A.4 Apparatus

A.4.1 Spectrophotometer or colorimeter

A sensitive spectrophotometer or colorimeter capable of operating within narrow spectral range with a maximum transmission at approximately 655 nm and fitted with 20 mm rectangular cuvettes.

A.4.2 Glassware

NOTE The quantities listed for items marked with * shall apply to the analysis of one sample only (in duplicate) and a blank.

a) *separating funnels*, *conical* — Fitted with a PTFE stopcock and glass stopper;

1 x 2 litre
6 x 500 mL *

b) *pipettes* — Bulb type;

2 x 10 mL capacity
2 x 20 mL capacity
2 x 25 mL capacity
2 x 50 mL capacity
2 x 100 mL capacity

c) *volumetric flasks* — Stoppered;

3 x 50 mL capacity *
2 x 1 000 mL capacity

d) *filter funnels* — Plain pattern;

3 x 50 mm diameter

e) *measuring cylinders* — Spouted type;

2 x 1 000 mL capacity
2 x 250 mL capacity
1 x 100 mL capacity
1 x 25 mL capacity

f) *micro-burette* — 10 mL capacity;

g) *glass wool* — Low in lead.

A.4.3 Cleaning of apparatus

All glassware used in this procedure must be scrupulously clean. The glassware used for the analysis of anionic surfactants shall be kept separate at all times. Use of sulphonate-based detergents in cleaning glassware shall be avoided and extreme precaution shall be taken to prevent contamination.

Glassware used for the analysis, i.e. separating funnels, stoppers, filter funnels, volumetric flasks and cuvettes, should be soaked overnight in the sulphuric acid/nitric acid mixture (see A.3) and thoroughly rinsed with either glass-distilled or deionized water before any analytical work is undertaken. The volumetric flasks and filter funnels shall be thoroughly dried prior to use.

A.5 Preparation of alkaline and acid methylene blue solutions

A.5.1 Alkaline methylene blue solution

The solution shall be prepared as follows:

a) charge a 2 litre separating funnel with the indicated volume of the following reagents for each separate analysis (including the blank):

   i) 100 mL distilled water;

ii) 20 mL alkaline borate solution (1:1 V/V mixture of 0.05M sodium tetraborate solution and 0.1 M sodium hydroxide solution);

iii) 10 mL methylene blue solution;

iv) 20 mL chloroform;

b) shake the mixture vigorously for 30 s and allow the phases to separate. Carefully draw off the chloroform layer and discard it;

c) add a further 20 mL per sample of chloroform to the mixture and repeat step b);

d) repeat step c), rinse the walls and the stopper with 20 mL to 30 mL of chloroform and discard the chloroform layer;

e) transfer 65 mL aliquots of this alkaline methylene blue solution to previously prepared (see A.4.3) 500 mL separating funnels.

A.5.2 Acid methylene blue solution

The solution shall be prepared as follows:

a) for each 65 mL of the aqueous phase remaining in the 2 litre separating funnel, add 50 mL of distilled water and 10 mL of chloroform. Shake the mixture vigorously for 30 s and allow the phases to separate. Draw off and discard the chloroform layer. Add 3 mL of 0.5 M sulphuric acid for every 65 mL of the original aqueous phase remaining in the separating funnel and mix.

b) transfer 118 mL aliquots of this acid methylene blue solution to a second series of 500 mL separating funnels.

A.6 Procedure

A.6.1 Calibration procedure

Take a 10 mL aliquot of the LAS stock solution and dilute to 1 litre with distilled water (concentration = 0.030 microequivalents of LAS per millilitre).

By means of a micro-burette, measure 0 mL, 3 mL, 5 mL, 7 mL, 9 mL, 11 mL, 13 mL and 15 mL of this diluted alkaline solution into a previously cleaned series of 500 mL separating funnels each containing 65 mL of the alkaline methylene blue/borate solution.

To each separating funnel add sufficient BOD water to produce a total volume of 315 mL. Using the analytical method as described in A.6.2, determine the corrected absorbance on the series of dilutions containing between 0.0 and 45 microequivalents of anionic soft surfactant standard. Prepare a calibration curve of LAS quantity (microequivalents) versus corrected absorbance.

A.6.2 Analytical method

A.6.2.1 Volume of sample

Select from Table A.1 the recommended volume of biodegradation solution for the expected concentration of the anionic surfactant under test.

Table A.1 — Recommended sample sizes

Expected LAS or TBS Concentration microequivalents/L	volume of sample, mL	volume of BOD solution mL
12 — 30	10	240
9 — 21	20	230
2.0 — 9	50	200
1.0 — 2.0	100	150
0.3 — 1.0	250	Nil

A.6.2.2 Extraction and colour development

a) Add volumes of sample and BOD solution as given in Table A.1 to the 500 mL separating funnel containing 65 mL of the chloroform-washed alkaline methylene blue solution. Add 15 mL of chloroform;

b) Simultaneously carry out a blank determination using 250 mL of BOD solution but omitting the sample;

c) Stopper the separating funnel and shake for 1 min. and allow the layers to separate. Excessive agitation may cause emulsion difficulties. Some samples require a longer period of phase separation than others. Before draining the chloroform layer, swirl the sample gently, then allow to settle. Draw off the chloroform layer into a second 500 mL separating funnel containing 118 mL of acid methylene blue solution. Rinse the delivery tube of the first separating funnel with approximately 2 mL of chloroform;

d) Stopper the second separating funnel containing the acid methylene blue solution and chloroform extracts and shake for 1 min. Emulsions do not form at this stage. Allow the phases to separate, swirl the contents and draw off the chloroform phase through glass wool, which has been previously wetted with chloroform, into a dry 50 mL volumetric flask. Rinse the delivery tube of the separating funnel with approximately 2 mL of chloroform collecting the washings in the volumetric flask;

(e) Repeat the extraction as in c) and d) using 15 mL of chloroform and rinse the stoppers, internal walls and delivery tubes of the separating funnels with 2 x 5 mL of chloroform. Finally, rinse the glass wool and filter funnel stem with 2 mL to 3 mL of chloroform collecting the washings in the volumetric flask. Dilute to the mark with chloroform and mix well.

A.6.2.3 Determination of absorbance

Determine the absorbance of the solution within 30 min. of final preparation at a wavelength of approximately 655 nm using an absorption cell of 20 mm against a blank of chloroform.

A.7 Calculations

a) correct the observed absorbance by means of the following equation:

Corrected absorbance = $R - B$

where

R = observed absorbance;
B = absorbance obtained with the 250 mL BOD solution blank.

NOTE The absorbance of the chloroform phase of the chemical blank measured against chloroform should not exceed the value of 0.008/10 mm of layer thickness (e.g. Klett Reading = 4). Higher absorbance figures may indicate imperfect washing of the glassware.

b) convert the corrected absorbance, after correction for the reagent blank value, to microequivalents of MBAS from the previously prepared calibration curve (see A.6.1);

c) calculate the concentration of MBAS in the test solution using the following formula.

$$C = \frac{E \times 1\,000}{V}$$

where

C = the MBAS concentration, in microequivalents/L;
E = the MBAS quantity (microequivalents) corresponding to the correct absorbance;
V = the volume of sample, in millilitres.

A.8 Report

The report shall state the concentration of surfactant remaining at various times *t.*

NOTE 1 The above method is based on that described by Longwell, J. and Maniece, W. D. *Analyst,* 80 (London) 1955, p. 167. Modifications are included attributable to Slack, J.G. *Analyst,* 84 (London).

NOTE 2 The concentration of the surfactant can, where circumstances dictate, be converted to either mgL, g/m^3 (p.p.m. *(m/V)* or p.p.b. *(m/V)* as follows:

$$\text{MBAS, mg/L, g/m}^3\ \{\text{ppm } (m/V)\} = \frac{E \times M}{V}$$

$$\text{MBAS, mg/m}^3\ \{\text{ppb } (m/V)\} = \frac{E \times M}{V \times 1000}$$

where

E = MBAS corresponding to the corrected absorbance, in microequivalents;
M = molar mass of a nominated standard surfactant e.g.

a) sodium dodecylbenzesulphonate ($C_{12}H_{25}C_6H_4SO_3Na$; M = 348);

b) manoxol OT or Aerosol OT (Na salt of *bis* (2-ethylhexyl) sulphosuccinate; M = 444).

V = is the volume of sample, in millilitres.

Annex B
(normative)

Method of analysis for non-ionic surfactants —TLC procedure

B.1 Introduction

This annex describes a procedure for the determination of non-ionic surfactants by means of thin layer chromatography.

B.2 Principle

The non-ionic actives shall be salted out with magnesium sulphate and extracted with chloroform. Subsequent treatment of the chloroform extracts with acid and alkaline sodium chloride solutions shall ensure the removal of potentially interfering substances. After washing, the extracts shall be evaporated to dryness and redissolved in a known volume of chloroform and analyzed by thin layer chromatography using a combination of selective solvents for the development of the thin layer chromatograms and a sensitive spray reagent for the detection of the non-ionics on silica gel thin layer plates. The non-ionics shall be quantified by comparing the chromatograms of the samples with those of identical compounds at known concentrations.

B.3 Reagents

B.3.1 General

Methanol — AR grade.
Chloroform — AR grade.
Magnesium sulphate heptahydrate — CP grade.
Sodium chloride — CP grade.
Hydrochloric acid, 1M solution sodium hydroxide — 2M solution.

B.3.2 Thin layer chromatography

Spray reagents — Prepare by mixing the following reagents in the order given:

a) 10 mL of modified burger reagent made by suspending 1.7 g of basic bismuth (III) nitrate AR ($BiO.NO_3.H_2O$) in 200 mL glacial acetic acid; add a solution of 40 g of potassium iodide in 100 mL water, mix well and then dilute with distilled water to 1 litre;

b) 1 mL orthophosphoric acid, 85 %;

c) 10 mL ethanol, AR;

d) 5 mL of an aqueous solution containing 200 g of barium chloride dihydrate per kilogram. TLC running solvent. Prepare a 90:10 mixture by volume of chloroform: methanol standard non-ionic solutions. Each candidate and reference standard shall be compared with a standard chloroform solution of its own non-degraded equivalent.

B.3.3 Wash solutions for the extraction of non-ionic surfactant

Prepare about 20 litres of each of the following:

- *magnesium sulphate* — Dissolve 600 g of magnesium sulphate heptahydrate in 1 litre of distilled water.

- *sodium chloride/hydrochloric acid solution* — Dissolve 250 g of sodium chloride in 1 litre of 1M hydrochloric acid.

- *sodium chloride/sodium hydroxide solution* — Dissolve 250 g of sodium chloride in 1 litre of 2M sodium hydroxide solution.

The above wash solutions shall be extracted with 2 x 50 mL portions of AR chloroform per litre of wash solution prior to the extraction of the non-ionic surfactant. This can conveniently be carried out by bulk extracting the appropriate volumes of magnesium sulphate (300 mL x number of samples in duplicate), acid and alkaline sodium chloride solutions (200 mL x number of samples in duplicate) using the 5 litre separating funnel. The separate solutions are shaken for 2 min, the phases allowed to separate and the chloroform discarded. This procedure is then repeated and the chloroform again discarded. Appropriate volumes of the wash solutions are then transferred to the requisite number of 1 litre and 500 mL separating funnels.

B.3.4 Standard stock solution

Prepare by dissolving 0.6250 ± 0.0005 g active matter (see footnote to 5.2) in 250 mL chloroform.

B.3.5 Standard spotting solution

Prepare by diluting a 10 mL aliquot of the standard stock solution to 100 mL with chloroform (concentration = 0.25 µg/µL of spotting solution). See Table B.1.

Table B.1 — Standard spot applications

Spotting volume, µl	Mass of standard non-ionic applied, µg
1	0.25
2	0.50
4	1.00
6	1.50
8	2.00
10	2.50
12	3.00
14	3.50
16	4.00
18	4.50
20	5.00

B.4 Apparatus

B.4.1 Glassware

NOTE The quantities listed for items marked with * apply to the analysis of one sample only (in duplicate).

a) separating funnels, spherical, and fitted with a PTFE stopcock and glass stopper;
1 x 5 litre*.

2 x 1 litre*.
2 x 500 mL*.

b) pipettes, bulb type;
1 x 1 mL capacity.

1 x 2 mL capacity.
1 x 50 mL capacity.
1 x 100 mL capacity.

c) measuring cylinders, spouted type;
1 x 1 000 mL capacity.
1 x 250 mL capacity.
1 x 50 mL capacity.

d) volumetric flasks, stoppered;
1 x 250 mL capacity.
1 x 100 mL capacity.

e) filter funnels, plain pattern;
2 x 50 mm diameter*.

f) flasks, round bottom;
2 x 250 mL capacity.

g) glass vials, 10 mL or 5 mL stoppered test tubes, glass rod, thin (5 mm), length approximately 300 mm;

i) glass wool;

k) filter paper, Whatman No 1 (110 mm).

B.4.2 Thin layer chromatography equipment

a) thin layer chromatography plate spreader;

b) chromatographic glass plates, 200 mm x 200 mm;

c) silica gel;

NOTE Commercially prepared silica gel TLC sheets may not be suitable for this analysis.

d) TLC plate rack;

e) *oven* — Capable of holding TLC plates and maintaining a temperature of 110 ± 2 °C;

f) stop clock or stop watch;

g) TLC spray unit;

h) disposable micropipettes, 2 µL capacity (e.g. drummond micro-caps);

j) tank, for developing TLC plates, shall be prepared as follows:

A glass tank measuring approximately 220 mm x 90 mm x 220 mm and fitted with a flat covering lid is required. Line the walls of the tank with filter paper and add running solvent to a depth of about 5 mm. Prepare the tank approximately 30 min before use to enable the solvent to ascend the filter paper and saturate the air in the tank. Maintain the developing tank at as low a temperature as possible; a temperature of 20°C has been found suitable.

Locate the tank in a draught-free position and not in direct sunlight.

B.5 Procedure

B.5.1 Procedure for extraction of non-ionic surfactant

a) Transfer 300 mL of the magnesium sulphate solution to each I litre separating funnel. Transfer 200 mL of the acidic wash solution to each of the first set of 500 mL separating funnels. Transfer 200 mL of the alkaline wash solution to each of the second set of 500 mL separating funnels;

b) Pipette 50 mL of the test solution into the magnesium sulphate solution. Add 50 mL of chloroform and shake vigorously for 1 min. Allow the solution to stand for 10 minutes;

NOTE 1 As the biodegradation progresses it will be necessary to use a larger test aliquot in order to recover a suitable amount of active material for analysis (see Table B.2 for recommended sample sizes) .

NOTE 2 If an emulsion persists, stir the chloroform layer gently with a thin glass rod. In most cases, the chloroform layer will separate sufficiently well to allow it to be run off.

c) draw off the chloroform layer into the acidic wash solution. Rinse the walls of the separating funnel with 10 mL of chloroform and swirl gently. Run this chloroform rinse into the cidic wash solution;

d) extract the test solution with three further 50 mL portions of chloroform;

NOTE 1 If the emulsion from the first chloroform extraction persists (see note 2 to step b) above), pass it through a pad of approximately 0.8 g of glass wool in a filter funnel. If a glass wool pad is used, run each chloroform extract in turn through the same pad into the acidic wash solution.

NOTE 2 If stable emulsions are formed thus preventing a reasonable separation, reject the first solution and start again, reducing the volume of the test solution by at least one-half by boiling it in a beaker. Cool the remaining solution and transfer it to a measuring cylinder. Wash the walls of the beaker with 20 mL of 1 M hydrochloric acid solution and transfer the washings to the measuring cylinder. Use this solution in place of the test solution as described in step a) above.

e) Shake the acidic wash solution and the combined chloroform extracts in the first 500 mL separating funnel for 1 min and then allow to stand until the phases separate. Run off the chloroform layer into the alkaline wash solution in the second 500 mL separating funnel. Shake the acid wash solution with a further 10 mL of chloroform and add this chloroform wash to the extracts in the alkaline wash solution;

f) Shake the alkaline wash solution and the combined chloroform extracts in the second 500 mL separating funnel for 1 min and then allow to stand until the phases separate. Run the chloroform layer through a No 1 filter paper into a clean 250 mL round bottom flask. Shake the solution with a further 10 mL of chloroform and pass this chloroform through the filter paper;

g) Evaporate the chloroform extracts to dryness on a water bath (fume cupboard). A gentle stream of dry oil-free air or nitrogen can be directed over the surface of the solution to accelerate evaporation. Allow the flask to cool to room temperature and dissolve the residue in 2 mL of chloroform. Transfer the solution to a stoppered vial or test tube.

B.5.2 Procedure for thin layer chromatography (TLC)

a) prepare thin layer chromatographic plates measuring 200 mm x 200 mm with the aim of obtaining a wet thickness of 0.5 mm;

NOTE 1 The manufacturer's instructions are usually followed in preparing the gel. In general, it shall be found that 30 g of the silica gel shaken for 75 s with 60 mL of distilled water will yield a slurry of the correct consistency for a number of commercially available plate spreaders.

NOTE 2 The slurry shall be immediately spread on the plates as undue delay will cause the mixture to set thus yielding a variable coating. The plates shall be allowed to dry in air overnight.

NOTE 3 The plates shall be activated by heating at 110 °C for 1 hour .

NOTE 4 Activated plates may be stored in a desiccator or suitable airtight container over silica gel, but plates that have been stored for more than 2 days must be reactivated as in Note 3, before use.

b) deliver a series of volumes of standard spotting chloroform solution to the TLC plate to contain 0.25 µg to 5.0 µg of BiAS (see Table B.1). The spots formed shall be used for comparison with the test sample. Reference spots shall bracket the test sample spots;

c) deliver 20 µg or 40 µl volumes of the test extract (see Table B.2 and following notes). The test samples shall be spotted in duplicate and on the same plate as the respective reference standard;

NOTE 1 To facilitate the estimation of the concentration of BiAS remaining on any test-day, the Table B.2 may be of assistance in selecting the appropriate test solution aliquots, chloroform take-up volumes for the extracts, TLC spotting volumes for the extracts, and the desired spotting volumes of the matching non-ionic standards.

NOTE 2 During the application of the solutions to the plates, marring of the thin layer shall be avoided and the size of the spots shall be kept to a minimum by directing a gentle stream of dry air or nitrogen towards the region of application to give rapid evaporation of the chloroform.

NOTE 3 Spotting shall be carried out about 20 mm from the bottom of the plate.

NOTE 4 Total volumes delivered to one spot shall be accomplished by overspotting with 2 µl increments.

NOTE 5 In overspotting it is necessary to ensure that the solvent from the previous application has evaporated before the next application is made. Consistent spot areas are desirable.

NOTE 6 The optimum mass loading for a 0.5 mm TLC layer for this work has been found to be between 0.5 mg and 5.0 µg.

Table B.2 — Quantities for sampling and spotting

Expected BiAS concentration mg/l	Test solution Sample aliquot mL	Chloroform take-up volume mL	TLC spot Volume for extract µl	Mass applied to TLC spot µg	Concentration factor *f*
5.5 – 10.0	50	2.0	20	2.5 — 5.0	1
2.5 – 5.0	50	1.0	20	2.5 — 5.0	2
1.0 – 2.5	50	0.5	20	2.0 — 5.0	4
0.5 – 1.0	50	0.5	40	2.0 — 4.0	8
0.0 – 0.5	100	0.5	40	0.0 — 4.0	16

d) Place the plate in the prepared developing tank and allow the chromatogram to run until the solvent front is between 100 mm to 150 mm above the starting line;

e) Remove the plate from the developing tank and leave on a rack until visibly dry. heat the plate in an oven at about 100 °C for 10 minutes. Allow the plate to cool in air and spray it with spray reagent (fume cupboard) whilst still warm;

NOTE A number of light sprayings is preferable to one heavy application.

f) Assess all tests plates immediately after colour development and, without undue delay, cover the chromatogram with a glass plate to prevent oxidation of the spray reagent.

B.6 Calculations

Calculate the concentration of non-ionic surfactant in the test sample by means of the following formula:

$$C = 2 \times m \times f$$

where

C = BiAS concentration, in milligrams per litre, at time t;

m = mass of BiAS, in micrograms, in the matching spot of standard spotting solution (see Table B.1);

f = concentration factor depending on chosen test conditions (see Table B.2).

B.7 Report

The report shall state the concentration of surfactant remaining at various times (t).

NOTE The method is broadly on the thin layer chromatographic (TLC) method described by Patterson, S.J.,S.J Hunt, E.C and Tucker, K.B.E(Laboratory of the Government Chemist, UK. Ministry of Technology) *J. Proc. Inst. Sew. Purif.,2* (London) 1966, P. 190.

Annex C
(normative)

Method of analysis for non-ionic surfactants wickbold procedure

C.1 Introduction

This Annex describes the wickbold method for the analytical determination of non-ionic surfactants. It is an alternative procedure to the thin layer chromatography method described in Annex B. It is considered to possess a legitimate status among the proven methods of analysis for non-ionics This method requires a larger biodegradation test vessel than the TLC method described in Annex B. The biodegradation procedure has been written around this larger vessel, but duplicates from degradation in the smaller vessel may be combined for analysis by means of the Wickbold procedure.

The method describes the determination of non-ionic surfactants of the alkyl phenol ethoxylate and alcohol ethoxylate types with chain lengths between 6 moles and 30 moles of ethylene oxide per mole of alkyl phenol or alcohol in aqueous solutions, for following the progress of bio-degradation of the stated surfactant type. The method is applicable to samples containing 100 µg to 800 µg of bismuth active substance (BiAS).

C.2 Principle

The non-ionic actives shall be extracted from the aqueous solution with ethyl acetate by a gas stripping technique. After phase separation and exaporation of the solvent, the non-ionics shall be precipitated in an aqueous solution as a bismuth complex. The precipitate shall be subsequently filtered and dissolved in an ammonium tartrate solution and titrated potentiometrically with a standard pyrrolidinedithiocarbamate solution in con-junction with platinum calomel electrodes (or silver/silver chloride).

C.3 Reagents

C.3.1 General

Unless otherwise indicated, all reagents shall be of analytical grade. All aqueous solutions shall be made up in distilled water.

Ethyl acetate — AR.

Sodium hydrogen carbonate ($NaHCO_3$) — AR.

Sodium chloride (NaCl) — AR.

Hydrochloric acid, 1 % (V/V) — by dilution of concentrated (10M) acid.

Methanol, AR.

Bromocresol purple indicator solution — Dissolve 0.10 g in 100 mL of methanol.

Precipitating reagent — A mixture of 2 volumes of solution A and 1 volume of solution B below. The mixture shall be stored in a brown bottle and can be used up to 1 week after mixing.

Solution A — Dissolve 1.70 g of basic bismuth (III) nitrate ($BiO.NO_3\ H_2O$) in 20 mL glacial acetic acid and make up to 100 mLwith water. Dissolve 65 g of potassium iodide (KI) in 100 mL of water. Mix these two solutions in a 1 000 mL measuring flask, add 200 mL of glacial acetic acid and make up to 1 litre with water.

Solution B — Dissolve 290 g of barium chloride dihydrate ($BaCl.H_2O$) in 1 litre of water.

NOTE Discard the precipitating reagent if a precipitate is formed on standing.

Glacial acetic acid, 99 – 100 % (lower concentrations are unsuitable).
Ammonia solution (ρ=0.880).

Ammonium tartrate solution — Mix 12.4 g of tartaric acid and 13.0 mL of ammonia solution (p = 0.880) and make up to 1 litre with water. (The equivalent amount of ammonium tartrate may also be used and made up to 1 litre.)

standard acetate buffer — Dissolve 40 g of solid sodium hydroxide in 500 mL of water in a 1000 mL volumetric flask. Add 120 mL of glacial acetic acid and mix thoroughly. Allow to cool and make up to 1 litre with water.

Pyrrolidinedithiocarbamate solution carbamate solution 0.0005M — Dissolve 103 mg of sodium pyrrolidinedithiocarbamate in about 500 mL of water. Add 10 mL of n-amyl alcohol and 0.5 g of sodium hydrogen carbonate and make up to 1 litre with water.

NOTE It is recommended that the contents of the flask be shaken thoroughly after the solution has been made up to 1 litre, as a cloudy solution can otherwise be obtained.

Copper sulphate solution — 0.00025M, for standardization of carbamate solution (see C.5.6).

C.3.2 Stock solution

Mix 1.249 g of cupric sulphate pentahydrate ($CuSO_4.5H_2O$) and 50 mL of 0.5M sulphuric acid and make up to 1 litre with water.

C.3.3 Standard solution

Mix a 50 mL aliquot of the stock solution and 10 ml of sulphuric acid (0.5M) and make up to 1 litre with water.

C.4 Apparatus

C.4.1 Glassware

NOTE The quantities in items marked * below apply to the analysis of one sample (in duplicate).

(a) *separating funnels,* spherical or conical, and fitted with a PTFE stopcock and glass stopper;
2 x 250 mL capacity.

(b) *pipettes,* bulb type;
1 x 5 mL capacity 2 x 10 mL capacity.
2 x 50 mL capacity.
2 x 100 mL capacity.

(c) *pipettes,* graduated;
2 x 5 mL capacity.
2 x 10 mL capacity.

d) *measuring cylinders,* spouted type;
1 x 1 000 mL capacity.
1 x 250 mL capacity.
2 x 50 mL capacity.

e) *beakers,* conical form, wide mouth with spout;
2 x 300 mL capacity*
2 x 2 000 mL capacity*

f) *filter funnels,* plain pattern;
2 x 50 mm diameter.

g) *flasks,* filter, with side arm, heavy wall, fitted with quickfit B24/29 neck;
2 x 1 000 mL capacity.
2 x 250 mL capacity.

h) *burette,* 50 mL capacity class A (for manual titrations);

j) *glass balls,* undrilled, approximately 4 mm diameter;

k) *glass wool;*

l) *filter paper*.

Whatman No 1, 110 mm diameter.
Whatman GF/A glass fibre paper, 21 mm diameter.

C.4.2 Other apparatus

a) gas stripping apparatus (see Figure C.1);

Either nominated size may be used. The diameter of the sintered disc must be the same as the internal diameter of the cylinder.

b) *scrubbing bottle,* 250 mL capacity (see Figure C.1);

c) *gooch filter adaptors,* quickfit, two with 24/29 cone and 34/35 socket;

d) *crucible,* gooch type with perforated base, diameter of base 21 mm;

e) *rubber ring adaptors,* 2 x size 4 to ensure a tight seal between the crucible and filter adaptors described above;

f) magnetic stirrer, capable of approximately 250 r/min minimum;

g) magnetic stirrer followers, encased in PTFE, length 40 mm;

h) gas flow meter, orifice or capillary type, capable of measuring gas flow rates of 0.5 to 1.5 L/min;

j) recording potentiometer, fitted with platinum and calomel (or silver/silver chloride) electrodes with a range of 250 mV, and an automatic burette capable of titrating at a minimum rate of 0.5 mL/min. Alternative manual equipment may be substituted if a recording type is not available.

C.5 Procedure

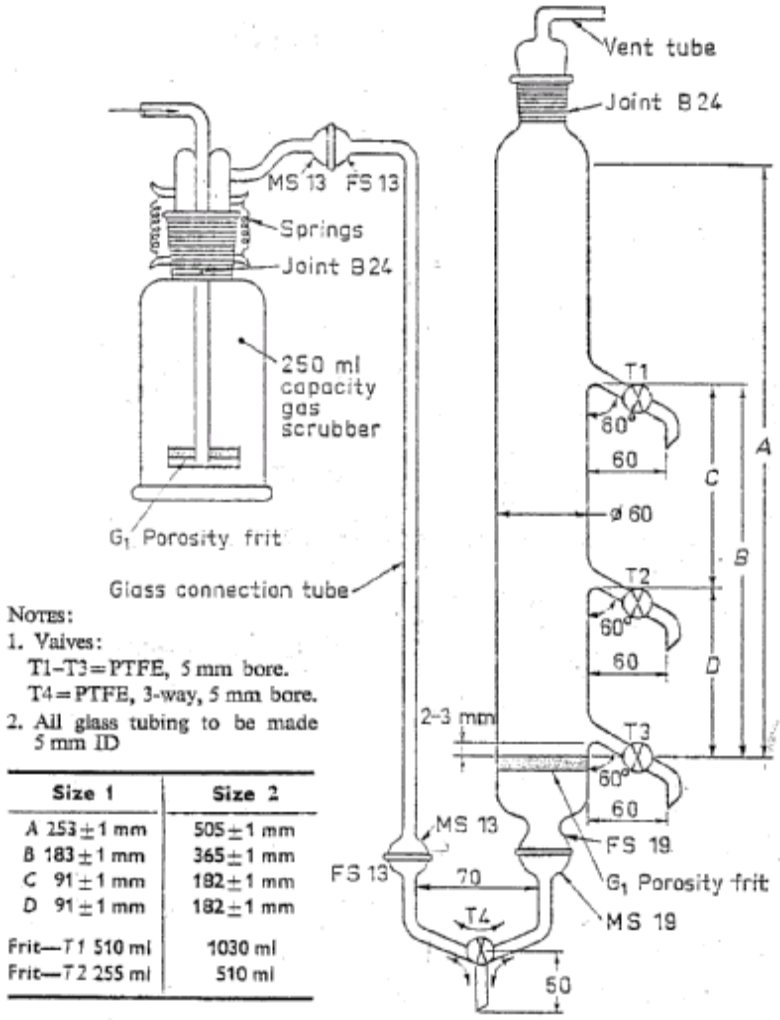
C.5.1 Procedure for extraction of non-ionic surfactant

a) Filter the aqueous sample through a qualitative filter paper;

b) Select the recommended volume of biodegradation solution for the expected concentration of the non-ionic surfactant under test from Table C.1;

Table C.1 — Recommended sample sizes

Expected concentration mg BiAS/L	Volume of test solution aliquot mL	BiAS content of test solution aliquot µg
5.0 – 10.0	50	250 – 500
2.5 – 5.0	50	125 – 250
1.0 – 2.5	100	100 – 250
0.25 – 1.0	500	125 – 500



NOTES:

1. Valves:
   T1–T3 = PTFE, 5 mm bore.
   T4 = PTFE, 3-way, 5 mm bore.
2. All glass tubing to be made 5 mm ID

	Size 1	Size 2
A	253 ± 1 mm	505 ± 1 mm
B	183 ± 1 mm	365 ± 1 mm
C	91 ± 1 mm	182 ± 1 mm
D	91 ± 1 mm	182 ± 1 mm
Frit—T1	510 ml	1030 ml
Frit—T2	255 ml	510 ml

Figure C.1 — Gas stripping apparatus

c) Charge the stripping apparatus, previously rinsed with ethyl acetate, with a test solution aliquot containing 100 µg to 800 µg of BiAS;

d) Add sufficient NaCl and $NaHCO_3$ to the stripping apparatus to give a final concentration equivalent to 100 g of NaCl and 5 g of $NaHCO_3$ per litre of solution in the apparatus. (This improves the separation effect during the extraction.);

NOTE 1 If the sample volume is small, the salts shall be dissolved in distilled water and then added to the stripping apparatus. When large sample volumes are used, it may be necessary to add the salts in solid form and dissolve them by passing air or nitrogen through the apparatus. If necessary, distilled water shall be added to raise the level of the solution to either stopcock T1 or T2 depending on the size of the apparatus.

NOTE 2 In cases where the sample volume required is too large for the apparatus, the sample shall be transferred to a 2 000 mL beaker and the volume reduced to approximately 500 mL by boiling on a moderate hotplate. After being cooled to room temperature, the residue shall then be transferred quantitatively to the stripping apparatus. In cases of rapidly degrading samples, it is advisable that mercuric chloride be added to give a concentration of 50 ppm in the solution to prevent further degradation.

e) Taking care to avoid excessive phase disturbance, add 100 mL of ethyl acetate to the surface of the solution in the apparatus. The scrubbing bottle (see Figure C.1) shall also be approximately two-thirds filled with ethyl acetate;

f) Pass air (or nitrogen) gas stream through the apparatus at a rate of 50 – 60 litres/h for a period of 5 minutes;

NOTE The gas flow rate must be maintained within the above range with the pre- scribed flow meter to ensure

that excessive phase separation does not occur. Failure to do so will result in interfacial turbulance and subsequent dissolution of ethyl acetate in the aqueous phase and return of surfactant to the aqueous phase.

g) Transfer the organic phase to a 250 mL separating funnel and rinse the delivery tube of the stripping apparatus with approximately 5 mL of ethyl acetate. Return any water carried over with the organic phase to the separating funnel back to the stripping apparatus;

NOTE If there is a reduction of more than 20 % by volume of the organic phase through solution in water, the mixture must be rejected and a fresh test solution aliquot obtained.

h) Charge the gas stripping apparatus with a further 100 mL of ethyl acetate and again pass air (or nitrogen) through the solution for 5 min at a rate of 50 – 60 litres/h. Combine the ethyl acetate extracts in the 250 mL separating funnel. Wash the walls and the delivery tube of the stripping apparatus with approximately 20 mL of ethyl acetate and transfer the washings to the separating funnel. Discard the aqueous phase from the separating funnel and pass the extract through a No 1 filter paper into a clean 400 mL beaker. Rinse the separating funnel and filter with 2 mL x 10 mL of ethyl acetate;

j) Evaporate the combined extracts to dryness on a water-bath (fume cupboard).

NOTE A gentle stream of dry, oil-free air or nitrogen can be directed over the surface of the solution to accelerate evaporation.

C.5.2 Precipitation and filtration of non-ionic surfactant

a) dissolve the residue {from C.5.1, step j)} in 5 mL of methanol. Then add 40 mL of water together with 3 – 5 drops of bromocresol purple indicator solution and stir the mixture with a magnetic stirrer. Add 1 % hydrochloric acid until the mixture turns yellow (1 – 3 drops are generally sufficient);

(b) add 30 mL of precipitating reagent (see C.3.1) from a measuring cylinder. Continuously stir the solution to allow the precipitate to form and then continue stirring for 10 min. Allow the mixture to stand for 5 minutes. Filter the precipitate through a Gooch crucible fitted with glass fibre filter paper and supported by the filter adaptor in the 1 000 mL litre flask;

NOTE Prior to the filtration, the filter mat shall be wetted under slight suction with approximately 2 mL of glacial acetic acid.

(c) thoroughly wash the beaker, magnet and crucible with 40 – 50 mL of glacial acetic acid (see notes below).

NOTE 1 It is not necessary to transfer the precipitate adhering to the sides of the beaker quantitatively to the filter mat as the solution of the precipitate for the titration is returned to the beaker and thus any precipitate remaining will then be dissolved.

NOTE 2 Care should be taken to ensure that the glass fibre filter mat is properly seated as losses can occur when even a small crease is present.

NOTE 3 It is important that all traces of the precipitating reagent be removed from the beaker and crucible. Failure to do so will yield high blank titres and erroneous surfactant levels.

C.5.3 Dissolution of the precipitate

a) Transfer the Gooch crucible containing the precipitate to the adaptor of the 250 mL filter flask. Apply gentle suction and add 3 x 10 mL aliquots of hot (50 °C – 60 °C) ammonium tartrate solution to dissolve the precipitate. Subsequently wash the Gooch crucible and glass adaptor with 50 —100 mL of distilled water;

b) Rinse the sides of the beaker used for the precipitation with 20 mL of hot ammonium tartrate solution to dissolve any remaining precipitate and then quantitatively transfer the contents of the filter flask to the beaker with the aid of approximately 100 mL of distilled water.

C.5.4 Titration

a) Stir the solution (from C.5.3, step b)) with a magnetic stirrer, add 3 – 5 drops of bromocresol purple indicator solution and add concentrated ammonia until the colour turns violet (0.2 – 0.5

mL are generally sufficient to neutralize the residual acetic acid). Then add 10 mL standard acetate buffer solution;

b) Place the beaker on the titration stand and adjust its position so that the electrodes are about half immersed. Commence stirring and allow the system to stabilize for approximately 3 – 5 minutes with the electrodes immersed. Fill the burette with the 0.0005M carbamate solution and place the burette in position in the titration assembly so that the tip extends approximately 25 mm below the surface of the liquid in the beaker. Adjust the speed of the stirrer to give vigorous stirring without splashing. Record the initial burette and meter (cell potential) readings;

c) Adjust the titration rate to 1.5 – 2.0 mL/min, and commence the titration. Continue the titration beyond the definite inflection point (see Figure C.2) and determine the end-point;

NOTE The end-point shall be defined by the intersection of the tangents to the two branches of the curve and is illustrated in Figure C. 2.

d) Remove the titrated solution, rinse the electrodes well with distilled water and wipe with a clean dry tissue. Burnish the platinum or silver electrode lightly with a fine emery cloth to remove any possible build-up of bismuth or copper dithiocarbamate deposits. Between successive determinations during the same day, immerse the electrodes in approximately 100 mL of distilled water containing 10 mL of the standard acetate buffer solution.

NOTE 1 Although it is important to wait for stability it is also important that the duration of the titration be as short as possible in order to avoid drifting, etc. Once commenced, the titration shall be not interrupted and resumed later.

NOTE 2 *Manual titrations.* In the case of a manual titration, suitable portions of carbamate solution are added and, after waiting until a constant potential has been established, the burette and meter readings are recorded for each addition. The potential is considered to be constant if it changes less than 5 mV/min.

At the start of a manual titration, when the change in potential is at a minimum for each increment or carbamate solution added, volumes as large as 1.0 – 2.0 mL can be added. When the rate of change of potential exceeds 5 mV /0.1 mL, the increments of solution are reduced to 0.05 mL. The titration is continued until the rate of change of cell potential is less than 3 mV per 0.1 mL of carbamate solution added. The cumulative volumes of carbamate solution are then plotted against the corresponding cell potentials and the end-point determined by the method described in step c) above.

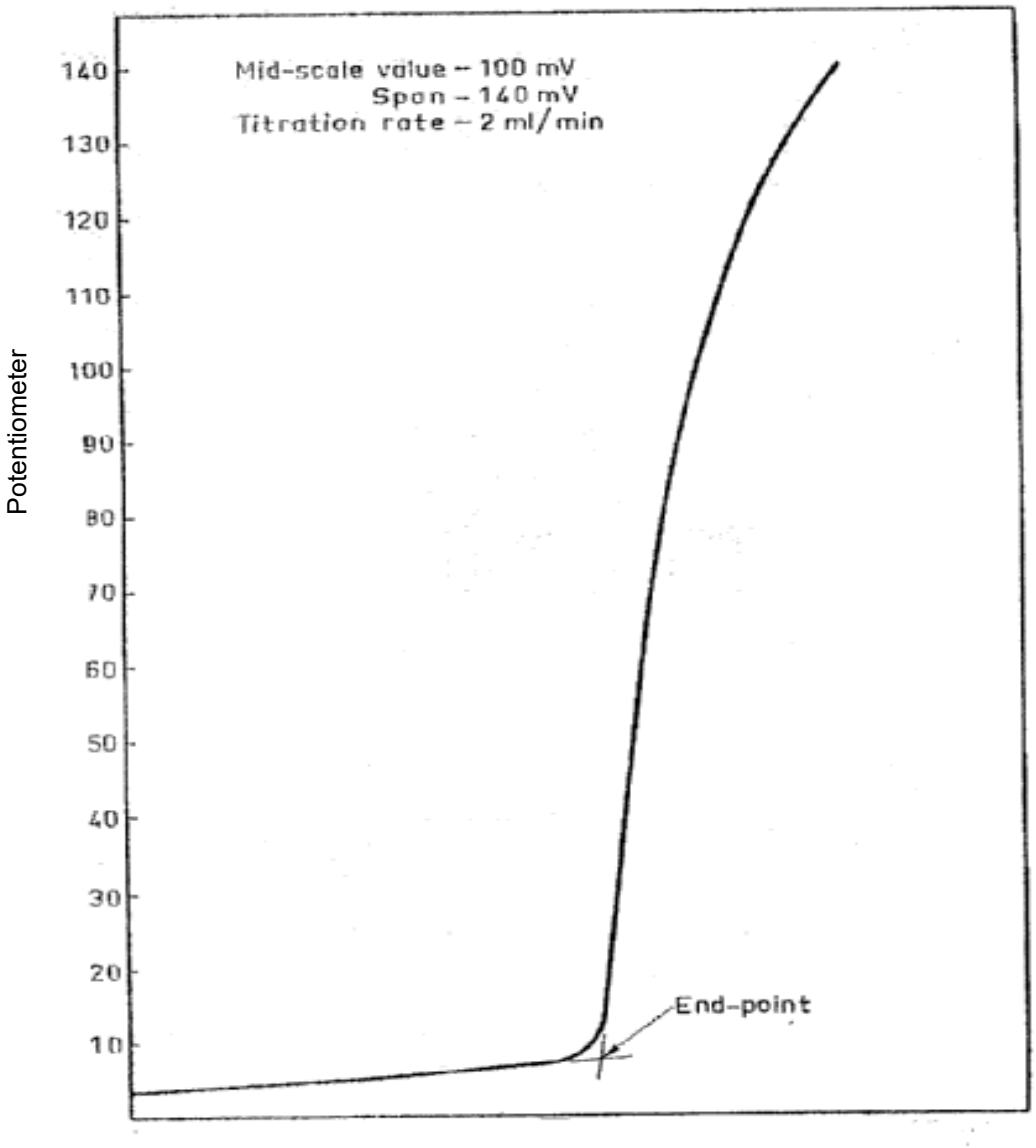


Figure C.2 — Example of titration curve

C.5.5 Blank determination

At the same time as the actual determination, run a blank determination with 5 mL methanol and 40 mL water, and continue according to the instructions described in C.5.2. The blank titre shall be below 1 mL, otherwise the purity of the reagents used is suspect, especially their heavy metals content. The blank titre must be taken into account for the calculation of the result.

C.5.6 Standardization of the carbamate solution

The carbamate solution must be standardized daily. A volume of 10.0 mL of the 0.00025M copper sulphate solution (see C.3.1) is titrated after the addition of 100 mL water and 10 mL standard acetate buffer (see C.3.1).

If the volume of carbamate solution = x mL, then the factor *f* is determined by the following formula:

$$f = \frac{10}{x}$$

All results of titrations are multiplied by this factor.

NOTE The standardization factor *f* of the carbamate solution shall be 1.0 ± 0.01 and if it drops to below 0.97 the solution shall be discarded and a fresh one prepared.

C.6 Calculations and expression of results

NOTE Since every non-ionic surfactant has a standard factor dependent on the length of the ethylene oxide chain, calculations are related to a standard substance. For this standard, a nonylphenol with 10 ethylene oxide units (NP10) is used. A conversion factor of 54 has been determined for this substance. By this conversion factor, the amount of detergent present in the quantity of sample taken is found and is expressed as micrograms of NP + 10EO (BiAS).

a) calculate the concentration of surfactant *C)* remaining in the test solution at time t_d using the formula:

$$C = \frac{(a-b)\times f \times 54}{V}$$

where

C = concentration of the surfactant, in milligrams of BiAS per litre;
a = volume of carbamate solution used by the sample, in millilitres;
b = volume of carbamate solution;
f = factor of carbamate solution;
V = volume of test solution aliquot taken at time t_d, in millilitres.

b) express the results in the following manner:

less than 1 mg BiAS/L .. .. to 2 decimal places;
more than 1 mg BiAS/L .. .. to 1 decimal places.

C.7 Report

The report shall state the concentration of surfactant remaining at various times *(t.)*

Bibliography

i. Working Group to identify and acknowledge useful literature used in the preparation of this standard.

ii. AS 1792:1976, *Method for determining the biodegradability of surfactants*

iii. EAS 814:2014, *Determination of biodegradability of surfactants — Test method*