II

(Acts whose publication is not obligatory)

COUNCIL

COUNCIL DIRECTIVE

of 31 March 1982

on the approximation of the laws of the Member States relating to methods of testing the biodegradability of non-ionic surfactants and amending Directive 73/404/EEC

(82/242/EEC)

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community, and in particular Article 100 thereof,

Having regard to the proposal from the Commission (1),

Having regard to the opinion of the European Parliament (2),

Having regard to the opinion of the Economic and Social Committee (3),

Whereas the methods of testing in force in the Member States, while pursuing the same objective, differ in certain respects and are thus detrimental to the proper functioning of the common market;

Whereas Council Directive 73/404/EEC of 22 November 1973 on the approximation of the laws of the Member States relating to detergents (4) provides in Article 4 for the adoption of Directives specifying methods of testing as well as relevant tolerances to enable compliance with the requirements of that Directive to be established; whereas Council Directive 73/405/EEC of 22 November 1973 on the approximation of the laws of the Member States relating to methods of testing the biodegradability of anionic surfactants (5) specified such methods and tolerances for anionic surfactants;

Whereas, to enable Member States to determine the level of biodegradability of non-ionic surfactants, it is advisable to employ methods of testing already in use for this purpose in certain Member States; whereas, however, in the event of disputes, biodegradability should be tested by a common reference method;

Whereas, in regard to the approximation of the laws of the Member States relating to detergents, suitable tolerances for measuring biodegradability should be laid down, as provided for in Article 4 of Council Directive 73/404/EEC, in order to safeguard against the unreliability of methods of testing which could lead to rejection decisions having considerable economic consequences; whereas a rejection decision must be taken only if the results obtained by an analytical method mentioned in Article 2 show a level of biodegradability lower than 80 %;

(1) OJ No C 104, 28. 4. 1980, p. 112.
(2) OJ No C 197, 4. 8. 1980, p. 66.
(3) OJ No C 310, 30. 11. 1981, p. 7.
(4) OJ No L 347, 17. 12. 1973, p. 51.
(5) OJ No L 347, 17. 12. 1973, p. 53.

Whereas for the time being small quantities of certain non-ionic surfactants of low biodegradability must be used for some purposes because of technical problems and in order to prevent other undesirable effects on health and the environment; whereas it will nevertheless be necessary to have the opportunity of reviewing the use of these surfactants of low biodegradability in the light of technical progress;

Whereas technical progress necessitates a rapid adaptation of the technical requirements specified by the Directives on detergents; whereas, to facilitate implementation of the necessary measures designed to achieve this, a procedure should be established providing for close collaboration between the Member States and the Commission by means of a Committee for the adaptation to technical progress of the Directives on the removal of technical barriers to trade in detergents,

HAS ADOPTED THIS DIRECTIVE:

Article 1

This Directive concerns the methods of testing the biodegradability of non-ionic surfactants present in the detergents defined in Article 1 of Directive 73/404/EEC.

Article 2

In accordance with the provisions of Article 4 of Directive 73/404/EEC, the Member States shall prohibit the placing on the market and use on their territory of a detergent if the level of biodegradability of the non-ionic surfactants contained in such detergent is less than 80 % determined in accordance with one of the following methods:

— the OECD method, published in the OECD technical report of 11 June 1976 on the 'Proposed Method for the Determination of the Biodegradability of Surfactants used in Synthetic Detergents',

— the method in use in Germany, established by the 'Verordnung über die Abbaubarkeit anionischer und nichtionischer grenzflächenaktiver Stoffe in Wasch- und Reinigungsmitteln' of 30 January 1977, published in the *Bundesgesetzblatt*, 1977, Part I, page 244, as set out in the Regulation amending that Regulation of 18 June 1980, published in the *Bundesgesetzblatt*, 1980, Part I, page 706,

— the method in use in France, approved by Decree of 28 December 1977 published in the *Journal officiel de la République française* of 18 January 1978, and experimental standard T 73-270 March 1974, published by the Association française de normalisation (AFNOR),

— the method in use in the United Kingdom called the 'Porous Pot Test', as described in Technical Report No 70/1978 of the Water Research Centre.

Article 3

Under the procedure laid down in Article 5 (2) of Directive 73/404/EEC, the laboratory opinion on non-ionic surfactants shall be based on the reference method (confirmatory test procedure) described in the Annex to this Directive.

Article 4

The amendments required for adapting the Annex to technical progress shall be adopted in accordance with the procedure laid down in Article 7b of Directive 73/404/EEC.

Article 5

The following Articles shall be inserted in Directive 73/404/EEC:

'Article 2a

1. Until 31 March 1986:

(a) Member States may exempt the following products from the requirements of paragraph 1 of Article 2: low-foaming alkene oxide additives on such substances as alcohols, alkylphenols, glycols, polyols, fatty acids, amides or amines used in dish-washing products;

(b) the requirements of paragraph 1 of Article 2 shall not apply to alkali-resistant terminally blocked alkyl and alkyl-aryl polyglycol ethers and substances of the type referred to in paragraph (a), used in cleaning agents for the food, beverage and metal-working industries.

2. Paragraph 1 shall apply to the abovementioned non-ionic surfactants which

come on the market after 30 September 1983 only if they have a higher level of biodegradability than existing products for the same application.

3. The use of the non-ionic surfactants benefiting from a temporary derogation mentioned in paragraphs 1 and 2 must not, under normal conditions of use, be harmful to human or animal health.

Article 7a

1. A Committee shall be established for the adaptation to technical progress of Directives for removing technical barriers to trade in the detergents sector, hereinafter called "the Committee", which shall consist of representatives of the Member States under the chairmanship of a representative of the Commission.

2. The Committee shall establish its rules of procedure.

Article 7b

1. Where recourse is had to the procedure defined in this Article, the matter shall be referred to the Committee by its chairman, on his own initiative or at the request of the representative of a Member State.

2. The Commission representative shall submit to the Committee a draft of the measures to be taken. The Committee shall deliver its opinion on the draft within a period which may be fixed by the chairman according to the urgency of the matter. A qualified majority of votes as laid down in Article 148 (2) of the Treaty shall be required before the Committee can deliver its opinion.

The chairman shall not vote.

3. (a) The Commission shall adopt the proposed measures where they are in accordance with the opinion of the Committee.

(b) Where the proposed measures are not in accordance with the opinion of the Committee, or if no opinion is delivered, the Commission shall submit to the Council without delay a proposal on the measures to be adopted. The Council shall decide by a qualified majority.

(c) If the Council has not acted within three months of the date on which the proposal was submitted, the proposed measures shall be adopted by the Commission.

Article 7c

1. In accordance with the procedure laid down in Article 7b,

— the references to test methods in the Directives referred to in Article 4 shall, if necessary, be brought up to date or supplemented by other references to test methods established in other Member States,

— the reference methods (confirmatory test) in the Annexes to the Directives referred to in Article 4 shall be modified in order to adapt them to technical progress.

2. These adaptations should not have the effect of modifying in a negative fashion the biodegradability requirements of surfactants, already laid down in accordance with Article 4.'

Article 6

1. The Member States shall bring into force the provisions necessary to comply with this Directive within a period of 18 months following its notification. They shall forthwith inform the Commission thereof.

2. Member States shall communicate to the Commission the text of the provisions of national law which they adopt in the field covered by this Directive.

Article 7

This Directive is addressed to the Member States.

Done at Brussels, 31 March 1982.

For the Council

The President

P. de KEERSMAEKER

ANNEX

DETERMINATION OF THE BIODEGRADABILITY OF NON-IONIC SURFACTANTS

Reference method (confirmatory test)

CHAPTER 1

1.1. **Definition**

Non-ionic surface active agents in the sense of this Directive are those surface active agents which, after passage through cationic and anionic ion exchangers, are determined as bismuth-active substance (BiAS) according to the analytical procedure described in Chapter 3.

1.2. **Equipment needed for measurement**

The method of measurement employs the small activated sludge plant shown in Figure 1, and in greater detail in Figure 2. The equipment consists of a storage vessel A for synthetic sewage, dosing pump B, aeration vessel C, settling vessel D, air-lift pump E to recycle the activated sludge, and vessel F for collecting the treated effluent.

Vessels A and F must be of glass or suitable plastic and hold at least 24 litres. Pump B must provide a constant flow of synthetic sewage to the aeration vessel; this vessel, during normal operation, contains three litres of mixed liquor. A sintered aeration cube G is suspended in the vessel C at the apex of the cone. The quantity of air blown through the aerator should be monitored by means of a flowmeter H.

1.3. **Synthetic sewage**

A synthetic sewage is employed for the test. Dissolve in each litre of tap water:

160 mg peptone,
110 mg meat extract,
30 mg urea $CO(NH_2)_2$,
7 mg sodium chloride NaCl,
4 mg calcium chloride, $CaCl_2 \cdot 2H_2O$,
2 mg magnesium sulphate, $MgSO_4 \cdot 7H_2O$,
28 mg of dipotassium hydrogen phosphate K_2HPO_4
and 10 ± 1 mg BiAS.

The BiAS is extracted from the product to be tested by the method given in Chapter 2. The synthetic sewage is freshly prepared daily.

1.4. **Preparation of samples**

1.4.1. Uncompounded surfactants may be examined in the original state. The BiAS content must be determined in order to prepare the synthetic sewage (1.3).

1.4.2. Formulated products are analyzed for BiAS, MBAS and soap content. They must be subjected to an alcoholic extraction and to a separation of the BiAS (see Chapter 2). The BiAS content of the extract must be known in order to prepare the synthetic sewage.

1.5. **Operation of equipment**

Initially, fill aeration vessel C and settling vessel D with synthetic sewage. The height of the vessel D should be so fixed that the volume contained in the seration vessel C is three

litres. Inoculation is made by introducing 3 ml of a secondary effluent of good quality, freshly collected from a treatment plant dealing with a predominantly domestic sewage. The effluent must be kept under aerobic conditions in the period between sampling and application. Then set the aerator G, air-lift E and dosing device B in operation. The synthetic sewage must pass through the aeration vessel C at a rate of one litre per hour; this gives a mean retention time of three hours.

The rate of aeration should be so regulated that the contents of vessel C are kept constantly in suspension and the dissolved oxygen content is at least 2 mg/l. Foaming must be prevented by appropriate means. Anti-foaming agents which inhibit the activated sludge or contain BiAS must not be used. The air-lift pump E must be set so that the activated sludge from the settling vessel is continually and regularly recycled to aeration vessel C. Sludge which has accumulated around the top of the aeration vessel C, in the base of the settling vessel D, or in the circulation circuit must be returned to the circulation at least once each day by brushing or some other appropriate means. When the sludge fails to settle, its settlebility may be increased by the addition of 2 ml portions of a 5 % solution of ferric chloride, repeated as necessary.

The effluent from the settling vessel D is accumulated in vessel F for 24 hours, following which a sample is taken after thorough mixing. Vessel F must then be carefully cleaned.

1.6. **Checking measuring equipment**

The BiAS content (in mg/l) of the synthetic sewage is determined immediately before use.

The BiAS content (in mg/l) of the effluent collected over 24 hours in vessel F should be determined analytically by the same method, immediately after collection: otherwise the samples must be preserved, preferably by freezing. The concentrations must be determined to the nearest 0·1 mg/l BiAS.

As a check on the efficiency of the process, the chemical oxygen demand (COD) or the dissolved organic carbon (DOC) of the glass fibre filtered effluent accumulated in vessel F and of the filtered synthetic sewage in vessel A is measured at least twice per week.

The reduction in COD or DOC should level off when a roughly regular daily BiAS degradation is obtained at the end of the running-in period shown in Figure 3.

The content of dry matter in the activated sludge contained in the aeration vessel should be determined twice a week in g/l. If it is more than 2·5 g/l, the excess activated sludge must be discarded.

The degradation test is performed at room temperature; this should be steady and kept between 292 and 297 K (19-24 °C).

1.7. **Calculation of biodegradability**

The percentage degradation of BiAS must be calculated every day on the basis of the BiAS content in mg/l of the synthetic sewage and of the corresponding effluent accumulated in vessel F.

The degradability figures thus obtained should be presented graphically as in Figure 3.

The degradability of the BiAS should be calculated as the arithmetic mean of the figures obtained over the 21 days which follow the running-in period, during which degradation has been regular and the operation of the plant trouble-free. In any event the duration of the running-in period should not exceed six weeks.

The daily degradation values are calculated to the nearest 0·1 % but the final result is given to the nearest whole number.

In some cases it may be permissible to reduce the frequency of sampling but at least 14 results collected over the 21 days which follow the running-in period should be used in calculating the average.

CHAPTER 2

PRELIMINARY TREATMENT OF PRODUCTS TO BE TESTED

2.1. Preliminary notes

2.1.1. *Treatment of samples*

The treatment of non-ionic surface active agents and formulated detergents prior to the determination of biodegradability in the confirmatory test is:

Products	Treatment
Non-ionic surfactants	None
Formulated detergents	Alcoholic extraction followed by separation of the non-ionic surfactants by ion exchange

The purpose of the alcoholic extraction is to eliminate the insoluble and inorganic ingredients of the commercial product, which in some circumstances might upset the biodegradability test.

2.1.2. *Ion-exchange procedure*

Isolation and separation of non-ionic surface active agents from soap, anionic and cationic surfactants is required for correct biodegradability tests.

This is achieved by an ion-exchange technique using a macro-porous exchange resin and suitable elutants for fractional elution. Thus soap, anionic and non-ionic surfactants may be isolated in one procedure.

2.1.3. *Analytical control*

After homogenizing, the concentration of anionic and non-ionic surfactants in the detergent is determined according to the MBAS and BiAS analytical procedure. The soap content is determined by a suitable analytical method.

This analysis of the product is necessary to calculate the quantities required to prepare fractions for the biodegradability tests.

Quantitative extraction is not necessary; however, at least 80 % of the non-ionic surfactants should be extracted. Usually, 90 % or more is obtained.

2.2. Principle

From an homogeneous sample (powders, dried pastes and dried liquids) an ethanol extract is obtained which contains the surfactants, soap and other alcohol-soluble constituents of the detergent sample.

The ethanol extract is evaporated to dryness, dissolved in an isopropanol/water mixture and the solution obtained is passed through a strongly acidic cation exchange/macro-porous anion exchange combination heated to 323 K (50 °C). This high temperature is necessary to prevent the precipitation of any fatty acids, which may be present in acidic media.

The non-ionic surfactants are obtained from the effluent by evaporation.

Cationic surfactants, which might upset the degradation test and the analytical procedure are eliminated by the cation exchanger placed above the anion exchanger.

2.3. Chemicals and equipment

2.3.1. Deionized water

2.3.2. Ethanol, 95 % (v/v) C_2H_5OH

(permissible denaturant: methyl-ethyl ketone or methanol)

2.3.3. Isopropanol/water mixture (50/50 v/v):

50 parts by volume isopropanol ($CH_3CHOH \cdot CH_3$) and
50 parts by volume water (2.3.1)

2.3.4. Ammonium bicarbonate solution (60/40 v/v):

0·3 mol NH_4HCO_3 in 1 000 ml of an isopropanol/water mixture consisting of 60 parts by volume isopropanol and 40 parts by volume water (2.3.1)

2.3.5. Cation exchanger (KAT), strongly acidic, resistant to alcohol (50-100 mesh)

2.3.6. Anion exchanger (AAT), macro-porous, Merck Lewatit MP 7080 (70-150 mesh) or equivalent

2.3.7. Hydrochloric acid, 10 % HCl w/w

2.3.8. 2 000 ml round-bottomed flask with ground glass stopper and reflux condenser

2.3.9. 90 mm diameter suction filter (heatable) for filter papers

2.3.10. 2 000 ml filter flask

2.3.11. Exchange columns with heating jacket and tap: inner tube 60 mm in diameter and 450 mm in height (Figure 4)

2.3.12. Water-bath

2.3.13. Vacuum drying oven

2.3.14. Thermostat

2.3.15. Rotary evaporator

2.4. Preparation of extract and separation of non-ionic active agents

2.4.1. *Preparation of extract*

The quantity of surface active agents necessary for the degradation test is about 25 g BiAS.

In preparing extracts for the degradation tests, the quantity of product to be used should be limited to a maximum of 2 000 g. Therefore it may be necessary to carry out the operation two or more times in order to obtain sufficient quantity for the degradation tests. Experience has shown that there are advantages in using a number of small extractions rather than one large extraction.

2.4.2. *Isolation of alcohol-soluble constituents*

Add 250 g of the synthetic detergent to be analyzed to 1 250 ml ethanol and heat the mixture to boiling point and reflux for one hour with stirring. Pass the hot alcoholic solution through a coarse-pored suction filter heated to 323 K (50 °C) and filter rapidly. Wash the flask and suction filter with approximately 200 ml hot ethanol. Collect the filtrate and filter washings in a filter flask.

In the case of pastes or liquid products to be analyzed, make sure that not more than 25 g anionic surfactants and 35 g soap are contained in the sample. Evaporate this weighed sample to dryness. Dissolve the residue in 500 ml ethanol and proceed as described above.

In case of powders of low apparent density (< 300 g/l) it is recommended to increase the ethanol ratio in the relation 20 : 1.

Evaporate the ethanolic filtrate to complete dryness, preferably by means of rotary evaporator. Repeat the operation if a greater quantity of extract is required. Dissolve the residue in 5 000 ml isopropanol/water mixture.

2.4.3. *Preparation of ion-exchange columns*

Cation-exchange column

Place 600 ml cation-exchange resin (2.3.5) in a 3 000 ml beaker and cover by adding 2 000 ml hydrochloric acid (2.3.7). Allow to stand for at least two hours, with occasional stirring. Decant the acid and transfer the resin into the column (2.3.11) by means of deionized water. The column should contain a glass wool plug. Wash the column with deionized water at a rate of 10-30 ml/min until the eluate is free of chloride. Displace the water with 2 000 ml isopropanol/water mixture (2.3.3) at a rate of 10-30 ml/min. The exchange column is now ready for operation.

Anion-exchange column

Place 600 ml anion-exchange resin (2.3.6) in a beaker and cover by adding 2 000 ml deionized water. Allow the resin to swell for at least two hours. Transfer the resin into the column by means of deionized water. The column should contain a glass wool plug.

Wash the column with 0·3 M ammonium bicarbonate solution (2.3.4) until free of chloride. This requires about 5 000 ml solution. Wash again with 2 000 ml deionized water. Displace the water with 2 000 ml isopropanol/water mixture (2.3.3) at a rate of 10-30 ml/min. The exchange column is now in the OH form and ready for operation.

2.4.4. *Ion-exchange procedure*

Connect the exchange columns so that the cation-exchange column is placed on top of the anion-exchange column. Heat the exchange columns to 323 K (50 °C) using thermostatic control. Heat 5 000 ml of the solution obtained in item 2.4.2 to 333 K (60 °C) and pass the solution through the exchanger combination at a rate of 20 ml/min. Wash the columns with 1 000 ml hot isopropanol/water mixture (2.3.3).

To obtain the non-ionic surface active agents, collect the filtrate and filter washings and evaporate to dryness, preferably by means of a rotary evaporator. The residue contains the BiAS. Add deionized water until a defined volume is obtained and determine the BiAS content as in item 3.3 in an aliquot. The solution is used as a standard solution of non-ionic surfactants for the degradation test. The solution should be kept at a temperature below 278 K (5 °C).

2.4.5. *Regeneration of ion-exchange resins*

The cation exchanger is rejected after use.

The anion-exchange resin is regenerated by passing about 5 000-6 000 ml of ammonium bicarbonate solution (2.3.4) down the column at a flow rate of approximately 10 ml/min until the eluate is free from anionic surfactants (methylene blue test). Then pass 2 000 ml isopropanol/water mixture (2.3.3) down the anion exchanger to wash. The anion exchanger is again ready for operation.

CHAPTER 3

DETERMINATION OF NON-IONIC SURFACE ACTIVE AGENT IN BIODEGRADATION TEST LIQUORS

3.1. **Principle**

Surface active agents are concentrated and isolated by gas stripping. In the sample used, the quantity of non-ionic surfactant should be in the range 250-800 µg.

The stripped surfactant is dissolved in ethyl acetate.

After phaser separation and evaporation of the solvent, the non-ionic surfactant is precipitated in aqueous solution with modified Dragendorff reagent ($KBiJ_4$ + $BaCl_2$ + glacial acetic acid).

The precipitate is filtered, washed with glacial acetic acid and dissolved in ammonium tartrate solution. The bismuth in the solution is titrated potentiometrically with pyrrolidinedithiocarbamate solution at pH 4-5 using a bright platinum indicator electrode and a calomel or silver/silver chloride reference electrode.

The method is applicable to non-ionic surfactants containing 6-30 alkylene oxide groups.

The titration result is multiplied by the empirical factor of 54 for conversion to the reference substance nonylphenol condensed with 10 mols ethylene oxide (NP 10).

3.2. **Reagents and equipment**

Reagents are to be made up in deionized water.

3.2.1. Pure ethyl acetate, freshly distilled.

3.2.2. Sodium bicarbonate ($NaHCO_3$) AR

3.2.3. Dilute hydrochloric acid (20 ml concentrated acid (HCl) diluted to 1 000 ml with water)

3.2.4. Methanol AR, freshly distilled, stored in a glass bottle.

3.2.5. Bromocresol purple, 0·1 g in 100 ml methanol.

3.2.6. Precipitating agent: the precipitating agent is a mixture of two volumes of solution A and one volume of solution B. The mixture is stored in a brown bottle and can be used for up to one week after mixing.

3.2.6.1. Solution A

Dissolve 1·7 g bismuth nitrate AR ($BiONO_3 \cdot H_2O$) in 20 ml glacial acetic acid, and make up to 100 ml with water. Then dissolve 65 g potassium iodide AR in 200 ml water. Mix these two solutions in a 1 000 ml measuring flask, add 200 ml glacial acetic acid (3.2.7) and make up to 1 000 ml with water.

3.2.6.2. Solution B

Dissolve 290 g barium chloride ($BaCl_2 \cdot 2H_2O$) AR in 1 000 ml of water.

3.2.7. Glacial acetic acid 99-100 % (lower concentrations are unsuitable).

3.2.8. Ammonium tartrate solution: mix 12·4 g tartaric acid AR and 12·4 ml of ammonia solution AR (d = 0·910 g/ml) and make up to 1 000 ml with water (or use the equivalent amount of ammonium tartrate AR).

3.2.9. Dilute ammonia solution: 40 ml ammonia solution AR (d = 0·910 g/ml) diluted to 1 000 ml with water.

3.2.10. Standard acetate buffer: dissolve 40 g solid sodium hydroxide AR in 500 ml water in a beaker and allow to cool. Add 120 ml glacial acetic acid (3.2.7). Mix thoroughly, cool and transfer to a 1 000 ml volumetric flask. Make up to the mark with water.

3.2.11. Pyrrolidinedithiocarbamate solution (known as 'carbate solution'): dissolve 103 mg sodium pyrrolidinedithiocarbamate ($C_5H_8NNaS_2 \cdot 2H_2O$) in about 500 ml water, add 10 ml of n-amyl alcohol AR and 0·5 g $NaHCO_3$ AR, and make up to 1 000 ml with water.

3.2.12. Copper sulphate solution (for standardization of 3.2.11).

Stock solution

Mix 1·249 g copper suphate ($CuSO_4 \cdot 5H_2O$) AR with 50 ml 0·5 M sulphuric acid and make up to 1 000 ml with water.

Standard solution

Mix 50 ml stock solution with 10 ml 0·5 M H_2SO_4 and make up to 1 000 ml with water.

3.2.13. Sodium chloride AR

3.2.14. Gas-stripping apparatus (see Figure 5).

The diameter of the sintered disc must be the same as the internal diameter of the cylinder.

3.2.15. Separating funnel, 250 ml.

3.2.16. Magnetic stirrer with magnet 25-30 mm.

3.2.17. Gooch crucible, diameter of the perforated base = 25 mm, Type G 4.

3.2.18. Circular glass-fibre filter papers, 27 mm diameter with fibre diameter 0·5-1·5 µm.

3.2.19. Two filter flasks with adaptors and rubber collars, 500 and 250 ml respectively.

3.2.20. Recording potentiometer fitted with a bright platinum indicator electrode and a calomel or silver/silver chloride reference electrode with a 250 mV range, with automatic burette of 20-25 ml capacity, or alternative manual equipment.

3.3. **Method**

3.3.1. *Concentration and separation of the surfactant*

Filter the aqueous sample through a qualitative filter paper. Discard the first 100 ml of the filtrate.

Into the stripping apparatus, previously rinsed with ethyl acetate, place a measured quantity of the sample, such that it contains between 250-800 µg non-ionic surfactant.

To improve the separation add 100 g sodium chloride and 5 g sodium bicarbonate.

If the volume of the sample exceeds 500 ml, add these salts to the stripping apparatus in solid form, and dissolve by passing nitrogen or air through.

If a smaller-sized sample is used, dissolve the salts in 400 ml water and then add to the stripping apparatus.

Add water to bring the level to the upper stopcock.

Cautiously add 100 ml ethyl acetate on top of the water.

Fill the wash-bottle in the gas-line (nitrogen or air) two-thirds full with ethyl acetate.

Pass a gas stream of 30-60 l/h through the apparatus; the inclusion of a rotameter is recommended. The rate of aeration must be increased gradually at the beginning. The gas rate must be so adjusted that the phases remain noticeably separate to minimize the mixing of the phases and the solution of the ethyl acetate in the water. Stop the gas flow after five minutes.

If there is a reduction of more than 20% in the volume of the organic phase through solution in water, the sublation must be repeated paying special attention to the rate of gas flow.

Run off the organic phase into a separating funnel. Return any water in the separating funnel from the aqueous phase — it should only be a few ml — to the stripping apparatus. Filter the ethyl acetate phase through a dry qualitative filter paper into a 250 ml beaker.

Put a further 100 ml ethyl acetate into the stripping apparatus and again pass nitrogen or air through for five minutes. Draw off the organic phase into the separating funnel used for the first separation, reject the aqueous phase and run the organic phase through the same filter as the first ethyl acetate portion. Rinse both the separating funnel and the filter with about 20 ml ethyl acetate.

Evaporate the ethyl acetate extract to dryness on a water-bath (fume cupboard). Direct a gentle stream of air over the surface of the solution to accelerate the evaporation.

3.3.2. *Precipitation and filtration*

Dissolve the dry residue from 3.3.1 in 5 ml methanol, add 40 ml water and 0·5 ml diluted HCl (3.2.3) and stir the mixture with a magnetic stirrer.

To this solution add 30 ml of precipitating agent (3.2.6) from a measuring cylinder. The precipitate forms after repeated stirring. After stirring for 10 minutes leave the mixture to stand for at least five minutes.

Filter the mixture through a Gooch crucible, the base of which is covered with a glass-fibre filter paper. First wash the filter under suction with about 2 ml glacial acetic acid. Then thoroughly wash the beaker, magnet, and crucible with glacial acetic acid, of which about 40-50 ml is necessary. It is not necessary to quantitatively transfer the precipitate adhering to the sides of the beaker, to the filter, because the solution of the precipitate for the titration is returned to the precipitating beaker, and the remaining precipitate will then be dissolved.

3.3.3. *Solution of the precipitate*

Dissolve the precipitate in the filter crucible by the addition of hot ammonium tartrate solution (about 80 °C, 353 K) (3.2.8) in three portions of 10 ml each. Allow each portion to stand in the crucible for some minutes before being sucked through the filter into the flask.

Put the contents of the filter flask into the beaker used for the precipitation. Rinse the sides of the beaker with a further 20 ml of tartrate solution to dissolve the rest of the precipitate.

Carefully wash the crucible, adaptor and filter flask with 150-200 ml water, and return the rinsing water to the beaker used for the precipitation.

3.3.4. *The titration*

Stir the solution using a magnetic stirrer (3.2.16), add a few drops of bromocresol purple (3.2.5) and add the diluted ammonia solution (3.2.9) until the colour turns violet (the solution is weakly acid from the residue of acetic acid used for rinsing).

Then add 10 ml standard acetate buffer (3.2.10), immerse the electrodes in the solution, and titrate potentiometrically with standard 'carbate solution' (3.2.11), the burette tip being immersed in the solution.

The titration rate should not exceed 2 ml/min.

The endpoint is the intersection of the tangents to the two branches of the potential curve. It will be observed occasionally that the inflection in the potential curve becomes flattened; this can be eliminated by carefully cleaning the platinum electrode (by polishing with emery paper).

3.3.5. *Blank determinations*

At the same time run a blank determination through the whole procedure with 5 ml methanol and 40 ml water, according to the instructions in 3.3.2. The blank titration should be below 1 ml, otherwise the purity of the reagents (3.2.3 — 3.2.7 — 3.2.8 — 3.2.9 — 3.2.10) is suspect, especially their content of heavy metals, and they must be replaced. The blank must be taken into account in the calculation of the results.

3.3.6. *Control of the factor of the 'carbate solution'*

Determine the factor for the carbate solution on the day of use. To do this, titrate 10 ml of the copper sulphate solution (3.2.12) with carbate solution after the addition of 100 ml water and 10 ml standard acetate buffer (3.2.10). If the amount used is 'a' ml, the factor f is:

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

and all the results of the titrations are multiplied by this factor.

3.4. **Calculation of results**

Every non-ionic surfactant has its own factor, depending on its composition, particularly on the length of the alkene oxide chain. The concentration of non-ionic surfactant is expressed in relation to a standard substance — a nonyl phenol with 10 ethylene oxide units (NP 10) — for which the conversion factor is 0·054.

Using this factor the amount of surfactant present in the sample is found expressed as mg of NP 10 equivalent, as follows:

$$(b\text{-}c) \cdot f \cdot 0{\cdot}054 = \text{mg non-ionic surfactant as NP 10}$$

where: b = volume of 'carbate solution' used by the sample (ml),

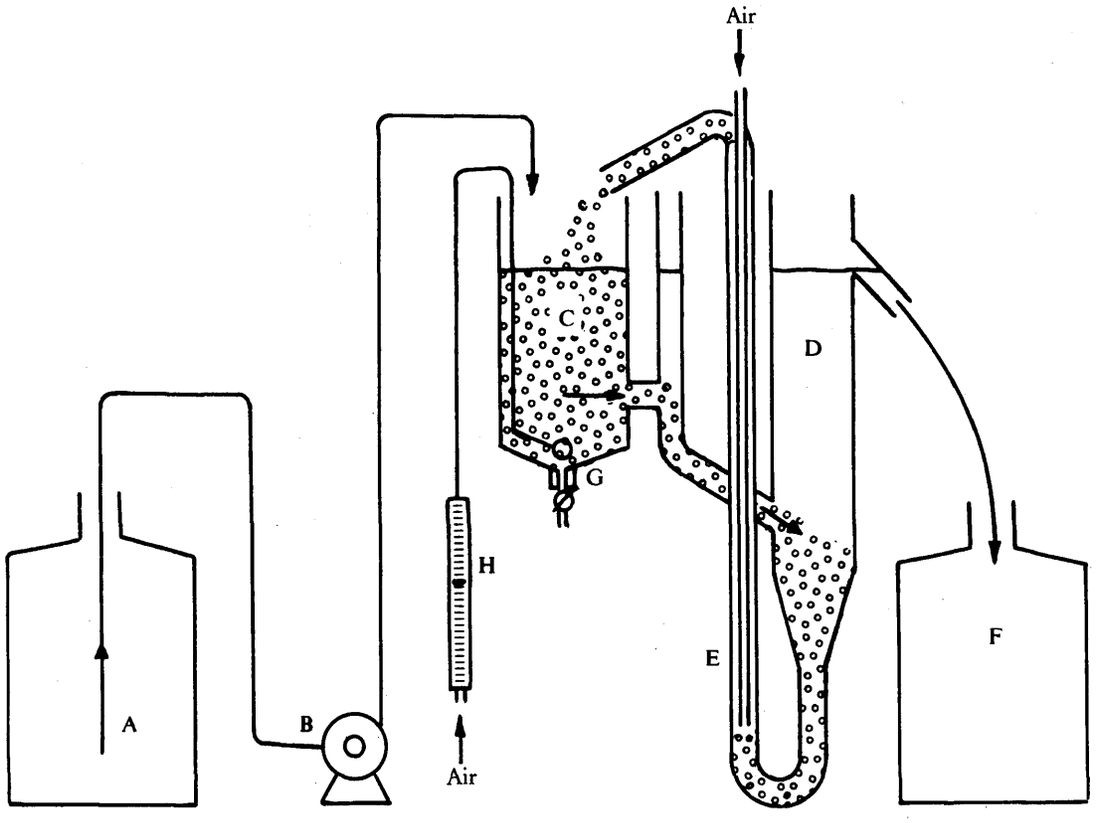
c = volume of 'carbate solution' used by the blank (ml),

f = factor of the 'carbate solution'.

3.5. **Expression of results**

Express the results in mg/l as NP 10 to the nearest 0·1.

Figure 1



A. Storage vessel
B. Dosing device
C. Aeration chamber (three litres capacity)
D. Settling vessel
E. Air-lift pump
F. Collector
G. Sintered aerator
H. Air-flow meter

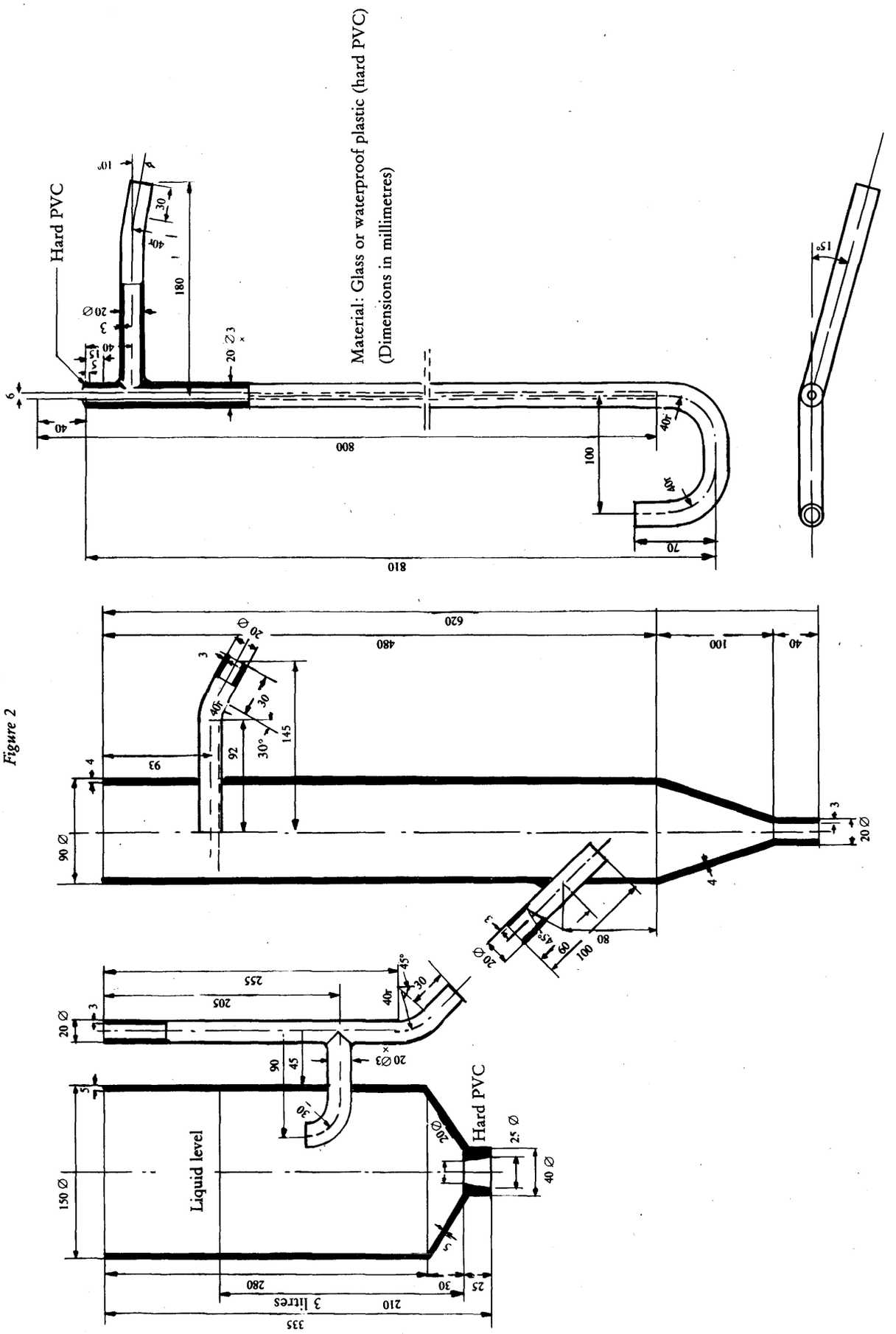

Material: Glass or waterproof plastic (hard PVC)
(Dimensions in millimetres)
Hard PVC
Liquid level
3 litres
Hard PVC

Figure 2

Figure 3

Calculation of biodegradability — Confirmatory test

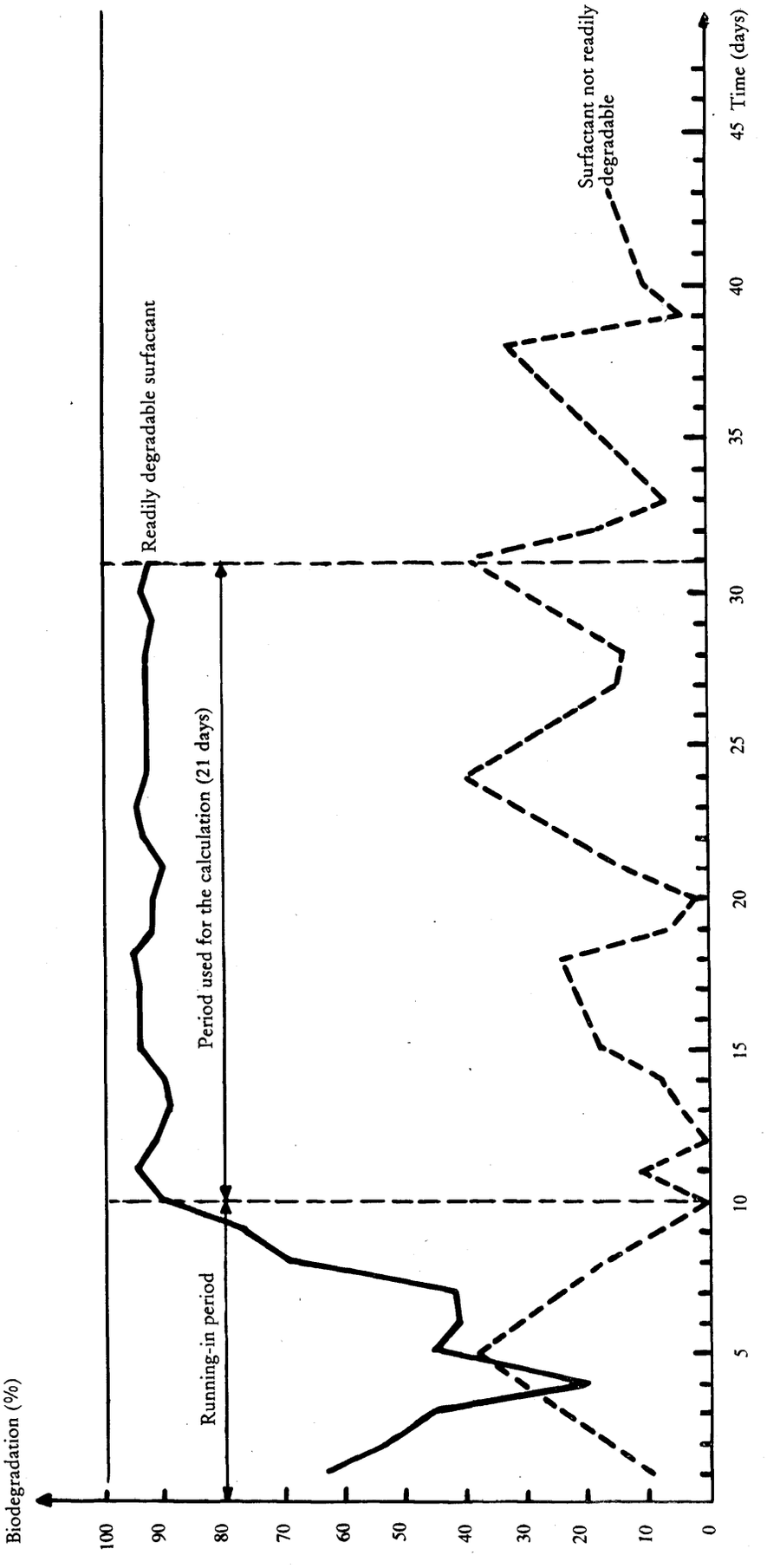


Figure 4

Heated exchange column

(Dimensions in millimetres)

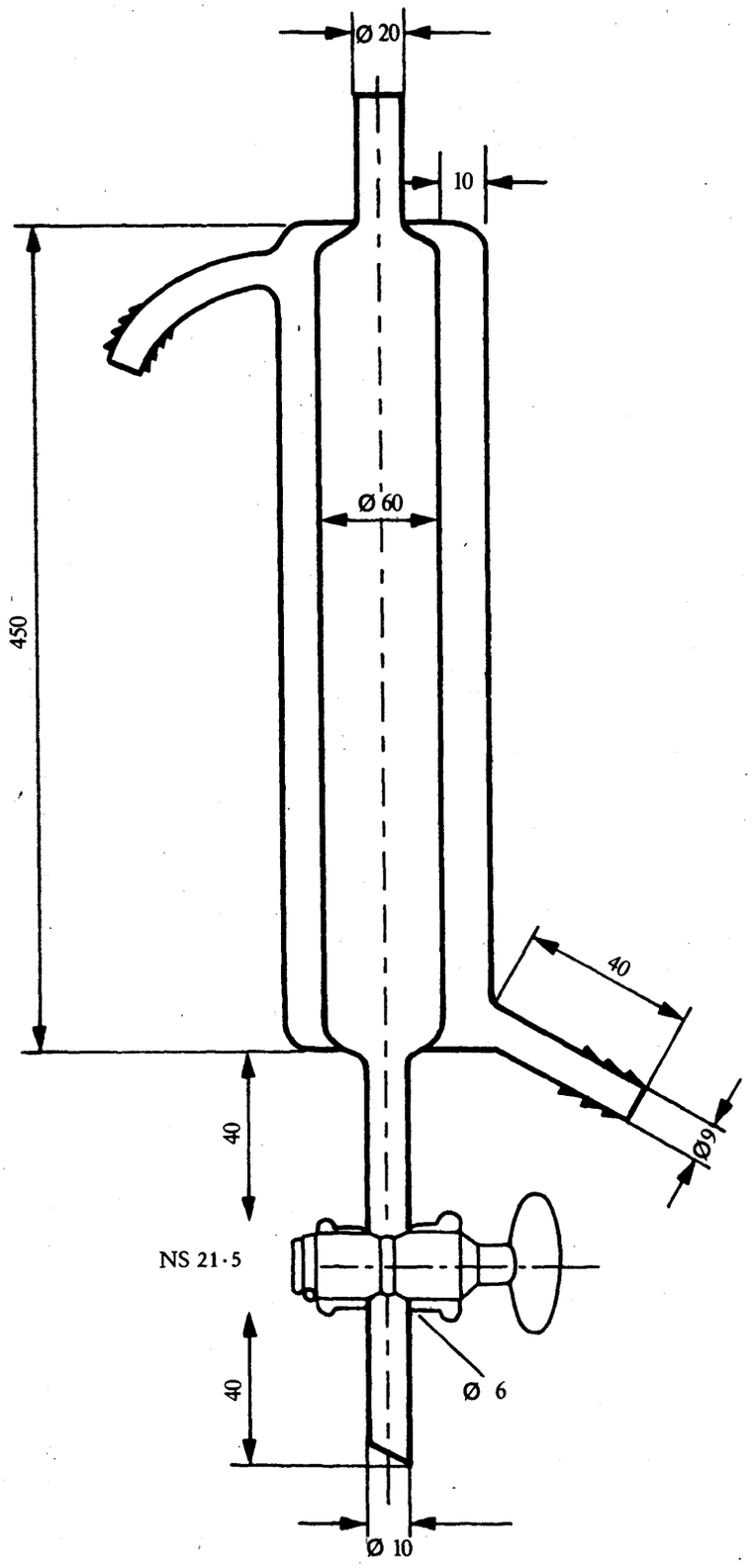


Figure 5

Gas-stripping apparatus

(Dimensions in millimetres)

