# COMMISSION OF THE EUROPEAN COMMUNITIES

COM (80) 40 final

Brussels, 8th February 1980

# PROPOSAL FOR A COUNCIL DIRECTIVE 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

(Submitted by the Commission to the Council)

COM (80) 40 final

# EXPLANATORY MEMORANDUM

## I. GENERAL CONSIDERATIONS

1. This proposal for a Council Directive comes under the General Programme for the removal of technical barriers to trade, which the Council approved on 28 May 1969, in line with which two Directives relating to the same sector have already been adopted. These are the Council Directive of 22 November 1973 relating to detergents and the Council Directive of the same date relating to methods of testing the biodegradability of anionic surfactants (1).

2. The measurement of the level of biodegradability by different methods can cause distortion of the Community market and have a direct effect on its operation. A Community solution proposing a single method is therefore necessary.

3. The biodegradability of detergents is one aspect of the much more wide-ranging and very topical problem of environmental pollution in general and water pollution in particular.

   For these reasons industry, in agreement with public authorities, has progressively shifted its production to detergents containing biodegradable surfactants. Nevertheless, for certain applications in the food industry, the metallurgical industries and in dishwasher detergents, small amounts of certain non-ionic surfactants of low biodegradability must be used for technical reasons: machines used in these industries work at high speed and have to use low-foaming and/or highly alkali-resistant surfactants of high detergency. For the time being there is no surfactant which is 80% biodegradable and has these properties.

   The amounts used in this way are small and the total EEC consumption is estimated at some 5 500 - 8 500 tonnes per year, i.e., less than 1 % of all the surfactants used in cleaning products.

   It should also be pointed out that these surfactants, although of low biodegradability, are not as far as it is known responsible for ecological problems in the Community and that, even if they were, such problems would be strictly local.

   Research is in progress in several Member States with a view to developing satisfactory substitute products which are sufficiently biodegradable to comply with the Directive.

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(1) OJ No L 347, 17 December 1973, pp. 51 and 53

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4. Technical progress necessitates a prompt adaptation of the technical standards in the methods for testing the biodegradability of surfactants. That is the reason why this Directive provides for the institution of a procedure of close collaboration between the Member States and the Commission within the framework of the Committee for the adaptation to technical progress of the directives relating to the removal of technical barriers to trade in detergents. This procedure is the one provided for by the Resolution of 28 May 1969 and subsequently adopted by the Council in different directives.

5. In accordance with the Opinions which the European Parliament and the Economic and Social Committee delivered on the basic "Detergents" Directive, the solution of "total" harmonization has been chosen.

## II. COMMENTS ON SPECIFIC ARTICLES

### Article 1

This Article establishes the scope of the Directive.

### Article 2

Article 2 stipulates that a detergent must not be placed on the market if it is determined that the level of biodegradability of the non-ionic surfactants it contains is less than 80 %, and provides that the methods used for this determination shall be those of the OECD, the Federal Republic of Germany, France and the United Kingdom.

### Article 3

This Article lays down the procedure to be followed in the event of a dispute.

### Article 4

This gives a temporary exemption for certain uses of non-ionic surfactants with a biodegradability of less than 80%.

### Article 5

This Article provides for a procedure for the adaptation to technical progress of the directive concerning detergents.

### Articles 6 and 7

These Articles are common to all directives.

## III. CONSULTATION OF THE SECTORS CONCERNED

When preparing this Directive, the Commission took note of the comments made by the representatives of the industries concerned.

## IV. CONSULTATION OF THE EUROPEAN PARLIAMENT AND THE ECONOMIC AND SOCIAL COMMITTEE

In accordance with the second paragraph of Article 100, the opinion of these two institutions is necessary.

PROPOSAL FOR A COUNCIL DIRECTIVE

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

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;

Having regard to the opinion of the European Parliament;

Having regard to the opinion of the Economic and Social Committee;

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 functioning of the common market;

Whereas Council Directive 73/404/EEC[1] relating to detergents provides in Article 4 for the adoption of directives specifying methods of testing in order to ascertain that the requirements of that Directive are being observed; whereas Council Directive 73/405/EEC of 22 November 1973[2] relating to methods of testing the biodegradability of anionic surfactants specified such methods and tolerances for anionic surfactants;

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

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[1] OJ No L 347, 17.12.1973, p. 51

[2] OJ No L 347, 17.12.1973, p. 53

Whereas in regard to the approximation of the laws of the Member States relating to detergents, suitable tolerances for measuring biodegradability should be determined, as provided for in Article 4 of Council Directive 73/404/EEC in order to take account of the unreliability of test methods which could lead to rejection decisions with 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%;

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 review 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 the introduction of the necessary measures, a system should be set up providing for close collaboration between the Member States and the Commission by means of a Committee for the adaptation, in the light of 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 used in detergents.

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Article 2

In accordance with the provisions of Article 4 of Directive 73/404/EEC, due account being taken of the unreliability of testing methods, 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 the Federal Republic of Germany, established by the "Verordnung über die Abbaubarkeit anionischer und nichtanionischer grenzflächenaktiver Stoffe in Wasch- und Reinigungsmitteln" of 30 January 1977, published in the Bundesgesetzblatt, Part I, page 244;

- 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" and described in Technical Report 78 (1977) by 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 "Confirmatory test procedure" described in the Annex.

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Article 4

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

"Article 2a

1. Until 31 December 1984, Article 2 shall not be applicable to the following non-ionic surfactants:

   a) low foaming polyalkene oxide addition products used in industrial cleaning agents and mechanical dish-washing products;

   b) alkali-resistant terminally-blocked alkyl and alkyl-aryl polyglycol ethers used in industrial cleaning agents for the food, beverage and metal working industries.

2. During that period of exemption, the exemption shall be re-examined and where appropriate adapted in the light of technical progress in accordance with the procedure laid down in Article 7 b.

3. In the event of industrial use of the surfactants referred to in paragrah 1, the user shall employ the best techniques available in order to limit the emission of these surfactants into the aquatic environment.

"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.

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" 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 majority of twelve votes shall be required before it can deliver its opinion, the votes of the Member States being weighted as laid down in Article 148(2) of the Treaty.

   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.

   (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

The appropriate methods of testing and tolerances, defined either directly or by reference to standards laid down outside the institutional framework of the Community, in the Directives referred to in Article 4 shall be modified, in particular in order to adapt them to technical progress in accordance with the procedure laid down in Article 7b."

Article 5

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

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

Article 6

This Directive is addressed to the Member States.

# 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 3 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
7 mg sodium chloride
4 mg calcium chloride, 2 $H_2O$
2 mg magnesium sulphate, $7^2H_2O$,
28 mg of dipotassium phosphate ($K_2HPO_4$)
and 10 $\pm$ 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 analysed 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 aeration vessel C is 3 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 3 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. Antifoaming 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 density may be increased by the addition of 2 ml portions of a 5 per cent solution of ferric chloride, repeated as necessary.

The efflent 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 be carefully cleaned.

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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 BiAS/l.

As a check on the efficiency of the process, the chemical oxygen demand (COD) or the dissolved organic carbon (DOC) of the 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 i.e. at the end of the running-in period shown in Figure 3.

The loss on ignition of the dry matter in the activated sludge in the aeration tank 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 which should be steady and kept between 291 and 298 K (18-25°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.

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 operation of the plant trouble-free.
In any case the duration of the running-in period should not exceed six weeks.

The daily degradation values are calculated to the nearest 0.1 per cent 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.

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# 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 biodegradatility 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 degradation 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 biodegradation tests.

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

2.I.3. Analytical control

After homogenizing, the concentration of anionic and non-ionic surfactants in the synthetic 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 per cent of the surfactants non-ionic should be extracted. Usually, 90 per cent and more is obtained.

2.2. Principle

From an homogeneous sample (powders, pastes, and dried liquids) an ethanol extract is obtained which contains the surfactants, soap and other alcohol-soluble constituents of the synthetic 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 temperature is necessary to prevent precipitation of fatty acids 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 on top of the anion exchanger.

2.3. Chemicals and equipment

2.3.1. Deionised water.

2.3.2. Ethanol, 95 vol. % $C_2H_5OH$

(permissible denaturant : methyl ethyl ketone or methanol).

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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) :
0,3 M $NH_4HCO_3$ 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 1 000 ml round-bottomed flask with ground glass stopper and reflux condenser.

2.3.9. 90 mm dia. suction filter (heatable) for paper filters.

2.3.10. 2 000 ml filter flask.

2.3.11. Exchange columns with heating jacket and cock :
Inner tube 60 mm in diameter and 450 mm in height (Fig. 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 max. 1 000 g. Therefore, it may be necessary to carry out the operation twice 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 1250 ml ethanol and heat the mixture to boiling point and reflux for 1 hour with stirring. Pass the hot alcoholic solution through a coarse-pored suction filter heated to 323 K (50°C) and suck off sharply. Wash the flask and suction filter with approx. 200 ml hot ethanol. Collect the filtrate and filter washings in a filter flask.

In the case of pastes or liquid products to be analysed, 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 all 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 in a 3 000 ml beaker and cover by adding 2 000 ml hydrochloric acid. Allow to stand for at least 2 hours stirring occasionally. Decant the acid and transfer the resin into the column (2.3.11.) by means of deionised water. The column should contain a glass wool plug. Wash the column with deionised 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 in a beaker and cover by adding 2 000 ml deionised water. Allow the exchanger to swell for at least 2 hours. Transfer the resin into the column by means of deionised 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 deionised 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 a thermostat. 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).

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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 deionised 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 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 anionics (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.

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# CHAPTER 3

## DETERMINATION OF NON-IONIC SURFACE ACTIVE AGENTS IN BIODEGRADATION TEST LIQUORS

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### 3.1. Introduction

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 phase separation and evaporation of the solvent, the non-ionic surfactant is precipitated in aqueous solution with modified Dragendorff reagent ($KBiI_4$ + $BaCl_2$ + 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 (NP10).

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3.2. Reagents and equipment

All reagents are to be made up in deionised water.

3.2.1. Pure ethyl acetate, freshly distilled.

3.2.2. Sodium bicarbonate $NaHCO_3$.A.R.

3.2.3. Dilute HCl (20 ml hydrochloric acid A.R. conc. per 1 000 ml water).

3.2.4. Methanol A.R., freshly distilled, kept 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 2 volumes of solution A and 1 volume of solution B. The mixture is stored in a brown bottle and can be used up to one week after mixing.

3.2.6.1. Solution A

Dissolve 1.7 g bismuth (III) nitrate A.R., ($BiO.NO_3.H_2O$) in 20 ml glacial acetic acid, and make up to 100 ml with water. Then dissolve 65 g potassium iodide A.R., in 200 ml water. Mix these two solutions in a 1 000 ml measuring flask, add 200 ml glacial acetic acid (3.3.7.) and make up to 1 000 ml with water.

3.2.6.2. Solution B

Dissolve 290 g $BaCl_2.2H_2O$ A.R. 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 and 12.4 ml of ammonia solution A.R. (d = 0.910) and make up to 1 000 ml with water (or use the equivalent amount of ammonium tartrate A.R.).

3.2.9. Dilute ammonia solution : 40 ml ammonia solution A.R. (d = 0.910) diluted to 1 000 ml with water.

3.2.10. Standard acetate buffer : dissolve 40 g solid sodium hydroxide A.R. in 500 ml water in a 1 000 ml volumetric flask and add 120 ml glacial acetic acid (3.2.7.). Mix thoroughly, cool and make up to the mark with water.

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3.2.11. Pyrrolidinedithiocarbamate solution (shortened to "Carbate solution") : dissolve 103 mg sodium Pyrrolidinedithiocarbamate ($C_5H_8NNa\ S_2.2H_2O$) in about 500 ml water, add 10 ml n-amyl alcohol A.R. and 0.5 g $NaHCO_3$ A.R., 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 sulphate A.R. ($CuSO_4.5H_2O$) 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 A.R.

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 ml 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.

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## 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 into the apparatus.

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 minimise the mixing of the phases and the solution of the ethyl acetate in the water. Stop the gas flow after 5 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.

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Put a further 100 ml ethyl acetate into the stripping apparatus and again pass nitrogen or air through for 5 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 min. leave the mixture to stand for at least 5 min.

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 (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.

×) (about 80°C, 353 K)

./.

3.3.4. The titration

Stir the solution with 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 daily. 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 the length of the ethylene oxide chain. The concentration of non-ionic surfactant 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-c) .f. 0.054 = mg non-ionic surfactant

where b = volume of "carbate solution" used by the sample (ml)

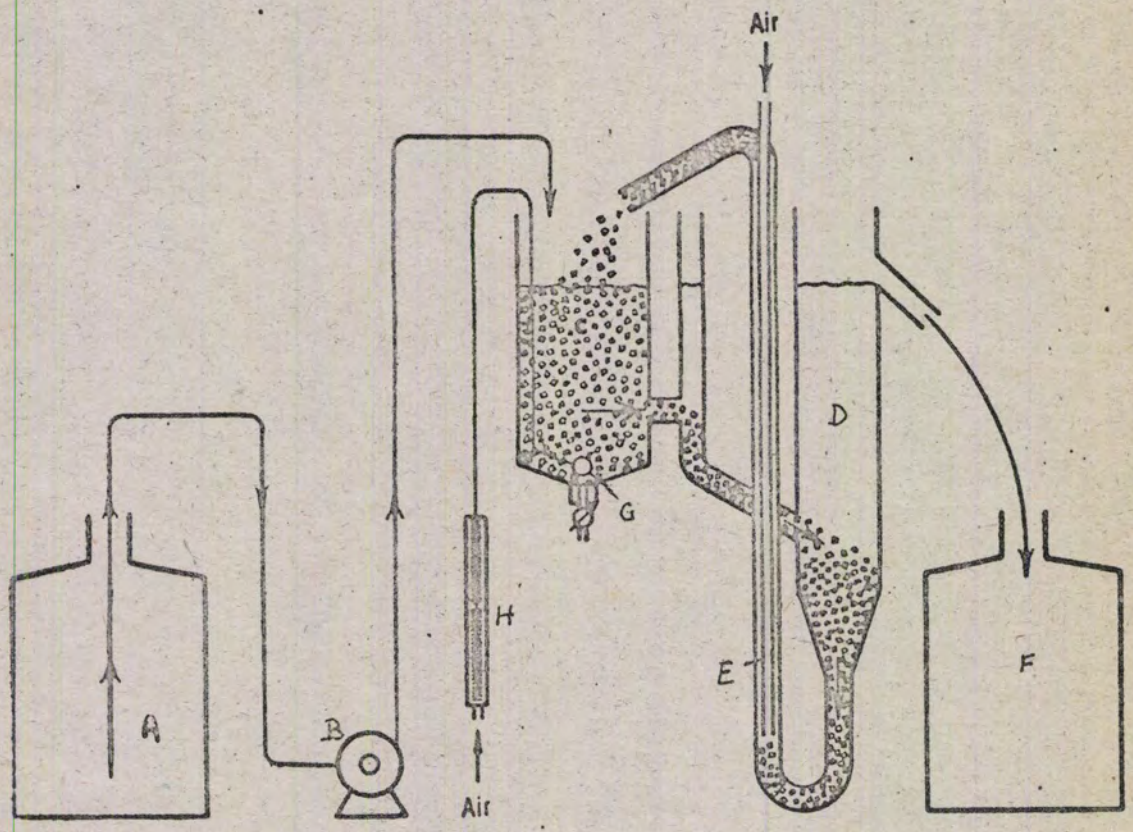
c = volume of "carbate solution" used by the blank (ml)

f = factor of the "carbate solution"

3.5. Expression of results

Express the results as mg BiAS/l to the nearest 0.1.

Figure 1




A. Storage vessel

B. Dosing device

C. Aeration chamber (3 l. capacity)

D. Settling vessel

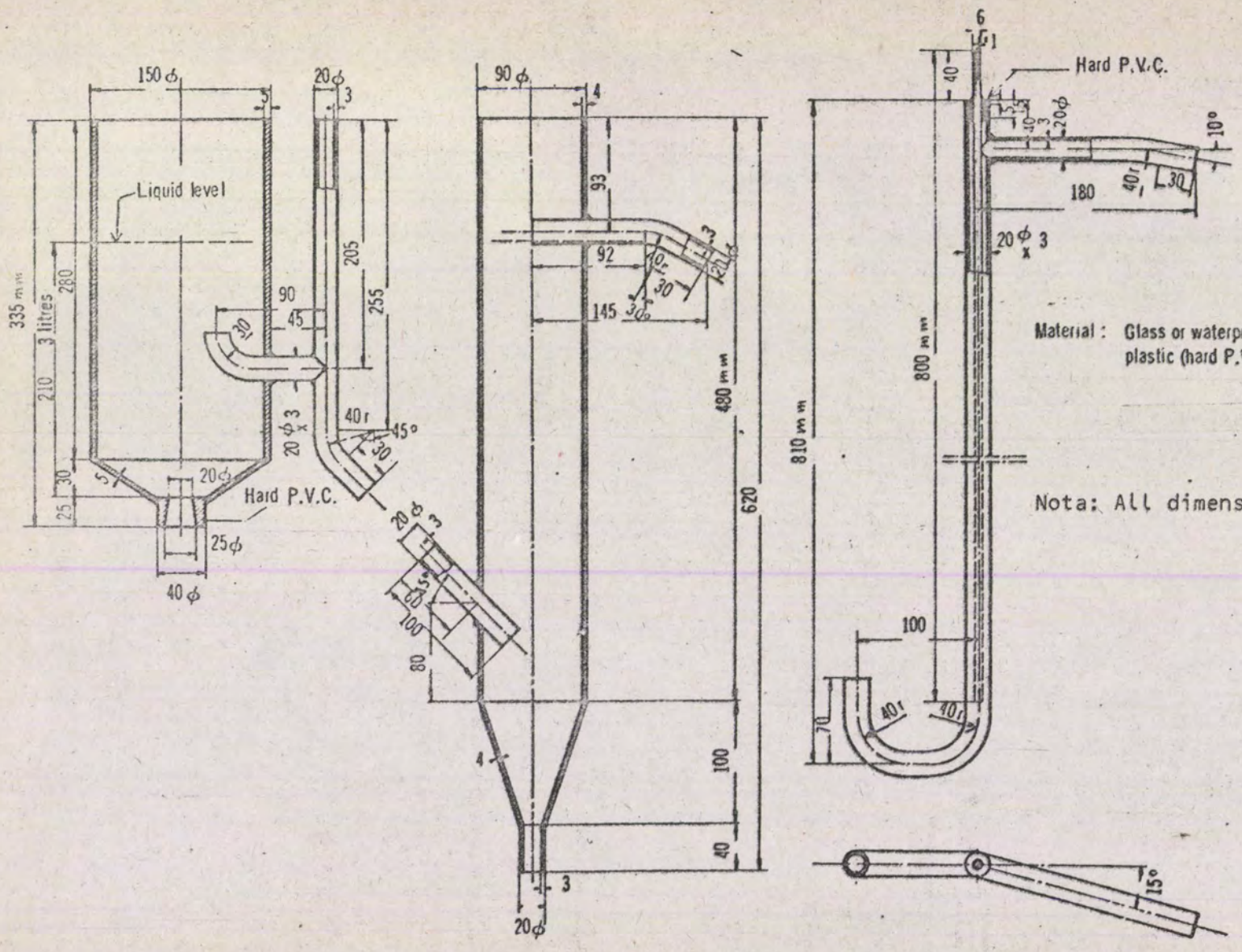
E. Air lift pump

F. Collector

G. Aerator

H. Air flow meter

Figure 2.




Material : Glass or waterproof plastic (hard P.V.C.)

Nota: All dimensions are given in mm

Figure 3

CALCULATION OF BIODEGRADABILITY - DYNAMIC SIMULATION TEST

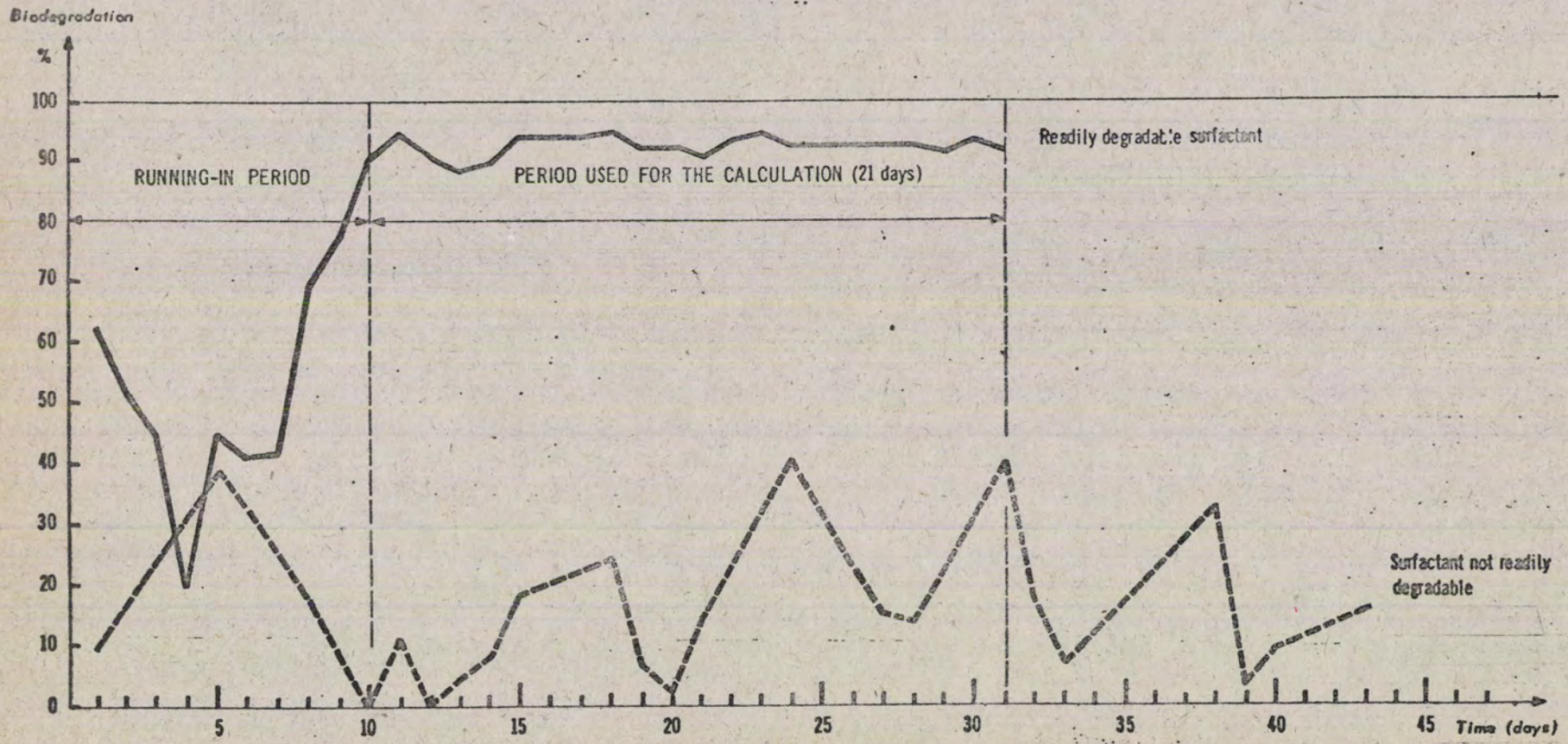


Fig. 4

Heated exchange column

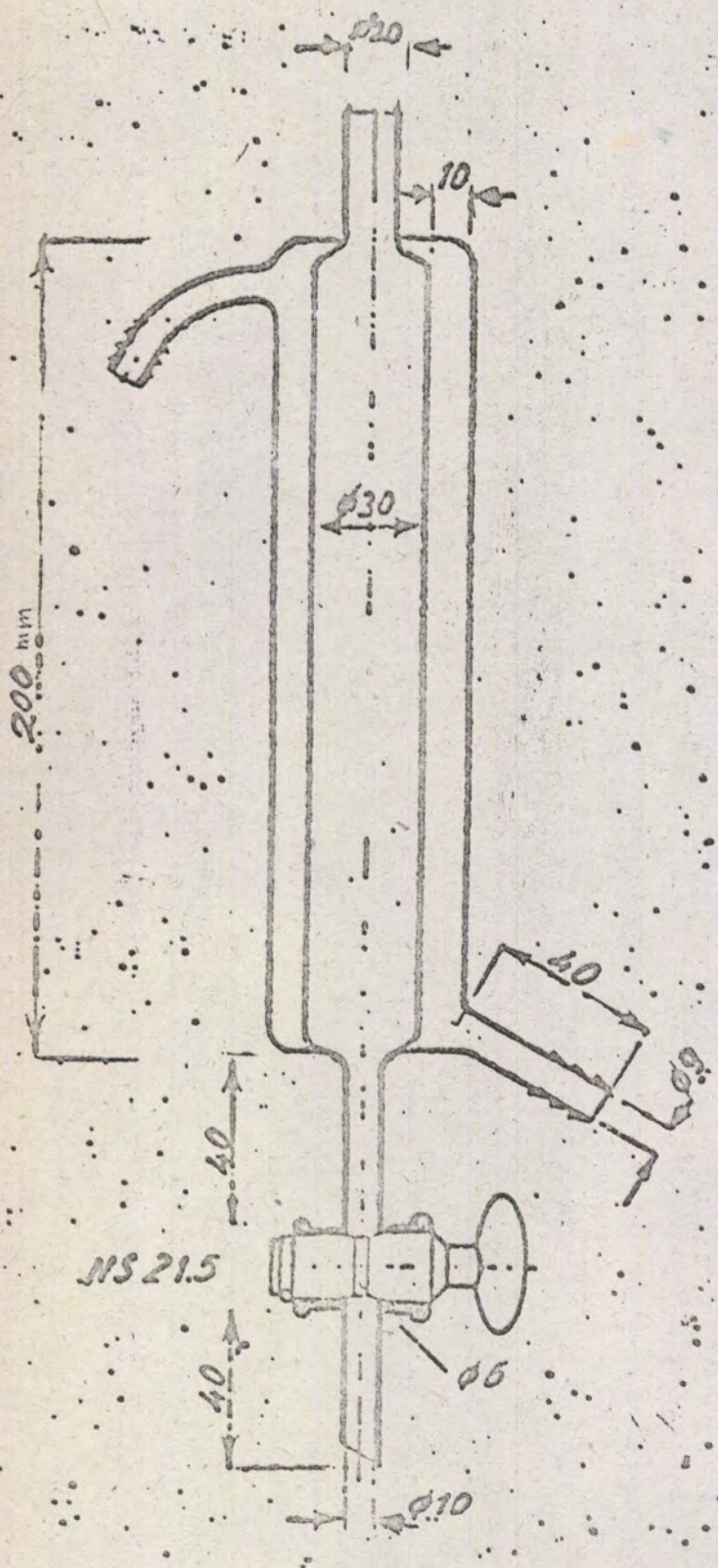


Figure 5

GAS - STRIPPING APPARATUS

(All dimensions are given in mm)