# **UGANDA STANDARD**

First Edition 2015-mm-dd

Hair shampoo— Part 1: synthetic detergent-based — Specification



Reference number DUS 1624-1: 2015

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## **Foreword**

Uganda National Bureau of Standards (UNBS) is a parastatal under the Ministry of Tourism, Trade and Industry established under Cap 327, of the Laws of Uganda. UNBS is mandated to co-ordinate the elaboration of standards and is

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Committee membership

# DUS 1624-1: 2015

# Hair shampoo —Part 2: synthetic detergent-based — Specification

## 1 Scope

This Draft Uganda Standard prescribes the requirements, methods of test and sampling for hair shampoo, synthetic detergent-based. This standard also includes shampoos with positive dermatological effect on the skin and neutralizing shampoos

# 3 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EAS 346, Labelling of cosmetics — General requirements

EAS 377-1, Cosmetics and cosmetic products — Part 1: List of substances prohibited in cosmetic products

EAS 377-2, Cosmetics and cosmetic products — Part 2: List of substances which cosmetic products must not contain except subject to the restrictions laid down

EAS 377-3, Cosmetics and cosmetic products — Part 3: List of colorants allowed in cosmetic products

EAS 377-4, Cosmetics and cosmetic products — Part 4: List of preservatives allowed in cosmetic products

EAS 377-5, Cosmetics and cosmetic products — Part 5: Use of UV filters in cosmetic products

ISO 24153, Random sampling and randomization procedures

#### 4 Classification

Synthetic detergent shampoo shall be classified into four types:

- a) General purpose shampoo This shall be for "Dry", "Oily" or "Normal" hair, and shall be indicated so.
- b) Treatment shampoo
- c) Baby shampoo
- d) Neutralizing shampoo

# 5 General requirements

5.1 The shampoo shall be in the form of a liquid, emulsion or paste. It may be coloured and perfumed.

- 5.2 The clear and /or transparent liquid shampoo, when examined visually shall be free from any sediment.
  - a) If in the form of an emulsion, it shall be homogenous and there shall be no visible signs of the emulsion having separated.
  - b) Shampoo in the form of a paste shall be free from any agglomerated particles.
- **5.3** The shampoo shall not be harmful to the user, and shall have no undesirable effect on the natural colour of the hair. (This does not include hair already treated with hair dyes).
- **5.4** The shampoo shall impart all the effects claimed (e.g. dandruff control).
- **5.5** The "General purpose shampoo" and the "Treatment shampoo" shall comply with the requirements given in Table 1
- **5.6** The "Baby shampoo" shall comply with the requirements given in Table 2.
- 5.7 The "Neutralizing shampoo" shall comply with the requirements given in Table 3.

# 6 Ingredients

- **6.1** All ingredients used including dyes, pigments and colours shall conform to EAS 377 (all parts).
- **6.2** Any product containing ingredients for which medicinal claims are made shall be registered with the relevant regulatory agency.
- **6.3** All essential oils/herbs used shall conform to the approved standards.
- **6.4** All products containing herbal extracts known to have antibacterial activity e.g. neem oil/extract and aloe oil/extract shall pass the test for antibacterial activity outlined in Annex F.
- **6.5** If neem oil is used as part of the formulation, the amount shall be 5 10 % m/m.
- **6.6** A list of ingredients conventionally used in the formulation of shampoos is given for guidance in Annex A.
- **6.7** All active ingredients including detergents, anti-bacterial or anti-dandruff agents shall be named. Any further information concerning the active ingredients shall be supplied by the manufacturer on request.
- **6.8** It shall be the responsibility of the manufacturer to ensure the dermatological safety of their formulations.
- 6.9 For baby shampoos, the active detergent, perfume and all other ingredients shall be of such nature and in such amounts as to leave the final baby product mild in nature. This is due to the sensitive nature of baby skin.

Table 1 — Requirements for general purpose and treatment shampoo, synthetic detergent based

SI no.	Characteristic	Requirement	Test method
i)	Active detergent matter content, % by mass, min.	6	Annex B
ii)	Matter insoluble in alcohol, % by mass, max.	2.0	Annex C
iii)	Lather volume for 1 % solution, mL, min.	100	Annex D

iv)	pH at 27 ± <b>2</b> °C	5.0 to 9.0	Annex E
v)	Antibacterial activity, min.	0.2 mm	Annex F

Table 2 — Requirements for baby shampoo, synthetic detergent based

SI no.	Characteristic	Requirement	Test method
(1)	(2)	(3)	(4)
i)	Active detergent matter content, % by mass, min.	5.0	Annex B
ii)	Matter insoluble in alcohol, % by mass, max.	2.0	Annex C
iii)	pH at 27±2 °C	5.0 to 7.0	Annex E
iv)	Lather volume for one % solution, mL, min	100	Annex D
v)	Antibacterial activity	0.2 mm	Annex F

Table 3 — Requirements for neutralizing shampoo, synthetic detergent-based

SI No.	Characteristic	Requirement	Test Method
(1)	(2)	(3)	(4)
i)	Active detergent matter content, percent by mass, min.	10.0	Annex B
ii)	Matter insoluble in alcohol, per cent by mass, max.	2.0	Annex C
iii)	pH at 27 ±2 °C	4.0 to 5.0	Annex E
iv)	Lather volume for 1 % solution, mL, min.	100	Annex D
v)	Antibacterial activity	0.2 mm	Annex F

# 7 Packaging

The synthetic detergent-based shampoo shall be packed in suitable containers.

# 8 Marking and labelling

In addition to the labeling requirements outlined in EAS 346, the package shall be legibly marked with the following information in or any other language as agreed between the manufacturer and the supplier.

a) type of shampoo;

# 9 Sampling

Random samples of the product shall be drawn for test in accordance with ISO 24153 from the market, factory or elsewhere

# Annex A (informative)

# List of ingredients conventionally used in formulation of synthetic detergent based shampoo

# A.1 Detergents

- a) Sodium or potassium or ethanolamine salts of lauryl sulphonic acid
- b) Lauryl ether sulphates
- c) Sulphated monoglycetides
- d) Sodium alkyl sulpho-acetate
- e) Alkyl benzene polyoxyethyl sulphonates
- f) Sodium n-lauryl sarcosinate
- g) Sodium alpha olefin sulthonates
- h) Other synthetic detergents

## A.2 Foam stabilizers

- a) Ethanolamides or isopropanolamides of fatty acids
- b) Amine oxides
- c) Cocobetaines
- d) Cocomidopropyl betaines

# A.3 Solubilizing agents

- a) Urea
- b) Aliphatic alcohols
- c) Sodium toluene sulphonate
- d) Sodium xylene sulphonate

### A.4 Preservatives

- a) Alcohols
- b) Formaldehyde
- c) Esters of p-hydroxybensoic acid
- d) Sorbic acid
- e) Imidozolidinyl urea

# A.5 Opacifying agents

- a) Higher fatty alcohols
- b) Ethylene / propylene glycol stearates
- c) Mono and di-stearates of glycerol
- d) Zinc, calcium and magnesium salts of fatty acids
- e) PEG distearates 6000
- f) Polyacrylates

# A.6 Inorganic salts

- a) Sodium chloride
- b) Sodium sulphate
- c) Sodium phosphate
- d) Ammonium sulphate
- e) Ammonium phosphate
- f) Ammonium chloride

# A.7 Emollients

a) Lanolin and its derivatives

# A.8 Thickening agents

- a) Sodium carboxymethyl cellulose
- b) Methyl cellulose
- c) Methyl isopropyl cellulose
- d) Guar gum

# A.9 Other groups of ingredients

- a) Perfumes
- b) cosmetic courants
- c) Conditioning agents
- d) Antidandruff agents
- e) Anti-bacterial agents
- f) Quaternary compounds
- g) Vitamins and proteins
- h) Vegetable oils and mineral oils.
- i) Silicones
- j) Sunscreens, etc.

# Annex B (normative)

# **Determination of active detergent content**

### **B.1** Outline of the method

When equivalent amounts of cationic and anionic detergents are present in a two-phase mixture of water and chloroform, methylene blue will colour the two phases to the same degree. Sodium alkyl benzene sulphonate and sodium lauryl sulphate or any other detergent can be titrated with a standard solution of cetyl trimethyl ammonium bromide.

## **B.1.1 Reagents**

### **B.1.1.1** Cationic solution (Solution A)

Weigh 1.5 " 0.001 g of cetyl trimethyl ammonium bromide into a 250 mL beaker. Add 100 mL of distilled water and stir until dissolved. Transfer quantitatively to a 1 litre volumetric flask and make to volume. Mix thoroughly and standardize against solution B. (See B.1.2.2).

#### **B.1.1.2** Anionic solution (Solution B)

Weigh accurately such amount of standard alkyl sulphate of known combined  $SO_3$  or active content so as to give exactly 0.320 g of combined  $SO_3$  into a 250 mL beaker. Dissolve in 100 mL to 200 mL of warm water. Transfer quantitatively to 1-litre volumetric flask and make to volume with water at room temperature. Mix thoroughly. This is the primary standard against which solution A, is standardized. Solution B is 0.004 N.

#### B.1.1.3 Methylene blue indicator

Dissolve 0.1 g of methylene blue in 100 mL of water. Transfer 30 mL of this solution to a 1 litre flask. Add 500 mL of water, 6.8 mL of concentrated sulphuric acid, 50 g of sodium phosphate ( $NaH_2PO_4H_2O$ ) and shake until solution is complete. Dilute to the mark.

### **B.1.1.4 Chloroform**

Analytical reagent grade

## **B.1.2 Procedure**

**B.1.2.1** Weigh accurately a sample of sufficient size to give approximately  $0.320 \, \mathrm{g}$  of combined  $\mathrm{SO}_3$  into a 250 mL beaker. Sample size is crucial (see Note). Use 700 mL to 800 mL of warm water to transfer quantitatively to a 1-litre volumetric flask. Warm on steam bath and shake gently until the sample is dissolved and solution is clear. Cool, dilute to the mark and mix thoroughly.

NOTE The titration value V should be as near as to 10 mL as possible, say between 8 mL and 12 mL but never outside 5 mL and 15 mL.

**B.1.2.2** Pipette 10.0 mL of the sample solution into a 100 mL glass stoppered cylinder (25 x 300 mm). Add 25.0 " 0.5 mL of methylene blue solution and 10 " 0.5 ml of chloroform (see Note). Titrate with solution A to the correct end point, shaking the cylinder carefully after such addition to avoid emulsion and maintaining temperature within prescribed limits of 20 °C to 30 °C by immersion in water bath, if necessary. As the end point is approached, the rate of transfer of colour increases and solution A shall be added dropwise with

vigorous shaking after each addition. If the approximate titration is known, before shaking since this avoids emulsion formation.

Application of vacuum to the titration cylinder may help to break some emulsions, if formed. The end point is reached when both layers have same colour intensity. The end point is very sharp and 0.05 mL will cause a distinct change in colour distribution at or near the equivalence point.

NOTE The titration value V should be as near to 10 mL as possible, say between 8 mL and 12 mL but never outside 5 mL and 15 mL.

**B.1.2.3** Pipette 10.0 mL of the sample solution into a 100 mL glass stoppered cylinder ( $25 \times 300$  mm). Add 25.0 " 0.5 mL of chloroform (see Note). Titrate with solution A to the correct end point, shaking the cylinder carefully after such addition to avoid emulsion and maintaining temperature within prescribed limits of 20EC to 30EC by immersion in water bath if necessary. As the end point solution A shall be added dropwise with vigorous shaking after each addition. If the approximate titration is known, 80 % of the required titrating solution should be added before shaking since this avoids emulsion formation.

Application of vacuum to the titration cylinder may help to break some emulsions, if formed. The end point is reached when both layers have same colour intensity. The end point is very sharp and 0.05 mL will cause a distinct change in colour distribution at or near equivalence point.

**NOTE** The volume of methylene blue solution and chloroform may be changed if found advantageous provided the same volumes are used in standardizing solutions A and B.

#### **B.1.2.4** Calculation

i) % combined SO<sub>3</sub> = 
$$\frac{V \times N \times 8.0}{M}$$

where,

V=volume, in mL of solution A used in the titration;

N=vormality of solution A; and

*M*=mass, in g of the sample in the aliquot.

ii) Per cent active detergent content = per cent combined SO<sub>3</sub> x Mol. weight of active detergent.

**NOTE** The molecular weight of active detergent shall be supplied by the manufacturer on request.

### B.2 Alternative method for determination of active detergent content

(To be used only if the first method (Clause B.1) fails to work on the product).

### **B.2.1 Field of application**

This method is applicable to the analysis of alkylbenzene sulphonates, alkyl sulphonate, sulphates and hydroxy-sulphates, alkylphenol and fatty alcohol ethoxysulphates and dialkyl sulphosuccinates and to the determination of active materials containing one hydrophilic group per molecule.

## **B.2.2 Principle**

Determination of anionic-active matter in a medium consisting of an aqueous and chloroform phase, by volumetric titration with a standard cationic-active solution (benzethonium chloride), in the presence of an indicator which consists of a mixture of a cationic dye (dimidium bromide) and an anionic dye (acid blue 1).

## **B.2.3 Reagent**

- **B.2.3.1** The water used shall be of distilled quality.
- **B.2.3.2** Chloroform, (sp. gravity = 1.48 g/m, distilling between 59.5E and 61.5E).
- **B.2.3.3** Sulphuric acid, 2.5 M solution.
- **B.2.3.4** Sulphuric acid, 0.5 M solution.
- **B.2.3.5** Sodium hydroxide, 1.0 M standard volumetric solution.
- **B.2.3.6** Sodium lauryl sulphate (sodium dodecyl sulphate) (CH<sub>3</sub>(CH<sub>2</sub>) (11 OSO<sub>3</sub>Na), 0.004 M standard volumetric solution.

Check the purity of the sodium lauryl sulphate and simultaneously prepare the standard solution.

**B.2.3.6.1** Determination of purity of sodium lauryl sulphate — Weigh to the nearest 1 mg, 5 " 0.2 g of the product into a 250 mL round bottom flask with ground glass neck. Add exactly 25 mL of the sulphuric acid solution (B.2.3.4) and reflux into a water condenser.

During the first 5 min to 10 min, the solution will thicken and tend to foam strongly; control this by removing the source of heat and swirling the contents of the flask.

In order to avoid excessive foaming, instead of refluxing the solution may be left on a boiling water bath for 60 min.

After a further 10 min the solution clarifies and foaming ceases . Reflux for further 90 min. Remove the source of heat, cool the flask and carefully rinse the condenser with 30 mL of ethanol followed by water.

Add a few drops of the phenolphthalein solution (B.2.3.8) and titrate the solution with the sodium hydroxide solution (B.2.3.5).

Carry out a blank test by titrating 25 mL of the sulphuric acid solution (B.2.3.4) with the sodium hydroxide solution (B.2.3.5).

The purity of the sodium lauryl sulphate, expressed as a percentage,

$$= \frac{28.84(V_1 - V_0) \ M_0}{M_1}$$

where,

 $V_0$  =Volume, in millilitres, of sodium hydroxide solution used for the blanktest;

 $V_1$ =Volume, in millilitres, of sodium hydroxide solution used for the sample;

 $m_1$ =mass, in grams, of the sodium lauryl sulphate to be checked; and

 $M_0$ =exact molarity of the sodium hydroxide solution.

**B.2.3.6.2** Weigh 0.004 M sodium lauryl sulphate standard volumetric solution. Weigh, to the nearest 1 mg between 1.14 g and 1.16 g of sodium lauryl sulphate and dissolve in 200 mL of water. Transfer to a ground glass stoppered 1 litre one-mark volumetric flask and dilute to the mark with water.

Calculate the molarity, M1, of the solution by means of the solution by means of the formula:

$$M_1 = \frac{m_2 \times purity(\%)}{288.4 \times 100}$$

where,

 $m_2$  = mass in grams of sodium lauryl sulphate.

#### **B.2.3.7** Benzethonium chloride 0.004 M standard volumetric solution

Weigh, to the nearest 1 mg, between 1.75 g and 1.85 g benzethonium chloride and dissolve in water.

Transfer to a ground glass-stoppered 1 litre one-mark volumetric flask and dilute to the mark with water.

NOTE In order to prepare a 0.004 M solution, dry the benzethonium chloride at 105EC, weigh 1.792 g, to the nearest 1 mg, dissolve in water and dilute to 1 litre.

**B.2.3.8** Phenolphthalein, ethanolic solution containing 10 g/L. Dissolve 1 g of phenolphthalein in 100 mL of 95 per cent (v/v) ethanol.

#### B.2.3.9 Mixed indicator

#### B.2.3.9.1 Stock solution

Weigh to the nearest 1 mg 0.5 " 0.005 g dimidium bromide into a 50 mL beaker, and 0.025 " 0.005 g of acid blue 1 into a second 50 mL beaker.

Add between 20 mL and 30 mL of hot 10 %. (v/v) ethanol to each beaker. Stir until dissolved and transfer the solutions to a 250-mL one mark volumetric flask. Rinse the beakers into the volumetric flask with ethanol and dilute to the mark with 10 % (v/v) ethanol.

## B.2.3.9.2 Mixed acid indicator solution

Take 20 mL of the stock solution prepared above, put it in a 500 mL one-mark volumetric flask. Add 200 mL of water, and 20 mL of 2.5 M sulphuric acid (B.2.3.2) mix and dilute to the mark with water. Store out of direct sunlight.

## **B.2.4 Apparatus**

Ordinary Laboratory apparatus, and

- a) Bottles, 200 mL, glass stoppered, or measuring cylinders, flask stoppered.
- b) Burettes, 25 mL and 50 mL.
- c) One-mark volumetric flask, 1-litre capacity glass stoppered.
- d) One-mark pipette, 25 mL.

#### **B.2.5 Procedure**

#### B.2.5.1 Standardization of benzethonium chloride solution

By means of the pipette transfer 25 mL of the 0.004 M sodium lauryl sulphate solution to a bottle or measuring cylinder, add 10 mL of water, 15 mL of the chloroform and 10 mL of the mixed indicator solution.

Titrate with the 0.004 M benzethonium chloride solution. Stopper the bottle or measuring cylinder after each addition and shake well. The lower layer will be coloured pink. Continue the titration with repeated vigorous shaking. As the end point approaches, the emulsions formed during shaking tend to break easily Continue the titration drop by drop. Shaking after each addition of titrant, until the end point is reached. This is at the moment when the pink colour is completely discharged from the chloroform layer, which becomes a faint greyish blue.

The molarity, M, of the benzethonium chloride solution is given by the formula:

$$M = \frac{M_1 \times 25}{V_2}$$

where,

 $M_1$ =molarity of the sodium lauryl sulphate solution; and

V<sub>2</sub>=Volume, in mollolitres, of benzethonium chloride added.

#### **B.2.5.2** Determination

Weigh to the nearest 1 mg a sample of 30 g, dissolve the test portion in water. Add a few drops of the phenolphthalein solution and neutralize to a faint pink colour with the sodium hydroxide solution or sulphuric acid solution as required.

Transfer to a 1 litre one-mark volumetric flask and dilute to the mark with water. Mix thoroughly and, by means of the pipette transfer 25 mL of this solution to a bottle or measuring cylinder, add 10 mL of water, and add 10 mL of water, and 15 mL of chloroform. Titrate with the benzethonium chloride solution as described in B.2.5.1.

### **B.2.6 Expression of results**

The content as a percentage by mass, of anionic-active matter

$$= \frac{V_3 \times M \times 1000 \times M_0 \times 100}{25 \times 1000 \times M_0}$$

$$= 4V_3M$$

The amount of active matter, expressed in milliequivalents per gram,

$$\frac{=40 \times V_3 \times M_1}{M_0}$$

where,

 $M_0$ =mass, in grams, of the test portion;

M=relative molar mass of anionic-active matter;

 $M_1$ =molarity of the benzethonium chloride solution;

V3=Volume, in millilitres, of benzethonium chloride solution used for the titration of a 25 ml aliquot of anionic-active matter solution.



# Annex C (normative)

# Determination of matter insoluble in alcohol.

#### C.1 General

This method may be used for the approximate determination of these constituents. As these salts are not completely insoluble in alcohol, separate portions of soap should be used for accurate determination, employing specific methods.

## C.2 Method

It consists in digesting the material in alcohol and filtering off the residue, which is dried and weighed.

## C.3 Reagents

- C.3.1 Phenolphthalein indicator, dissolve 1 g in 100 mL of 95 % rectified spirit.
- **C.3.2** Ethyl alcohol or rectified spirit, freshly boiled and neutral to phenolphthalein.

#### C.4 Procedure

- **C.4.1** Weigh accurately 2 g to 10 g of the sample and digest with 200 mL of freshly boiled ethyl alcohol in a covered vessel on a steam bath until the soap is dissolved. Filter into a filter flask through a tared, dried and counterpoised filter paper till neutral to phenolphthalein, or through a tared and dried Gooch or sintered glass crucible with suction, protecting the solution from carbon dioxide and other acid fumes during the operation by covering with a watch glass. The filter paper and Gooch crucible shall be prepared as per the method given under C4.2.
- **C.4.2** Wash it several times with hot ethyl alcohol to remove all the alcohol solubles. Dry the filter paper or the crucible with residue at  $100 \pm 2$  °C for 3 hours and cool. Weigh the total matter insoluble in alcohol.
- **C.4.3** Place filter paper in a weighing bottle and dry in an air oven at  $105 \pm 2$  °C, with cover removed. Remove from the oven, replace cover, cool to room temperature in desiccator and weigh. Prepare the Gooch crucible with a pad of asbestos fibre. Wash the pad with water, alcohol and ether and then dry to constant mass at  $105 \pm 2$ °C, cool to room temperature in a desiccator and weigh.

## **C.5 Calculation**

Matter insoluble in alcohol,

% by mass = 
$$\frac{100M_1}{M_2}$$

where,

 $M_1$  = mass, in g of matter insoluble alcohol;

 $M_2$  = mass, in g of the material taken for the test.

# Annex D (normative)

## Determination of lather volume.

#### D.1 General

Strict attention shall be paid to all details of the procedure in order to ensure concordant results. Particular care should be taken to invert the cylinder exactly as described.

#### D.2 Outline of the method

A suspension of the material in standard hard water is taken in a graduated cylinder and given 12 inversions under prescribed conditions. The volume of the foam formed is observed after keeping the cylinder for minutes.

# D.3 Reagents

- **D.3.1** Calcium chloride (CaCl<sub>2</sub>2H<sub>2</sub>0, chemically pure.
- **D.3.2** Magnesium sulphate (MgSO<sub>4</sub>.7H<sub>2</sub>0), chemically pure.
- **D.3.3** Distilled water.

### D.4 Apparatus

- a) Graduated cylinder Glass stoppered with graduations from 0 ml to 25 mL, with 2 mL divisions. Overall height about 35 cm and the height of the graduated portion about 20 cm.
- b) A 100 mL glass beaker.
- c) Thermometer of range 0 °C to 110 °C.

# D.5 Preparation of standard hard water

Dissolve 0.220 g of chemically pure calcium chloride, and 0.246 g of chemically pure magnesium sulphate in distilled water. Dilute to 5 litres with distilled water.

NOTE This standard hard water has a hardness of approximately 50 pm calculated as calcium carbonate.

#### D.6 Procedure

Weigh 2 g of the shampoo accurately in a 100 mL glass beaker. Add 10 mL of the standard hard water. Cover the beaker with a watch glass and allow to stand for 30 minutes. This operation is carried out to disperse the shampoo. Stir the contents of the beaker with a glass rod and transfer the slurry to a 250 mL graduated cylinder ensuring that not more than 2 mL foam is produced. Repeat the transfer of the residue left

in the beaker with further portions of 20 mL of standard hard water ensuring that all the matter in the beaker is transferred to the cylinder.

Bring the contents of the cylinder to 30 °C. Stir the contents of the cylinder with a glass rod or thermometer to ensure a uniform suspension.

As soon as the temperature of the contents of the cylinder reach 30  $^{\circ}$ C, stopper the cylinder and give it 12 complete inversions, each inversion comprising movements in a vertical plane, upside down and vice versa. After the 12 inversions, let the cylinder stand for 15 minutes. Take the following readings as shown in Figure D.1.

- a) Foam plus water (V<sub>1</sub>mL)
- b) Water only (V<sub>2</sub>mL)

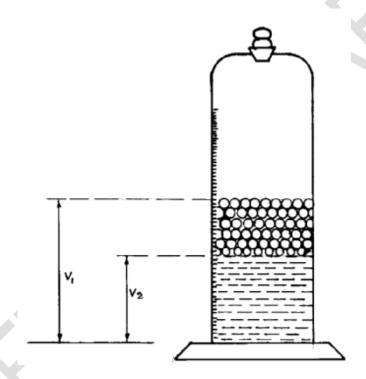


Figure D.1 — Measurement of foam

## D.7 Calculation

Lather volume =  $V_1 - V_2$ 

where,

 $V_1$  = volume, in mL of foam and water;

 $V_2$  = volume, in mL of water only.

# Annex E (normative)

# **Determination of pH**

# **E.1 Apparatus**

E.1.1 pH meter equipped with glass electrode.

# **E.2 Procedure**

Determine the pH at a temperature of  $27\pm2$  °C. In the case of a shampoo in the form of a powder or paste, mix 1 g of the sample with 9 mL of water and determine the pH of the resulting solution.

# Annex F (normative)

# **Antibacterial test**

#### F.1 Procedure

Prepare nutrient agar for bacterial growth by dispersing 28 g of nutrient agar powder in 1 litre of de-ionized water. Allow to soak for 10 minutes, swirl to mix and then heat gently with stirring to ensure uniformity. Sterilize by autoclaving for 15 minutes at 121 °C, cool at 47 °C, mix well and then pour to sterilized petridishes. Leave it to solidify undisturbed. Plant *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* on the so prepared nutrient agar in petri dishes.

Meanwhile, prepare filter paper discs and sterilize them by autoclaving. Dip in various samples. Place in the petri dishes containing bacteria culture agar mixture. Incubate the petri dishes at  $35\,^{\circ}\text{C}$  for  $48\,$  hours. Determine bacteria growth inhibition zones.

# F.2 Antifungal test

Dissolve potato dextrose agar (39 g) in 1 dm<sup>3</sup> of distilled water. Use the same procedure as for bacterial test

(F.1 ) above. Test the cream against fusarium fungi. Obtain the results after 4 days and the temperature of incubation should be  $25\,^{\circ}$ C.

## F.3 Results

The inhibition zone shall be at least 0.2 mm in diameter.

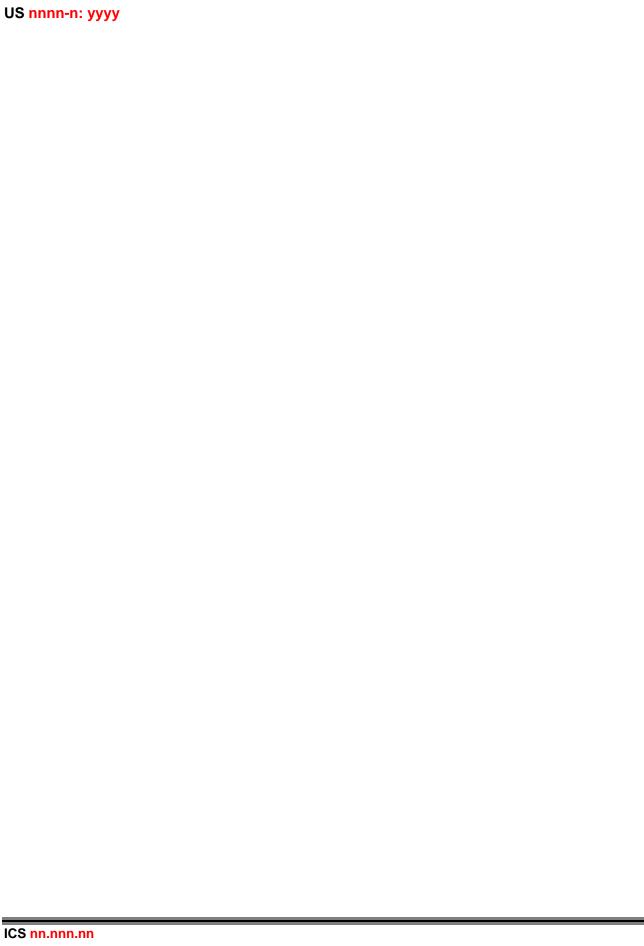
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