INTERNATIONAL STANDARD

ISO 11609

Second edition
2010-09-01

Dentistry — Dentifrices — Requirements, test methods and marking

Médecine bucco-dentaire — Dentifrices — Exigences, méthodes d'essai et marquage

Reference number
ISO 11609:2010(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 11609 was prepared by Technical Committee ISO/TC 106, *Dentistry*, Subcommittee SC 7, *Oral care products*.

This second edition of ISO 11609 cancels and replaces the first edition (ISO 11609:1995), which has been technically revised.

Introduction

Dentifrices should not cause any adverse reactions to the oral soft tissues when used in accordance with the manufacturer's recommendation for frequency and duration of use, nor cause any known side effects.

Guidelines on assessing the claimed or implied efficacy of dentifrices for the prevention or control of oral conditions can be found through the US Food and Drug Administration[3], the American Dental Association[4] and the Commission Work Project (8-95) of the FDI World Dental Federation[16].

Dentistry — Dentifrices — Requirements, test methods and marking

1 Scope

This International Standard specifies requirements for the physical and chemical properties of dentifrices and provides guidelines for suitable test methods. It also specifies requirements for the marking, labelling and packaging of dentifrices.

This International Standard applies to dentifrices, including toothpastes, destined to be used by the public on a daily basis with a toothbrush to promote oral hygiene.

Specific qualitative and quantitative requirements for freedom from biological and toxicological hazards are not included in this International Standard. These are covered in ISO 7405[1] and ISO 10993-1[2].

2 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.

ISO 1942, *Dentistry — Vocabulary*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 8601, *Data elements and interchange formats — Information interchange — Representation of dates and times*

International Nomenclature of Cosmetic Ingredients (INCI), *in International Cosmetic Ingredient Dictionary and Handbook*[1)]

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 1942 and the following apply.

3.1
dentifrice
any substance or combination of substances specially prepared for the public for hygiene of the accessible surfaces of teeth and surrounding tissues

3.2
toothpaste
any semi-solid dentifrice preparation presented in the form of a paste, cream or gel

NOTE The products' common constituents are abrasives, humectants, binders, surfactants, flavourings, fluorides and other agents for oral health benefits.

1) Nomenclature developed by the Personal Care Products Council (Formerly CTFA). Available at: http://www.ctfa.org/council-bookstore.

3.3
single-unit container
container of dentifrice marketed to individual consumers

3.4
primary container
container that is in contact with the product

4 Requirements relative to the physical and chemical properties of dentifices

4.1 Total fluoride

4.1.1 Total fluoride concentration

The total fluoride concentration shall not exceed a mass fraction of 0,15 % when tested in accordance with one of the procedures given in Annex C.

Other validated methods of similar sensitivity and accuracy may be used (see References [5] to [12], [28] and [29]).

4.1.2 Total fluoride in a single-unit container

The amount of total fluoride in a single-unit container shall not exceed 300 mg.

This requirement does not apply to containers of dentifrice to be dispensed under supervised conditions in community-based caries prevention programmes such as school tooth brushing programmes.

4.2 Heavy metals

The total maximum concentration shall not exceed 20 mg/kg.

Test in accordance with References [13], [14] or [15], or another validated method of similar sensitivity and accuracy.

4.3 pH

When tested in accordance with 5.1, the dentifrice shall have a pH below 10,5.

4.4 Microbiology

Testing for microbiological contamination shall be carried out according to References [17] to [22] and [31] to [38] or any other validated method of equivalent sensitivity, accuracy and specificity.

4.5 Abrasivity

The abrasivity of the dentifrice shall not exceed the following limits for dentine:

— 2,5 times that of the primary reference material, if using the procedure specified in Annex A; or

— 2 times that of the primary reference material, if using the procedures specified in Annex B.

The abrasivity of the dentifrice shall not exceed the following limits for enamel:

— 4 times that of the primary reference material, if using the procedure specified in Annex A; or

— 4 times that of the primary reference material, if using the procedures specified in Annex B.

Test in accordance with 5.2 or 5.3 or any other validated method of similar sensitivity and accuracy.

4.6 Stability

The dentifrice shall show no deterioration that may affect compliance with this International Standard after being subjected to one of the ageing procedures specified in 5.4 or after 30 months of storage at room temperature. If deterioration is detected, the dentifrice shall be labelled with an expiry date.

4.7 Readily fermentable carbohydrates

The dentifrice shall not contain readily fermentable carbohydrates. Compliance shall be established by the absence of such compounds in the complete formula or by performing tests in accordance with commonly used analytical methods.

5 Test methods

5.1 Determination of pH

Suspend one part by mass of the dentifrice into three parts by mass of water for analytical laboratory use complying with ISO 3696 (grade 3). Determine the pH of the suspension within 10 min, using a pH-meter and electrode assembly.

5.2 Determination of dentine abrasivity

Determine the mean relative abrasivity compared to the primary reference sample, or any other reference material calibrated to the primary reference sample for human dentine, using one of the methods specified in Annex A or B.

Other validated measurement methods on dentine of similar sensitivity and accuracy may be used; see for example References [23] and [24].

5.3 Determination of enamel abrasivity

Determine the mean relative abrasivity compared to the primary reference sample, or any other reference material calibrated to the primary reference sample for human enamel, using one of the methods specified in Annex A or B.

Other validated measurement methods on enamel of similar sensitivity and accuracy may be used; see for example References [23] and [24].

5.4 Determination of stability

For the accelerated ageing procedure, the dentifrice shall be stored in its original container at 40 °C ± 2 °C at 75 % ± 5 % relative humidity for three months or at such conditions of time and temperature as will simulate storage at room temperature for 30 months[25]. Following storage, test the product according to this International Standard.

6 Marking and labelling

With the exception of small single units (less than 10 ml), all primary containers shall be marked with the following information:

a) the word “dentifrice” or equivalent (see Clause 3);

b) the trade name;

c) the name and contact information of the manufacturer or responsible distributor;

d) the tracking code that includes an intelligible production date;

e) a complete list of ingredients according to the International Nomenclature of Cosmetic Ingredients (INCI);

f) the concentration and type of fluoride, if present, expressed in micrograms per gram, or percent by mass, or both;

g) the net volume, in millilitres, or net mass in grams, or both;

h) the expiry date, expressed according to ISO 8601, if the period of stability (shelf-life) is less than 30 months;

i) a safety notice regarding the use, by children below six years of age, of dentifrices containing concentrations of fluoride of 1 000 µg/g or more.

7 Packaging

The product shall be packaged in such a way that under normal conditions of handling and transport, the container or dispensing system, or both, shall not contaminate or permit contamination of the dentifrice inside, so as to affect its compliance with this International Standard, after being subjected to the ageing procedure described in 5.4.

Annex A
(informative)

Abrasivity test procedure — American Dental Association (ADA) method

A.1 General

This annex identifies the specific procedures for determination of the dentifrice abrasivity using the ADA laboratory method [26].

A.2 Sampling

A representative sample shall be taken from at least two batches.

A.3 Procedure

A.3.1 Standard reference abrasive

The primary reference abrasive is from a specific lot of calcium pyrophosphate[2]. An alternate, silica reference abrasive[3] is also available[27]. For the procedure specified in BSI 5136[30], a chalk reference dentifrice is also available.

A.3.2 Apparatus

A.3.2.1 Brushing machine

A cross-brushing machine is the apparatus of choice[4]. The apparatus should have eight positions for holding specimens. A toothbrush shall be positioned to pass reciprocally at a small angle (≈ 5°) over the mounted specimens, with a designated tension on the brush, while immersed in a dentifrice slurry. The distance traversed by the brush should not be longer than the brush head so that the specimen does not lose contact with the brush. The mechanism for holding the dentifrice slurry may vary with different machine designs, but should allow for easy removal of the slurry sample. It is important to have some mechanism for the agitation of the slurry while the brushing is taking place. A convenient method to accomplish this is to attach rubber mixing vanes just below the brush head. As the brushing takes place, these vanes will prevent the abrasive from settling to the bottom of the slurry container.

---

2) Reference calcium pyrophosphate is available from Odontex Inc., 3030 Campfire Dr., Lawrence, KS 66049, USA, http://www.odontexusa.com. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

3) Alternate reference silica (Sident®) is available from Evonik, Rodenbacher Chaussee 4, 63457 Hanau Wolfgang, Germany, Arnold.Storeck@EVONIK.com. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

4) An acceptable product is available from Sabri Dental Enterprises, Inc., 1404 Brooke Dr., Downers Grove, IL 60515, USA, http://www.sabridentalresearch.com. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

A.3.2.2 Radioactivity detector

The two recommended methods for the determination of the radioactivity of the used dentifrice slurries are a Geiger-Müller planchet counter and a liquid scintillation detector. The use of the Geiger counter requires that the samples be dried under defined controlled conditions. The liquid scintillation method has the advantage of reading directly from the slurry.

Counting should be done for a period expected to reduce the alpha value for counting error to less than 2 %. Counting should be performed for a minimum of 1 000 counts and for at least 1 min. The number of brushing strokes may be increased if counting times become too long.

A.3.3 Preparation of tooth specimens

A.3.3.1 Dentine specimens

A.3.3.1.1 Selection

Human root dentine of extracted permanent teeth are used as the substrate. Single-rooted teeth that were vital at extraction should be selected. An exception, because of the small size, are mandibular incisors: these should not be used. The specimen should be at least 14 mm long and 2 mm wide at the narrow end. All roots shall be caries-free and free of anatomical defects. After extraction, the roots should be stored in a neutralized solution that disinfects but does not alter the physical properties.

A.3.3.1.2 Preparation

Scrape the roots clean of all soft tissue and as much cementum as possible. Then remove the crown and the root tips using a separating disc under a flow of water.

A.3.3.1.3 Irradiation

For each set of eight specimens to be irradiated, add one or two extra roots for use in correction factors. Pack the specimens in disinfection solution and submit to a nuclear reactor for irradiation. The neutron flux should be sufficient to produce about 1 mCi of ^{32}P beta radiation after several hours. Elevated temperatures in the reactor (above 65 °C) should be avoided. A specific position shall be requested to shield the samples from fast neutrons and gamma radiation. Handling of the irradiated specimens should be done with care using good laboratory practice. The specimens should not be used during the first half-life because of excess radiation and should be used before the end of the third half-life because of lack of activity. The half-life of ^{32}P is 14,3 days so the usable life span of a set of teeth is four weeks.

A.3.3.1.4 Mounting of specimens

Mount the specimens individually in a mould in cold-cure methyl methacrylate resin such that either the buccal or lingual surface protrudes at least 2 mm above and parallel to the resin. Orient the mould in the brushing machine such that the direction of brushing is perpendicular to the long dimension of the root. Store the mounted specimens in a neutralized solution that disinfects but does not alter the physical properties.

NOTE The type and configuration of the mould depends on the holder of the brushing machine.

A.3.3.2 Enamel specimens

A.3.3.2.1 Selection

Selection criteria for enamel specimens are the same as for dentine. The enamel specimens should be obtained from human maxillary incisors.

A.3.3.2.2 Preparation

The entire labial surface of the specimen is used after removing the root. Clean the enamel in the same way as the root.

A.3.3.2.3 Irradiation

Irradiation of the enamel is identical to the method used with the roots. The roots and enamel specimens may be packed together for submission to the reactor.

A.3.3.2.4 Mounting

Mount the enamel specimens in the same way as the roots. The labial surface shall protrude 2 mm and be parallel to the resin surface.

A.3.4 Toothbrushes

The toothbrushes[5)] used should have nylon filaments about 10 mm in length. Filament ends should lie in a plane.

Store the brushes in water overnight prior to their first use and then keep them in water until they are discarded. Use a new set of brushes for each set of teeth. Do not remove the brushes from the machine between runs but raise the tufts off the specimen so as not to bend the bristles. At the beginning of each run, set the tension of the brush on the specimen to 150 g using a Chatillon spring gauge or equivalent. This tension should be rechecked at least twice daily. The method of adjusting the tension will vary depending upon the type of mechanism on the brushing machine.

A.3.5 Reference diluent

The diluent is a 0,5 % carboxymethylcellulose (7MF CMC)[6)] solution in 10 % glycerine. To prepare 1 l of the diluent, heat 50 ml of glycerine to 60 °C and add 5 g of CMC while stirring. When the mixture is homogeneous, add another 50 ml of heated glycerine and continue stirring for 60 min. Transfer the solution to a 1 l flask and add 900 ml of distilled water. Allow to cool but continue stirring slowly overnight. To stabilize the viscosity, allow the solution to stand overnight before using. This solution is used to make up slurries of the reference abrasive or any powder being tested.

A.3.6 Reference abrasive slurry

Using the reference material described in A.3.1, dilute 10 g of the abrasive with 50 ml of the diluent (A.3.5). The same ratio is used for all powders. It is possible for the reference abrasive to be used as a dentifrice. If this is the case, it shall be made up as a 40 % abrasive dentifrice with the rest of the constituents being conventional dentifrice components. The slurry is then made using 25 g of reference dentifrice and 40 ml of water.

---

5) Acceptable toothbrushes are available from Odontex Inc., 3030 Campfire Dr., Lawrence, KS 66049, USA, info@Odontexusa.com. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

6) An acceptable CMC is available from Hercules Incorporated, Aqualon Division, 1111 Hercules Road, Hopewell, VA 23860-2782, USA, http://www.herc.com/aqulon/. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

A.3.7 Dentifrice slurries

To prepare the test slurries, add 40 ml of water to 25 g of each dentifrice. For the machine, prepare eight slurries of each dentifrice. This dilution produces a final slurry volume and concentration similar to those of the reference abrasive slurry. All slurries (reference and test) should be used shortly after preparation and after vigorous mechanical stirring to prevent particles from settling.

A.3.8 Preconditioning of tooth specimens

A.3.8.1 Dentine

To reduce the variation caused by dentine surface differences, precondition the specimens prior to each use. The preconditioning treatment consists of brushing with a slurry of the reference abrasive but not taking a sample. The first time dentine specimens are used, the preconditioning should be for 6 000 strokes. Each successive daily run should begin with a shorter preconditioning brushing of 1 000 strokes. The tension of the toothbrush on the roots shall be 150 g.

Discard the preconditioning slurries.

A.3.8.2 Enamel

Preconditioning of the enamel is similar to that of the dentine, except that 10 000 strokes are used prior to the first use and 1 000 strokes are given at the beginning of each day.

Discard the preconditioning slurries.

A.3.9 Test design

A.3.9.1 Test design for dentine

The test design may be either a sandwich design or a Latin Square design. The sandwich design is such that a set of reference slurries is run (pre-test), followed by a set of the first test slurries. These are followed by a second set of reference slurries (post-test). This second set of reference slurries then acts as the pre-test slurries for the next test group. This continues until all the test groups are run.

The Latin Square design is such that a set of reference slurries is run first. All the test groups are randomized over the eight brushing heads for the next few runs (depending on the number of test groups). Then a post-test reference set of slurries is run as the final procedure.

In both test designs, the brush tension is set at 150 g and brushing is performed for 1 500 to 3 000 strokes, depending on the radioactivity level of the specimens.

A.3.9.2 Test design for enamel

The test design for enamel is identical to that for dentine, except that the number of strokes is 5 000 to 7 500 depending on the activity of the specimens.

A.3.10 Sampling of slurries

The sampling of the slurries following the brushing is identical for both dentine and enamel. An aliquot of each slurry is removed immediately following brushing. The size of the aliquot will depend upon the counting method and equipment, but 3 ml is usually adequate to provide a detectable level of radioactivity. A convenient method for removing the sample is a syringe fitted with a blunt needle. Take care to ensure there is no carry-over between samples. This can best be done by a complete rinsing of the syringe between samples. It is also important to remove the same quantity of sample from each slurry. Dry the sample if a planchet counter system is being used to detect the radioactivity. If drying is needed, the samples should be air-dried for a least 1 h and then dried in an oven at 60 °C with forced air overnight.

A.3.11 Correction factors

A.3.11.1 General

Correction factors are needed for both dentine and enamel abrasion tests when using the planchet counting method and are identically prepared in both methods. When testing dentifrices with abrasive systems different from the reference material, the self-absorption and backscattering characteristics of the abrasives for beta radiation may also differ. Real differences in abrasivity may then be significantly distorted. The correction factor is a means of reducing this variable. The correction factor is determined in different ways depending on the counting method used.

A.3.11.2 Preparation of correction factor slurries for Geiger-Müller planchet counting

Dissolve one piece of irradiated dentine (or enamel) in 5 ml of concentrated HCl. Transfer the solution to a 250 ml volumetric flask and add water to the mark. Add 1,0 ml of this radioactive solution to slurries of the reference abrasive and to each of the test abrasives prepared in the same manner as in the test. To neutralize the acid, add 1,0 ml of 0,5 mol/l NaOH. Mix the slurries thoroughly, sample, and dry the samples along with those from the test runs. Do not brush with these correction factor slurries.

These samples are counted along with the test samples.

A.3.11.3 Calculation of correction factors

The correction factor, C_f, to be applied to all count values of the test sample is calculated as in Equation (A.1):

$$C_f = \frac{C_r}{C_t} \tag{A.1}$$

where

C_r are the mean counts for four reference samples;

C_t are the mean counts for four test samples.

A.3.11.3.1 Correction factors for liquid scintillation counting

The correction is determined with regard to the amount of sample mixed with the scintillation cocktail. Each sample is weighed and the net count per minute (CPM) is divided by the mass to get a net CPM per gram of slurry. These net CPM-per-gram values are then used in calculating abrasivity in place of net CPM values according to A.3.13, and there is no C_f term.

A.3.11.3.2 Correction factors for liquid scintillation detection

Self-absorption and backscatter are less of a concern because of the liquid medium being used. Most modern liquid scintillation equipment will automatically colour-correct, so this is not a problem. The differences in mass of the samples do need to be accounted for in the calculation. To do this, each sample taken after brushing needs to be weighed to an accuracy of 0,01 g.

A.3.11.3.3 Applying the correction factor

Before calculating the relative abrasion values, the net CPM of each slurry is divided by the mass of the slurry used to get a net CPM per gram of slurry. These values are then used in the calculation of relative abrasive values.

A.3.12 Calculation of abrasivity using Geiger-Müller counting

A.3.12.1 Dentine abrasivity

The dentine abrasivity, A_D, of the test dentifrices (or abrasives) is calculated as in Equations (A.2) and (A.3):

$$C_{mr} = \frac{C_{pre} + C_{post}}{2} \tag{A.2}$$

where

C_{mr} is the mean reference net CPM;

C_{pre} is the pre-net CPM;

C_{post} is the post-net CPM.

$$A_D = \frac{C_f \times 100 \times C_{mt}}{C_{mr}} \tag{A.3}$$

where

A_D is the dentine abrasivity;

C_f is the correction factor;

C_{mt} is the mean test dentifrice net CPM;

C_{mr} is the mean reference net CPM.

A.3.12.2 Enamel abrasivity

The enamel abrasivity, A_E, of the test dentifrices (or abrasives) is calculated as in Equations (A.4) and (A.5):

$$C_{mr} = \frac{C_{pre} + C_{post}}{2} \tag{A.4}$$

where

C_{mr} is the mean reference net CPM;

C_{pre} is the pre-net CPM;

C_{post} is the post-net CPM.

$$A_E = \frac{C_f \times 10 \times C_{mt}}{C_{mr}} \tag{A.5}$$

where

A_E is the enamel abrasivity;

C_f is the correction factor;

C_{mt} is the mean test dentifrice net CPM;

C_{mr} is the mean reference net CPM.

A.3.13 Calculation of abrasivity using liquid scintillation

A.3.13.1 Dentine abrasivity

The dentine abrasivity, A_D, of the test dentifrices (or abrasives) is calculated as in Equations (A.6) and (A.7):

$$G_{mr} = \frac{G_{pre} + G_{post}}{2} \tag{A.6}$$

where

G_{mr} is the mean reference net CPM per mass of slurry, in grams;

G_{pre} is the pre-net CPM per mass of slurry, in grams;

G_{post} is the post-net CPM per mass of slurry, in grams.

$$A_D = \frac{100 \times G_{mt}}{G_{mr}} \tag{A.7}$$

where

A_D is the dentine abrasivity;

G_{mt} is the mean test dentifrice net CPM per mass of slurry, in grams;

G_{mr} is the mean reference net CPM per mass of slurry, in grams.

A.3.13.2 Enamel abrasivity

The enamel abrasivity of the test dentifrices (or abrasives) is calculated as in Equations (A.8) and (A.9):

$$G_{mr} = \frac{G_{pre} + G_{post}}{2} \tag{A.8}$$

where

G_{mr} is the mean reference net CPM per mass of slurry, in grams;

G_{pre} is the pre-net CPM per mass of slurry, in grams;

G_{post} is the post-net CPM per mass of slurry, in grams.

$$A_E = \frac{10 \times G_{mt}}{G_{mr}} \tag{A.9}$$

where

A_E is the enamel abrasivity;

G_{mt} is the mean test dentifrice net CPM per mass of slurry, in grams;

G_{mr} is the mean reference net CPM per mass of slurry, in grams.

Annex B
(informative)

Determination of relative dentifrice abrasivity to enamel and dentine by a surface profile method

B.1 General

This method, based on a modification of BS 5136:1981[30], may be used as an alternative to the radio-tracer method (see Annex A).

B.2 Apparatus

B.2.1 Contact profilometer, or similar instrument with a sensitivity to < 0,1 µm [e.g. Surfometer or Surftest SV-2000[7)]], or a **non-contact profilometer**, or similar instrument with a sensitivity to < 0,1 µm [e.g. Proscan 2000[8)]].

B.2.2 Lapping and polishing unit [e.g. the Kent 3 automatic lapping and polishing unit[9)]], with sequential silicon carbide disks up to P1200.

NOTE Other methods of polishing enamel and dentine to conform with the baseline requirements for specimens can be used (e.g. diamond powder).

B.2.3 Brushing machine as detailed in A.3.2.1.

NOTE Some machines have less than eight specimen positions, but can be used provided the n value for specimens/treatment is reached.

B.2.4 Manual toothbrush as detailed in A.3.4.

B.2.5 Standard reference abrasive as detailed in A.3.1, being either pyrophosphate or silica abrasives. Do not use the BSI chalk reference, i.e. BS 5136[30] abrasive, in an attempt to harmonize the relative dentine abrasivity (RDA) measurement radio-tracer and profilometry methods.

B.3 Preparation of enamel and dentine specimens

B.3.1 Use human caries-free, erupted or unerupted permanent teeth from subjects under 40 years old.

NOTE 1 Lower incisors may be unsuitable because of their small size.

NOTE 2 In certain countries human teeth may not be readily available, in which case bovine teeth can be used.

---

7) Surfometer is the trade name for a product supplied by Planar Products Ltd., Sunbury on Thames, UK; and Surftest SV-2000 is the trade name for a product supplied by Mitutoyo, Andover, UK. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

8) Proscan 2000 is the trade name for a product supplied by Scantron Industrial Products Ltd., Monarch Centre, Venture Way, Taunton, UK. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

9) Kent 3 is the trade name for a product supplied by Kermet International Ltd, Maidstone, UK. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

B.3.2 Remove all soft tissue remnants by scraping with a suitable instrument (e.g. curette, scalpel, etc.), sterilize the teeth with sodium hypochlorite for at least 24 h and store in a suitable medium (e.g. sterile 0,9 % saline).

NOTE Other antimicrobial and storage solutions can be used providing they are known not to alter the physico-chemical properties of enamel and dentine.

B.3.3 Section the teeth at the amelo-cemental junction with a dental bur or disc. Use the coronal portion for enamel specimens and the radicular portion for dentine specimens.

NOTE Depending on the size and morphology of the crown and roots, between two and four specimens each of enamel and dentine may be obtainable from each tooth.

B.3.4 Section the crown vertically in either a bucco-lingual or a mesio-distal direction. Similarly, section the root portion vertically in half or quarters so that an outer portion of root surface is available for polishing.

NOTE In the case of molars, buccal, lingual, mesial and distal slices of enamel can be obtained. This is facilitated by a diamond-edged annular, circular cutting instrument.

B.3.5 Place the enamel and dentine portions, outer face down, in moulds measuring 25 mm $\times$ 25 mm $\times$ 3 mm and embed in epoxy resin [e.g. Stycast 1266[10)]]. Allow the resin to set for at least 24 h.

NOTE Other specimen-embedding resins can be used and the overall final dimensions of specimens can differ depending on the dimensions of the specimen-holding positions of the brushing machine used.

B.3.6 Place the specimens on the automatic lapping and polishing machine with the outer face of the enamel or dentine on the polishing discs. Surface the specimen with coarse abrasive paper, e.g. P180, followed by fine abrasive paper of P1200.

NOTE If necessary, intermediate grit can be used, e.g. P600.

B.3.7 Ensure that the polishing procedure results in a flat surface with no more than an average profile of $\pm$0,3 µm measured by a profilometer.

B.3.8 Use at least six enamel and six dentine specimens for each dentifrice being evaluated and for the reference dentifrice. Place two pieces of adhesive tape, e.g. PVC, parallel to each other to expose a window of enamel or dentine approximately 2 mm wide. Keep the specimens hydrated during all preparation, abrasion and measurement procedures.

B.4 Preparation of reference dentifrice

B.4.1 Reference diluent

Prepare the reference diluent as described in A.3.5.

B.4.2 Reference abrasive slurry

Use either the pyrophosphate or the silica reference abrasives listed in A.3.1. Prepare the slurry as described in A.3.6 using the reference diluent (see A.3.5) or as a 40 % abrasive dentifrice.

10) Stycast 1266 is the commercial name for a product supplied by Hitek Electronic Material Ltd., Scunthorpe, UK. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

B.5 Preparation of test dentifrice slurries

Prepare the test dentifrice slurries as described in A.3.7.

B.6 Reference dentifrice and test dentifrice brushing procedure

B.6.1 Place the dentine or enamel specimens in the countersunk wells of the brushing machine. Place a sufficient volume of dentifrice slurry in the brushing machine reservoir to ensure that specimens are covered by at least 3 mm of slurry. Brush specimens for a total of 10 000 strokes with a load of 150 g.

NOTE One stroke corresponds to the forward and backward movement of the brush heads over the specimens.

B.6.2 Remove the specimens from the wells, wash under running tap water, remove the tape and measure the depth of abrasion by profilometry as described below.

a) If the chosen n value for enamel or dentine specimens per dentifrice slurry cannot be achieved in a single brushing cycle, then repeat the cycle for that dentifrice with the remaining specimens placed into the machine and new brush heads and dentifrice slurry.

b) If a second cycle or more cycles are necessary to achieve the n value, but not all counter-sunk wells are required for specimens, place resin blank specimens in the remaining wells in order to ensure a smooth traverse of the brush heads across the specimen chamber.

B.7 Profilometry method

B.7.1 After dentifrice slurry brushing, remove the tape from specimens and re-measure using the operating method for the particular profilometer.

a) For two-dimensional contact profilometers, take the profile from just inside the previously taped zone of the specimen, across the exposed zone, and just into the previously taped zone opposite. Calculate the mean from at least 100 z values across the scan. Measure three scans from different points along the exposed zone and calculate the mean of the three measurements.

b) For 3-D contact and non-contact profilometers, take a length in the X axis of the exposed window, such as 1 mm. Scan at several micrometres along this zone, again from the edge of the previously taped zone, across the treated zone, to the edge of the other previously taped zone. Calculate the mean of these averaged z values in micrometres, to the nearest 2 µm, to give the abrasion value for that specimen.

B.7.2 Depending on the number of specimens of enamel and dentine allocated to each reference and test dentifrice, calculate a mean abrasive depth across the respective specimen group.

B.8 Calculation of relative dentine abrasivity (RDA) and relative enamel abrasivity (REA) of test dentifrices using profilometry measurements

The reference dentifrice is considered to have an RDA value of 100 and an REA value of 10 respectively.

The following Equations (B.1) and (B.2) are used to calculate the RDA and REA respectively of the test dentifrice:

$$A_{\mathrm{D}} = \frac{A_{\mathrm{mt}} \times 100}{A_{\mathrm{mr}}} \tag{B.1}$$

$$A_{\mathrm{E}} = \frac{A_{\mathrm{mt}} \times 10}{A_{\mathrm{mr}}} \tag{B.2}$$

where

A_{D} is the relative dentine abrasivity (RDA) of the test dentifrice;

A_{E} is the relative enamel abrasivity (REA) of the test dentifrice;

A_{mt} is the mean abrasion of the test dentifrice;

A_{mr} is the mean abrasion of the reference dentifrice.

Annex C
(informative)

Testing of total fluoride in dentifrices

C.1 General

This annex describes two different methods for testing the total fluoride content of dentifrices containing fluoride. See References [28] and [29] for other methods.

C.2 Methods

C.2.1 Total fluoride in dentifrice (paste and gel): ADA method

C.2.1.1 Background

This procedure implements a diffusion technique which extracts the fluoride as HF from the dentifrice matrix and then allows measurement of the total fluoride using an ion-specific electrode. Sample preparation is designed to account for the presence of calcium fluoride or silica-bound fluoride, or both, in the dentifrices using ethylene diamine tetraacetic acid (EDTA).

C.2.1.2 Procedure

Coat the inside of polystyrene Petri dish covers (60 mm × 15 mm) with sodium hydroxide by placing 0,3 ml of 0,5 mol/l sodium hydroxide in ethanol and allowing the alcohol to evaporate. Accurately weigh approximately 1 g of paste. Using a dilution ratio 1:10, add 10 ml of 0,1 mol/l EDTA solution with the pH previously adjusted to 8,0 by adding NaOH as necessary. Homogenize the mixture for 1 min and centrifuge 4 ml of the slurry at 14 000 r/min for 5 min using a centrifuge. Transfer 2,0 ml of the supernatant to the bottom of a Petri dish. Add 4,0 ml of 70 % $HClO_4$ and cover immediately with a sodium-hydroxide-coated Petri dish cover.

CAUTION — This latter step shall be done extremely carefully so that foam formed after adding $HClO_4$ does not wet the Petri dish cover.

Place the Petri dish in an oven at 60 °C ± 2 °C for at least 6 h.

Remove the Petri dishes from the oven and allow them to cool to room temperature. Remove the Petri dish cover and wash with 5,0 ml deionised water twice, resulting in a total volume of the solution of 10,0 ml. Transfer 1,0 ml of this solution to a 3 ml to 5 ml plastic beaker and add 1 ml of TISAB II. Analyse the solution for fluoride using an ion-specific electrode. Prepare a five-point calibration curve and use it to determine the fluoride content of each dentifrice slurry.

C.2.2 Total fluoride in dentifrice: IS 6356

NOTE This method is based on Indian Standard IS 6356[8].

C.2.2.1 Principle

Sodium monofluorophosphate or fluoride ions are extracted with water from toothpaste and the extract is fused with sodium carbonate to convert it into sodium fluoride. The fluoride content is then determined potentionmetrically with the help of a fluoride-ion-sensitive electrode.

C.2.2.2 Apparatus

C.2.2.2.1 pH-meter (potentiometer).

C.2.2.2.2 Fluoride-ion-sensitive electrode.

C.2.2.2.3 Saturated calomel electrode as reference electrode.

C.2.2.3 Reagents

C.2.2.3.1 Sodium fluoride, reagent grade.

C.2.2.3.2 Triethanolamine, pure.

C.2.2.3.3 EDTA, disodium salt, dihydrate, reagent grade.

C.2.2.3.4 Sodium carbonate, reagent grade.

C.2.2.3.5 Hydrochloric acid, reagent grade.

C.2.2.4 Standard solutions and reagent solutions

C.2.2.4.1 Triethanolamine buffer solution.

Dissolve 149 g of pure triethanolamine in 600 ml of distilled water and, with concentrated hydrochloric acid, adjust the pH to 7,0 using a pH-meter. Cool the solution to room temperature and test the pH; if necessary, re-adjust it; then dilute to 10 000 ml with distilled water.

C.2.2.4.2 EDTA, 0,1 mol/l solution.

Weigh accurately 37,224 g of EDTA and dissolve it in distilled water; make the solution up to 1 000 ml in a volumetric flask.

C.2.2.4.3 Standard sodium fluoride solution, 0,1 mg F/ml.

Dry the sodium fluoride at 110 °C for 4 h and transfer accurately 0,222 g to a 100 ml volumetric flask. Add distilled water to dissolve the sodium fluoride and make up to the mark (solution A). Take 10 ml of solution A in the 1 000 ml volumetric flask and make up this volume to the mark (solution B). Each millilitre of solution B contains 0,01 mg of fluoride ion. Transfer solutions A and B to polythene bottles for storing.

C.2.2.5 Preparation of standard solutions of sodium fluoride

Pour 1 ml, 2 ml, 5 ml, 10 ml, 20 ml and 25 ml of solution B (C.2.2.4.3) into 100 ml volumetric flasks marked A, B, C, D, E and F respectively. To each, add 25 ml of EDTA and 10 ml of triethanolamine hydrochloride buffer solution and make up the volume to 100 ml with distilled water. Now the solutions A, B, C, D, E and F contain 0,01 mg, 0,02 mg, 0,05 mg, 0,1 mg, 0,2 mg and 0,25 mg of fluoride ion per 100 ml respectively. Transfer the solutions to 150 ml polythene beakers to measure the potential difference.

C.2.2.6 Test solution

Weigh accurately 5,0 g of toothpaste in a 150 ml beaker and add 50 ml of distilled water. Stir over a magnetic stirrer at about 40 °C for 30 min and cool. Centrifuge the solution for 10 min at 15 000 r/min, wash and collect the washings. Transfer the supernatant liquid and the washing to a 100 ml volumetric flask and fill up to the mark. Transfer the solution to a polythene bottle. Take 5 ml of this supernatant solution into a 25 ml capacity platinum crucible and add 1 ml of a volume fraction of 10 % solution of sodium carbonate. Heat the crucible

over a flame to dryness. Transfer the crucible to a muffle furnace and heat to 600 °C ± 2 °C. After cooling the crucible in a desiccator, add 25 ml of 0,1 mol/l EDTA and boil for some time. Cool and transfer to a 100 ml volumetric flask (if necessary, filter the solution). Add 10 ml of triethanolamine hydrochloride buffer solution and make up to 100 ml. Transfer the solution to a 150 ml polythene beaker for measurement of potential difference. Stir the standard solutions and the test solutions over the magnetic stirrer and measure the steady potential difference, in millivolts. Take the readings for standard and test solutions simultaneously.

C.2.2.7 Calculation

A graph is plotted for content of fluoride ion against potential difference on semi-logarithmic paper. The potential difference, in millivolts, is plotted on the X axis and the fluoride ion, in milligrams, on the Y axis (on the logarithmic scale).

From the calibration curve, determine the concentrations, c, of fluorides in the toothpaste, in parts per million in the test solutions using Equation (C.1):

$$c = \frac{2a \times 10\,000}{m} \tag{C.1}$$

where

c is the fluoride concentration, in parts per million;

a is the content of fluoride ion, in milligrams, from the calibration graph;

m is the mass of the sample, in grams.

Bibliography

[1] ISO 7405, *Dentistry — Evaluation of biocompatibility of medical devices used in dentistry*

[2] ISO 10993-1, *Biological evaluation of medical devices — Part 1: Evaluation and testing within a risk management process*

[3] U.S. Food & Drug Administration, Anticaries Drug Products for Over-the-Counter Human Use; Final Monograph, Final Rule. Federal Register, 21 CFR Part 310, 1995, p. 52474

[4] Clinical caries trials: Proceedings of a conference of the American Dental Association on May 17-19, 1983. *J. Dent. Res*., **63**, 1984, pp. 693-828

[5] EC-OJ No. L 291/37-40, *Determination of total fluorine in dental creams*, 1983

[6] U.S. Food & Drug Administration, *Standards for fluoride dentifrices*. Federal Register, **45**, 1980, p. 62

[7] AS 2827:1985, *Method for determining soluble fluorine derivatives and total fluorine derivatives in toothpaste*

[8] IS 6356:2001, *Toothpaste — Specification*

[9] THEMELIS, D.G., TZANAVARAS, P.D. Simultaneous spectrophotometric determination of fluoride and monofluorophosphate ions in toothpastes using a reversed flow injection manifold. *Analytica Chimica Acta*, 2001, 429:111-116

[10] WANG, T., LI, SAM F.Y. Development of a capillary electrophoresis method with indirect photometric detection for the determination of anions related to anticaries. *Journal of Chromatography*, A, 1997, 781: 457-466

[11] BIEMER, T.A., ASRAL, N., SIPPY, A., Ion chromatographic procedures for analysis of total fluoride content in dentifrices. *Journal of Chromatography*, A, 1997, 771:355-359

[12] LOUIS, D., WILKES A.J., TALBOT, J.M., Optimization of total fluoride analysis by capillary gas chromatography. Part I: Silica-based dental creams. *Belg. Pharm. Acta Helv.*, 1996, 71:273-277

[13] US Pharmacopeia, *Heavy metal analysis in toothpastes*, 1990, pp. 1523-1524

[14] British Pharmacopeia, *Heavy metal analysis in toothpastes*, 1990, pp. 87-88

[15] Japanese Pharmacopeia, *Heavy metals limit test.* 14th ed., 2001, pp. 43-44

[16] Lignes directrices pour l'évaluation de l'efficacité des dentifrices, Projet de Travail (8-95) de la Commission FDI. *Journal dentaire international*, **49**, 1999, pp. 311-316

[17] CTFA, *Determination of adequacy of preservation of cosmetics and toiletry formulations*, 1973

[18] CTFA, *Microbiological limit guidelines for cosmetic and toiletries*, 1973

[19] CTFA, *Microbial quality management*, 1990

[20] JSQI: 2006, *Japanese Standard of Quasi-Drug Ingredients*

[21] SABS 1302:1980, *Standard specification for toothpastes*

[22] EEC 88/667, *Guidelines for analysis methods of cosmetic products*

[23] *Le Chirurgien Dentiste de France,* French dentistry organization*,* **193**, 1983, pp. 49-51

[24] *Regulations concerning the certification and technical agreement relating to toothpastes.* French Official Journal Décret 80/524, July 1982

[25] POPE, D.G. Accelerated stability testing for production of drug product stability. *Drugs and Cosmetics*, 1980, pp. 54-62

[26] HEFFERREN, J.J. A Laboratory Method For Assessment of Dentifrice Abrasivity. *J. Dent. Res.*, **55**, 1976, pp. 563-573

[27] HEFFERREN, J.J., SCHEMEHORN, B.R., STORECK, A., LERCH, M., LI, N. Silica as a reference for laboratory dentifrice assessment methods: Multiple site collaborative study, *J. Clin. Dent.*, 2007, 18: 12-16

[28] *Recommended assay procedure for the testing of fluoride containing dentifrices*. American Dental Association, 1989

[29] HATTAB, F.N. Analytical methods for the determination of various forms of fluoride in toothpastes. *J. Dent.*, **17**, 1989, pp. 77-83

[30] BS 5136:1981, *Specification for toothpastes*

[31] ISO 16212, *Cosmetics — Microbiology — Enumeration of yeast and mould*

[32] ISO 18416, *Cosmetics — Microbiology — Detection of Candida albicans*

[33] ISO 21148, *Cosmetics — Microbiology — General instructions for microbiological examination*

[34] ISO 21149, *Cosmetics — Microbiology — Enumeration and detection of aerobic mesophilic bacteria*

[35] ISO 21150, *Cosmetics — Microbiology — Detection of Escherichia coli*

[36] ISO 22717, *Cosmetics — Microbiology — Detection of Pseudomonas aeruginosa*

[37] ISO 22718, *Cosmetics — Microbiology — Detection of Staphylococcus aureus*

[38] ISO 29621, *Cosmetics — Microbiology — Guidelines for the risk assessment and identification of microbiologically low-risk products*

ICS 97.170

Price based on 20 pages