INTERNATIONAL STANDARD

ISO 10993-14

First edition
2001-11-15

Biological evaluation of medical devices —

Part 14: Identification and quantification of degradation products from ceramics

Évaluation biologique des dispositifs médicaux —

Partie 14: Identification et quantification des produits de dégradation des céramiques

Reference number
ISO 10993-14:2001(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.ch
Web www.iso.ch

Printed 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 3.

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 part of ISO 10993 may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 10993-14 was prepared by Technical Committee ISO/TC 194, *Biological evaluation of medical devices*.

ISO 10993 consists of the following parts, under the general title *Biological evaluation of medical devices*:

— *Part 1: Evaluation and testing*

— *Part 2: Animal welfare requirements*

— *Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity*

— *Part 4: Selection of tests for interactions with blood*

— *Part 5: Tests for in vitro cytotoxicity*

— *Part 6: Tests for local effects after implantation*

— *Part 7: Ethylene oxide sterilization residuals*

— *Part 8: Selection and qualification of reference materials for biological tests*

— *Part 9: Framework for identification and quantification of potential degradation products*

— *Part 10: Tests for irritation and delayed-type hypersensitivity*

— *Part 11: Tests for systemic toxicity*

— *Part 12: Sample preparation and reference materials*

— *Part 13: Identification and quantification of degradation products from polymeric medical devices*

— *Part 14: Identification and quantification of degradation products from ceramics*

— *Part 15: Identification and quantification of degradation products from metals and alloys*

— *Part 16: Toxicokinetic study design for degradation products and leachables*

— *Part 17: Establishment of allowable limits for leachable substances using health-based risk assessment*

— *Part 18: Chemical characterization of materials*

Introduction

This part of ISO 10993 consists of two tests for the biological evaluation of medical devices: an extreme solution test and a simulation solution test. The extreme solution test is developed as a worst-case environment and the simulation test is developed as a very common environment.

Degradation products covered by this part of ISO 10993 are formed primarily by dissolution in an aqueous environment. It is recognized that additional biological factors such as enzymes and proteins can alter the rate of degradation. Degradation by such outside factors is not addressed in this part of ISO 10993.

It should be kept in mind that a ceramic device might have extraneous chemical phases and/or elements in extremely minor amounts. Whilst these components might not be named in the original specification, they can often be suspected by the relationship that the material in question has to other materials and the expected history of the material's processing.

Once identified and quantified, the chemical composition of the degradation products form the basis for risk assessment and, if appropriate, biological safety studies according to the principles of ISO 10993-1.

Biological evaluation of medical devices —

Part 14: Identification and quantification of degradation products from ceramics

1 Scope

This part of ISO 10993 specifies two methods of obtaining solutions of degradation products from ceramics (including glasses) for the purposes of quantification. It also gives guidance on the analysis of these solutions in order to identify the degradation products. Because of the generalized nature of this part of ISO 10993, product specific standards, when available, that address degradation product formation under more relevant conditions of use, should be considered first.

This part of ISO 10993 considers only those degradation products generated by a chemical dissociation of ceramics during *in vitro* testing. No degradation induced by mechanical stress or external energy is covered. It is noted that while ISO 6872 and ISO 9693 cover chemical degradation tests, they do not address the analysis of degradation products.

Because of the range of ceramics used in medical devices and the different requirements for accuracy and precision of the results, no specific analytical techniques are identified. Further, this part of ISO 10993 provides no specific requirements for acceptable levels of degradation products.

Although these materials are intended for biomedical applications, the biological activity of these degradation products is not addressed in this part of ISO 10993.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this part of ISO 10993. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this part of ISO 10993 are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 3310-1, *Test sieves — Technical requirements and testing — Part 1: Test sieves of metal wire cloth*

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

ISO 5017, *Dense shaped refractory products — Determination of bulk density, apparent porosity and true porosity*

ISO 6474, *Implants for surgery — Ceramic materials based on high purity alumina*

ISO 6872:1995, *Dental ceramic*

ISO 10993-1, *Biological evaluation of medical devices — Part 1: Evaluation and testing*

ISO 10993-9, *Biological evaluation of medical devices — Part 9: Framework for identification and quantification of potential degradation products*

3 Terms and definitions

For the purposes of this part of ISO 10993, the terms and definitions given in ISO 10993-1 and ISO 10993-9 as well as the following apply.

3.1
ceramics
typically crystallized materials that are physically nonmetallic and chemically inorganic

3.2
blank disc
noncoated circular plate made of the substrate material to be used in the finished device

3.3
retentate
undissolved solids remaining in the filter paper after filtration

3.4
filtrate
solution which passes through the filter paper

4 Test procedures

4.1 Principle

This part of ISO 10993 consists of two tests. The first test, an extreme solution test conducted at low pH, serves as a screen for most ceramics for the observation of possible degradation products. The second test simulates a more frequently encountered *in vivo* pH. A flowchart of the decision process for using these test methods is given in Figure 1.

The test methods described in this part of ISO 10993 shall be used for ceramics in bulk and granular form as well as ceramic coatings.

When deviations from the recommended test specimen or solution volumes are used, full justification shall be provided.

4.2 Testing of dental devices

4.2.1 General

This part of ISO 10993 is intended to simulate worst-case exposure to tissue environments. For dental ceramics exposed to the oral cavity (e.g. ceramic veneering material), a more appropriate test environment is given in ISO 6872. However, for dental devices not exposed to the oral cavity, such as dental implant stems, the specifications given in 4.4 of this part of ISO 10993 shall apply.

4.2.2 Test methods for dental devices exposed to the oral cavity

For dental devices exposed to the oral cavity, the method given in 8.4 of ISO 6872:1995 shall be used as the extreme solution test.

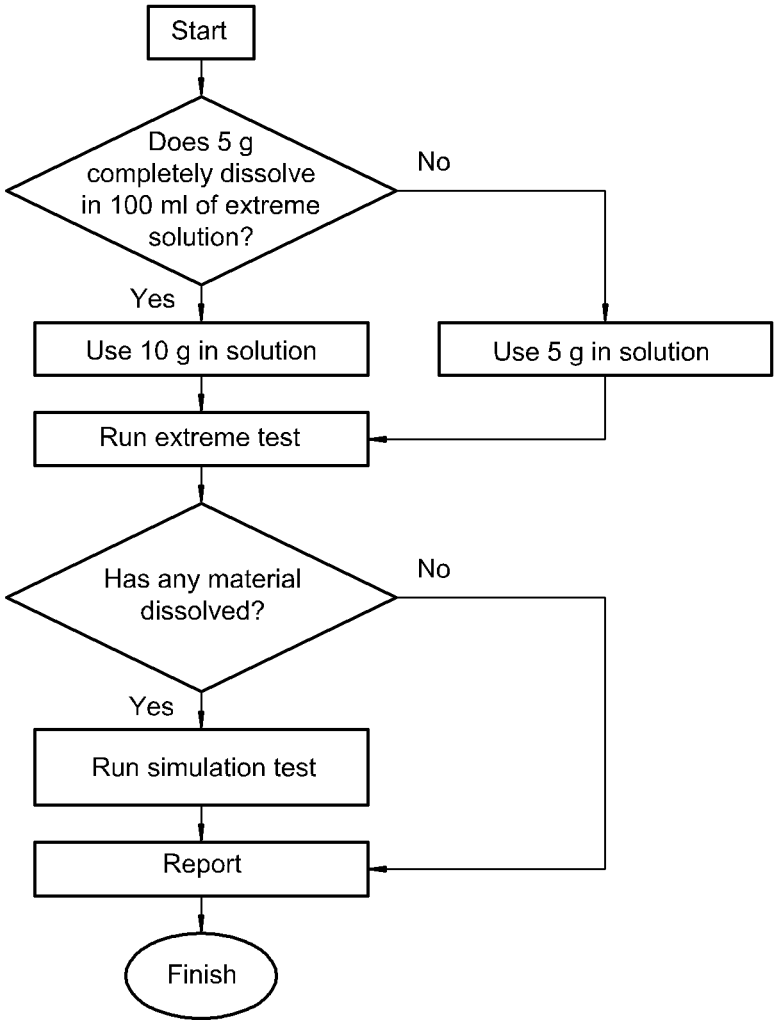

Figure 1 — Flowchart of the decision-making process for the extreme solution test and the simulation solution tests (see text for details)

4.2.3 Specimen characterization

The specimen shall be characterized as described in 4.4.4. If the specimen density is greater than 99 % of the theoretical maximum density, and the specimen has an average surface roughness (Ra) of less than 5 µm, the surface area may be calculated by direct geometrical measurement.

Low surface roughness is required for geometrical measurement in order to avoid grossly underestimating the surface area.

4.2.4 Analysis

The filtrate for analysis shall be separated from the retentate as described in 4.4.7.6 to 4.4.7.11.

4.3 General testing techniques

4.3.1 Mass determination

Mass shall be determined using a balance with an accuracy of no less than 0,000 5 g. All mass determinations shall have 6 replicates.

4.3.2 Drying techniques

Drying in an oven at a temperature of (100 ± 2) °C shall continue until a mass change of < 0,1 % occurs between mass determinations. This is normally accomplished by drying overnight and weighing at 2-h intervals the next day.

4.4 Extreme solution test

4.4.1 Principle

The extreme solution test is a test based on a low pH citric acid buffer solution. The pH value of 3 is defined here as a worst-case low-end service environment. For devices exposed to an environment where the pH is lower than 3, an alternative lower pH solution shall be used and justification shall be provided. In the event of a chemical reaction between the extreme solution and the test specimen, an alternative extreme test at similar pH shall be justified and performed.

4.4.2 Application range

This test is applicable to all ceramics. It should be noted that the mechanisms of degradation may not be the same for all materials at low pH as they are at blood pH (approximately pH 7,35 to pH 7,45). Nonetheless, as an extreme condition for the production of possible degradation products, this severe test can serve as a screen for most materials.

It is expected that materials will dissolve up to their solubility limit in the solution. To accelerate the test to the solubility limit endpoint, the test is carried out on a granulated specimen (see 4.4.3.3).

4.4.3 Extreme test sample preparation

4.4.3.1 Specimen configuration

Specimens shall be granulated from a specimen manufactured according to the method intended for material use. If the specimen is a ceramic coating, it shall be removed from the substrate material and granulated to an appropriate size. Under some circumstances (e.g. thin coatings), insufficient ceramic material is available to perform the extreme test. In these cases a scaled-down test may be used in which a sample may be prepared using the ratio of 1 g per 20 ml of test solution. When this is done, the precision and accuracy of the mass determination shall be appropriately scaled and justified in order to accommodate the alternative sample size.

4.4.3.2 Granulation

Granulation shall be accomplished by grinding with a tungsten carbide mortar and pestle.

4.4.3.3 Sizing

The granulated specimen shall pass through a 400 µm screen but be retained on a 315 µm screen using a dry screen method such as that described in ISO 3310-1.

If it is not possible to produce granules of this size (e.g. due to the grinding of a coating), granules of a size smaller than that defined in this part of ISO 10993 shall be used, and the size shall be reported.

NOTE The use of a particle size smaller than that specified in this clause is likely to lead to increased dissolution and therefore is not expected to reduce the yield of dissolution products and not expected to compromise risk analysis for biological safety.

4.4.3.4 Specimen preparation

The mass of starting material is dependent upon the solubility of the material as determined by the solubility characterization in 4.4.4.3:

— for low solubility granulated material, (5,00 ± 0,05) g shall be used;

— for high solubility granulated material, (10,00 ± 0,05) g shall be used.

4.4.4 Extreme test sample characterization

4.4.4.1 Surface area characterization

The specimen shall be characterized by gas adsorption in accordance with an appropriate method, e.g. such as those given in ASTM D4780.

4.4.4.2 Density

The specimen shall be characterized for density in accordance with ISO 5017.

4.4.4.3 Solubility characterization

From information about the material available from the producer or other sources, the materials shall be characterized as "high" or "low" solubility materials in the following manner.

— Consult Figure 1 for a flowchart of the decision-making process.

— If (5,00 ± 0,05) g of the material are expected to totally dissolve in 100 ml during testing as described in 4.4.7.1 to 4.4.7.5, the material shall be considered as high solubility.

— If (5,00 ± 0,05) g of the material are not expected to totally dissolve in 100 ml, the material shall be considered as low solubility.

— If the information is unavailable, the material shall be considered as high solubility material.

4.4.4.4 Microstructural and X-ray characterization

X-ray diffraction shall be performed with an X-ray diffractometer. The 2Θ resolution and reproducibility shall be better than 0,02°. Microstructure analysis shall conform with that specified in ISO 6474.

4.4.5 Test equipment

4.4.5.1 Test container

A 250 ml polypropylene or high-density polyethylene container shall be used. A fresh specimen container shall be used for each test. Glass containers shall not be used since they may contaminate test solutions.

4.4.5.2 Büchner funnel

A Büchner or similar type funnel fitted appropriately to retain undissolved particles shall be used.

4.4.6 Citric acid buffer solution

The buffered citric acid solution shall be freshly prepared and have a pH of 3,0 ± 0,2 at a temperature of (37 ± 1) °C. The solution shall be prepared as follows:

Dissolve 21 g of citric acid monohydrate in 500 ml water (ISO 3696, grade 2) in a 1 000 ml volumetric flask. Add 200 ml of 1 mol/l sodium hydroxide solution and dilute to the mark with water (ISO 3696, grade 2). Mix 40,4 ml of this solution with 59,6 ml of 0,1 mol/l hydrochloric acid yielding the buffered citric acid solution.

4.4.7 Test procedure

4.4.7.1 Weigh the container without the top.

4.4.7.2 Weigh the container and specimen. Report the difference in mass between the container with specimen and the container without specimen as the mass of the specimen.

4.4.7.3 Add (100 ± 1) ml of buffered citric acid. Care should be taken to ensure that all of the specimen is in contact with the solution.

4.4.7.4 Place the container with specimen in a controlled-temperature environment at (37 ± 1) °C for (120 ± 1) h. The container shall be agitated at 2 Hz using a longitudinal or circular movement. If the test specimen is totally dissolved before 120 h, terminate the test and note the time in the test report.

4.4.7.5 Remove the container and specimen and allow them to cool to room temperature.

4.4.7.6 Weigh the filtering medium (e.g. filter paper) to determine its mass without retentate.

4.4.7.7 Remove the specimen via filtration and retain the filtrate for analysis. Filtrate should not be stored in glass containers.

4.4.7.8 Rinse the filtering medium and retentate three times with small amounts of water (ISO 3696, grade 2) to remove the citric acid buffer.

4.4.7.9 Dry the specimen and filtering medium with retentate to a constant mass (see 4.3.2).

4.4.7.10 Weigh the filtering medium with retentate. The difference in mass between the filtering medium with and without retentate is the mass of the retentate.

4.4.7.11 The difference between the mass of the specimen and the mass of retentate is the mass of the dissolved material.

4.5 Simulation solution test

4.5.1 Principle

The simulation test is based on a buffer solution of pH 7,4 ± 0,1 as defined in 4.5.6. This will simulate the body's normal pH level.

4.5.2 Application range

This test is applicable to all ceramics.

NOTE The mechanism of degradation in this test may not be the same as in the extreme test.

4.5.3 Simulation test specimen configuration

4.5.3.1 Coated ceramics

4.5.3.1.1 Blank discs

Test specimens shall be prepared as coatings on blank discs.

Blank discs shall be of diameter (36 ± 1) mm and thickness of (2 ± 0,1) mm using the same substrate material and preparation techniques as in the finished device.

4.5.3.1.2 Coated discs

Blank discs shall be coated on all sides using coating techniques that are used in the production of the finished device.

NOTE Because of the reduced surface area to volume ratio, the sensitivity of the test will be reduced using this method.

4.5.3.2 All other ceramics

Test specimens shall be granulated using the methods described in 4.4.3.2 and 4.4.3.3 from a specimen manufactured by methods used to produce the finished device.

4.5.4 Simulation test sample characterization

4.5.4.1 General

For coated samples, surface area, microstructure and X-ray characterization shall be recorded. For all other ceramics, density, surface area, microstructure and X-ray characterization shall be recorded.

4.5.4.2 Density

The specimen shall be characterized for density in accordance with ISO 5017.

4.5.4.3 Microstructural and X-ray characterization

X-ray diffraction shall be performed with an X-ray diffractometer. The resolution and reproducibility shall be better than 0,02°. Microstructure analysis shall conform with that specified in ISO 6474.

4.5.4.4 Surface area characterization

The specimen shall be characterized by gas adsorption in accordance with an appropriate method, e.g. such as those given in ASTM D4780.

4.5.5 Test equipment

4.5.5.1 Test container

A 250 ml polypropylene or high-density polyethylene container shall be used. A fresh specimen container shall be used for each test. Glass containers shall not be used since they may contaminate test solutions.

4.5.5.2 Büchner funnel

A Büchner or similar type funnel fitted appropriately to retain undissolved particles shall be used.

4.5.6 Buffer solution

The solution shall be freshly prepared TRIS-HCl buffer. It shall be prepared by dissolving 13,25 g of tris(hydroxymethyl)aminomethane in 500 ml of water (ISO 3696, grade 2). Adjust the pH with an appropriate amount of 1 mol/l hydrochloric acid to pH 7,4 ± 0,1 at a temperature of (37 ± 1) °C. Make up to 1 000 ml with water (ISO 3696, grade 2).

4.5.7 Coated disc test procedure

4.5.7.1 General

Both the coated and uncoated discs are exposed to the simulation test solution in order to determine whether degradation products are generated under simulated test conditions.

4.5.7.2 Blank disc test

4.5.7.2.1 Place the blank disc in the test container for the exposure test.

4.5.7.2.2 Add (100 ± 1) ml of buffer solution to the container with the blank disc. Care shall be taken to ensure that the entire blank disc is in contact with the solution.

4.5.7.2.3 Maintain the container with blank disc at (37 ± 1) °C in a controlled-temperature chamber for (120 ± 1) h. The container shall be agitated at 2 Hz using a longitudinal or circular movement.

4.5.7.2.4 Remove the container with specimen and allow them to reach room temperature.

4.5.7.2.5 Filter the solution and retain the filtrate for analysis (see clause 5).

4.5.7.3 Coated disc test

4.5.7.3.1 Determine the mass of the ceramic coating by subtracting the mass of the coated disc from the mass of the blank disc for each test specimen.

Each disc shall be weighed before and after coating to determine the mass of the coating.

4.5.7.3.2 Place the coated disc in a test container for the exposure test.

4.5.7.3.3 Add (100 ± 1) ml of buffer solution to the container with the coated disc. Care shall be taken to ensure that the entire coated disk is in contact with the solution.

4.5.7.3.4 Maintain the container with coated disc at (37 ± 1) °C in a controlled-temperature chamber for (120 ± 1) h. The container shall be agitated at 2 Hz using a longitudinal or circular movement.

4.5.7.3.5 Remove the container with specimen and allow them to cool to room temperature.

4.5.7.3.6 Weigh the filtering medium (e.g. filter paper).

4.5.7.3.7 Filter the solution and retain the filtrate for analysis (see clause 5).

4.5.7.3.8 Rinse the filtering medium and retentate three times with small amounts of water (ISO 3696, grade 2).

4.5.7.3.9 Dry the coated disc and filtering medium with retentate to a constant mass.

4.5.7.3.10 Weigh the filtering medium with retentate. The difference in mass between the filtering medium with and without retentate is the mass of the retentate.

4.5.7.3.11 The difference between the original mass of the coating and the mass of the retentate is the mass of the materials dissolved.

4.5.8 Test procedure (all other ceramics)

4.5.8.1 Weigh the container without the top.

4.5.8.2 Weigh the container and specimen. Report the difference in mass between the container with specimen and the container without specimen as the mass of the specimen.

4.5.8.3 Add (100 ± 1) ml of buffer solution. Care shall be taken to ensure that the entire specimen is in contact with the solution.

4.5.8.4 Place the container with the specimen in a controlled-temperature environment at (37 ± 1) °C for (120 ± 1) h. The container shall be agitated at 2 Hz using a longitudinal or circular movement. If the test specimen is totally dissolved before 120 h, terminate the test and note the time in the test report.

4.5.8.5 Remove the container and specimen and allow them to cool to room temperature.

4.5.8.6 Weigh the filtering medium (e.g. filter paper) to determine its mass without retentate.

4.5.8.7 Remove the specimen via filtration and retain the filtrate for analysis. Filtrate should not be stored in glass containers.

4.5.8.8 Rinse the filtering medium and retentate three times with small amounts of water (ISO 3696, Grade 2).

4.5.8.9 Dry the specimen and filtering medium with retentate to a constant mass (see 4.3.2).

4.5.8.10 Weigh the filtering medium with retentate. The difference in mass between the filtering medium with and without retentate is the mass of retentate.

4.5.8.11 The difference between the mass of the specimen and the mass of retentate is the mass of the dissolved material.

5 Analysis of filtrate

5.1 General

After each experiment a qualitative and quantitative analysis of the solution shall be performed in triplicate.

The number of test methods, practices, accuracy and precision on analytic techniques is large and changeable. Samples should be analysed using Inductively Coupled Plasma Spectroscopy (ICP) if possible. Other tests, such as Atomic Absorption Spectroscopy (AAS), whilst less useful, may provide information at the desired concentration levels.

5.2 Choice of chemicals or elements to be analysed

The chemicals or elements to be analysed in the filtrate solutions should include both chemical constituents known to exist in the material and possible impurities such as small amounts of elements that are due to commonly known substitutions in the raw material and possible additions to the material during processing. The volume of filtrate shall be made up to a fixed volume of 125 ml or 250 ml, depending on the starting volume. Larger volumes shall be justified.

5.3 Sensitivity of the analysis method

Applied analysis methods shall be of adequate sensitivity (e.g. at least 10^{-6} by atomic absorption or mass spectroscopy). Record only compositional constituents that have been detected above the limits of quantification. Hazardous materials shall be recorded in accordance with the appropriate International Standards, if available.

6 Test report

The test report shall include all data identified in accordance with this part of ISO 10993 during characterization, testing and analysis:

a) test institution;

b) date of measurement;

c) a statement that this test was conducted in accordance with ISO 10993-14 and describing any deviations from the standard protocols, with justifications;

d) description of test material including batch or lot number;

e) type of test:

   1) high solubility,

   2) low solubility,

   3) extreme (intra-oral),

   4) extreme (10993-14),

   5) simulated;

f) surface area and method;

g) sample density, microstructure and X-ray diffraction pattern;

h) duration of test;

i) test results:

   1) specimen mass,

   2) volume of solution added,

   3) drying time,

   4) mass of retentate,

   5) mass of material dissolved,

   6) volume of filtrate,

   7) chemical analysis and method (for coated specimens, the degradation products from the ceramic are differentiated from the substrate by comparing the analysis of filtrate of the blank disc with that from the coated disc),

   8) for each element identified in the filtrate, calculation of mass dissolved per total surface area.

Bibliography

[1] ISO 9693, *Metal-ceramic dental restorative systems*

[2] ISO 10993-12, *Biological evaluation of medical devices — Part 12: Sample preparation and reference materials*

[3] ISO 10993-16, *Biological evaluation of medical devices — Part 16: Toxicokinetic study design for degradation products and leachables*

[4] ISO 10993-17, *Biological evaluation of medical devices — Part 17: Establishment of allowable limits for leachable substances using health-based risk assessment*

[5] ASTM C92, *Standard Test Methods for Sieve Analysis and Water Content of Refractory Materials*

[6] ASTM D4780, *Standard Test Method for Determination of Low Surface Area of Catalysts by Multipoint Krypton Adsorption*

ICS 11.100

Price based on 11 pages