INTERNATIONAL STANDARD

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Implants for surgery — Copolymers and blends based on polylactide — *In vitro* degradation testing

Implants chirurgicaux — Copolymères et mélanges à base de polylactide — Essais de dégradation in vitro

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Contents

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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.

International Standard ISO 15814 was prepared by Technical Committee ISO/TC 150, *Implants for surgery*, Subcommittee SC 1, *Materials*.

Annex A of this International Standard is for information only.

Implants for surgery — Copolymers and blends based on polylactide — *In vitro* degradation testing

1 Scope

This International Standard is applicable to copolymers and/or blends based on polylactide, in bulk or processed forms, used for the manufacture of surgical implants.

This International Standard describes methods for the determination of chemical and mechanical changes in the properties of these copolymers and/or blends under *in vitro* degradation testing conditions.

The test methods specified in this International Standard are intended to determine the degradation rate and the changes in material properties of polylactide-based copolymers and/or blends with various comonomers (for example glycolid, trimethylene carbonate, ε-caprolactone) *in vitro*. These *in vitro* methods cannot be used to predict definitely the behaviour of these materials under in *vivo* conditions.

The purpose of this International Standard is to compare and/or evaluate materials or processing conditions.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard 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 178:1993, *Plastics — Determination of flexural properties.*

ISO 180:1993, *Plastics — Determination of Izod impact strength.*

ISO 527-1:1993, *Plastics — Determination of tensile properties — Part 1: General principles.*

ISO 527-2:1993, *Plastics — Determination of tensile properties — Part 2: Test conditions for moulding and extrusion plastics.*

ISO 537:1989, *Plastics — Testing with the torsion pendulum.*

ISO 604:1993, *Plastics — Determination of compressive properties.*

ISO 1184:1993, *Plastics — Determination of tensile properties of films.*

ISO 1628-1:1998, *Plastics — Determination of the viscosity of polymers in dilute solution using capillary viscometers — Part 1: General.*

ISO 1805:1973, *Fishing nets — Determination of breaking load and knot breaking load of netting yarns.*

ISO 2062:1993, *Textiles — Yarns from packages — Determination of single-end breaking force and elongation at break.*

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*.

ISO 5081:1977, *Textiles — Woven fabrics — Determination of breaking strength and elongation (Strip method).*

ISO/TR 10993-9:1994, *Biological evaluation of medical devices — Part 9: Framework for identification and quantification of potential degradation products.*

ISO 13781:1997, *Poly(L-lactide) resins and fabricated forms for surgical implants — In vitro degradation.*

ISO 14130:1997, *Fibre-reinforced plastic composites — Determination of apparent interlaminar shear strength by short-beam method*.

3 Terms and definitions

For the purposes of this International Standard, the terms and definitions given in ISO 13781, ISO/TR 10993-9 and the following apply.

3.1
copolymer
polymeric material which consists of different monomer units

3.2
blend
polymeric material which consists of different polymers mixed intensively together

3.3
resorption
loss of morphology and loss of mass

4 Degradation methods

4.1 General

The initial values for the following tests shall be determined directly before starting the degradation test (time zero). The tests shall be carried out on the degraded samples at each test period.

4.2 Reagents and apparatus

4.2.1 Soaking solution (phosphate buffer solution; Sörensen buffer)

For the *in vitro* degradation study, the test sample shall be immersed in a "Sörensen" buffer solution (pH 7,4) consisting of potassium dihydrogenphosphate and disodium hydrogenphosphate in analytical water Grade 2 in accordance with ISO 3696.

a) 1/15 mol/l KH_2PO_4: 9,078 g KH_2PO_4 per litre H_2O

b) 1/15 mol/l Na_2HPO_4: 11,876 g $Na_2HPO_4 \cdot 2H_2O$ per litre H_2O

Prepare the solution by mixing 18,2 % (volume fraction) from solution a) and 81,8 % (volume fraction) from solution b).

No other additives shall be used for the solution.

Salts used for the preparation of the buffer solution shall be of analytical grade and dried to constant mass.

The minimum volume of the buffered solution used shall be 10 ml. The ratio of the volume, in millilitres, of buffer solution to the test sample mass, in grams, shall be greater than 30:1. The buffer capacity shall be equal or greater than the maximum calculated acid concentration. The test sample shall be completely immersed in the soaking solution.

4.2.2 Inert plastic or glass container (e.g. bottle, jar, vial, etc.) capable of holding the test sample for each material and time period and the required volume of soaking solution. Each container shall be sealed against loss of solution by evaporation and to prevent microbial contamination.

4.2.3 Constant-temperature bath or oven capable of maintaining the sample containers at degradation temperature (37 ± 1) °C at any place for the specified test duration (for example circulating-air dryer).

4.2.4 pH-Meter, for controlling the pH value.

4.2.5 Analytical balance with an accuracy of 0,1 mg.

4.3 Control of the buffer solution

4.3.1 Changes in pH value

The pH value shall be measured in two different containers at each test period. In extended test periods, the pH value shall be measured additionally in at least two containers every four weeks.

If in one container the pH value has shifted beyond the limits, measure the value in all containers and adjust to pH 7,4 ± 0,3 using 0,1 mol/l NaOH solution.

4.3.2 Clouding of buffer solution

Clouding of the buffer solution may indicate contamination with microorganisms. Discard the test sample if any clouding is visible which cannot be related directly to the material itself or its degradation products.

It is recommended that the containers and soaking solutions be sterilized in order to avoid contamination with microorganisms.

4.4 Degradation test

4.4.1 Temperature

The test sample shall be maintained at physiological temperature of (37 ± 1) °C.

4.4.2 Test period

The test samples shall be completely immersed in the soaking solution.

For materials intended to degrade over a short time, remove the test samples from the soaking solutions at the following numbers of weeks after time zero: 2, 4, 8, 16 and 26.

For materials intended to degrade over a long time, remove the test samples from the soaking solutions on at least six occasions, including at 6, 12, 26, 39 and 52 weeks after time zero.

5 Mechanical tests

5.1 General

Each test sample shall be used for one mechanical test only. During all test periods, do not agitate the solution. To prevent slippage of the test sample in the grips, carefully dry the surfaces to be gripped, using e.g. a paper towel.

At least three test samples shall be tested at each period.

For statistical analysis, at least six samples at each test period are required.

NOTE Mechanical properties change with temperature. The glass transition temperature of amorphous or semicrystalline polymers depends on the content of water in the material. Drying of test samples, especially of fibre-reinforced and/or degraded materials, may affect the properties.

5.2 Test procedures

5.2.1 Conditioning of the test sample

The type of conditioning used shall be given in the test report.

a) Type A: Soak the samples in analytical grade water for 60 min ± 3 min at a temperature of 37 °C ± 1 °C. Carry out the mechanical tests in a water bath at 37 °C ± 1 °C.

b) Type B: Soak the samples in the solution for 60 min ± 3 min. Carry out mechanical tests at room termperature (22 °C ± 1 °C) 10 min after removal from the solution.

5.2.2 Test periods

For materials intended to degrade in a short time, at least seven test periods are required, including those at the following number of weeks after time zero: 2, 4, 8, 16 and 26.

For materials intended to degrade in a long time, at least seven test periods are required, including those at the following number of weeks after time zero: 6, 12, 26 and 52.

5.2.3 Test method

Select the test methods to simulate the intended loading conditions of the device, taking into account the shape of the test sample. Determine the mechanical properties using one of the test procedures given in Table 1.

The test method to be used shall be specified by the parties submitting the sample.

The test parameters to be determined shall be those specified in the appropriate standard listed in Table 1, those specified by the parties submitting the sample, or such other parameters as shall be determined by the test house, provided that in each case the selection of these parameters is justified and reported.

NOTE 1 As the shape and the structure of the test sample may have a strong influence on the degradation kinetics, where applicable the test sample should be comparable to the final products in shape and structure.

NOTE 2 Other test methods for specific structures (e.g. cellular plastics) or specific requirements (e.g. tensile creep) may be used, if applicable.

Table 1 — Test methods

Form	Test methods
Rigid material	ISO 178 ISO 180 ISO 527-1, ISO 527-2 ISO 537 ISO 604 ISO 14130
Film, foil, sheet	ISO 1184
Fibres, textiles	ISO 2062 ISO 1805 ISO 5081

6 Physicochemical tests

6.1 Loss of substance mass

6.1.1 Apparatus

6.1.1.1 Balance, a calibrated mass device capable of measuring the mass of the test sample to an accuracy of 1 % of the total sample mass.

6.1.1.2 Desiccator containing a desiccant to absorb moisture for drying the test samples. For example, silica gel beads containing an indicator can be used.

6.1.1.3 Vacuum pump, capable of producing a vacuum of at least 5 kPa (50 mbar) in the desiccator.

6.1.1.4 An appropriate apparatus for the separation of the debris produced during the degradation study. This may involve an inert filter, a temperature-controlled centrifuge, or a combination thereof. The apparatus shall be described and defined in the test report.

6.1.2 Number of test samples

At least three test samples shall be tested at each period. A separate container shall be used for each sample.

For statistical analysis, at least six samples at each test period are required.

6.1.3 Shape and structure of the test samples

As the shape and the structure of the test sample may have a strong influence on the degradation kinetics, where applicable the test samples shall be comparable to the intended products in dimensions and structure.

6.1.4 Procedure

6.1.4.1 Separation of sample and debris by means of a filter

Dry a filter under vacuum at room temperature to constant mass. Determine the mass of the filter.

Separate sample, possible debris and the degradation solution by means of the weighed filter. For filtering, a water-jet blast can be used. Wash the contents of the filter three times with analytical water.

6.1.4.2 Separation of sample and debris by means of a centrifuge

Determine the mass of a clean dry centrifuge tube. Transfer the degradation test sample solution into the centrifuge tube and close the tube prior to separation. Spin the tube in the centrifuge to obtain a firm debris pellet. Carefully decant the supernatant solution into a container. Resuspend the pellet with analytical water and spin again.

Decant again the supernatant solution and add this solution to the container. Repeat this procedure a further two times.

6.1.4.3 Measurement of initial mass

Dry the test sample under vacuum at room temperature to constant mass. Determine the initial mass of the test sample with an accuracy of 1,0 % of the total mass.

6.1.4.4 Determination of mass of degraded samples

Rinse the sample with small amounts of analytical water and add the rinse solution to the degradation solution. Combine the sample and any debris obtained from the filter or the centrifuge and dry to constant mass. Then determine the mass balance.

6.1.5 Reusability of test samples

The dried samples used for the mass loss measurement shall not be used for mechanical testing.

Test samples for the determination of mass loss should be used for further testing [e.g. loss of molecular weight, changes in differential scanning calorimetry (DSC) curves, changes in molecular weight distribution [by gel permeation chromatography (GPC)], structural analysis [by scanning electron microscopy (SEM)].

The following analytical methods are suggested, if appropriate:

a) DSC, in accordance with ISO 3146;

b) atomic absorption spectroscopy (AAS) for catalyst content;

c) size exclusion chromatography (SEC), GPC for change in molecular weight distribution; ASTM D 3536 is suitable;

d) gas chromatography (GC) for monomer content;

e) X-ray diffraction for analysis of crystallinity and structure;

f) SEM for morphological structure analysis, propagation of resorption, and validation of breakage mechanism particularly for reinforced materials;

g) determination of optical activity.

For these analyses, the dried samples used for the mass measurements may be used.

6.2 Determination of limiting viscosity

Dry samples to constant mass. Determine the inherent viscosity of the undegraded and degraded materials in accordance with ISO 1628-1 at a test temperature of (25 ± 0,1) °C.

The solvent shall be chloroform. If the copolymer or blend is not soluble in chloroform, use hexafluoroisopropanol (HFIP).

The concentration of the polymer solution shall be 0,1 %, with a mass concentration of (50 ± 2) mg/50 ml solution.

7 Test termination

Terminate testing of degraded samples if:

a) the predetermined time has been reached; or

b) a minimum of 50 % mass loss has been reached.

Stop mechanical tests when test values are insignificantly small, or measurement is technically impossible.

8 Test report

The test report shall include the following information:

a) test material description, batch or lot number and dimensions;

b) type of conditioning (see 5.2.1);

c) detailed description of test methods used, including, where appropriate, specificity, sensitivity, detection and quantification limits;

d) test environment;

e) method used to determine mass loss, including precision and standard deviation;

f) mass/volume ratio;

g) sample pretreatment and drying method;

h) test temperature;

i) test periods;

j) deviations from test protocol;

k) reason for test termination;

l) test results:

    1) test sample masses, expressed as an average percentage loss (initial and subsequent) by time period;

    2) inherent viscosity (initial and subsequent) by time period;

    3) mechanical properties, if measured: initial and by time period; test parameters (for example deformation speed, distance between the clamps);

    4) appearance of test samples after the different exposure-time periods.

Annex A
(informative)

Accelerated degradation test

A.1 Principle

The accelerated test is a screening test for the degradation behaviour of the material to be tested. There is no correlation to *in vivo* behaviour.

A.2 Temperature

The test sample shall be maintained at increased temperature of (70 ± 1) °C.

A.3 Test period

Remove the test samples from the soaking solution and test after at least two soaking time periods, for example 24 h and one week.

Bibliography

[1] ISO 3146:1985, *Plastics — Determination of melting behaviour (melting temperature or melting range) of semi-crystalline polymers.*

[2] ASTM D 3536, *Standard test method for molecular weight averages and molecular weight distribution by liquid exclusion chromatography (gel permeation chromatography — GPC).*

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