# INTERNATIONAL STANDARD

# ISO 13781

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# Poly(L-lactide) resins and fabricated forms for surgical implants — *In vitro* degradation testing

*Résines et éléments à base de poly(L-lactide) pour implants chirurgicaux — Essais de dégradation in vitro*

Normen-Download-Beuth-Forschungszentrum Karlsruhe GmbH in der Helmholtz-Gemeinschaft-KdNr.32548-LfNr.4543402001-2009-07-30 12:01

Reference number
ISO 13781:1997(E)

## 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.

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 13781 was prepared by Technical Committee ISO/TC 150, *Implants for surgery*, Subcommittee SC 1, *Materials*.

Annexes A and B of this International Standard are for information only.

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# Poly(L-lactide) resins and fabricated forms for surgical implants — *In vitro* degradation testing

## 1 Scope

This International Standard describes methods for the determination of chemical and mechanical changes in properties of poly(L-lactide) under *in vitro* degradation testing conditions.

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

This International Standard applies to poly(L-lactide) in various forms, used for the manufacture of surgical implants, including:

a) bulk material;

b) processed material;

c) finished products (packaged and sterilized implants).

The test methods specified in this International Standard are intended to determine the degradation rate and the changes in material properties of poly(L-lactide) *in vitro.* These *in vitro* methods cannot be used to predict definitely the behaviour under *in vivo* conditions.

NOTE — It is recommended that copolymers and/or blends from poly(L-lactide) and/or its stereoisomers also be tested according to this International Standard as long as no relevant International Standard is available.

## 2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 178:1993, *Plastics — Determination of flexural properties.*

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

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 527-3:1995, *Plastics — Determination of tensile properties — Part 3: Test conditions for films and sheets.*

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

ISO 1628-1:1984, *Guidelines for the standardization of methods for the determination of viscosity number and limiting viscosity number of polymers in dilute solution — Part 1: General conditions.*

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

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

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

ISO 6721-2:1994, *Plastics — Determination of dynamic mechanical properties — Part 2: Torsion-pendulum method.*

ASTM D 4475:1990, *Test method for apparent horizontal shear strength of pultruded reinforced plastic rods by the short-beam method.*

DIN 53137:1977, *Testing of filter paper, determination of rate filtration on freely suspended filters.*

## 3 Definitions

For the purposes of this International Standard, the following definitions apply:

**3.1 degradation:**
Loss of mechanical properties and/or chemical integrity through chemical changes in the material induced by environmental conditions, which can be followed by mass loss and morphological change.

**3.2 *in vitro* degradation:**
Degradation induced by storage in physiological fluids or simulating environment.

**3.3 resorbable polymer:**
Polymer deliberately designed to be transformed *in vivo* to nonharmful byproducts which are subsequently eliminated from the organism.

**3.4 poly(L-lactide):**
Polymeric material which consists of L-lactide units.

## 4 Degradation methods

### 4.1 General

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

### 4.2 Apparatus and reagents

**4.2.1 Soaking solution** (phosphate buffer solution; Sörensen buffer), consisting of potassium dihydrogen phosphate and disodium hydrogen phosphate in sterile double-distilled water.

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

Solution a) 1/15 mol/l $KH_2PO_4$, prepared by dissolving 9,078 g $KH_2PO_4$ in 1 litre water.

Solution b) 1/15 mol/l $Na_2HPO_4$, prepared by dissolving 11,876 g $Na_2HPO_4 \cdot 2H_2O$ in 1 litre water.

The buffer comprises a mixture of a volume fraction of 18,2 % from solution a) and a volume fraction of 81,8 % from solution b).

No other additives shall be used for the solution.

The pH value of the buffer solution shall be 7,4 ± 0,2.

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**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 sealable against loss of solution by evaporation.

**4.2.3 Constant-temperature bath, or oven,** capable of maintaining the sample containers at the specified degradation temperature ± 1 °C for the specified test duration.

**4.2.4 pH-meter**, for controlling the pH value in the range between pH 6 and pH 8 ± 0,2.

## 4.3 Control of buffer solution

### 4.3.1 Changes in pH

The pH of the buffer solution shall be measured in at least two different containers at each test period. In extended test periods, the pH shall be measured additionally in two containers at the following intervals:

a) real-time degradation test: every six weeks;

b) accelerated degradation test: weekly.

If in at least one container the pH value has shifted beyond the specified limits, all containers shall be measured and adjusted to pH 7,4 ± 0,2, using a solution of $c(\mathrm{NaOH}) = 0{,}1$ mol/l.

### 4.3.2 Clouding of buffer solution

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

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

## 4.4 Real-time degradation

Place the test sample in a container (4.2.2), cover with soaking solution (4.2.1) and seal the container. The minimum volume of the buffer solution used shall be 10 ml. The ratio of volume of the buffer solution, in millilitres, to the test sample mass, in grams, shall be greater than 30:1. The test sample shall be fully covered by the soaking solution.

Using the constant-temperature bath or oven (4.2.3), maintain the test sample at physiological temperature of (37 ± 1) °C.

Remove the test samples from the soaking solution and test at predetermined intervals.

For a real-time degradation test, at least six tests are required, at periods including 4 weeks, 26 weeks and 104 weeks of soaking.

## 4.5 Accelerated degradation

### 4.5.1 Principle

A test after accelerated degradation is a screening test for the quality control of the processed material. No correlation exists with *in vivo* behaviour. This test can be used only to obtain information concerning degradation of one material in a short time.

### 4.5.2 Test procedure

Place the test sample in a container (4.2.2), cover with soaking solution (4.2.1) and seal the container. The minimum volume of the buffer solution used shall be 10 ml. The ratio of volume of the buffer solution, in millitres, to

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the test sample mass, in grams, shall be greater than 30:1. The test sample shall be fully covered by the soaking solution.

Using the constant-temperature bath or oven (4.2.3), maintain the test sample at a temperature of (70 ± 1) °C.

Remove the test samples from the soaking solution and test at predetermined intervals.

For an accelerated degradation test, at least two tests are required, for example after periods of 24 h and one week.

## 5 Physicochemical tests

NOTE — The following analytical methods are suggested, if appropriate:

- differential scanning calorimetry (DSC) in accordance with ISO 3146;
- atomic absorption spectrometry (AAS) for catalyst content;
- size exclusion chromography (SEC) and gas phase chromatography (GPC) for change in molecular weight distribution;
- gas chromatography (GC) for monomer content;
- X-ray diffraction for analysis of crystallinity and structure;
- scanning electron microscopy (SEM) (morphological structure analysis, propagation of resorption, validation of breakage mechanism particularly for reinforced materials).

For these analyses, dried samples from mass loss measurements may be used.

### 5.1 Loss of mass of degraded samples

#### 5.1.1 Apparatus

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

**5.1.1.2 Desiccator**, containing a desiccant, for example silica gel beads, to absorb moisture, for drying the test samples.

**5.1.1.3 Vacuum pump**, capable of producing a vacuum in the desiccator.

**5.1.1.4 Filter**, for qualitative and quantitative analysis, having a filtration time measured according to Herzberg (see DIN 53137) of 160 s.

NOTE — Filters 589/4 yellow in accordance with DIN 12448 have been found suitable.

#### 5.1.2 Preparation of test samples

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

NOTE — For statistical analysis, more than three samples at each test period are required.

As the shape and the structure of the test sample may have a strong influence on the degradation kinetics, where applicable the initial test sample should be comparable to the intended product in shape and structure, i.e. the test sample may be fibrous, a film or a bulk material, as appropriate.

#### 5.1.3 Test procedure

Dry the undegraded test sample in the desiccator (5.1.1.2) under vacuum (5.1.1.3) at room temperature to constant mass. Determine the mass of the test sample, using the balance (5.1.1.1), to an accuracy of 1,0 % of the total mass.

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Carry out the desired degradation procedure.

Dry a filter (5.1.1.4) under vacuum at room temperature to constant mass. Determine the mass of the filter to an accuracy of 1,0 % of the total mass. Rinse the degraded test sample in deionized water three times, using the filter (5.1.1.4) for retaining possible debris. For filtering, a water jet blast can be used.

Dry the degraded test sample and the filter under vacuum at room temperature to constant mass. Determine the mass of the test sample and the filter together, to an accuracy of 1,0 % of the total mass.

### 5.1.4 Reusability of test samples

Dried samples from the measurement of mass loss shall not be used for mechanical testing.

NOTE — Test samples used for the determination of mass loss may be used for further physicochemical testing (e.g. loss in molecular weight via changes in differential scanning calorimetry curves, changes in molecular weight distribution via gas phase chromatography, structural analysis via scanning electron microscopy).

## 5.2 Determination of inherent viscosity

The inherent viscosity of the undegraded and degraded materials shall be determined in accordance with ISO 1628-1 using an Ubbelohde capillary Oc viscometer.

Samples shall be dried to constant mass before testing.

The solvent shall be chloroform. The concentration of the polymer solution shall be 0,1 % with a mass of 50 mg ± 2 mg polymer per 50 ml of solvent.

# 6 Mechanical tests

## 6.1 General

Mechanical tests shall be performed on test samples not altered by previous testing.

During all test periods the solution shall not be agitated.

The test samples shall be kept wet after removal from the soaking solution and during testing.

NOTE — Testing should be carried out preferably in a water bath. Test samples for each test period may be placed in one container if desired.

## 6.2 Conditioning of test samples

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

Therefore mechanical tests shall be performed on test samples conditioned at a temperature of 37 °C ± 1 °C in distilled water.

### 6.2.1 Before measurement of initial values

For measuring the initial value, the samples shall be conditioned in distilled water for 60 min ± 15 min at a temperature of 37 °C ± 1 °C. Each single sample shall be tested directly after removal from the water. To prevent slippage of the test sample in the grips, the surface shall be dried carefully at the ends using e.g. a paper towel.

### 6.2.2 During real-time degradation testing

At the respective degradation intervals, each individual test sample shall be tested directly after removal from the soaking solution. To prevent slippage of the test sample in the grips, the surface shall be dried carefully at the ends, e.g. using a paper towel.

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### 6.2.3 During accelerated degradation testing

At the respective degradation intervals, the test sample shall be removed from the container and conditioned in distilled water for 1 h at 37 °C ± 1 °C.

## 6.3 Preparation of test samples

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

NOTE — For statistical analysis more than three samples at each test period are required.

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, i.e. the test sample may be fibrous, a film or a bulk material if appropriate.

## 6.4 Test methods

The test methods depend on the loading conditions of the intended mechanical testing device, if used, and on the test sample shape. The test results shall include data on strength, elongation and elastic modulus. The tensile test shall be used.

The mechanical properties shall be tested using the test methods referenced in table 1.

If necessary, the mechanical test parameters specified in the methods referenced in table 1 may be changed, but any change shall be justified and reported.

NOTE — Test samples used for the determination of mechanical properties may be used for further physicochemical testing (e.g. loss of molecular weight via changes in differential scanning calorimetry curves, changes in molecular weight distribution via gas phase chromatography, structural analysis via scanning electron microscopy). However, when mechanical properties are measured, energy is put into the samples and directional loads are applied. Both can alter the physicochemical properties of the material and therefore influence the results of the investigations carried out after mechanical testing.

**Table 1 – Mechanical test methods**

| Form | Test method reference |
|---|---|
| Rigid material | ISO 178<br>ISO 180<br>ISO 527-1 and 527-2<br>ISO 6721-2<br>ISO 604<br>ASTM D 4475 |
| Film, sheet | ISO 527-3 |
| Fibre, textile | ISO 2062<br>ISO 1805<br>ISO 5081 |
| NOTE — Other test methods for specific structures (e.g. cellular plastics) or specific requirements (e.g. tensile creep) may be used, if applicable. | |

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## 7 Test termination

Testing of degraded samples shall be terminated when one or more of the following has occurred:

a) a predetermined time has been reached;

b) 100 % mass loss has been reached or degradation is so advanced that mechanical testing becomes meaningless or technically impossible;

c) an inherent viscosity $\leqslant$ 0,1 dl/g has been reached.

## 8 Test report

The test report shall include the following information:

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

b) test environment;

c) deviations from the specified temperature and pH value with time, if applicable;

d) test sample mass expressed as an average percentage mass loss — initial and subsequent by time period;

e) inherent viscosity — initial and subsequent by time period;

f) mechanical properties, if measured — initial and by time period, including applicable test parameters such as deformation speed, distance apart of the clamps, etc.;

g) change in appearance of test samples after different exposure periods, by visual inspection (and SEM if appropriate);

h) reason for the test termination.

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# Annex A
(informative)

# Rationale

With the development of resorbable polymers for use in implantable devices, there is a need to define standard test methods to evaluate the behaviour of bulk material or devices under simulated physiological environments. On the other hand, the behaviour of resorbable materials and devices *in situ* depends on the conditions in which the material is implanted. These conditions differ, so that the behaviour of the material can differ. The interpretation of *in vitro* test results therefore has to be considered very carefully, also taking into account any correlation of test results under *in vitro* and *in vivo* conditions. Only functional *in vivo* tests with the final product can provide answers about actual degradation behaviour *in situ*.

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# Annex B
(informative)

## Bibliography

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

[2] DIN 12448:1977, *Paper laboratory ware — Filter paper.*

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