

N01-02: REPORT***IN VITRO DEGRADATION AND STRENGTH RETENTION OF HIGH-MOLECULAR WEIGHT POLY(L-LACTIDE-CO-D,L-LACTIDE) 70:30; BATCH RES-0023*****1. Aim of the Study**

Poly(L-lactide-co-D,L-lactide) 70:30 is an resorbable polyester.

High-molecular weight polymers of this type might be applicable for manufacturing of medical implants for temporary fixation of bone fractures or other medical devices.

After implantation into living tissues or after contact with water poly(L-lactide-co-D,L-lactide) 70:30 degrades by hydrolytic reaction of the polymer backbone. Initially, this results in a reduction of molecular weight and in changes of mechanical properties. As soon as sufficiently low molecular weight species (lactic acid or oligomers) have been generated, a weight loss can be observed and the degradation products are resorbed by the body.

The aim of this *in vitro* study is to investigate degradation kinetics of massive injection molded parts and the speed of changes in mechanical properties.

2. Experimental**2.1. Materials**

During this study a high molecular weight poly(L-lactide-co-D,L-lactide) 70:30 was investigated. The following analytical data were obtained on RESOMER® LR 70x batch # RES-0023:

- inherent viscosity: 3.6 dl/g (c = 0.1 %, CHCl₃, 25 °C)
- residual lactide: not detectable
- water: 0.19 %
- comonomer composition: L-lactide: D,L-lactide = 70:30
- thermal properties: peak maximum: approx. 95 °C
glass transition temperature: 59 °C

2.2. Sample Preparation

The test bars were manufactured in the production facility of Biovision GmbH, Ilmenau.

For the injection molding of the test bars an Arburg 170 CMD Allrounder injection molding machine with 18 mm diameter screw was used.

Prior to molding, the materials were dried in an on-line dryer at 40 °C under vacuum.

The injection molded test bars were analyzed and characterized as follows:

Analysis:	Flexural test bars	Tensile test bars
Inherent viscosity:	2.8 dl/g	3.1 dl/g
Monomer content (Lactide):	0.01 %	0.01 %
Glass transition temperature:	55 °C	55 °C

The injection molded test bars were translucent and amorphous.

2.3. Sterilization

The test bars were sterilized by Willy Rüsçh AG, Kernen-Rommelshausen.

The molded test bars were sterilized with 27.8 kGy of γ -irradiation.

The sterilized test bars were analyzed and characterized as follows:

Analysis:	Flexural test bars	Tensile test bars
Inherent viscosity:	1.4 dl/g	1.5 dl/g
Monomer content (Lactide):	not detectabel	not detectabel
Glass transition temperature:	54 °C	54 °C

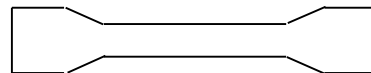
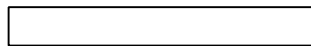
The sterilized test bars were translucent and amorphous.

2.4. Mechanical testing

The material was injection molded into miniaturized tensile test bars according to DIN 53452 (flexural test) and according to DIN 53455 (tensile test):

Dimensions:	Flexural test bars	Tensile test bars
Weight:	appr. 0.5 g	appr. 0.7 g
Thickness:	appr. 2.0 mm	appr. 1.6 mm
Width:	appr. 5.0 mm	appr. 4 mm
Length:	appr. 40.3 mm	appr. 60 mm
Cross section:	appr. 10 mm ²	appr. 6.2 mm ²

The shape of the test bars is illustrated below:



non sterile:

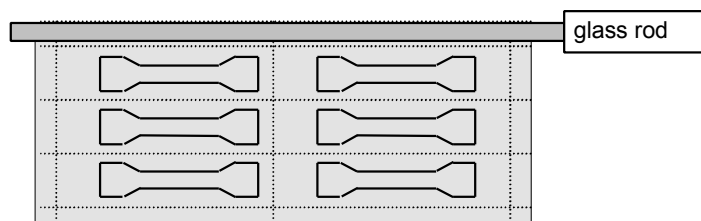
Stress at maximum load:	110.0 MPa	74.3 Mpa
Distance at maximum load:	4.35 mm	
Stress at break:	appr. 97 MPa	appr. 67 MPa
Distance at maximum load:	8.10 mm	
Elongation at maximum load:		18.9 %
Modulus of elasticity:		393 MPa

sterile:

Stress at maximum load:	109.0 MPa	73.0 MPa
Distance at maximum load:	4.60 mm	
Stress at break:	appr. 99 MPa	appr. 68 MPa
Distance at maximum load:	8.55 mm	
Elongation at maximum load:		16.9 %
Modulus of elasticity:		432 MPa

2.5. Hydrolysis

The test specimens were accurately weighed, numbered and a few of them were separately sealed in polypropylene gauze. The filled polypropylene bags were fixed on a glass stick (see figure) and immersed into a buffer solution of pH 7.4 at a temperature of 37 (\pm 0.5) °C.



The buffer solution was prepared as described in European Pharmacopeia (Pharm. Eur. 4.1.3. [1998] BUFFER SOLUTIONS, 4004600). The buffer solution consists of 0.60 g potassium dihydrogen phosphate, 6.40 g disodium monohydrogen phosphate, 5.85 g sodium chloride and 1000 ml of purified water.

Once a month the pH value of the buffer solution was checked. If the pH value does not correspond to the ordered value, the buffer was replaced by fresh buffer solution.

Periodically, the test bars were removed from the buffer, rinsed with demineralized water and cut out of the polypropylene bags. The surface of the specimens was carefully dried with filtration paper. Then the bars were dried at 35 °C in a vacuum oven to constant weight.

The dried bars were subjected to the following characterizations:

- Determination of dimensions
- Determination of inherent viscosity
- Determination of water uptake (prior to drying)
- Determination of weight loss
- Flexural / Tensile testing

All these experiments were carried out in triplicate.

Selected samples were analyzed by Differential Scanning Calorimetry (DSC).

2.6. Analytical Methods and Characterization

The analytical work was done in the QC-laboratory of Boehringer Ingelheim Pharma KG, Ingelheim, except the testing of the mechanical properties which was carried out by Biovision GmbH, Ilmenau.

The results are documented under different registration numbers in the QC-laboratory of RESOMER®.

Dimensions (M 364):

The thickness, length and width of the test bars were determined with a slide gauge.

DSC measurement (M 337):

Performed with a Netzsch, Model DSC 204 with a heating rate of 10 °C/min. The peak maximum was determined from the 1st heating, the glass transition temperature was determined from 2nd heating.

The starting values from the unsterilized and sterilized test bars are determined with a Mettler DSC 20. These values are not comparable with those received with the DSC 204.

Inherent viscosity (M 006):

Measured in chloroform in 0.1 % (w/v) concentration at 25 °C using an Ubbelohde-type viscometer with a capillary size 0c.

Flexural / Tensile test (M 363):

Performed with a tensile tester type AGS-G at Biovision GmbH, Ilmenau with a cross head speed of 10 mm/min.

Water uptake (M 364):

Difference between the weight of the degraded test bars prior to drying and after drying.

Weight loss (M 364):

Difference between the weight of the dried test bars (after hydrolysis) and initial weight.

Residual monomer content (M 308):

Gas chromatographic determination on a capillary column.

Solvent: Chloroform; Internal Standard: γ -decalactone; Stationary phase: DB-225 fused silica; Detection: Flame ionization detector; Limit of Detection: < 0.01 % lactide.

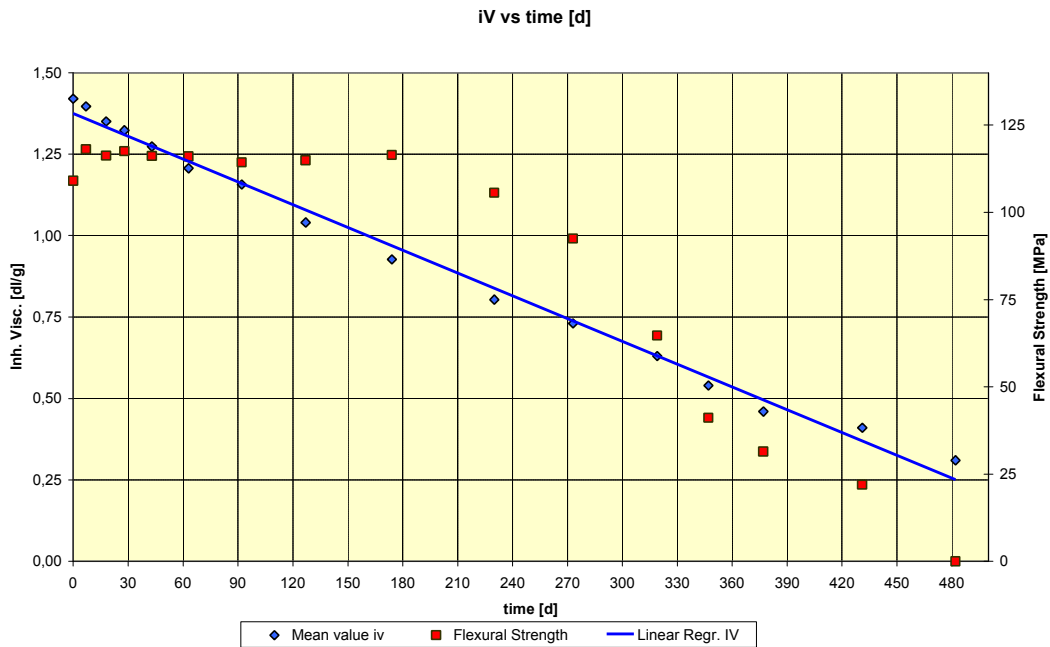
☞ This test was only done for characterization of the starting material and the test bars, but not during the degradation study.

3. Results

3.1. Flexural and tensile tests

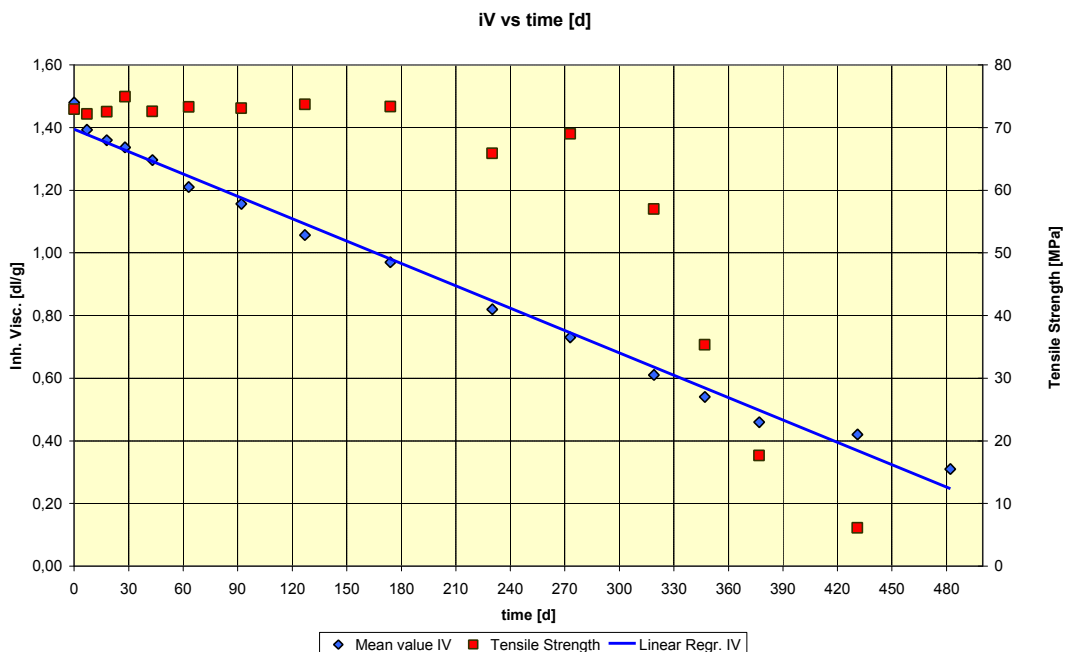
The results of inherent viscosity and the flexural / tensile tests (mean values) are shown in the following graphs:

Flexural Test:



The linear regression of the inh. viscosity can be expressed by the following equation:
 $y = -0,00235x + 1,37632$ $R^2 = 0,98667$

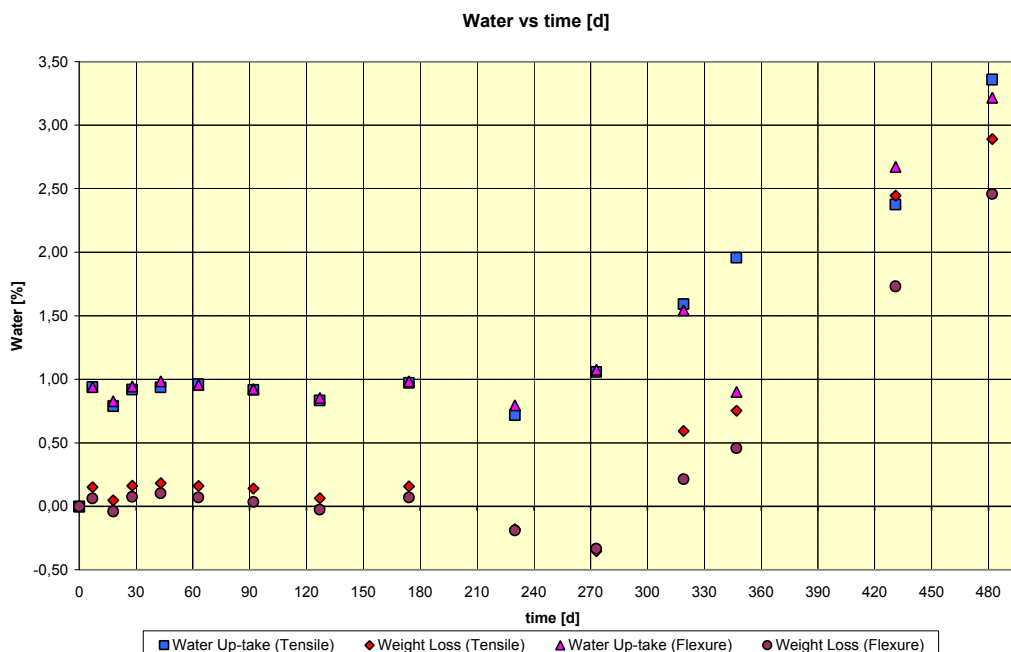
Tensile Test:



The linear regression of the inh. viscosity can be expressed by the following equation:
 $y = -0,00238x + 1,39492$ $R^2 = 0,98763$

3.2. Water Uptake and Weight Loss

The results of the water uptake and weight loss (mean values) are shown in the following graph:



4. Comments and Conclusions

4.1. Dimensions

After sterilization no significant change in dimensions of the test bars can be observed.

After contact with water and subsequent drying a slight change in dimensions of the test bars can be observed.

The width increases by approx. 0.1 mm (= + 2 %) and the length shrinks by approx. 3.4 mm (= - 8 %)

This effect can be explained by a relaxation of mechanical stress introduced by orientation of the polymer during injection molding process.

4.2. DSC measurement

The glass transition temperature varies about 59 ± 1 °C. No significant change of the glass transition temperature can be observed during the degradation time.

4.3. Viscosity

As described in many studies on degradation of solid specimens of resorbable polyesters, the decrease in molecular weight resp. the decrease in inherent viscosity starts immediately after contact with water.

Within the observation period of this study of 480 days the inherent viscosity decrease from approx. 1.5 to 0.3 dl/g

The viscosity data may be analyzed according to a kinetic law frequently described in the literature^{1, 2}:

$$\ln \eta = \ln \eta^{\circ} - k \times t$$

where η is the inherent viscosity, η° is the initial inherent viscosity, k is the apparent rate constant for the decrease in η , and t is the reaction time.

This relatively simple pseudo first-order rate law is based on the autocatalytic mechanism of the hydrolytic degradation of resorbable polyesters in the solid state.

With the apparent rate constant the half life of the material can be calculated:

$$\text{Half life} = \ln 2 / k$$

Applying this rate law to the findings of the present study results in:

Results:	Flexural test bars	Tensile test bars
Rate constant k :	0.00235 d ⁻¹	0.00238 d ⁻¹
Half life:	appr. 290 days	appr. 290 days
Regression coefficient:	0.987	0.988

This calculation is derived from linear regression of $\ln \eta$ versus time with experimental data obtained up to total hydrolysis.

4.4. Water Uptake and Weight Loss

Up to day 270 the water up-take is nearly constant at a level of approx. 1 % and no significant weight loss can be observed.

Then the water up-take and the weight loss increased up to approx. 3 % until the end of the study.

4.5. Mechanical Properties

In the initial phase of degradation up to 180 days the strength values keep nearly at the initial level of approx. 72 MPa (tensile strength) and approx. 115 MPa (flexural strength). Then the material loses its tensile and flexural strength.

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¹ B. Buchholz, Accelerated Degradation Test on Resorbable Polymers, in: H. Planck, M. Dauner, M. Renardy (Eds.), Degradation Phenomena on Polymeric Biomaterials (Springer-Verlag, Berlin), p. 67-76

² C. G. Pitt, Z. W. Gu, J. Controlled Rel. 4, p. 283-292, 1987