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Comparative study of the degradation of polylactide and polytartrate implants *in vitro*

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Poly(D/L-lactide (PLA) and 2′,3′-(1′,4′-diethyl-L-tartryl)-poly-(2,3-*O*-isopropylidene)L-tartrate (PTA) were investigated with respect to their hydrolytic degradation. Implants were made of compressed polymer and melted polymer. They were immersed in a buffer solution (pH 7.4) and stored in a water bath at 30 °C. Change of surface morphology, content of water, glass transition temperature, existence of crystalline degradation products and decrease of average molecular weight were used to assess the extent of hydrolytic degradation. Glass transition temperature of PTA devices, which is influenced by content of absorbed water, decreased more rapidly in comparison to the implants made of PLA. The importance of non-freezing water accumulated at the ester linkages for the hydrolytic degradation could be shown. Decrease in average molecular weight was determined by measuring intrinsic viscosities and using the Staudinger equation. The average molecular weight of PLA implants decreased for compressed and melted devices from 17400 to 5000 and 8500, respectively, during 7 weeks of immersing. Implants made of PTA (average molecular weight of about 23000) provided an average molecular weight of approximately 1000 after 3 weeks storage in the buffer solution.

1. Introduction

Biodegradable polymers are widely used to develop controlled-release drug delivery systems. The properties of biodegradable polymers for pharmaceutical applications such as polylactic acid, polyglycolic acid, polyhydroxybutyric acid, polyorthoesters and polyanhydrides are determined during formation via the polycondensation reaction. Change of their properties can only be achieved by co-condensation or by variation of the molecular weight. An example is the generation of polylactic-co-glycolic acid. In the case of polytartaric acid derivatives, the biodegradation of these polymers takes place by hydrolysis of the ester side groups, the ketal groups or the main-chain ester groups depending on the pH value of the media. The system can be tuned for specific applications by the appropriate choice of the ester groups or the ketal groups or even the substitution of one condensation component for other non-tartartic elements. Variation of the molecular weight and co-condensation components provides materials with a broad property profil (Ahlers et al. 1992). The sustained release of peptide drugs with their poor stability and short *in vivo* half life can be achieved by preparing biodegradable microspheres for parenteral application or producing cylindrical devices and tablet-like implants as long-time acting formulations. Release of drugs from biodegradable matrices can be based on three phenomena, namely diffusion through channels, dissolution and then diffusion through connected channels in the matrix and release because of matrix degradation. The release of drugs from a polymer with a really high hydrophilic character is also influenced by a change of polymer quality. Water molecules immigrate into the matrix to form a swollen layer and its borderline moves to the inside of the polymer device, during immersion into aqueous media. This polymer is mechanically stressed at this border area which is shown by deformation of the devices. The higher the amount of water absorbed, the bigger the extent of softening. During incubation, when the glass transition temperature decreases to the same order of magnitude as the temperature of the incubation fluid, the polymer changes from the glassy state to the rubbery state. The diffusion coefficient of embedded drugs in a rubbery matrix is bigger than in a glassy one, resulting in an increased rate of drug liberation. The determination of water-soluble acid distribution in poly(lactide-co-glycolide was carried out (Ding and Schwendeman 2004). The hydrolytic degradation of polylactide and polytartrate occurs in opposite to the synthesis, additionally ring opening in the case of polytartrate. The four basic parameters of hydrolytic degradation of ester bonds are cleavage rate constant, extent of water absorbed, diffusion coefficients of the created chain fragments in the matrix and their solubility in the surrounding aqueous medium. Bulk hydrolysis starts immediately after incubation of the polymer matrix in the aqueous medium because the water molecules have a higher rate of diffusion compared to the rate of ester cleavage. Sometimes surface erosion is seen. In this case a quicker cleavage of ester bonds is possible. Water soluble chain fragments formed inside are released more slowly than fragments from the surface. Therefore, they are able to autocatalyse the hydrolysis through their carboxylic chain ends. Polymer degradation is influenced by the properties of the incubation medium such as: pH value, temperature, ionic strength, the presence of structure breaking substances or the presence of esterase en-

zymes. Hydrolytic reactions are accelerated in the case of high pH values and retarded in the case of low ones. Therefore, investigations of polyester degradation should be done in buffer solutions with sufficient buffer capacity, and a change in the pH value should not be used to measure the polymer degradation. The higher the temperature of incubation, the higher is the rate of degradation. The relation of incubation temperature to glass transition temperature of the used polymer plays an important role because the rubbery matrix chain fragments, and incorporated drugs show higher diffusion coefficients. Ionic strength does not influence the cleavage rate constant but it rises the part of chain fragments with a middle molecular weight. Structure breaking substances, such as urea or hydrophilic polymers, increase the so-called concentration of fault points. This is the reason for a decrease in cluster dimension, it is called the hydrotropic effect, and it only causes a small increase in cleavage rate constant. Also the enzyme esterase in biological tissues has some influence, and it proceeds a cleavage of ester bonds at the surface of the polymer matrix. The hydrolytic degradation of poly (DL-lactide) materials was investigated in order to elucidate the effects of temperature and acidity of the external medium on the degradation characteristics (Li and McCarthy 1999).

The purpose of this study was to compare the degradation of compressed implants and melted implants of polytartrate (cPTA, mPTA) as well as compressed implants and melted implants of polylactide (cPLA, mPLA). The polytartaric acid derivative 2′,3′-(1′,4′-diethyl-L-tartryl)-poly-(2,3-*O*-isopropylidene)L-tartrate produced by Krone and coworker (Krone et al. 1992) was used. The implants were immersed in buffer solution. Changes in surface morphology, the content of freezing and non-freezing water and glass transition temperature were studied in dependence on the immersion time.

2. Investigations and results

2.1. Macroscopic characterisation of implants

In order to assess the degradation of polymer devices, the macroscopic description can be used as a basic method. The rate and extent of polyester cleavage can be estimated from a change in the shape and surface of the devices stored in a buffer solution. Before incubation cPLA-implants showed a white and smooth surface without any visible pores. Translucent areas can be seen because of partially sintering during the preparation with high hydraulic pressure. The mPLA implants had some air bubbles enclosed in their clear matrix. After three weeks of incubation, the compressed implants became clearer and had a glassy surface. After 6 weeks of incubation, the number of air bubbles was increased. The devices of mPLA showed only little optical change during the incubation period, and their clear-looking matrix became slightly cloudy. Both kinds of preparation suffered from deformation because of the mechanical stress produced from the swollen polymer during degradation. Implants made of PTA were yellowish after preparation and the compressed devices had translucent areas like corresponding PLA implants. A rapid change was found in implants made of PTA during the incubation. A blistered surface characterised the compressed devices after two days of storage whereas melted implants became cloudy and scarred. During the second week of storage both types of PTA converted into a clear gel with air bubbles. This gel was transformed into a salt-like residue. In summary, PLA implants were more stable than the devices made of PTA unaffected by their production method.

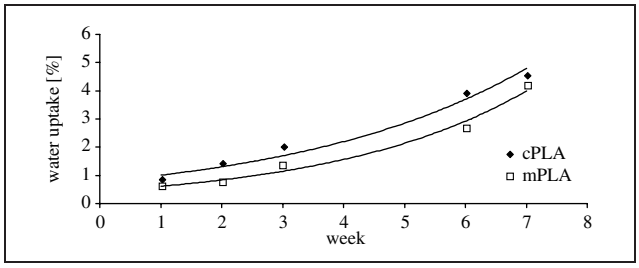

Fig. 1: Water uptake of immersed PLA implants in dependence on the immersion time

2.2. Content of water

An important parameter for hydrolytic cleavage of ester bonds is the content of water. Therefore, it was distinguished between the part of freezing water and the part of non-freezing water. Total water was determined through increasing implant mass during incubation. This simple method can be used because there was found to be no mass loss resulting from emigrating chain fragments. The amount of water uptake was investigated in cPLA- and mPLA-implants for 7 weeks. An exponential increase was found for both types of PLA implants – as shown in Fig. 1. The only compressed implants absorbed more water than the melted ones because of their higher porosity. Devices made of cPTA and mPTA could be investigated for one week only. After this time they converted from the glassy state to the rubbery state, thus, the implants could not be removed completely. The implants made of PTA had a content up to 25 percent (cPTA) and 23 percent (mPTA) of water respectively. This is twenty times the amount of absorbed water in comparison with the PLA devices after one week and it defines a more hydrophilic character in the novel polymer. The amount of non-freezing water can be calculated from the difference between the total water and the freezing water. The quantities of freezing water and non freezing water are shown in Table 1. The non-freezing water is accumulated on the ester linkages, so that the degree of polyester degradation is dependent on the content of non-freezing water.

2.3. Glass transition temperature

The transformation from the glassy to the rubbery state is a characteristic value of amorphous and partly amorphous substances. Diffusion coefficients of polymer chain fragments or embedded drugs are influenced by polymer softening if decreasing glass temperature reaches the temperature of storage. The decrease of glass transition temperature is shown in Fig. 2. The melted implants had a lower glass transition after preparation in comparison to the pressed ones. The energy, which is necessary to compensate the relaxation enthalpy of a non-heated polymer, could be the

Table 1: Contents of freezing and non-freezing water of immersed implants (pressed and melted implants; n = 4)

PLA after	Total water [%] (mean ± SD)	Freezing water [%] (mean ± SD)	Non-freezing water [%] (calculated)
3 weeks	1.59 ± 0.39	1.45 ± 0.03	0.14
7 weeks	4.31 ± 0.33	1.71 ± 0.16	2.60
PTA after			
2 days	9.58 ± 1.26	0.62 ± 0.07	8.96
4 days	17.62 ± 1.73	0.67 ± 0.21	16.95
7 days	24.06 ± 1.90	0.90 ± 0.08	23.16

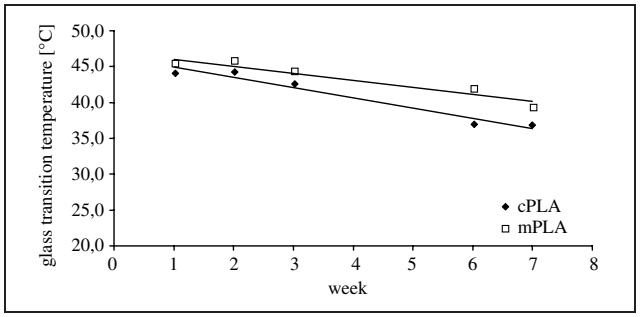

Fig. 2: Glass transition temperature of pressed and melted PLA implants in dependence on the immersion time

reason for this difference. A linear relationship was found between decreasing glass transition temperature and the time of storage in the immersion liquid. The glass transition temperature of pressed PLA decreases faster than that of the melted one. Water can reduce the intermolecular force of attraction of the polymer chains, leading to increasing chain agility. Glass temperatures of PTA could be determined in the first week. An accelerated degradation of PLA was found while immersing of microparticles above their glass transition temperature in comparison to storage below it (Aso et al. 1994). This phenomenon could not detected for the PLA- or PTA-implants. The different water content and the surface/volume ratio of implants and microparticles could be responsible for these results.

2.4. Weight-average molecular weight and cleavage rate constant

Macromolecular substances increase the viscosity of a solution in dependence on their molecular weight. The reduced specific viscosity is used to eliminate influences of polymer concentration and solvent viscosity. The molecular weight is viscosimetrically calculated based on the Staudinger equation. The decrease of weight – average molecular weight of pressed and melted PLA and PTA implants, in dependence on the storage time in immersion liquid, can be seen in Fig. 3 and Table 2. The faster decrease of weight – average molecular weight, found in pressed PLA-implants compared to the melted ones corresponds with the water uptake and glass transition temperature in dependence on the storage time in immersion liquid. The hydrolytic cleavage of PTA ester bonds occurs much faster because of its greater hydrophilic character. The PLA and PTA degradations obey a first order kinetic and prove a bulk hydrolysis. The question of reduction in M_w during melt processing is conflictingly discussed in published works. Bhardwaj and Blanchard (1997) did not find a decrease of M_w by melt extrusion (operating temperature 45–70 °C) for poly (D/L-lactide-co-glycolide)-based implants. But Schmitt et al. (1993) described a reduction while the melt-pressing at 56–57 °C from 91000 to 64000 using poly (D/L-lactide-co-glycolide). In this study, the polymers were melted at 150 °C for 15 min to prepare implants, and no decrease in weight – average molecular weight was found. The cleavage rate constant is an important index to assess the hydrolytic degradation, and it is determined using the following equation:

$$\ln\left(\frac{M_0}{M_t}\right) = k \cdot t \qquad (1)$$

The cleavage rate constants and their correlation coefficients are presented in Table 3. Reich (1997) published a cleavage rate constant of $5 \cdot 10^{-2}$ d^{-1} for poly (D/L lactide) with a M_w of 14500. This agrees with the values which we detected for pressed and melted PLA implants.

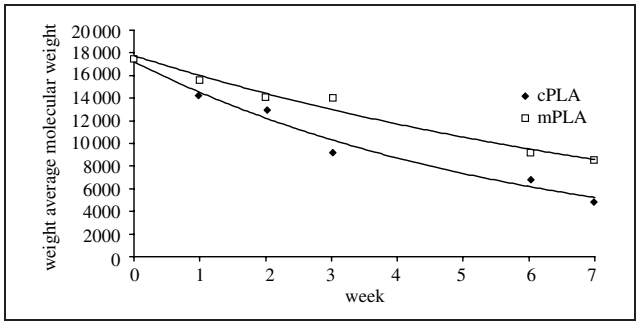

Fig. 3: Weight – average molecular weight of pressed and melted PLA implants in dependence on the storage time in immersion liquid

Table 2: Weight-average molecular weight of pressed and melted PTA implants in dependence on the storage time in immersion liquid

Week	cPTA	mPTA
0	23000	23000
0.275	14864	13545
0.55	10053	6641
1	7504	6417
2	3751	2303
3	1028	699

2.5. Crystallised oligomeric degradation products

A heterogeneous degradation of PLA yielding a crystalline residual material was described in the literature (Li et al. 1990). The cleavage of ester bonds between D-α-lactic acid and L-α-lactic acid is preferred to cleavage of bonds between two monomers in the same kind of enantiomer. This is because the polymer contains sections with a higher content of one enantiomer as a result of the synthesis, the cleavage leads to D- or L-enriched chain fragments. In the final stage of hydrolytic degradation crystallised degradation products were found, and Li and Vert (1994) identified them as an oligomeric stereocomplex made of equimolar poly (D-lactic acid) and poly (L-lactic acid). In the present study, crystallised degradation products could not be determined for PLA based implants after seven weeks of immersing by means of DSC. Taking other studies into account, it could be expected that they were formed in a later state of degradation probably when average molecular weight decreases to less than 1500. The residues of PTA implants after three weeks of incubation were investigated using DSC. Crystallised degradation products could be found for both pressed and melted devices. This is in accordance with the macroscopic characterisation of the implants. The melting points, which were determined from the DSC graphs, were 167.5 °C respectively 172.6 °C for pressed and melted implants. This agrees with pure tartaric acid having a melting

Table 3: Cleavage rate constants and their correlation coefficients after immersion into buffer solution

Implant	Cleavage rate constant (d)	Correlation coefficient r
cPLA	$2.431 \cdot 10^{-2}$	0.989
mPLA	$1.486 \cdot 10^{-2}$	0.990
cPTA	$13.683 \cdot 10^{-2}$	0.991
mPTA	$15.489 \cdot 10^{-2}$	0.984

point between 168 °C and 174 °C. The residues of PTA devices showed the proof of tartaric acid according to the European pharmacopoeia, too. An IR spectrum of this residues has also been done and shows a good similarity to pure tartaric acid. It can be concluded that the crystallised degradation products of PTA based implants consist of tartaric acid and their salts with buffer components.

3. Discussion

This study compares PLA to PTA, which is a new biodegradable polyester. Two kinds of implants, a pressed and a melted one, are investigated for both polymer types. The PTA is characterised by a more hydrophilic character. As a result, the hydrolytic degradation is more rapid in comparison to the commercially available PLA. It will be an addition to the lipophilic polyesters currently available for developing controlled-release drug delivery systems and should open the possibility to prepare implants with a decreased "burst release" and protect embedded proteins from denaturation. The differences between the melted and pressed implants of each polymer are really small and do not justify the complicated production of the melted ones. An incorporated drug can be destroyed while melting the polymer to prepare the implants, or it can separate and promote an inhomogeneous distribution in the implants. In the opposite, pressed implants can be prepared after improving the bad flow characteristics of the polymers on simple tablet machines and should have preference in the development of new implantable formulations.

4. Experimental

4.1. Materials

Poly (D/L-lactide) (Resomer® R 202, M_w 17 400, Batch-No. 15027) was gifted by Boehringer Ingelheim KG (Ingelheim/Germany). Polytartrate, (2′,3′-(1′,4′-diethyl)-L-tartryl poly-(2,3-*O*-isopropylidene)-L-tartrate; M_w 23 000, Batch-No. 002 GF) was donated by Hoechst AG (Frankfurt am Main/Germany). All other chemicals used were of analytical grade.

4.2. Preparation of implants

Two kinds of cylindrical implants were produced. Compressed implants were prepared with a hydraulic press (Perkin-Elmer, Überlingen/Germany) using a pressure of 77 kN/cm^2 [cPLA/cPTA]. The other implants were formed by low pressure (7.7 kN/cm^2), and then they were melted at a temperature of 150 °C for 15 min in an aluminium pan [mPLA/mPTA]. The implants had a diameter of 13 mm.

4.3. Degradation study

The weighted implants (m_1) were immersed in 10 ml of the phosphate buffer R1 (pH 7,4) containing sodium chloride (European pharmacopoeia), and they were stored in a water bath at 30 °C. The devices were withdrawn at appropriate intervals.

4.4. Water uptake

Water uptake was determined gravimetrically. The implants were dried at a temperature of 30 °C for 30 minutes, and then they were weighted correctly (m_2). The water uptake was determined by using the following equation:

$$\text{water uptake}\,[\%] = \frac{m_2 \cdot 100}{m_1} - 100 \qquad (2)$$

The results presented are the average of two samples.

4.5. Freezing water

Polymers dried as above-mentioned were scanned by DSC (Polymer Laboratories Ltd, Amherst / USA) from −100 °C to 40 °C at a heating rate of 10 K/min. The amount of freezing water was calculated from the area under the melting curve of water.

4.6. Glass transition temperature

Thermal analysis was conducted by using a DSC. Samples between 4 and 8 mg were heated in sealed aluminium pans at 10 K/min. They were scanned from 15 °C to 60 °C. The glass transition temperature was determined from the mid-point of the transition curve. The experiments were carried out in duplicate.

4.7. Crystallised oligomeric degradation products

The melting point was also determined by DSC. Samples were scanned from 20 °C to 200 °C by using a heating rate of 10 K/min.

4.8. Intrinsic viscosity

The polymers were dissolved in dichlormethane (0.00015 mg/ml; 0.0003 mg/ml; 0.00045 mg/ml; 0.0006 mg/ml). The viscosities of these solutions were determined by means of a Ubbelohde viskosimeter at 25.0 °C and the median of three samples was used. The reduced specific viscosities were plotted against the polymer concentrations. The intrinsic viscosities were obtained by extrapolation to the limit concentration zero.

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