From the experiment conducted it is found that in the group of the animals implanted with type B ceramics, the maximum accumulation of the radio pharmacological preparation was seen after 14 days and was statistically higher as against that in the control group (236.7±.8%). During sequential observations (after 56 days) the values of the relative level of the preparation accumulation decreased to 148.2±9.8%.

In the group of the animals implanted with type D ceramics, the maximum accumulation of the preparation (371.0±31.9%) was registered after 21 days since surgery. After 56 days since surgery, there was seen a gradual decrease in the measured value up to 175.7±15.4%.

In the control group, the maximum accumulation of the osteotropic radio pharmacological preparation was registered after 14 days since surgery and corresponded to (183.9±12.3%). In the subsequent periods of observation (after 56 days) the relative level of fixation of the preparation at the place of the defect gradually decreased up to 120±5.6%.

Analysis of the obtained results is evident of the fact that for the animals implanted with type B ceramics (1st group) or with D type ceramics (2nd group) the conditions for filling the defect with the primary bone callus were better than in the case when a blood clot (3rd group) was present in the defect cavity.

Thus, the from the investigations carried out it follows that the activity of metabolic processes during healing of the bone defect implanted with calcium phosphate ceramics "Kafam" was more intense as against the control group.

References

[1] Blijdorp P.A., Vanasche B.J.E., De Lange G.L. The hydroxylapatite - bone interface. Studies on a human biopsy. *Int. J. Oral Maxillofac. Sur.*, 17 (1988), 354-357.

[2] Iordanashvili A.K. Surgical treatment of periodontitis and maxilla cysts. S.-Petersburg, Published by Normed , 2000. 224 p.

[3] Ulyanova T.M., Titova L.V., Evtukhov V.L., Chudakov O.P., Bykadorova L.G. A new bioactive bone-replacing material for maxillofacial surgery. *J. Engineering of Biomaterials*, 5(2002), 23-25, 7.

● ● ● ● ● ● ● ● ●

COPOLYMERS OF L-LACTIDE AND TRIMETHYLENE CARBONATE EVALUATION OF SURFACE PROPERTIES AND DEGRADATION IN AQUEOUS MEDIUM

Elżbieta Pamuła*, Elodie Gaugain**, Aurélie Dessart**, Piotr Dobrzyński***

*AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Department of Biomaterials, Al. Mickiewicza 30, 30-059 Kraków, Poland
epamula@agh.edu.pl

**Université du Maine, Institut Universitaire de Technologie du Mans, Avenue Olivier Messiaen, 72-085 Le Mans, France

***Centre of Polymer Chemistry, Polish Academy of Sciences, Ul. Curie-Skłodowskiej 34/20, 41-819 Zabrze, Poland

Abstract

Two poly(L-lactide-co-trimethylene carbonates)s having the carbonate content of 15% and 50%, respectively were prepared by ring-opening copolymerization with the use of zirconium acetylacatonate as an initiator. The copolymers were characterized by NMR, GPC, DSC and AFM. Wettability and surface energy of the copolymers were also evaluated. The copolymers were submitted to degradation in phosphate buffered saline at 37°C for 10 weeks. It was shown that degradation mechanism and kinetics depend on chemical structure of the copolymers.

Introduction

Polycarbonates and poly(a-hydroxy acids) have attracted much attention recently, because they degrade both in vitro and in vivo through hydrolysis reaction. Their big advantage is that their degradation products, namely carbonates and carboxylic acids might be metabolized by the living body [1]. Polymers derived from carbonic acid show, however less tendency towards hydrolysis than that with an ester linkage [2]. On the other hand, degradation of poly(a-hydroxy acids) often leads to local increase of acidity what may be harmful to the surrounding tissue, while polycarbonates do not cause pH decrease [3]. Therefore, copolymerization of both types of monomers may result in a wide variety of resorbable materials which might be attractive for medicine, pharmacy and tissue engineering.

In this paper we characterize two recently synthesized copolymers of L-lactide and trimethylene carbonate and study their degradation in aqueous medium in order to evaluate their usefulness in medical and tissue engineering applications.

Materials and methods

Copolymers synthesis and foils preparation

1,3-trimethylene carbonate (Boeringer Ingelheim, Germany) and L-lactide (Purac, The Nederland) were purified by recrystallization from dry ethyl acetate in a vacuum dryer at a room temperature. Copolymerization was performed in bulk with a $Zr(acac)_4$ initiator (Aldrich, Germany) at 110°C by a conventional method using a vacuum line for degassing and sealing of the ampoules. The copolymers were purified by dissolution in chloroform and precipitation in methanol. Subsequently, the copolymers were dried under reduced pressure at 25°C until constant weight was obtained.

Copolymer foils, having the thickness of 0.18±0.01 mm, were obtained by slip casting from 10 % (w/v) copolymer solution in methylene chloride (POCH S.A., Gliwice, Poland), followed by air and vacuum drying under reduced pressure at 25°C for at least 24h.

Characterization methods

The composition of the copolymers was determined by ^{1}H NMR measurements (Varian Unity Inowa spectrometer). Molecular masses Mn and Mw of the copolymers were determined by gel permeation chromatography with the Physics SP 8800 chromatograph.

Thermal properties, such as glass-transition temperature (T_g), melting temperature (T_m) and heat of melting (ΔH_m) were studied by differential scanning calorimetry with DuPont 1090B apparatus calibrated with gallium and indium.

Topography measurements were performed with an Explorer atomic force microscope (ThermoMicroscopes, Vecco, USA). Contact mode topographic images were recorded using Si_3N_4 tips with a spring constant of 0.05 N/m and a nominal radius of curvature of 20 nm (Vecco NanoProbe™ Tips, model MLCT-EXMT-A). The images were recorded at scan areas 100 μmx100 μm for three aleatory chosen places (300x300 data points) and with scan rate of 3 lines/s. All images were flattened using a third order polynominal algorithm provided with the instrument. Ra roughness was also calculated.

The contact angle was measured by sessile drop method by an automatic drop shape analysis system DSA 10 Mk2 (Kruss, Germany). UHQ-water (produced by Purelab UHQ, Elga, Germany) and diodomethan (Aldrich, Germany) droplets of the volume of 0.2 μl were put on each sample and the contact angle was obtained by averaging the results of 10-12 measurements. Surface energies of the copolymers (total, dispersive part and polar part) were calculated according to the Owens-Wendt-Rabel-Kaelble method.

Degradation study

Degradation of copolymer foils was performed in 0.9% sodium chloride solution buffered with 24 mM Na_2HPO_4 and 16 mM KH_2PO_4 (PBS-solution) of pH=7.0 at 37°C. Foils weighing 0.2 g were incubated in 100 ml of buffer in plastic vials for 10 weeks. The buffer was exchanged every week. After every week foils were washed ten times in UHQ water (PureLab UHQ, Elga, Germany) and dried in a vacuum oven at 25°C for 72 h.

Another set of samples (0.5 g) was incubated in 100 ml of UHQ water at 37°C and pH and conductivity of water, at 25°C, were evaluated once a week.

Results and discussion

Properties of copolymers

sample	N_{TMC} [mole %]	M_n [kDa]	d	T_g [°C]	T_m [°C]	ΔH_m [J/g]	χ [%]
I (85:15)	15	35	2.2	32.7	191.3	65.9	70
II (50:50)	50	58	1.8	20.8	166.5	15.7	17

N_{TMC}– molar fraction of trimetylene carbonate; M_n- number-average molecular weight; d – polydispersion index, d = M_n/M_w; T_g – glass transition temperature; T_m – melting temperature; ΔH_m – heat of melting; χ - degree of crystallinity;

TABLE 1. Structural properties of the poly(L-lactide-co-trimethylene carbonates)s.

TABLE 1 presents structural properties of two copolymers of L-lactide and trimethylene carbonate P(L-TMC). A molar fraction of trimethylene carbonate in the copolymers is 15 and 50, respectively, as determined by ^{1}H NMR. GPC results show, that the copolymer 85:15 has lower number
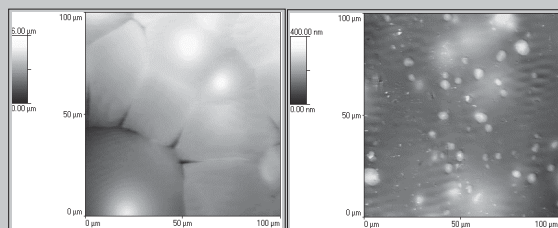

FIG. 1. AFM topographical images of P(L-TMC): a) 85:15 (x=y=100μm, z=6μm) and b) 50:50 (x=y=100μm, z=400 nm).

sample	R_a [nm]	θ [°]	γ [mJ/m^2]	γ_d [mJ/m^2]	γ_p [mJ/m^2]
I (85:15)	380 (100)	96 (3)	39.7 (0.9)	39.5 (0.8)	0.2 (0.1)
II (50:50)	50 (13)	76 (3)	42.9 (1.2)	37.4 (0.9)	5.5 (0.3)

R_a – roughness, standard deviation in brackets, n=3; θ – water contact angle; γ - total surface energy; γ_d - dispersive part of surface energy; γ_p - polar part of surface energy (standard deviation in brackets, n=12)

TABLE 2. Surface properties of the poly(L-lactide-co-trimethylene carbonates)s

average molecular weight and higher polydispersion than the copolymer 50:50. The copolymer 85:15 has also higher glass transition and melting temperatures as well higher heat of melting, what indicates that it is also much more crystalline than the copolymer 50:50. The heat of melting of the copolymers can be compared to the value of the heat of melting of the crystalline regions of poly-L-lactide, ΔH_m=93.1J/g [5]. Hence, the relative degree of crystallinity of the copolymer 85:15 is 70% while that of copolymer 50:50 is only 17%.

FIG. 1 presents AFM topographical images of the surface of foils prepared from two copolymers. On the surface of the copolymer 85:15 ringed spherulitic crystalline structures are visible. The height of the structures attains 6 μm, while their diameter ranges from 20 to 60 μm. On the other hand, the surface of 50:50 copolymer is more smooth and only small ringed structures of the height of hundreds nanometers and diameter of 3-10 μm are visible. The surface roughness (R_a) of the copolymer 85:15 is much higher than that of 50:50 [TABLE 2].

TABLE 2 presents the results of water contact angle and surface energy of the two copolymers. The surface of the copolymer 85:15 is very hydrophobic (contact angle of 96°), while that of 50:50 is much hydrophilic (contact angle of 76°). Interestingly, the surface free energy of 85:15 is low and consist only of a dispersive component, whereas that of 50:50 consist of both dispersive and polar parts.
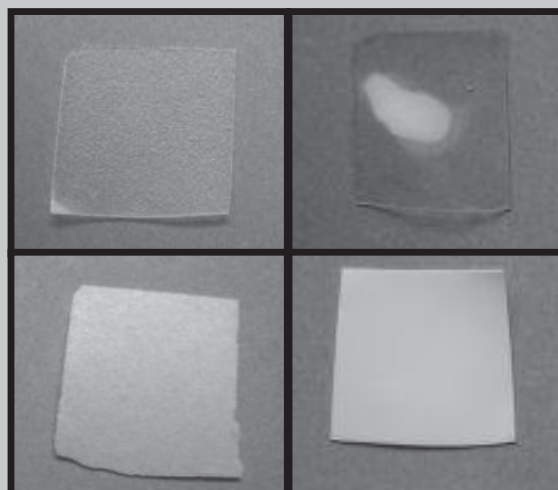

FIG. 2. Gross morphology of P(L-TMC) foils (squares ca. 1cmx1cm) before A-85:15, B-50:50 and after incubation in PBS for 7 weeks: C-85:15, D-50:50.
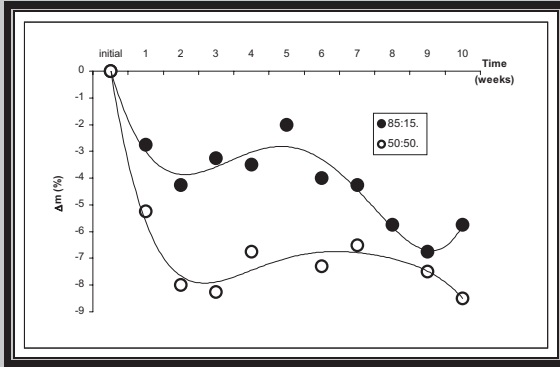
FIG. 3. Relative mass changes of P(L-TMC) foils as a function of incubation time in PBS.
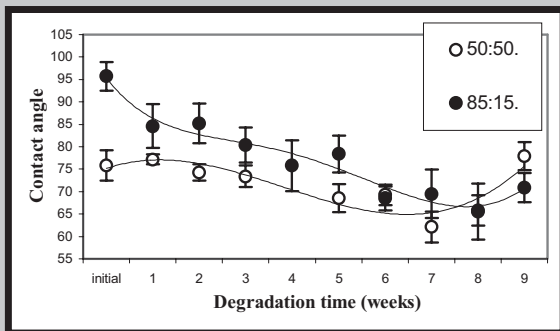

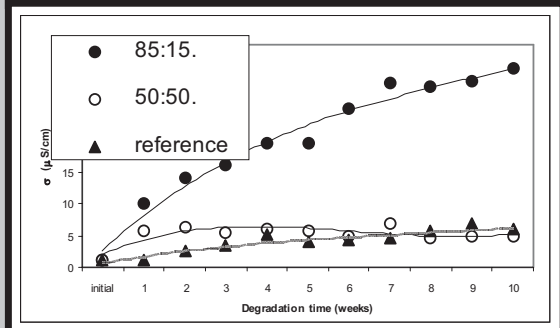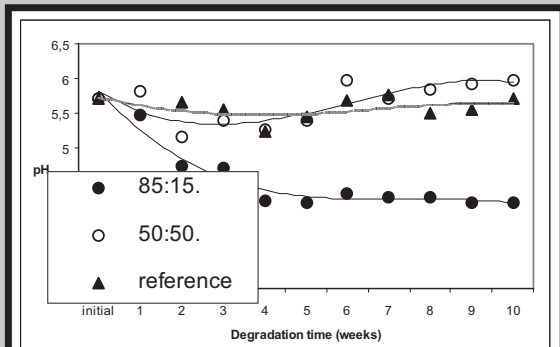**Fig. 4. Evolution of water contact angle of P(L-TMC) foils as a function of degradation in PBS.**

FIG. 5. Evolution of pH and conductivity of UHQ water with P(L-TMC) foils as a function of degradation time in PBS.

Degradation study

FIG.2 shows a gross morphology of P(L-TMC) foils before and after incubation in PBS. The foils 85:15 are less transparent than that of 50:50 in accordance to their crystalline structure. After incubation the foils become opaque, suggesting an increase in crystallinity. It is caused by relatively higher mobility of short polymeric chains, which can organize much more easily. The foils 85:15 are dimensionally stable for 5 weeks, afterwards they become fragile with negligible strength, while the foils made of copolymer 50:50 are stable for the whole experimental period, e.g. 10 weeks. FIG.3 and 4 present the evolution of mass change and contact angle, respectively as a function of incubation in PBS. During two weeks of degradation the sample 50:50 loses already 8% of its mass. Afterwards the mass of the foil does not change considerably. Loss of mass in the case of sample 85:15 is much more uniform. The contact angle of 50:50 do not change considerably during the whole experimental period, while that of 85:15 decreases from 95° to about 75°. FIG.5 presents evolution of pH and conductivity of UHQ water containing analyzed copolymer foils as a function of incubation time. Foils made of the 50:50 copolymer do not have an impact on pH and conductivity, while foils made of 85:15 cause a decrease of pH and an increase of conductivity. This may suggest that degradation products, having acidic properties are released from the P(L-TMC) 85:15 to the liquid environment.

The results show that hydrolytic stability depends on structure and composition of the poly(L-lactide-co-trimethylenecarbonate)s. The copolymer having the carbonate content of 15% degrades very fast despite its high crystallinity and hydrophobicity. After the 5-week incubation it becomes brittle and loses its integrity. Moreover it brings about a decrease of pH of the incubation medium. Note that degradation of homopolymer of L-lactide is very slow and takes at least 2 years [6]. The copolymer having 50% of trimethylene carbonate degrades more slowly: the foils retain their form for 10 weeks, however they turned opaque. It may suggest that degradation proceeds in bulk.

Acknowledgement

References

[1] Bezwada R. S., Jamiolkowski D. D., Lee I., Agarwal V., Persivale J., Trenka-Benthin S., Erneta M., Suryadevara J., Yang A., Liu S., "Monocryl suture, a new ultra-pliable absorbable monofilament suture" Biomaterials 16 (1995) 1141-1148.
[2] Acemoglu M. "Chemistry of polymer biodegradation and implications on parental drug delivery". International Journal of Pharmaceutics 277 (2003), 133-139.
[3] Matsumura S., Tsukada K., Toshima K., "Novel lipase-catalyzed ring-opening copolymerization of lactide and trimethylene carbonate forming poly(ester carbonate)s" International Journal of Biological Macromolecules 25 (1999) 161-167.
[4] Södergärd A., Stolt M., "Properties of lactic acid based polymers and their corelation with composition". Progress in Polymer Science 27 (2002), 1123-1163
[5] Fisher E.W., Sterzel D. J., Wegner G., Kolloid-Z. u. Z. Polymere 251 (1973) 980-990
[6] Chandra R., Rustgi R., "Biodegradable Polymers" Progress in Polymer Science 23 (1998) 1273-1335.