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Novel method to characterize the hydrolytic decomposition of biopolymer surfaces

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Abstract

Present pharmaceutical research is focused on the development, modification and characterisation of new drug delivery systems. Among the many different substances, biodegradable polymers and copolymers are of practical importance, especially if their degradation byproducts are non-toxic. The polymeric drug carriers are not easily wettable by water or aqueous solutions, i.e. they are hydrophobic. This surface hydrophobicity is unfavourable for keeping drug carriers circulating in the blood long enough to release the drug so that it reaches its target. Therefore, copolymers with components of different hydrophobicity were introduced, to make them less hydrophobic and hence more suitable for drug delivery in the human body. Exploratory experiments with one homopolymer, D,L-poly(lactic acid), D,L-PLA and two of its copolymers, D,L-poly(lactic/glycolic acid), and D,L-PLGA with 85/15 and 50/50 copolymer ratios were carried out. Films of these substances were prepared by dip coating onto hydrophobic and hydrophilic substrates. The changes in wettability of the polymer layers, caused by the direct contact with an aqueous environment (soaking the samples in distilled water), have been studied to model the hydrolytic decomposition of polymer surfaces and to follow the changes in their wettability by dynamic contact angle measurements in a non-destructive manner. It was found that each polymer film became less hydrophobic (dynamic contact angles decreased) and more heterogeneous as the decomposition progressed with time. Increasingly significant decreases in contact angles were observed for the copolymer films containing 15 and 50% glycolic acid, during the 50–80-day-long study. These findings were supported by gel chromatographic analysis of the soaking liquids. It was concluded that the homopolymer layer of D,L-PLA was the most resistant to hydrolysis and the stability of copolymer films decreased with increasing glycolic acid ratio in the copolymers. This is accordance with the fact that the less crystalline poly(glycolic acid) is more hydrophilic and hence less resistant to hydrolytic decomposition, than the poly(lactic acid). The effect of pH on the rate of hydrolysis of polymer films was also established; the influence of pH on the decomposition was best demonstrated, again, for the copolymer with 50/50 component ratio. The outcome of these experiments showed that the contact angle measuring method enables us to detect, follow and interpret the hydrolytic decomposition of biopolymer substances in a non-invasive manner.

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1. Introduction

The focus of present pharmaceutical research is the development of new, biodegradable, non-toxic, stable, reproducible and well-characterised drug delivery systems. The critical points in the design of drug delivery devices (such as various implants) and other drug carriers (e.g. microspheres, nanoparticles) are the understanding of the mechanisms of both polymer surface degradation/bulk erosion and drug release as well as the clarification of any differences existing between these processes in vitro and in vivo. Although biodegradable polymers are in the center of interest for delivering anticancer agents [1–6], hormones [7,8], polypeptides, proteins [9–15], antigens, vaccines [16–18], antibiotics [19] and non-steroidal antiinflammatory drugs (NSAIDs) [20,21] for the possible treatment of various diseases, still only limited knowledge exists regarding their degradation, erosion and the mechanisms of drug release from the carriers. Appropriate techniques are necessary for monitoring these processes (which likely proceed simultaneously in most of the systems) without disturbing the sample, but unfortunately, the usually applied analysis methods do not always meet that requirement.

One of the most frequently studied biodegradable polymers of pharmaceutical interest is poly(-lactic acid) (PLA) and its copolymers with glycolic acid (PLGA). Most of the above cited, selected publications referred to poly(L or D,L-lactic acid) polymers or poly(D,L-lactic/glycolic acid) copolymers as drug carriers. The major advantages of these copolymers are that due to the different crystallinity and hydrophobicity of the lactic and glycolic acid components, their application permits the preparation of 'custom made' carriers according to the specific needs. By varying both the ratio of the components [11,22] and the copolymer composition [18], or by modifying the polymer surface chemically [4,5], the properties of the carriers (including the hydrophobicity of their surfaces) can be changed and adjusted to achieve suitable degradation behavior and well-designed drug release. Another advantage of the poly(lactic/glycolic acid) copolymers is that when they decompose in the human body due to enzymatic, hydrolytic or other reactions, non-toxic lactic and glycolic acids will be produced. There were a great number of studies published in the literature referring to surface hydrolysis, polymer erosion and drug release for empty (without drug) and drug loaded polymeric microspheres both in vitro and in vivo. Different approaches were applied (a) to initiate and/or (b) to characterize the hydrolytic decomposition of PLA and PLGA biopolymers, as well as (c) to determine, vary and optimize those major parameters which effect the hydrolysis of these polymeric substances.

1.1. Initiation of hydrolysis

Hydrolysis occurred when the polymer samples were exposed to water or aqueous solutions. This process might be catalysed by varying the pH [7,23,24] and/or the temperature [23,24]. The mode of scission in the hydrolysis of biodegradable polymers could be completely random regarding the backbone bonds or it could be 'chain-end-unzipping' mechanisms. It was found that the base catalysed hydrolysis of the poly(D,L-lactic acid) demonstrated a random process, while the acid catalysed hydrolysis followed a fast chain-end scission [23]. It was also established that the hydrolysis rate is higher in acidic than in neutral media [7]. The hydrolytic polymer degradation was found to be dependent on the degradation temperature, namely, whether it was above or below the glass transition temperature (T_g) of the selected polymer sample [24]. The degradation became a bulk process above the glass transition temperature of the polymer, while at temperatures below T_g (where the polymer microspheres or films are in amorphous, glassy state) the degradation of the polymer matrix was restricted to its surface. These findings also showed that the temperature (both indirectly and directly) influenced

the process of drug release when the biodegradable polymers were used as drug delivery systems [24].

In other studies, while keeping the biopolymers at constant pH (usually pH 7.4, using a phosphate buffer saline, PBS) and temperature (at 37°C), samples were exposed to $_{60}$Co-gamma-irradiation [25–28], to investigate its effect on the decomposition of polymers. Gamma irradiation is the most expedient method applied presently for parenteral products to terminally sterilize moisture and heat sensitive substances, including biopolymers. Early studies indicated that gamma irradiation induced dose dependent chain scission and molecular weight loss [26,27]; another paper reported a lag prior to the zero order loss of the polymer mass in vitro [25]. The onset times for mass loss (where the degradation started) decreased with increasing irradiation dose [28]. The effect of ultrasound irradiation was also investigated [19,29], in which case chemical reactions (degradation) were introduced by mechanical stress. Orthopedic polymeric implant samples were irradiated by ultrasound at different frequencies for different irradiation times and the degradation of these specimens was compared with a control set of samples subjected to hydrolytic degradation at pH 7.4 and 37°C. It was confirmed that both the polymer degradation and the drug release significantly increased due to ultrasound irradiation [29]. The ultrasound signal caused the polymeric solution to expand and to form gas bubbles. When these bubbles collapsed they generated an ultrasonic wave which caused the scission of molecular chains.

1.2. Characterisation of hydrolytic decomposition

The progress of hydrolysis/degradation of the polymer samples could be followed in time by measuring the mass loss of the sample [19,24,28,30] by a microbalance within a selected time interval (usually up to 40–65 days), by determining the molecular weight reduction of the polymer by means of gel permeation chromatography (GPC) [7,19,24,28,30–34], or by calculating the M_w average from the intrinsic viscosity data (obtained from the relative viscosity of the aqueous soaking liquid/supernatant containing the dissolved polymer degradation products) [25]. A qualitative graphical method was also developed for studying the mode of the hydrolysis of biodegradable polymers. This approach requires the determination of molar fraction of the monomer by nuclear magnetic resonance (H-NMR) or high pressure liquid chromatography (HPLC) methods and the degree of degradation by H-NMR [23]. In another study, the supernatant was used to determine the concentration of the water soluble L-lactic acid formed during the hydrolysis by using a lactic acid assay kit from Sigma [32]. It was found that the fractional lactic acid content increased with time and increasing glycolic acid ratio. Differential scanning calorimetry (DSC) was also applied to determine the glass transition temperatures of polymers and hence characterize the polymer degradation [24,30,32]. It was established that the hydrolytic scission of the ester bonds primarily targeted the linkage between the lactic and glycolic acids [32]. Non-invasive nuclear magnetic resonance imaging (NMRI) [22,23,28,34] for poly(ortho esters), polyanhydrides, and electron paramagnetic resonance (EPR) spectroscopy [22], were introduced for testing the degradation of biodegradable polyanhydride implants in vitro and in vivo.

H-NMR monitors the local concentration and physical state of protons, therefore it can potentially be used to characterize the penetration of water into the polymer which is the key event both in the degradation of polymer and the release of drug. EPR studies indicated that the water penetration into the polymer induced changes of the microenvironment inside the implants [22]. The NMR studies showed that first the polymer eroded around the intact implant core, which was followed by the deformation of the implant samples [22]. The changes in surface area and porosity of polymer microspheres, films and implants due to hydrolytic degradation was followed by N_2 sorption analysis (NSA) [31] and any variation in the surface morphology was visually detected by using optical [6] or

scanning electronmicroscopy (SEM) [11,12,15,-18,30–32].

1.3. Influencing parameters

Among those influencing parameters, which play a major role in the hydrolytic degradation of biopolymers and their copolymers, the molecular weight (polymers with low M_w degrade faster) [7,20,33,35], crystallinity, chemical structure of the components [8,18,22,34], the copolymer composition of the polymers [7,11,18,22,32], and also the production parameters, such as solvent used [25], speed of solvent removal [15], presence of electrolyte (NaCl) in the aqueous phase [11], stirring rate, applied shear force [12], and both the concentration [34] and chemical nature/hydrophobicity of the encapsulated drug [7,18,21] have to be mentioned. The production parameters affect the porosity, morphology and the size of the microspheres formed, which in turn, control the water accessibility to the ester linkage. All of these factors directly or indirectly influence the surface degradation, bulk erosion and as the outcome, the drug release.

The purpose of the present work was to perform model experiments with limited number of components under simple, well defined and controlled conditions in order to follow the progress of the hydrolytic decomposition of poly(lactic acid) and its copolymers with poly(glycolic acid). Contact angle measurement was selected to characterize the hydrophobicity of the model surfaces and also to follow any changes in wettability of these surfaces which might occur. Studies based on contact angle determination (by means of sessile drop and dynamic contact angle measurements, or using axisymmetric drop shape analysis, ADSA) have been widely applied to reveal the hydrophobic or hydrophilic character of the surfaces of such diverse substances as polymers [36–45], coal [46,47], bitumen [48], wood [49] and biological surfaces [50–56], just to refer to very few, randomly selected examples.

Our hypothesis was that during hydrolysis, the change in the hydrophobicity of the polymer and copolymer films was induced by the composition and structural changes which occurred in both the surface and bulk phases of the biopolymers. To follow these changes, dynamic contact angles of water were measured on the polymer surfaces (soaked in distilled water) by a Wilhelmy plate method at given intervals of an extended time period. Any change in the contact angles was supposed to be solely due to the surface degradation process.

This approach, if it results in evaluable and reproducible data, provides an independent and non-destructive tool to follow the polymer degradation while the simultaneously released drug concentration from the same, drug-loaded samples could be analysed by properly selected analytical means. This method, based on the wettability change of the decomposing biosurface, may also be the first step towards our future work aiming at the surface modification of biodegradable drug delivery carriers.

2. Experimental

2.1. Materials

The selected model biopolymers were: one homopolymer, poly(D,L-lactic acid) (PLA) ($M_w \sim 26\,000$), and two poly(D,L-lactic-co-glycolic acid) (PLGA) copolymers (M_w 50 000–75 000) with 85/15 and 50/50 lactic/glycolic acid component ratios. PLA was obtained from Polysciences (Warrington, PA, USA) and the PLGA samples were purchased from Aldrich (Milwaukee, WI, USA). Structural compositions of the copolymer components, PLA and PGA, are illustrated in Fig. 1.

Acetone and dichloromethane of analytical grade were supplied by Sigma (St. Louis, MO, USA) and Chemolab (Budapest, Hungary) and were used for sample-film preparation. Doubly distilled water obtained from a Wagner + Munz Muldestor SE (München, Germany) equipment was used as soaking water for the polymer films and also as immersion liquid for dynamic contact angle measurements. The quality of distilled water was checked daily by conductivity (< 5 μS) and surface tension (> 72 mN/m) measurements at 25°C.

2.2. Sample preparation

Glass plates (microscope cover glasses: 22 × 40 mm, Menzel-Glaser, Germany) were used as hydrophilic substrates for the polymer films formed. The glass surfaces were freshly cleaned in concentrated hydrogen peroxide/sulfuric acid solution, rinsed with doubly distilled water and vacuum dried for 16 h. Glass plates, previously hydrophobised by silylation, were also used as substrates carrying the polymer films deposited on them. In the latter case, dimethyldichlorosilanes were dissolved in diethylether (5 v/v% concentration) and the previously cleaned glass surfaces were exposed to this solution for 2 h at room temperature. The silylation was followed by thoroughly rinsing the plates with water, then the plates were conditioned at 150°C for 1 h and stored in a vacuum chamber. Hydrophilicity and hydrophobicity of the prepared surfaces were tested by wettability measurements. Based on these results the plates with advancing contact angle of water below 15° were selected as hydrophilic substrates, and those with contact angles of water above 90° as hydrophobic substrates.

The polymers and copolymers (PLA, PLGA85/15 and PLGA50/50) were dissolved in acetone and dichloromethane at concentration of 1 g/dm^3. Thin layer of each polymer film (with at least 5-nm thickness) was prepared by dip coating the glass surfaces in the solution of each polymer/copolymer. Dipping (immersion and withdrawal) was performed at constant speed, 10 cm/min, and was repeated once following a drying period of 1 min.

2.3. Methods

The wettability of the polymer layers was characterised by dynamic water contact angles determined by using a Wilhelmy-type electronic tensiometer (Herceg, Hungary). Advancing (Θ_A) and receding (Θ_R) contact angles were measured at a given contact line velocity of 1.19 cm/min. The immersion cycle was repeated once so as to compare the values obtained first on a 'dry' surface and then on a 'wet' surface. The capillary force acting on the water/air contact line at the perimeter of the suspended solid plate was measured with a sensitivity of 1 μN, which allowed us to deduce the contact angles with an accuracy of $\pm 0.2°$. This is significantly lower than the standard deviation of the contact angle values (typically $\pm 2°$) originating from macroheterogeneity and the reproducibility of the sample preparation. The force curves were analysed by a Newscale computer program. Mechanical or chemical homogeneity (or heterogeneity) of the surface is reflected by the shape of the force curve, each point of which represents an average value of wetting force along the contact line. Since uniform force curves with a constant slope (corresponding to buoyancy) were obtained for all of surfaces studied here, the advancing and receding

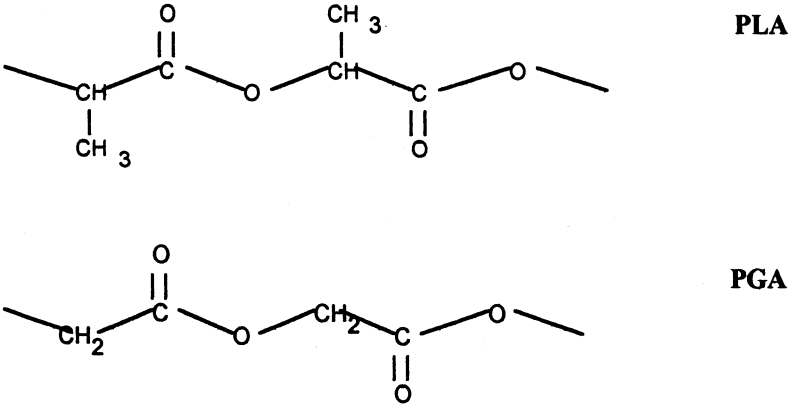

Fig. 1. Chemical composition of polylactic acid and polyglycolic acid components of the polymer and copolymers.

contact angles were calculated over the total immersed area of the polymer film from the corresponding parts of the force curves. The results presented here were obtained by averaging the data measured as duplicates on at least four sample films of each polymer.

The prepared samples were soaked in doubly distilled water for up to 50 or 80 days. At given time intervals both the advancing and receding contact angles of water were measured on rinsed and then air-dried surfaces. Buffer solutions (Clark and Lubs buffer systems, Merck Laboratory Products) in the pH range 2.0–11.8 were also used to measure their contact angles on several hydrolysing film samples.

The water in which the sample was soaked for the given time, was analysed by gel permeation chromatography [57] at the end of the hydrolysis procedure to check for the appearance of any water soluble degradation products of the polymer layer. The experimental conditions of the gel permeation chromatography were the following. Device: Biotronik BT 8100 pump; Biotronik BT 8200 UV-Vis detector; Biotronik BT 8300 System Controller; ISCO Chemresearch data handling system; Rheodyne 7125 injector; column (300×7.5 mm with a guard column of 50×7.5 mm); V_0 (void volume): 7.0 ml; detection: 205 nm, ABS range: 0.16. Samples were filtered through Spartan 13 disposable filters with a pore size of 0.45 μm (Schleicher and Schuell). The sample volume was 50 μl; the eluent was water with a flow rate of 1.0 ml/min.

3. Results and discussion

3.1. Wetting characteristics of polymer films on different substrates prior to hydrolysis

3.1.1. Effect of substrate

Polymer films were prepared on solid substrates which were either hydrophilic ($\Theta_A < 15°$) or hydrophobised ($\Theta_A > 90°$) glass plates. As solvent for the preparation of the polymer films dichloromethane was used. The water contact angles obtained on various polymer layers are summarised in Tables 1 and 2. The advancing and receding water contact angles, as average values of two measurements performed on four separate polymer samples for hydrophilic and hydrophobic substrates, are displayed together with the standard deviations in Table 1.

Table 1
Average values of advancing and receding water contact angles on polymer films formed on hydrophilic and hydrophobic substrates prior to hydrolysis[a]

Polymer	Substrate	Θ_A	Θ_R
PLA	Hydrophilic	81.5 ± 0.6	59.3 ± 0.3
	Hydrophobic	79.6 ± 1.1	56.9 ± 2.4
PLGA85/15	Hydrophilic	81.2 ± 2.0	57.7 ± 1.8
	Hydrophobic	81.4 ± 1.8	57.1 ± 3.4
PLGA50/50	Hydrophilic	80.8 ± 0.5	48.6 ± 2.1
	Hydrophobic	80.8 ± 1.4	49.8 ± 1.6

[a] Solvent: dichloromethane.

The deposition of both homopolymer and copolymer films was indicated by the significant changes in wettability of the solid plates (substrates). The surfaces covered by the polymer films became uniformly hydrophobic surfaces showing a considerable hysteresis ($\Delta\Theta \approx 20–30°$). The hysteresis might be related to the microheterogeneity of the polymer films.

It is noticeable that the contact angles measured on the polymer films deposited on hydrophilic or hydrophobic substrates did not differ from each other (Table 1), proving that the wettability data obtained were characteristic of the polymer films themselves and consequently the substrates could be considered as surfaces completely covered by the polymer layers.

Table 2
Average values of advancing and receding water contact angles on polymer films formed on hydrophilic substrates prior to hydrolysis[a]

Polymer	Substrate	Θ_A	Θ_R
PLA	Hydrophilic	79.3 ± 1.2	57.7 ± 1.3
PLGA85/15	Hydrophilic	47.5 ± 2.2	19.2 ± 5.7
PLGA50/50	Hydrophilic	54.5 ± 8.1	20.8 ± 11.1

[a] Solvent: acetone.

The composition of the polymer and copolymer substances had seemingly no influence on the wettability data. The only exception was the receding angle values obtained for PLGA50/50 polymer film. These contact angles were significantly lower than the corresponding values of PLA and even that of the PLGA85/15 samples, and this lower value might be related to the least hydrophobic character of the PLGA50/50 copolymer.

3.1.2. Effect of the solvent used for polymer film formation

In Table 2 the advancing and receding water contact angles as the average values of two measurements performed on four individual polymer films deposited on hydrophilic substrates are summarised. The solvent used for the formation of polymer layers was in this case acetone. We can see by comparing the results given in Tables 1 and 2 that the nature of the solvent used had a significant effect on the wettability characteristics of the copolymer layers formed. The probable reason for the low contact angles obtained on copolymer films deposited from acetone solutions (shown in Table 2) might be that only a partial coverage of the hydrophilic substrate was reached. This assumption was supported by the fact that e.g. freshly cleaved mica, being a highly hydrophilic surface, did not adsorb PLA and PLGA copolymers from acetone solution either. The deposition of these copolymers from acetone solution onto highly oriented pyrolytic graphite (HOPG), as a typical hydrophobic substrate, also resulted in microscopically inhomogeneous layers. Both homopolymer and copolymer films formed from acetone solutions showed characteristic patchy structures observed under microscope at $800\times$ magnification. The 'patch'-like nature of the polymer films was more expressed in film samples with higher ratio of the glycolic component of the PLGA copolymer [58]. Since the polymer and copolymer films prepared from dichloromethane solutions did not show morphological inhomogeneity in the microscopic scale, this solvent was selected for further sample preparation. These polymer layers were used to investigate the effect of the hydrolytic decomposition of the biopolymers on the hydrophobicity of the films by measuring the changes in surface wettability of these layers.

3.2. Wetting characteristics of biopolymers during surface hydrolysis

When the polymer and copolymer layers were exposed to highly purified (both ion and organic free) distilled water (used as soaking liquid) for an extended period of time, at the start of the hydrolysis (when the soaking of the polymer layers began), the experimental model system consisted of only two components: the polymer film and distilled water. In the quoted references, in most of the cases, PBS solution (phosphate buffer saline) was applied to keep the degrading system at a constant pH 7.4 value. Since the buffer is a multicomponent solution, we rather used water because we preferred to minimize the number of components to be able to clarify the influencing factors on the hydrolytic decomposition.

The polymer films at given time intervals were taken out of the soaking liquid, rinsed (to eliminate any hydrolytic byproducts which might accumulated on the film surface) and air dried; then the advancing and receding contact angles of distilled water were measured under the same experimental conditions as done for the freshly prepared (non-hydrolysed) samples.

The advancing and receding contact angles measured on homopolymer (PLA) films deposited on both hydrophilic and hydrophobic substrates from dichloromethane are shown in Fig. 2. The contact angles decreased with time, i.e. the hydrophilicity of the surface increased. There might be two reasons for the increasing wettability of the surface films: (a) it could be caused by a slow mechanical thinning of the polymer layer with time, hence the original hydrophilicity of the glass substrate would be less and less 'shadowed' by the polymer film; or (b) another explanation might be that, due to hydrolytic decomposition of the polymer with time, the layer itself became more hydrophilic and also heterogeneous. The increase in the hydrophilic character of the layer is supposed to be in connection with the appearance of carboxylic and hydroxyl groups due to the hydrolysis of ester bonds in the polymer chain.

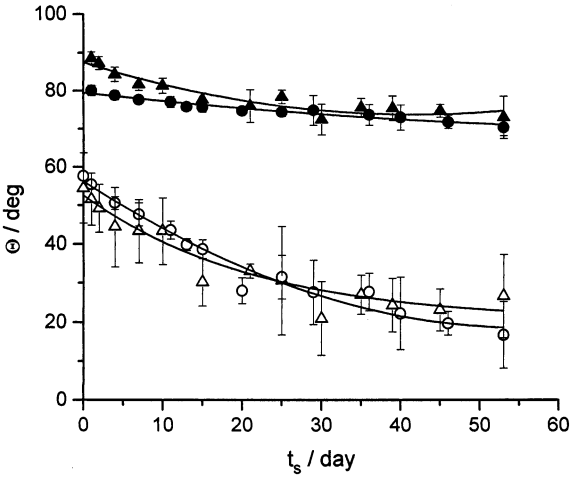

Fig. 2. Advancing (filled symbols) and receding (open symbols) water contact angles on D,L-PLA films prepared on hydrophobic (△) and hydrophilic (○) substrates. Solvent: acetone.

3.2.1. Effect of substrate

To justify one of the reasons, samples of the homopolymer PLA films were prepared on both hydrophobic (silylated glass) and hydrophilic (highly purified, not coated glass) surfaces. We speculated that if the contact angles decreased with time because the hydrophilic surface 'showed through' due to mechanical thinning of the films, then the films deposited onto the hydrophobic carrier should have behaved differently. The opposite was clearly proved; the curves were very similar in character in both cases (Fig. 2). The similar character of the curves indicated that the observed decrease of contact angles, i.e. the increasing wettability of the surfaces, was indeed the direct consequence of the hydrolytic decomposition process which occurred when the polymer films were exposed to the soaking liquid (water) for an extended period of time.

3.2.2. Effect of the copolymer composition

As was expected, the time dependence of contact angles measured on copolymer layers differed from those obtained on homopolymer surfaces (Fig. 3). For these studies the polymer and copolymer films were deposited on hydrophobic (silylated) glass surfaces. It was observed that the decrease of both the advancing and receding contact angles caused by hydrolysis was faster for copolymer than for homopolymer surfaces. In order to characterize the difference in the hydrolytic stability of the polymer films as a function of their composition, two parameters (t_A and t_R) had been selected: t_A means the soaking time (in days) required to reduce the advancing contact angle by 5° and t_R means the soaking time (in days) required to reduce the receding contact angle by 20°.

These two parameters were selected to describe the effect of the soaking time in water on the wettability, or rather on the magnitude of change in wettability of the polymer films

Film	t_A(days)	t_R(days)
D,L-PLA	27	29
D,L-PLGA 85/15	9	19
D,L-PLGA 50/50	4	13

Comparing the t_A and t_R values among the substances studied it can be concluded that (a) the homopolymer layer (PLA) was the most resistant to hydrolysis (the longest time was required to reach the advancing and receding contact angle decrease by 5° and 20°, respectively), and (b) the stability of the copolymer layers was significantly lower, and it decreased with increasing glycolic

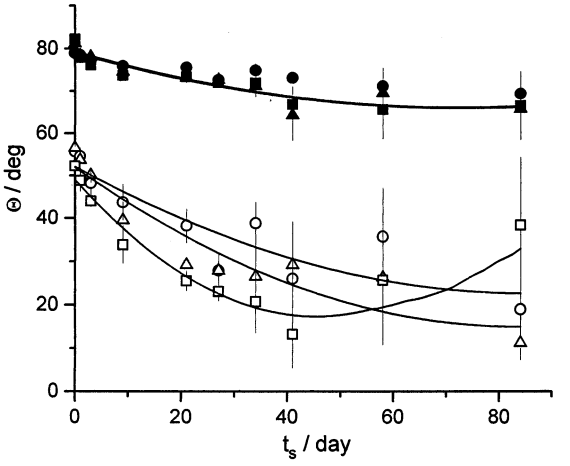

Fig. 3. Advancing (filled symbols) and receding (open symbols) water contact angles on D,L-PLA (○), D,L-PLGA85/15 (△) and D,L-PLGA50/50 (□) polymer layers deposited on hydrophobic substrate. Solvent: dichloromethane.

acid ratio in the copolymers. These findings were in accordance with the already established fact that the polylactic acid exhibited a more crystalline structure, and hence was less easily decomposable than the less crystalline copolymers [59]. The crystallinity correlates closely with other morphological and physicochemical properties of the polymer, such as molecular weight, porosity, surface roughness and surface area, which, in turn, greatly affect the hydrolysis and drug release rate [59,60]. It should also be mentioned here that the more hydrophobic character of the PLA is due to the $-CH_3$ groups in the molecule (Fig. 1). These methyl groups are substituted by hydrogen in the glycolic acid molecule, making it less hydrophobic, and therefore with increasing PGA content the wettability of the copolymer increases.

3.2.3. Effect of pH

Another justification of the above thinking might be provided by the contact angle measurements on the homopolymer and two copolymer films as a function of pH of the measuring liquids. It is known from the literature [61] that the protonated form of acid groups rendered the surfaces less hydrophilic, hence less wettable. We can speculate that in acidic media the hydrolytically decomposed lactic and glycolic acids became protonated, while in the higher pH range they remained in dissociated (hence more hydrophilic) form. This speculation was supported by our results shown in Fig. 4. The contact angles on each surface were measured at zero time (before soaking the samples in distilled water) and after 4 and 8 days soaking. The dynamic contact angles were measured in six different buffer solutions at pH values of 2.0, 3.5, 5.5, 8.3, 9.5 and 11.8.

During the period involved (up to 8 days) no pH dependence of the advancing contact angle on the homopolymer (PLA) surfaces was noticeable. For the copolymer, which contained 85% of the hydrophobic PLA, again, no pH-induced change, only a large scatter of the data was observed after the start of the soaking process. However, after 8 days a slight difference in contact angles measured in the acidic and alkaline ranges appeared for the copolymer with 85/15 component ratio. For the second copolymer with the 50/50 ratio of PLA/

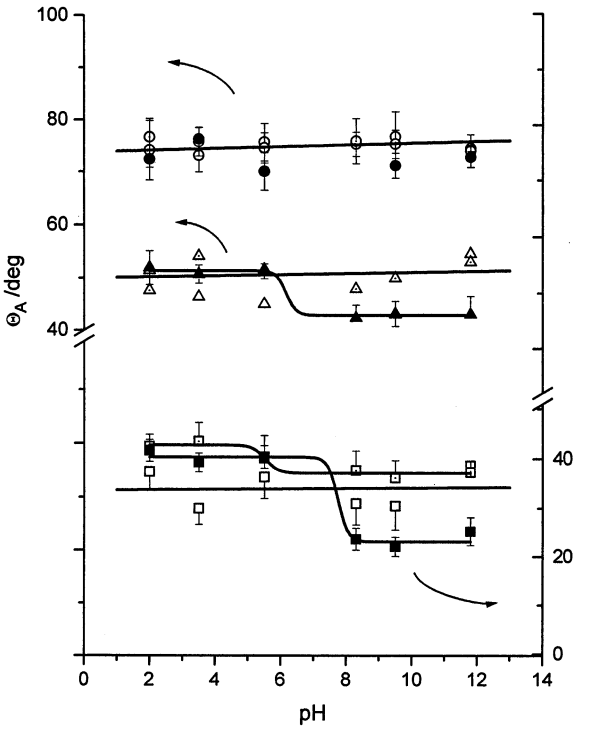

Fig. 4. Advancing contact angles of buffer solutions on D,L-PLA (○), D,L-PLGA85/15 (△) and D,L-PLGA50/50 (□) polymer layers before soaking (open symbols) and following 4 (dotted symbols) and 8 days (filled symbols) of soaking in distilled water.

PGA, only the zero time measurements showed no pH dependence (but an extremely high scatter in data). After 4 days a very small but noticeable difference, after 8 days a significant change was observed for contact angles obtained within the acidic and alkaline ranges (Fig. 4). As mentioned earlier, it is known that poly(lactic acid) is more resistant to hydrolytic decomposition than glycolic acid. That was supported by our observations, namely that the copolymer 85/15 showed pH dependence later in time than the copolymer with 50/50 composition and in the case of the latter, this effect was found to be more significant.

3.2.4. Analysis of soaking liquids

Gel chromatographic analysis was performed for the liquids in which the polymer films were soaked for 30 day. The results related to the different polymers are shown in Fig. 5, where the

absorbances at 280 nm are plotted against time. It can be seen that chromatograms of the soaking liquids used for PLA homopolymer and PLGA85/15 copolymer samples did not differ from that of the pure water. There was no detectable mass of degradation products in these soaking liquids. On the other hand, chromatogram of soaking liquid of the PLGA50/50 copolymer films indicated the presence of low molecular weight, water soluble components. The appearance of degradation products with low molecular weight in the soaking liquid of PLGA50/50 copolymer film and the absence of such compounds in the case of PLA and PLGA85/15 polymers (up to 30 days) supported our previous findings on the difference in hydrolytic stability of the homo- and copolymers. The gel chromatographic results also indicated the accelerating effect of the ratio of the more hydrophilic PGA component on the decomposition of the biopolymer films.

4. Conclusions

Changes in surface properties of biopolymers during hydrolytic decomposition (caused by direct contact with aqueous environment) can be detected, followed and interpreted by dynamic contact angle measurements. It was established that the solvent used for polymer film preparation influenced the mechanical homogeneity of the layers, but the hydrophobic or hydrophilic character of the substrate (if the film thickness reached a threshold value) did not affect the initial contact angles measured on the homopolymer and copolymer films. There was no effect of the hydrophobicity of substrate on the wettability of polymer films observed during the hydrolysis process either. The hydrolytic decomposition showed a time dependence, as expected, and the parameters, which had major effect on the rate of decomposition, were the biopolymer composition and the pH. Difference was found between the progress of hydrolysis of the homopolymer D,L-PLA and its copolymers with D,L-PGA. It was also proven that with increasing glycolic acid component in the copolymer films the hydrolysis rate increased significantly.

The introduction of the non-invasive contact angle measurements to monitor the wetting behavior of biopolymer surfaces due to hydrolytic decomposition could be of importance in biomedical and pharmaceutical applications.

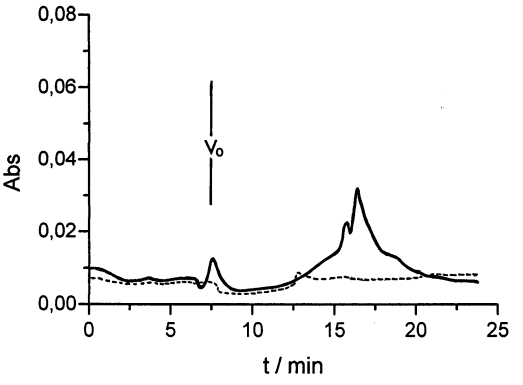

Fig. 5. Chromatogram of pure water and soaking liquids of different polymer layers. Dotted line: pure water, as well as soaking liquids of D,L-PLA and D,L-PLGA85/15 (the three curves overlap each other). Solid line: soaking liquid of D,L-PLGA50/50.

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