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Functionalized surfaces of polylactide modified by Langmuir–Blodgett films of amphiphilic block copolymers

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To modify the surface of poly(L-lactide) (PLA) supports, we have investigated the feasibility to deposit on the PLA surface Langmuir–Blodgett films of amphiphilic block copolymers based on poly(L-lactide). AB and ABA block copolymers were prepared with PLA as the A block and either poly(ethylene oxide), α-methoxy-ω-hydroxy poly(ethylene oxide), α-carboxy-ω-hydroxy poly(ethylene oxide) or poly(L-aspartic acid) as the B blocks. Films with phase-separated hydrophilic and hydrophobic blocks in a bilayer "brush" structure were prepared by compression of the copolymer Langmuir films on the water/air interface. The interfacial behavior of the monolayers and the effect of the copolymer composition on the phase separation was followed by measurements of the surface-pressure/area isotherms using a Langmuir trough and by contact angle measurement of deposited Langmuir–Blodgett (LB) films. The phase separation of the hydrophilic and PLA blocks is more effective in diblock AB copolymers compared with triblock ABA copolymers. The presence of ionic groups in the hydrophilic chains facilitates penetration of hydrophilic segments into the water subphase. Dynamic contact angle measurements were used to study the stability of the LB-films transferred on the PLA support and the changes in the surface properties upon incubation of surfaces in water.

1. Introduction

The feasibility of enhancing adhesion and proliferation of cells through modification of surfaces by introduction of bioactive groups (such as binding ligands or charges) and their structuring in surface domains are very desirable features of polymer biomaterials for tissue engineering. Current research trends in surface modification focus on (1) the ability to suppress nonspecific adsorption of extracellular matrix proteins [1, 2], and (2) the formation of a "clustered" surface topography of binding ligands, reflecting spatial distribution of cell membrane adhesive receptors [3]. In this respect, the most frequently used biodegradable polymer biomaterials, the lactone-based polyesters, such as poly(lactic acid) (PLA), poly(ε-caprolactone), and poly(lactide-co-glycolide), offer only a limited possibility of chemical modification. The modification of the polyester homopolymers by their amphiphilic block copolymer derivatives represents a way of making the surfaces of the polymer matrix more hydrophilic and capable of carrying functional groups.

Amphiphilic copolymers have been introduced into the homopolymer matrix by blending in solution and subsequent casting of films [4], by adsorption from copolymer solution [5], spin coating [6] or adsorption of the copolymers from solvent–nonsolvent mixtures on the polymer surface [7]. During the processing, the phase separation of hydrophilic and hydrophobic blocks usually leads to the exposition of hydrophilic blocks at the film surface. A well-organized "brush-like" structure of hydrophilic blocks on the homopolymer surface would be particularly desirable. Nevertheless, the feasibility of controlling and predicting the surface morphology in these processes remains to be understood.

The Langmuir–Blodgett (LB) technique can be used for the preparation of well-organized closely-packed films by compression of a copolymer monolayer formed at liquid/air or liquid/liquid interfaces. The LB-technique has been used in biomaterial research for studies of protein–ligands interactions [8] or of the degradation mechanisms of polymer supports [9, 10]. Cho *et al.* [11] have studied fibroblast adhesion on poly(γ-benzyl L-glutamate)/poly(ethylene oxide) diblock copolymer surfaces on glass slides.

Our goal is to use the LB-technique to prepare well-organized stable brush structures of amphiphilic block copolymers containing a polyester (PLA) block for the surface modification of polyester-based supports such as PLA. The PLA chains separated in one layer of the LB film are supposed to provide for a good adhesion of the brush to the surface of the PLA matrix, while the hydrophilic chains should be exposed to the aqueous environment and thus be available for presentation of special moieties eventually bound to them and capable to control protein and cell adhesion. In the present study, we use the LB-technique as a tool to analyze how the molecular parameters of block copolymers contribute to phase separation of blocks at water/air and water/solid interfaces. The effect of the copolymer composition on the phase separation was followed by measurement of the surface-pressure/surface area (π-A) isotherms using a Langmuir trough and by contact angle measurement of LB films. Dynamic contact angle measurements were used to study the stability of surfaces modified by LB films and the changes occurring at the modified surfaces during their incubation in water.

2. Materials and methods

2.1. Materials

The monomer, L-lactide (L-LA), was prepared by known procedures [12] and stored over P_2O_5. It was freshly recrystallized from dry toluene and dried in vacuum at 60 °C shortly prior to use. Tin(II) octanoate (Fluka) was purified by distillation in vacuum and stored in sealed ampoules under N_2 at $-20\,°C$. Dioxane (Fluka) was dried by refluxing with sodium and distillation under inert atmosphere before use for polymerizations. Ultrapure water for LB measurements was obtained from a Milli-Q UF Plus (Millipore) apparatus. Poly(ethylene oxide) PEO6 ($M_{n(\mathrm{GPC})} = 6110$, $M_w/M_n = 1.08$) was purchased from Fluka, AG, and α-methoxy-ω-hydroxy polyethylene oxide M-PEO5 ($M_{n(\mathrm{GPC})} = 5070$, $M_w/M_n = 1.10$) and α-carboxy-ω-hydroxy polyethylene oxide C-PEO5 ($M_{n(\mathrm{GPC})} = 5100$, $M_w/M_n = 1.12$) were products of ShearWaters Polymers, USA. PEO samples were dialyzed against water, lyophilized, dried in vacuum at 25 °C and stored over P_2O_5 in vacuum prior to use. Hydroxy-terminated poly(γ-benzyl L-aspartate) PBA was prepared by controlled living polymerization of N-carboxyanhydride of γ-benzyl L-aspartate terminated with 2-aminoethanol [13, 14].

2.2. Polymers

Amphiphilic block copolymers of L-LA were prepared by controlled ring-opening polymerization of L-LA using hydroxy-terminated PEO derivatives or PBA as macroinitiators. The polymerization was carried out in dioxane solution at 60 °C, with tin(II) octanoate as a catalyst. The polymerization of L-LA initiated with α,ω-dihydroxy PEO resulted in ABA block copolymer (PLA/PEO/PLA), while α-methoxy-ω-hydroxy-PEO (M-PEO5), α-carboxy-ω-hydroxy-PEO (C-PEO5) and hydroxy-terminated poly(γ-benzyl L-aspartate) PBA were used as macroinitiators for the synthesis of diblock copolymers M-PEO5/PLA, C-PEO5/PLA and PBA/PLA, respectively. The details of the synthesis are described in previous papers [4, 14]. The protecting γ-benzyl ester groups from poly(γ-benzyl L-aspartate) block of PBA/PLA were removed by hydrogenation on Pd/C, yielding an amphiphilic block copolymer with ionic poly(aspartic acid) block (PAA), carrying carboxylic groups in side chains (PAA/PLA) [13]. The high-molecular-weight poly(L-lactide) (PLA) was prepared by an analogous procedure but with no co-initiator added.

2.3. Methods

Polymer analysis. The composition of copolymers was determined from NMR spectra recorded on a Bruker Avance DPX-300 spectrometer in $CDCl_3$.

Size exclusion chromatography (SEC) was performed on a Waters HPLC-SEC modular system, using PLgel 10^3Å, 10 μm and Plgel Mixed C ($2 \times 7.5 \times 600\,\mathrm{mm}^3$) columns in THF, with Waters 410 RI detector. The columns were calibrated with PEO standards (Chrompack) for PEO measurements. For measurements of PLA samples, columns were calibrated using polystyrene standards and the molecular-weight/elution volume relationships were recalculated for PLLA with Mark–Houwink coefficients for polystyrene and PLLA [15], using a universal calibration concept. The calibration for PLA/PEO copolymers was derived as a linear combination of contributions made by PEO and PLA blocks. This calibration dependence was very close to that for PLA alone.

Measurement of π-A isotherm and LB-technique. The dependence of surface pressure (π) vs. area (A) (π-A isotherms) were obtained using a Langmuir film balance (Lauda FW2, Germany). A typical procedure was as follows: 20 μl of the polymer solution in chloroform (1 mg/ml) was spread on the surface of the aqueous subphase using a microsyringe. Ultrapure water (neutral pH), 0.01 M solution of Na_2HPO_4 (pH 8) or 0.01 M solution of HCl (pH 2.1) were used as subphases. After evaporation of chloroform (about 15 min), the monomolecular polymer film was compressed at a constant rate of $46\,\mathrm{cm}^2/\mathrm{min}$. Multiple measurements were performed for each sample and the data consistent for at least three successive measurements were taken.

The PLA support for adsorption of the LB-film was prepared by spin-casting a PLA solution in chloroform (1%) on silanized glass slides and both sides of the glass slide were coated. While monitoring the π-A isotherm, the copolymer monolayer was compressed to reach a desired value of the surface pressure π corresponding to the transition from the plateau region to the condensed film (see description in Fig. 2). The compressed film was transferred onto one side of the PLA-coated glass slide by horizontal touching (speed 1 mm/min), as it is schematically depicted in Fig. 1. After drying the modified PLA support, the other portion of the LB film was transferred onto the other side of the PLA-coated glass slide. The modified surfaces were dried in vacuum and kept in a grease-free desiccator under vacuum until next experiments.

Contact angle measurement. Dynamic contact angle measurements of PLA modified surfaces were carried out by the Wilhemy-plate method using a tensiometer KRÜS, model K12, Germany. The advancing contact angle (θ_A) and the receding contact angle (θ_R) were measured after incubation of the samples in water for a given period.

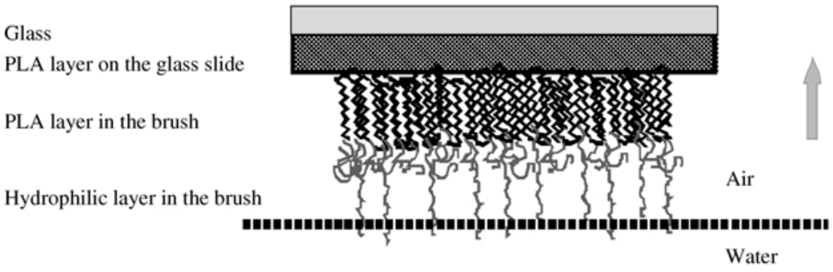

Figure 1 Schematic view of the horizontal deposition of the compressed LB film on the PLA support.

3. Results and discussion

3.1. Polymers

To eliminate the effect of polymer heterogeneity on the monolayer behavior during compression at the air/water interface, well-defined amphiphilic block copolymers of PLA were prepared by controlled ring-opening polymerization of L-LA in the presence of tin(II) octanoate as a catalyst. The polymerization of L-LA initiated with α,ω-dihydroxy-PEO resulted in a triblock copolymer with a central hydrophilic block (PLA/PEO/PLA). When macroinitiators with only one terminal hydroxy group (M-PEO5, C-PEO5 and hydroxy-terminated poly(γ-benzyl-L-aspartate) (PBA) were used, diblock copolymers M-PEO5/PLA, C-PEO5/PLA and PBA/PLA, respectively, were obtained. Starting from macroinitiators with narrow molecular-weight distribution and applying the conditions of living ring-opening polymerization of lactide in solution [4, 14], well defined block copolymers with low heterogeneity in block length were obtained. Composition and molecular parameters of the copolymers were determined by ^{1}H NMR spectroscopy and were in good accord with the values found by SEC. The prepared polymers with their characteristics are listed in Table I.

3.2. Behavior of copolymers at the water/air interface: π-*A* isotherms

The formation of a brush film of amphiphilic block copolymers at the water/air interface depends on desorption of hydrophilic and hydrophobic blocks into two phase-separated layers during compression of the copolymer monolayer. Considering the fact that the surface pressure developed during compression of the film depends on the arrangement of polymer chains at the interface, valuable information about the phase separation in the copolymer monolayer can be derived from features of π-*A* isotherms and their comparison with those described for other systems [16, 17, 18]. The hypothesized behavior of copolymers at different stages of the π-*A* isotherm is schematically depicted in Fig. 2.

The π-*A* isotherms, i.e. the dependences of the measured surface pressure π vs. the area (A) occupied by an average monomer structural unit of the polymer in the film, are presented in Figs. 3–5. For each copolymer, an average value for the molar mass of the monomer structural unit was calculated from the monomer masses of lactic acid and ethylene oxide and/or aspartic acid and the mole fraction of the respective unit in the copolymer. Characteristics of the isotherms are summarized in Table II.

At low surface pressures (π, up to about 5 mN/m), a typical behavior of the liquid-expanded monolayers with a continuous slight increase in π with compression of the film area is observed for all samples. At this stage, a two-dimensional ''pancake'' form of the film is maintained, assuming polymer chains lying flat on the water/air interface [19]. Somewhat higher pressure in this region for the copolymer M-PEO5/PLA2, as compared to other neutral polymers in this series (Fig. 3), may indicate a stronger interaction of these copolymers with the subphase due to a relatively high content of the

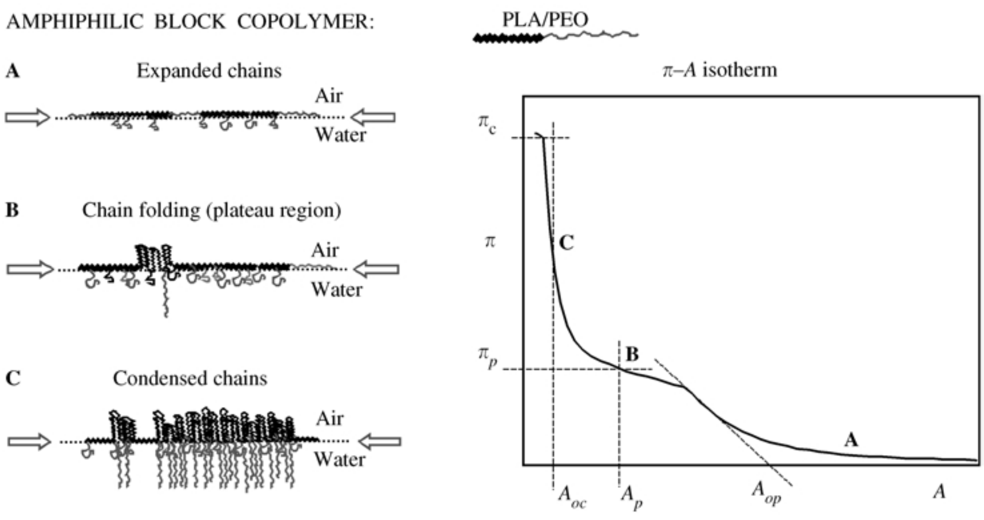

Figure 2 Schematic view of organization of hydrophilic and hydrophobic segments during compression of the copolymer monolayer at the water/air interface at different stages of the π-A isotherm.

TABLE I Characteristics of polymers under study

Polymer	Initiator	M_n^a initiator	M_n^a PLLA block	M_n NMR[a] copolymer	M_w/M_n^b copolymer
PLA12/PEO/PLA12[c]	PEO6	6110	23 560	29 670	1.15
M-PEO5/PLA2[d]	M-PEO5	5070	2050	7120	1.11
M-PEO5/PLA5[d]	M-PEO5	5070	5130	10 200	1.22
C-PEO5/PLA5[d]	C-PEO5	5100	5570	10 670	1.14
PAA/PLA9[d]	PBA	3100	9100	12 200	1.15
PLLA[e]	—	—	—	550 000	—

[a] From NMR analysis.
[b] From GPC analysis.
[c] ABA triblock copolymer with the central hydrophilic block.
[d] AB diblock copolymer.
[e] Homopolymer, the molecular weight (M_v) obtained by viscometry.

TABLE II Characteristics of the π-A isotherms*

Polymer	Fraction[a] wt %	A_{OP}^{b} Å^2/mon	A_p^c Å^2/mon	A_{OC}^{d} Å^2/mon	π_p mN/m	π_c mN/m
M-PEO5/PLA2	68	32	12	NA	11	24
C-PEO5/PLA5	50	NA	NA	1.8	NA	52
PAA/PLA9	25	20	10	3.6	10.5	56
PLA12/PEO6/PLA12	25	19	11.5	3.6	8.5	58
PLLA	0	17.5	13	4.5	7.5	63

* Measurements carried out using the water subphase.
[a] Content of the hydrophilic component in the block copolymer.
[b] A_{OP}, the limiting ''pancake'' area per monomer unit.
[c] The area occupied by the monomer unit in the inflection point of the plateau region.
[d] A_{OC}, the limiting area per monomer unit in condensed film.
NA, not applicable.

hydrophilic PEO phase in the copolymer (68%, see Table II).

By further compression, the surface pressure increases until a plateau region is reached (π in a range of 8–12 mN/m). The intercept of a linear extrapolation of this part of the curve taken in its inflection point on the zero-pressure axis characterizes the limiting pancake area A_{OP} per monomer unit (see Fig. 2 for a scheme), when the interaction between segments has still a negligible effect on the monolayer behavior. The existence of the plateau is described as a consequence of the transition from liquid-expanded (LE) to liquid-condensed (LC) type of the monolayer, LE-LC transition [19]. For homopolymer PLA and copolymer PLA12/PEO6/PLA12 (Fig. 3), the plateau is flat with a constant surface pressure π for a range of A. This behavior has been described as the first-order transition and attributed to conformational changes of the chains that occur due to lateral pressure on the film [19]. An inclined plateau region with slightly increasing π with compression, i.e. with a decrease in A, is observed for diblock copolymers M-PEO5/PLA2 (Fig. 3) and PAA/PLA9 (Fig. 5). This indicates a diffuse first-order transition, where the conformational changes are accompanied also by chain overlapping. In the case of copolymer C-PEO5/PLA5 (Fig. 4), no characteristic transition was observed, suggesting the highest interaction of segments with the aqueous subphase.

For all samples, with further compression, the plateau region is followed by an abrupt steep increase in the surface pressure, as a result of high resistance of closely-packed chains forming a liquid-condensed (LC) film (part C of the isotherm: see Fig. 2 for a scheme). By still further compression, the film collapses. The limiting surface area per one monomer unit in the LC state, A_{OC} (Table II), was determined from the inflection point of the steep part of the isotherm (see Fig. 2 for a scheme). In the LC state, a ''brush formation'' regime with the polymer chains most stretched in the direction normal to the surface is assumed [20]. For block copolymers, the surface pressure at which the film collapses (πc; see Fig. 2 for a scheme) is lower than for the PLA homopolymer (Table II). The decrease in the pressure at collapse with the decreasing length of PLA blocks and/or with increasing length of hydrophilic block is in accord with the view that the stability of the monolayer derives primarily from the interaction between PLA blocks.

The interaction of hydrophilic blocks with the aqueous

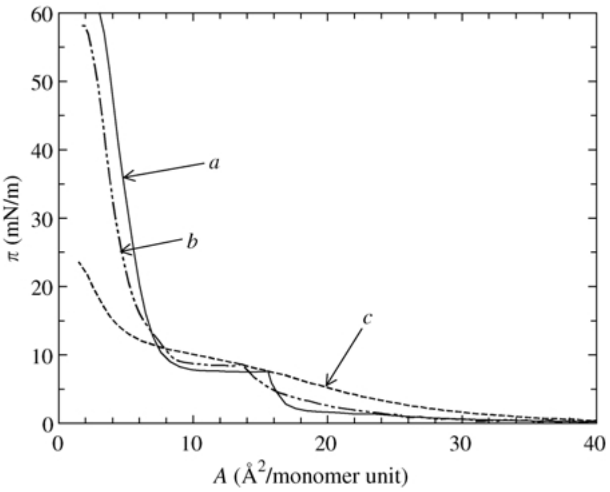

Figure 3 π-A isotherms of polymer monolayers spread on water as a subphase: (a) homopolymer PLA (————); (b) ABA triblock copolymer PLA12/PEO6/PLA12 (—· ·—· ·); (c) AB diblock copolymer M-PEO5/PLA2 (— — —).

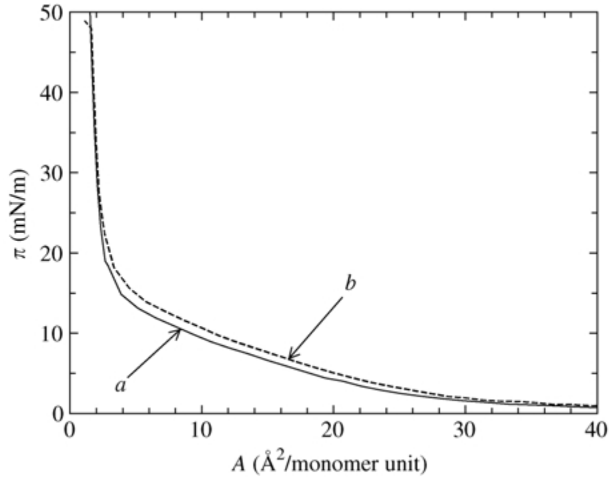

Figure 4 π-*A* isotherms of monolayers of the AB diblock copolymer C-PEO5/PLA5 spread: (a) on water (pH 6.7) (————); (b) Na_2HPO_4 (pH 8) (— — — —) as a subphase.

subphase is revealed in experiments with copolymers carrying ionic groups, such as C-PEO5/PLA5 (Fig. 4) and PAA/PLA9 (Fig. 5). While the shape and position of the isotherms on neutral (water pH 6.9) and acidic (pH 2.1) subphases are almost identical, the isotherm for the alkaline subphase (pH 8.0) is significantly shifted. At pH 8, the carboxyl groups are ionized, carrying negative charge and, therefore, the chains are more solvated by the subphase. The average monomer unit thus occupies larger space, leading to an increase in the A_{OP} value for sample PAA/PLA9 from 20 to 22 Å^2 per monomer unit as well as an increase in the area corresponding to the plateau inflection from 10.0 to 12.5 Å^2 (Table II). Also, the repulsion of negatively charged chains of the immersed hydrophilic blocks is reflected in higher pressure at plateau. The pH dependence of the isotherm parameters confirms that hydrophilic blocks of copolymers are separated from PLA chains and are immersed in the aqueous subphase.

For the copolymers, the measured surface pressure and limiting pancake area A_{OP} increase with increasing content of the hydrophilic phase in the copolymer what indicates, that the hydrophilic segments are active on the subphase surface and contribute to the surface area of the film. When reaching the plateau region (Fig. 2, phase B), LE-LC transition, the polymer segments approach upon further compression closer to each other and chain reorientation starts as a result of steric requirements. The first-order transition has been observed also for poly(DL-lactide) (PDLLA) and lactide copolymers with glycolic acid and was associated with forming a three-dimensional (3D) polymer layer, composed of condensed polylactide micro-domains by stacking of 5–6 lactide units remaining on the subphase surface [21, 22]. A similar behavior of hydrophobic blocks of amphiphilic copolymers has been already described for amphiphilic block copolymers of PEO with poly(styrene) [23, 24]. The results obtained from characterization of LB films lead to the expectation that a phase-separated PLA layer can be formed in the plateau region of the isotherm, which is desirable for a good adhesion of the copolymer brush after it has been deposited on the PLA support.

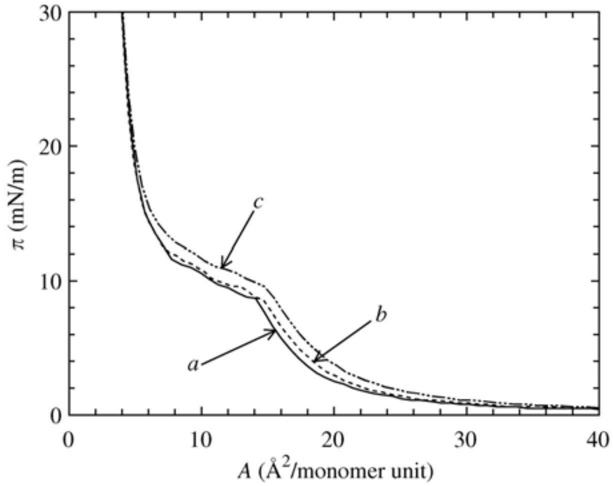

Figure 5 π-*A* isotherms of monolayers of the AB diblock copolymer PAA/PLA9 spread on: (a) 0.1 M HCl, pH 2.1 (————); (b) water (— — — —); (c) 0.1 M Na_2HPO_4, pH 8 (— · · — · ·) as subphases.

3.3. Dynamic contact angle measurements

Dynamic contact angle measurements were used to study the hydrophilization of the PLA support incurred by the deposition of LB films. When polymer scaffolds for tissue regeneration are to be implanted into a living body, they perform in contact with body fluids and aqueous environment of the extracellular matrix. Therefore, changes on the polymer surface developing during incubation in water were studied.

In the case of chemically heterogeneous surfaces, such as those in the present study, the advancing contact angle (θ_A) is attributed predominantly to low-free-energy components on the surface and it characterizes wettability of the solid, while the receding contact angle (θ_R) is associated with the advance of the liquid phase over the well-wetted high-free-energy phase of the surface [25]. The evolution of the contact angles with time provides information about the structure changes and adaptation in the uppermost layer of the material, incurred by interactions with the aqueous environment.

The θ_A and θ_R contact angles for the modified PLA supports and their evolution with the incubation time are presented in Figs. 6 and 7. The values of θ_A and θ_R measured on dry samples, i.e. without pre-incubation in water, are lower at higher contents of the hydrophilic phase in the copolymer, as it was expected; nevertheless, relatively high values for copolymers PLA12/PEO6/PLA12 ($\theta_A = 83.1°$), PAA/PLA9 ($\theta_A = 84.9°$), M-PEO5/PLA5 ($\theta_A = 93°$) and C-PEO5/PLA5 ($\theta_A = 77.8°$) indicate rather a hydrophobic character of the surface. We suppose, that during the drying of the modified PLA support, the hydrophobic PLA segments within the outermost layer reorient towards the surface to minimize the interfacial free energy between the solid and air [26]. The immediate contact angle values on dry samples may not reflect the true organization of the LB-films on the water/air interface before their transfer onto the PLA support.

When the PLA surfaces are placed in water, the polymer–water interface restructure to minimize the interfacial free energy, by both reorientation of polymer

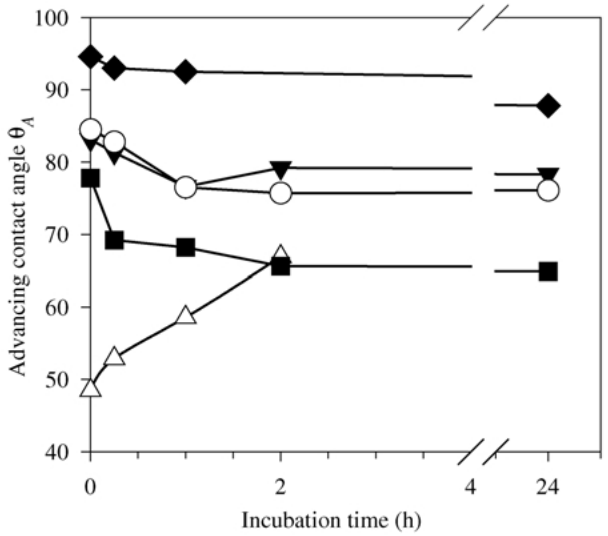

Figure 6 The evolution of advancing contact angle (θ_A) for PLA surfaces modified by LB-films of copolymers after incubation in water: (◆) M-PEO5/PLA5, (▼) PLA12/PEO/PLA12, (○) PAA/PLA9, (■) C-PEO5/PLA5, (△) M-PEO5/PLA2.

chains and penetration of water [25]. For most of the samples, the structural changes are manifested in the changes of contact angle observed during the incubation in water.

An increase in the θ_A and θ_R values with incubation (the polymer surface becomes more hydrophobic) for copolymer M-PEO5/PLA2 indicates desorption of the LB film from the support. Apparently, short PLA segments (M_n of PLA block is 2000), though forming a 3D PLA layer at the water/air interface as indicated by a plateau LE–LC transition in π-A isotherm (Fig. 3), are not long enough to anchor the LB film to the PLA surface.

For the other samples, the θ_A values decrease with the incubation time, indicating that the surfaces become more hydrophilic due to penetration of water, with a steady state being reached within ca. 2 h of the incubation. For ABA block copolymer PLA12/PEO6/PLA12 (25% of the hydrophilic component), the smallest decrease in θ_A (from 83.1° to 79°) and no changes in θ_R point to only a limited hydration. It indicates that the high energy components – PEO segments – are not active on the surface, probably because the central PEO segments are well fixed between PLA segments in compressed LB film, thus preventing reorientation after contact with water. This explanation is supported by the presence of a flat plateau in the π-A isotherm, characteristic of a rigid film structure, observed also for the PLA homopolymer (Fig. 3).

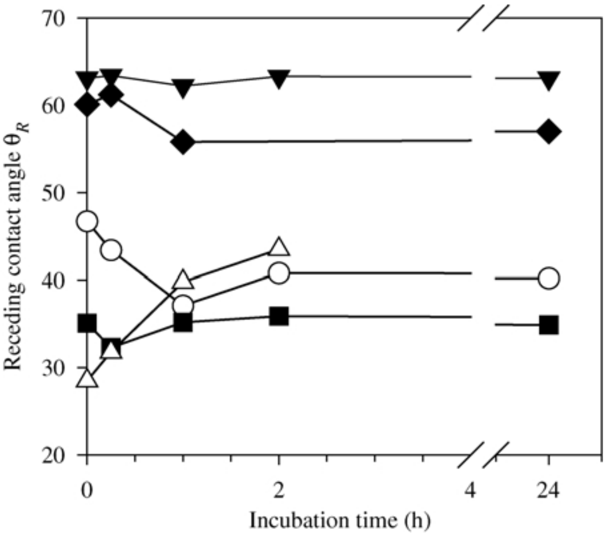

Figure 7 The evolution of receding contact angle (θ_R) for PLA surfaces modified by LB-films of copolymers after incubation in water: (◆) M-PEO5/PLA5, (▼) PLA12/PEO/PLA12, (○) PAA/PLA9, (■) C-PEO5/PLA5, (△) M-PEO5/PLA2.

On the other hand, the significant decrease in θ_A $(-10°)$ as well as θ_R $(-8°)$ for the copolymer PAA/PLA9 with the same hydrophilic content suggests an exposure of PAA hydrophilic chains at the surface and indicates reorientation towards the solid/water interface. A higher mobility of hydrophilic chains in diblock copolymer can be assumed, as compared to the triblock copolymer, and this behavior can be related to the ascending character of the LE-LC transition in the π-A isotherm (Fig. 5).

The high and stable values for θ_A and θ_R for the AB block copolymer M-PEO5/PLA5 (50% of the hydrophilic component) could be attributed to an incomplete coverage of the surface by the LB films (it means by the hydrophilic segments), with hydrophobic PLA segments of the polymer support dominating in the modified surface.

The most hydrophilic character and the largest θ_A decrease $(-14°)$ was observed for copolymer C-PEO5/PLA5 with the carboxylic end-group (50% of the hydrophilic component), indicating the most effective hydration of the modified surface. No change in θ_R during the incubation shows a stable presence of the hydrophilic components on the polymer surface. It leads to the conclusion that this copolymer provides for a LB film with the best-separated PEO and PLA layers out of the studied copolymers. Interestingly enough, the π-A isotherm shows no plateau corresponding to the formation of PLA micro-domains during the monolayer compression (Fig. 4). Nevertheless, this does not exclude the presence of a stable PLA layer because the monolayer collapses at a surface pressure π_c, which is an approximately same as for other copolymers, close to the collapse of the PLA homopolymer monolayer.

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