Colloid Polym Sci 275:580–586 (1997)
© Steinkopff Verlag 1997

ORIGINAL CONTRIBUTION

N. Vila Romeu
J. Miñones
E. Iribarnegaray
O. Conde
M. Casas

Influence of the solvent on the spreading of poly[(D,L-lactic acid)-*co*-(glycolic acid)] monolayers

Received: 14 October 1996
Accepted: 7 January 1997

Dr. N. Vila Romeu (✉) · J. Miñones
E. Iribarnegaray · O. Conde · M. Casas
Universiad de Santiago de Compostela
Facultad de Farmacia
Departamento de Quimica-Fisica
15706 Santiago de Compostela, Spain

Abstract The properties of mono layers of poly[(D,L-lactic acid)-*co*-(glycolic acid)] (PLA/GA) are strongly conditioned by the nature of the solvent from which they are spread. In this work, we studied the properties of PLA/GA films deposited on water from acetonitrile (a poor spreading solvent) and chloroform (a good one), observing marked differences with regard to the influence of the pH, temperature and ionic strength of the subphase. These differences were attributed to the structure of PLA/GA at the air/water interface, being pre-determined by its structure in the bulk spreading solvent (closely coiled in acetonitrile, unfolded in chloroform). Viscosity measurements on acetonitrile and chloroform solutions of PLA/GA, and the calculation of the corresponding intermolecular expansion factor, α, confirmed that PLA/GA was better solvated by chloroform than by acetonitrile, in which intramolecular interactions between polymer segments, and thus coiling, will therefore have predominated over polymer–solvent interactions.

Key words Poly[(D,L-lactic acid)-*co*-(glycolic acid)] – drug forms – monolayers – air/water interface

Introduction

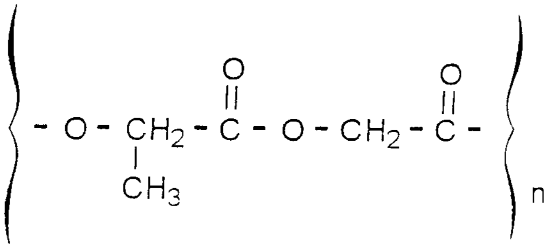
In recent years, research aimed at the sustained release of drugs has investigated drug forms in which the active principle is contained in a microsphere or nanoparticle with a biocompatible, biodegradable polymeric coating and/or matrix [1–5]. Because these drug forms have large specific surface areas, their behavior in the organism depends largely on surface phenomena, and useful insight into this behavior may be obtained by studying their components as monolayers on aqueous substrates. Hitherto, such studies have provided information on the behavior, orientation and packing of various microsphere polymers at the air/water interface [6–11], and on the interactions between these polymers and certain drugs [12].

Copolymers of D,L-lactic acid and glycolic acid are widely used in microsphere drug systems [13, 14], the properties of which are largely determined by the nature of the solvent and stabilizing agent used in their preparation [11, 15]. In view of this, in this work, we examined the effects of the spreading solvent on the behavior of monolayers of poly [(D,L-lactic acid)-*co*-(glycolic acid)] (PLA/GA) at the air/water interface. The results afford insight into the interactions occurring within and among PLA/GA molecules, and provide a basis for the consideration of how these interactions might affect an incorporated drug and condition its release from the microsphere and its biological effects.

Experimental techniques

Two 50:50 PLA/GA polymer samples, one of mean molecular weight 34 000 (commercial name Resomer 503), and the other of mean molecular weight 98 000 (Resomer

506), were used as supplied by Boehringer (Ingelheim, Germany).



$$\left[-O-\underset{\underset{CH_3}{|}}{CH}_2-\overset{\overset{O}{\|}}{C}-O-CH_2-\overset{\overset{O}{\|}}{C}- \right]_n$$

Spreading solutions containing approximately 0.8 mg/ml (unless otherwise stated) of Resomer 503 were prepared in chloroform, dichloromethane, acetonitrile and 4:1 (v/v) mixtures of chloroform with methanol or ethanol (procedural hint: dissolve the PLA/GA in chloroform and then add the cosolvent). All solvents were Merck p.a. grade products. Pending use (always within 5 or 6 days of preparation), the spreading solutions were stored at 4 °C in a desiccator under an atmosphere saturated with the corresponding solvent.

Subphases were Theorell–Stenhagen buffers (pH 7 unless otherwise stated) [9], which were prepared in water of resistivity 18 MΩcm obtained from a Millipore Milli-RO, Milli-Q reverse osmosis system (Millipore Corp.).

Compression isotherms were recorded in a Lauda (Germany) FW-1 surface balance; the balance trough was thermostatted to 25° ± 1 °C by water circulating from a Grant LC10 thermostat. The initial area available to the deposited film was 562 cm^2. The number of polymer molecules spread was 4.96×10^{14} for the chloroform solution, and 1.66×10^{15} for the acetonitrile solution. After spreading, the solvent was allowed to evaporate over 10 min, and then the film was compressed at a barrier speed of 97.5 cm^2/min (the isotherms obtained coincided in the range of barrier speeds 25.5–97.5 cm^2/min). Surface pressure (π) was measured to within ± 0.1 mN/m. The isotherms were highly reproducible, coinciding to within ± 0.5 $Å^2$/monomer at all surface pressures, in three repetitions of each experiment.

The viscosities of the PLA/GA (Resomer 503 and Resomer 506) solutions were determined in a Cannon–Fenske Ostwald viscometer, which was immersed in a water bath thermostatted at 25° ± 0.1 °C. The standard technique was followed (ASTM D-446-74). The value of k, the characteristic constant of the viscometer, was estimated by linear interpolation between the values given by the manufacturer at two temperatures. From the relative viscosities of series of solutions of concentration between 1 and 6 mg/ml, the specific viscosities n_{sp} were calculated [16] and then the intrinsic viscosities $[\eta]$ were estimated by fitting a suitable equation to plot n_{sp}/c against c [16, 17]. Of the various models available for estimation of $[\eta]$, our data were fitted best by Martin's equation [18]

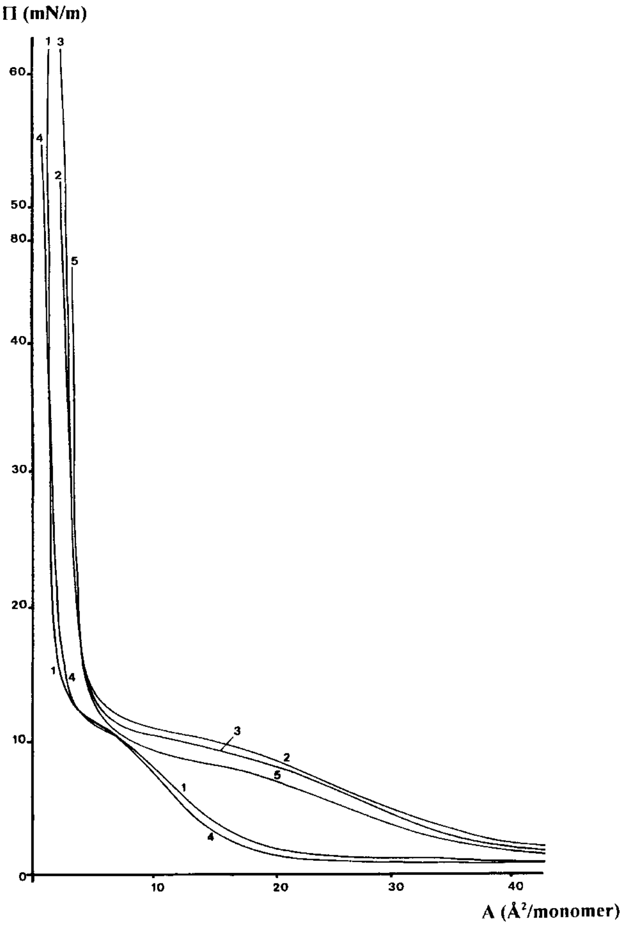
$$\log n_{sp}/c = \log[\eta] + k[\eta]c \, .$$

Results

Influence of the spreading solvent

For each of the five spreading solvents, Fig. 1 shows the surface pressure – area (π–A) curves obtained by continuous compression of PLA/GA films at the air/water interface. The two poorest spreading solvents were acetonitrile and 4:1 chloroform/ethanol (Curves 1 and 4, respectively), for both of which the limiting areas of the expanded and condensed states of the film (around 18 and 2 $Å^2$/monomer, respectively) were much smaller than for similar polymers [19].

Fig. 1 Comparison of π–A isotherms for PLA/GA films compressed on a subphase of pH 7 at 25 °C after spreading from: 1) acetonitrile; 2) chloroform; 3) dichloromethane; 4) 4:1(v/v) chloroform/ethanol; 5) 4:1 (v/v) chloroform/methanol



As for the polyvinylacetate the best spreading solvent was chloroform [20]) (Curve 2), which like dichloromethane (curve 3) allowed expanded phase films with limiting areas of around 42 Å^2/monomer, in keeping with other values obtained for polymers [19]. The isotherm obtained with 4:1 chloroform/methanol as spreading solvent (Curve 5) was similar to Curves 2 and 3, but with rather smaller specific areas for surface pressures lower than 17 mN/m.

Influence of the concentration of the spreading solution

Figure 2a shows the π–A isotherms for PLA/GA films spread from chloroform at three different concentrations (0.31, 0.155 and 0.031 mg/ml). Dilution of the spreading solution did not significantly alter the shape of the isotherm. Figure 2b shows the results of similar experiments in which PLA/GA was spread from acetonitrile (classified above as a poor spreading solvent), this time at four concentrations (4.46, 2.23, 0.86 and 0.446 mg/ml). In this case, the specific areas of the polymer increased significantly as its concentration in the spreading solution decreased.

Influence of subphase temperature

Figures 3a and b show the isotherms obtained for continuous compression of PLA/GA films spread from chloroform and acetonitrile, respectively, onto subphases with temperatures between 10 °C and 35 °C. For chloroform, the specific areas of the expanded film were barely affected by the subphase temperature, whereas for acetonitrile the

Fig. 2 (**A**) Influence of the concentration of the spreading solution on the π–A isotherms for PLA/GA films compressed on a subphase of pH 7 at 25 °C after spreading from: chloroform (xxxx: $c = 0.31$ mg/ml, oooo: $c = 0.155$ mg/ml, : $-c = 0.031$ mg/ml). (**B**) Inset: acetonitrile (A: $c = 4.46$ mg/ml, A′: $c = 2.23$ mg/ml, A″: $c = 0.86$ mg/ml, A‴: $c = 0.446$ mg/ml)

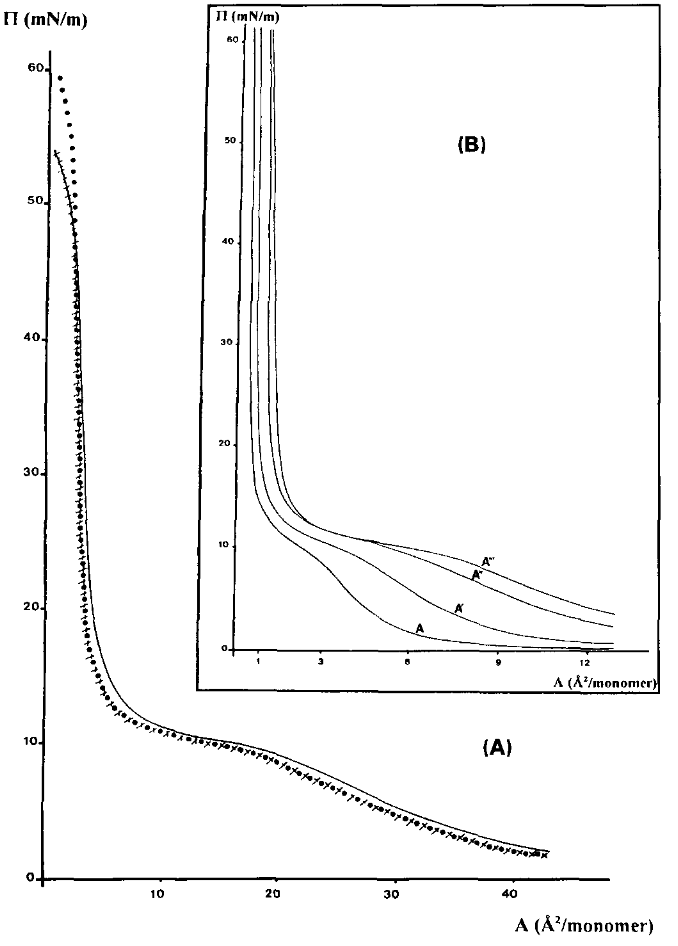
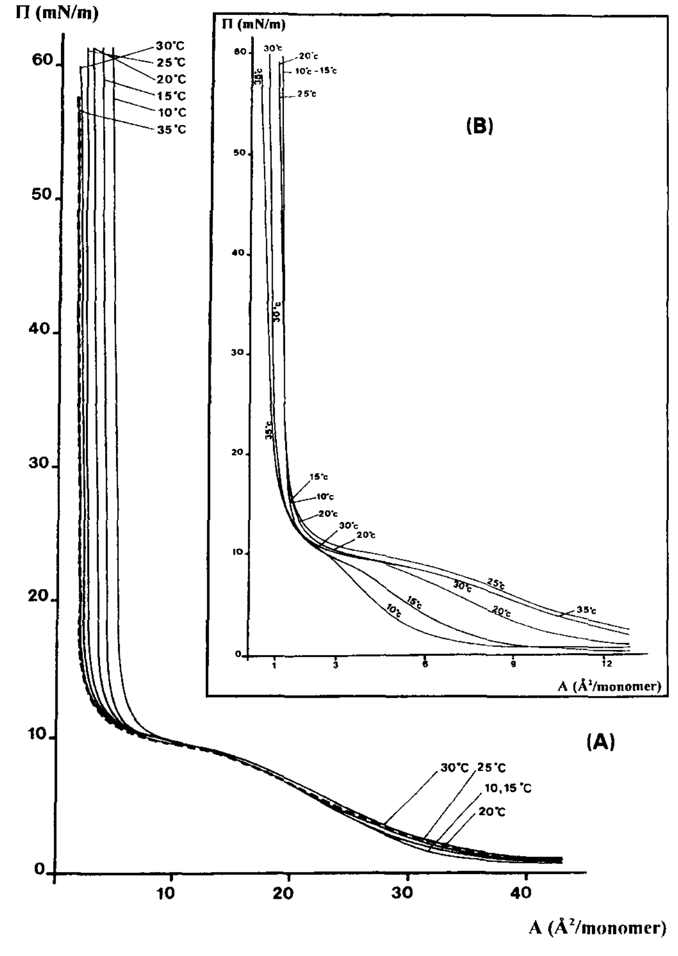


Fig. 3 Influence of the subphase temperature on the π–A isotherms for PLA/GA films compressed on a subphase of pH 7 after spreading from: **A**) chloroform; **B**) acetonitrile



specific area increased considerably with temperature in the range studied. For both solvents, the onset of the transition between expanded and condensed states barely shifted with temperature, although the length of the corresponding plateau increased, as a result the specific areas of the condensed film decreased with increasing temperature.

Influence of subphase pH

Figures 4a and b show the isotherms for PLA/GA films spread from chloroform and acetonitrile, respectively, onto subphases of between pH 1.5 and 12. Again, the results for the two spreading solvents differed: for chloroform, the shape of the isotherm barely altered in the pH range 2–9, whereas for acetonitrile the specific areas of the expanded film increased considerably in the same pH range. For both solvents, the isotherms obtained at pH 11 and pH 12 (the latter especially) were suggestive of dissolution of the PLA/GA in the subphase.

Influence of the ionic strength of the subphase

The effects of increasing the ionic strength of the subphase (by addition of NaCl) are shown in Figs. 5a and b for films spread from chloroform and acetonitrile, respectively. For both the solvents, the specific areas of the expanded and condensed PLA/GA films increased with ionic strength in the range studied, but this effect was much more marked for acetonitrile.

Fig. 4 Influence of the pH of the subphase (numbers on curves) on the π–A isotherms for PLA/GA films compressed at 20 °C after spreading from: a) chloroform; b) acetonitrile

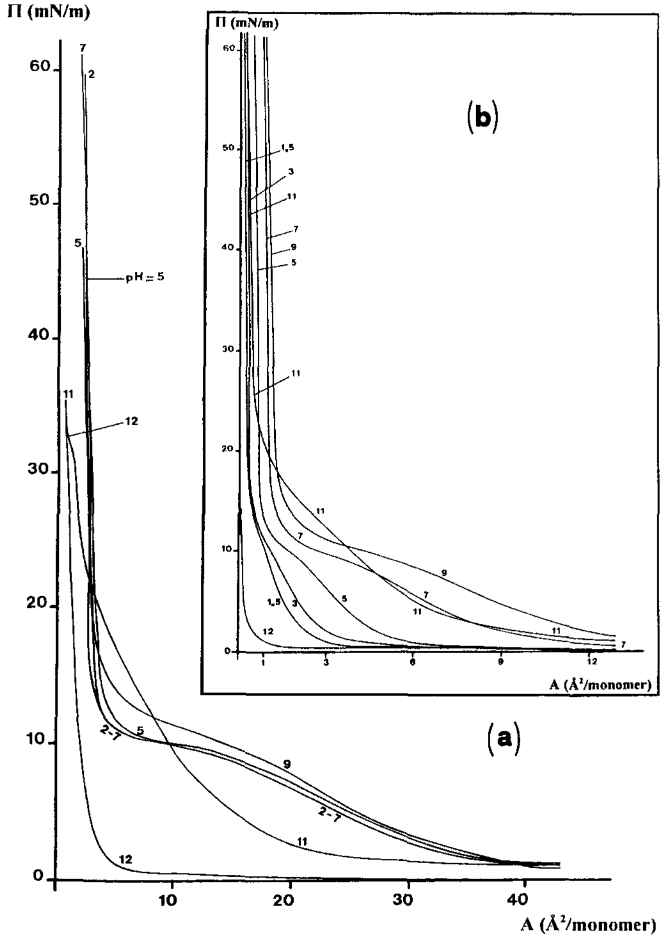
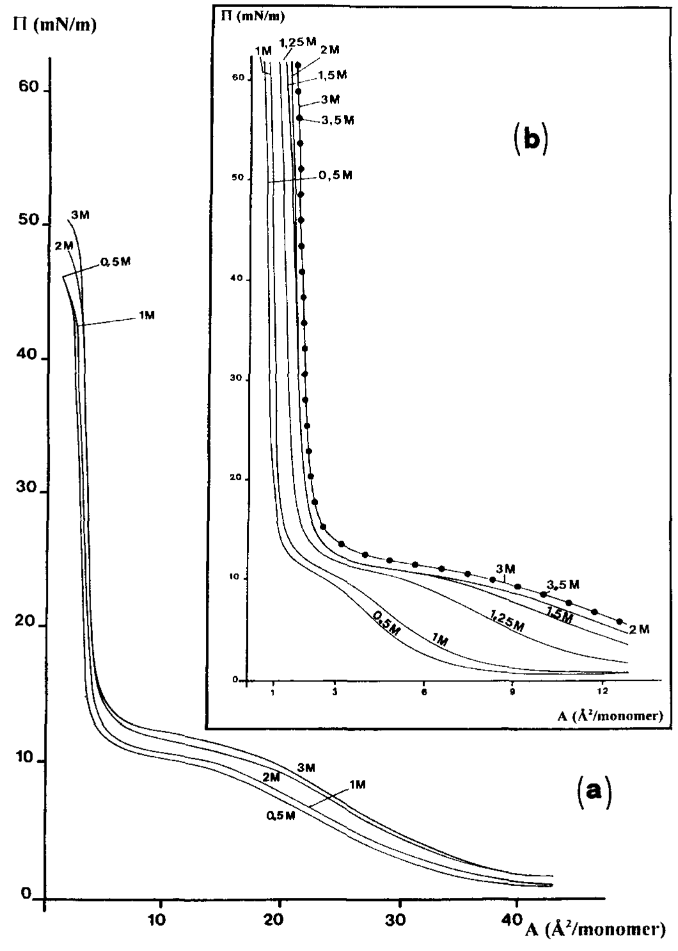


Fig. 5 Influence of the ionic strength of the subphase (molarities on curves) on the π–A isotherms for PLA/GA films compressed on a subphase of pH 7 at 25 °C after spreading from: a) chloroform; b) acetonitrile



Discussion

Influence of the spreading solvent

For the best two spreading solvents, chloroform and dichloromethane, the specific areas of the expanded PLA/GA films did not depend on the concentration of PLA/GA in the spreading solution (Figs. 1 and 2a). This, and the large specific areas occupied, suggests that in these solvents PLA/GA has an uncoiled structure in the spreading solution (regardless of concentration). By contrast, the specific areas of the PLA/GA film spread from acetonitrile were small and increased as the concentration of PLA/GA concentration in the spreading solution decreased (Figs. 1 and 2b), suggesting that in this solvent the polymer chains had a coiled structure resulting in small specific areas and enhanced probability of dissolution in the subphase; the unfolding of these structures through solvation of the polymer chain would explain why the specific film areas increased with the ratio of solvent to polymer molecules. The evidence for the coiling of the polymer chains in acetonitrile also came from the fact that the specific areas of the PLA/GA film increased with the time elapsing etween preparation of the spreading solution and spreading itself, especially for concentrated solutions (results not shown). No such effect was observed for the chloroform solutions.

Indirect evidence of structural differences between PLA/GA in chloroform and PLA/GA in acetonitrile comes from the viscosity measurements, since the viscosity of a polymer solution increases with solvation of the polymer [21]. According to the Mark–Houwink power law,

$$[\eta] = KM^{\alpha}$$

(where M is the molecular weight of the polymer and K is constant for a given polymer), the intrinsic viscosity of a solution of a polymer in an organic solvent depends on the intermolecular expansion factor α, which reflects polymer–solvent interaction and hence varies with the solvent. In a solvent that interacts strongly with the polymer, α is greater than in a poor solvent in which the polymer interacts mainly with itself and so becomes coiled [22, 23]. The experimental values of $[\eta]$ obtained for solutions of the two different types of PLA/GA in chloroform and in acetonitrile (Table 1) are of the same order of magnitude as for other polymers [7]. The fact that α was greater in chloroform confirms that PLA/GA interacts more strongly with this solvent than with acetonitrile, and suggests that in the latter solvent the polymer must form dense coils that prevent its full extension when spread at the surface of a subphase [23–25]. Although α was <1 in both solvents (indicating that the polymer chains were not fully unfolded even in chloroform), it should be borne in mind that these α values are for bulk-phase solutions of the PLA/GA, and thus do not necessarily faithfully reflect the behavior of the polymer at the air/water interface, where surface forces will play an important role in unfolding the polymer chains.

Table 1 Intrinsic viscosities $[\eta]$ and parameters of the Mark–Houwink power law, estimated for the two PLA/GA samples

Molar weight	Acetonitrile $[\eta]$[ml/g]	Chloroform $[\eta]$[ml/g]	Acetonitrile α	Chloroform α
34.000	30.05	41.02		
98.000	36.91	71.39	0.19	0.52

Influence of temperature

The specific areas of PLA/GA films spread from acetonitrile increased with temperature at low surface pressures but decreased with increasing temperature at high surface pressures (Fig. 3b). The increase of specific areas with temperature at low surface pressures may be attributable to a combination of increased molecular agitation [26] and thermally induced unfolding of the polymer chains (presumed coiled in acetonitrile), effects which in the expanded film must have predominated over the film-shrinking effect of increased polymer solubility. At high surface pressures, lack of available space may be assumed to have limited unfolding and the film-expanding effect of increased agitation, and the predominant effect will therefore have been the thermally facilitated submersion of polymer coils in the substrate, which explains the observed (anomalous [27]) decrease in surface area as temperature increased.

Qualitatively similar behavior was exhibited by PLA/GA films spread from chloroform (Fig. 3a), but in this case the area of the expanded film increased with temperature very much less than in the case of acetonitrile. This difference is probably due to the absence of any polymer unfolding effect, the polymer chains being highly unfolded at all temperatures when deposited from chloroform, and to PLA/GA molecules having very short side chains, which means that expansion due to increased thermal agitation is less than that reported for amphiphiles with long apolar side chains. For films spread from chloroform, the decrease in surface area with increasing temperature at high surface pressures may be attributed to increased submersion of individual polar groups (following their reorientation during the transition from the expanded to the condensed state [9]), rather than to increased submersion of coiled segments as in the case of films spread from acetonitrile. At pH 9 the anomalous temperature dependence of the area of the condensed film is even more marked than at pH 7 (results not shown), which is attributable to increased solvation of carboxyl groups due to their increased ionization. Further light can be thrown on this question by the use of other surface techniques such as ellipsometry or surface potencial measurements.

Influence of pH

The small areas of the PLA/GA films spread from acetonitrile onto subphases of pH 1.5 or 3 (Fig. 4b) are attributed to the coiling of the PLA/GA being more pronounced at acid pH, at which the polymer is unionized. Above pH 4.3 (the pK_a of the terminal carboxyl groups of PLA/GA), ionization of the polymer causes the film to expand through intra- and intermolecular repulsions. Above pH 11, the compressibility of the film increases with increasing pH and the transition between expanded and condensed states disappears, which is attributed to the increasing solubility [19] of the PLA/GA; at pH 12, the solubility of the PLA/GA film is so high that the monolayer essentially does not form. For films spread from chloroform, pH hardly affects compression behavior over the pH range 2–9 because the polymer chains are unfolded in this solvent regardless of their ionization state, but above pH 9 the solvation of polar groups due to their electric charge results in the solubilization of the monolayer (Fig. 4a).

Influence of ionic strength

The specific areas of films spread on substrates of pH 7 increased with the ionic strength of the substrate, especially in the case of films spread from acetonitrile (Fig. 5). This may be attributed to a salting-out effect: competition for solvent molecules between the inert electrolyte and the ionized PLA/GA groups will have meant that increasing the concentration of the former will have reduced the solvation of the polymer and increased the number of monomers at the interface and the surface area of the film. That the effect was less marked for films spread from chloroform than for films spread from acetonitrile will have been simply due to the fact that the former were already highly expanded even on substrates of low ionic strength, so that little further expansion was possible upon increasing ionic strength. In the condensed film region, the effect of salting-out was that the collapse pressure of the films increased with ionic strength, especially in the case of films spread from chloroform.

Increasing the ionic strength may also be expected to have reduced the thickness of the electric double layer around polar PLA/GA groups, and hence the repulsive forces among them. By itself, this effect would lead to smaller surface areas, but it appears to have been too small to outweigh the salting-out effect except in the case of condensed films spread from chloroform (Fig. 5a).

General conclusions

1) The properties of PLA/GA monolayers depend on the nature of the spreading solvent. Of the solvents used in this work, PLA/GA was spread worst by acetonitrile and 4:1 chloroform/ethanol, and best by chloroform and dichloromethane.
2) The above differences are attributed to the structure of PLA/GA at the air–water interface depending on its structure in the bulk spreading solution (closely coiled in acetonitrile, unfolded in chloroform).
3) Indirect evidence of the difference between the structures of PLA/GA in chloroform and acetonitrile is afforded by the values of the viscosity of solutions of PLA/GA in the two solvents. The greater values of $[\eta]$ for chloroform solutions indicate significant polymer–solvent interaction.
4) The properties of PLA/GA monolayers vary significantly with temperature, pH and the ionic strength of the substrate on which they are spread. This behavior can be explained in terms of the influence of these variables on polymer–substrate interactions.

Further work must be carried out to gain a better understanding of the interactions of PLA/GA with solvents and with other molecules involved in the preparation of PLA/GA-based microsphere or nanoparticle drug forms, and of the implications of these interactions for the behavior of such drug forms.

References

1. Beck LR, Flowers CE Jr, Pope VZ, Wilborn WH, Tice T (1983) Am J Obset Ginecol 147:815
2. Peters F, Del Pozo E, Cont A, Breakwaldt M (1986) Obstet Gynecol 67:82
3. Tice TR, Rowe CE, Stterstrom JA (1984) Proc Int Symp Cont Rel Bioact Mater 6:11
4. Baker RW, Krisko EA, Kochinke F, Grassi M, Armitage G, Robertson PA (1988) Proc Int Symp Cont Rel Bioact Mater 15:238

5. Tice TR, Lewis DH, Cowsar DR, Beck LR (1985) US Patent 4,542,025
6. George L, Gaines Jr (1991) Langmuir 7:834–839
7. Ringard-Lefebvre C, Baszkin A (1994) Langmuir 10:2376–2381
8. Gericke A, Simon-Kutscher J, Hühnerfuss H (1993) Langmuir 7:2119–2127
9. Miñones J, Iribarnegaray E, Varela C, Vila N, Conde O, Cid L, Casas M (1992) Langmuir 8:2781–2784
10. Baglioni P, Gallori E, Gabrielli G, Ferroni CJ (1982) Colloid Interface Sci 88:221
11. Boury F, Olivier JE, Proust JE, Benoit JP (1993) J Colloid Interface Sci 160:1
12. Miñones J, Yebra-Pimentel, Conde O, Iribarnegaray E, Casas M, Vila Jato Jl, Seijo B (1994) Langmuir 10:1888–1893
13. Christel PS, Vert M, Chabot F, Abols Y, Leray JL (1984) In: Ducheyne P, Van der Perre G, Aubert AE (eds) Biomaterials and Biomechanics 1993. Elsevier, Amsterdam, p 1
14. Benita S, Benoit JP, Puisieux F, Thies C (1984) J Pharm Sci 73:1721
15. Spenlehauer G, Vert M, Benoit JP, Boddaert A (1989) Biomaterials 10:557
16. Van Krevelen DW (1990) Properties of Polymers. Elsevier, Amsterdam
17. Opota O, Maillols H, Acquier R, Delonca H, Fortune R (1988) Pharm Acta Helv 63:26
18. Martin AF (1942) ACS Meeting, Memphis
19 Crisp DJ (1958) In: Surface Phenomena in Chemistry and Biology. Pergamon Press, London, pp 23–54
20. Gabrielli G, Baglioni P, Ferroni E (1979) Colloid Polymer Sci 257:121–127
21. Crisp DJ (1946) J Colloid Sci 1:49:16112
22. Flory PJ (1971) In: Principles of Polymer Chemistry. Cornell University Press, Ithaca and London, p 266
23. Israelaachvili JN (1991) In: Intermolecular and Surface Forces. Academic Press, London/San Diego/New York/Boston/Sydney/Tokyo/Toronto, p 228
24. Gaines JL Jr (1991) Langmuir 7:834
25. Morawetz HH (1965) In: Macromolecules in Solution. Interscience Publishers, New York/London/Sydney, p 161
26. Adam NK (1941) In: The Physics and Chemistry of Surfaces. Oxford University Press, London, p 59
27. Caminati G, Senatra D, Gabrielli G (1991) Langmuir 7:604–607