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Monolayer studies on poly(isobutylcyanoacrylate)-ampicillin association

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Surface pressure-area isotherms (π-A) of poly(isobutylcyanoacrylate) monolayers with or without glucose and dextran as polymerization adjuvants used in the preparation of nanoparticles have been derived from measurements made at the air-water interface with the subphase pH at 2·7, 5·5 or 8·8. The isotherms were characteristic of the expanded type of polymer monolayer curves, yielding unusually low limiting area values compared with those of other known poly(acrylate) derivatives. This behaviour may be explained by the folding of polar moieties of the polymer groups in the water subphase. Ampicillin incorporated during preparation of nanoparticles had a negligible effect on the general behaviour of adjuvant-free monolayers, but a significant one on the adjuvant-loaded polymer monolayer system which showed an increase in surface area throughout the compression cycle, as compared with the surface area of the adjuvant-free polymer system although the collapse pressure was practically the same at 67 mN m^{-1}.

Polyalkylcyanoacrylates as submicroscopic particles are able to carry biologically active substances (Couvreur et al 1979a, b), since such nanoparticles having a diameter < 0·3 μm can sorb a wide variety of drugs. They are biocompatible and biodegradable, the degradation being effected by ester hydrolysis catalysed by enzymes with the release of drug (Lenaerts et al 1984). The fate of poly(alkylcyanoacrylate) nanoparticles and their distribution in the body, depend to a large extent on the surface charge, hydrophilicity, molecular weight, length of the alkyl chain and particle size (Vansnick et al 1985; Douglas et al 1984; Grislain et al 1985) of the material. In this respect, there is insufficient information concerning the physicochemical characteristics of the polymer and, in particular, about the surface interactions between the polymer and the stabilizing agents used in the polymerization process and between the polymer and associated drug.

Studies on polymer monolayers spread at the air-water interface, using the surface balance technique, can provide an insight into the effect of polymer structure on monolayer interfacial behaviour and on the interactions of polymer chains with other constituents of either the aqueous phase or with the polymer film forming additives (Glazer 1954; Nakamae et al 1982).

We describe experiments with poly(isobutylcyanoacrylate) monolayers obtained by dissolution of either unloaded or ampicillin-loaded nanoparticles in organic solvents. Ampicillin was chosen because of the extent of its attachment to nanoparticles and of its efficiency after linking to nanoparticles (Henry-Michelland et al 1987).

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MATERIALS AND METHODS

Preparation of nanoparticles

Poly(isobutylcyanoacrylate) nanoparticles were prepared using the emulsion polymerization procedure described by Vansnick et al (1985). Four different aqueous polymerization media were used: (I) HCl 10^{-3} M leading to the unloaded polymer (P); (II) HCl 10^{-3} M containing 1% dextran 70 and 5% glucose w/w used as polymerization adjuvants, giving adjuvant-loaded polymer (PAd); (III) HCl 10^{-3} M containing ampicillin (in the weight ratio polymer/drug equal to 100/7·5) leading to ampicillin-loaded polymer (PAm); (IV) HCl 10^{-3} M with polymerization adjuvants as in (II) and ampicillin as in (III), leading to adjuvant and ampicillin-loaded polymer (PAdAm).

Isobutylcyanoacrylate monomer was purchased from Ethnor (Paris, France) and used for polymerization as received. Ampicillin was from Negma (Buc, France), glucose and dextran were Prolabo (Paris, France) products.

Surface pressure-area isotherms

The autorecording Langmuir film balance (MCN Lauda, Germany) was used to measure the surface pressure (π)-area (A) isotherms. The polymer was dissolved in a mixure of dimethylsulphoxide–chloroform (9 : 1 v/v). The polymer solutions were spread

on the aqueous substrate on the maximum available area (780 cm^2) by means of a syringe (20 μL) and left for 20 min before being compressed at a constant rate of 0·38 $cm^2\ s^{-1}$ at room temperature (20 ± 2 °C). Control experiments were performed to test the reproducibility of the system. Varying the compression rate from 0·38 to 1·0 $cm^2\ s^{-1}$ and spreading the monolayer from 15 or 30 μL, caused no observed effects.

The pH dependency of the system was assessed by measurement of the surface pressure-area curves when it was spread on aqueous substrates containing HCl or NaOH. The 1 M HCl and NaOH solutions used for pH adjustments of the aqueous subphases were purified by foaming with a stream of N_2 to relieve them of fatty impurities (Hendrikx & Mari 1980). The surface pressure measurements were replicated and the results were reproducible within experimental error not exceeding ± 2%. π-A isotherms were recorded for the four systems described.

All reagents used were Merck (Darmstadt, FRG) analytical grade reagents. Water was triple distilled from a permanganate solution using Pyrex apparatus.

RESULTS

Pressure-area (π-A) curves of poly(isobutylcyanoacrylate) monolayers at various subphase pH values are shown in Fig. 1. Area is expressed in terms of area per monomer unit. The collapse pressure was virtually independent of subphase pH over the range 2·5 to 8·8 and occurs at about 67 mN m^{-1}. On compression of the system, first there appeared kinks characteristic of the beginning of transition from the expanded to the condensed state, and then, after plateaux, steep linear regions of pressure increase, corresponding to a precollapse state.

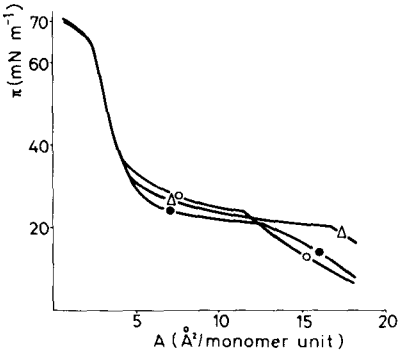


FIG. 1. Surface pressure (π)-area (A) isotherms of poly(isobutylcyanoacrylate). Effect of subphase pH. △ pH = 2·5; ● pH = 5·5; ○ pH = 8·8.

The shapes of the π-A isotherms of PAd (Fig. 2) and PAm (Fig. 3) monolayers were similar to those representative of the unloaded polymer. However, there was noticeable difference in their compression parameters (Table 1).

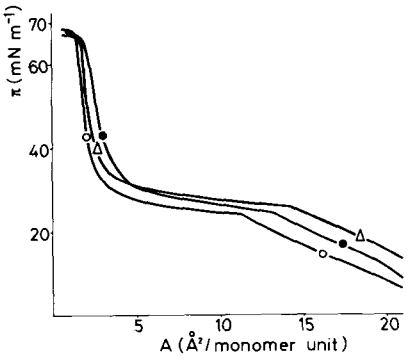


FIG. 2. Surface pressure (π)-area (A) isotherms of adjuvant-loaded poly(isobutylcyanoacrylate)-PAd. Symbols as in Fig. 1.

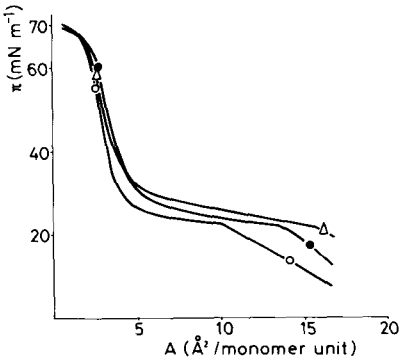


FIG. 3. Surface pressure (π)-area (A) isotherms of ampicillin-loaded poly(isobutylcyanoacrylate)-PAm. Symbols as in Fig. 1.

The π-A isotherms of the PAdAm monolayers (Fig. 4) had a second kink in the precollapse region; other compression parameters are given in Table 1.

The collapse pressure of all the systems, at the three pH values, was essentially the same (67 mN m^{-1}).

DISCUSSION

All pressure-area isotherms are representative of expanded (gaseous) polymer monolayer curves, as described by Glazer (1954). Such isotherms are

Table 1. Film characteristics of the different poly(isobutylcyanoacrylates) (areas in Å²/monomer unit; pressures in mN m⁻¹).

		pH 2·5		pH 5·5		pH 8·8	
		Area	Pressure	Area	Pressure	Area	Pressure
Polymer	1st kink	16·8	21	12·7	21	11·6	23
	collapse	2·3	67	2·3	67	2·3	67
Polymer + adjuvants	1st kink	14·2	26	13·2	25	11·2	24
	collapse	1·95	67	1·95	67	1·5	67
Polymer + ampicillin	1st kink	15·6	22	13·3	22	10	23
	collapse	2·2	67	2·4	67	2·0	67
Polymer + adjuvants + ampicillin	1st kink	17·1	24·5	15·7	25	14·5	25·5
	2nd kink	6·3	53	6·0	53	5·3	56
	collapse	1·85	67	1·85	67	1·85	68·5

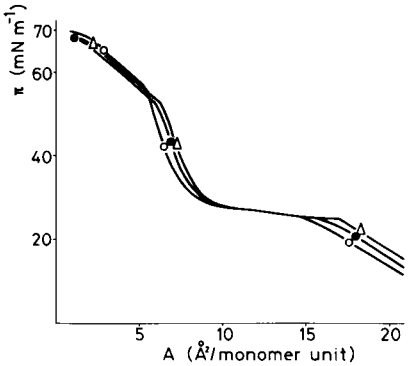
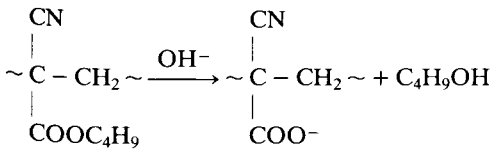


FIG. 4. Surface pressure (π)-area (A) isotherms of adjuvant- and ampicillin-loaded poly(isobutylcyanoacrylate)-PAdAm. Symbols as in Fig. 1.

characteristic of polymer, exhibiting low intermolecular monolayer cohesion, associated with high adhesion to the aqueous subphase.

For the highly expanded state of the monolayer at the same surface pressure, a reduction in the surface area with the pH increase was observed for all the systems. This may most probably be attributed to the reorganization of polymer/water interface induced by the change in pH.

Considering that poly(isobutylcyanoacrylate) degrades faster at basic pH values, according to the reaction (Lenaerts et al 1984)



the increase in subphase pH could induce ionization of carboxyl groups in the monolayer and thus increase the net electrical charge of the system. The repulsion between the charged groups would, in turn, cause the polymer segments to occupy a larger surface area than that corresponding to polymer segments in an uncharged film at the same surface pressure. However, ionized polymer groups should solvate and consequently tend to penetrate into the aqueous phase, thereby reducing the number of polymer segments at the water-monolayer interface. The degree of penetration at each surface pressure would, thus, depend on the balance of these two opposing effects. When the attraction of charged groups towards water overcomes the coulombic repulsive forces between the ionized carboxylic polymer functions, the monolayer would assume an accordion conformation in which ionized groups enter into the bulk water phase. At low subphase pH, the polymer is less ionized, e.g. more hydrophobic, so a higher degree of spreading is expected and was found with the systems studied. The appearance of the kink at pH 2·7 (Figs 1, 2, Table 1) was seen first at surface areas greater than those at which the kink at pH 5·5 occurred and these in turn appeared before the kink at pH 8·8.

On compression, the balance of repulsion-dissolution effects on the film may be changed. Thus, for the unloaded polymer (Fig. 1) in the highly expanded state, the surface area per monomer unit was lower at pH 8·8 than at the other pH values, but the situation was reversed in the plateau region.

The limiting area of poly(isobutylcyanoacrylate) found by extrapolation of the steep linear portion of the isotherm in Fig. 1 to the abcissa, is 6·4 Å²/monomer unit. This value is lower than that obtained by Florence et al (1976), who reported 11 Å² for the butylcyanoacrylate monomer polymerized in-situ at the air/water interface. Although the overall shape of the surface pressure area isotherm obtained by Florence et al (1976) is similar to the isotherms in Fig. 1, there are two differences: (i) the

surface pressures corresponding to surface areas per monomer unit are much higher in our experiments, and (ii) there was an absence of kinks on the π-A isotherm of the in-situ polymerized butylcyanoacrylate. These differences may be explained by the viscoelastic properties of the in-situ polymerized monomer differing from those of the polymer we have used and may result from different folding–unfolding transitions on compression of the monolayer.

The small limiting area of the isobutylcyanoacrylate in the P monolayer system may be explained by the oligomeric nature of the polymer. Indeed, as reported by Vansnick et al (1985), the average chain length of the polymer is five monomeric units.

In compression, oligomeric units penetrate into the water phase and give rise to the high collapse pressures (about 67 mN m^{-1}). The high collapse pressure of poly(isobutylcyanoacrylate) clearly indicates that its hydrophobic groups are oriented to the air phase, while the polar groups are in the aqueous phase. The collapse pressure is close to that obtained with dipalmitoyl phosphatidylcholine monolayers, which may be considered as a model of a close packed aliphatic chain arrangement at the air-water interface (Tabak & Notter 1977).

From considerations of the points made, the most likely arrangements of poly(isobutylcyanoacrylate) chains at the monolayer/water interface would seem to be those represented in Fig. 5.

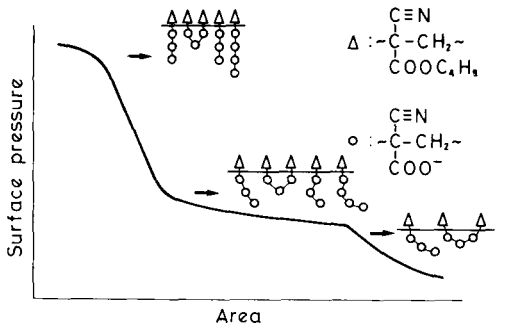


FIG. 5. Arrangements of poly(isobutylcyanoacrylate) chains at the monolayer-water interface.

The use of adjuvants in the polymerization medium caused clearly distinguishable effects on the behaviour of the PAd monolayers, compared with that of the unloaded polymer. For the PAd system, in the expanded region of the monolayer, at a given pressure, the area occupied by a monomer unit was higher than for the P monolayer system, at the three pH values. However, on compression, in the steep region of the π-A curves, the PAd system occupied smaller areas compared with the unloaded polymer. The resulting low limiting area values (Table 2) probably indicate that the adjuvants enhance the penetration of polar moieties into the water bulk.

Analysis of the PAm isotherms (Fig. 3, Tables 1, 2) revealed the existence of slight differences in their general character compared with that of the unloaded polymer. If any association of ampicillin with the poly(isobutylcyanoacrylate) existed, it would be very weak. Only at pH 5·5 did the kink surface area increase compared with the ampicillin-free polymer system. At the two other pH values, ampicillin caused a decrease in the kink surface areas (Table 1). This difference is most probably due to ampicillin's existing at pH 5·5 as a zwitterion and its two pK_a values being 2·66 and 7·25 (Hou & Poole 1971).

Table 2. The limiting areas of studied system (Å²/monomer unit).

	pH 2·5	pH 5·5	pH 8·8
Polymer	6·3	6·3	6·3
Polymer + adjuvants	3·5	4·8	3·2
Polymer + ampicillin	5·9	6·3	5·0
Polymer + adjuvants + ampicillin	11·0	10·8	10·0

The effect of ampicillin on the adjuvant-loaded polymer system (PAdAm) was much more pronounced (Fig. 4). Not only was there a change in the limiting area, which increased from an average value of about 3·8 Å²/monomer unit to about 10·6 Å²/monomer unit, but also the first kink was shifted towards higher surface areas at all the pH values (Table 1). Such an increase in surface areas would indicate that the combined effect of ampicillin and the adjuvants is stronger than the sum of their separate effects. However, the existence of the second kink in the PAdAm isotherms, followed by a decrease in surface areas, clearly shows that this combined effect is decreased on further compression. The high pressures observed in the precollapse region would then result from the released hyrophilic groups penetrating into the aqueous phase. This is supported by the fact that the order of appearance of the second kink varies with the pH in the same manner as the first kink, thereby showing dependency on the number of hydrolysed ester groups.

Conclusion

Measurements of surface pressure-area isotherms (π-A) of poly(isobutylcyanoacrylate) monolayers have shown that, in the presence of polymerization adjuvants, incorporation of ampicillin during preparation of nanoparticles modifies the shape of the π-A curves by increasing the surface area compared with the unloaded polymer system. As the collapse pressure of both monolayer systems is practically the same, the hypothesis of mechanical entrapment of the drug inside the polymeric network of nanoparticles is favoured, rather than one of covalent linkage with the polymer or with the adjuvants. This model is, moreover, consistent with the fact that antibiotic activity of ampicillin was kept intact after linkage to nanoparticles (Henry-Michelland et al 1987).

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