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Molecular interactions at biointerfaces: a study of lipid adsorption

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The study of the behaviour of biointerfaces is of great interest because it enables us to gain a much better understanding of the interactions between different biological compounds. Superficial processes are strongly dependent on such interactions. In the present work, we have focused our attention on the adsorption of a cationic lipid onto different colloidal polymer systems. Subsequently, the coadsorption of this lipid and an immunoprotein ($F(ab')_2$) was performed trying to achieve stable latex particles. The aim was to obtain a structured interface similar to that of a simple biological membrane. Mainly, we have placed emphasis on the study of interaction forces that govern lipid adsorption when we change the dielectric constant in the medium. In order to obtain homogeneous aqueous lipid solutions some ethanol was added to samples. The adsorption isotherms were carried out at different experimental conditions, changing the ethanol contents and the pH of the environment. Moreover, the electrokinetic behaviour and the colloidal stability of these biocomplexes were studied, and both yielded highly compatible results. The adsorption of lipid onto polymeric sorbents is an irreversible process that takes place rapidly. The preferential interaction between the lipid and polymeric surfaces is electrostatic. Only in those samples with low alcohol concentration, hydrophobic forces take place weakly. Lipid-surface interactions are influenced by the nature of buffer ions. The colloidal stability of the systems decreases as the amount of the adsorbed lipid is higher. Sequential coadsorption experiments showed that the lipid molecules adhere both to the polymer surface and to the previously adsorbed immunoprotein.

1. Introduction

The development of immunodiagnostic tests based on latex particles immunoagglutination processes (LIA) is an important research field that has broad applications in biomedicine [1, 2]. The colloidal system is made up by polymer particles (latex), which act as carriers of antibodies or antigens. The microspheres with adsorbed antibody (or antigen) will begin to aggregate because of the presence of the conjugate protein. However, the most serious problem in these kind of diagnostic tests is that the system can lose its colloidal stability in absence of antigen (or antibody), due to the physicochemical conditions of the reaction medium. One should obtain stable latex-immunoprotein complexes unable to collapse under the environmental conditions; but this is a difficult task since the adsorption of immunogamma globulines G (IgG) onto latex particles leads to unstable systems [3, 4]. The isoelectric point (i.e.p.) of most polyclonal IgG molecules is in the range 6.5 to 8.5; in addition, they present a low charge density. Therefore, when the particles are covered by IgG the agglutination process takes place under physiological conditions (pH 7.4 and ionic strength of $I \approx 0.15$), since there is almost no electrostatic repulsion between them.

Some researchers have pointed out different strategies to preserve the colloidal stability of such immunocomplexes. There are five alternatives that can help to solve this problem and are briefly described below:

– Coadsorption of surfactants and immunoproteins. However, the use of surfactants could present some disadvantages, since such molecules can both unfold polipeptide structures and desorb the previously adsorbed protein [5].

– Adsorption of monoclonal IgG with i.e.p. far from the physiological pH. The stability of such systems improves considerably [6], but the use of monoclonal antibodies would increase the cost of immunotests.

– Covalent coupling of polyclonal IgG onto hydrophilic latexes (instead of onto hydrophobic surfaces) [7]. Nevertheless, one has to link the immunoprotein

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molecules in the interface chemically, since the physical (and spontaneous) adsorption is energetically unfavoured.

– Coadsorption of albumin and IgG. The use of serum albumin molecules to create IgG-BSA-latex complexes has been studied by Peula *et al.* [8], who have demonstrated that these colloidal systems possess high stability keeping high quality immunoresponses.

– Finally, the coadsorption of lipids and proteins onto polymer surfaces has recently been proposed [9, 10] in order to obtain interfacial structures that are similar to biological membranes.

As we have just mentioned, little research has been carried out on the adsorption of lipids. This is why we have made an attempt to adsorb a (cationic) lipid and the $F(ab')_2$ fragment (obtained from polyclonal IgG molecules) onto different polymer latexes in order to study the main interactions that exist among these materials and the macromolecules. Additionally, we have studied the electrokinetic behaviour of the complexes (by means of electrophoretic mobility measurements) and their colloidal stability at neutral pH. This last property will be useful to check if such systems can be applied to the development of immunodiagnostic tests.

2. Materials and methods

2.1. Preparation of latexes

Five different latexes of polystyrene were employed in this work. Styrene monomer was purchased from Merck. It was distilled at reduced pressure at 40 °C. The purified monomer was stored at −5 °C until required. **JL2** was synthetized by copolymerization of styrene (Merck) and sodium styrene sulfonate (NaSS) (Fluka), following the recipes reported by Bastos *et al.* [11]. **JL4** and **JL7** are homopolymers of styrene, that were synthetized using different amounts of initiator: 4,4′ azobis (cyanovaleric acid) (ACPA) (Aldrich); therefore, there are only carboxyl groups on the surface of these colloidal systems [12], although they differ in their surface charge densities. **JL8** is a cationic latex, whose surface charged groups come from the initiator used in its synthesis [13]: azo N,N′ dimethylene isobutyr amidine hydrochloride (AMBDA) (Bayer). **JL10** is a copolymer of styrene and hydroxyethyl methacrylate (HEMA) (Merck) synthetized using a "core-shell" method [14], where the "core" was made up with polystyrene and the "shell" was a mixture of polystyrene and polyHEMA. The initiators and other reagents, such as sodium bicarbonate, potassium carbonate, sodium chloride, and hydrochloric acid, were of analytical grade and were used without further purification. Double-distilled and deionized (DDI) water was used throughout.

Finally, the latex samples were cleaned by repeated cycles of centrifugation–decantation–redispersion, and afterwards by using serum replacement with DDI water. After these processes, the specific electrical conductivity was found to be constant below 10 $\mu S\,cm^{-1}$. A complete characterization of each latex was carried out. The main results, such as size, polydispersity index (P.D.I.), surface polar groups, critical coagulation concentration (C.C.C.) and hydrophobicity degree, are shown in Table I. The quantification of the hydrophobicity character are based on the amount of a non-ionic surfactant (Triton X100) (Merck) that can be adsorbed onto the different polymer surfaces [15, 16].

2.2. Lipid

In this work we have used a cationic commercial lipid, namely distearoyl dimethyl ammonium bromide (DSDMA), which was purchased from Fluka. Orange II–Chloroform method [9] was employed in order to calculate the amount of lipid that remains adsorbed onto the polymer particles. The Orange II (SIGMA) makes up a complex ion with the DSDMA, which is soluble in an apolar phase.

2.3. Immunoprotein

$F(ab')_2$ antibody fragments from rabbit polyclonal IgG were kindly donated by Biokit S.A. (Spain). They were obtained by pepsin digestion of IgG, followed by a gel filtration chromatography (Superose 12 HR 10/30 Pharmacia) and a Protein-A chromatography, HiPAc (ChromatoChem), to remove undigested IgG. Purity was checked by SDS-Page electrophoresis. The isoelectric point (i.e.p.) of $F(ab')_2$ molecules was determined by isoelectric focusing, and the i.e.p. values were found in the range 4.6–6.0. The molecular weight is 102 kD.

We used an indirect method to calculate the lipid or protein coverage degree of the latex particles. We added the necessary amount of latex solution to a certain volume of DSDMA or $F(ab')_2$ solution of known concentration, the final polymer area being 0.3 m^2. Total adsorption volume was 10 ml. Temperature was

TABLE I Main features of bare latexes

Latex	Surface polar group(s)	Size (nm)	P.D.I.	σ_0 ($\mu C\,cm^{-2}$)	C.C.C. (mM KBr)	Triton X100 ($\mu mol\,m^{-2}$)
JL2	sulphonate	201 ± 10	1.013	−10.5 ± 0.7	700 ± 40	1.06 ± 0.22
JL4	carboxyl	337 ± 9	1.002	−12.1 ± 0.2	790 ± 40	1.13 ± 0.23
JL7	carboxyl	337 ± 7	1.002	−19.0 ± 0.4	1000 ± 100	1.52 ± 0.12
JL8	amidine	191 ± 5	1.002	+5.3 ± 0.2	160 ± 10	2.32 ± 0.07
JL10	hydroxyl, carboxyl, sulphate	636 ± 15	1.002	−10.3 ± 0.4	—	0.56 ± 0.16

fitted at 25 °C. Incubation time always lasted four hours. This time was more than enough for the lipid adsorption to take place, since previous kinetic experiments showed that the same amount of DSDMA could be adsorbed onto the latex particles if the incubation lasted half an hour or a day (figure not included). Later, the samples were spun, the pellets were redispersed at neutral pH to perform electrokinetic and stability studies, and the supernatants were analysed following the Orange II–Chloroform method for the lipid, and using UV spectrometry for the $F(ab')_2$ ($\Sigma = 1.48\ \text{ml mg}^{-1}\ \text{cm}^{-1}$ at $\lambda = 280$ nm). The values of the adsorbed amount of such macromolecules could be calculated from the difference between the initially added amount and what remains in the bulk. All the experiments were carried out at low ionic strength ($I = 0.002$).

3. Results

Although Arai [9, 10] managed to dissolve DSDMA in water at concentration near 10^{-4} M, we could not solubilize this lipid in pure water, at least at concentrations $\geq 1.5 \times 10^{-6}$ M. We had to use some ethanol to avoid the presence of lipid multilamellae or other organized macrostructures, such as micelles and vesicles. The reason is that one cannot separate these structures from the latex particles by centrifugation, a process that is necessary for calculating the amount of adsorbed DSDMA by means of the indirect method. We decided to carry out the adsorption experiments in different mixtures of ethanol/water: 1%, 50% and 95% (volume/volume). Let us start with the last one.

3.1. Adsorption isotherms of DSDMA in 95% ethanol/water mixtures (v/v)

Fig. 1 shows the result of adsorption of the cationic lipid onto the anionic particles when the medium is rich in ethanol. There was no adsorption of DSDMA onto the cationic latex particles.

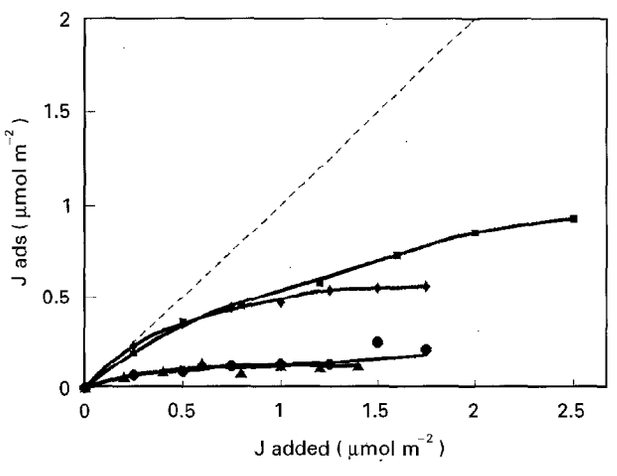

Figure 1 Adsorption isotherms of DSDMA, in 95% ethanol/water solutions (v/v), for JL2 latex (■), JL4 (▲), JL7 (●) and JL10 (◆).

3.2. Adsorption isotherms of DSDMA in 50% ethanol/water mixtures (v/v)

Fig. 2 shows the amount of adsorbed lipid versus the initial amount added to the anionic latexes, when the aqueous solution was at pH 7. Again, there was no adsorption onto the cationic latex. As can be seen, one always obtains well defined "plateaus". In these experiments, when the particles had maximum coverage (J_{pl}), e.g. $2.10 \pm 0.05\ \mu\text{mol m}^{-2}$ (for latex JL2), $1.30 \pm 0.05\ \mu\text{mol m}^{-2}$ (JL10), $0.88 \pm 0.03\ \mu\text{mol m}^{-2}$ (JL4) and $1.40 \pm 0.04\ \mu\text{mol m}^{-2}$ (JL7), colloidal systems were completely unstable. Similar stability results were obtained when the adsorptions were performed at different pHs. Fig. 3 shows the maximum amounts of DSDMA adhered onto the anionic latex versus the pH of the aqueous solution in the final mixture. As can be seen, J_{pl} depends on the pH in the same way the charge of the superficial ionic groups of the latexes does [17].

3.3. Adsorption isotherms of DSDMA in 1% ethanol/water mixtures (v/v)

The amount of adsorbed lipid as a function of the initially added amount is plotted in Fig. 4. No defined

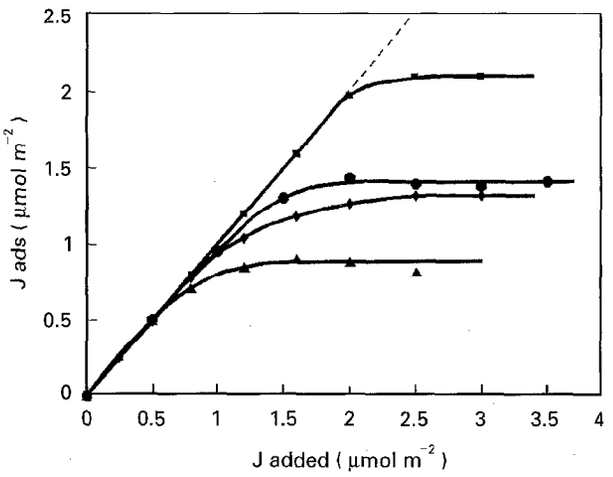

Figure 2 Adsorption isotherms of DSDMA, in 50% ethanol/water solutions (v/v), for JL2 latex (■), JL4 (▲), JL7 (●) and JL10 (◆).

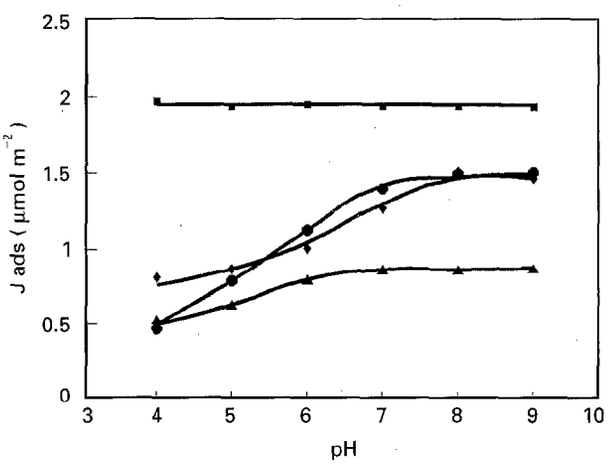

Figure 3 Maximum amounts of DSDMA adsorbed versus pH, in 50% ethanol/ water solutions (v/v), for JL2 latex (■), JL4 (▲), JL7 (●) and JL10 (◆).

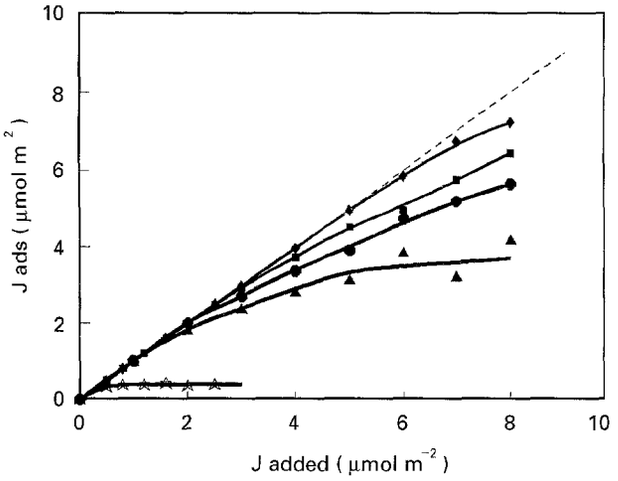

Figure 4 Adsorption isotherms of DSDMA, in 1% ethanol/water solutions (v/v), for JL2 latex (■), JL4 (▲), JL7 (●), JL8 (★) and JL10 (◆).

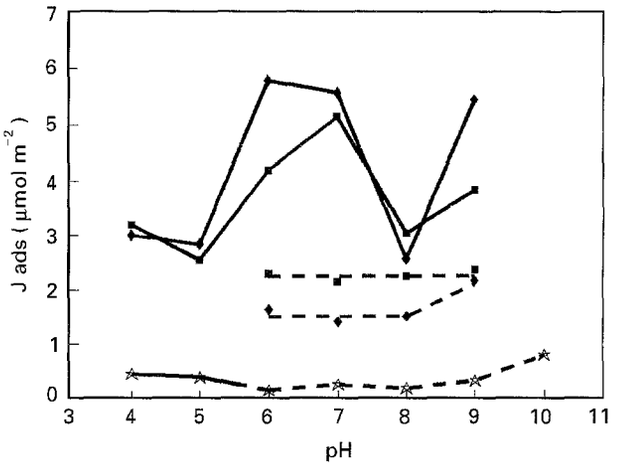

Figure 5 Maximum amounts of DSDMA adsorbed versus pH, in 1% ethanol/water solutions (v/v), for JL2 latex (■), JL8 (★) and JL10 (◆). (——): pH 6–7 (phosphate) and pH 8–9 (borate). (- - -): pH 6–7 (BIS-TRIS) and pH 8–9 (TRIS).

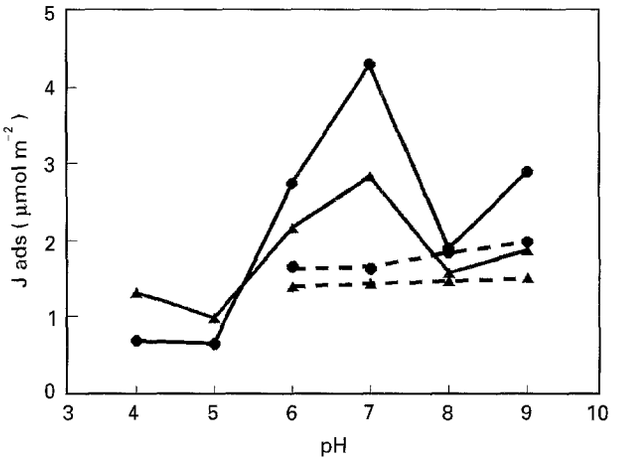

Figure 6 Maximum amounts of DSDMA adsorbed versus pH, in 1% ethanol/water solutions (v/v), for JL4 latex (▲) and JL7 (●). (——): pH 6–7 (phosphate) and pH 8–9 (borate). (- - -): pH 6–7 (BIS-TRIS) and pH 8–9 (TRIS).

"plateaus" were obtained. It was impossible to increase the lipid concentration in the original samples for higher values since this would result in the formation of DSDMA macrostructures. Those samples that had lipid amounts near the J_{pl} obtained in the 50% ethanol/water mixtures were also completely aggregated. Those samples with higher coverage degrees seemed to be stable, at least by sight. In these (rich in water) solutions, the cationic latex adsorbed little amounts of DSDMA ($\approx 0.4\ \mu mol\ m^{-2}$). When performing the experiment of J_{pl} versus the adsorption pH, we found amazing and reproducible results, which are depicted in Figs 5 and 6. The values of the adsorbed lipid amounts depend on the buffer nature, in other words, on the ionic species in the bulk. Initially, solutions were buffered with acids: $AH \rightleftarrows A^- + H^+$; 4 and 5 pHs with acetate; 6 and 7 with phosphate; and 8 and 9 with borate. But if solutions of pH 6 to 9 were buffered with bases: $B + H_2O \rightleftarrows BH^+ + OH^-$ (BIS-TRIS (6 and 7) or with TRIS (8 and 9)), the results changed completely. We checked that none of the buffer ions employed in these experiments affected on the Orange II–Chloroform method.

Some desorption isotherms were carried out both by diluting the samples in the same buffer solution where the initial adsorption was performed, and by changing the medium to pH 8. Results indicate that the adsorbed lipid amount, which still remained on the particle's surface after the desorption process had finished, is higher than 97% in the dilution experiments, and higher than 90% when the pH was changed.

3.4. Electrophoretic mobility and stability

As particles were covered with lipid their mobilities decreased continuously. However, once the i.e.p. of complexes was passed, the electrophoretic mobility kept constant, in spite of the fact that more lipid molecules were adhered onto the polymer surface. The mobility measurements were carried out at pH 7, $I = 0.002$, in absence of ethanol. The results obtained with JL2 and JL7 latexes as well as the stability results of such samples (expressed as critical coagulation concentration (C.C.C.) in KBr solutions) are depicted in Figs 7 and 8. As can be seen, a high degree of compatibility exists between both measurement series. The results obtained with the JL4 and JL10 samples were completely similar to those described above.

3.5. Coadsorption of $F(ab')_2$ and DSDMA

Initially, particles of the JL2, JL4, JL7 and JL8 latexes were covered with $F(ab')_2$ anti-CRP at three different degrees of coverage. In a second step, the cationic lipid was added to these samples at pH 7, low ionic strength and 1% ethanol/water solutions. No protein desorption took place when the samples were redispersed under these conditions. The results obtained were quite different from what could be expected. Arai's works indicate that the higher the protein coverage, the lower the amount of adsorbed DSDMA. However, as can be seen in Figs 9–12, when there is no $F(ab')_2$ on the polystyrene surface the amount of adsorbed lipid is lower than when protein molecules are previously adhered onto the particles. This occurs with every latex, no matter what the nature of the

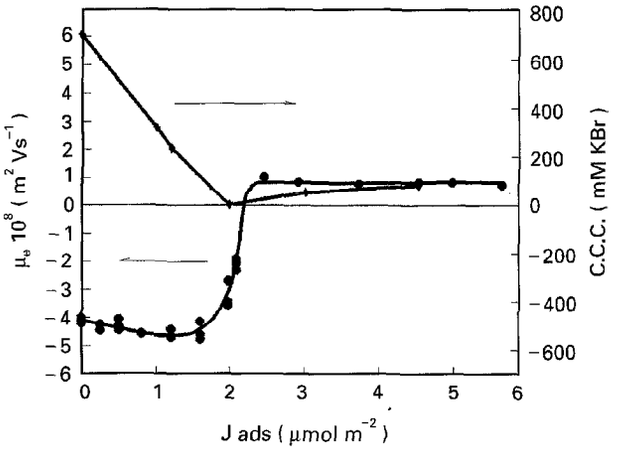

Figure 7 Electrophoretic mobility (●) and stability (◆), in aqueous solution at pH 7, for DSDMA–JL2 complexes versus adsorbed lipid amount.

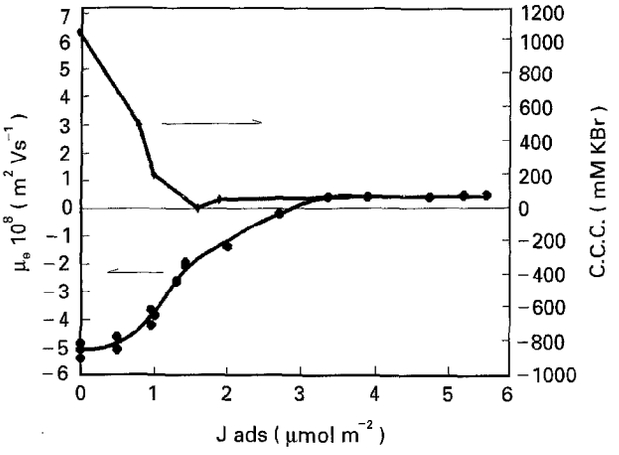

Figure 8 Electrophoretic mobility (●) and stability (◆), in aqueous solution at pH 7, for DSDMA–JL7 complexes versus adsorbed lipid amount.

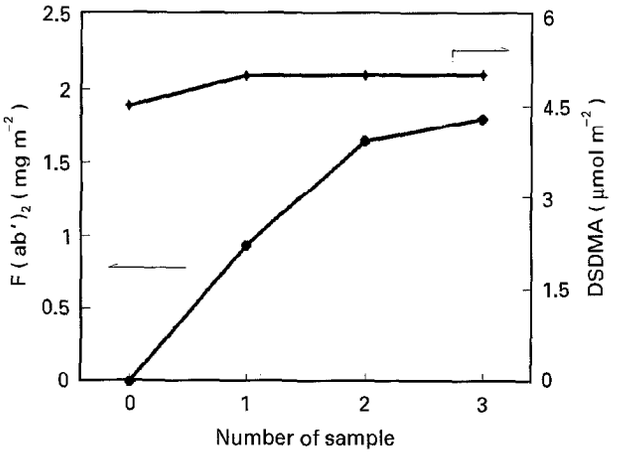

Figure 9 Adsorbed amounts of $F(ab')_2$ (●) and DSDMA (◆) in JL2 particles.

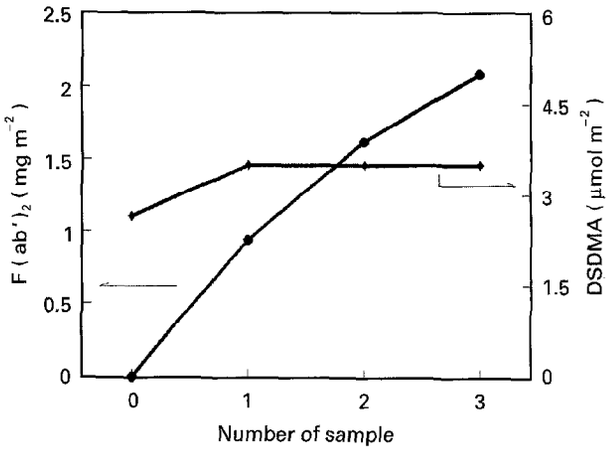

Figure 10 Adsorbed amounts of $F(ab')_2$ (●) and DSDMA (◆) in JL4 particles.

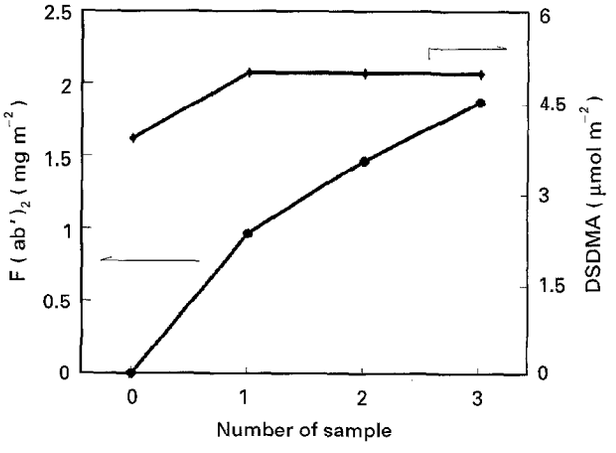

Figure 11 Adsorbed amounts of $F(ab')_2$ (●) and DSDMA (◆) in JL7 particles.

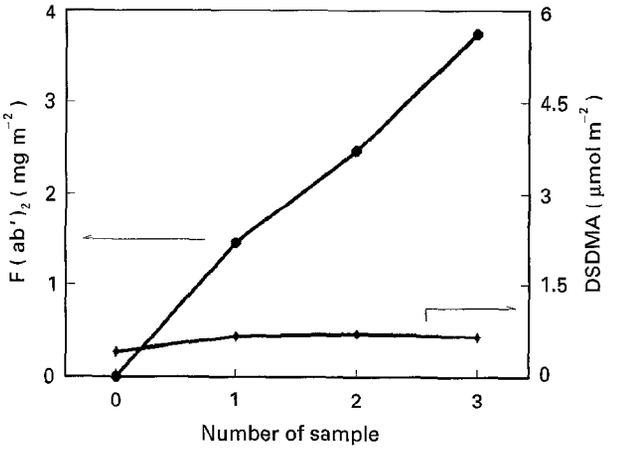

Figure 12 Adsorbed amounts of $F(ab')_2$ (●) and DSDMA (◆) in JL8 particles.

superficial polar groups is. It seems to indicate that DSDMA adsorbs not only onto the bare polymer surface, but also onto the previously adsorbed $F(ab')_2$. This was confirmed by latex immunoaggregation reactions. Using CRP as antigen, the suspension turbidity increased faster in the free DSDMA samples than in the lipidic ones (figures not included).

3.6. Electrophoretic mobility and stability of the latex-$F(ab')_2$-DSDMA complexes

In Figs 13 and 14 we show the electrophoretic mobility measurements together with the stability results of the JL2 and JL8 samples. The electrokinetic and stability behaviour of the other anionic latexes (JL4, JL7 and JL10) was exactly the same as that of the JL2.

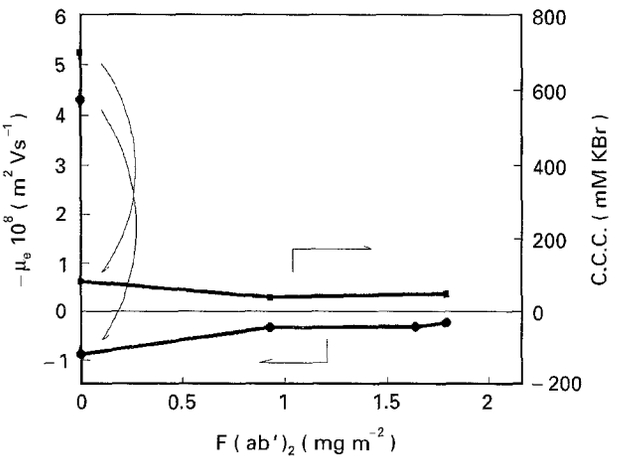

Figure 13 Electrophoretic mobility (●) and stability (■), in aqueous solution at pH 7, for $F(ab')_2$–DSDMA–JL2 complexes versus adsorbed protein amount.

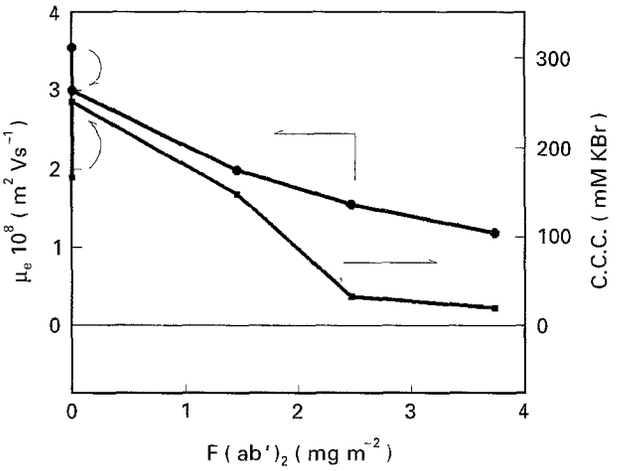

Figure 14 Electrophoretic mobility (●) and stability (■), in aqueous solution at pH 7, for $F(ab')_2$–DSDMA–JL8 complexes versus adsorbed protein amount.

4. Discussion

As for the adsorptions that were carried out with 50% ethanol/water mixtures, the results suggest that the main interaction between lipid molecules and polymer surfaces comes from coulombic attractions. This is due to several reasons: (i) no DSDMA adsorption takes place onto the cationic latex, although it possesses the most hydrophobic surface; (ii) the adsorbed lipid amounts vary in the same way the surface charge of the particles does; and (iii) those samples with a maximum coverage are completely unstable at low ionic strengths, indicating that the anionic groups (carboxyl, sulphonate or sulphate) of the latexes are neutralized by the positive charge of the lipid molecules. However, the electrostatic attraction is not the unique interaction that takes place in these adsorption processes. It is more than likely that, although initially the DSDMA molecules are situated on the polymer surface with their polar heads oriented toward the particles, in a second step the molecules can rotate and adhere their hydrophobic tails to the polystyrene surface, avoiding lipid desorption. This mechanism has already been proposed by Carmona-Ribeiro [18].

The differences among the results obtained when experiments were carried out in the 50% ethanol/water solutions and the 95% ones depend on this hydrophobic interaction, since in rich ethanol environments the dielectric constant is quite low (as compared to pure water) and the electrostatic attraction between ionic groups of opposite charge sign would be strong. Nevertheless, ethanol is a good solvent for hydrocarbon chains, and this is why the lipid apolar tails and the latex surface do not merge. It is probable that, in these cases, DSDMA–particle interactions are quite weak and desorption can happen easily. This latter explanation justifies why only little amounts of lipid can be adsorbed in solutions with high ethanol content. As a matter of fact, it makes no sense to talk about hydrophobic interaction when one adsorbs substances in almost free water solutions. What can be checked is that the higher the water/ethanol ratio, the more important the hydrophobic interaction. At least, little amounts of DSDMA can be adsorbed onto the most hydrophobic latex (JL8) in 1% ethanol/water mixtures, even under conditions where electrostatic repulsions occur.

The values of the i.e.p. of the samples obtained by mobility measurements, which coincide with the J_{pl} values of Fig. 2, reinforces the idea that the main driving force during the lipid adsorption process is the electrostatic interaction. In spite of these conclusions, there is a striking result, since the ratio between J_{pl} and the number of surface charged groups (Γ_e) should be "1". As can be seen in Table II, there is no case where this ratio equals the unity. With regard to these values, the DSDMA–JL2 complexes should be stable, since its value seems to indicate that the amount of DSDMA molecules almost doubles that of the number of sulphonated groups [9, 10]. We can explain this result by taking into account the method employed to calculate the σ_0 values. It is easy and reliable to get σ_0 by means of conductometric and potentiometric back titrations in latexes that only possess weak acid (or basic) surface groups [17]; but if one wants to know the surface charge density in colloid particles with strong acid groups, the counterions of such groups have to be H_3O^+. Achieving this purpose is a difficult task, since the counterions are generally Na^+ or K^+. This is why it is necessary to incubate the latex samples with ion exchange resins before titrations [19]. In our experiments we did so, but JL2 and JL10 latexes suffered only one incubation process. It is more than likely that we had underestimated the surface charge density values in these samples, this error being more marked in the former than in the latter, since JL2 has only sulphonate groups on its surface while JL10 has sulphate and carboxyl groups.

TABLE II Maximum amounts of DSDMA adsorbed onto different latexes, and lipid molecules surface charged groups ratios of particles totally covered

Latex	J_{pl} (μmol m^{-2})	J_{pl}/Γ_e (molecules/surface charged)
JL2	2.10 ± 0.05	1.92 ± 0.15
JL4	0.88 ± 0.03	0.70 ± 0.07
JL7	1.40 ± 0.04	0.71 ± 0.07
JL10	1.30 ± 0.05	1.22 ± 0.10

In the JL4 and JL7 latexes, the ratio $\sigma_{0\,JL7}/\sigma_{0\,JL4}$ has the same value as the ratio $J_{pl\,JL7}/J_{pl\,JL4} = 1.6$, which is another fact that supports the hypothesis that the driving force in the DSDMA adsorptions is the coulombic interaction when the medium has enough ethanol. The value $J_{pl\,JL7}/\sigma_{0\,JL7} = J_{pl\,JL4}/\sigma_{0\,JL4} = 0.7$ can be explained on the basis of the fact that weak acid groups are less dissociated in solutions with low dielectric constants than in pure water. This is why the lipid adsorption in 95% ethanol/water mixtures is almost zero in these two carboxylated latexes.

Another striking result comes from the values of J_{pl} versus adsorption pH in 1% ethanol/water solutions. Buffer ions play an important role on the amount of DSDMA adhered onto the particles. It seems that phosphate ions (mainly PO_4H^{2-}) and borate ($BO_3H_2^-$) foster the lipid adsorption. This idea can explain the fact that higher amounts of lipid can be adsorbed at pH 7 and pH 9 than at pH 6 (except for JL10 latex) and pH 8, respectively. Figs 5 and 6 show that if the type of buffer ions is changed, the J_{pl} values vary completely. After the surface charges of the particles are neutralized by the adsorption of a determined number of DSDMA molecules (i.e.p. in Figs 7 and 8), a coadsorption of low mass ions (such as PO_4H^{2-}) is necessary to be able to adsorb higher amounts of lipid. This is why the electrophoretic mobility remains at a constant value once the i.e.p. is surpassed, since (although the amount of adsorbed DSDMA was higher) the electrokinetically transported charge is the same. A simple mechanism of phosphate ions and lipid molecules coadsorption is depicted in Fig. 15. Moreover, the stability of the covered particles is rather low. In fact, according to DLVO theory, this result should be expected considering the electrokinetic behaviour of the samples.

With regard to the latex–$F(ab')_2$–DSDMA complexes, we have discussed some aspects above. (i) The adsorption of DSDMA onto the cationic latex (JL8), which takes place in rich water solutions, can slightly increase the C.C.C. of these particles (as the bare latex has a C.C.C. = 160 mM at pH 7, and complexes JL8–DSDMA (0.4 $\mu mol\,m^{-2}$) have a C.C.C. = 250 mM in the same medium, as can be seen in Fig. 14). However, as higher amounts of $F(ab')_2$ cover the particles, the colloidal stability becomes very low. (ii) In the anionic latexes, the instability is caused by the adsorbed lipid layer. Therefore, it has not been possible to stabilize latex–$F(ab')_2$ complexes under physiological conditions adsorbing a cationic lipid, and this is why we cannot develop immunodiagnosis tests based on LIA reactions with these systems. Even so, in this work we have successfully gone into depth in the study of interactions between lipid molecules and hydrophobic polymer surfaces.

5. Conclusions

The DSDMA adsorption onto polymeric sorbents is an irreversible process, and it takes place rapidly. In 50% ethanol/water mixtures, the preferential interaction is of an electrostatic type, although after the first contact point between lipid molecules and polymer surface is established, the hydrophobic interaction becomes important. The latter strengthens the lipid–latex union avoiding the reversibility of the process. The role played by this interaction is more effective in 1% ethanol/water solutions. The amounts of adsorbed DSDMA are influenced by the presence of buffer ions, phosphate and borate ions being the cause of maximum lipid adsorption. The colloidal stability of anionic latexes decreases as the surface is covered by

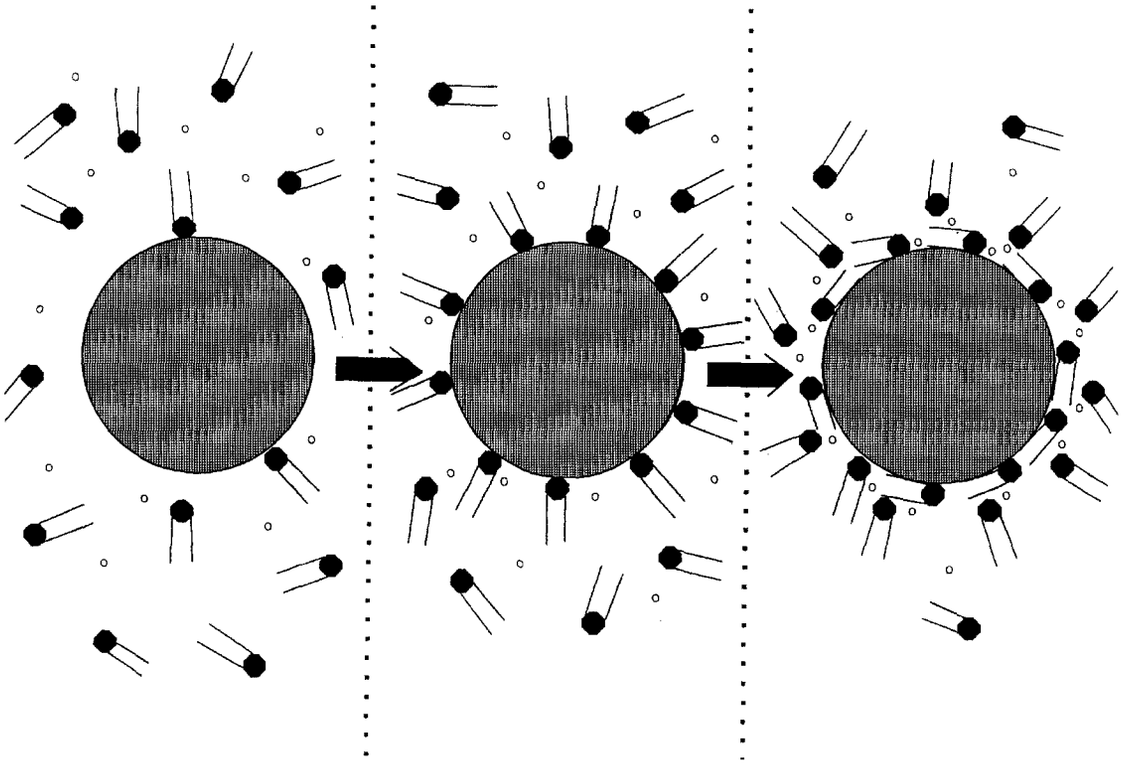

Figure 15 Scheme of the coadsorption of phosphate ions and DSDMA onto an anionic polymer surface.

DSDMA molecules; only positively charged particles slightly improve their stability.

When performing sequential adsorption of $F(ab')_2$ and DSDMA, the lipid is always adsorbed both onto the bare latex surface and onto the previously adhered protein molecules. Therefore, the immunoreactivity of these systems is rather low in comparison with $F(ab')_2$–BSA–latex complexes.

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