Colloid Polym Sci 275:198–202 (1997)

SHORT COMMUNICATION

J.M. Peula-Garcia
R. Hidaldo-Alvarez
F.J. de las Nieves

Protein co-adsorption on different polystyrene latexes: electrokinetic characterization and colloidal stability

Received: 27 March 1996
Accepted: 1 September 1996

J.M. Peula-Garcia · R. Hidalgo-Alvarez
Biocolloid and Fluids Physics Group
Department of Applied Physics
University of Granada
18071 Granada, Spain

Prof. F.J. de las Nieves (✉)
Complex Fluid Physics Group
Department of Applied Physics
University of Almería
04120 Almería, Spain

Abstract The latex agglutination immunoassay technique uses polymer colloids as a carrier for the adsorbed proteins to enhance the antigen–antibody reaction. Competitive co-adsorption of IgGaCRP and m-BSA proteins on polystyrene latexes with different functionality (sulfate and sulfonate groups) was carried out looking for the increase in the immunoreactivity and colloid stability of latex–protein complexes. The preferential adsorption of a protein is also studied, comparing both surface types.

Regarding the application in the development of a diagnostic test system, it is necessary to study the latex–protein complexes from an electrokinetic and colloid stability point of view.

The presence of protein on the surface latex shifts the iso-electric point (i.e.p.) of the latex–protein complexes to pH values near the i.e.p. of the protein which is the majority. Thus by the adsorption of m-BSA we can obtain complexes with the i.e.p. near pH 5 and, therefore, with a significant electrostatic repulsion at neutral pH.

Due to the higher surface charge density of the sulfonate latexes there is a higher adsorption of both proteins, which can provide a better colloidal stability (by the adsorption of m-BSA) and a better immunoreactivity (by the adsorption of IgG).

Key words Polystyrene latex – protein coadsorption – electrokinetic – colloidal stability

Introduction

Recently, the latex agglutination immunoassay has been widely studied, favored by the recent advances in colloid science that have made it possible to produce uniform latex with control of the more important characteristics such as particle size, surface charge density and functionality. In this way, polymer colloids are used as a carrier for the adsorbed protein molecules to enhance the antigen–antibody reaction. Adsorption of a single protein is a complex process in which several physico–chemical factors are involved. The amount and way in which that protein is adsorbed is dependent on the nature of the protein, characteristics of the solid surface and solution conditions [1–4].

One of more important requirements of a protein–latex complex for its application in the field of clinical diagnostics is the stability of the system from a colloidal point of view. Another requirement for their application is the immunoreactivity of the protein molecules after adsorption. Immuno–gamma–globulin (IgG) is the protein adsorbed in our work. The active sites of this molecule must to be directed towards the solution to react with the antigens present in the solution. The C-reactive protein (CRP) is the specific antigen for our IgG (IgGantiCRP). However, the IgG molecules adsorbed on the surface of the latex particles lead to colloidal

aggregation of the system in absence of their specific antigen, CRP [5].

To suppress non-specific interactions of the complementary antigen, non-occupied parts of the latex particles have to be covered with a second protein by sequential co-adsorption. The monomeric bovine serum albumin (m-BSA) is the protein used in this way. This method emphasizes the colloidal stability and degree of agglutination of the latex agglutination immunoassay [6] because the m-BSA protein leads to an additional stabilization of the latex particles [7]. The co-adsorption of protein couple (IgGaCRP/m-BSA) on polymer colloids (*competitive co-adsorption*) has found considerable interest in the immunodiagnostic field [8]. Thus, the high colloidal stability by the m-BSA and the good immunological reactivity by the IgGaCRP can be combined. The adsorption phenomena become even more complicated when two types of proteins are in the medium. In this work we compare the co-adsorption process on two polystyrene latexes of different functionality. One of these is a conventional latex with sulfate groups on their surface. The other is a sulfonated latex that presents important advantages already studied [9, 10]. The sulfonated latexes with a relatively high surface charge density can absorb a higher amount of protein [11] and, at the same time, can improve the colloidal stability of a latex covered by a protein [12]. Regarding the application of these systems in the development of a diagnostic test, the latex–protein complexes were characterized from an electrokinetic and colloidal stability point of view, by measuring the electrokinetic mobility and the critical coagulation concentration of the complexes from both latexes. Finally, the immunological response of the complexes with a good colloidal stability were studied by turbidity experiments.

Experimental

All chemicals used in this study were analytical grade and were used without further purification. Water used in all experiments was double distilled and deionized with a Millipore Q Water Purification System (Millipore).

Two polystyrene latex were used in this study: a conventional sulfate sample from Rhone–Poulenc (R–P), with an average particle diameter of 297 ± 7 nm, and a surface charge density of $-6.9 \pm 0.2\ \mu C/cm^2$, and a sulfonate latex obtained by a "shot-growth" emulsion polymerization technique (SN9) [9] with an average particle diameter of 196 ± 10 nm and a surface charge density of $-14.2 \pm 1.2\ \mu C/cm^2$. The methods and procedures to characterize the latexes were described in previous works [8, 13].

The preparation methods and characteristics of the proteins (IgGaCRP, CRP and m-BSA) used in this work were indicated in ref. [8], which presents the different methods to calculate the concentration of both proteins, IgGaCRP and m-BSA, when they are isolated or mixed, and the experimental co-adsorption process used to obtain the latex–proteins complexes.

In this work, we compare the results obtained for the two previously indicated latexes. On the basis of previous results with the same proteins [8], specific experimental conditions (pH and ionic strength, where both proteins were significantly adsorbed) were used. In this way, latexes–proteins complexes with different degree of coverage of both proteins were obtained.

The electrophoretic mobility was obtained with a Zeta-Sizer IV (Malvern Instruments, UK) by calculating the average of six measurements at the stationary level in a cylindrical cell.

The electrolyte concentration at which the latex particles coagulate, CCC, is obtained by a static method. The latex particles were resuspended in water, sonicated and mixed with the NaCl solutions. These suspensions were kept at room temperature for 24 h and the CCC determined visually as the highest NaCl concentration that did not produce coagulation of the latex particles. The experiments were developed at pH 5, 7 and 9 using acetate, phosphate and borate buffers respectively.

Finally, the immunological reaction of some complexes was studied by a turbidity method by measuring the absorbance increments at 570 nm of a dispersion containing the latex–proteins complex and different concentrations of CRP ranging from 2 μg/ml to 12.5 ng/ml diluted in a reaction medium containing 1 mg/ml of BSA and an ionic strength of 150 mM after 5 min.

Results and discussion

Competitive co-adsorption experiments at specific experimental conditions were realized for both latexes. Table 1 shows the co-adsorbed amounts of each protein on both latexes at pH 5 and low ionic strength, 2 mM, when the initial concentration of m-BSA in solution is constant and the IgGaCRP concentration is increasing. These conditions have to favor the adsorption of the protein with isoelectric point (i.e.p.) near the experimental pH, in this case the m-BSA. As can be seen in Table 1, the adsorbed amount of m-BSA is always higher than IgGaCRP. However, those values cannot be used to study preferential adsorption because the protein–surface affinity depends on the protein, the surface and the sensitized medium. Using the mass fraction of each protein on the surface for a co-adsorption experiment, $\phi_{protein}$, makes it possible to know the protein that resists preferentially adsorbed under the experimental conditions. Equation (1) shows the

Table 1 Co-adsorbed amount of both proteins on sulfate (R–P) and sulfonate (SN9) latexes. Co-adsorption experiment: pH 5, $I = 2$ mM, $[\text{m-BSA}]_{\text{initial}} = 5.50\ \text{mg/m}^2$

Initial [IgGaCRP]	Co-adsorbed amount on R–P latex, (mg/m^2)		Co-adsorbed amount on SN9 latex (mg/m^2)	
(mg/m^2)	IgGaCRP	m-BSA	IgGaCRP	m-BSA
0.52	0.10	2.41	0.13	4.01
1.03	0.15	2.30	0.28	3.73
1.91	0.49	2.24	1.04	3.70
3.00	0.51	2.09	1.40	3.12
5.10	0.76	2.00	1.40	2.80
7.16	1.10	1.77	1.20	2.75

Table 2 Surface composition of latex–proteins complexes on both latexes as a function of the IgGaCRP mass fraction

Latex	Jadd IgGaCRP (mg/m^2)	Jadd m-BSA (mg/m^2)	ϕ_{IgGaCRP}	$\phi_{\text{p1, IgGaCRP}}$
RP	7.16	5.22	0.38	0.58
SN9	7.16	5.45	0.30	0.55

expression for this parameter:

$$\phi_{\text{protein1}} = \frac{\Gamma_{\text{protein1}}}{\Gamma_{\text{protein1}} + \Gamma_{\text{protein2}}} \tag{1}$$

where Γ_{protein} is the adsorbed amount of this protein in mg/m^2. If the diffusion condition ([IgGaCRP]/[mBSA] = Diffusion coefficients ratio = 1.3) and the surface affinity of each protein are the same, it would result in a surface composition directly related with the single adsorption of those proteins. Considering:

$$\phi_{\text{pl, protein1}} = \frac{\Gamma_{\text{pl, protein1}}}{\Gamma_{\text{pl, protein1}} + \Gamma_{\text{pl, protein2}}} \tag{2}$$

where $\Gamma_{\text{pl, protein}}$ shows the maximum adsorbed amount of a single protein at the same experimental conditions; if $\phi_{\text{protein}} = \phi_{\text{pl, protein}}$ there is not preferential adsorption. Some deviation of a fraction with regard to the other means preferential adsorption of a protein.

Table 2 shows the mass fraction of the IgGaCRP protein in both experiments: competitive co-adsorption and single adsorption, at pH 5 and low ionic strength. In this table J_{add} means the initial amount of the protein in the adsorption medium. The maximum amounts of both proteins adsorbed by a single adsorption process on the RP latex were $2.5\ \text{mg/m}^2$ for the m-BSA and $3.5\ \text{mg/m}^2$ for the IgGaCRP [8], while for the SN9 latex the final amounts adsorbed after the desorption step were $3.80\ \text{mg/m}^2$ for the m-BSA and $4.72\ \text{mg/m}^2$ for the IgGaCRP [13]. With these values it is possible to calculate $\phi_{\text{p1, IgGaCRP}}$. On the other hand, using the values of Table 1 for an initial amount of IgGaCRP of $7.16\ \text{mg/m}^2$, we can obtain ϕ_{IgGaCRP}. In this way, the ratio between the initial amount of each protein in the co-adsorption medium is 1.3 and the diffusion conditions are the same for both proteins. As can be seen ϕ_{IgGaCRP} was always lower than $\phi_{\text{p1, IgGaCRP}}$ although the latexes have different surface groups (sulfate and sulfonate). There is preferential adsorption of the m-BSA because pH 5 is near the i.e.p. of the m-BSA. These results show the importance of the hydrophobic forces in the co-adsorption process which are the main interaction [1, 11, 14, 15]. Protein–protein interactions have been mentioned by several authors [15, 16]. These interactions could be very important because these can affect the surface affinity of the proteins. Furthermore, these interactions could have influence on the position of the adsorbed protein molecules on the surface. The difference between the latexes used is only the higher adsorbed amount of both proteins on the sulfonate latex, that is the latex with a higher surface charge density. The special surface structure of the sulfonate latex synthesized by a "shot-growth" process can be responsible for the higher adsorption of this type of particles. Higher amounts of both m-BSA and IgGaCRP proteins on non-rigid particles have been found by several authors [17–19]. These particles have flexible surfaces due to the presence of chemically bound oligomers as have been shown in a previous work and by other authors [9, 10, 20]. The complexes of sulfate and sulfonate latex obtained by the competitive co-adsorption process have an important amount of m-BSA and a significant amount of IgGaCRP that can give very interesting results in the immunodiagnostic field. In this way, it is necessary to study the electrokinetic, colloidal stability and immunoreactivity behavior of these complexes.

Figure 1 shows the electrophoretic mobility values of several complexes for the sulfonated latex when these are redispersed at different pH buffered by acetate, phosphate and borate. All the mobility measurements were carried out at an ionic strength of 2 mM, which was adjusted by adding NaCl. As a consequence of the strong acid nature of the surface groups of this latex, the mobility of the bare particles remains constant in the interval of pH shown in the figure. Latex–protein complexes with only an adsorbed protein are compared with a complex obtained by the previous co-adsorption experiment. The presence of a protein on the latex surface shifts the i.e.p. of the latex–protein complexes to pH values near the i.e.p. of the protein adsorbed. Thus the particles covered by m-BSA show an i.e.p. near pH 4.7 (the i.e.p. of this protein) and will have a high mobility at neutral and basic pH that indicates high colloidal stability at these pH. On the other hand, a complex with IgGaCRP show an i.e.p. higher than pH 5 (the

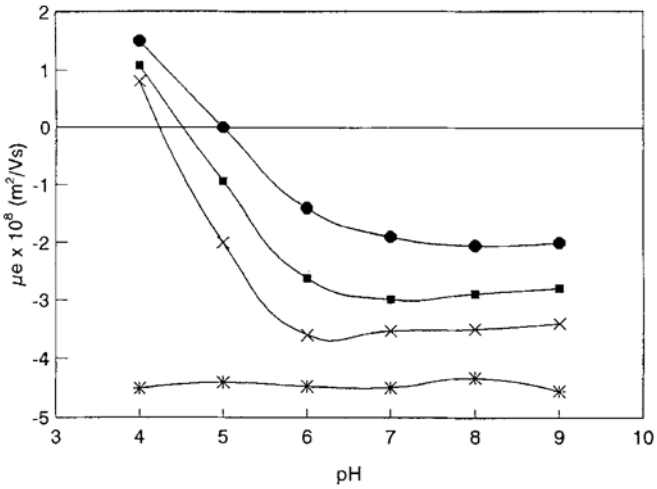

Fig. 1 Electrophoretic mobility of m-BSA/SN9, IgGaCRP/SN9 and m-BSA-IgGaCRP/SN9 complexes as a function of pH. *, bare latex; ×, 3.53 mg(m-BSA)/m^2; ●, 4.63 mg(IgGaCRP)/m^2; ■, 2.80 mg(m-BSA)/m^2 – 1.40 mg(IgGaCRP)/m^2

IgG is a polyclonal sample with an i.e.p. between 6 and 8) but the mobility values at neutral and basic pH are lower than the bare latex indicating low colloidal stability. These results have been normally found in previous works [8, 17]. Finally, the co-adsorbed complex with 2.80 mg/m^2 of m-BSA and 1.40 mg/m^2 of IgGaCRP shows an i.e.p. intermediate between previous single complexes as a consequence of the presence of both proteins on surface latex particles. The electrokinetic behavior of this complex seems to indicate that it can be colloidally stable at neutral and basic pH.

The next step is the study of the colloidal stability by calculating the critical coagulation concentration for the latex–proteins complexes. Tables 3 and 4 show this parameter for the sulfate and sulfonate latexes, respectively, at different degrees of coverage and several pH. A higher percentage of m-BSA on the surface and lower adsorption of IgGaCRP made the complexes stable at pH 7 and 9 and not stable at pH 5. When the amount of IgGaCRP on sulfate latex surface increases up to a value between 0.49 and 0.81 mg/m^2 (see Table 3) the stability decreases at pH 7. However, the sulfonate latex, as a consequence of the higher adsorbed amount of m-BSA, presents complexes highly stable (see Table 4), from a colloidal point of view, with a higher amount of IgGaCRP on the surface. Therefore, the colloidal stability appears when the degree of coverage by m-BSA is high and at a pH in which this protein was negatively charged [12]. In this way, the sulfonate complexes shown in Table 4 with an amount of BSA higher than 2.8 mg/m^2 and an amount of IgG lower than 1.4 mg/m^2 display a high colloidal stability at pH 7 and 9 where the BSA molecules are negatively charged.

Table 3 Colloidal stability (CCC values for [NaCl]) for co-adsorbed complexes of R-P latex at several pH

(IgGaCRP/m-BSA)-RP (mg/m^2)	CCC pH 5 (mM)	CCC pH 7 (mM)	CCC pH 9 (mM)
0.10/2.41	2	2000	2000
0.25/2.30	2	2000	2000
0.49/2.14	2	2000	2000
0.81/2.09	4	50	2000
0.76/2.00	4	50	2000
1.10/1.77	4	50	100

Table 4 Colloidal stability (CCC values for [NaCl]) for co-adsorbed complexes of SN9 latex at several pH

(IgGaCRP/BSA)-SN9 (mg/m^2)	CCC pH 5 (mM)	CCC pH 7 (mM)	CCC pH 9 (mM)
0.13/4.01	2	1000	1000
0.28/3.37	2	1000	1000
1.04/3.70	2	1000	1000
1.40/2.80	2	1000	1000

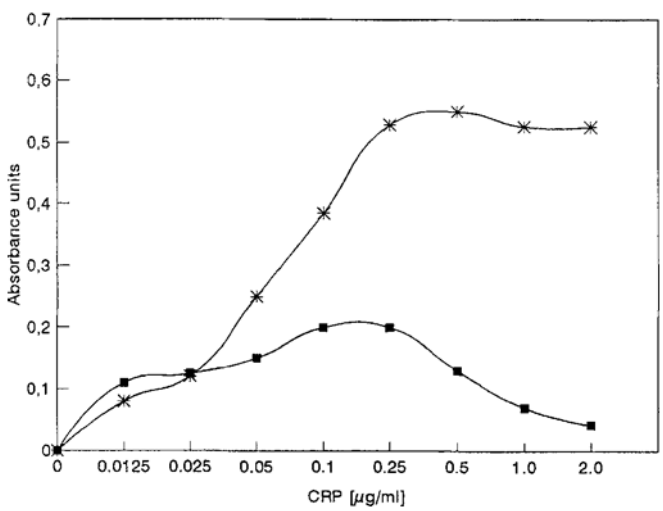

Fig. 2 Immunoreactivity of a co-adsorbed complex, 2.8 mg(m-BSA)/m^2 – 1.4 mg(IgGaCRP)/m^2, of SN9 latex. *, 4.2 × 10^{10} part/ml; ■, 1.1 × 10^{11} part/ml

As the final step of this work, the immunoreactivity of several complexes was studied in order to know the minimum amount of IgGaCRP that permits a good immunological response. Only the complexes which were colloidally stable have been studied since the complexes unstable at the immunological reaction conditions (150 mM) cannot be used to detect the presence of antigen (CRP) in the medium due to colloidal aggregation produced in absence of the coaggulating agent. Figure 2 shows,

after 5 min of the reaction, the change in the optical absorbance of the dispersion as a function of the CRP concentration, for one of the complexes obtained with the sulfonate latex. As a reference sample the same complex concentration redispersed at the same solution but without the presence of antigen was used. The turbidity of the references was constant which is an indication of the stability of this complex at the physiological ionic strength (150 mM). As can be seen in this figure, the increments in the absorbance were very high, especially for the sample with a higher particle concentration. The shape of the curve shows the typical Heidelberger–Kendall curve of the immunoprecipitine reaction [21]. The minimum CRP antigen concentration detected was 12.5 ng/ml with an optical response in the range of the immunodiagnostic test commercially available. However, the results obtained for the RP complexes (not shown) present an important decrease in the response, which indicates that the maximum adsorbed amount of IgGaCRP in a stable complex is not sufficient to display a good immunological behavior.

Therefore, the latex–proteins complex properties depended on the percentage of m-BSA or IgGaCRP adsorbed and on the electric state of the proteins at the redispersion pH. It is necessary to find the adequate equilibrium between the amount of IgGaCRP which produces a good immunological response and the amount of m-BSA responsible for the colloidal stability. The sulfonated latex, due to the special surface structure and higher surface charge density, allows adsorption of higher amounts of both proteins improving the colloidal stability and the immunoreactivity of the latex–proteins complexes.

Acknowledgments This work is supported by the Comisión Interministerial de Ciencia y Tecnología (CICYT), project MAT 96-1035-C03-03. We would like to express our sincere thanks to Dr. Delfina Bastos for kindly supplying the sulfonated latex.

References

1. Norde W, Lyklema J (1978) J Colloid Interface Sci 66:257
2. Zsom RLJ (1986) J Colloid Interface Sci 111:434
3. Shirahama H, Suzawa T (1985) Colloid Polym Sci 263:141
4. Andrade JD, Hlady VC, Van Wagenen RA (1984) Pure Appl Chem 56:1345
5. Martin A, Puig J, Galisteo F, Serra J, Hidalgo-Alvarez R (1992) J Dispersion Sci Techn 13(4):399
6. Puig J, Fernández-Barbero A, Bastos D, Serra J, Hidalgo-Alvarez R (1994) In: West R, Batts G (eds) Surface Properties of Biomaterials. Butterworth-Heinemann, Oxford
7. Tamai H, Hasegawa M, Suzawa T (1990) Colloid Surfaces 51:271
8. Peula JM, Puig J, Serra J, de las Nieves FJ, Hidalgo-Alvarez R (1994) Colloid and Surfaces A: Physicochem Eng Aspects 92:127
9. de las Nieves FJ, Daniels ES, El-Aasser MS (1991) Colloids Surfaces 60:107
10. Bastos D, de las Nieves FJ (1994) Colloid Polym Sci 272:592
11. Peula JM, de las Nieves FJ (1993) Colloid Surfaces A: Physicochem Eng Aspects 77:199
12. Peula JM, de las Nieves FJ (1994) Colloid Surfaces A: Physicochem Eng Aspects 90:55
13. Peula JM, Hidalgo-Alvarez R, de las Nieves FJ (1995) J Biomater Sci Polym Edn 7(3):231
14. Tilton R, Robertson CR, Gast A (1991) Langmuir 7(11):274
15. Elgersma AV, Zsom RLJ, Norde W, Lyklema J (1991) J Colloid Interface Sci 54:859
16. Beissinger RL, Leonard EF (1982) J Colloid Interface Sci 85:521
17. Galisteo F, Puig J, Martin A, Serra J, Hidalgo-Alvarez R (1994) Colloids Surfaces B: Biointerfaces 2:435
18. Suzawa T, Shirahama H, Fujimoto T (1982) J Colloid Interface Sci 86(1):144
19. Betton F, Theretz A, Elaissari A, Pichot C (1993) Colloid Surfaces B: Biointerfaces 1:97
20. Chow RS, Takamura K (1988) J Colloid Interface Sci 125:226
21. Jefferis R, Deverill I (1991) In: Price CP, Newman DJ (eds) Principles and Practice of Immunoassays. Stockton press, UK