

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/8110973>

# On the Restabilization of Protein-Covered Latex Colloids at High Ionic Strengths

ARTICLE *in* LANGMUIR · FEBRUARY 2005

Impact Factor: 4.46 · DOI: 10.1021/la0481795 · Source: PubMed

---

CITATIONS

6

---

READS

17

3 AUTHORS, INCLUDING:



Marian Manciu

University of Texas at El Paso

80 PUBLICATIONS 1,209 CITATIONS

SEE PROFILE

# On the Restabilization of Protein-Covered Latex Colloids at High Ionic Strengths

Haohao Huang, Marian Manciú,<sup>†</sup> and Eli Ruckenstein\*

Department of Chemical and Biological Engineering, State University of New York at Buffalo, Buffalo, New York 14260

Received July 20, 2004. In Final Form: October 6, 2004

Recent experiments on restabilization of protein-covered latex colloids at high ionic strengths reported by Lopez-Leon et al.<sup>1</sup> revealed strong specific anion effects. The same authors also emphasized that a recent polarization model, which involves both hydration and double layer forces, can account only for some of their experimental results but are in disagreement with other experimental results. The aim of the present paper is to show that most experimental results of ref 1 can be described, more than qualitatively, when the association equilibria for all the ions (with both the acidic and basic sites of the protein) are taken into account. As the traditional Poisson–Boltzmann approach, the polarization model neglects additional interactions between ions, and ions and surfaces, not included in the “mean field” electrical potential; therefore, a complete quantitative agreement should not be expected. While many of the discrepancies between calculations and experiment occur at low ionic strengths ( $10^{-4}$ – $10^{-2}$  M), in the range of validity of the traditional DLVO theory, the latter can neither explain them. It is suggested that the structural modifications of the protein configuration induced by the electrolyte are responsible for some of the disagreements between experiment and calculations.

## 1. Introduction

The traditional Derjaguin–Landau–Verwey–Overbeek (DLVO) theory regards the stability of colloids as a balance between a repulsive double layer interaction due to the surface charges and an attractive van der Waals interaction.<sup>2</sup> At high electrolyte concentrations, the electrostatic interactions are screened and the van der Waals attraction leads to the coagulation of colloids. While describing qualitatively the behavior of most colloids, this theory is in disagreement with numerous experimental results. For example, it is known that the neutral lipid bilayers<sup>3</sup> and the strong hydrophilic colloids (e.g., silica)<sup>4</sup> are stable regardless of the ionic strength and an additional repulsion is required to explain those experiments. This repulsion was sometimes associated with the structuring of water in the vicinity of an interface (hence called “hydration force”),<sup>4</sup> but there is still no consensus regarding its microscopic origins.

One of the models for the hydration force, the polarization model,<sup>5</sup> assumes that the hydration force is generated by the local correlations between neighboring dipoles present on the surface and in water. The macroscopic continuum theory, in which water is assumed to be a homogeneous dielectric, predicts that there is no electric field above or below a neutral surface carrying a uniform dipolar density. However, at microscopic level the water is hardly homogeneous, and the electric interactions

between neighboring molecules are much less screened than the interactions between remote molecules; consequently, a polarization is induced in water in the vicinity of dipolar surfaces.<sup>5–7</sup> It should be noted that Molecular Dynamics simulations reported also a polarization of water in the presence of surface dipoles,<sup>8</sup> which is not proportional to the electric field,<sup>9</sup> as assumed in the traditional macroscopic theory. Both results support the predictions provided by the polarization model regarding the hydration interactions.

The surface dipoles are sometimes generated by the association of the cations and anions with the negative and positive sites, respectively, of the surface. These associations decrease the surface charge but increase the surface dipole densities, and the polarization induced by them has been used<sup>10,11</sup> to explain the experimental data regarding the restabilization of protein-covered polystyrene colloids at high ionic strengths reported by Molina-Bolivar and Ortega-Vinuesa.<sup>12</sup>

The present article was stimulated by the recent experimental data on protein-covered latex colloidal systems immersed in various electrolyte solutions: NaCl, NaNO<sub>3</sub>, NaSCN and Ca(NO<sub>3</sub>)<sub>2</sub>, which showed strong specific anionic effects on the restabilization curves.<sup>1</sup> In the opinion of López-León et al.,<sup>1</sup> the above polarization model for double layer/hydration forces could explain only some of their experiments, but not all of them. However, they assumed that at pH = 10 the adsorption of anions was negligible; hence “specific anion effects” could not be predicted by their association with the positive sites of the surface. Furthermore, at pH = 4 they assumed the

\* Corresponding author: e-mail address, feaeliru@acsu.buffalo.edu; phone, (716) 645 2911/2214; fax, (716) 645 3822.

<sup>†</sup> Permanent address: Physics Department, University of Texas at El Paso.

(1) López-León, T.; Gea-Jódar, P. M.; Bastos-González, D.; Ortega-Vinuesa, J. L. *Langmuir* **2005**, *21*, 87–93.

(2) Deryaguin, B. V.; Landau, L. *Acta Physicochim. URSS* **1941**, *14*, 633. Verwey, E. J.; Overbeek, J. Th. G. *Theory of stability of lyophobic colloids*; Elsevier: Amsterdam, 1948.

(3) Rand, R. P.; Parsegian, V. A. *Biochim. Biophys. Acta* **1989**, *988*, 351.

(4) Israelachvili, J. *Intermolecular and Surface Forces*; Academic Press: New York, 1992.

(5) Schiby, D.; Ruckenstein, E. *Chem. Phys. Lett.* **1983**, *95*, 435.

(6) Manciú, M.; Ruckenstein, E. *Langmuir* **2001**, *17*, 7061.

(7) Ruckenstein, E.; Manciú, M. *Langmuir* **2002**, *18*, 7584.

(8) Perera, L.; Essmann, U.; Berkowitz, M. L. *Prog. Colloid Polym. Sci.* **1997**, *103*, 107.

(9) Faraudo, J.; Bresme, F. *Phys. Rev. Lett.* **2004**, *92*, 236102–1.

(10) Huang, H.; Manciú, M.; Ruckenstein, E. *J. Colloid Interface Sci.* **2003**, *263*, 156.

(11) Ruckenstein, E.; Huang, H. *Langmuir* **2003**, *19*, 3049.

(12) Molina-Bolivar, J. A.; Ortega-Vinuesa, J. L. *Langmuir* **1999**, *15*, 2644.

adsorption of cations to be negligible and the “specific cation effects”, observed experimentally, could not be predicted.<sup>1</sup>

Strong specific anion effects were reported particularly at low electrolyte concentrations ( $10^{-4}$ – $10^{-2}$  M),<sup>1</sup> a range in which the DLVO theory is considered accurate. However, as shown later, the present experimental data cannot be reproduced by the traditional theory in this range of electrolyte concentrations. In the past, no agreement could be obtained, on the basis of the traditional theory, because small changes in the values of the parameters, caused by the nonuniformity of the particles, affected strongly the stability ratio.<sup>13</sup> The polarization model provides similar results in the above range of electrolyte concentrations, when the dipole densities are sufficiently low and cannot explain the data.

It is clear that a perfect agreement with experiment cannot be provided by a theory which ignores the additional interactions between ions, and ions and surfaces, not included in the mean field potential (such as image forces,<sup>14</sup> excluded volume effects,<sup>15</sup> and ion-dispersion<sup>16</sup> or ion-hydration forces<sup>17</sup>). However, it will be shown that the experimental results reported by López-León et al.<sup>1</sup> can be more than qualitatively reproduced for univalent electrolytes by the present polarization model for hydration/double layer forces, if one accounts for the association equilibria with the surface sites for all the ions present in the electrolyte ( $H^+$ ,  $OH^-$ , anions, and cations).<sup>11</sup> Some additional reasons for the quantitative disagreements, involving the structural modifications of the adsorbed protein layer and the nonuniformity of the colloidal particles, will be also noted.

## 2. Review of the Theoretical Framework

If one assumes that all the interactions between ions are mediated by a “mean field” potential  $\psi$  and that the ions are Boltzmannian distributed, the charge density  $\rho$  is given by

$$\rho = -e(c_E + c_{OH}) \exp\left(\frac{e\psi}{kT}\right) + e(c_E + c_H) \exp\left(-\frac{e\psi}{kT}\right) \quad (1)$$

where  $e$  is the protonic charge,  $c_E$  is the bulk electrolyte concentration (assumed for the sake of simplicity univalent),  $c_{OH}$  and  $c_H$  are the concentrations of  $OH^-$  and  $H^+$  in the bulk, respectively,  $k$  is the Boltzmann constant, and  $T$  is the absolute temperature. The macroscopic electric field  $E \equiv -\nabla\psi$  obeys the Poisson equation

$$\nabla(\epsilon_0 E + P) = \rho \quad (2)$$

where  $\epsilon_0$  is the vacuum permittivity and  $P = m/v_0$  is the polarization, with  $m$  being the average dipole of a water

molecule of volume  $v_0$ . Equations 1 and 2 can be combined into

$$\epsilon_0 \frac{\partial^2 \psi(z)}{\partial z^2} = e(c_E + c_{OH}) \exp\left(\frac{e\psi(z)}{kT}\right) - e(c_E + c_H) \exp\left(-\frac{e\psi(z)}{kT}\right) + \frac{1}{v_0} \frac{\partial m(z)}{\partial z} \quad (3)$$

In a typical “macroscopic” assumption of proportionality between polarization and applied electric field,  $P = \epsilon_0(\epsilon - 1)E$ , where  $\epsilon$  is the dielectric constant, and eq 3 reduces to the traditional Poisson–Boltzmann equation (the concentrations  $c_H$  and  $c_{OH}$  being in general much smaller than  $c_E$ ). However, if the correlations between neighboring dipoles are taken into account, the following constitutive equation relating the polarization to the “macroscopic” electric field is obtained<sup>7</sup>

$$\lambda_m^2 \frac{\partial^2 m(z)}{\partial z^2} = m(z) + \epsilon_0 v_0 (\epsilon - 1) \frac{\partial \psi(z)}{\partial z} \quad (4)$$

where  $z$  is the direction normal to the planar surface containing the dipoles. If the “dipole correlation length”  $\lambda_m = 0$ , the usual macroscopic constitutive relation  $P = \epsilon_0(\epsilon - 1)E$  (and, consequently, the traditional Poisson–Boltzmann theory) is recovered.

The solutions for  $\psi(z)$  and  $m(z)$  of the system of eqs 3 and 4 depend not only on the surface charge density but also on the surface dipole densities. The boundary conditions are related to the surface charge and the surface dipoles generated by the association of the cations and  $H^+$  with some of the  $N_A$  acidic surface sites per unit area of the surface and of the anions and  $OH^-$  with some of the  $N_B$  basic surface sites per unit area. The details are given elsewhere,<sup>11</sup> and the results will only be briefly reviewed here.

The surface charge density is given by

$$\sigma = \frac{eN_B}{1 + \left(\frac{c_{OH}}{K_{OH}} + \frac{c_E}{K_A}\right) \exp\left(\frac{e\psi_S}{kT}\right)} - \frac{eN_A}{1 + \left(\frac{c_H}{K_H} + \frac{c_E}{K_C}\right) \exp\left(-\frac{e\psi_S}{kT}\right)} \quad (5)$$

where  $K_A$  and  $K_C$  are the association-equilibria constants for anions and cations, respectively. The average areas occupied by anions,  $A_A$ , and cations,  $A_C$ , on the surface are provided by

$$A_A = \frac{1 + \frac{K_A}{K_{OH}} \frac{c_{OH}}{c_E} + \frac{K_A}{c_E} \exp\left(-\frac{e\psi_S}{kT}\right)}{N_B} \quad (6a)$$

$$A_C = \frac{1 + \frac{K_C}{K_H} \frac{c_H}{c_E} + \frac{K_C}{c_E} \exp\left(\frac{e\psi_S}{kT}\right)}{N_A} \quad (6b)$$

(13) Prieve, D. C.; Ruckenstein, E. *J. Colloid Interface Sci.* **1980**, *73*, 539.

(14) Jönsson, B.; Wennerström, H. *J. Chem. Soc., Faraday Trans.* **1983**, *79*, 19.

(15) Levine, S.; Bell, G. M. *Discuss. Faraday Soc.* **1966**, *42*, 69; Ruckenstein, E.; Schiby, D. *Langmuir* **1985**, *1*, 612.

(16) Boström, M.; Williams, D. M. R.; Ninham, B. W. *Phys. Rev. Lett.* **2001**, *87*, 168103.

(17) Manciu, M.; Ruckenstein, E. *Adv. Colloid Interface Sci.* **2003**, *105*, 63; Ruckenstein, E.; Manciu, M. *Adv. Colloid Interface Sci.* **2003**, *105*, 177.

The two boundary condition at the surface ( $z = 0$ ) are given by

$$\epsilon_0 \frac{\partial \psi(z)}{\partial z} \Big|_{z=0} - \frac{m(z)}{v_0} \Big|_{z=0} = -\sigma \quad (7a)$$

and

$$(1 - \epsilon_0 v_0 (\epsilon - 1)(C_0 + C_1))m(z) \Big|_{z=0} - \epsilon_0 v_0 (\epsilon - 1) \Delta C_1 \frac{\partial m}{\partial z} \Big|_{z=0} + \epsilon_0 v_0 (\epsilon - 1) \frac{\partial \psi}{\partial z} \Big|_{z=0} = \epsilon_0 v_0 (\epsilon - 1) \left[ \left( \frac{p_A}{\epsilon'} \right) \frac{1}{2\pi\epsilon_0} \frac{1}{\left( \frac{A_A}{\pi} + \Delta'^2 \right)^{3/2}} + \left( \frac{p_C}{\epsilon'} \right) \frac{1}{2\pi\epsilon_0} \frac{1}{\left( \frac{A_C}{\pi} + \Delta'^2 \right)^{3/2}} \right] \quad (7b)$$

where  $p_A/\epsilon'$  and  $p_C/\epsilon'$  represent the effective dipoles generated by the association of the anions and cations with the positive and negative sites of the surface (note that the fields generated by the two types of dipoles are oriented in opposite directions), respectively,  $\epsilon'$  is an effective dielectric constant of water in the vicinity of the surface,  $\Delta = 3.68 \text{ \AA}$  is the distance between two icelike water layers in the liquid,  $\Delta' = 2 \text{ \AA}$  is the distance between the surface dipoles and the first water layer, and the coupling parameters  $C_0$  and  $C_1$  are given by

$$C_0 = - \frac{3.766}{4\pi\epsilon_0\epsilon''l^3} \quad (8a)$$

$$C_1 = \frac{1.827}{4\pi\epsilon_0\epsilon''l^3} \quad (8b)$$

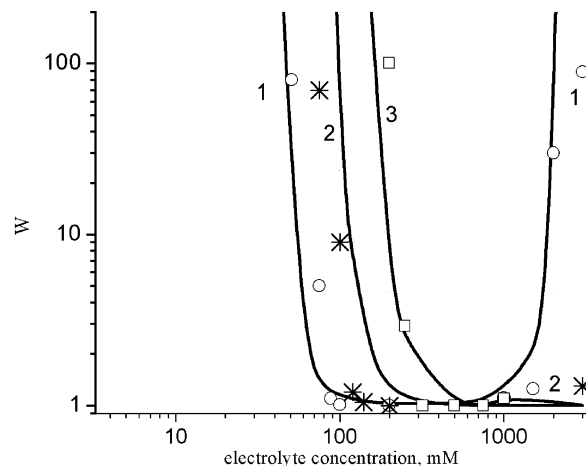
$l = 2.76 \text{ \AA}$  being the shortest distance between two water molecules and  $\epsilon'' = 1$  the effective dielectric constant for the electrostatic interactions between neighboring water molecules. The boundary condition (7b) takes explicitly into account the polarization generated by the surface dipoles, while eq 7a is a consequence of the Poisson equation (2).

For two identical surfaces immersed in an electrolyte separated by a distance  $2d$ , the symmetry provides the other two boundary conditions at the middle distance  $z = d$

$$\frac{\partial \psi(z)}{\partial z} \Big|_{z=d} = 0 \quad (7c)$$

$$m(z) \Big|_{z=d} = 0 \quad (7d)$$

Once the system of eqs 3 and 4 is solved under the boundary conditions (7a–d), the total free energy of the system can be calculated by adding the van der Waals interactions between the surfaces to the double layer free energy composed of electrostatic, entropic, and chemical contributions,<sup>11</sup> and the stability ratio can be calculated in the usual manner.<sup>18</sup>



**Figure 1.** Experimental values of the stability ratio of protein-covered latex particles as a function of electrolyte concentration, at pH = 4.0, reported by Lopez-Leon et al.,<sup>1</sup> compared to those calculated from the polarization-based hydration model, for the following parameter values:  $N_A = 1.2 \times 10^{18}$  sites/m<sup>2</sup>,  $N_B = 2.31 \times 10^{18}$  sites/m<sup>2</sup>,  $A_H = 0.5 \times 10^{-20}$  J,  $K_H = 10^{-6}$  M,  $K_{OH} = 8.95 \times 10^{-8}$  M,  $K_{Na} = 0.021$  M,  $(p/\epsilon')_{Na} = 1.8$  D; (1)  $K_{SCN} = 0.65$  M,  $(p/\epsilon')_{SCN} = -1.65$  D; (2)  $K_{NO_3} = 0.62$  M,  $(p/\epsilon')_{NO_3} = -1.8$  D; (3)  $K_{Cl} = 0.76$  M,  $(p/\epsilon')_{Cl} = -2.3$  D; circles, NaSCN; squares, NaCl; stars, NaNO<sub>3</sub>.

### 3. Coagulation of Protein-Covered Latex Particles

Let us first analyze the stability ratio for particles immersed in an electrolyte of pH = 4. The densities of acidic and basic sites, as well as their dissociation constants, are unknown. While one of these parameters could be determined as a function of the others from the isoelectric point (IP), even the latter is uncertain:  $6.1 < IP < 8.5$ .<sup>19</sup> The following values have been selected for the parameters:  $N_A = 1.2 \times 10^{18}$  sites/m<sup>2</sup>,  $N_B = 2.31 \times 10^{18}$  sites/m<sup>2</sup>,  $K_H = 10^{-6}$  M, and  $K_{OH} = 8.95 \times 10^{-8}$  M. The calculated stability ratio is plotted in Figure 1, using for the cation ( $Na^+$ )  $K_{Na} = 0.021$  M,  $(p/\epsilon')_{Na} = 1.8$  D, while for the various anions:  $K_{Cl} = 0.76$  M,  $(p/\epsilon')_{Cl} = -2.3$  D,  $K_{NO_3} = 0.62$  M,  $(p/\epsilon')_{NO_3} = -1.8$  D, and  $K_{SCN} = 0.65$  M,  $(p/\epsilon')_{SCN} = -1.65$  D. As shown in Figure 1, a reasonable agreement between experiment and calculations was obtained for uni-univalent electrolytes by selecting a rather low value for the Hamaker constant of the van der Waals interactions between colloidal particles,  $A_H = 0.5 \times 10^{-20}$  J.

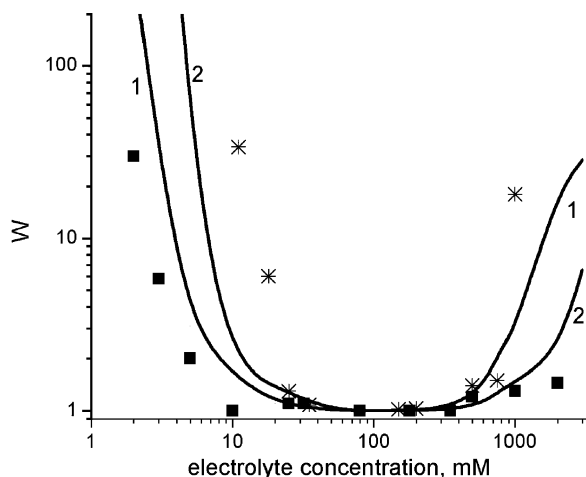
The calculations at pH = 10.0 are compared in Figure 2 with the experimental data provided by ref 1 for NaNO<sub>3</sub> and NaCl. The only modifications in the parameter values selected above are for the number of basic sites per unit area and for the Hamaker constant, which were selected in this case as  $N_B = 1.62 \times 10^{18}$  sites/m<sup>2</sup> and  $A_H = 0.9 \times 10^{-20}$  J. Again, qualitative agreement was obtained. There is no doubt that better agreement with experiment could have been obtained by other fittings of the values of the parameters to the experimental data. However, this is not very meaningful. The large number of parameters of unknown values involved in calculations raises doubt whether a good fit can be obtained only for the appropriate values of the parameters. As a matter of fact, because of the large number of parameters, a previous paper reported a good fit for the stability ratio of one of the above systems for different parameter values.<sup>10</sup>

In Figure 3, the experimentally determined stability ratio<sup>1</sup> is plotted for low concentrations of NaSCN and pH

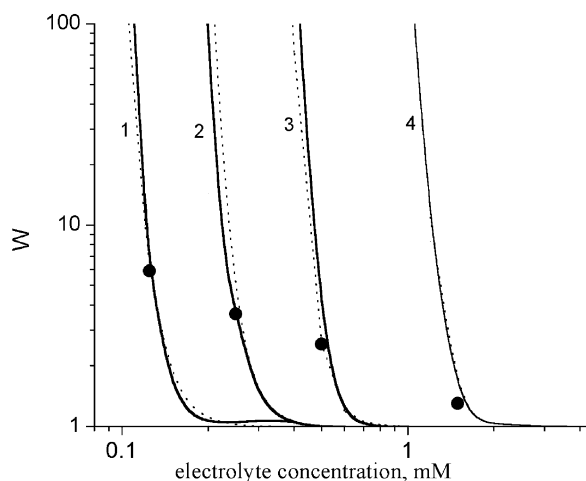
(18) Hunter, R. J. *Foundations of Colloid Science*; Oxford Science Publications: New York, 1987.

(19) López-León, T.; Jódar-Reyes, A. B.; Ortega-Vinuesa, J. L.; Bastos-González, D. Submitted for publication in *J. Colloid Interface Sci.*





**Figure 2.** Experimental values of the stability ratio of protein-covered latex particle as a function of electrolyte concentration, at pH = 10.0, reported by Lopez-Leon et al.,<sup>1</sup> compared to those calculated from the polarization-based hydration model, for the following parameter values:  $N_A = 1.2 \times 10^{18}$  sites/m<sup>2</sup>,  $N_B = 1.62 \times 10^{18}$  sites/m<sup>2</sup>,  $A_H = 0.9 \times 10^{-20}$  J,  $K_H = 10^{-6}$  M,  $K_{OH} = 8.95 \times 10^{-8}$  M,  $K_{Na} = 0.021$  M,  $(p/\epsilon')_{Na} = 1.8$  D; (1)  $K_{Cl} = 0.76$  M,  $(p/\epsilon')_{Cl} = -2.3$  D; (2)  $K_{NO_3} = 0.62$  M,  $(p/\epsilon')_{NO_3} = -1.8$  D; stars, NaNO<sub>3</sub>; squares, NaCl.



**Figure 3.** Stability ratios at low concentrations of NaSCN at pH = 10. The calculations have been carried out for  $N_A = N_B = 0.5 \times 10^{18}$  sites/m<sup>2</sup> and various dissociation constants  $K_{Na}$  based on the present model (thick lines) and on the DLVO theory, with different values for  $K_{Na}$  (dotted lines), predict almost identical results but varies much more rapidly with electrolyte concentration than the experimental values reported in ref 1 (circles).  $K_H = K_{OH} = 10^{-6}$  M,  $(p/\epsilon')_{Na} = 1.8$  D,  $K_{SCN} = 0.8$  M,  $(p/\epsilon')_{SCN} = -0.8$  D,  $A_H = 0.1 \times 10^{-20}$  J. (1) polarization model,  $K_{Na} = 12.5 \times 10^{-6}$  M; DLVO,  $K_{Na} = 2.5 \times 10^{-6}$  M. (2) polarization model,  $K_{Na} = 23.1 \times 10^{-6}$  M; DLVO,  $K_{Na} = 5.83 \times 10^{-6}$  M. (3) polarization model,  $K_{Na} = 54.7 \times 10^{-6}$  M; DLVO,  $K_{Na} = 12.6 \times 10^{-6}$  M. (4) polarization model,  $K_{Na} = 164 \times 10^{-6}$  M; DLVO,  $K_{Na} = 42 \times 10^{-6}$  M.

= 10. One can see that this ratio varies extremely slowly over a wide range of electrolyte concentrations. The low values of electrolyte concentrations, at which relatively rapid coagulation occurs (e.g.,  $1.25 \times 10^{-4}$  M), suggest a low surface charge density. Therefore, in the calculations based on the polarization model (solid lines), the values  $N_A = N_B = 0.5 \times 10^{18}$  sites/m<sup>2</sup> were selected, and the value of the association constant for Na,  $K_{Na}$ , was varied to fit at least one experimental point, all the other parameter values remaining unchanged. All the calculations predict a stability ratio that varies much more rapidly with the ionic strength than the experimental one. It is

therefore unlikely to find a set of parameters that can reproduce accurately this experimental behavior.

At low ionic strengths, the polarization model predicts results which are qualitatively similar to those obtained from the traditional DLVO theory. The main differences are due to the different boundary conditions, generated by the surface dipoles (ignored in the traditional theory). To obtain a quantitative agreement between the two theories, an additional surface charge should be employed in the DLVO calculations, to compensate for the field generated by the surface dipoles. The calculation of the stability ratio was repeated in the framework of the DLVO theory (dotted lines), with the  $K_{Na}$  selected to fit each of the experimental data points. As shown in Figure 3, there is good agreement (at low ionic strengths) between the results of the two treatments, and none of them can predict the slow variation of the stability ratio observed experimentally. It should be noted that at large ionic strengths the results of the two theoretical models are drastically different and that the traditional one cannot predict restabilization.

One might expect the additional interactions, not included in the electrical potential, between the large structure-breaking SCN<sup>-</sup> and interface to be responsible for this effect. However, it was shown that these additional interactions (such as ion-dispersion<sup>16</sup> or ion-hydration<sup>17</sup> forces) are, in general, important only at sufficiently high electrolyte concentrations (above about 0.05 M) but negligible below about 0.01 M. A modality to include in the framework of the polarization model the additional interactions between ions and surfaces, not accounted for by the mean field potential  $\psi$ , was recently proposed.<sup>20</sup> The previous conclusion, that these additional interactions are negligible at low electrolyte concentration,<sup>17</sup> is supported by the new model.<sup>20</sup>

We are therefore inclined to believe that the non-DLVO behavior reported in the recent experiments at low ionic strengths<sup>1</sup> are mainly due to the nonuniformity of the colloidal particles and to the configurational changes of the adsorbed protein, triggered particularly by the added electrolyte. Let us first emphasize that the nonuniformity of the particles affects strongly the stability ratio.<sup>13</sup> The proteins were adsorbed on latex particles at pH = 6, then the pH of the system was changed to either pH 4 or 10 and stored up to 1 week before the light scattering experiments.<sup>19</sup> It was observed that the amount of protein adsorbed decreased with time,<sup>19</sup> and even if the latex particles were uniformly covered initially (which is unknown), one would expect some "voids" to be generated until the light scattering experiments. The stability ratio depends strongly on the coverage of the latex particles at the points of closest approach, and experiment provides only a statistical average over a polydisperse system. The statistical average might vary slowly with the electrolyte concentration, whereas the stability ratio calculated for identical particles (with constant numbers of acidic and basic sites), which are plotted in Figure 3, varies much more rapidly.

An important issue is whether the structure of the adsorbed protein is modified by the addition of electrolyte. The electrolyte is added only at the beginning of the light scattering experiment,<sup>1</sup> and one might expect that during the experiment (about 2 min), and at low electrolyte concentrations, not much modification in the structure of the adsorbed layer can occur. This expectation, however, is probably not accurate. The strong specific ionic effects

(20) Manciu, M.; Ruckenstein, E. *Adv. Colloid Interface Sci.*, **2004**, *112*, 109.

determined experimentally at low electrolyte concentrations, which cannot be explained by the existing theories, can be easily understood if one assumes that each type of electrolyte triggers different structural configurations of the adsorbed protein layer. Moreover, electrophoresis experiments, performed on the same system, revealed a rather low IP for the protein-covered latex particles immersed in low ionic strength electrolyte solutions ( $c_E = 6 \times 10^{-3}$  M).<sup>19</sup> When a protein with an IP  $\sim 7$  is adsorbed on a polystyrene particle with an IP  $\sim 10.5$ , one expects an intermediate IP to result. The experimental values obtained for the IP (between 5 and 6 for the electrolytes used in the experiment)<sup>19</sup> suggest either that many anions collapsed on the surface (perhaps on other places than on the empty basic sites) or that the structure of the protein was in such a manner modified that much more basic sites were exposed to water. A lowering by two units of the IP indicates a quite large change (by orders of magnitude) of the number of surface acidic and basic sites, and such large changes are possible only if structural modifications of the adsorbed layer occur.

It should be noted that the Hamaker constant of the van der Waals interaction between particles is strongly affected by the properties of the external layer of the particles. Because the colloidal particles interact through water, the incorporation of water molecules into the adsorbed protein layer decreases strongly the van der Waals interactions between particles. It was previously reported<sup>21,22</sup> that large decreases in the effective Hamaker constant (by orders of magnitude) could be achieved if water penetrates the external layer of the particles. Therefore, the relatively low value for the Hamaker constant in our calculations might suggest that the layer adsorbed on the latex particle contains a large amount of water.

If the structure of the adsorbed layer is modified during experiment, then a more accurate model is required to take into account the complex structure of the particles. Therefore, one should not expect a perfect match between calculations and experimental results.

An important issue that has to be emphasized is that the experimentally determined dependence of the stability ratio on electrolyte concentration, at low ionic strengths, exhibits (at least for NaSCN) a strongly non-DLVO behavior, in a range in which the DLVO theory is considered fairly accurate. Therefore, we are inclined to believe that the electrolyte (even at low ionic strength) induces indeed structural modifications of the adsorbed protein layer at least near the interface.

Another issue which has to be clarified is the criticism in ref 1 that the hydration/double layer model fails because it assumes an icelike layered structure of water in the vicinity of an interface, while the structure-breaking ions (such as  $\text{SCN}^-$ ) probably destroy this layered structure.<sup>1</sup> First, the hydration/double layer model assumes only correlations between neighboring dipoles, not necessarily an icelike structure. The correlations are perhaps stronger when the water is organized in icelike layers (and might even lead to an oscillatory behavior of the polarization)<sup>23</sup> but do not vanish in less organized structures; they are only weaker. In fact, the value for the dipole correlation length  $\lambda_{\text{TP}}$  was derived assuming that only a cluster of about 5 Å around the central water molecule preserves locally the structure of ice.<sup>7</sup> The influence of disorder on dipole correlation length was discussed elsewhere.<sup>23</sup>

The destruction of the icelike ordering by  $\text{SCN}^-$  might lower the hydration force, although such an effect was not reported for other systems (e.g., neutral lipid bilayers in water). However, the decrease in hydration due to the destruction of the icelike layers cannot explain the experiments of López-León et al.<sup>1</sup> The rapid coagulation at low electrolyte concentrations ( $10^{-4}$ – $10^{-3}$  M) is not due to the decrease in the hydration force: at these ionic strengths, even a small surface charge controls the coagulation. The decrease in the hydration force should affect coagulation at high ionic strength; however, at high ionic strength, the system was restabilized by adding NaSCN.

Furthermore, the restabilization achieved by the addition of NaSCN is even stronger than that obtained through the addition of NaCl. However, the opposite behavior should have been observed, because the stronger structure-breaking  $\text{SCN}^-$  is expected to destroy the icelike structure more than  $\text{Cl}^-$  and, consequently, would lower more the hydration repulsion. Of course, one might suspect that the strong restabilization by NaSCN might be due to an unusually large negative charge generated on the surface by the accumulation of  $\text{SCN}^-$ , but this hypothesis is invalidated by the electrophoretic experiments, which indicated an almost neutral surface at high ionic strengths.<sup>19</sup>

#### 4. Conclusion

It was recently suggested<sup>1</sup> that some of the strong specific anionic effects observed regarding the coagulation of protein-covered latex particles cannot be predicted by a theory of hydration/double layer interaction,<sup>5,7</sup> which accounts for the correlation between neighboring dipoles and the formation of dipoles on the surface.<sup>10,11</sup>

Here it is shown that the above theory can in most cases predict at least qualitatively the experimental behavior. The unusual strong specific anion effects observed for  $\text{SCN}^-$  at low ionic strengths are in disagreement with both the present and the traditional DLVO theory. The present theory is an extension of the traditional theory and predicts qualitatively similar results for sufficiently charged surfaces at low ionic strengths. Notable differences occur between the two treatments either at low surface charges or at high ionic strengths, at which the double layer is screened.

The strong discrepancy between experiment and the traditional DLVO theory at low ionic strengths (where the latter theory is considered to be accurate) cannot be explained by additional interactions between ions and surfaces, because they are negligible below 0.01 M. Therefore, we are inclined to believe that the structural modification of the adsorbed protein by the addition of a structure breaking ion, such as  $\text{SCN}^-$  is mainly responsible for the quantitative disagreement between experiment and model calculations. The nonuniformity of the colloidal particles may be also responsible for the disagreement.

It is well-known that the structure-breaking anions generate strong specific ion effects for proteins (while the type of cations does not matter much).<sup>24</sup> In contrast, the structure-making cations (e.g.,  $\text{K}^+$ ,  $\text{Li}^+$ ,  $\text{Cs}^+$ ) have strong specific effects for the interactions between latex<sup>25</sup> or mica<sup>26</sup> surfaces. An explanation for this apparent paradox might be that the structure-breaking ions are more likely to be

(21) Johnson, R. E.; Morrison, W. H. *Adv. Ceram.* **1987**, *21*, 323.

(22) Ruckenstein, E. *Colloids Surf.* **1993**, *63*, 271.

(23) Manciu, M.; Ruckenstein, E. *Langmuir* **2001**, *17*, 7582.

(24) Cacace, M. G.; Landau, E. M.; Ramsden, J. J. *Q. Rev. Biophys.* **1997**, *30*, 241.

(25) Healy, T. W.; Homola, A.; James, R. O.; Hunter, R. J. *Faraday Discuss. Chem. Soc.* **1978**, *65*, 156.

(26) Pashley, R. M. *J. Colloid Interface Sci.* **1981**, *83*, 531.

expelled from the bulk water toward the surface, whereas the structure-making ions are in general depleted from the interface.<sup>17</sup> The unstable surfaces, such as those of the proteins, which can be easily modified, are particularly sensitive to the ions that can approach them closely, such as  $\text{SCN}^-$ , and modify the water properties near them. In contrast, the interactions between stable surfaces are particularly sensitive to the counterions depletion, because the screening of the double layer is much weaker in this case. Therefore, for negatively charged stable surfaces, one expects strong specific effects for the structure-making cations, such as  $\text{K}^+$ ,  $\text{Li}^+$ , or  $\text{Cs}^+$ . The strong specific effects obtained experimentally for structure-breaking ions,<sup>1</sup> such as  $\text{SCN}^-$ , suggest that the structure of the surface is

modified by the ions; the structural modifications are also expected to occur for multivalence ions, such as  $\text{Ca}^{2+}$ . Therefore, we suggest that the departure of the experimental data reported in ref 1 from the predictions of DLVO theory (at low ionic strengths) and from the polarization model (at all ionic strengths) are mainly due to the structural modification of the adsorbed protein layer, induced by electrolyte. This conclusion is also supported by electrophoretic experiments performed on the same systems, which showed a drastic change in the isoelectric points of the particles, dependent on the nature of the added electrolyte.<sup>19</sup>

LA0481795