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Hofmeister Effects in the Restabilization of IgG−Latex Particles: Testing Ruckenstein's Theory

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The hydration interaction is responsible for the colloidal stability observed in protein-coated particles at high ionic strengths. The origin of this non-DLVO interaction is related not only to the local structure of the water molecules located at the surface but also to the structure of those molecules involved in the hydration of the ions that surround the colloidal particles. Ruckenstein and co-workers have recently developed a new theory based on the coupling of double-layer and hydration interactions. Its validity was contrasted by their fitting of experimental data obtained with IgG−latex particles restabilized at high salt concentration. The theory details the important role played by the counterions in the stability at high salt concentrations by proposing an ion pair reaction forming surface dipoles. These surface dipoles are responsible of repulsive interactions between two approaching surfaces. This paper checks the theory with recent data where some ions associated with the Hofmeister series (NO_3^-, SCN^- and Ca^{2+}) restabilize the same kind of IgG−latex systems by means of hydration forces. Surprisingly, these ions induce stability acting even as co-ions, likely by modifying the water structure at the surface, but not forming surface ion pairs. Therefore, this experimental evidence would question Ruckenstein's theory based on the surface dipole formation for explaining the observed restabilization phenomena.

Introduction

Repulsive hydration forces between hydrophilic surfaces have been extensively observed in many systems for the last 25 years. These forces were first studied between clay surfaces by van Olphen.[1] Israelachvili and Adams[2] were the first who directly measured the hydration forces between two mica surfaces immersed in aqueous KNO_3 solutions. The origin of this force has been associated with the structuring of water in the vicinity of the surfaces. It is a structural force that arises from the local order of water layers adjacent to the surface. It not only is correlated to the hydrophilicity degree of the surface but also strongly depends on the nature and concentration of the hydrated counterions that surround the surface.[3−7] In fact, this force is usually attributed to the hydration shells of (i) adsorbed counterions and (ii) ionic and/or polar functional groups in the surface. Despite its perception as a short-range force, compared to the classical forces accounted in the DLVO theory,[8,9] its effective range goes from 0 to as much as 5−10 nm.[3,10] This "relatively" long-range interaction is believed to arise from a cooperative mechanism of water−water H-bonding.[11] The hydrophilic surface restructures the first adjacent water layer, which in turn modifies the H-bonding network of subsequent water molecules. This cooperative mechanism propagates the hydration forces a few nanometers from the surface. Experimentally, an exponential decay of the hydration interactions has been often found.[2,3,5,12−15] The decay length is in the 0.2−1.1 nm range,[16] while the preexponential factor varies by more than an order of magnitude, depending on the hydration of the surface.

Regarding hydration in colloidal dispersions, the effects of different electrolytes on the hydration forces between colloidal particles can determine whether they will coagulate or not. Highly hydrophilic surfaces (e.g., silica, lecithin)[3,17] cannot be coagulated by changing the ionic conditions. Less hydrophilic surfaces, for example, polystyrene particles totally covered by globular proteins, show destabilization with increasing ionic strength, as predicted by the DLVO theory, but restabilization at sufficiently large electrolyte concentrations.[7,15,18,19] The role played by the presence of hydrated ions at the solid/water interface is crucial in the restabilization process. Cations, but not anions, have been usually considered responsible for the hydration restabilization when they act as counterions. Actually, the hydration numbers are bigger in cations than in anions. It has been experimentally confirmed that the hydration forces increase with the hydration number of the cations.[3]

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There have been several theoretical approaches to included the hydration interaction in the colloidal stability framework. Most of them are based on adding a "hydration" term (V_H), given by an exponential equation, to the usual repulsive (V_R) and attractive (V_A) terms shown by the DLVO theory.[20,15] V_R considers the repulsive interactions coming from the overlapping of the double layers, while V_A is related to the attractive van der Waals interactions. Thus, the total interaction can be described by the algebraic sum of three potentials

$$V_T = V_A + V_R + V_H \tag{1}$$

Nevertheless, Ruckenstein et al.[21−25] have recently demonstrated that this simple equation would be incorrect, as double layer and hydration interactions are interrelated and they cannot be considered independent terms. These authors demonstrated an elaborated theory based on a set of coupled equations dependent on the polarization of water molecules, where the electric fields generated by the surface charges and surface dipoles, as well as the field due to the neighboring dipoles in structured water layers, were taken into account. These equations were satisfactorily used in refs 24 and 25 to explain the restabilization phenomena observed at large ionic strengths for colloidal particles covered by IgG molecules shown in ref 18. The average field and polarization generated by the dipoles on the surface, which act on the first water layer, is the cornerstone of the theory. This polarization is transmitted from the surface to the liquid medium through dipole−dipole interactions giving a gradient of polarization. The gradient polarization, related to the water structure, affects via Poisson equation the macroscopic electric field and, hence, the double layer interaction. According with this theory, the restabilization of IgG−latex complexes is triggered by the formation of surface dipoles, which are originated by the formation of ion pairs (dipoles) between fixed negative charged groups on the surfaces and the cations that act as counterions. It must be pointed out that this theory combines simultaneously the two parameters affecting the hydration forces: the water structure and the presence of ions.

On the other hand, it is worth mentioning that there are a plethora of phenomena associated to the correlation between ions and water structure. Over a century ago, Hofmeister and Lewith[26,27] published accounts of great differences between the minimum concentration of various salts required to precipitate a given protein from solution. The specific ionic effects are nonnegligible at all, and they are always present in intermolecular or surface interactions. Polymer cloud points, protein solubility, chromatographic selectivity, critical micelle concentrations, etc., are dependent on how the ions specifically interact and/or structure the surrounded water molecules. Many ions have been ranked in sequences called *Hofmeister series*, depending on their specific effects (usually referred to as *Hofmeister effects*) on different experimental systems. Despite the broad spectrum of results existing at this point, the molecular origin of the Hofmeister effects still remains unraveled, as shown in comprehensive reviews.[28,29] Whatever the molecular mechanism is subjacent, they do emerge from a combination of effects in which the water structure and/or specific ionic adsorption or exclusion from a surface are the cornerstones.[30] Therefore, the Hofmeister effects and the hydration forces just discussed above must be related. However, there is a controversial point. The Hofmeister effects are dominated by anions instead of cations,[28] while hydration forces between interfaces are associated mainly to cations.[3] To elucidate this controversial item, we very recently designed different experiments where anions associated to the Hofmeister series were used to restabilize latex particles covered by IgG at high ionic strength.[31] Restabilization induced by anions was found, and to our knowledge, these experiments become the first report where anions are responsible for hydration forces in this kind of system. This is a clear example that corroborates the interrelation between the Hofmeister effects and hydration interactions.

The goal of the present paper is, therefore, to apply the theory developed by Ruckenstein et al.[21−25] to colloidal restabilization phenomena given by some Hofmeister anions. As previously commented, Hofmeister effects are associated with molecular mechanisms related to water structure and/or ion adsorption or exclusion. Ruckenstein's theory also is based on the same points. So, it could be interesting to test this theory with novel results concerning colloidal restabilization.

Materials and Methods

NaCl, NaNO₃, NaSCN, and Ca(NO₃)₂ salts were of analytical grade and purchased from Sigma and Scharlau. Deionized water was used throughout. Freshly nonbuffered solutions were obtained when required by adding HCl or NaOH to water in the desired quantity to get pH 4 or pH 10, respectively.

Polystyrene latex particles were synthesized, cleaned, and characterized in our laboratories. Details are given in ref 30. Size and surface charge density data are briefly described below. The mean diameter, obtained by transmission electron microscopy, was 320 ± 15 nm, having a polydispersity index extremely close to unity (1.007). It showed an anfoteric nature with positive charge in the pH 4−10 range and had an isoelectric point (iep) at pH equal to 10.5. Consequently, the positive surface charge density (σ_0) was not constant. The σ_0 values at the two pHs tested in the present work were equal to 18.3 ± 1.2 $\mu C/cm^2$ at pH 4 and 4.0 ± 0.6 $\mu C/cm^2$ at pH 10, respectively.

Polyclonal IgG from rabbit was obtained, purified, and kindly donated by Biokit S.A. (Spain). The isoelectric point determined by isoelectric focusing was in the 6.1−8.5 range. An excess of protein was added to the latex particles in order to cover the polystyrene surface with IgG as much as possible. The protein loads of the latex particles were around 6 mg/m². Details are given in ref 31.

Particle aggregation studies were carried out using a low-angle light-scattering technique. Scattering light intensity was observed at 10° for 120 s. The scattering cell was rectangular with a 2 mm path length. Equal volumes (1 mL) of salt and IgG−latex solutions were mixed and introduced into the cell by an automatic mixing device. The light scattered at 10° behaves linear for the first steps of the coagulation. The stability ratio, also called Fuch's factor (W), is a criterion broadly used to study the stability of colloidal systems. It can be obtained by the following expression

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$$W = k_r/k_s \tag{2}$$

in which the rate constant "k_r" corresponds to rapid coagulation kinetics and "k_s" is the rate constant for the slow coagulation regime. The scattered light intensity at low angles increases linearly with time during a coagulation process.[32] Thus, the coagulation rate constants can be easily obtained from the slopes. The critical coagulation concentration "CCC", defined as the minimum salt concentration needed to rapidly aggregate the colloidal system, can be obtained by plotting the logarithm of W versus the logarithm of the salt concentration and locating that point where log W reduces to zero. Likewise, the critical stabilization concentration (CSC) is defined as the minimum salt concentration at which restabilization starts.

The Basic Equations

It should be noted that the following theoretical framework must be thought of as a very brief summary. Complete information can be found in refs 23 and 24. It should be also noted that the vectorial notation of vectors has been intentionally removed for the sake of clarity, as only one spatial dimension is considered.

Traditional double-layer theory combines the Poisson equation with the assumption that the polarization is proportional to the macroscopic electric field. However, this is only applicable when the electric field is sufficiently uniform and, hence, when the polarization gradient is sufficiently small. When this condition is not satisfied, the polarization gradient generates an additional electric field, because the fields acting on a water molecule due to the neighboring water dipoles do not compensate each other any longer. Actually, a large polarization gradient is generated in an aqueous electrolyte solution, either when the surface charge density is large and/or when there are dipoles on the surfaces. Ruckenstein's theory assumes that near a surface the water is composed of a succession of icelike layers. The electric field E_p that acts on a water molecule and that is generated by the neighboring structured water molecules through their dipoles can be approximately expressed as[23,24]

$$E_p \approx C_1 \Delta^2 \frac{\partial^2 m}{\partial z^2} \tag{3}$$

where Δ is the distance between the centers of two adjacent water layers, $m(z)$ is the dipole moment as a function of z, which is the normal component of the distance, and C_1 is given by

$$C_1 = 1.827/4\pi\epsilon_0\epsilon''l^3 \tag{4}$$

l is the distance between the centers of two adjacent water molecules and ϵ'' is the dielectric constant for the interaction between neighboring molecules, taken as unity in the present calculations. It should be noted that only the fields of the neighboring dipoles are important because those caused by more remote ones are screened. This is why the electric constant is unity between two adjacent dipoles but very large for the remote ones.

On one hand, by introducing the field E_p (eq 3) in the traditional Lorentz−Debye theory and relating the dipole moment m with the local electric field via the water polarizability, one finally obtains[23,24]

$$m(z) = \epsilon_0 v_0(\epsilon - 1)E(z) + \epsilon_0 v_0(\epsilon - 1)C_1\Delta^2 \frac{\partial^2 m}{\partial z^2} \tag{5}$$

where v_0 is the volume of a water molecule, ϵ is the dielectric constant of water, and $E(z)$ is the macroscopic electric field.

On the other hand, assuming Boltzmann distributions for the ions, one can write

$$\rho = -2c_E e \, \sinh\left(\frac{e\Psi}{kT}\right) \tag{6}$$

where ρ is the volume charge density, c_E the bulk electrolyte concentration, Ψ the electrical potential, and k, T, and e have its usual meaning. Introducing eq 6 in the Poisson equation, this last one becomes

$$\frac{\partial^2 \Psi(z)}{\partial z^2} = \frac{2ec_E}{\epsilon_0} \sinh\left(\frac{e\Psi}{kT}\right) + \frac{1}{\epsilon_0 v_0} \frac{\partial m(z)}{\partial z} \tag{7}$$

Equations 5 and 7 (see Appendix) constitute a system of differential equations for Ψ and m. To obtain the solutions, some boundary conditions must be set. These conditions, described in ref 24, are based in electroneutrality and symmetry impositions when two surfaces approach each other. The key for solving the system of eqs 5 and 7 is given by the average electric field E_s generated by dipoles on the surface and that is acting in the first water molecules. It affects the boundary conditions and it is indirectly used as the fitting parameter to explain the experimental results. Its expression is given by[22]

$$E_s = \left[\frac{(p/\epsilon')}{2\pi\epsilon_0\left(\frac{A}{\pi} + \Delta'^2\right)^{3/2}}\right] \tag{8}$$

where p is the normal component of the dipole moment of the surface dipoles, ϵ' is the local dielectric constant of the medium, A is the surface area per dipole of the surface ($1/A$ is the surface dipole density), and Δ' is the distance between a surface dipole and the center of the first layer of water molecules. As will be shown afterward, (p/ϵ') and A will be actually the two fitting parameters of this theory, while many other parameters will be set at constant values.

Once the system of nonlinear equations is numerically solved, the potential interaction between two colloidal particles can be obtained from the free energy of the system per unit area F, which contains three contributions

$$F = F_{chem} + F_{ele} + F_{entropy} \tag{9}$$

where F_{chem} is the chemical, F_{ele} the electrostatic, and $F_{entropy}$ the entropic contribution. Their values depend on $E(z)$, $\Psi(z)$, and $m(z)$, and their analytical expressions are given in refs 33, 24, and 34, respectively. By using the Derjaguin approximation, the repulsive interaction energy between two identical spheres of radius a is given by

$$V_R = \pi a \int_{H_0}^{\infty} (F - F_\infty) \, dH \tag{10}$$

where H_0 is the minimum distance between the surfaces of two approaching spheres and F_∞ is the repulsive free energy per unit area between two infinitely separated

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plates. The van der Waals attractive interaction between two spherical particles has the form[35]

$$V_A =$$
$$-\frac{A_H}{6}\left[\frac{2a^2}{H(4a+H)} + \frac{2a^2}{(2a+H)^2} + \ln\frac{H(4a+H)}{(2a+H)^2}\right] \quad (11)$$

A_H being the Hamaker constant.

The total interacting free energy between the two particles is therefore given by

$$V_T = V_A + V_R \quad (12)$$

The main difference between this last equation and eq 1 is that hydration interactions and the electrical term associated to the overlapping of the double layers are now dependent. The surface dipoles polarize the nearby water molecules, which in turn polarize the next layer and so on. Thus, water and ionic effects are simultaneously considered in this theory in the V_R term.

The experimental value of the stability ratio W is easily accessible through aggregation experiments monitored by optical devices (see eq 2). To be able to compare theory and experiments, a theoretical expression for such a factor is required. The stability ratio is related to the interaction potential via the expression[36,37]
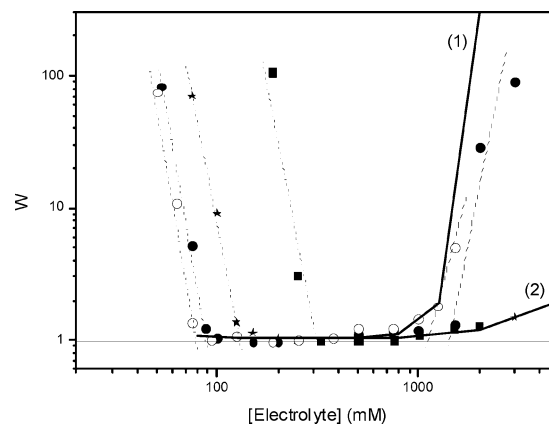
$$V_R = \frac{\int_0^\infty \frac{\beta(u)}{(u+2)^2}\exp\left(\frac{V_T}{kT}\right)du}{\int_0^\infty \frac{\beta(u)}{(u+2)^2}\exp\left(\frac{V_A}{kT}\right)du} \quad (13)$$

where β is a hydrodynamic correction factor and $u = H_0/a$. For the sake of simplicity, the hydrodynamic factor has been taken as unity.

Finally, as our colloid particles are covered by protein, some parameters related to the IgG molecules are needed to definitively quantify the theoretical value of W. They are the surface density of basis sites (N_B), the surface density of the acidic sites (N_A), and the acidic constant of the acidic sites (K_H). Although they are not known, Ruckenstein et al.[24] assumed some constant values that match in typical experimental ranges. These variables control the value of the surface charge density, that is, a parameter that appears in the boundary conditions used to solve the system of equations (5 and 7). In addition, authors mentioned above consider that surface dipoles are formed through the adsorption of counterions, forming the following ion pairs

$$S_A^- + C^+ \rightarrow S_A-C$$

$$S_B^+ + A^- \rightarrow S_B-A$$
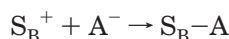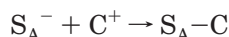
where S_A^- is a dissociated acidic site of the surface, C^+ is a cation, S_B^+ is a dissociated basic site, A^- is an anion, and S_A-C and S_B-A are the corresponding ion pairs. The first reaction is proposed for negatively charged surfaces and the second one for positive. These authors quantify this surface dipole formation proposing an equilibrium constant (K_C for a cation union and K_A for anions), which is used as a fitting parameter in their theory. Actually, the

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Figure 1. Stability ratio (Fuch's factor) versus electrolyte concentration for IgG−latex complexes buffered at pH 4: NaSCN (★), NaNO₃ (○), NaCl (●), and Ca(NO₃)₂ (■). Dotted and segmented lines are used only to guide the eye to get the CCC and CSC values, respectively. Solid lines correspond to theoretical predictions using K_A and (p/ϵ') as the fitting parameters. $(p/\epsilon') = 3$ D; (1) $K_A = 0.8$ M; (2) $K_A = 1.4$ M.

value of K_C (or K_A) controls the value of the dipole density on the surface ($1/A$), which in turn, and together with p/ϵ', governs the E_s value via eq 8. As previously commented, the E_S value is the key for solving the system of eqs 5 and 7 and then for obtaining the theoretical W values.

Results and Dicussions

NaCl, NaNO₃, and NaSCN were chosen to analyze the effect of three anions located in different positions of the Hofmeister series. The specific effects shown by ions usually present an inversion at about Cl⁻ (for anions) and Na⁺ (for cations) in the Hofmeister series. So, these two ions are usually considered as null points in the specific ionic effects. Thiocyanate is a strongly chaotropic ion. That is, it is a weakly hydrated anion that greatly disturbs the order of the surrounding water molecules.[28,29] Nitrate behaves as SCN⁻, although its chaotropic character is less pronounced. Ca(NO₃)₂ was used to analyze the specific effects of calcium comparing the experimental results with those obtained with NaNO₃. As both salts share the same anion, experimental differences must be attributed to the cations. Calcium is strongly hydrated. It belongs to the so-called kosmotropic ions. Its strong interaction with water molecules also modifies the local order of the solvent around it. It should be noticed that the effect of kosmotropic anions is opposite to that of the kosmotropic cations. Although both of them interact with water molecules strongly, the orientation of the oxygen and hydrogen atoms toward the ions surface is opposite due to its difference in charge sign. However, the water structure induced by a chaotropic anion produces results similar to that of a kosmotropic cation, and vice versa.[28,29] This is why SCN⁻ and Ca²⁺ usually show similar specific effects.

The experimental stability ratio W was obtained in aggregation experiments adding independently the chosen salts to samples containing the IgG−latex particles. Figure 1 shows the results obtained at pH 4, at which particles exhibit positive charge. The results obtained at pH 10, particles showing a negatively charged surface, are plotted in Figure 2. The corresponding CCC and CSC values are given in Table 1. As shown, restabilization phenomena at high salts concentrations (not explained by the DLVO theory) are observed at both pH values. These patterns are a consequence of the hydration forces. This can be confirmed taking into account that the ions that most change the water structure, that is those located at the
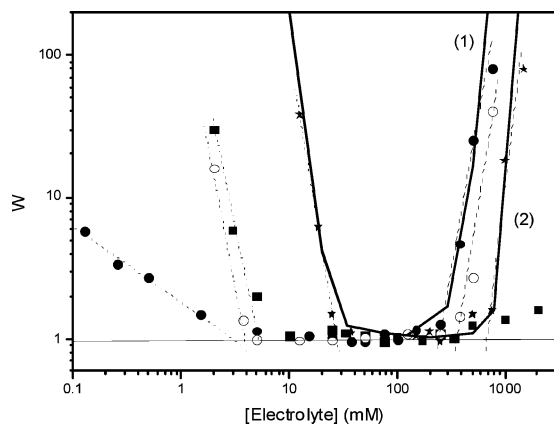
Figure 2. Stability ratio (Fuch's factor) versus electrolyte concentration for IgG−latex complexes buffered at pH 10: NaSCN (★), NaNO₃ (○), NaCl (●), and Ca(NO₃)₂ (■). Dotted and segmented lines are used only for a guide to get the CCC and CSC values, respectively. Solid lines correspond to theoretical predictions using K_A and p/ϵ' as the fitting parameters. $(p/\epsilon') = 3$ D; (1) $K_A = 0.2$ M, (2) $K_A = 0.5$ M.

Table 1. Critical Coagulation Concentration (CCC) and Critical Stabilization Concentration (CSC) for the IgG−Latex Particles

	IgG-latex at pH 4				IgG-latex at pH 10			
	SCN⁻	NO₃⁻	Cl⁻	Ca²⁺	SCN⁻	NO₃⁻	Cl⁻	Ca²⁺
CCC (mM)	90	130	270	80	4	25	6	4
CSC (mM)	1500			1100	260	700		350

extreme of the Hofmeister series (SCN⁻ and Ca²⁺), show the most restabilizing effects. Surprisingly, not only cations but also anions can induce colloidal restabilization. Thus, these data support the relation between hydration forces and Hofmeister effects. If the 1:1 electrolytes are compared, the observed differences in CCC and CSC values can be only attributed to the specific effects of the anions. As SCN⁻, NO₃⁻, and Cl⁻ specifically modify the surrounding water molecules structure, despite possessing identical valency, only hydration interactions must be responsible for the stability differences, discarding any electrical origin. The structure of water molecules at the surface must be specifically modified by the presence of these ions, causing observable changes between the surface interactions of two approaching particles. A more extensive discussion about this item can be found elsewhere.[30,31]

The goal of this paper actually aims to the application of the theory developed by Ruckenstein and co-workers summarized before. These authors attained to explain the restabilization of IgG−latex particles induced independently by sodium and calcium at high concentrations.[24,25] Restabilization was theoretically explained by considering the adsorption of sodium (or calcium) on a negative surface forming ion pairs. The concentration at which the ion pair formation starts was selected by controlling the value of the equilibrium constant K_C. The adsorption of cations onto negatively charged groups of the surface, which is stimulated by higher salt concentrations, increases the density of surface dipoles. The surface dipoles polarize the nearby water molecules, which in turn polarize the next layer and so on. The force generated by the overlap of the polarization layers of the two plates (hydration force) is responsible for the restabilization of the colloidal system as soon as the salt concentration becomes higher than a critical stabilization concentration. Therefore, the theory combines adsorption of ions and structuration of water (dipole−dipole interactions). Actu-
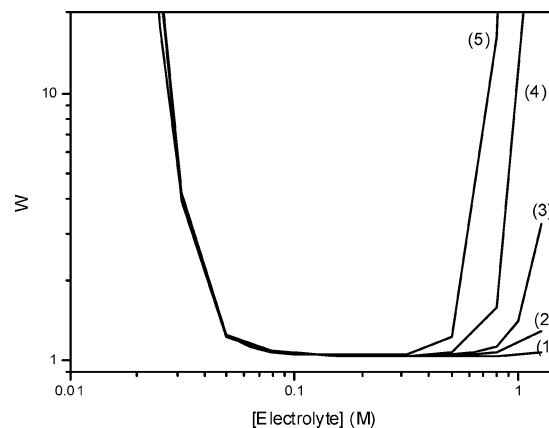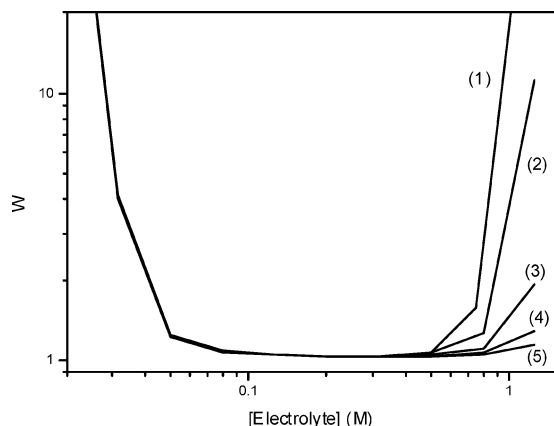

Figure 3. Theoretical stability ratio versus electrolyte concentration: $l = 2.76$ Å, $\Delta = 3.68$ Å, $\Delta' = 1.0$ Å, $N_A = N_B = 2 \times 10^{18}$ m⁻², $K_H = 8 \times 10^{-10}$ M, $A_H = 3.2 \times 10^{-20}$ J, pH 10, $K_A = 1.0$ M; (1) $(p/\epsilon') = 3$ D, (2) $(p/\epsilon') = 5$ D, (3) $(p/\epsilon') = 7$ D, (4) $(p/\epsilon') = 10$ D, (5) $(p/\epsilon') = 15$ D.

ally, these are the same mechanisms usually proposed for explaining Hofmeister effects.

Unfortunately, the theory has many parameters that must be set. Some of them are not known. As an example, Δ (the distance between the centers of two adjacent water layers), l (the distance between the centers of two adjacent water molecules), Δ' (the distance between a surface dipole and the center of the first layer of water molecules), p (the normal component of the dipole moment of the surface dipoles), ϵ' (the *local* dielectric constant of the medium), A_H (the Hamaker constant), N_B (the surface density of basic sites in the IgG molecule), N_A (the surface density of the acidic sites), K_H (the acidic constant of the acidic sites), and K_C (the equilibrium constant for surface dipole formation for cation adsorption). The last four parameters are needed to get a value for $1/A$, which will be responsible for colloidal restabilization. This important drawback of the theory is solved by Ruckenstein et al. keeping constant many of these parameters giving them plausible values and only using (p/ϵ') and K_C (for cation adsorption) as fitting parameters. Then, we will apply their theory using the same procedure, as our experimental system is completely similar to that used by them. However, K_A instead of K_C will be used for explaining the effects caused by the anions. Consequently, most of the following data were taken from refs 21 and 24: $l = 2.76$ Å; $\Delta = 3.68$ Å; $\Delta' = 1.0$ Å; $N_A = N_B = 2 \times 10^{18}$ m⁻²; $K_H = 8 \times 10^{-10}$ M. $A_H = 3.2 \times 10^{-20}$ J. The data shown in Figures 1 and 2 were theoretically fitted optimizing the values of (p/ϵ') and K_A. Both of them control the E_s and subsequently the W values at high ionic strengths. On one hand, Figure 3 shows the influence of the (p/ϵ') parameter for a given K_A value. On the other hand, Figure 4 shows the K_A effect keeping constant (p/ϵ').

The theoretical description based on surface dipole formation could satisfactorily explain the restabilization at pH 4 (Figure 1) induced by the presence of SCN⁻. Particle surfaces are positive at this acidic pH, and this anion, acting as counterion, could form ion pairs in the surface triggering the stabilizing mechanism previously discussed. However, the theory would fail for justifying the stabilizing effect given by the calcium. In this case, calcium acts as co-ion, and the formation of surface dipoles to restabilize the colloidal particles makes no sense. It should be highlighted that it is the co-ion calcium and not the counterion nitrate that is responsible for this non-DLVO phenomenon, since restabilization did not take place when NaNO₃ was used. The same problem appears

Figure 4. Theoretical stability ratio versus electrolyte concentration: $l = 2.76$ Å, $\Delta = 3.68$ Å, $\Delta' = 1.0$ Å, $N_A = N_B = 2 \times 10^{18}$ m^{-2}, $K_H = 8 \times 10^{-10}$ M, $A_H = 3.2 \times 10^{-20}$ J, pH 10, $(p/\epsilon') = 3$ D; (1) $K_A = 0.5$ M, (2) $K_A = 0.6$ M, (3) $K_A = 0.8$ M, (4) $K_A = 1.0$ M, (5) $K_A = 1.2$ M.

at pH 10 (Figure 2). At this basic pH the particles clearly exhibit a negatively charged surface.[31] Now, both thiocyanate and nitrate acting as co-ions provoke restabilization. The ion pair formation on negative surfaces $(S_A^- + C^+ \rightarrow S_A-C)$, which is one of the cornerstones of the Ruckenstein theory, cannot be the mechanism subjacent to this colloidal stability at high SCN$^-$ and NO$_3^-$ concentrations. Again, the stability cannot come from the counterion (sodium), as the restabilization does not happen when using NaCl. This corroborates that SCN$^-$ and NO$_3^-$ (but not Na$^+$) are the stabilizing ions. At this basic pH, only calcium could form the ion pairs. Therefore, there would be only two cases supporting the Ruckenstein theory (SCN$^-$ at pH 4 and Ca^{2+} at pH 10), while three exceptions are found (Ca^{2+} at pH 4, NO$_3^-$ and SCN$^-$ at pH 10). Therefore, the theory should be revised. It should be noted that considering the ion valency in the theory for application to the calcium experiments complicates the mathematical equations.[25] This additional effort was not made for fitting our data, since (shown) the theory fails to explain restabilization by means of ion pair formation even for the 1:1 electrolytes.

The value of E_s, obtained from the fitting parameters via eq 8, is crucial to control the theoretical behavior of the stability ratio W. Therefore, any modification affecting this equation could be planned at least as a first approximation. However, the fact that the ions that strongly modify the water structure (those located at the extreme of the Hofmeister series), show higher stabilizing features would question another important point of this theory. The theory is based on a succession of icelike water layers parallel to the surface.[22] However, it is known that these specific ions disrupt the structure of water,[28,29] and thus, it is more than likely that the virtual structured water layers proposed by the theory do not actually exist. This point would also invalidate the theory for explaining the restabilization given by chaotropic and kosmotropic ions at high concentrations.

Conclusions

As shown, the formation of surfaces dipoles can hardly be accepted as an explanation of the colloidal stability of protein−latex particles at high ionic strengths. The hydration interaction must be thought of as coming from changes in the structure of water molecules induced by specific ions. The repulsion that originated for the overlapping of structured water layers when two particles

approach could have different, but interrelated, origins. On one hand, there could be a steric component associated with the rupture of the liquid structure at the surface when two particles collapse. This sterical repulsion not only would account for entropic contributions but also would include the energy needed to dehydrate the ions near the surfaces before contacting. In addition, one could also take into account repulsive solvation forces of the solvent molecules behaving as hard sphere forces.[38,39] In our case, however, these hard sphere forces should be only associated to the ions, but not to the water molecules, as restabilization only occurs in the presence of high salt concentrations, while the system aggregates at intermediate ionic strengths. On the other hand, there could be an electrical component coming from structured dipoles. These orientated dipoles would generate an additional electric field, as shown by Ruckenstein et al., yielding electrical repulsive forces and preventing aggregation. Both mechanisms are not independent as they are based on the local orientation of the water molecules. This is why the hydration interaction could be thought of as electrosteric origin. What can be inferred is that the hydration force is not of a simple nature, and it may be fair to say that it is probably the least understood of all the forces in water.

Appendix

Equations 5 and 7 are basic equations used in this paper. They can be obtained from other elementary equations of physics, as demonstrated below.

Equation 5 of the main text comes from a known relation for dielectric materials[40]

$$\mathbf{D} = \epsilon_0\mathbf{E} + \mathbf{P} = \epsilon_0\epsilon\mathbf{E} \tag{A.1}$$

where "$\mathbf{D}$" is the electric displacement vector, "ϵ_0" the vacuum permittivity, "$\mathbf{E}$" the macroscopic electric field, "$\mathbf{P}$" the polarization of the medium, and "ϵ" the electric constant of the medium.

So, the polarization vector can be expressed as

$$\mathbf{P} = \epsilon_0\epsilon\mathbf{E} - \epsilon_0\mathbf{E} = \epsilon_0(\epsilon -1)\mathbf{E} \tag{A.2}$$

Polarization is defined as the average dipole moment per unite volume

$$\mathbf{P} = \mathbf{m}/v_0 \tag{A.3}$$

Thus, eq A.2 can be written as

$$\mathbf{m} = \epsilon_0 v_0(\epsilon -1)\mathbf{E} \tag{A.4}$$

If only one spatial dimension is considered, this equation reduces to

$$m(z) = \epsilon_0 v_0(\epsilon - 1)E(z) \tag{A.5}$$

Ruckenstein's theory suggests that the electric field acting on a water molecule located at "z" position must include

(38) Qin, Y.; Fichthorn, K. A. *J. Chem. Phys.* **2003**, *119*, 9745.
(39) Henderson, D.; Trokhymchuk, A. D.; Wasan, D. T. *J. Mol. Liq.* **2004**, *112*, 21.
(40) Albella-Martín, J. M.; Martínez-Duart, J. M. *Física de Dieléctricos*; Marcombo: Barcelona, 1984.

the field $\mathbf{E_p}$ generated by the neighboring dipoles. If this new term is added, then

$$m(z) = \epsilon_0 v_0 (\epsilon - 1)[E(z) + E_p(z)] \qquad (A.6)$$

The expression for $E_p(z)$ is shown in eq 3 (see main text) and demonstrated by Ruckenstein et al. in refs 23 and 24. Consequently, finally one obtains

$$m(z) = \epsilon_0 v_0 (\epsilon - 1)E(z) + \epsilon_0 v_0 (\epsilon - 1)C_1 \Delta^2 \frac{\partial^2 m}{\partial z^2} \qquad (A.7)$$

which is the first of the differential equations for Ψ and m, knowing that

$$E(z) = -\frac{\partial \Psi}{\partial z} \qquad (A.8)$$

The second differential equation (eq 7) can be obtained from the Maxwell equations. The Gauss law in differential form says

$$\nabla \cdot \mathbf{D} = \rho \qquad (A.9)$$

where "ρ" is the volume charge density. Taking into account eq A.1, the Gauss law becomes

$$\epsilon_0 \nabla \cdot \mathbf{E} + \nabla \cdot \mathbf{P} = \rho \qquad (A.10)$$

which is the Poisson equation for dielectric materials

$$\epsilon_0 \nabla \cdot \mathbf{E} = \rho - \nabla \cdot \mathbf{P} \qquad (A.11)$$

If only one dimension is considered and multiplying everything by -1, one can write

$$-\epsilon_0 \frac{\partial E}{\partial z} = -\rho + \frac{\partial P}{\partial z} \qquad (A.12)$$

Changing E and P for their corresponding expressions, that is, using eqs A.8 and A.3, one obtains

$$\frac{\partial^2 \Psi}{\partial z^2} = -\frac{\rho}{\epsilon_0} + \frac{1}{\epsilon_0 v_0} \frac{\partial m}{\partial z} \qquad (A.13)$$

If a Boltzmann distribution for the ions is assumed (see eq 6 in the main text), the sought equation is finally obtained

$$\frac{\partial^2 \Psi}{\partial z^2} = \frac{2ec_E}{\epsilon_0} \sinh\left(\frac{e\Psi}{kT}\right) + \frac{1}{\epsilon_0 v_0} \frac{\partial m}{\partial z} \qquad (A.14)$$