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Colloidal stability of Pluronic F68-coated PLGA nanoparticles: A variety of stabilisation mechanisms

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Abstract

Poloxamers are a family of polypropylene oxide (PPO) and polyethylene oxide (PEO) tri-block copolymers that are usually employed in the micro- and nanoparticulate engineering for drug delivery systems. The aim of this work is to study the electrophoretic mobility (μ_e) and colloidal stability of complexes formed by adsorbing a poloxamer (Pluronic F68) onto poly(D,L-lactic-*co*-glycolic acid) (PLGA) nanoparticles. A variety of stabilisation mechanisms have been observed for the Pluronic-coated PLGA nanoparticles, where DLVO interactions, solvent–polymer segment interactions and hydration forces play different roles as a function of the adsorbed amount of Pluronic. In addition, the μ_e and stability data of these complexes have been compared to those obtained previously using a PLGA–Pluronic F68 blend formulation. As both the μ_e and the stability data are identical between the two systems, a phase separation of both components in the PLGA–Pluronic blend formulation is suggested, being the PLGA located in the core of the particles and the Pluronic in an adsorbed shell.
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1. Introduction

The use of adsorbing macromolecules to modify the aggregation state, sedimentation behaviour, and rheological properties of colloidal dispersions represents an industrially significant, although largely empirical, technology [1]. The pharmaceutical industry also develops and works with colloidal systems for drug delivery purposes. In this case, any successful pharmaceutical application requires adjustment of the surface properties of the polymeric drug delivery system to be compatible with the biological environment. Thus, the use of biocompatible macromolecules adsorbed onto biodegradable nanoparticles is necessary, not only to avoid spontaneous particle aggregation under certain physico-chemical conditions of pH, ionic strength and temperature, but also to prevent the rapid uptake of intravenously injected particulate drug carriers by the cells of the reticuloendothelial system [2]. It has been proven that surface modification of the carriers by adsorption of non-ionic amphiphilic macromolecules (i.e., poloxamers, poloxamines or PEG derivatives) helps to overcome such a drawback [3]. In addition, the presence of these macromolecules in the drug carrier composition may also help to improve the release of the encapsulated materials (drugs, proteins, DNA, and so on) and even protects the proteins encapsulated into the carriers against partial or total denaturation [4,5]. This protective action can be explained as follows. The PLGA degradation is governed by hydrolytic processes and leads to the formation of acidic oligo- and monomers that cause an acidic microclimate. The use of protective excipients (i.e., polaxamers and poloxamines) could possibly prevent unwanted interactions between the drug and the PLGA as well as neutralise the acidity generated in the course of polymer degradation.

Although adsorption of this kind of surfactants is the most wide known procedure to modify the surface characteristics of

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the primitive carriers, the incorporation of these copolymers into the particles during the manufacturing process has become an alternative strategy. Successful incorporation of polypropylene oxide–polyethylene oxide (PPO–PEO) copolymers into poly(D,L-lactic-*co*-glycolic acid) (PLGA) particles has been recently reported [5,6]. The extent of incorporation depends strongly on both the hydrophobic/hydrophilic degree of the carrier matrix and the hydrophilia–lipophilia balance (HLB) values of the PPO–PEO-derivatives. Therefore, whereas the incorporation of surfactants with high hydrophilicity (high HLB values) into hydrophobic nanoparticle matrixes is probably limited, an effective mixture and homogeneous distribution would be expected for surfactants with large hydrophobic and short hydrophilic moieties (low HLB values). This statement have been recently confirmed by Kiss et al. [7,8] who have studied the distribution of different poloxamers (Pluronics) into poly(lactic acid) (PLA) and PLGA blend films.

In the present work, the adsorption of Pluronic F68 on PLGA nanoparticles has been studied and, subsequently, the electrophoretic mobility (μ_e) and colloidal stability of these complexes have been analysed. As will be shown, different stability mechanisms have been observed as a function of the surfactant coverage, which suggests different spatial conformations of the poloxamer molecules in the adsorbed layer. In addition, a comparison between the μ_e and stability of our complexes with those of other particles obtained in another work [9] (where they were manufactured by simultaneously mixing PLGA and Pluronic F68 during the formulation of the particles) is presented. As electrophoretic mobility and colloidal stability are exclusively dependent on the surface characteristics, the comparison may help us to gain an insight into the exact location of the polyoxide copolymers in the blend formulations. That is, if pure PLGA particles covered by Pluronic and blended PLGA–Pluronic particles present similar mobility and stability properties, it could be inferred that, in the blend formulation, a large part of the poloxamer must be located on the particle surface.

2. Materials and methods

2.1. Materials

The polymer poly(D,L-lactic acid/glycolic acid) 50/50 (PLGA) was purchased from Boehringer–Ingelheim, under the commercial name of Resomer RG 503. Its average molecular weight was 35,000 Da. The poloxamer Pluronic F68 was obtained from Sigma Aldrich. It is a polyoxyethylene–polyoxypropylene–polyoxyethylene type polymer (see Fig. 1) with a molecular weight equal to 8500 Da. All other solvents and chemicals used were of the highest grade commercially available. Buffered solutions presented a constant ionic strength of 0.002 M.

2.2. Preparation of PLGA nanoparticles

The PLGA nanoparticles were prepared by a modified emulsion-solvent diffusion technique. First, 50 mg of PLGA was dissolved in 2 ml of dichloromethane and this organic

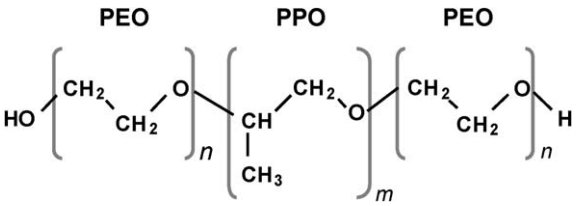

Fig. 1. Chemical structure of a poloxamer. It presents a central hydrophobic fragment of polyoxypropylene (PPO) and identical hydrophilic chains of polyoxyethylene (PEO) at both sides. For the Pluronic F68, $n = 75$ PEO units and $m = 30$ PPO units.

solution was mixed for 30 s with 0.2 ml of pure water by vortex (2400 min^{-1}, Heidolph). This first volume of water is in pharmaceutical applications used to dissolve drugs to be incorporated in the particles. Then, the obtained emulsion was poured under moderate magnetic stirring onto a larger polar phase (25 ml ethanol), leading to immediate polymer precipitation in the form of nanoparticles. This sample was diluted with 25 ml MilliQ water and the stirring was maintained for 10 more minutes. Finally, the organic solvents (both ethanol and dichloromethane) were eliminated under vacuum at 30 °C (Rotavapor Büchi R-114, Flawil).

2.3. Adsorption isotherm

The adsorption of Pluronic F68 onto PLGA particles was performed as follows: 7.6 mg of PLGA (with a total surface area of 0.2 m^2) was added to 8 ml of buffered solutions at pH 7 containing different concentrations of poloxamer. These solutions were poured in stoppered cylinders, which were then gently rotated end-over-end and set in cabinets agitated on a rotating plate at constant temperature (25 °C) for 20 h. The adsorbed amounts reach a steady value well within this time [10]. The dispersions were centrifuged at 15,000g for 15 min and the supernatants were analysed with the molybdophosphoric acid reagent [11]. It should be noted that the quantification of the poloxamer concentration by means of this reagent is only reliable for polymer concentrations below its critical micelle concentration (CMC). Consequently, the poloxamer concentration was spectrophotometrically determined using calibration curves constructed in the 0–400 mg/L range, where the errors are usually in the 5–10% range.

2.4. Electrophoretic mobility

The electrophoretic mobility measurements were carried out with a Zeta-Sizer IV (Malvern Instruments). The PLGA–Pluronic particles were diluted in the desired buffered solutions for 10 min just prior to measuring. Final particle concentration was equal to 3×10^9 particles/ml. Then, the mobility data were taken from the average of six measurements at the stationary level in a cylindrical cell.

2.5. Hydrodynamic diameter and colloidal stability

The average size of stable PLGA and PLGA–Pluronic particles was analysed by photon correlation spectroscopy (PCS)

using a 4700c System (Malvern Instruments). The same apparatus was used to study the aggregation of these particles in saline media, using NaCl and Ca_2Cl as aggregating electrolytes. The PCS instrument had an Argon laser ($\lambda_0 = 488$ nm) with a perpendicular polarisation and a power rating of 75 mW. Two millilitres of very diluted samples were poured into a cylindrical cell and 1 ml of the saline solution at the desired ionic strength was then added and rapidly stirred. The scattered intensity autocorrelation function measured at 60° was then analysed by the computer software. The aggregation measurements took about 10 min. Information about the aggregation kinetics was obtained by plotting the average diameter of the particles versus time.

3. Results and discussion

Four sets of experiments were performed to determine different properties of our Pluronic-coated PLGA particles: adsorption isotherm, Pluronic adlayer thickness, electrokinetic behaviour of the complexes versus the pH, and colloidal stability as a function of the electrolyte concentration.

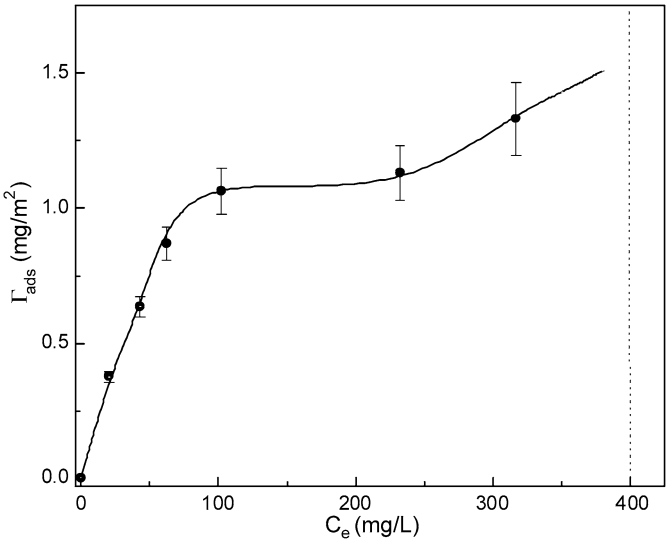

Fig. 2. Adsorption isotherm of Pluronic F68 onto PLGA particles. The adsorbed amount (Γ_{ads}) is plotted versus the poloxamer equilibrium concentration (C_e). Vertical dotted line indicates the Pluronic CMC (400 mg/L).

Adsorption isotherm results are shown in Fig. 2. As expected from the work of Kayes and Rawlins [12], the specific adsorption values for Pluronic F68 reach a clear plateau value at bulk polymer concentrations below its CMC. It is instructive to compare the plateau surface coverage determined in the present work to those reported in literature. Tadros and Vincent [10] and Baker and Berg [13] obtained a plateau adsorption value of approximately 8.5×10^{-4} and 9.5×10^{-4} g/m^2 respectively working with polystyrene particles. These values coincide with our plateau adsorption values (which are around 10×10^{-4} g/m^2), although PLGA instead of polystyrene surfaces are used. Nevertheless, the isotherm suffers a little change just below the CMC. AFM studies [14–18] have shown that surfactants do not always form homogeneous layers at any interface. Actually, non-ionic surfactants can form micellar-like surface aggregates under certain conditions [19,20], presenting different regimes for the formation of surface aggregates. For such surfactants, a step-isotherm is often found, which has also been explained theoretically [21,22]. Therefore, the observed change in the isotherm curve might be caused by an adsorption of hemimicelles onto the PLGA surface, as illustrated in Fig. 3. It should be noted that if experimental errors are taking into account, it is difficult to discern if this last point belongs to a single plateau or if it is out of this plateau. This conflictive point can be solved analysing differences on some colloidal properties associated to the surface characteristics (i.e., electrophoretic mobility or colloidal stability). As will be shown afterward, differences in the colloidal stability exist, and thus, it is more than likely that this last complex has a higher poloxamer coverage than that of the plateau.

The hydrodynamic diameter (∅) was measured by photon correlation spectroscopy for the complexes sensitised below the CMC. Results are shown in Fig. 4. It is possible to obtain the plateau adlayer thickness of the Pluronic F68 comparing the ∅ values of the complexes with those of the bare particles. The calculated thickness was around 20 nm. This value is higher than that reported by Baker and Berg [13] using a 56-nm-diameter polystyrene latex, who obtained a 6-nm thickness for the same tri-block copolymer using the same optical technique. It should be noted that adsorption is driven by hydrophobic forces between the PPO moiety and the adsorbent surface. PLGA is not as hydrophobic as polystyrene, and thus, the adhesion of the surfactant onto the surface may be less

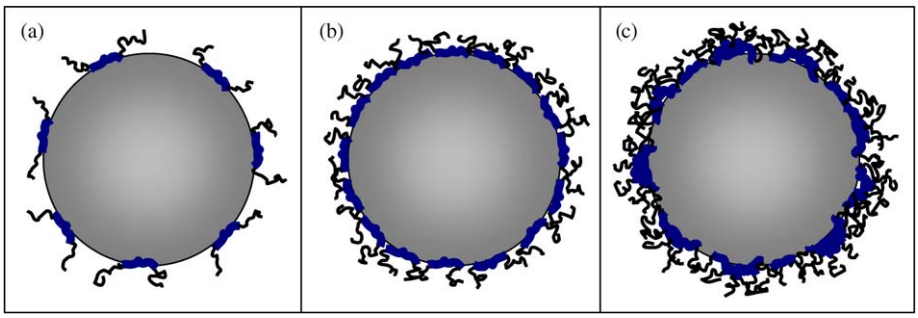

Fig. 3. Scheme of PLGA–Pluronic complexes. Particles and surfactant molecules are represent at different scale. The PPO block anchors the poloxamer to the PLGA surface, while the PEO chains extend to the solution. (a) Low coverage (below the adsorption plateau). (b) Coverage at the adsorption plateau. (c) Hemimicelles adsorbed on the PLGA surface when using Pluronic concentrations around the CMC.

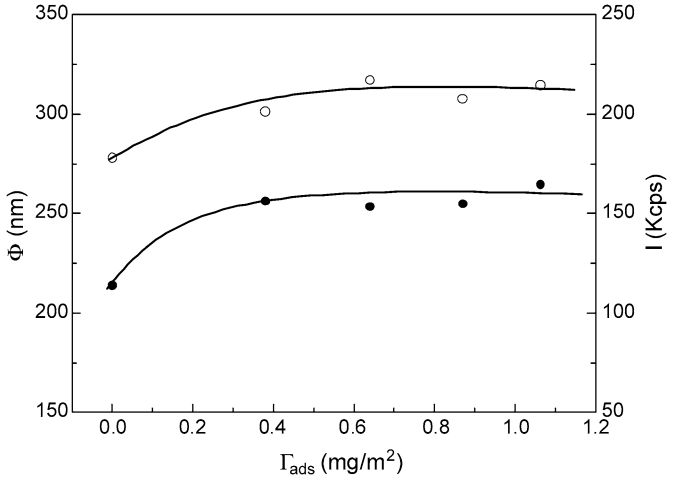

Fig. 4. Hydrodynamic diameter (●) and scattered light intensity (at 60°) (○) of different PLGA–Pluronic complexes.

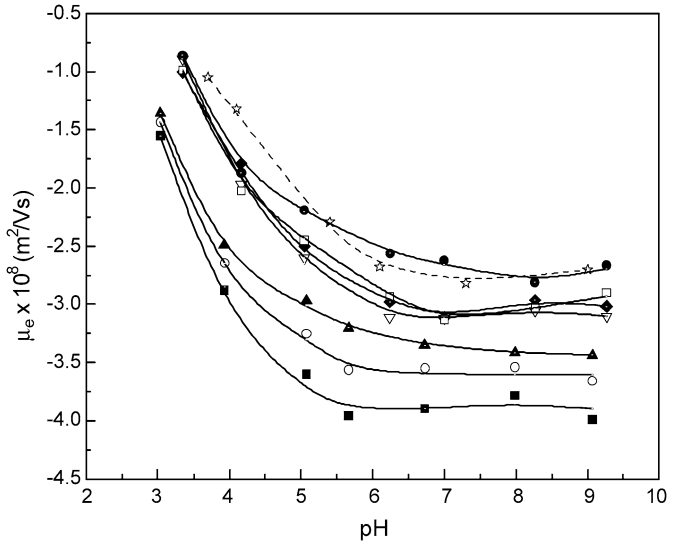

Fig. 5. Electrophoretic mobility versus pH of different PLGA–Pluronic complexes. Bare PLGA particles (■). $\Gamma_{ads} = 0.38$ mg/m^2 (○). $\Gamma_{ads} = 0.87$ mg/m^2 (▲). $\Gamma_{ads} = 1.06$ mg/m^2 (▽). $\Gamma_{ads} = 1.13$ mg/m^2 (◆). $\Gamma_{ads} = 1.33$ mg/m^2 (□). Above the CMC, $\Gamma_{added} = 1.5 \times$ CMC (●). PLGA–Pluronic blend formulation (☆), dash line.

compact in the former than in the latter. This reasoning would explain, at least qualitatively, the above differences.

The electrokinetic behaviour was evaluated by measuring the electrophoretic mobility (μ_e) versus pH at different surfactant coverage. It should be noted that PLGA chains that form the particles have carboxylic groups at the extreme of them. It is more than likely that this charged groups tend to be located in an aqueous environment, that is, at the particle/water interface, creating a surface charge density capable of generating colloidal stability even without adsorbed Pluronic molecules. These weak acid groups explain the electrokinetic behaviour and the colloidal stability of the particles in absence of poloxamer. The (μ_e) results are shown in Fig. 5. Bare particles present a negative mobility value that increases in absolute value up to a certain pH (pH 5.5); this is due to the deprotonation of the superficial carboxyl groups. For pH values above 5.5, a plateau is reached. By adding Pluronic, the mobility of the complex remains negative. However, as a general rule, the mobility value decreases in absolute value by increasing the Pluronic load. The samples with coverage around the adsorption plateau present a similar electrokinetic behaviour, even the last point of the isotherm. However, differences are found when working with complexes obtained at concentrations above the CMC (see 1.5 × CMC data), which can be due to structural changes at the surface. In the case of the complex obtained just below the CMC ($\Gamma_{ads} = 1.33$ mg/m^2), hemimicelles might start appearing on the surface, and the polymer adsorbed amount increases slightly. Nevertheless, changes on the electrokinetic behaviour are not observed when comparing with the data corresponding to the plateau ($\Gamma_{ads} = 1.06$ and 1.13 mg/m^2). Only when the complex is formed at concentrations well above the CMC, the polymer adsorbed amount on the surface has reached its maximum value, which gives rise to differences in the electrokinetic behaviour that could come from structural changes of the adsorbed poloxamer chains. Mobility comes from a combination of electrical and frictional forces. The adsorption of this non ionic surfactant onto the PLGA particles affects both forces, as detailed below. The absorbed non-ionic Pluronic layer partially screens the surface charge of the PLGA particles, and thus, reduces the ζ-potential. The adsorption also changes a smooth PLGA surface into a rough one (with extended PEO chains into the solution) [23], which shifts the shear plane outward, diminishing the ζ-potential and causing an increase in the hydrodynamic friction when particles are in motion. Both effects simultaneously cause a decrease in mobility by increasing the surfactant coverage, as experimentally observed. Finally, the mobility curve of a PLGA–Pluronic F68 sample obtained by blending both components during the particle synthesis process [9] is also shown in Fig. 5 (dashed line). The electrokinetic behaviour of this last sample resembles that of pure PLGA particles totally coated by adsorbed Pluronic F68. This feature would support that in a binary mixture of PLGA and Pluronic F68 (50:50 w/w) there must be a phase separation where PLGA forms the hydrophobic core of the particles and the poloxamer tends to accumulate at the solid/water interface. It is noteworthy that some theoretical considerations based on the Kiss et al.'s studies [7,8] support the following hypothesis: nanoparticles obtained by blending Pluronic F68 and PLGA (50/50), (adding the same mass of both components during the manufacturing process), are formed by two phases: a core rich in PLGA and an external part consisting of an adhered poloxamer shell. If this heterogeneous distribution occurs, the electrokinetic behaviour of the blend formulation particles and those obtained by adsorbing the poloxamer onto a PLGA core should coincide, as experimentally found.

The colloidal stability of the complexes was analysed as ionic strength was increased, using independently NaCl and $CaCl_2$ as aggregating electrolytes. Stability was evaluated by calculating the Fuchs factor (W) [24] versus the salt concentration from aggregation processes monitored by an optical technique. If $W = 1$, the system is completely unstable, while $W = \infty$ indicates total stability. Details about how to get experimental W values can be found elsewhere [25]. Figs. 6a

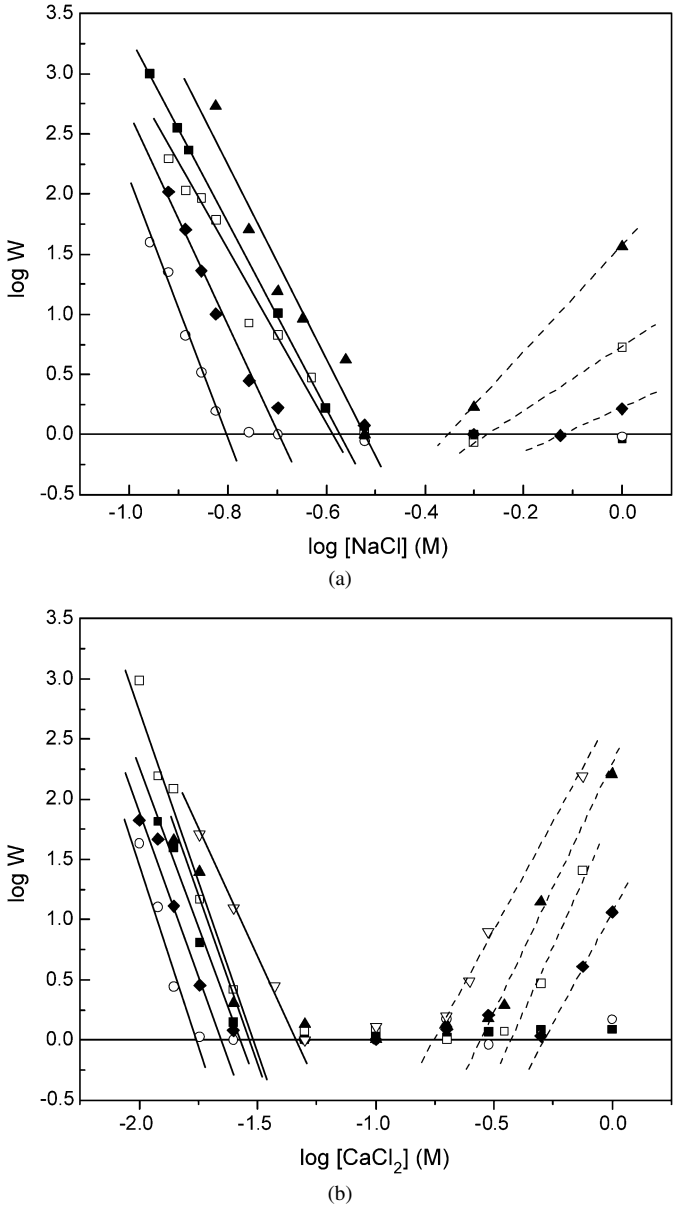

Fig. 6. Stability factor versus NaCl (a) and $CaCl_2$ (b) concentration. Bare PLGA particles (■). $\Gamma_{ads} = 0.38$ mg/m^2 (○). $\Gamma_{ads} = 0.64$ mg/m^2 (◆). $\Gamma_{ads} = 0.87$ mg/m^2 (□). $\Gamma_{ads} = 1.06$ mg/m^2 (▲). $\Gamma_{ads} = 1.13$ mg/m^2 (▽).

and 6b show the results obtained with NaCl and $CaCl_2$, respectively. Stability results for those complexes obtained by adding poloxamer at concentrations above the CMC are not shown, as these complexes were completely stable at any salt concentration. This extreme stability was also found with the last point of the isotherm (see Fig. 2), but not with the other complexes located at the plateau. Therefore, differences in the poloxamer coverage actually must exist between the former and the latter. In addition, the total stability shown by the particles coated with poloxamer at concentrations above the CMC also coincides with that found for the PLGA–Pluronic blend formulations [9], and it is caused by a steric mechanism produced by the external PEO chains that protrude into the solution. It is therefore observed that not only the electrophoretic mobility but also the stability results suggest a heterogeneous distribution of PLGA and Pluronic F68 in blend formulations. In addition, it is worth to highlighting that the PLGA–Pluronic particles show a relevant variety of stability mechanisms, depending on the surfactant coverage. Initially, the bare PLGA particles exhibit a stability behaviour that can be explained by the DLVO theory [24], which is applicable to lyophobic colloids. It is widely known that this theory considers the total interaction potential (V_T) between two approaching particles dependent on two terms, one attractive (V_A) and another repulsive (V_E). V_A corresponds to the interaction energy caused by the van der Waals dispersion forces, while V_E represents the repulsive interaction created by the overlapping of the electrical double layers of the charged particles. The value of this repulsive term depends on the surface electrostatic potential (which in turn depends on the surface charge density of the particles), and it is modulated by the ionic strength of the medium in such a way that the higher the salt concentration, the lower the V_E value. Those interested in reading further about the topic can find the analytical expressions of all these energetic terms in Supplementary material that can be found in the web page of this journal. V_T can be obtained simply by summing V_A and V_E. Thus, the colloidal stability of the bare PLGA particles can be reduced by diminishing the V_E term (and thus V_T) by adding electrolytes, as shown in Fig. 7a. This destabilisation by salt is observed in Figs. 6a and 6b, where W diminishes to unity when salt concentration increases. The critical coagulation concentration (CCC), that is the minimum salt concentration needed to rapidly aggregate the system, can be obtained from these figures locating that point where log W reduces to zero. The CCC values so obtained are shown in Table 1. The screening effect exerted by calcium is more significant than that of the sodium. That explains why the CCC values obtained in presence of $CaCl_2$ are lower than those of NaCl.

If PLGA particles are covered by small amounts of poloxamer, the surface electrostatic potential is partially reduced (in absolute value) when comparing it with that of the bare particles, as some charged carboxyl groups are screened by the surfactant adsorption. This agrees with the electrokinetic results shown in Fig. 5, where the μ_e of the complex (and thus the ζ-potential) decreased by adsorbing Pluronic. In addition, a low surface poloxamer concentration is still not capable of stabilising the system by a steric hindrance mechanism. Actually, Li et al. [23] suggested that a low surface concentration allows a non-extended PEO chain conformation (as depicted in Fig. 3a). All this gives rise to more unstable particles (lower CCC values), as experimentally observed. Nevertheless, if the Pluronic surface concentration is increased, other stabilising mechanisms appear. On the one hand, a crowded surfactant surface exhibits a more extended PEO layer [23] (see Fig. 3b), which favours the steric stabilisation. This can explain that, once the minimum observed in the CCC values is surpassed, the stability increases when the complex coverage increases (see Table 1). However, the most surprising result is obtained at very high salt concentrations, where the system begins to re-stabilise (that is, W increases) for high Pluronic coatings. Neither the DLVO theory nor the steric hindrance of PEO chains predicts this sort of re-stabilisation. This is caused by another stabilisation mechanism

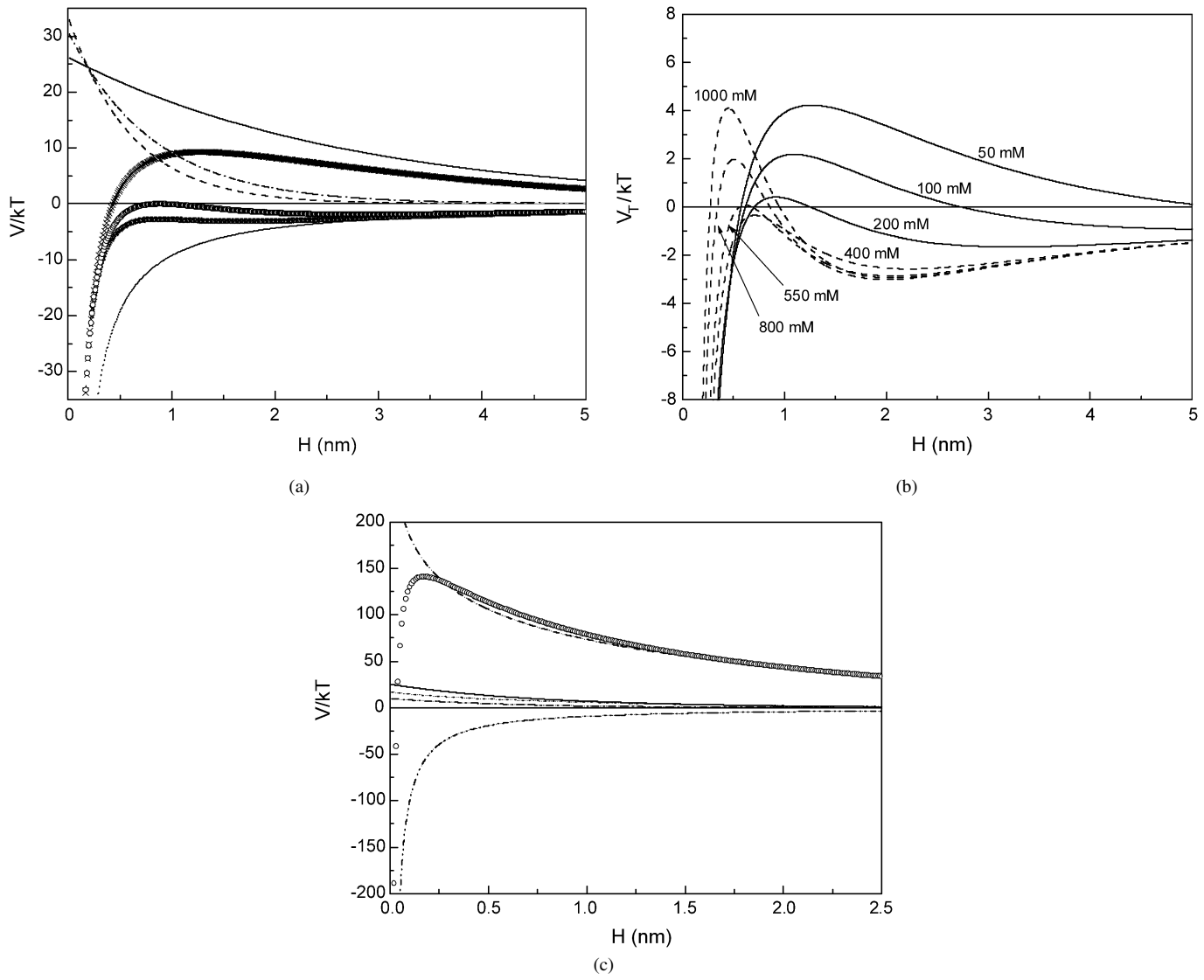

Fig. 7. (a) Interaction potentials versus distance according to the DLVO theory. Dotted line: attractive potential (V_A) using a Hamaker constant $A = 0.5 \times 10^{-20}$ J. Different repulsive potentials (V_E) using a Stern potential $\Psi_\delta = 15.3$ mV at the following NaCl concentrations: 25 mM (solid line), 270 mM (CCC) (dashed–dotted line), and 500 mM (dashed line). Total interaction potentials (V_T) at 25 mM (×), 270 mM (CCC) (○), and 500 mM (☆). (b) Total interaction potential versus distance including the hydration interaction term (V_H). The following parameters were set at the values shown below: $A = 0.5 \times 10^{-20}$ J, $\Psi_\delta = 13.7$ mV, $C_h = 0.2 \times 10^{-20}$ J, and $\lambda = 0.6$ nm [32]. (c) Interaction potentials for PLGA–Pluronic complexes represented in Fig. 3c. Attractive potential (V_A) (dash–dot–dot line). Repulsive potential (V_E) (solid line). Osmotic term (V_{osm}) (dashed line). Elastic-steric repulsion (V_{vr}) (dotted line). Hydration potential (V_H) (dashed–dotted line). Total interaction potentials (V_T) (○). The following parameters were set at the values shown below: $A = 0.5 \times 10^{-20}$ J, $\Psi_\delta = 13.7$ mV, $C_h = 0.2 \times 10^{-20}$ J, and $\lambda = 0.6$ nm [32], $\chi = 0.48$ [34], $\delta = 6$ nm [13], $\phi_2 = 0.01$.

based on repulsive hydration forces, which become significant when great amounts of hydrated ions are accumulated at the proximities of any hydrophilic surface. The physical origin of this type of force has been studied for the last 25 years and has been associated with the structuring of water in the vicinity of hydrophilic surfaces [26]. It is a structural force that arises from the local order of water layer adjacent to the surface. It is not only correlated to the hydrophilicity of the surface but also depends strongly on the nature and concentration of the hydrated counterions that surround the surface [27–29]. There are several theoretical approaches that try to include 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 [30,31], to the usual DLVO V_E and V_A terms, that is: $V_T = V_A + V_E + V_H$. The dependence of this new V_T on the electrolyte (NaCl) concentration has been depicted in Fig. 7b. Therefore, the adsorption of a Pluronic F68 has converted a hydrophobic surface (PLGA) into a hydrophilic one, promoting new stabilising mechanisms at very high salt concentrations. The critical stabilisation concentration (CSC) is defined as the minimum salt concentration at which re-stabilisation by hydration forces starts. These data are also shown in Table 1. As expected, the presence of calcium (a highly hydrated cation) favours the re-stabilisation more than sodium, a less hydrated ion.

Finally, if adsorption of the poloxamer is carried out at concentrations around or higher than the CMC, the complexes obtained in such a manner are completely stable at any salt con-

Table 1
Critical coagulation concentration (CCC) and critical stabilisation concentration (CSC) of different PLGA–Pluronic complexes in presence of NaCl and $CaCl_2$

	Aggregating salt: NaCl		Aggregating salt: $CaCl_2$	
	CCC (mM)	CSC (mM)	CCC (mM)	CSC (mM)
Bare PLGA	270	–	27	–
0.38 mg/m^2	160	–	17	–
0.64 mg/m^2	200	750	22	520
0.87 mg/m^2	260	550	31	370
1.06 mg/m^2	300	440	30	280
1.13 mg/m^2	–	–	45	175

centration. The absence of aggregation phenomena by saline effects suggests that stability must be now controlled by steric interactions. For that reason, other non-DLVO stabilising mechanism appears in the PLGA–Pluronic system. If a layer of structured polymer chains is located at the solid/liquid interface, stability by means of polymer-induced forces may occur. These forces can be enthalpically or entropically driven and are mainly dependent on the nature of the solvent–polymer segment interactions [24]. The steric stabilisation effect usually includes two contributions: osmotic and polymer coil compression [33]. Thus, two new terms can be added to the total interaction potential (V_T). The first one (V_{osm}) considers the osmotic effects that take place when the adsorbed polymer layers of dispersed particles overlap. The local concentration of polymers in the overlap zone exceeds that of the external regions, leading to a driving force for the spontaneous flow of solvent into the interparticle region, which pushes the particles apart. Of course, this potential is highly dependent on the solvent solubility of the polymer chains, represented by χ (the Flory–Huggins solvency parameter). The second term is a restriction volume potential (V_{vr}). If two approaching particles are closer than a distance shorter than the adhered polymer thickness, some polymer molecules will be forced to undergo elastic compression. Thermodynamically, this compression produces a net loss in configurational entropy, which renders repulsion between the particles, and in turn improves the colloidal stability. Fig. 7c reflects a combination of all the aforementioned interaction potentials, where we have used a value of $\chi = 0.48$ for the solvent solubility of the PEO fragments of the Pluronic, which is in line with the calculations reported by Einarson and Berg for a similar polymer [34]. As can be seen, the osmotic contribution exerted by the adsorbed polymer chains immersed in good solvents becomes the most stabilising term. It makes the potential barrier high enough regardless of the salt concentration, yielding extremely stable particles.

It is now possible to use these stability results and the adsorption isotherm data (Fig. 2) to support our hypothesis on the Pluronic layer structure (shown in Fig. 3). That is, the steric stabilisation mechanism only appears in the case of complexes formed above certain surfactant concentration around and above the CMC, where a step in the adsorption isotherm was observed. This result indicates a structural change in the surfactant adsorbed layer (i.e., the formation of surface aggregates that gives a highly enriched polymer layer). On the contrary, below the CMC single Pluronic molecules adsorb on the surface forming a monolayer, and a clear adsorption plateau is obtained. In these cases, no strong steric stabilising phenomena appear.

4. Conclusions

In this work has been demonstrated that both the electrophoretic mobility and the colloidal stability results of PLGA nanoparticles fully coated by Pluronic F68 practically coincide those obtained with PLGA–Pluronic blend formulations. This would suggest a two phase separation of both components in PLGA–Pluronic (50:50 w/w) mixtures, which has also been predicted theoretically analysing the miscibility of these polymers at a molecular scale, where PLGA would form the core and the poloxamer would be located at the particle surface. On the other hand, the Pluronic-coated PLGA nanoparticles have exhibited different stability patterns depending on the poloxamer coverage. At null or low coverage, pure DLVO interactions take place. Intermediate coatings stabilise the system at very high ionic strength by means of hydration forces. Finally, in the case of complexes formed above certain poloxamer concentration just below and above the CMC, strongly stabilising steric mechanisms do manifest. The electrokinetic and stability results together with the adsorption behaviour of the system suggest a structural change in the surfactant layer at high poloxamer concentrations (i.e., the formation of surface poloxamer aggregates).

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Supplementary material

The online version of this article contains additional supplementary material.

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