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First Steps in the Formulation of Praziquantel Nanosuspensions for Pharmaceutical Applications

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Abstract

Praziquantel, a broad spectrum anthelmintic drug, cannot be found in acceptable dosage forms for elderly patients, paediatric patients, and for veterinary use. In fact, very little has been done up to now in the formulation of liquid dosage forms, being they always formulated for parenteral administration. To beat this important challenge, it was accomplished a comprehensive analysis of the influence of two elementary physicochemical aspects, i.e. surface thermodynamic and electrokinetic properties, on the colloidal stability of praziquantel nanosuspensions. The hydrophobic character of the drug, intensely determining the flocculation curves, was confirmed by the thermodynamic characterization. The electrophoretic characterization, in combination with the sedimentation and relative absorbance *versus* time curves, highlighted that the electrical double layer thickness and the surface charge can play an essential role in the stability of the pharmaceutical colloid. Finally, it was demonstrated that controlling the pH values and the incorporation of electrolytes can help in formulating praziquantel aqueous nanosuspensions with appropriate stability and redispersibility behaviours for pharmaceutical use.

Keywords: correlation zeta potential – sedimentation; electrophoresis; human and veterinary liquid dosage forms; paediatric drug formulations; praziquantel; surface thermodynamics.

1. Introduction

Helminths are parasitic worms characterized by a bilateral symmetry and by an elongated, flat, or round morphology. Depending on the specie, the size of these invertebrates varies from millimeters to meters. In the group, nematodes include soil-transmitted helminthes or the so-called intestinal worms commonly responsible for helminthiasis, and filarial worms that are described to cause lymphatic filariasis and onchocerciasis. With respect to platyhelminths, trematodes or flukes (i.e. schistosomes) and cestodes or tapeworms are frequently defined to be responsible for parasitic infections in humans and animals (Hotez et al., 2008; Hotez and Aksoy, 2017). Helminth infestation causes morbidity and mortality, affecting cognitive processes, compromising the nutritional status, inducing tissue reactions and causing intestinal obstruction or rectal prolapse. Preschool children and school-aged children, including adolescents, are commonly infected by intestinal worms and schistosomes, experiencing stunted growth and cognitive and memory impairments (resulting in cognitive and educational deficits) (Crompton and Nesheim, 2002; Hotez and Aksoy, 2017).

The neglected tropical disease Bilharzia, or schistosomiasis, is an acute and chronic parasitic infection that can result in serious damage to internal organs. It is caused by trematodes belonging to the genus *Schistosoma*, principally by *Schistosoma mansoni*, *S. japonicum*, and *S. haematobium* (Lustigman et al., 2012; Raso et al., 2007). The disease is a significant public health issue with severe socio-economic impacts in developing countries (King et al., 2005; Steinmann et al., 2006). The acute phase of the disease is generally asymptomatic, but fever, cough, diarrhoea, a red and itchy rash, and joint and abdominal pains are usually described (Olveda et al., 2013; Ross et al., 2013). On the opposite, acute local inflammatory lesions are reported in chronic schistosomiasis to be produced by the remaining trematode eggs trapped in tissues, while massive infestations are characterized by pseudopolyps in bladder and intestinal light (Colley et al., 2014).

Praziquantel (PZQ) is a broad spectrum and very effective anthelmintic drug being not only used in the treatment of schistosomiasis, but also in the prevention and early treatment of schistosomal infections. In fact, this is the chemotherapy agent of choice for an efficient treatment

in all species of *Schistosoma* (Caffrey, 2007), and it is included in the World Health Organization (WHO) Model List of Essential Medicines for Children (Hoppu and Sri Ranganathan, 2015). Additionally, PZQ is a highly lipophilic agent characterized by a low aqueous solubility and a high permeability, which is thus classified as a Class II drug according to the Biopharmaceutics Classification System (BCS) (Linderberg et al., 2004). The hydrophobic character of the compound has limited the development of acceptable (i.e. liquid) dosage forms for elderly patients, paediatric patients, and for veterinary use. Indeed, the common marketed formulations of PZQ are oral solid dosage forms (e.g. tablets) that frequently are improper to those groups of patients and for a flexible drug dosing (Conway et al., 2013). Regarding the formulation of liquid PZQ dosage forms, little has been performed heretofore: the active agent is only described in oily suspensions (Qian et al., 2017; Tang et al., 2016), or loaded to liposomes (Mourão et al., 2005) and nanoparticulate systems, e.g. solid lipid nanoparticles (Xie et al., 2011), poly(methyl methacrylate) nanoparticles (Malhado et al., 2016), and chitosan nanoparticles (Torabi et al., 2018), being they always formulated for parenteral administration.

The present contribution has been devoted to the definition of a basic aspect in the formulation of PZQ nanosuspensions as oral liquid dosage forms for human and veterinary use, i.e. the control and prediction of the stability and redispersibility properties of the colloid. Given their relevance to these characteristics (Arias et al., 2009; Gallardo et al., 2000, 2003), the surface thermodynamics (hydrophilic/hydrophobic nature) and the electrical properties of the drug/liquid interface were analysed, additionally evaluating the effects of both pH and electrolyte content of the dispersion medium.

2. Materials and methods

2.1. Materials

Chemicals were of analytical quality from Panreac Química S.A.U. (Spain), except for PZQ (Guinama S.L.U., Spain; complying the *European Pharmacopoeia* requirements), formamide (Carlo Erba, Italy) and diiodomethane (Merck KGaA, Germany). Water used was previously deionized and filtered (Milli-Q Academic System, Millipore, Spain).

2.2 Methods

2.2.1. Preparation of the PZQ aqueous nanosuspensions

Aqueous nanodispersions of the anthelmintic drug (0.5%, w/v) were obtained by progressive incorporation of the solid to water under sonication. Prior to the addition of PZQ, the microtip of the sonicator was immersed in the aqueous phase, leaving one cm distance from the bottom of the flask without touching its walls. Pulsed mode, with a cycle of 50%, was used because it facilitated a better temperature control than continuous mode operation, and avoided a temperature increase in the dispersion. For further temperature control, flasks were surrounded with ice during the process.

An alternative procedure, that could be easily scalable at the industrial scale, consisted in the dropwise addition of the drug particles, under mechanical stirring (3,000 rpm) to the aqueous dispersion medium. In accordance to the sonication method, it also assured the generation of homogeneous PZQ nanosuspensions.

2.2.2. Size of the PZQ particles

Mean hydrodynamic diameters (and polydispersity index, PdI) of the anthelmintic particles were determined by dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments Ltd., UK) (n = 12). The scattering angle was set at 90º, and the measurements of the aqueous dispersions (≈ 0.1%, w/v) were done after equilibration during 2 min to 25.0 ± 0.5 ºC.

The technique was further used to evaluate the evolution with time (up to six months) of the particle size in nanosuspensions stored under standard conditions (4.0 ± 0.5 ºC).

2.2.3. Hydrophobic/hydrophilic character

To define the wettability (hydrophobic / hydrophilic character) of PZQ particles, it was used a surface thermodynamic model capable of evaluating the surface-free energy components of the solid, γ_S (Adamson and Gast, 1997; van Oss, 2006). This is a well-known model that has clearly demonstrated its suitability in colloids and suspensions formulated for pharmaceutical applications (Arias et al. 2008, 2009; Cózar-Bernal et al., 2010; Viota et al., 2010). Measurements of the contact angle (θ) of three liquids (water, formamide, and diiodomethane) on pellets of powdered PZQ solids (diameter ≈ 1 cm) were done at 25.0 ± 0.5 ºC, using a goniometer (model 100/07/00, Ramé-Hart, Inc., USA) equipped with a charge-coupled device (CCD) camera and a digital image analysis of drop pictures. Pellets were prepared by compressing the dry powder in a hydraulic press (Specac™, UK) set to 2 Ton during 5 min.

2.2.4. Stability of the aqueous nanosuspensions

Complementarily to the electrophoretic study (see Section 2.2.5.), two methods were used to characterize the stability of PZQ nanosuspensions (0.5%, w/v). The first one consisted in the measurement of the sediment volume, V_s, after keeping these dispersions in 0.1 L cylinders (inner radius ≈ 1.2 cm) at 25.0 ± 0.5 ºC. The flocculation ratio (F%) was then calculated as V_s/V_0 (%), where V_0 is the initial volume of the suspension (Matthews and Rhodes, 1970).

The second method, suitable for dilute pharmaceutical dispersions (Arias et al. 2009; Cózar-Bernal et al., 2010), was based on the measurement as a function of time of the optical absorbance (A) of drug nanosuspensions (λ = 500 nm; PerkinElmer UV/Vis Lambda 25 spectrophotometer, PerkinElmer, USA) at room temperature.

Finally, redispersibility of the PZQ sediments was evaluated by visual inspection of the nanosuspensions after placing them in an ultrasonic bath (model Branson 5200E4, Branson, USA; set at 40 kHz, with a sonic power of 100W) (Arias et al. 2009; Cózar-Bernal et al., 2010). Results were found to be equal to moderate hand shaking of the suspensions (≈ 3 min).

2.2.5. Surface electrical properties of PZQ particles

The electrophoretic properties of the FBZ colloids (≈ 0.05%, w/v) were analysed (Zetasizer Nano ZS device) as a function of both electrolyte concentration (ionic strength), i.e. NaCl, $CaCl_2 \cdot 4H_2O$, or $AlCl_3 \cdot 6H_2O$ (concentrations from 0.01 mM to 0.1 M), and pH (adjusted with either NaOH or HCl). These well-known electrolytes (NaCl, $CaCl_2$, and $AlCl_3$) are classically used to stabilize aqueous pharmaceutical suspensions (Abrahamsson and Odman, 2008; Arias et al., 2008, 2009; Cózar-Bernal et al., 2010; Gallardo et al., 2003). NaCl and $CaCl_2$ were approved by the Food and Drug Administration (F.D.A.) and, despite $AlCl_3$ has not been yet approved by this Administration, it was included in the study to fully analyse the effect of the type of electrolyte and its concentration on the stability of PZQ suspensions. Measurements were done after 24 h of contact at room temperature under mechanical stirring (100 rpm), and after 2 min equilibration time at 25.0 ± 0.5 ºC. The experimental uncertainty of the measurements was < 3%.

3. Results and discussion

3.1. Size and surface thermodynamics of PZQ particles

PZQ particles were found to be of colloidal size and moderately monodisperse. The average diameter (± standard deviation) and polydispersity index were 610 ± 70 nm and 0.089, respectively.

Regarding the wettability of the anthelmintic drug, it is important to define this behaviour in aqueous media when preparing a nanosuspension. With that aim, the evaluation of the surface thermodynamics of drug particles has demonstrated to be of great help, especially when considering the data coming from the non-polar Lifshitz–van der Waals (γ_S^{LW}), the electron-donor (γ_S^-), and the

electron-acceptor (γ_S^+) components to elucidate the nature of a pharmaceutical solid (Adamson and Gast, 1997; Arias et al. 2008; Cózar-Bernal et al., 2010). This study started with the quantification of the contact angles (θ) of water, formamide, and diiodomethane on dry PZQ pellets: 83º ± 2º, 53º ± 1º, and 20º ± 2º, respectively.

The θ data was used to calculate the γ_S components by applying the Young's equation (Adamson and Gast, 1997):

$$2\sqrt{\gamma_S^{LW}\,\gamma_L^{LW}} + 2\sqrt{\gamma_S^{+}\gamma_L^{-}} + 2\sqrt{\gamma_S^{-}\gamma_L^{+}} = (1 + \cos\,\theta)\,\gamma_L^{TOT} \quad (1)$$

The value of the γ_S^{LW}, γ_S^{-}, and γ_S^{+}, components were 47.613 ± 0.542 mJ/m^2, 2.471 ± 0.754 mJ/m^2, and 0.021 ± 0.008 mJ/m^2, respectively. Hence, PZQ was found to be a monopolar electron-donor solid (van Oss, 2006): this anthelmintic agent can have acid–base interactions with phases of whatever polarity (γ_S^- and γ_S^+ different from zero), even though the acid–base forces do not contribute to its cohesion free energy.

These γ_S components further manifest themselves in the hydrophobic/hydrophilic character of the drug particles, and they can be used to evaluate the free energy of interaction (ΔG_{SLS}, not considering the electrostatic component) between the solid phases immersed in the liquid (van Oss, 2006). This quantity is written as follows per unit area of interacting particles:

$$\Delta G_{SLS} = -2\cdot\gamma_{SL}^{TOT} = -2\cdot\left(\gamma_{SL}^{LW} + \gamma_{SL}^{AB}\right) = -2\cdot\left(\gamma_{SL}^{LW} + 2\sqrt{\gamma_S^{+}\gamma_S^{-}} + 2\sqrt{\gamma_S^{-}\gamma_S^{+}} - 2\sqrt{\gamma_S^{+}\gamma_L^{-}} - 2\sqrt{\gamma_S^{-}\gamma_L^{+}}\right) \quad (2)$$

The negative value of ΔG_{SLS} for the PZQ particles (-78.37 ± 5.48 mJ/m^2) characterized the hydrophobic nature of the drug, this postulating that interfacial interactions will support attraction of the nanoparticles to each other in the aqueous media.

3.2. Effects of electrolyte type and concentration on the stability and redispersibility of PZQ aqueous nanosuspensions

Introducing electrolytes in the composition of the dispersion medium has been described to help in the control of the electrical surface charge of pharmaceutical formulations based on colloids and suspensions (Arias et al. 2008, 2009; Cózar-Bernal et al., 2010; Gallardo et al., 2000, 2003). Thus, the significance of characterizing how the stability of the PZQ aqueous nanosuspension depended on the electrolyte type and concentration.

Figs. 1a, 2a, and 3a gather the sedimentation (*F*%) *versus* time curves of the PZQ aqueous nanosuspensions at their natural pH (pH 5), when NaCl, $CaCl_2$, and $AlCl_3$ were incorporated in concentrations up to 10^{-1} M. Independently of the electrolyte type and concentration, the initial sediment volume was very low and progressively increased with time to get stable sediments after ≈ 60 h. Additionally, it was observed a small tendency toward greater flocculation ratios and faster settling rates as both the valence of the electrolyte and concentration increased. The hydrophobic character of the anthelmintic drug (see Section 3.1.), determining particle aggregation, could be behind the absence of more significant differences in the sedimentation profiles even at the very low electrolyte concentrations were the drug would tend to settle as individual particles (high zeta potential, ζ, see below).

This behaviour was confirmed by the relative absorbance (optical absorbance *A*, relative to its initial value, A_0) *versus* time curves (Figs. 1b, 2b, and 3b). The ratio A/A_0 decreased with time, as PZQ particles disappeared out of the light beam during sedimentation. Faster settling rates were characteristic of the slightly more voluminous (heavy) sediments probably generated at the greatest electrolyte concentrations.

These stability and redispersibility profiles could be qualitatively explained in the context of the DLVO classical theory of the stability of hydrophobic colloids (Hunter, 2001), by considering the evolution of the electric double layer properties of the PZQ nanoparticles when varying the electrolyte type and concentration (Fig. 4). Regarding the ionic strength, an increase may favour the accumulation of counterions closer to the drug surface, and hence a reduction in the electric double layer thickness. The resulting lower electrical potential in the shear plane defined low $|\zeta|$ values for

the PZQ nanoparticles, and accordingly weak electrostatic repulsions between the drug particles that determine the fast sedimentation rates and the formation of voluminous sediments (Figs. 1, 2, and 3), which complete redispersion was slightly easier. As these repulsions were maximized when the electrolyte concentration was reduced, the opposite occurred for the high $|\zeta|$ values characteristic of the particles, and slow sedimentation of individual particles may be facilitated (Arias et al., 2009; Gallardo et al., 2000, 2003). The average particle-particle distance could thus be small, and particles will be at the range of van der Waals attractions, packing into compact sediments, difficult to redisperse.

With respect to the valence of the counterion, a similar effect was observed when it was increased. The highly charged counterion Al^{3+} was more efficient in reducing the ζ values than the divalent Ca^{2+} cation, which in turn was more efficient than Na^{+} (Fig. 4), probably a consequence of the Schulze-Hardy rule (briefly described below). In fact, the ζ values were found to be positive almost within the range of concentrations investigated for the trivalent cation. As well, the very low ζ values generated even at weak ionic strengths for $AlCl_3$ could explain the formation of slightly more voluminous aggregates, settling lightly faster (Fig. 3).

Given the incomplete coverage of the drug surface by counterions at low electrolyte concentrations, Cl^{-} ions could reach the solid/liquid interface and an additional increase in the negative ζ could take place. This effect was compensated by the double-layer compression coming from increasing counterion concentrations. Adsorption of these co-ions may be prevented or reduced when the electrolyte concentration (number of cations) was increased. Thanks to the greater charge of Al^{3+}, this cation was more efficient in this coating effect (strong interaction with the PZQ surface), and ζ data moved to positive values (Cózar-Bernal et al., 2010; Ruiz et al., 2004).

Finally, total redispersion was possible after ≈ 4 and ≈ 1 min of ultrasonic shaking of the nanosuspensions with small and high ionic strengths and/or valence of the counterion, respectively. In addition, it was observed the maintenance of the original mean particle size (≈ 600 nm) up to five months in the flocculated PZQ nanosuspensions (see Table 1). This probably meaning the absence

of irreversible particle aggregation (generation of macroaggregates) under the standard storage conditions (4.0 ± 0.5 ºC).

3.3. Effects of pH on the stability and redispersibility of PZQ aqueous nanosuspensions

The pH of the dispersion media has been classically described to display a significant effect on the electrical surface charge of many drugs (and polymers) of pharmaceutical interest (Arias et al. 2008, 2009; Cózar-Bernal et al., 2010; Ruiz et al., 2004). Therefore, it was relevant to analyse how the colloidal stability of the anthelmintic drug depended on $[H^+]$.

Fig. 5a exemplifies the type of time evolution registered for the *F* (%) of the PZQ nanosuspensions at three pH values (sedimentation *versus* time curves). Generally speaking, V_0 was very low and progressively increased with time, so that ≈ 60 h were needed to get a constant low V_s. It can be additionally identified in the figure a slight trend towards lower sedimentation values as the pH was increased. Again and as previously postulated in Section 3.2., the hydrophobic character of the PZQ particles (see Section 3.1.) could be behind the small differences found in the sedimentation profiles.

These results were verified by the relative absorbance *versus* time curves (Fig. 5b): the slight trend for lower sedimentation values as the pH increases (plotted in Fig. 5a) was confirmed, possibly a consequence of the lower weight of these lightly less voluminous flocculi.

These results could be qualitatively explained in the context of the DLVO classical theory of the stability of hydrophobic colloids (Hunter, 2001). As can be observed in Fig. 6, PZQ nanoparticles showed a well-defined isoelectric point (pH_{iep} or pH of zero ζ) in the vicinity of pH 3.7. As pH became more acidic, the decrease in $|\zeta|$ values could come from neutralization of the negative regions at the drug surface by adsorption of increasing quantities of H^+ ions (Arias et al. 2008, Cózar-Bernal et al., 2010; Ruiz et al., 2004). This dependence of the surface electrical charge of the solid with the pH of the dispersion media may explain the stability and redispersibility of the PZQ dispersions above described. At pH 3, the low ζ values (and hydrophobic character) of the particles

may favour aggregation of voluminous flocculi, also characterized by a large weight, by van der Waals attraction, and these aggregates generated visible sediments in a short period of time (fast sedimentation). Despite the hydrophobic attraction between the solids, the average distance between them could be slightly large and the nanosuspensions would be easily redispersible even by mild shaking. Concretely, complete redispersion of the sediments was possible after ≈ 1 min of ultrasonic shaking at acid pHs.

On the contrary, at higher pH values (basic pHs), electrostatic double layer repulsions between the drug particles should take place due to their larger electrokinetic potential (Fig. 6). The nanosuspension could now be considered colloidally stable, this determining lower F (%) values in Fig. 5a and the slightly higher A/A_0 values in Fig. 5b. PZQ solids are expected to settle as individual particles that will pack efficiently into a compact sediment, smaller than at acid pHs (despite the hydrophobic character of the drug), slightly difficult to redisperse (total redispersion after ≈ 4 min of ultrasonic shaking).

Finally, and similarly to what was described in Section 3.2., the original particle size was kept constant (≈ 600 nm) up to five months in the flocculated PZQ nanosuspensions (see Table 2), presumably meaning the absence of irreversible aggregation under the standard storage conditions (4.0 ± 0.5 ºC).

4. Conclusions

Stability (and redispersibility) of praziquantel nanosuspensions is essentially controlled by: *i*) the hydrophobic character of this anthelmintic drug; and, *ii*) the electrokinetics of the particles and the thickness of their ionic double layers. Although the former property intensely determines the flocculation curves, modification of pH and incorporation of electrolytes may contribute to the control of the stability of the colloids. The combined use of moderate or even low concentrations of $AlCl_3$ and acidic pHs should allow the formulation of praziquantel aqueous nanosuspensions with very appropriate stability and redispersibility profiles for pharmaceutical applications. Formulation

of this BCS Class II drug in the form of a nanosuspension could be a promising method to improve the poor solubility limiting its *in vivo* bioavailability due to a low dissolution rate in gastrointestinal fluids following oral administration. Additional preformulation studies are needed to include additional excipients in a final liquid PZQ dosage form, e.g. flavouring agents for enhancing patient acceptance (given the disgusting taste of pure drug and electrolytes), and polymers and surfactants (e.g. poloxamers, polyvinyl alcohol, polyvinylpyrrolidone, or sodium lauryl sulfate, to gain the benefits of a steric stabilization).

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Figure Captions

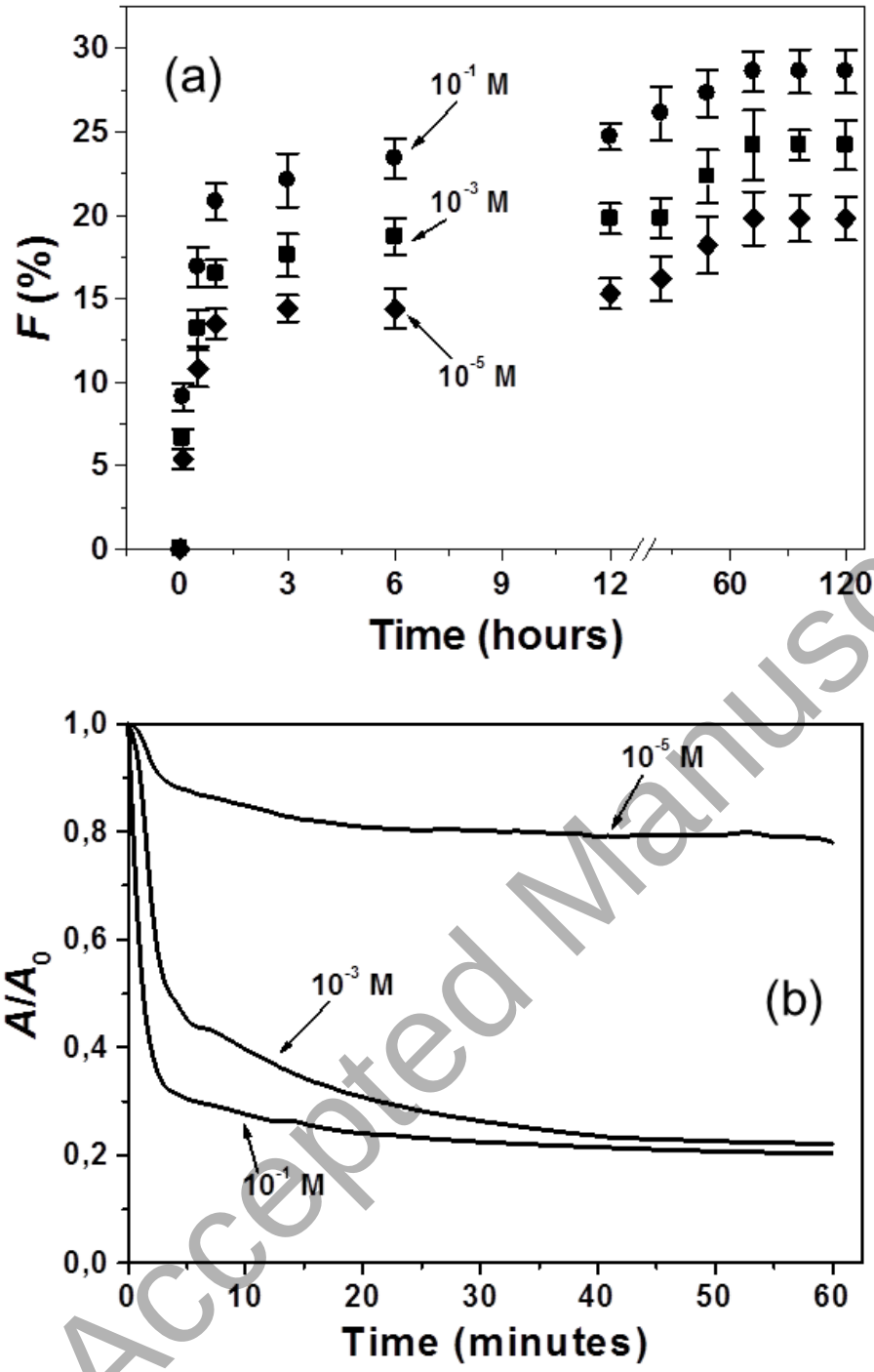


Figure 1. Flocculation ratio (F, V_s/V_0, %) (*a*), and (*b*) optical absorbance A (relative to its initial value, A_0), as a function of time for PZQ aqueous dispersions (pH 5) at the NaCl molar concentrations: 10^{-1} (●), 10^{-3} (■), and 10^{-5} (♦).

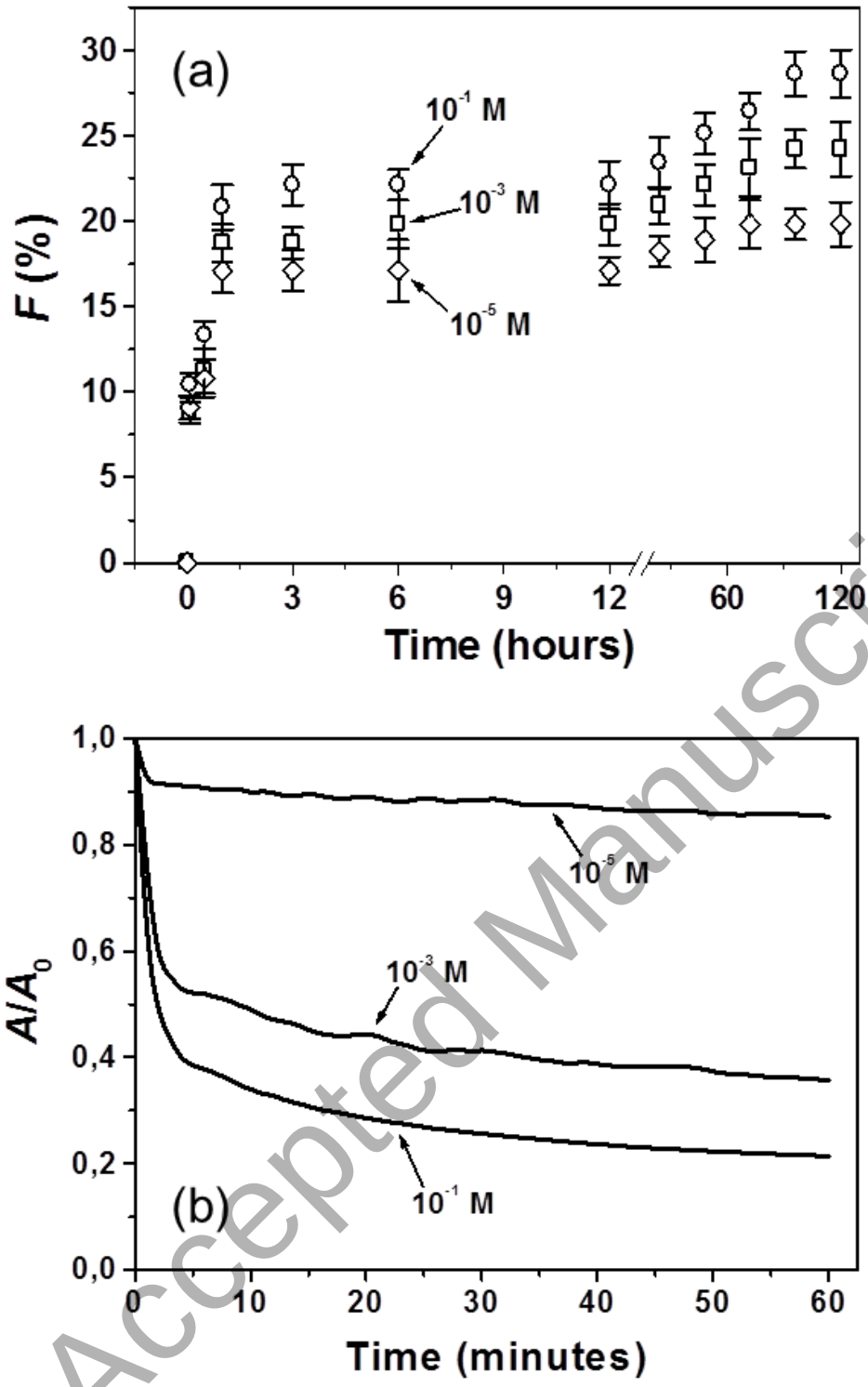


Figure 2. Flocculation ratio (F, V_s/V_0, %) (*a*), and (*b*) optical absorbance A (relative to its initial value, A_0), as a function of time for PZQ aqueous dispersions (pH 5) at the $CaCl_2$ molar concentrations: 10^{-1} (○), 10^{-3} (□), and 10^{-5} (◊).

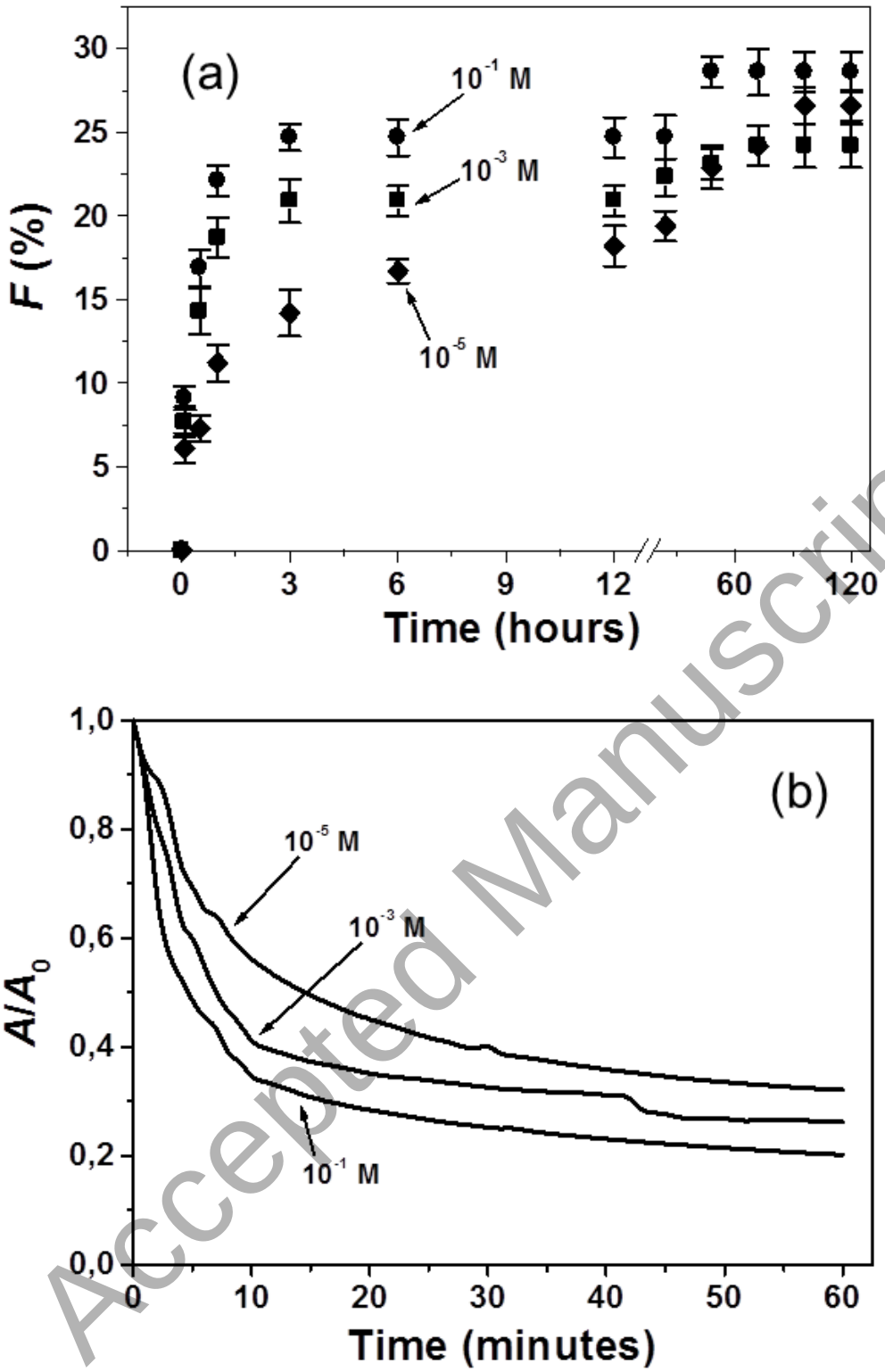


Figure 3. Flocculation ratio (F, V_s/V_0, %) (*a*), and (*b*) optical absorbance A (relative to its initial value, A_0), as a function of time for PZQ aqueous dispersions (pH 5) at the $AlCl_3$ molar concentrations: 10^{-1} (●), 10^{-3} (■), and 10^{-5} (♦).

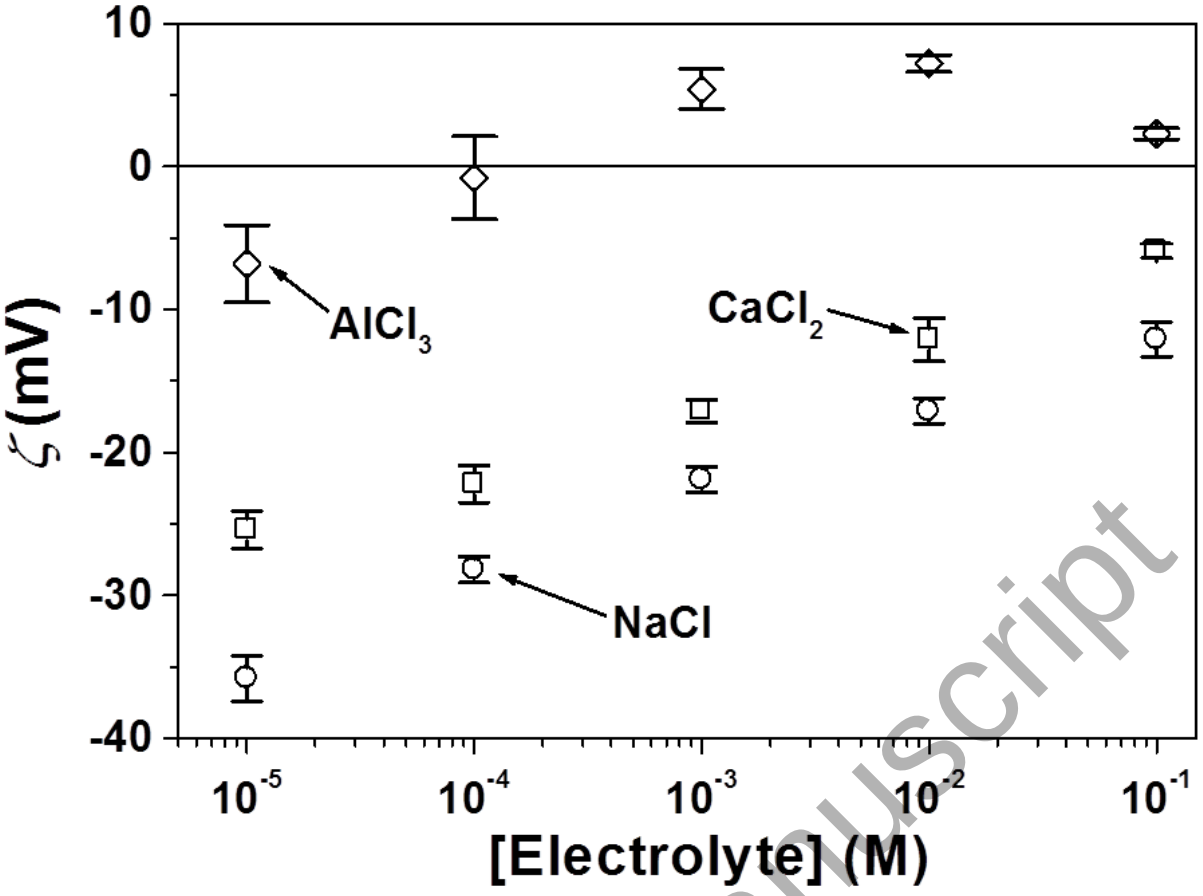


Figure 4. Zeta potential (ζ, mV) of PZQ nanoparticles as a function of the concentration of NaCl (○), $CaCl_2$ (□) and $AlCl_3$ (◊) at pH 5.

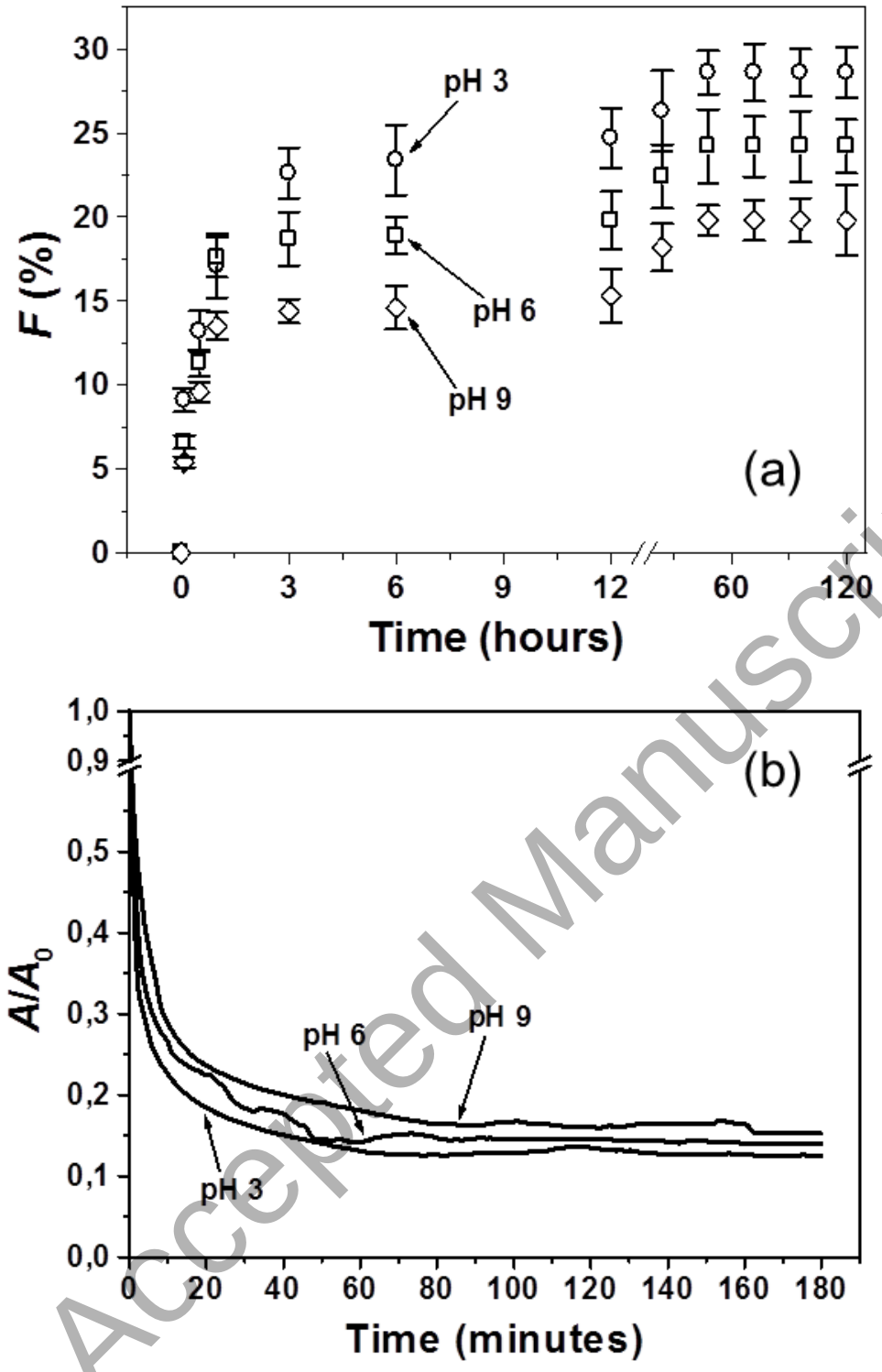


Figure 5. Flocculation ratio (F, V_s/V_0, %) (*a*), and (*b*) optical absorbance A (relative to its initial value, A_0), as a function of time for PZQ aqueous dispersions at pH 3 (○), 6 (□), and 9 (◊), in the presence of 10^{-3} M NaCl.

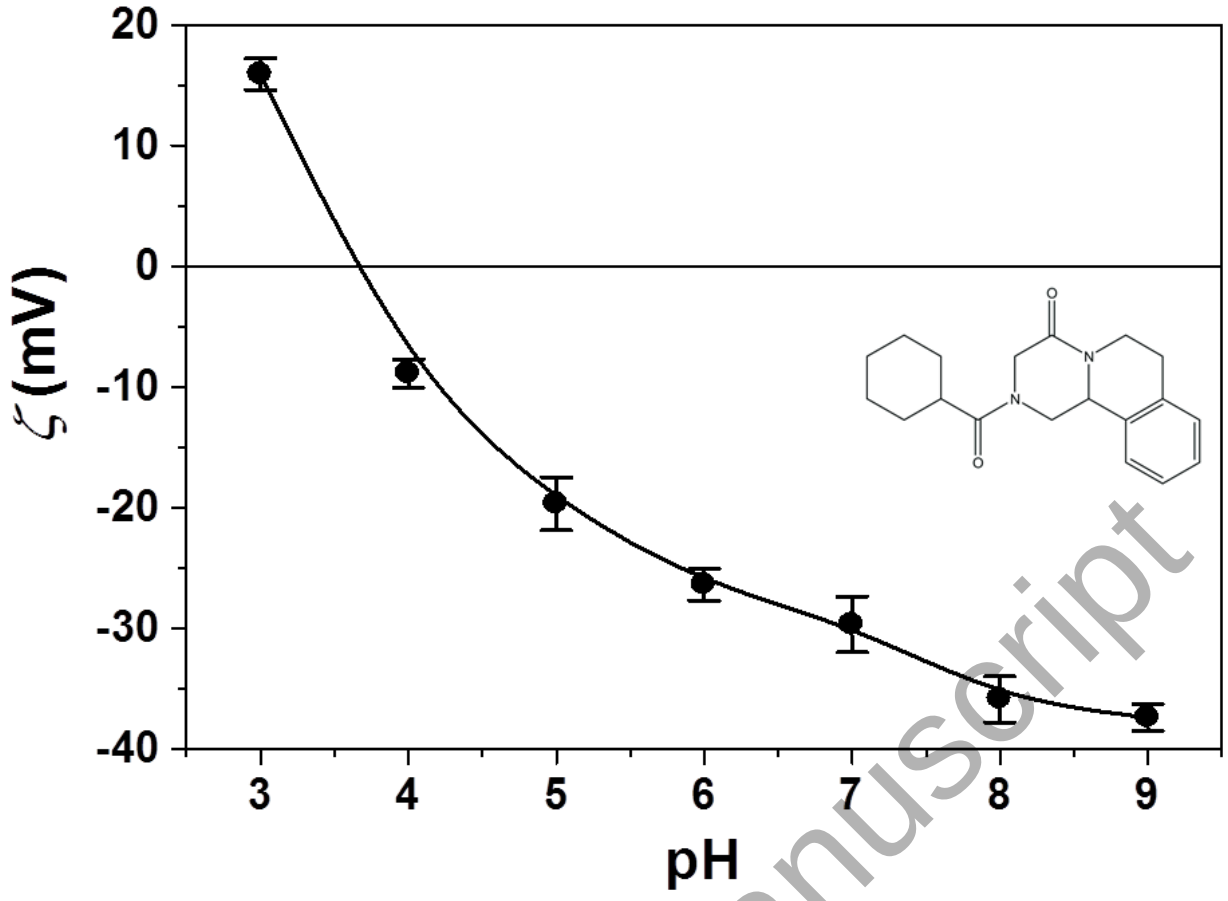


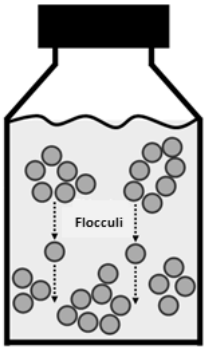
Figure 6. Zeta potential (ζ, mV) of PZQ nanoparticles as a function of pH in the presence of 10^{-3} M NaCl. Inset: chemical structure of this anthelmintic drug.

Table 1. Effects of electrolyte type and concentration on the redispersibility of PZQ aqueous nanosuspensions stored under standard conditions (4.0 ± 0.5 ºC): aspect on visual inspection, and evolution with time of the particle size. The values are the mean ± standard deviation (SD) of triplicate experiments.

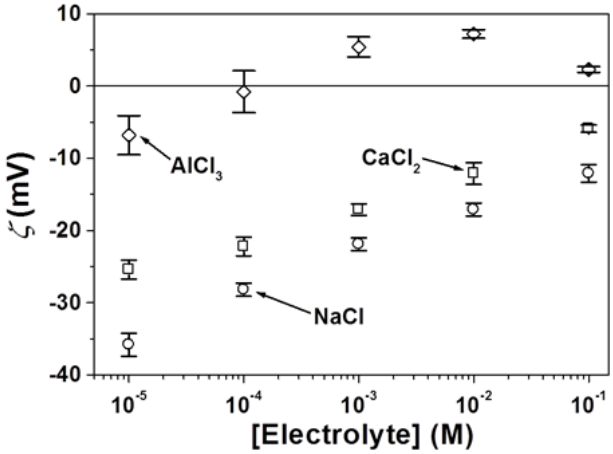
Electrolyte type and concentration	**Size (nm)**					
	Month 1	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
10^{-5} M NaCl	Macroaggregates	Macroaggregates	Macroaggregates	Macroaggregates	Macroaggregates	Macroaggregates
10^{-5} M $CaCl_2$	930 ± 140 (complete redispersion, homogeneous aspect)	Macroaggregates	Macroaggregates	Macroaggregates	Macroaggregates	Macroaggregates
10^{-5} M $AlCl_3$	630 ± 80 (complete redispersion, homogeneous aspect)	670 ± 70 (complete redispersion, homogeneous aspect)	640 ± 60 (complete redispersion, homogeneous aspect)	610 ± 60 (complete redispersion, homogeneous aspect)	750 ± 120 (complete redispersion, homogeneous aspect)	Macroaggregates

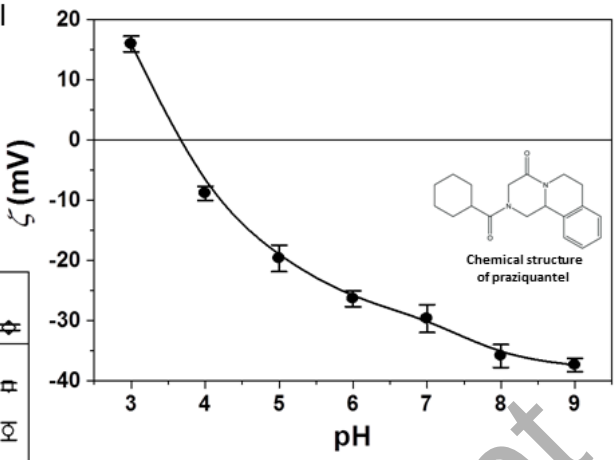
Table 2. Effects of pH on the redispersibility of PZQ aqueous nanosuspensions stored under standard conditions (4.0 ± 0.5 ºC): aspect on visual inspection, and evolution with time of the particle size. The values are the mean ± standard deviation (SD) of triplicate experiments.

pH	Size (nm)					
	Month 1	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
3	600 ± 60 (complete redispersion, homogeneous aspect)	630 ± 90 (complete redispersion, homogeneous aspect)	620 ± 80 (complete redispersion, homogeneous aspect)	710 ± 90 (complete redispersion, homogeneous aspect)	790 ± 110 (complete redispersion, homogeneous aspect)	Macroaggregates
6	880 ± 160 (complete redispersion, homogeneous aspect)	Macroaggregates	Macroaggregates	Macroaggregates	Macroaggregates	Macroaggregates
9	Macroaggregates	Macroaggregates	Macroaggregates	Macroaggregates	Macroaggregates	Macroaggregates



Low ζ values determining flocculated praziquantel nanosuspensions





High ζ values determining deflocculated praziquantel nanosuspensions