molecular pharmaceutics

Article

pubs.acs.org/molecularpharmaceutics

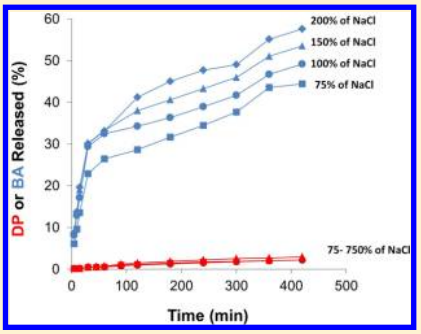
Eudragit E100 as a Drug Carrier: The Remarkable Affinity of Phosphate Ester for Dimethylamine

M. L. Guzmán, R. H. Manzo, and M. E. Olivera*

Department of Pharmacy, Faculty of Chemical Sciences, Pharmaceutical Technology Research Unit (UNITEFA-CONICET), National University of Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria (5000), Córdoba, Argentina

Supporting Information

ABSTRACT: Therapeutic agents containing phosphate groups in their molecules have increasing therapeutic impact. The object of this study was to characterize the cationic polyelectrolyte Eudragit E100 (EuE100) as a carrier for drugs containing phosphate groups, using dexamethasone phosphate (DP) as a model. A series of EuE100-DP complexes was obtained by acid−base reaction in which DP neutralized 12.5−75% of the basic groups of EuE100. The solids obtained after solvent evaporation revealed by spectroscopic characterization the complete reaction between the components through the ionic interaction between the amine groups of EuE100 and the phosphate groups of DP. The reversibility of the counterion condensation, evaluated through the proton-withdrawing effect produced by the ionic exchange generated by titration with NaCl, showed a remarkable high affinity between EuE100 and DP. In line, drug delivery in bicompartimental Franz cells toward water as receptor medium was very slow (2% in 6 h). However, it was increased as water was replaced by NaCl solution, which upon diffusion generates ionic exchange. A sustained release of DP with noticeable zero order kinetics accounted for a remarkable high affinity, mainly due to the electrostatic attraction. The release rate remains constant regardless of the saline concentration of the media. Besides, the delivery control is maintained even in gastric simulated fluid, a property not informed previously for EuE100 complexes.



KEYWORDS: *phosphate ester, controlled release, cationic polymethacrylate*

INTRODUCTION

The use of polymers as carriers in drug delivery has rapidly developed over the last decade.[1] The design of dosage forms for new or existing drugs poses challenges in terms of formulation needs and the desired release profiles.

Polyelectrolytes (PEs) under the form of ionic exchange resins (insoluble PE) or dispersible hydrophilic polymers (soluble PE) have been largely used in pharmaceutical formulations.[2,3] The unique properties arising from the interaction of PE with inorganic or organic counterions have been exploited for a variety of purposes such as drug delivery modulation, taste masking, and drug compatibility/stability improvement, among others.[4−6]

The knowledge about the factors that determine the interaction between ionic or ionizable drugs and PE is relevant in the design of pharmaceutical dosage forms.

Basic PEs, such as chitosan, chitosan derivatives, poly(glycoamideamine)s, and cationic polymethacrylates, are able to interact with oppositely charged compounds, which results in spontaneous association due to the formation of strong but reversible electrostatic links.[7−12] The interaction between the basic groups of the PEs (mostly amines) with carboxylic acid moieties was reported.[13] Besides, several potentialities are claimed for basic PEs concerning their ability to interact with peptides or to act as nonviral delivery vehicles for plasmid DNA.[9−12] They have also showed a capacity to bind and to compact nucleic acid nanoparticles.[12] In some cases, it has also been reported that cationic polymers can help nucleic acid therapeutics survive the different extracellular and intracellular pathways during cellular uptake and intracellular trafficking.[14,15]

In all of these cases, little (if so) explanation about the polymer−drug interactions was given. In most of them, the negative charges interacting with the basic PEs are provided by phosphate moieties. Phosphate groups are also present in a variety of drugs and drug derivatives. Therefore, the study of the interactions that occur between the basic groups of PE and phosphate acidic groups is relevant.

Eudragit E100 (EuE100) is a cationic polymer based on dimethylaminoethyl methacrylate (DMAEMA) and other neutral methacrylic acid esters. EuE100 is widely used in oral and topical formulations and is generally regarded as nontoxic, nonirritant, and essentially safe in humans.[16] It is currently used as coating in solid pharmaceutical dosage forms for taste masking and for protection. Its use as a dissolution modifier and as a granulating agent has also been explored.[17,18] Several transdermal drug delivery systems were also reported.[19] Modified drug release is achieved due to the interaction

Received: August 17, 2011
Revised: July 16, 2012
Accepted: July 18, 2012

dx.doi.org/10.1021/mp300282f | *Mol. Pharmaceutics* XXXX, XXX, XXX−XXX

between reactive groups of copolymer pairs in combination with polyanionic polymers containing carboxylic acids such as EuL100 or EuS100.[20]

We have previously reported some results in relation to the properties of the complexes between the cationic polymer EuE100 and some drugs possessing carboxylic acid groups.[13] When Cl^- is used as an additional counterion, clear optically isotropic aqueous dispersions are produced. This increase in aqueous compatibility allows the loading of acid drugs and generates systems with a high condensation ratio between the drugs and the polymer. This property allows the increase of the aqueous compatibility of low solubility acid drugs in the range of pH in which solubility is determined by the undissociated species. They also have the capability to control the release rate since EuE100 behaves as a carrier that slowly releases the studied model drugs containing carboxylic acid groups as it is dispersed in water but increases the delivery rate in saline solutions simulating biological fluids. All of these properties make these complexes interesting for developing systems for nonparenteral routes in which a controlled release is desired. At present, studies concerning the interaction of EuE100 with moieties containing phosphate ester groups are not reported.

This work is focused on the ionic interaction between the amine groups of EuE100 and the phosphate groups of a selected model drug, dexamethasone phosphate (DP), whose structures are presented in Figure 1. This study is relevant not

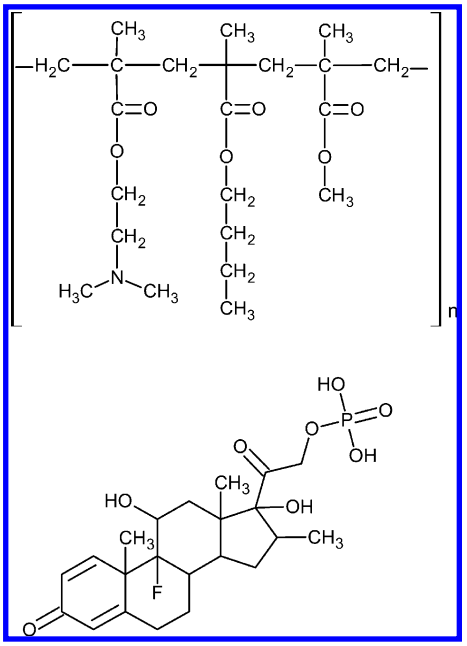


Figure 1. Structural formula of EuE100 monomer (up) and DP (down).

only to increase the potentiality of EuE100 to load new groups of drugs but also to approach the study of the interaction with phosphate groups in DNA molecules, nucleotides, or plasmids which have an increasing therapeutic impact. In this context, EuE100 has been used in techniques for precipitation/isolation of proteins, protein encapsulation, among others.[21,22]

MATERIALS AND METHODS

Materials. Poly(butyl methacrylate, (2-dimethyl aminoethyl)methacrylate, methyl methacrylate) 1:2:1 (Eudragit E100, Pharmaceutical grade, Rohm, Germany) was a gift from Etilfarma S.A. (Buenos Aires, Argentina). DP was obtained by neutralization of the sodium salt ($DPNa_2$) with 1 M HCl solution. The obtained solid melts at 205 °C. Benzoic acid (BA, Parafarm) and diclofenac (DI, Cicarelli) pharmaceutical grade were used. Simulated gastric fluid without pepsin (SGF) was prepared according to USP 34 Ed.[23]

Preparation of Solid Complexes. Before complexation, EuE100 was milled and passed through 40 and 70 mesh sieves. The equivalents of amine groups per gram of EuE100 (3.10×10^{-3}) were assayed by acid−base titration, which is equivalent to 23.8% of the ionogenic group DMAEMA in the polymer (theoretical value 20.8−25.5).[24]

A series of complexes were prepared by dispersing EuE100 in a mortar with 8 mL of ethanol per gram of polymer. Variable amounts of HCl 0.1 M were added to obtain an initial partial neutralization of the amine groups of EuE100. The mixture was kept for 30 min at room temperature to favor hydration and relaxation of the polymer. After that, DP was added as a powder to neutralize a given fraction of the amine groups of EuE100. Then, the solvent was evaporated under vacuum at 45 °C. This series was regarded as EuE100-$Cl_{(x)}$$DP_{(y)}$, in which (x) and (y) refer respectively to the mole percent of the incorporated HCl or DP. Additionally, a complex EuE100-$DP_{(25)}$, in which HCl was not added, was prepared. Physical mixtures (PM) were prepared by mixing in a mortar EuE100 and DP in the same proportion used to obtain the complex.

Spectroscopic and Thermal Characterization. Solid products EuE100-$DP_{(25)}$, EuE100-$Cl_{(25-75)}$$DP_{(12.5-75)}$, EuE100, DP, and their respective PM, were characterized through Fourier transform infrared spectroscopy (FTIR), thermal analysis, and X-ray powder diffraction (XRPD).

FTIR. The infrared spectra of the samples dispersed at 1% of DP in KBr discs were recorded in a NICOLET FTIR (360 FTIR ESP, Thermo Nicolet, Avatar) spectrometer. The samples were scanned from 4000 to 400 cm^{-1}. The recording conditions were resolution, 8.0; sample scan, 40. Data were analyzed using OMNIC software.

Thermal Analysis. The samples were subjected to differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), from room temperature to 350 °C. The DSC standard cell of an A2920 MDSC (TA Instruments) equipped with a data station (Universal Analysis, TA Instruments) was used to determine DSC curves. The temperature axis and the cell constant of the DSC cell were calibrated with indium (24 mg, 99.99% pure, peak maximum at 156.66 °C, and heat of fusion 28.71 J/g, cell constant 1.2375). The samples (0.9−1.7 mg) were heated in nonhermetic aluminum pans with a pine hole, under nitrogen flux (60 mL/min). Samples were run at 10 °C/min ramps. The areas of the endothermic peaks were found by drawing a line from the point where the thermogram first departs from the baseline (onset) to the point where the baseline was resumed following fusion (recovery). The fusion and dehydration temperatures were taken as the extrapolated onset temperature of the endothermic peak. A 2950 TGA HR thermogravimetric analyzer (TA Instruments) linked to a data station was used. The samples (approximately 2 mg) were placed in open aluminum pans and heated under the same conditions used in their respective DSC analysis.

For the determination of glass transition temperature (T_g) additional DSC experiments were carried out. Samples (4−7 mg) of EuE100, EuE100-$Cl_{(25-100)}$, and EuE100-$Cl_{(25)}$$DP_{(25-75)}$ were accurately weighed into aluminum pans, which were

dx.doi.org/10.1021/mp300282f | Mol. Pharmaceutics XXXX, XXX, XXX−XXX

sealed and perforated. The samples were heated (20 °C/min), under nitrogen flux (60 mL/min) from room temperature to 100 °C and maintained at this temperature for 15 min to avoid moisture effect on the T_g measurement. Then the samples were cooled to 0 °C and reheated (20 °C/min) to 200 °C. The T_g was determined from the second heating cycle.

XRPD. This technique was used to identify crystalline phases and to qualitatively examine changes in crystallinity. Measurements were done with a Rigaku Miniflex diffractometer (The Woodlands, TX) having a copper target X-ray tube, equipped with specific software (Standard monitoring 3.2), operated in scan mode over 5−80° 2θ, steps of 0.05, and scan speed of 0.66° 2θ/s. The accelerating voltage was 30 kV, and the current was 15 mA.

^{1}H NMR Experiments. Solution ^{1}H NMR spectra were recorded at 25 °C on Bruker Advance II Ultra Shield TM spectrometer operating at 400.16 MHz. A sample of EuE100-$Cl_{(30)}DP_{(40)}$ that had a final DP concentration of 9 mM was prepared by dispersing the complex in a mixture of H_2O/D_2O 80:20. In addition, samples of both DP and EuE100-$Cl_{(30)}$ at the same respective concentration as that of the complex were recorded. The ionic strength was regulated by adding NaCl to DP and EuE100-$Cl_{(30)}$ samples to obtain values equivalent to that of the EuE100-$Cl_{(30)}DP_{(40)}$ dispersion. TSP (trimethylsilylpropionic acid-*d*4, sodium salt) was used as an internal reference. Assignments of DP were done by comparing the values with those in the literature.[25]

DP Displacement by Ion Exchange. The effect of the addition of sodium chloride on ionic equilibrium of complex dispersions was evaluated through the titration of 20 mL of an EuE100-$Cl_{(25)}DP_{(40)}$ aqueous dispersion (3.5×10^{-5} mole of DP) with NaCl 0.9%. The pH of the dispersions was plotted against NaCl concentration, referred to as percentage of DP in the complexes. Dispersions of complexes of EuE100-$Cl_{(25)}BA_{(40)}$ and EuE100-$Cl_{(25)}DI_{(40)}$, at the same molar concentration as that of DP, were titrated and used for comparison as a model systems containing drugs with carboxylic acid groups.

Drug Release. Aqueous dispersions of complexes of EuE100-$Cl_{(25)}DP_{(38)}$ and EuE100-$Cl_{(25)}DP_{(50)}$ (4 mL, equivalent to 22 and 28 mg of DP, respectively) were subjected to drug release analysis. The release rate from an aqueous solution with an equivalent concentration of $DPNa_2$ was used as a reference. A dispersion of EuE100-$Cl_{(25)}BA_{(50)}$, at the same molar concentration as that of DP (4 mL, equivalent to 7.87 mg of BA), was used for comparison.

Experiments were performed in a modified Franz diffusion assembly at 37 ± 1 °C. A semipermeable acetate cellulose membrane (Sigma 12 000) was placed between the donor and the receptor compartments. Complex dispersions were placed in the donor compartment. The receptor compartment was filled with 70 mL of water, 0.9% NaCl solution, or SGF and stirred with a Teflon-coated magnetic stirring bar. At selected times, 4 mL aliquots were withdrawn and replaced by the same volume of receptor medium, prewarmed at 37 °C. Data were corrected for dilution. DP and BA concentrations were determined by UV spectroscopy (Thermo-Electronic Corporation, Evolution 300 BB, England) at 241 nm and 230 nm, respectively.

The pH values of the receptor compartments were 6.15, 5.80, and 1.20 (in water, NaCl, and SGF, respectively) at the beginning of the release experiments and 4.98 ± 0.31, 5.80 ± 0.23, and 1.20 ± 0.05 (in water, NaCl, and SGF, respectively) at the end.

To confirm sink conditions, solubility determinations of DP were conducted. An excess of DP was placed into suitable stoppered containers and added with SGF, water, or 0.9% NaCl. The samples (in triplicate) were immersed in a thermostatized water bath at 37 °C and periodically shaken. Once the equilibrium was reached, the pH of the supernatant was recorded. Aliquots of the filtrate, properly diluted with the same solvent, were analyzed by UV spectrophotometry at the maximum wavelengths (λ = 241 nm). The solubility of DP was 3.01 ± 0.03 (water, pH = 1.30 ± 0.05), 3.04 ± 0.01 (NaCl, pH = 1.13 ± 0.02), and 1.22 ± 0.09 mg/mL (SGF, pH = 0.97 ± 0.04). The BA solubility at 25 °C in 0.15 M KCl has been reported as 3.2 mg/mL (pH = 1) and 29.5 mg/mL (pH = 5).[26] Hence, under the conditions used (volume of the receptor compartment, sampling frequency, and concentration of drugs in the donor compartment), sink conditions (defined as the volume of medium at least greater than three times that required to form a saturated solution of a drug substance[27]) prevailed in all studies.

In addition, a set of experiments were run in which NaCl was added to the donor compartment and the receptor was filled with water. In these cases, the dispersions of EuE100-$Cl_{(25)}DP_{(38)}$ or EuE100-$Cl_{(25)}BA_{(50)}$ were added with 0.5 mL of a NaCl solution containing between 50 and 750% of the equivalents of DP or BA present in the dispersions. All of the assays were run in triplicate and followed for 6 h.

RESULTS AND DISCUSSION

Table 1 reports the series of EuE100-$Cl_{(x)}DP_{(y)}$ complexes prepared in the solid state, in which the proportions of DP and HCl were systematically modified. The complexes were easily obtained in the solid state as described in the experimental

Table 1. EuE100-$Cl_{(x)}DP_{(y)}$ Complexes Obtained in Solid State

EuE100-$Cl_{(x)}DP_{(y)}$	HCl[a] (%)	DP (%)[a,b]	total neutralization[a] (%)	pH of 1% (w/v) aqueous dispersion
EuE100-$Cl_{(75)}DP_{(25)}$	75	25	100	1.96
EuE100-$Cl_{(50)}DP_{(50)}$	50	50	100	3.17
EuE100-$Cl_{(25)}DP_{(75)}$	25	75	100	*c*
EuE100-$Cl_{(50)}DP_{(25)}$	50	25	75	4.45
EuE100-$Cl_{(25)}DP_{(50)}$	25	50	75	4.88
EuE100-$Cl_{(30)}DP_{(40)}$	30	40	70	5.12
EuE100-$Cl_{(25)}DP_{(38)}$	25	38	63	5.45
EuE100-$Cl_{(25)}DP_{(25)}$	25	25	50	5.90
EuE100-$Cl_{(25)}DP_{(12.5)}$	25	12.5	37.5	6.08
EuE100-$DP_{(25)}$		25	25	*c*

[a]Expressed as equivalents of dimethylamine groups of EuE100. [b]Considering 1 equiv/mol. [c]pH values not registered due to the low solubility of the 1% (w/v) complexes dispersions.

dx.doi.org/10.1021/mp300282f | *Mol. Pharmaceutics* XXXX, XXX, XXX−XXX

section. Most of the systems are hydrophilic enough to obtain 1% (w/v) dispersions.

Spectroscopic and Thermal Characterization of the Complexes. EuE100 is a strong polybase and acts as proton acceptor. The average pK_a of the basic monomer is 8.4.[28] Besides, DP presents values of pK_{a1} and pK_{a2} of 1.89 and 6.4, respectively.[29]

In the FTIR analysis, the indicative bands of the acid−base interaction were investigated for changes arising as a result of the association between EuE100 and DP. The appearance of new bands was also investigated. The regions of the stretching modes associated with the phosphate group of DP (P−OH st, and asymmetric st mode of the POO^-) and the bands of dimethylamine groups of EuE100 were analyzed in the complexes, DP, EuE100, and their PM. The FTIR spectra of the solid complexes showed some remarkable differences when they were compared to that of the reactants (Figure 2).

The absorption bands of the nonprotonated dimethylamine groups appear at 2770 and 2816 cm^{-1} in EuE100 and the PM. In the complexes, these bands are significantly reduced which indicates the presence of protonated and nonprotonated dimethylamine groups.[30,31] The reduction is related to the neutralization percentages of the samples, and the bands disappear when all of the dimethylamine groups of EuE100 are protonated in EuE100-$Cl_{(50)}DP_{(50)}$. Interestingly, the dimethylamine bands of the complex EuE100-$Cl_{(50)}DP_{(25)}$ have a stronger intensity than those of the complex EuE100-$Cl_{(25)}DP_{(50)}$, although they have the same neutralization percentage. This suggests that, in the solid state, each phosphate group could be interacting with more than one dimethylamine group of EuE100. This is possible since the pK_{a2} of this group is about 2 units lower than that of EuE100.

In DP, a band at 970 cm^{-1} ascribed to the P−OH st of the phosphate group was observed.[32] This band is also present in the PM (973 cm^{-1}) but disappears in all of the complexes and $DPNa_2$. Besides, the appearance of a new band at 987 cm^{-1} associated with the asymmetric stretching mode of POO^- groups was observed in all of the complexes and $DPNa_2$ (Figure 2). This points out the presence of phosphates ionically bound to protonated amines. The absence of unreacted DP in the complexes also suggests that the preparation method allows a complete reaction between EuE100 and DP and all of the dimethylamine groups of EuE100 would be able to interact with the phosphate groups, giving complexes with high loading capacity.

Representative XRPD patterns are shown in Figure 3. The patterns clearly show the crystalline character of DP given by different peaks at fixed angles. Their specific values are presented in the Supporting Information. On the other hand, EuE100 shows an amorphous character without defined peaks in the XRPD pattern. The PM showed the characteristic peaks of DP and a profile similar to its sum with EuE100. In line with the ionic interaction observed by FTIR, no reflections of crystalline DP were present in the complexes, which showed characteristic profiles of amorphous compounds. The disappearance of the peaks of DP suggests that it exists in an amorphous form and a complex was formed between EuE100 and DP. These results are in line with those observed by FTIR and those previously reported.[3,13] Notice that three sharp reflections at 37°, 45°, and 70° 2θ appear in the complexes in a region where crystalline DP has no signals. This behavior was previously observed in some EuE100-DI complexes and should

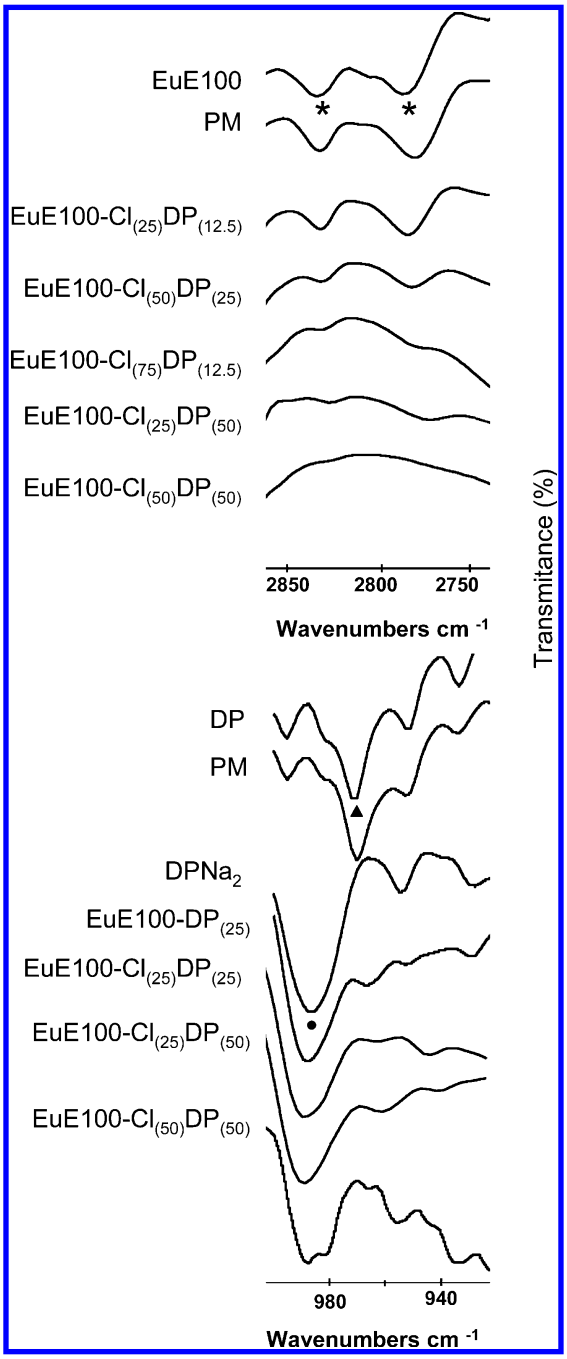


Figure 2. Representative FTIR spectra of DP, $DPNa_2$, EuE100, PM, and some EuE100-$Cl_{(x)}DP_{(y)}$ complexes. Bands corresponding to: *, stretching of C−H groups from the dimethylamine N−CH_3 of EuE100 (2770 and 2816 cm^{-1}); ▲, symmetric and assymmetric stretching of P−OH group (970 cm^{-1}); ●, symmetric stretching of POO^- group (987 cm^{-1}). For clarity, only some representative spectra were included in the figure.

be related to some crystalline points in the samples. However, they are attributable to neither the DP nor EuE100.[13]

Figure 4 shows the DSC and TGA thermograms of EuE100, DP, their PM, and a representative complex. DP presents an endothermic peak (onset: 57.17 °C, maximum peak: 94.04 °C, recovery: 118.42 °C) paralleled by 0.49% of mass loss in TGA which was assigned to water loss. The melting endothermic peak (onset: 185.67 °C, maximum peak: 204.94 °C) was followed by an exothermic event above 211 °C, attributable to a

dx.doi.org/10.1021/mp300282f | Mol. Pharmaceutics XXXX, XXX, XXX−XXX

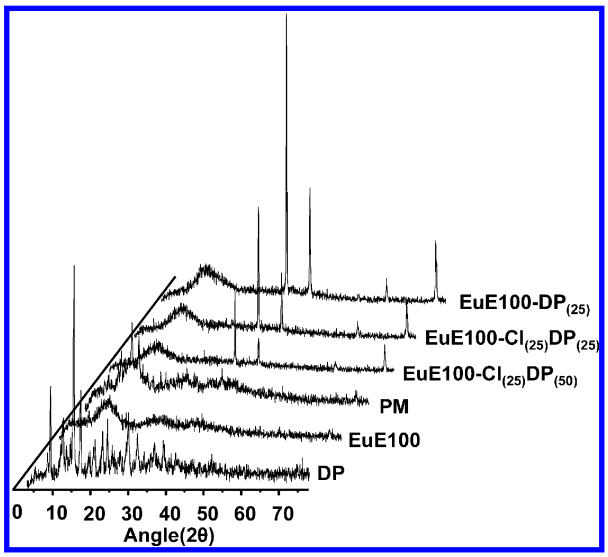


Figure 3. X-ray powder diffraction patterns of some EuE100-$Cl_{(x)}DP_{(y)}$ complexes, the reactants, and their PM.

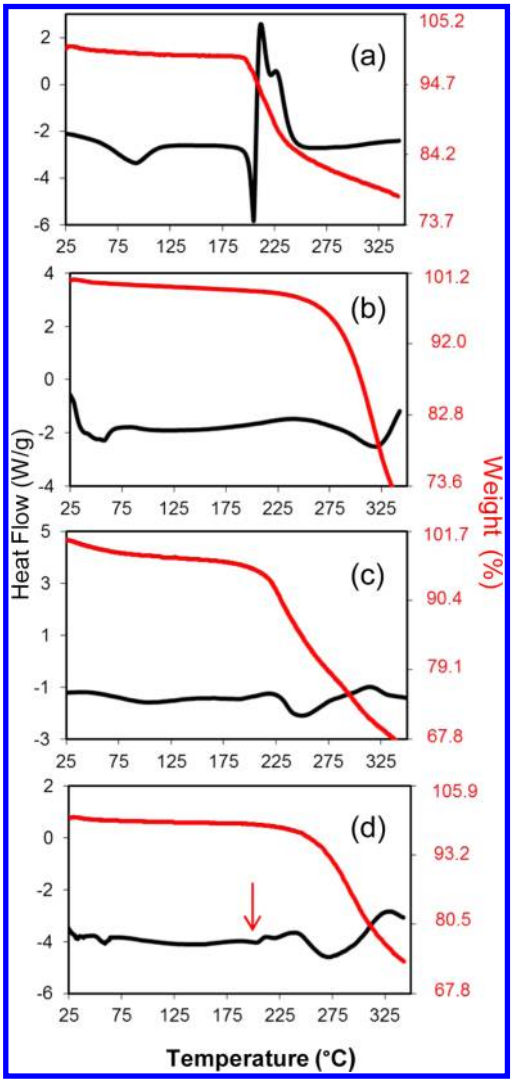


Figure 4. DSC (black) and TGA (red) curves for: (a) DP, (b) EuE100, (c) EuE100-$Cl_{(25)}DP_{(25)}$; (d) EuE100-$DP_{(25)}$ PM. The arrow shows the melting of DP in the PM.

decomposition process. In the complexes, the melting peak of DP was absent confirming the complete reaction between the EuE100 and the DP in all of the proportions evaluated. All of the complexes exhibited decomposition after 240 °C. The PM shows a small endothermic peak corresponding to DP melting, which is substantially lower than the expected one for the amount of DP in the sample. This behavior could be related to partial reaction, probably favored by the assay heat. In fact, from the ratio between the DP experimental melting enthalpy in the PM and the expected one for the pure form, the proportion of material that is in molecular dispersion or in crystalline form could be estimated (92% and 8%, respectively).

Measuring the T_g of solid dispersions can give a wealth of information pertaining to the physical properties of these systems, such as the physical state (amorphous or not) of the drug/carrier, miscibility of the drug with the carrier, or potential for specific interactions between them.[33,34] In agreement with published results, EuE100 showed a T_g of 44.5 °C and decomposition after 320 °C.[35] The drug-loaded systems, EuE100-$Cl_{(25)}DP_{(25-75)}$, showed one single T_g indicating that DP is molecularly distributed. As DP percentage goes from 25 to 75%, a significant increase in T_g was observed (Figure 5)

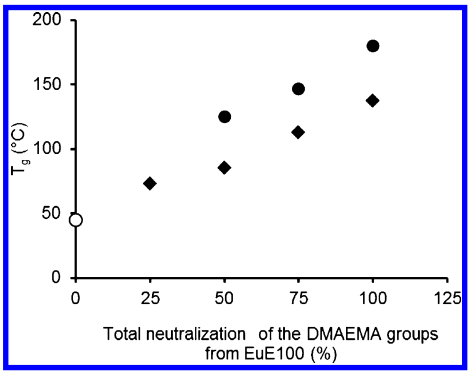


Figure 5. Glass transition temperatures (T_g) of EuE100 (○), EuE100-$Cl_{(25-100)}$ (♦) and EuE100-$Cl_{(25)}$-$DP_{(25-75)}$ (●). The *x*-axis shows the total DMAEMA groups of EuE100 neutralized by Cl or DP.

suggesting ionic interactions between phosphates and protonated amines as observed by FTIR. A similar behavior was informed after salt formation between poly(acrylic acid) and three basic model compounds, which resulted in high T_g values.[36] The heteromolecular forces in the salt are ionic while homomolecular interactions occurring in the pure polymer are not. Since ionic interactions are stronger, this leads to an increase in density and hence a decrease in free volume. Higher temperatures, therefore, are needed to bring such material above its T_g. The neutralization of EuE100 with HCl to obtain EuE100-$Cl_{(25-100)}$ showed the same tendency (Figure 5). Interestingly, T_g values of EuE100-$Cl_{(25)}DP_{(25-75)}$ were higher than EuE100-$Cl_{(25-100)}$ at the same complexation ratio. It has been shown that the size of the electrostatic forces in amorphous salts determines the rise of the T_g values,[37] suggesting that the magnitude of the electrostatic forces between the dimethylamine and phosphate groups are stronger than dimethylamine and Cl^-.

^{1}H NMR Experiments. As expected, when ^{1}H NMR of EuE100, DP, and EuE100-$Cl_{(30)}DP_{(40)}$ spectra were compared (Figure 6), EuE100 signals were wide and unresolved due to efficient spin-net relaxation, usually observed in this systems.[38] On the contrary, ^{1}H NMR spectrum of DP is well-resolved.

dx.doi.org/10.1021/mp300282f | *Mol. Pharmaceutics* XXXX, XXX, XXX−XXX

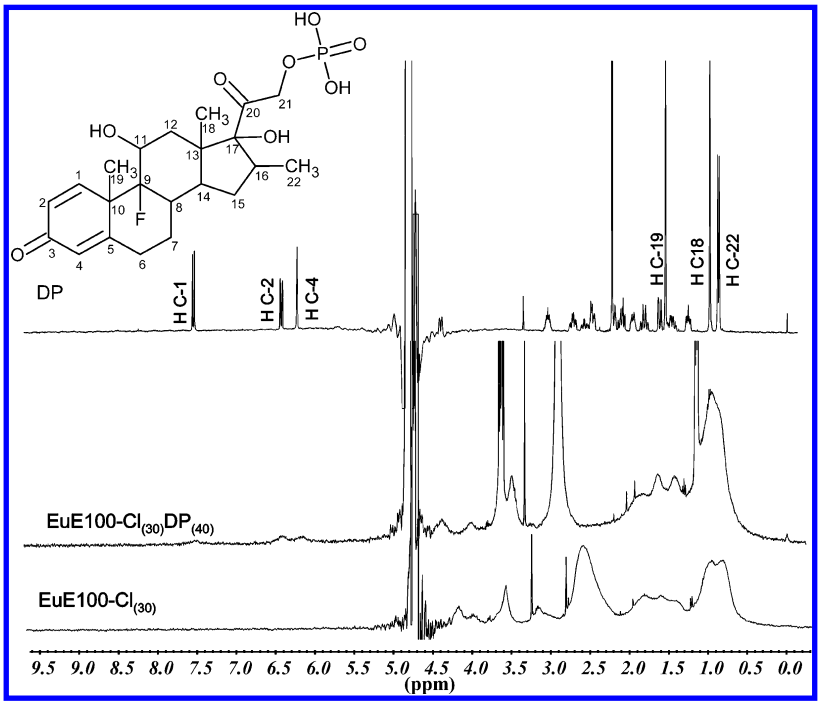


Figure 6. ^{1}H NMR spectra of DP, EuE100-Cl$_{(30)}$, and EuE100-Cl$_{(30)}$-DP$_{(40)}$ in H_2O/D_2O (80:20).

The signals ascribed to the vinyl conjugated system between 6.2 and 7.4 ppm and the three methyl signals with different environments in C-18, C-19, and C-22, which are close to data found in literature, were observed.[25] In the ^{1}H NMR spectrum of the complex, important differences can be observed in DP and EuE100 signals, the widening of the vinyl system signals of DP being the most noticeable one. It suggests a reduction in its relaxation times due to the interaction with the polymeric net. Important modifications are also observed between 1 and 4 ppm in shape and location of the signals corresponding to EuE100, which indicate a perturbation in the environment owing to the presence of DP. All of the observations are strong evidence of the interaction/association between EuE100 and DP.

DP Displacement by Ion-Exchange. The titration with NaCl of the fraction of drug condensed with the PE has been successfully performed in dispersions of complexes of acidic PE with basic drugs as well as in dispersions of complexes of EuE100 and some drugs possessing carboxylic acid groups.[6,13] The addition of NaCl to EuE100-Cl$_{(x)}$DP$_{(y)}$ systems should produce the equilibria depicted in eq 1. Then, under conditions in which the shift to the right of equilibrium shown in eq 2 would be significant to produce a proton withdrawing effect that overcome the deprotonation of R-N$(CH_3)_2H^+$, a plot of pH versus [NaCl] would account for the degree of DP displacement.

$$[\text{EuE100H}^{+}\text{D}-\text{O}-\text{PO}_3\text{H}^{-}] + \text{NaCl} \rightleftharpoons [\text{EuE100H}^{+}{}^{-}\text{Cl}] + \text{D}-\text{O}-\text{PO}_3\text{H}^{-}{}^{+}\text{Na} \quad (1)$$

$$\text{D}-\text{O}-\text{PO}_3^{2-} \rightleftharpoons \text{D}-\text{O}-\text{PO}_3\text{H}^{-} + \text{OH}^{-} \rightleftharpoons \text{D}-\text{O}-\text{PO}_3\text{H}_2 + \text{OH}^{-} \quad (2)$$

$$\text{EuE100}H^{+} \rightleftharpoons \text{EuE100} + \text{H}^{+} \quad (3)$$

Figure 7 shows the increase of pH against the percentage of NaCl relative to the moles of DP in the complexes. In this way,

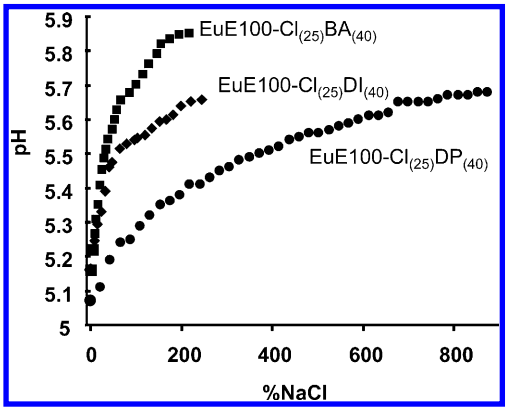


Figure 7. pH changes in aqueous dispersions of the complexes due to displacement of drugs by ion exchange with NaCl.

the overall proton withdrawing effect produced by the addition of NaCl on the complexes EuE100-Cl$_{(25)}$DP$_{(40)}$ should be ascribed not only to the shifting of equilibrium in eq 2 but also to a secondary contribution due to the shifting to the right of equilibria in eq 3. Previous studies showed that pH changes produced by ion-exchange of EuE100 and NaCl are negligible.[13] Therefore, it is clear that the ionic exchange between Cl^- and anionic species of DP produces the main contribution to the pH shift. For comparison, Figure 7 also shows the profiles obtained from complexes of EuE100-Cl$_{(25)}$BA$_{(40)}$ and EuE100-Cl$_{(25)}$DI$_{(40)}$ as systems containing model drugs with carboxylic acid groups. It has been shown that these complexes have a degree of counterion condensation as high as 97.9%, which was reversible by titration with NaCl.[13] Notice that, in the EuE100-Cl$_{(25)}$DP$_{(40)}$ complex, a plateau is reached after addition of more than 800% of NaCl while, in complexes with carboxylic acid moieties, it is achieved after only

dx.doi.org/10.1021/mp300282f | Mol. Pharmaceutics XXXX, XXX, XXX−XXX

200% NaCl. This fact would suggest that the interaction between EuE100 and DP is stronger than that with model drugs with carboxylic acid groups.

Drug Release. Preliminary experiments (unshown results) revealed that tableted EuE100-$Cl_{(x)}DP_{(y)}$ solid complexes rapidly dissolve in acidic media as well as in water when assayed by dissolution apparatus. As a consequence, the released DP cannot be easily differentiated from complexed DP. Based on that, release toward biorelevant fluids in bicompartimental Franz cells from complex aqueous dispersions (rather than in the solid state) was selected to study the release behavior of the complexes. The same approach has been previously used by other authors to obtain mechanistic and quantitative information regarding rate and kinetics of release.[39−41]

Diffusion of DP from the complexes EuE100-$Cl_{(25)}DP_{(38)}$ and EuE100-$Cl_{(25)}DP_{(50)}$ was evaluated. Dispersions of complex EuE100-$Cl_{(25)}BA_{(50)}$, as a model system containing a drug with a carboxylic acid group, were also assayed for comparison. It was selected because BA has good water solubility, and hence sink conditions are warranted. When the receptor compartment was filled with water, a fast diffusion of DP from the reference solution was observed (Figure 8). However, release rates of DP

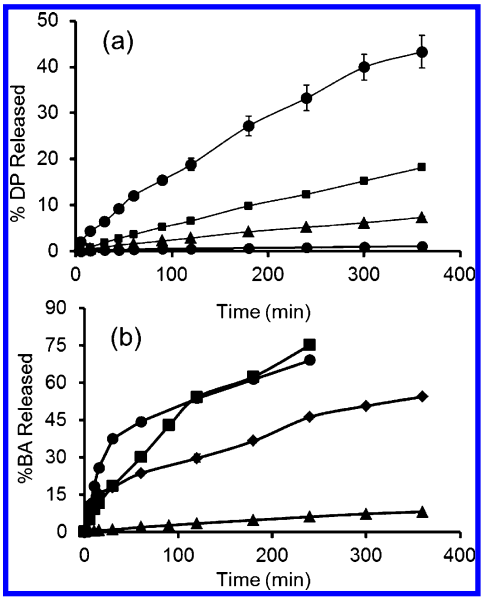


Figure 8. Delivery of (a) DP and (b) BA from EuE100-$Cl_{(25)}$-$DP_{(50)}$ and EuE100-$Cl_{(25)}$-$BA_{(50)}$ complexes toward: (▲) water, (■) SGF, and (♦) NaCl 0.9%; (●) DP and BA reference solutions toward water.

from the aqueous dispersions of both complexes were substantially slower. The diffusion of anionic species of DP from the complexes is substantially prevented by the macroion electrostatic attraction; then, the release should occur mainly through the diffusion of the free DP species (see Figure 9a). Therefore, the slow release of DP observed in Figure 8a (<2% in 6 h) is a consequence of the low concentration of free DP due to the counterionic condensation on one hand and the high degree of ionization of the phosphate group of DP on the other. As also depicted in Figure 8, when water in the receptor compartment was replaced by a solution of NaCl, release rates from the DP complexes were increased as a consequence of the diffusion of Na^+ and Cl^- to the donor compartment generating a ionic exchange that produces free DP and also its salts, which are able to diffuse (Figure 9b). The release in SGF drives to an additional increase in DP release rate due to a shift to the right in eq 2. However, the release is still controlled (Figure 8a). In addition, the delivery rate from EuE100-$Cl_{(25)}DP_{(38)}$ and EuE100-$Cl_{(25)}DP_{(50)}$ approaches zero order kinetics, and no burst effect was observed.

Notice that, the increase of DP release rates in the complexes due to the replacement of water by NaCl solution in the receptor compartment was markedly lower than those observed with BA complexes (Figure 8b). It was also lower than those observed in aqueous dispersions of EuE100 complexes with other drugs containing a carboxylic acid groups[13] and with the highly condensed acid PE-basic drug aqueous dispersions of carbomer.[40] In contrast, EuE100-$Cl_{(25)}BA_{(50)}$ dispersions failed to control release of BA in acidic media showing the same release profile as its reference solution. This behavior is in line with that observed in the displacement by ion-exchange, suggesting that the interaction of phosphate groups with EuE100 is stronger than those of the carboxylic acid groups. In agreement with FTIR observations, this could be due to the ability of phosphate groups in DP to interact with more than one protonated dimethylamine group from EuE100, whereas in the case of drugs with carboxylic acid groups such as BA, each molecule would establish an ionic interaction with only one protonated dimethylamine group. Therefore, the drug release rate would be much slower in the case of E100-$Cl_{(x)}DP_{(y)}$ complexes. To get further information to clarify this point, a set of comparative experiments between the release behavior of EuE100-$Cl_{(25)}DP_{(38)}$ and EuE100-$Cl_{(25)}BA_{(50)}$ dispersions added with increasing proportions of NaCl in the donor compartment was performed. The results shown in Figure 10 clearly demonstrate the remarkable affinity of EuE100-$Cl_{(25)}DP_{(38)}$ whose release rate remains constant regardless of the saline concentration.

It should be considered that a PE-drug complex dispersed in water creates a microenvironment with a number of properties such as electrokinetic potential, intra- and interchain interactions, and a number of interactions with the bulk medium that are expected to be modified during the process of delivery. In fact, polybasic polymers as chitosan and EuE100 exhibit a pH-sensitive behavior due to the large quantities of amine groups on their chains. Besides, their solubility in aqueous media also depends on temperature and ionic strength of the dissolving medium.[42,43] Therefore, the interaction of the complexes with different physiological fluids (due, for example, to different administration routes) can have a significant impact on its performance, and further detailed studies will be necessary to get a more complete description of the delivery in selected conditions. In addition, for drugs having phosphate ester moieties, the enzymatic action of phosphatases is an additional factor which could influence the *in vivo* release rate.

■ CONCLUSIONS

The methodology employed to prepare the complexes allows the complete reaction of the components. The products obtained are amorphous solids with a high loading capacity. In the complexes EuE100-$Cl_{(x)}DP_{(y)}$ a remarkable high affinity between phosphate ester groups and dimethylamine groups was evident, which is also higher than that observed for compounds interacting through carboxylic acid groups. The interaction between EuE100 and the phosphate groups of DP is ionic and probably involves the two acidic groups of the molecule. Such results also support the conclusion that the main contribution

dx.doi.org/10.1021/mp300282f | *Mol. Pharmaceutics* XXXX, XXX, XXX−XXX

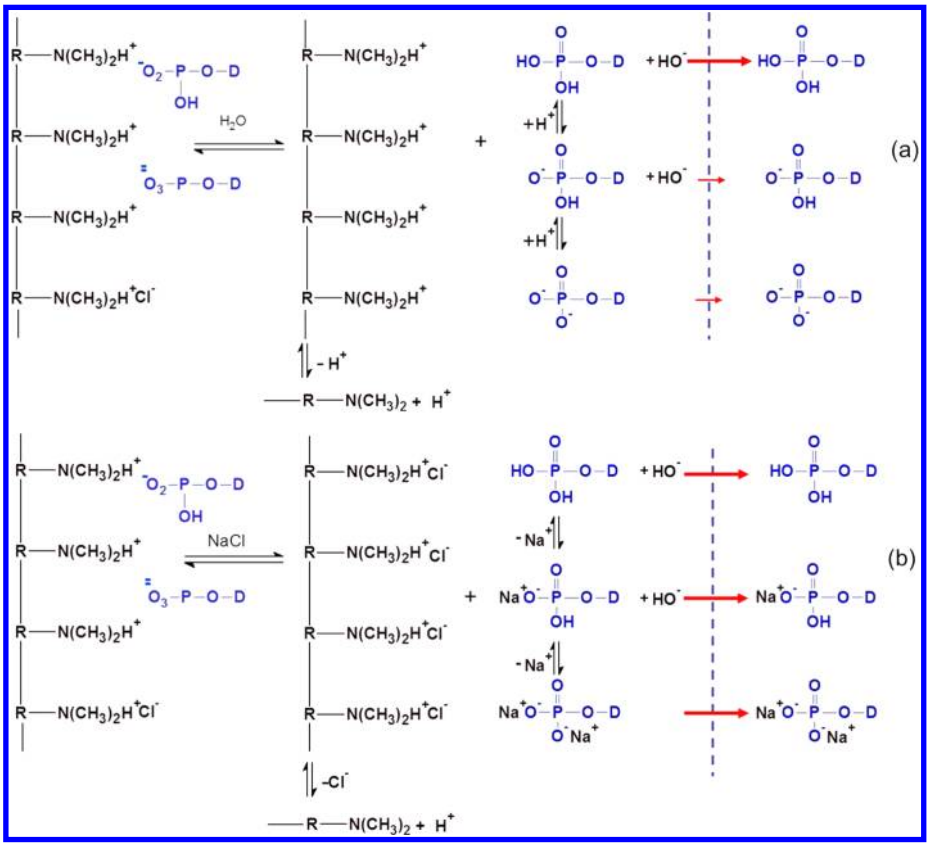


Figure 9. Ionization equilibria of dimethylamine groups of EuE100 (black) and phosphate groups of DP (blue) in aqueous dispersions of complexes EuE100-$Cl_{(x)}DP_{(y)}$: (a) in water, (b) produced by the addition of NaCl. EuE100 and DP are subjected to the acid−base equilibria described in eqs 1 and 2. The hydrolysis of DP released produces an increase in pH. The dashed line represents the semipermeable membrane which can be passed through by the free DP species. The bigger the arrow, the higher the diffusion rate.

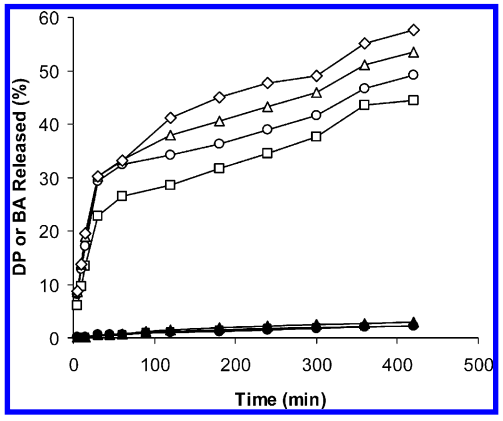


Figure 10. Release of DP (filled symbols) and BA (empty symbols) from EuE100-$Cl_{(25)}DP_{(38)}$ and EuE100-$Cl_{(25)}BA_{(50)}$ aqueous dispersions added with (□) 75%, (○) 100%, (△) 150%, (◊) 200%, and (●) 750% of NaCl in the donor compartment. NaCl is referred to as the molar percentage of DP or BA in the complexes.

to the overall interaction arises from the electrostatic attraction. Aqueous dispersions of EuE100-$Cl_{(x)}DP_{(y)}$ are physically stable systems with a high degree of counterion condensation between EuE100 and DP. EuE100 behaves as a carrier loaded with DP and could function like "smart" systems designed to release slowly the drug as it is dispersed in water. The delivery rate increases in saline solutions simulating biological fluids. A noticeable zero order kinetics with no burst effect was also observed. More research is necessary to determine if the high affinity between the dimethylamine groups of EuE100 and phosphate groups could be exploited to develop new systems with therapeutic agents containing phosphate groups in their molecules, such as DNA, nucleotides, or plasmids.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Specific values for intensity of X-ray powder diffraction for DP. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*National University of Córdoba, Pharmaceutical Technology Research Unit (UNITEFA-CONICET), Department of Pharmacy, Faculty of Chemical Sciences, Haya de la Torre y Medina Allende, Ciudad Universitaria (5000), Córdoba, Argentina. Fax: +54-351-4334127. Phone: +54-351-4334163 ext 111. E-mail: meoliver@fcq.unc.edu.ar.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Fu, Y.; Kao, W. J. Drug release kinetics and transport mechanism of non-degradable and degradable polymeric delivery systems. *Expert Opin. Drug Delivery* **2010**, *7*, 429−433.

(2) Jantzen, G. M.; Robinson, J. R. Ion-exchange systems. *Modern Pharmaceutics* **1996**, *3*, 592−593.

(3) Jimenez-Kairuz, A. F.; Llabot, J. M.; Allemandi, D. A.; Manzo, R. H. Swellable drug-polyelectrolyte matrices (SDPM) Characterization and delivery properties. *Int. J. Pharmaceutics* **2005**, *288*, 87−99.
(4) Ramírez Rigo, M. V.; Allemandi, D. A.; Manzo, R. H. Swellable drug−polyelectrolyte matrices (SDPM) of alginic acid: Characterization and delivery properties. *Int. J. Pharmaceutics* **2006**, *322*, 36−43.
(5) Agresti, C.; Tu, Z.; Ng, C.; Yang, Y.; Liang, J. F. Specific interactions between diphenhydramine and α-helical poly(glutamic acid)—A new ion-pairing complex for taste masking and pH-controlled diphenhydramine release. *Eur. J. Pharm. Biopharm.* **2008**, *70*, 226−233.
(6) Jimenez-Kairuz, A. F.; Allemandi, D. A.; Manzo, R. H. A strategy to improve the chemical stability of a model basic drug by ion pairing with acid groups of polyelectrolytes. *Int. J. Pharmaceutics* **2004**, *269*, 149−156.
(7) Luppi, B.; Bigucci, F.; Cerchiara, T.; Zecchi, V. Chitosan-based hydrogels for nasal drug delivery: from inserts to nanoparticles. *Expert Opin. Drug Delivery* **2010**, *7*, 811−828.
(8) Kamiński, K.; Szczubiałka, K.; Zazakowny, K.; Lach, R.; Nowakowska, M. Chitosan derivatives as novel potential heparin reversal agents. *J. Med. Chem.* **2010**, *53*, 4141−4147.
(9) Vijay, P.; Taori, Y. L.; Theresa, M. R. DNA delivery in vitro via surface release from multilayer assemblies with poly-(glycoamidoamine)s. *Self-Assembling Biomater.* **2009**, *5*, 925−933.
(10) Esposito, E.; Sebben, S.; Cortesi, R.; Menegatti, E.; Nastruzzi, C. Preparation and characterization of cationic microspheres for gene delivery. *Int. J. Pharmaceutics* **1999**, *189*, 29−41.
(11) Lu, Z.-Z.; Wu, J.; Sun, T.-M.; Ji, J.; Yan, L.-F.; Wang, J. Biodegradable polycation and plasmid DNA multilayer film for prolonged gene delivery to mouse osteoblasts. *Biomaterials* **2008**, *29*, 733−741.
(12) Morille, M.; Passirani, C.; Vonarbourg, A.; Clavreul, A.; Benoit, J. P. Progress in developing cationic vectors for non-viral systemic gene therapy against cancer. *Biomaterials* **2008**, *29*, 3477−3496.
(13) Quinteros, D. A.; Ramirez Rigo, M. V.; Jimenez Kairuz, A. F.; Olivera, M. E.; Manzo, R. H.; Allemandi, D. A. Interaction between a cationic polymethacrylate (Eudragit E100) and anionic drugs. *Eur. J. Pharm. Sci.* **2008**, *33*, 72−79.
(14) Anwer, K; Rhee, B. G.; Mendiratta, S. K. Recent progress in polymeric gene delivery systems. *Crit. Rev. Ther. Drug Carrier Syst.* **2003**, *20*, 249−293.
(15) Akhtar, S. Beyond delivery. *Gene Ther.* **2006**, *13*, 739−740.
(16) Rowe, R. C.; Sheskey, P. J.; Owen, S. C. Polymethacrylates. In *Handbook of Pharmaceutical Excipients*, 5th ed.; American Pharmaceutical Association and Pharmaceutical Press: London, 2006; pp 525−533.
(17) Raob, V. M.; Engha, K.; Qiu, Y. Design of pH-independent controlled release matrix tablets for acidic drugs. *Int. J. Pharmaceutics* **2003**, *252*, 81−86.
(18) Pereira de Souza, T.; Martínez-Pacheco, R.; Gómez-Amoza, J. L.; Petrovick, P. R. Eudragit E as Excipient for Production of Granules and Tablets From Phyllanthus niruri L Spray-Dried Extract. *AAPS PharmSciTech* **2007**, *8*, Article 34 (http://www.aapspharmscitech.org).
(19) Hu, Y; Wu, Y. Y.; Xia, X. J.; Wu, Z.; Liang, W. Q.; Gao, J. Q. Development of drug-in-adhesive transdermal patch for α-asarone and in vivo pharmacokinetics and efficacy evaluation. *Drug Delivery* **2011**, *18*, 84−9.
(20) Mustafin, R. I. Interpolymer combinations of chemically complementary grades of Eudragit copolymers: a new direction in the design of peroral solid dosage forms of drug delivery systems with controlled release. *Pharm. Chem. J.* **2011**, *45*, 285−295.
(21) Boeris, V.; Romaninia, D.; Farruggi, B. Interaction and complex formation between catalase and cationic polyelectrolytes: Chitosan and Eudragit E100. *Int. J. Biol. Macromol.* **2009**, *45*, 103−108.
(22) Haining, W. N.; Anderson, D. G.; Little, S. R.; von Berwelt-Baildon, M. S.; Cardoso, A. A.; Alves, P.; Kosmatopoulos, K.; Nadler, L. M.; Langer, R.; Kohane, D. S. pH-Triggered Microparticles for Peptide Vaccination. *J. Immunol.* **2004**, *173*, 2578−2585.
(23) *United States Pharmacopoeia*. The National Formulary and Dispensing Information (USP 34-NF 29); USP: Rockville, MD, 2011.
(24) Specifications and test methods for EUDRAGIT E 100, EUDRAGIT E PO and EUDRAGIT E 12,5. Degussa. Röhm GmbH & Co. KG, 2011. http://eudragit.evonik.com/product/eudragit/Documents/evonik-specification-eudragit-e100,-eudragit-e-po-and-eudragit-e-12,5.pdf.
(25) Midelfart, A.; Dybdahl, A.; Muller, N.; Sitter, B.; Gribbestad, I. S.; Krane, J. Dexamethasone and Dexamethasone Phosphate Detected by ^{1}H and ^{19}F NMR Spectroscopy in the Aqueous Humour. *Exp. Eye Res.* **1998**, *66*, 327−337.
(26) Avdeef, A.; Berger, C. M. pH-metric solubility. Dissolution titration template method for solubility determination. *Eur. J. Pharm. Sci.* **2001**, *14*, 281−291.
(27) In-vitro and in-vivo evaluation of dosage forms <1088>. *United States Pharmacopoeia, The National Formulary and Dispensing Information (USP 30-NF 25)*; USP: Rockville, MD, 2007.
(28) Menjoge, A. R.; Kulkarni, M. G. Mechanistic investigations of phase behavior in Eudragit E blends. *Int. J. Pharmaceutics* **2007**, *343*, 106−121.
(29) Cázares-Delgadillo, J.; Balaguer-Fernández, C.; Calatayud-Pascual, A.; Ganem-Rondero, A.; Quintanar-Guerrero, D.; López-Castellano, A. C.; Merino, V.; Kalia, Y. N. Transdermal iontophoresis of dexamethasone sodium phosphate in vitro and in vivo: Effect of experimental parameters and skin type on drug stability and transport kinetics. *Eur. J. Pharm. Biopharm.* **2010**, *75*, 173−178.
(30) Moustafine, R. I.; Zaharov, I. M.; Kemenova, V. A. Physicochemical characterization and drug release properties of Eudragit E PO/Eudragitw L 100−55 interpolyelectrolyte complexes. *Eur. J. Pharm. Biopharm.* **2006**, *63*, 26−36.
(31) Moustafine, R. I.; Kemenova, V. A.; Van den Mooter, G. Characteristics of interpolyelectrolyte complexes of Eudragit E100 with sodium alginate. *Int. J. Pharmaceutics* **2005**, *294*, 113−120.
(32) Herlinger, A. W.; Ferraro, J. R.; Garcia, J. A.; Chiarizia, R. A far-infrared study and investigation of the difference between the asymmetric and symmetric POO- stretching frequencies in metal-diphosphonate complexes. *Polyhedron* **1998**, *17*, 1471−1475.
(33) Baird, J. A.; Taylor, L. S. Evaluation of amorphous solid dispersion properties using thermal analysis techniques. *Adv. Drug Delivery Rev.* **2012**, *64*, 396−421.
(34) Siepmann, F.; Le Brun, V.; Siepmann, J. Drugs acting as plasticizers in polymeric systems: a quantitative treatment. *J. Controlled Release* **2006**, *115*, 298−306.
(35) Goddeeris, C.; Willems, T.; Houthoofd, K.; Martens, J. A.; Van den Mooter, G. Dissolution enhancement of the anti-HIV drug UC 781 by formulation in a ternary solid dispersion with TPGS 1000 and EudragitE100. *Eur. J. Pharm. Biopharm.* **2008**, *70*, 861−868.
(36) Weuts, I.; Kempen, D.; Verreck, G.; Peeters, J.; Brewster, M.; Blaton, N.; Van den Mooter, G. Salt formation in solid dispersions consisting of polyacrylic acid as a carrier and three basic model compounds resulting in very high glass transition temperatures and constant dissolution properties upon storage. *Eur. J. Pharm. Sci.* **2005**, *25*, 387−393.
(37) Lee, H.-K.; Hajdu, J.; McGoff, P. Propranolol: methacrylic acid copolymer binding interaction. *J. Pharm. Sci.* **1991**, *80*, 178−180.
(38) Scheler, U. NMR on polyelectrolytes. *Curr. Opin. Colloid Interface Sci.* **2009**, *14*, 212−215.
(39) Qiu, Y.; Park, K. Environment-sensitive hydrogels for drug delivery. *Adv. Drug Delivery Rev.* **2001**, *53*, 321−339.
(40) Vilches, A. P.; Jimenez Kairuz, A. F.; Alovero, F.; Olivera, M. E.; Allemandi, D.; Manzo, R. H. Release Kinetics and up-take studies of model fluoroquinolones from Carbomer hydrogels. *Int. J. Pharmaceutics* **2002**, *246*, 17−24.
(41) Jimenez-Kairuz, A. F.; Allemandi, D. A.; Manzo, R. H. Equilibrium properties and mechanism of kinetic release of metoclopramide from carbomer hydrogels. *Int. J. Pharmaceutics* **2003**, *250*, 129−136.

(42) Wu, J.; Luan, M.; Zhao, J. Trypsin immobilization by direct adsorption on metal ion chelated macroporous chitosan-silica gel beads. *Int. J. Biol. Macromol.* **2006**, *39*, 185−191.
(43) Hori, M.; Onishi, H.; Machida, Y. Evaluation of Eudragit-coated chitosan microparticles as an oral immune delivery system. *Int. J. Pharmaceutics* **2005**, *297*, 223−234.