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Research paper

Self-organized drug-interpolyelectrolyte nanocomplexes loaded with anionic drugs. Characterization and *in vitro* release evaluation

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ABSTRACT

The current study is focused on physicochemical characterization and *in vitro* drug delivery evaluation of self-organized nanoparticles based on drug-interpolyelectrolyte complexes (DIPEC). The interaction between two oppositely charged polymethacrylates, Eudragit® EPO and L100, and four non-steroidal anti-inflammatory drugs, Salicylic Acid, Benzoic Acid, Ketoprofen and Naproxen was studied.

DIPEC nanoparticles produce translucent and stable aqueous dispersions where a remarkably high proportion of drug (between 57 and 95%) is condensed with polyelectrolyte (PE) under the form of ionic pairs. They exhibited particle size in the range of 120–198 nm and high positive zeta potential, contributing to physical stability. The sign of zeta potential shifted from positive to negative by changes in composition of DIPEC. Thermal analysis and X-ray diffraction patterns of freeze dried DIPEC showed the amorphous state of products and the complete interaction of PE and drug. Solid complexes were easily redispersed in water yielding nearly the same parameters of fresh dispersions. *In vitro* release experiments showed that DIPEC nanodispersions behave as drug reservoirs, exhibiting a slow drug release rate in water, which significantly increased in simulating physiological fluids, promoted by ionic exchange. They also exhibited a remarkable robustness towards simulated physiological media of different pH.

1. Introduction

Over the past decade, significant progress has been made in the development of new pharmaceutical technology platforms based on polyelectrolyte-ionizable drugs (PE-D) complexes, both in aqueous dispersions and solid material forms, with potential drug delivery applications [1–7]. The ionic interaction between PE and acid or basic drugs (D) is a valuable resource to obtain new materials with improved physicochemical, pharmaceutical and biopharmaceutical properties in comparison to their precursors. The acid-base interaction between carboxylic groups of a PE and a basic group of a drug yields a high degree of counterionic condensation [1]. Likewise, the interaction between protonable amino groups of a PE and anionic drugs has been described [8–12].

PE-D complexes in solid state or aqueous dispersions have a number of unique and favorable properties such as drug delivery modulation [9,13], bioavailability enhancing [14], drug targeting [15], taste masking [16], and drug compatibility/stability improvement [17,18], among others.

However, in relation to the oral route of administration, the performance of (PE-D) complexes in physiological environments, like gastric or intestinal fluids, should be improved since the drug release is highly dependent on pH values [9,11,19]. In this line, self-organized drug-interpolyelectrolyte complexes (DIPEC) may be a successful alternative. Complexes based on DIPEC can be obtained in aqueous dispersions by spontaneous association of oppositely charged PE, due to strong and reversible electrostatic interactions; their properties have drawn increasing attention for different medical and pharmaceutical applications [12,20–24]. Currently, there is an important amount of reports dealing with PE and drugs under salt form [20]. Despite this, scarce information about the interaction characteristics of PE and drugs in neutral form is available. In particular, DIPEC nanodispersions exhibited improved drug delivery behavior, compared to homologs binary PE-D

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complexes, showing a remarkable robustness towards changes of pH in release media [25].

Based on these considerations, two biocompatible PE, Eudragit® L100 (EL) and Eudragit® EPO (EE) were selected as anionic and cationic PE, respectively. These acrylic polymers are extensively used for the preparation of modified release dosage forms, including enteric coating, colonic release, micro- and nano-capsules and matrix systems [24]. Four acid model drugs, benzoic acid, salicylic acid, ketoprofen and naproxen were selected upon consideration of their structure and acid-base behavior as reported in Table 1.

Therefore, the aim of this work was to obtain and evaluate DIPEC loaded with model acid drugs. The study deals with the characterization of some relevant physicochemical properties and *in vitro* release performance using simulated physiological fluids to evaluate the potential use of these nanodispersions in drug delivery design for oral or topical administrations.

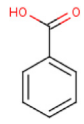
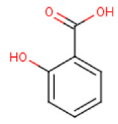
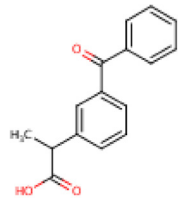
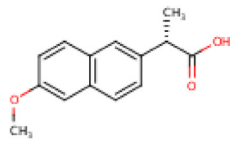
In this article, the strategy used to obtain DIPEC aqueous dispersions involved two oppositely charged polyions and an ionizable acid D. Scheme 1 shows cooperative reactions. Two main assumptions are involved in this strategy: 1) It is implicit that the affinity of D^- for protonated dimethylamine groups of EE is higher than that of Cl^-, and 2) it is expected that the addition of increasing proportions of the counter PE, EL, generates a hindering effect on D^-, modifying the properties of the binary (EE-D_x) complexes.

2. Materials and methods

2.1. Materials

Eudragit® L-*100* (*MW* = 135,000) and Eudragit® EPO (*MW* = 150,000) were gently supplied by Etilpharma® SA (Bs. As., Arg.). The proportions of ionizable groups of the two PE were determined by potentiometric titration and the equivalents of carboxylic groups or amino groups, expressed as mmol/g of PE, were 4.85 and 3.10 for EL and EE respectively. Salicilic Acid (Sal), Benzoic Acid (Ben), Ketoprofen (Ket) and Naproxen (Nap) (USP-grade, Parapharm®, Bs. As., Arg.).

Table 1
Relevant physicochemical properties of acid model drugs [30].

Drug	Molecular structure	pKa	*MW*	Log P	S_{ap} (mg/mL)
Benzoic acid		4.19	122.12	1.87	3.40
Salicylic acid		2.97	138.12	2.26	2.24
Ketoprofen		4.45	254.28	3.12	0.51
Naproxen		4.15	230.26	3.18	$1.59 \cdot 10^{-2}$

[30] Drug Bank, Open data of drug and drug target database. Web site: http://www.drugbank.ca/ [last accessed: 08/25/2015].

2.2. Preparation of drug-interpolyelectrolyte complexes

Four series of (EE-D_{50}) complexes (D = Ben, Sal, Ket and Nap) were prepared by neutralizing 1.0% aqueous dispersion of EE with an appropriate amount of drug under constant stirring. The subscript "50" refers to mole % of D that neutralizes the ionizable dimethylamine pending groups of EE. A proportion of 15 mL 1.0 M HCl solution per gram of PE was added, to obtain an initial partial neutralization of 50% of the amine groups of EE to favor hydration and relaxation of the polymer. The drug was then added as a powder and mixed until complete dissolution. Appropriate amounts of drug to neutralize 50 mol% of the amine groups of EE were added. Under these conditions all translucent dispersions were obtained. Likewise, the dispersion of the counter PE, EL, should be appropriately neutralized with an amount of 1.0 M NaOH solution equivalent to the HCl used in the first step.

The DIPEC was obtained by mixing appropriate amount of dispersions of (EL-Na_{50}) with (EE-D_{50}-Cl_X) under continuous and simultaneous agitation at 10,000 r.p.m. (Ultraturrax® homogenizer, T18-basic, IKA Works Inc., USA) and sonication for 15 min (Elma® Ultrasonic bath, LC30H, Ger.)

The increase in aqueous compatibility allows loading the same concentration of the four acid drugs (7.5 mM).

Ultrapure water was used as solvent. After preparation, DIPEC dispersions were kept at room temperature for 24 h before physicochemical and release characterization. Thus, the series DIPEC (EE-D_{50})-EL_X prepared have a proportion of NaCl with concentrations in the range of: 3.8, 7.6, 11.3 and 15.0 mM for 25, 50, 75 and 100 mol% of EL-Na_{50} added.

Additionally, DIPEC in solid state were obtained by freeze-drying of their aqueous dispersions frozen at −18 °C (Labconco® Freeze Dry System, FreezeZone-6, MO, USA). The solid samples were stored in tight containers at room temperature.

2.3. Optical density measurement

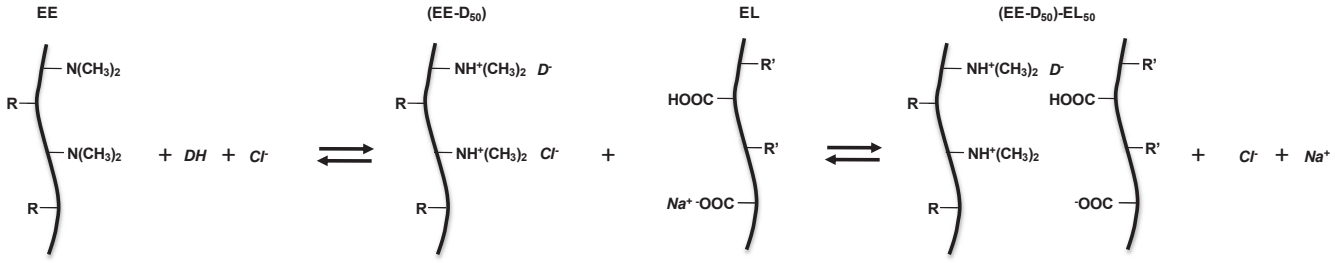
Optical Density (OD) of DIPEC dispersions, without dilution, was measured spectrophotometrically at 600 nm using a UV–Vis spectrophotometer (Thermo®, Evolution 300, Eng.). At this wavelength, absorption due to the PEs and drug did not occur.

2.4. Species distribution and ionic-pair condensation

At equilibrium conditions, the species distribution in aqueous phase was calculated according to previously published methodology [2,26].

The partition equilibrium of Ket and Nap in DCE/Water was carried out in order to determine the true partition coefficient. Samples of aqueous dispersions of DIPEC, containing Ket and Nap, were shake-flask partitioned with 1,2-dichloroethane (DCE, Cicarelli®, Arg.), using a DCE/aqueous dispersion at 1:2 volume ratio in tightly closed test tubes. The samples were kept during 24 h with appropriate agitation at room temperature (25 °C) to reach equilibrium. It should be noted that the organic solvent selectively extracts the free undissociated species fraction. Drug concentrations in organic phase were spectrophotometrically assayed at 253 nm and 274 nm for Ket and Nap, respectively, and the pH of each aqueous dispersion was recorded before partition test and after equilibrium (pH_{eq}).

The concentration of *DH* and pH_{eq} of aqueous phase allowed calculating D^-. Sal and Ben were not assayed due to their high



Scheme 1. Scheme of cooperative reactions involving opposite charged polyions, EE and EL, and acidic drugs, *DH*, in aqueous medium. R and R′ are non-ionized monomer units.

hydrophilicity.

Complementarily, the proportion of drug condensed with PE was determined by ultrafiltration and expressed as apparent association efficiency (AE_{app}), considered an approximation of drug proportion condensed with PE. Freshly-prepared DIPEC dispersions were centrifuged at 20,000 rpm for 30 min at 25 °C (Hermle®, Z36HK, Labrtechnik Gmbh, Ale.), using ultra-centrifugal filters (Amicon® Ultra-4, 10 kDa, Millipore, MA, USA). In order to adjust pH above 7, two drops of 1.0 M NaOH solution was added on filtered solution. The quantity of free drug ($D_{Free} = D^- + DH$) remaining in solution was assayed spectrophotometrically at maximum wavelength for each drug immediately after being processed. All samples were measured in triplicate. The AE_{app} was calculated as:

$$AE_{app}(\%) = (D_{Total} - D_{Free})/D_{Total} \times 100 \quad (1)$$

where D_{Total} is the total amount of drug incorporated in preparation of DIPEC.

2.5. Particle size distribution and electrokinetic potential measurement

Diffusion coefficients (DC) and zeta potential (ζ) of DIPEC aqueous dispersions as function of increasing proportions of EL-Na_{50} were measured by photon correlation spectroscopy (PCS) and electrophoretic light scattering using a DelsaNano-C instrument (Beckman Coulter, Osaka, Jp.). The PCS measurements were carried out at 165° scattering angle and a laser diode of 658 nm. Hydrodynamic diameters (d_H) were calculated from DC values, using cumulants method (DelsaNano 2.20 software, Beckman Coulter, Osaka, Jp.). The ζ was calculated from Smoluchowski equation provided by the software. All measurements were performed in triplicate at 25 °C.

In addition, atomic force microscopy (AFM) was employed to characterize the morphology of DIPEC using a Bruker-Innova® Atomic Force Microscope (Bruker, Santa Barbara, CA US). DIPEC dispersions were diluted with ultrapure water (1/10 v/v), placed on a glass surface and allowed to air-dry. AFM scanning was performed in tapping mode to avoid damage of the sample surface.

2.6. Characterization of solid materials

Solid products (EE-D_{50})-EL_X and their respective physical mixtures (PM) were characterized through thermal analysis and X-ray powder diffraction (XRPD). The PM were prepared in the same proportions as those in the complexes.

Thermal behavior was evaluated by differential scanning calorimetry (DSC) (TA-Instruments Modulated-DSC 2920, Universal Analysis-NT software). The temperature axis and cell constant of the DSC cell were calibrated with indium. Samples of 81 mg were heated in non-hermetic aluminum pans with a pine hole, under nitrogen flux. Samples were run at 10 °C/min ramps.

Powder X-ray diffraction (PXRD) patterns were recorded in a Philips X'pert Pro instrument using a CuKα lamp (λ = 1.5408 Å) at 40 kV and 40 mA between 3° and 65° (2θ) in step mode (0.05°),

Table 2
Physicochemical properties of DIPEC nanodispersions.

DIPEC	NaCl (M)	pH	OD	AE	ζ (mV)	DC (cm^2/s)	d_H (*nm*)	*PI*
(EE-Ben_{50})-Cl_{50}	–	4.76 ± 0.01	<0.005	–	42.4 ± 1.9	nd	nd	nd
(EE-Ben_{50})-EL_{25}	$3.8 \cdot 10^{-3}$	4.55 ± 0.06	0.016 ± 0.004	nd	41.2 ± 1.9	2.50E-08	197.9 ± 19.5	0.280 ± 0.027
(EE-Ben_{50})-EL_{50}	$7.6 \cdot 10^{-3}$	4.70 ± 0.01	0.028 ± 0.007	85.9 ± 0.1	37.3 ± 1.6	3.13E-08	161.0 ± 26.4	0.264 ± 0.018
(EE-Ben_{50})-EL_{75}	$11.3 \cdot 10^{-3}$	4.81 ± 0.02	0.066 ± 0.009	74.8 ± 0.9	32.5 ± 2.5	3.20E-08	156.9 ± 13.6	0.262 ± 0.013
(EE-Ben_{50})-EL_{100}	$15.0 \cdot 10^{-3}$	4.87 ± 0.05	0.137 ± 0.015	57.2 ± 1.1	30.7 ± 1.2	3.32E-08	148.8 ± 12.6	0.272 ± 0.018
(EE-Sal_{50})-Cl_{50}	–	3.97 ± 0.02	<0.005	–	35.6 ± 2.1	nd	nd	nd
(EE-Sal_{50})-EL_{25}	$3.8 \cdot 10^{-3}$	4.35 ± 0.15	0.027 ± 0.005	nd	31.7 ± 1.1	3.82E-08	116.6 ± 37.4	0.248 ± 0.033
(EE-Sal_{50})-EL_{50}	$7.6 \cdot 10^{-3}$	4.52 ± 0.19	0.048 ± 0.004	90.5 ± 0.5	30.8 ± 0.6	3.56E-08	139.4 ± 11.4	0.264 ± 0.017
(EE-Sal_{50})-EL_{75}	$11.3 \cdot 10^{-3}$	4.62 ± 0.16	0.107 ± 0.009	86.7 ± 0.5	29.3 ± 1.1	3.43E-08	147.3 ± 20.6	0.265 ± 0.038
(EE-Sal_{50})-EL_{100}	$15.0 \cdot 10^{-3}$	4.89 ± 0.08	0.250 ± 0.024	79.9 ± 0.8	28.6 ± 1.0	3.47E-08	145.3 ± 4.5	0.272 ± 0.026
(EE-Ket_{50})-Cl_{50}	–	4.05 ± 0.01	<0.005	–	38.5 ± 3.2	nd	nd	nd
(EE-Ket_{50})-EL_{25}	$3.8 \cdot 10^{-3}$	4.60 ± 0.07	0.015 ± 0.005	nd	33.3 ± 0.2	3.80E-08	159.6 ± 30.6	0.254 ± 0.026
(EE-Ket_{50})-EL_{50}	$7.6 \cdot 10^{-3}$	4.72 ± 0.04	0.036 ± 0.003	89.5 ± 0.7	30.7 ± 0.5	4.85E-08	173.9 ± 13.6	0.237 ± 0.017
(EE-Ket_{50})-EL_{75}	$11.3 \cdot 10^{-3}$	4.86 ± 0.06	0.088 ± 0.010	88.7 ± 0.7	29.7 ± 0.9	3.60E-08	144.0 ± 3.8	0.208 ± 0.015
(EE-Ket_{50})-EL_{100}	$15.0 \cdot 10^{-3}$	5.04 ± 0.07	0.199 ± 0.022	81.8 ± 0.6	29.9 ± 0.2	4.11E-08	119.7 ± 7.4	0.215 ± 0.012
(EE-Nap_{50})-Cl_{50}	–	4.71 ± 0.04	<0.005	–	37.1 ± 1.9	nd	nd	nd
(EE-Nap_{50})-EL_{25}	$3.8 \cdot 10^{-3}$	4.52 ± 0.11	0.022 ± 0.007	nd	36.7 ± 3.8	3.04E-08	158.7 ± 17.0	0.265 ± 0.030
(EE-Nap_{50})-EL_{50}	$7.6 \cdot 10^{-3}$	4.69 ± 0.09	0.039 ± 0.005	95.1 ± 0.4	30.8 ± 0.8	3.91E-08	197.3 ± 6.3	0.337 ± 0.027
(EE-Nap_{50})-EL_{75}	$11.3 \cdot 10^{-3}$	4.80 ± 0.08	0.111 ± 0.013	80.1 ± 0.8	28.7 ± 0.6	3.46E-08	142.7 ± 13.7	0.255 ± 0.017
(EE-Nap_{50})-EL_{100}	$15.0 \cdot 10^{-3}$	4.97 ± 0.04	0.237 ± 0.016	68.1 ± 1.2	25.4 ± 0.8	3.12E-08	159.0 ± 16.4	0.256 ± 0.032

The final concentration of EE in DIPEC nanodispersions was: 0.5% v/v ($1.55 \cdot 10^{-2}$ M).
The subscript "x" in DIPEC nomenclatures indicates the proportions of EL and different drugs, expressed as mol%.
OD: optical density; AE: association efficiency; ζ: zeta potential; DC: diffusion coefficient; d_H: hydrodynamic diameter; PI: polydispersity index; nd: not determined.

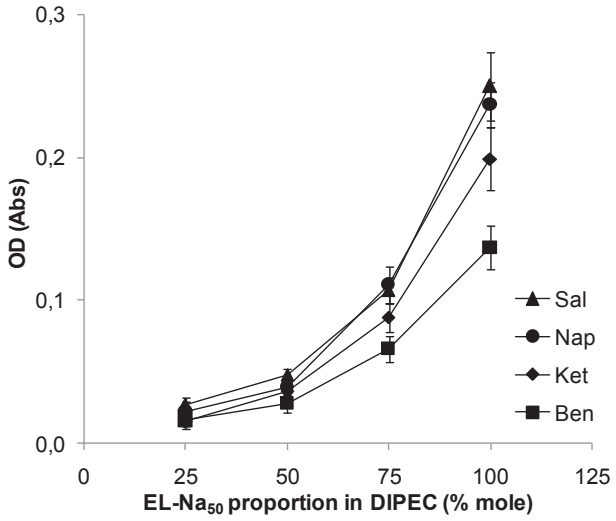


Fig. 1. Optical density of DIPEC dispersions resulting from increasing proportions of EL-Na_{50} added to (EE-D_{50}).

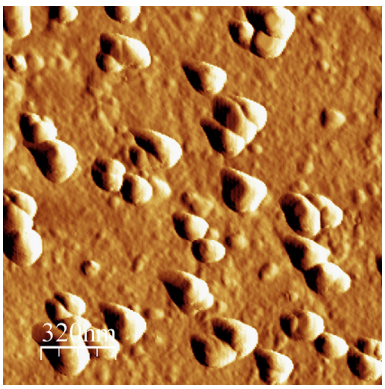


Fig. 2. AFM image of dried (EE-Ket_{50})EL_{50} complexes.

with an scan speed of 1° 2θ/s.

2.7. Stability of DIPEC dispersions

The stability of nanodispersions was evaluated by measuring d_H and ζ of samples stored at room temperature for a month.

2.8. Drug release

Drug release from aqueous DIPEC aqueous dispersions was performed in a bicompartimental diffusion device (Franźs cells) mounted with a semi synthetic cellulose membrane (12,000 Da, Sigma–Aldrich, St Louis, MA, USA). The effective diffusion area was 1.25 cm^2. The donor compartment was filled with 1 mL of each DIPEC dispersion and kept in contact with 15 mL of receptor medium at 37 °C. Water, 0.9% NaCl solution, and pH 1.2 and 6.8 USP buffer solutions were used as receptor media. Samples of 1.0 mL of receptor medium were withdrawn at predetermined time intervals and replaced with equal quantities of fresh medium. The concentration of drug released was assayed spectrophotometrically at 260, 330, 272 and 296 nm for Ket, Nap, Ben and Sal, respectively. All experiments were carried out in triplicate.

Release data were processed using the diffusion equation proposed by Peppas [27] in order to evaluate the kinetic and mechanism of drug release:

$$\frac{M_t}{M_\infty} = kt^n \tag{2}$$

where M_t is the amount of drug permeated at time t; M_∞ is the initial amount of drug in the donor compartment; k is the kinetic constant and n the diffusion exponent which characterizes release mechanism. Equation (2) is valid in the release interval up to 60% of the released drug.

3. Results and discussion

3.1. Preparation of complexes

According to Scheme 1, self-organized DIPEC aqueous dispersions were prepared by acid-base interaction between the protonable pending groups of EE partially neutralized with HCl and a drug having anionic groups (*DH*), followed by the addition of different proportions of the counter PE, EL, and appropriately neutralized with NaOH. The mixing of solutions of both PE leads to the spontaneous formation of interpolyelectrolyte complexes under release of the small counterions [20]. Under these conditions translucent DIPEC dispersions were obtained.

3.2. Characterization of ionic interactions

As Table 2 reports, the addition of increasing proportions of EL-Na_{50} to the binary EE-D_{50} dispersions produced an increase of OD accompanied by a modest rise of pH. Although a progressive decrease in positive ζ was also observed, DC showed no significant changes.

Fig. 1 shows the progressive increase of OD in (EE-D_{50}) complexes containing Ben, Sal, Ket or Nap as a function of the addition of EL-Na_{50}. All DIPEC presented similar patterns and absence of particle sedimentation along time (at least for 1 month).

Samples of nanocomplexes assayed by PCS showed a unimodal distribution rendering DC in the interval of $1.3–3.8 \cdot 10^{-8}$ cm^2 s^{-1}, which remained virtually unchanged regardless of the interpolyelectrolyte composition (Table 2). As reported, the dominating process is the generation of new particles with increasing mixing ratio, rather than an increase in polyelectrolyte complex particles [28]. The d_H of DIPEC dispersions was in the range of 116–198 nm. All measurements showed polydispersity indexes between 0.250

Table 3
Species distribution in aqueous dispersions of DIPEC after partition with DCE.

DIPEC	$[D]_{ini}$ (M)	pH_{eq}	$[D]_{eq}$ (M)	Species distribution (%)		
				(*DH*)	(D^-)	*Ionic pairs*
(EE-Ket_{50})-EL_{25}	7.47 E^{-3}	5.41 ± 0.08	4.38 E^{-3}	0.5 ± 0.1	4.4 ± 0.8	95.1 ± 0.8
(EE-Ket_{50})-EL_{50}		5.29 ± 0.01	2.42 E^{-3}	1.4 ± 0.1	9.8 ± 0.8	88.8 ± 0.9
(EE-Nap_{50})-EL_{25}	8.25 E^{-3}	5.26 ± 0.02	3.85 E^{-3}	0.2 ± 0.1	1.8 ± 0.2	98.0 ± 0.2
(EE-Nap_{50})-EL_{50}		5.14 ± 0.02	2.15 E^{-3}	0.4 ± 0.1	3.8 ± 0.4	95.8 ± 0.5

$[D]_{ini}$ and $[D]_{eq}$ = Respective anionic drug concentrations in initial aqueous phase, remaining stable after partition equilibrium.

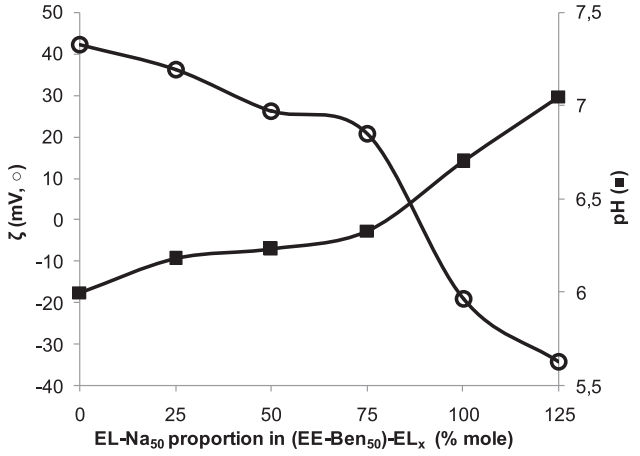
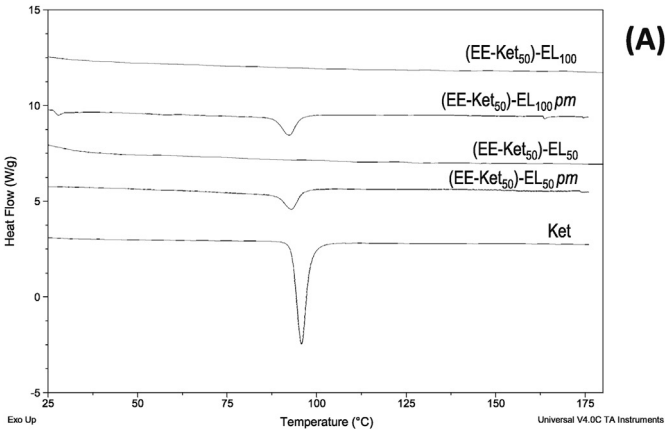


Fig. 3. Changes in pH and zeta potential of (EE-Ben_{50}) nanocomplexes with increasing addition of EL-Na_{50}.

Table 4
d_H and ζ of lyophilized and redispersed DIPEC.

CIPEF	d_H ± DE (nm)	ζ ± DE (mV)
(EE-Sal_{50})-EL_{25}	316 ± 6	29.9 ± 0.2
(EE-Sal_{50})-EL_{50}	312 ± 35	27.9 ± 2.3
(EE-Sal_{50})-EL_{75}	*nr*	
(EE-Sal_{50})-EL_{100}	*nr*	
(EE-Nap_{50})-EL_{25}	250 ± 11	28.9 ± 0.6
(EE-Nap_{50})-EL_{50}	284 ± 24	27.7 ± 0.9
(EE-Nap_{50})-EL_{75}	*nr*	
(EE-Nap_{50})-EL_{100}	*nr*	
(EE-Ket_{50})-EL_{25}	222 ± 39	28.7 ± 0.5
(EE-Ket_{50})-EL_{50}	266 ± 11	27.4 ± 1.7
(EE-Ket_{50})-EL_{75}	*nr*	
(EE-Ket_{50})-EL_{100}	*nr*	
(EE-Ben_{50})-EL_{25}	635 ± 94	30.4 ± 1.1
(EE-Ben_{50})-EL_{50}	271 ± 14	31.1 ± 0.28
(EE-Ben_{50})-EL_{75}	*nr*	
(EE-Ben_{50})-EL_{100}	*nr*	

nr: not redispersed by simple agitation.



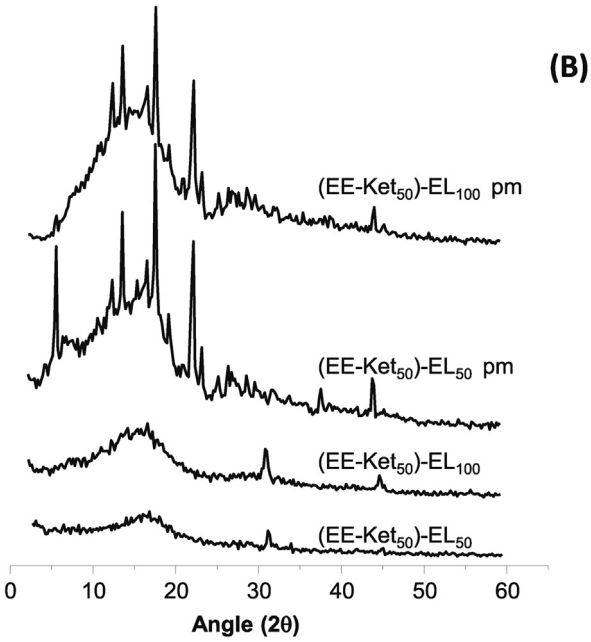


Fig. 4. DSC thermograms and powder X-ray spectra of (EE-Ket_{50})-EL_X complexes and homolog physical mixtures (*pm*).

and 0.362. Although these values are high to consider them as monodisperse nanoparticulate systems, they are in agreement with those frequently reported for similar polymeric nanocomplexes [22,29]. On the other hand, the relative low standard deviations of reported d_H reflect the reproducibility and robustness of DIPEC preparation method.

High values of polydispersity indexes could be attributed to non-spherical colloidal structures produced by random ionic interactions and self-organized condensation between two linear PE [20]. The AFM image of dehydrated nanocomplexes containing Ket showed non-spherical structures and particle size around 200–250 nm (Fig. 2). This result was observed in all DIPEC complexes (data not shown). It is necessary to clarify that the slight growth of particle size can be attributed to drying process of dispersion before the determination.

The distribution of free species *DH and* D^- and drug condensed with PE in the aqueous dispersions was determined through the selective extraction of *DH* species with DCE according to the methodology previously reported [2,8,26]. Table 3 reports species distribution in complexes with Ket and Nap having two compositions (EE-D_{50})-EL_{25} and (EE-D_{50})-EL_{50}. In both cases, the complexes exhibited a degree of condensation higher than 88%. These results are in line with those previously reported by Quinteros et al. [8] for binary (EE-D_x) complexes with the same drugs. However, this methodology yielded no reliable results with complexes having

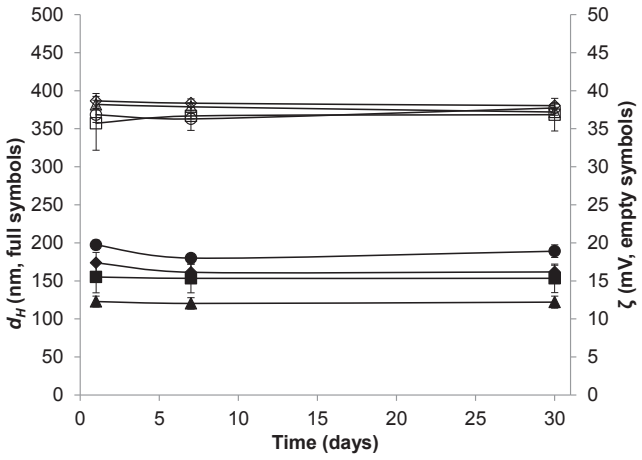


Fig. 5. Stability profiles of (EE-D_{50})-EL_{50} nanocomplexes stored at room temperature for a month. Ben (*square*), Sal (*triangle*), Ket (*diamond*) and Nap (*circle*).

higher EL/(EE-D_{50}) ratios.

Complementarily, AE_{app} was calculated as an approximation to ionic degree of condensation obtained by solvent partition. For complexes having low proportions of EL, both methods, solvent extraction and ultrafiltration, gave similar and comparative results. As Table 2 reports, AE_{app} decreases with the increase of the counter EL, noting a drop in both the condensed fraction from the average of 90% until 57% and pH values. These results suggest that the increase of NaCl concentration resulting from the preparation of DIPEC nanodispersion promotes ionic exchange and drop of pH.

In addition, due to drug condensation in DIPEC, the concentration of Ket and Nap in aqueous medium was 20 and 100 times higher than that of respective aqueous solubility at the same pH. This property allowed increasing the aqueous compatibility of low solubility acid drugs which is an important issue in the design of liquid dosage forms.

On the other hand, DIPEC dispersions showed high positive ζ (Table 2) that slightly decreased with the addition of EL-Na_{50}. However, it remained above 25 mV in the whole composition range, contributing to their physical stability. The addition of EL-Na_{50} produced a slight increase of pH, which remained close to 4.8 even at the highest proportion of 100%, which suggests that, in the resulting DIPEC, the degree of protonation of amino groups of EE remains higher than the degree of ionization of carboxylic groups of EL along the interval of EL-Na_{50} added.

Since the interaction between EE and Ben yields stable dispersions (EE-Ben_{50}) without the addition of HCl, this system was selected to determine ζ under the effect of the addition of EL-Na_{50} in a range from 25 to 125%. Fig. 3 shows that high proportions of EL-Na_{50} led to an increase of pH above 7, which is accompanied by a shifting of ζ from positive to negative sign, without disturbing the stability of dispersion.

This behavior suggests that, under such conditions, the rise of pH increases the degree of ionization of carboxylic groups of EL

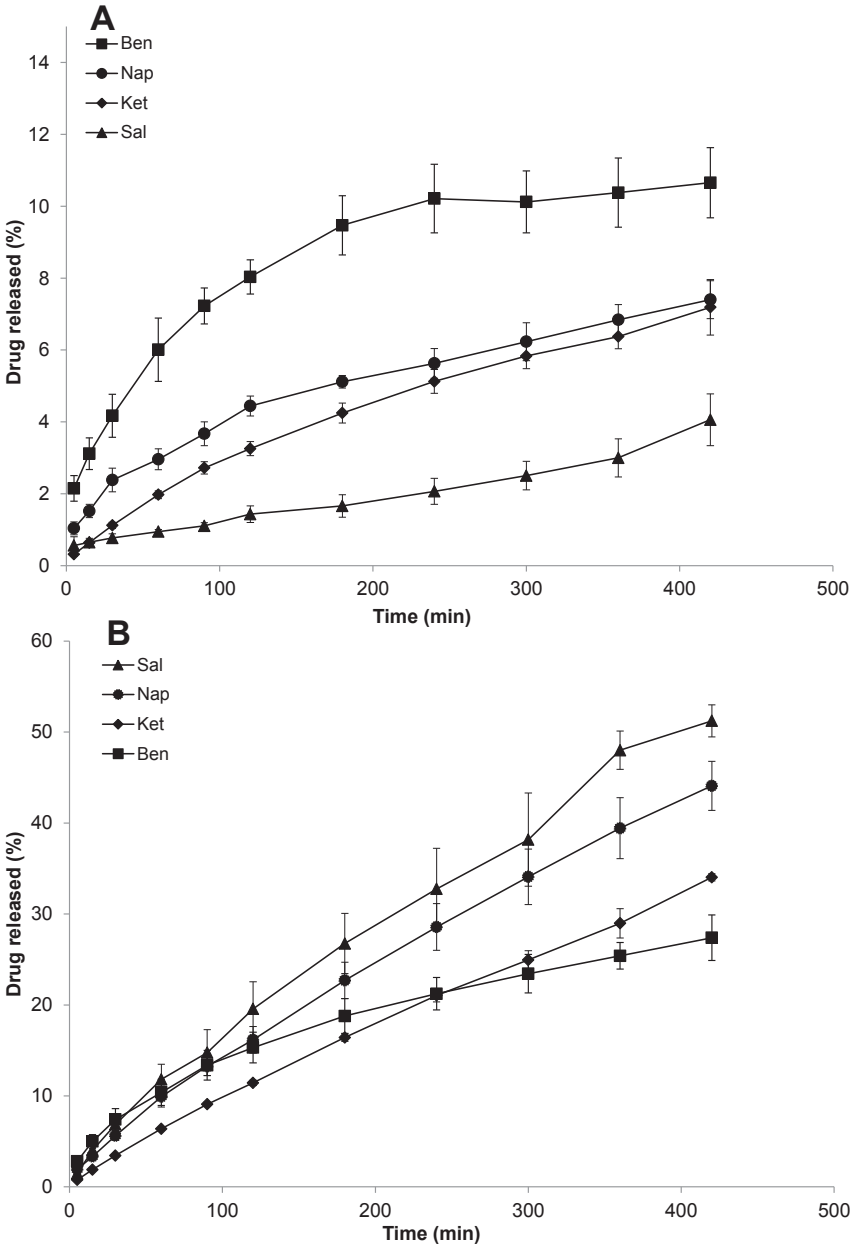


Fig. 6. *In vitro* release profiles of anionic drugs from (EE-D_{50})-EL_{50} nanodispersions, using Franz cells, towards water (A) or 0.9% NaCl soln. (B) as receptor media.

overcoming that of dimethylamino groups of EE. Therefore, these nanocomplexes may be seen as a self-organized zwitterionic macromolecular system, where the sign of ζ can shift from negative to positive one by appropriate changes in their composition. These results are in agreement with our previous studies using homolog nanocomplexes of (EL-D_{50})-EE_X loaded with basic model drugs [25].

Thermal analysis of DIPEC, such as solid material, obtained by centrifugation of freshly dispersions and subsequent freeze-drying did not reveal the presence of free drug. In DSC thermograms, the typical melting endotherms of free drugs were not observed (Fig. 4). Complementarily, X-ray diffraction patterns showed the amorphous state of DIPEC products since they exhibited absence of significant signals present in crystalline drug powders and also in physical mixtures of drug and two PE. The overlapping of the characteristic bands in FT-IR spectra revealed no conclusive data about ionic interactions between the two PE and drugs (data not shown).

The reversibility from solid state to aqueous dispersions is a relevant property in pharmaceutical formulation in terms of stability and flexibility of dosage form design. Freeze dried complexes without the addition of any anti-aggregate or cryoprotectant agent were easily redispersed in water by simple mixing. All nanocomplexes remained well dispersed after redispersion, showing a similar ζ than those in situ prepared. However, an increase in d_H was observed which could be attributed to particle aggregation during drying (Table 4). This behavior is a common property of other nano or micro particulate drug carrier systems, in which different adjuvants are necessary in order to prevent particle aggregation or other irreversible physical changes.

3.3. Stability of nanodispersions

Fig. 5 shows that DIPEC dispersions remained stable and no significant changes in ζ and particle size distribution were observed on dispersions stored at room temperature for 30 days. In addition, pH and optical density of nanocomplex dispersions also remained unchanged over this period (data not shown).

3.4. Drug release

In order to gain additional knowledge on the release properties of anionic drugs from this kind of nanocomplexes, dispersions of (EE-D_{50})-EL_{50}, containing equivalent concentration of each drug were prepared to perform release studies in bicompartimental diffusion cells.

The release mechanism of ionizable drugs from binary PE-D complexes has been extensively studied. The diffusion of neutral species of drug from donor compartment to water as a receptor medium is the main mechanism of drug release. The diffusion of ionic species is prevented by the macroion electrostatic attraction with opposite charges. The presence of ions dissolved in the receptor medium (like NaCl) promotes ion exchange with the macromolecular complex, increasing significantly drug release rates [1,8,26]. DIPEC dispersions exhibited a slow release of drug when water was the receptor medium. Although they contain about 25 mol% of NaCl, the fraction released after 6 h did not exceed 12% in any case. When water was replaced by 0.9% NaCl solution in the receptor compartment (Fig. 6), the release rates of all drugs were significantly raised, in the range of 5.0–14.5 times for Ket and Sal, respectively. A lower effect was observed for Ben exhibiting an increase of 2.9 times, which could be attributed to its higher hydrophilicity.

In order to evaluate the effect of ζ on release profiles, comparative release experiments using (EE-Ben_{50})-EL_{50} having negative and positive charge were performed. Fig. 7 shows the similarity of both release profiles suggesting that a negative ζ does not promote displacement of the anionic drug toward the medium.

On the other hand, drug release from DIPEC dispersions using two simulated physiological media of pH 1.2 and 6.8 was performed. Under both conditions, modified drug release from dispersions was observed (Fig. 8A and B). A negligible effect of pH on release rate of Ben, Sal and Ket could be observed showing similar release profiles at both pH conditions. However, DIPEC containing Nap exhibited a significant decrease in release rate at pH 1.2 probably due to its small aqueous solubility at pH above 4.

The remarkable robustness of drug release toward changes of receptor media pH and composition of nanocomplexes in comparison with homologs (PE-D) binary complexes could be attributed to two opposite effects, 1) an "*encapsulating or buffering effect*" of the loaded drug by the incorporation of the second PE with the consequent lowering of release rates, and 2) a "*promoting effect*" on the neutral species release improved by proton exchange in presence of the DIPEC. This behavior was also reported in DIPEC nanocomplexes based on EL loaded with cationic model drugs and different proportions of counter ionic EE [25].

Finally, the kinetic analysis of release profiles showed a good fit to Peppas equations ($R^2 \geq 0.95$) with exponent n values between 0.43 and 0.55 for the small hydrophilic molecules, Ben and Sal, indicating that the diffusion of the D from the nanocomplexes is the main factor controlling the release (Table 5). However, the release of drug with high molecular weight, Ket and Nap, showed higher n values suggesting a non-fickian or anomalous release behavior. Therefore, Ket and Nap diffusion from nanocomplexes could not be the unique mechanism that controls drug delivery, highlighting the importance of the process of dissolution and dissociation of ionic pairs at this high pH value.

In vivo studies of DIPEC formulations are necessary to evaluate performance in terms of pharmaceutical efficacy and security. However, these *in vitro* results could be considered as a good approximation to potential *in vivo* performance. Hence, these self-organized nanocomplexes are promising materials for the design of drug controlled release systems for oral and topical administration.

4. Conclusions

Aqueous dispersions of self-organized nanoparticulate carrier, based on drug-interpolyelectrolite nanocomplexes, were prepared

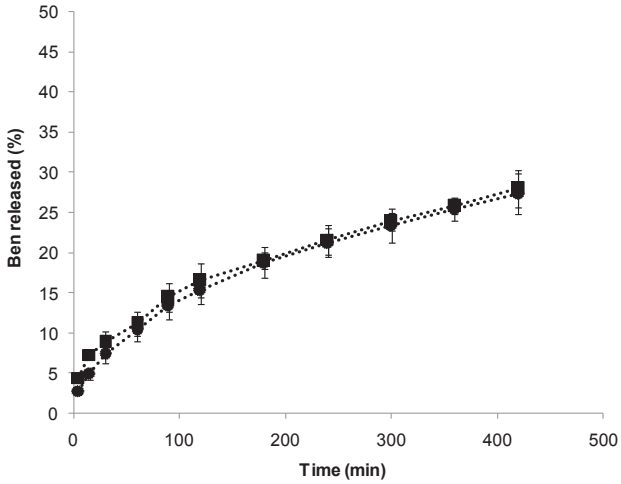


Fig. 7. *In vitro* release profiles of Ben from (EE-Ben)$_{50}$-EL_{50} complex dispersions with (●) positive and (■) negative ζ using NaCl 0.9% as receptor medium.

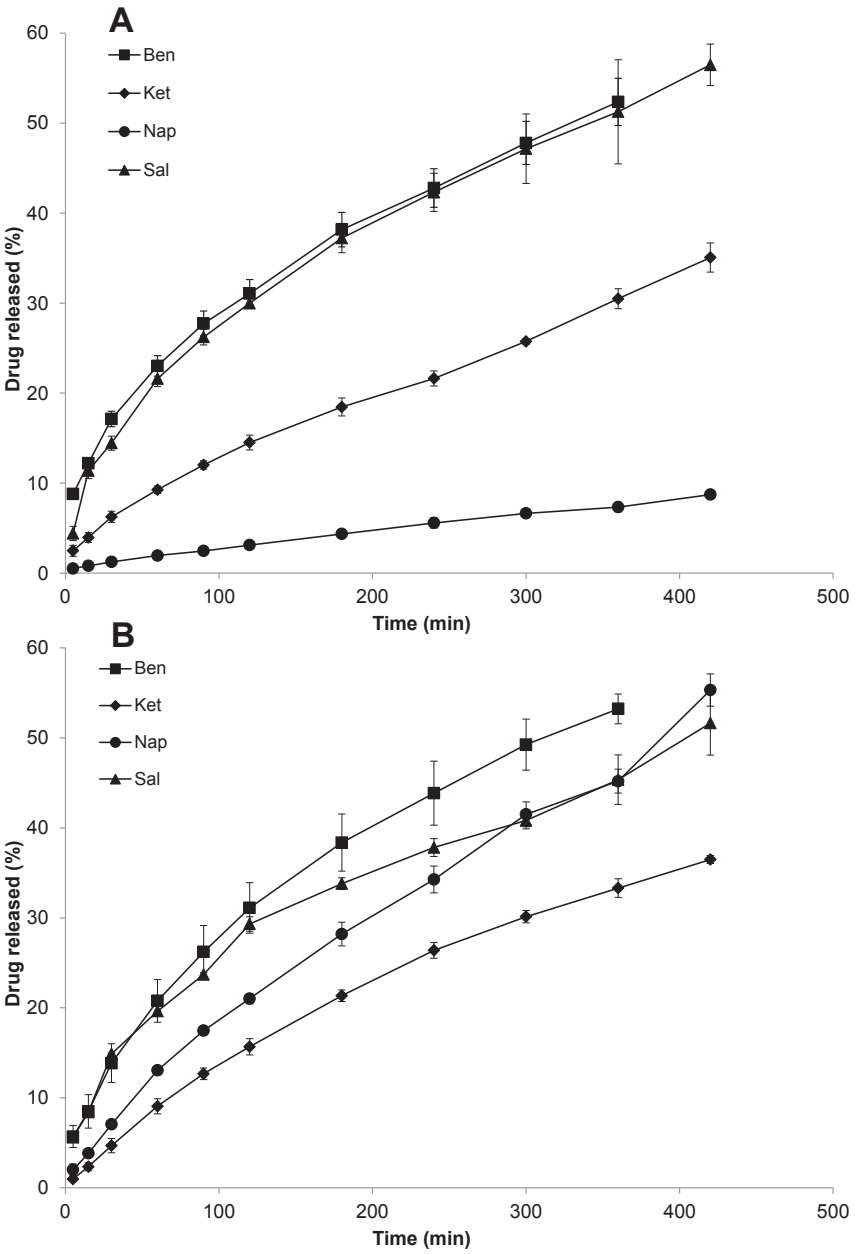


Fig. 8. *In vitro* release profiles of anionic drugs from (EE-D_{50})-EL_{50} nanodispersions, using Franz cells, towards pH = 1.2 buffer soln. (A) or pH = 6.8 phosphate buffer soln. (B) as receptor media.

Table 5
Release kinetic data obtained from drug permeation studies using Peppas equation.

DIPEC	Receptor media											
	Water			NaCl 0.9%			HCl pH = 1.2			PBS pH = 6.8		
	K	*n*	R^2	*k*	*n*	R^2	*k*	*n*	R^2	*k*	*N*	R^2
EE-Ben_{50}-EL_{50}	1.304	0.37	0.955	1.286	0.51	0.998	4.086	0.43	0.996	2.188	0.55	0.995
EE-Sal_{50}-EL_{50}	0.034	0.76	0.956	0.498	0.77	0.998	2.897	0.49	0.998	2.411	0.51	0.987
EE-Ket_{50}-EL_{50}	0.116	0.69	0.995	0.188	0.86	0.999	0.674	0.64	0.996	0.379	0.77	0.993
EE-Nap_{50}-EL_{50}	0.525	0.44	0.994	0.398	0.78	0.999	0.094	0.74	0.997	0.583	0.75	0.997

k expressed as ($\%\cdot min^{-n}$).

using a simple method. Freeze dried nanocomplexes were easily redispersed in water.

The DIPEC nanodispersions were physically stable at least for a month. They could be seen as zwitterionic macromolecular systems that may shift the sign from positive to negative one by appropriate changes in composition.

A remarkably high proportion of drug condensed with PEs under the form of ionic pairs allowed the system to behave as a

reservoir of the acidic drug that releases the drug slowly in water and improves aqueous compatibility at low pH values.

The delivery rate increases in saline solutions simulating biological fluids due to ionic exchange. In addition, D delivery from DIPEC exhibited a remarkable robustness toward changes of pH of receptor media.

In summary, DIPEC systems exhibited interesting properties to design nanoparticulate drug delivery systems for oral and topical routes.

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