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Development of iron/ethylcellulose (core/shell) nanoparticles loaded with diclofenac sodium for arthritis treatment

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ABSTRACT

Diclofenac sodium is a non-steroidal anti-inflammatory drug of choice to treat arthritis because of its potential anti-inflammatory and analgesic activity. Because of its shorter biological half-life, it is needed to be given frequently and at high doses to elicit the required therapeutic activity, simultaneously leading to severe side effects. We hypothesized that the efficient delivery of diclofenac sodium to inflammation using a magnetic colloid could reduce the dose required to bring out sufficient therapeutic response. Hence, we have developed a diclofenac sodium-loaded magnetic nanomedicine, consisting of a magnetic core (iron) and a biocompatible polymeric shell (ethylcellulose) for parenteral administration. These core/shell nanoparticles were synthesized by an emulsion solvent evaporation process. Two drug loading methods were analyzed: the first one being drug addition prior to the emulsion solvent evaporation process (leading to drug entrapment into the polymeric network), and the second method based on diclofenac sodium surface adsorption onto the preformed nanoparticles. Compared to drug adsorption, the entrapment of this active agent into the polymeric matrix yielded a higher drug loading and a slower drug release profile. Such nanocomposites possessed very important characteristics such as unusually high drug loading, enhanced magnetic susceptibility and prolonged drug release, indicating their potential use as nanocarriers for efficient delivery of diclofenac sodium to inflammation sites.

1. Introduction

Many of the diseases in the musculoskeletal system are characterized by local inflammatory processes and are currently treated with non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids. Diclofenac sodium [sodium(*O*-((2,6-dichlorophenyl)-amino)-phenyl)-acetate] is a NSAID that has been shown to be effective in the treatment of acute pain and a broad variety of inflammatory processes including arthritis, because of its potential anti-inflammatory and analgesic activity. Despite its efficient activity, this NSAI agent suffers from several drawbacks, mainly a short biological half-life (due to a very rapid metabolism), a high percentage of protein binding and a very high pre-systemic metabolism. This generating the need of using high doses, simultaneously leading to severe dose-limiting side effects (including cardiac, gastrointestinal, hepatic and renal adverse events) (Carson et al., 1989).

Despite recent sustained release formulations have tried to maintain effective diclofenac sodium concentrations for prolonged periods, they still fail in reducing efficiently the pre-systemic metabolism and the severe side effects (Davies, 1999). Therefore, new drug delivery strategies are needed for enhancing the accumulation of diclofenac sodium at the inflammation region, while minimizing its biodistribution. The main purpose is always to achieve the highest therapeutic effect with minimal toxicity, and a protection of the loaded drug from *in vivo* metabolization and elimination. Active targeting (or specific targeting) of drugs to targeted locations can be achieved by a number of strategies that are based on a specific recognition mechanism (ligand- or receptor-mediated targeting) or by means of stimuli-sensitive drug carriers (Reddy, 2005; Couvreur and Vauthier, 2006; Wei et al., 2006; Hood et al., 2007; Farokhzad and Langer, 2009). The latter are drug delivery systems that generally alter their physical properties under exposure to an external stimulus (e.g., pH, ultrasound, light, enzymes, magnetic fields, etc.). This special property is widely used to trigger drug release at the target site, but can also be utilized to accumulate the drug at the targeted region before allowing its release. In both cases, the systemic distribution of the drug is clearly kept to a minimum (and, subsequently, the undesired side effects) and its therapeutic activity is considerably enhanced (Arias, 2008; Bawa et al., 2009).

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One of the very promising applications of magnetic colloids is focused on the musculoskeletal system. Magnetic nanocarriers could be engineered for local drug delivery to inflammatory sites under the guidance of a magnetic field, assuring therapeutic concentrations while at the same time reducing the incidence of unwanted side effects (Saravanan et al., 2004; Neuberger et al., 2005). Hence, the present work will focus on the formulation of iron/ethylcellulose (core/shell) nanoparticles (NPs) loaded with diclofenac sodium. Ethylcellulose (EC) was selected as the biocompatible polymeric shell on the magnetic particles, responsible for the diclofenac sodium transport and release. This is a hydrophobic polymer, widely used in pharmaceutical technology, chemically stable under storage, and characterized by a great tolerability and lack of toxicity for patients (Dubernet et al., 1990; Kato et al., 1996; Zinutti et al., 1996; Grattard et al., 2002; DeMerlis et al., 2005). Iron (Fe) was chosen as a magnetic nucleus because of its high initial magnetic susceptibility and magnetic saturation (Arias et al., 2007), very low toxicity (LD_{50}: 50 g/kg) (Chua-anusorn et al., 1999; Whittaker et al., 2002) and biodegradability [most Fe NPs of ≈350 nm prepared in this investigation will be eliminated mainly by renal filtration] (Okon et al., 1994). Even more, the inclusion of magnetic systems into polymeric materials does not increase the toxicity of the latter, as have been reported in clinical phase I trials (Ibrahim et al., 1983; Lübbe et al., 1996a,b).

It is therefore of great interest to investigate the applications of iron/ethylcellulose (Fe/EC) nanocomposites in drug delivery to the musculoskeletal system. In this article, a reproducible technique for the preparation of core/shell NPs consisting of a magnetic nucleus and a polymeric shell and loaded with the NSAID diclofenac sodium is described. The amount of drug loaded to the magnetic nanoplatform, either by entrapment in the polymeric network or by single surface adsorption, has been investigated according to the drug, polymer and surfactant concentrations. Finally, the *in vitro* drug release profiles were also evaluated depending on the drug loading procedure.

2. Materials and methods

2.1. Materials

Water used in the experiments was deionized and filtered with a Milli-Q Academic System (Millipore, France). All chemicals used were of analytical quality from Panreac (Spain), except for diclofenac sodium (Guinama, Spain), iron (BASF, Germany), ethylcellulose 9004-57-3 (ICN Biomedical Inc., USA) and pluronic® F-68 (Sigma–Aldrich Chemical Co., France).

2.2. Methods

2.2.1. Preparation of ethylcellulose and iron/ethylcellulose (core/shell) nanoparticles

EC NPs were prepared following an emulsion solvent evaporation method (Arias et al., 2007). Briefly, EC (0.2 g) was dissolved in a mixture of organic solvents (0.2 g ethanol and 1 g benzene). After 24 h at room temperature, 0.1 g of *n*-decane were added to the polymeric phase, and it acted as a stabilizer of the emulsion prepared by adding this organic solution to 4 g of a 10^{-3}N HNO_3 aqueous solution, containing 0.125% (w/v) sodium dodecyl sulphate and 0.375% (w/v) polyethylene glycol 4000. Both phases were heated at 70.0 ± 0.5 °C before mixing, and the incorporation of the aqueous phase to the polymeric one was done under mechanical stirring at 12,000 rpm during 15 min. After that, the organic solvents were completely evaporated using a Rotavapor® to obtain the aqueous suspension of pure EC NPs. This formulation was then subjected to a cleaning procedure that included repeated cycles of centrifugation (30 min at 20,000 rpm, Centrikon T-124 high-speed centrifuge, Kontron, France) and re-dispersion in water, until the conductivity of the supernatant was ≤10 μS/cm.

Fe NPs were selected from 0.3% (w/v) Fe aqueous suspensions (BASF, Germany) by gravitational separation (Arias et al., 2006, 2007). Briefly, during 10 min the suspensions were sonicated in 1 L flasks (internal diameter of ≈90 mm) and settling under gravity was carried out during 60 min, before taking the upper 10 mm of supernatant. The particles were then dried at 60.0 ± 0.5 °C in a vacuum oven and stored until their use.

Finally, the method followed to obtain the magnetic nanocomposites was similar to that above described for the preparation of EC NPs, except that the aqueous phase contained the Fe nuclei [3.75%, w/v] (Arias et al., 2007). Cleaning of the core/shell NPs was achieved by repeated magnetic separation and re-dispersion in an aqueous medium containing pluronic® F-68 (1%, w/v), until the conductivity of the supernatant indicated that the suspensions were sufficiently clean (≤10 μS/cm). For the current investigation, these NPs were freshly prepared and used within 24 h (conservation at 4.0 ± 0.5 °C).

Diclofenac sodium loading onto these materials was achieved by using two procedures. The first one involved single drug surface adsorption onto preformed NPs. Briefly, a suspension of Fe, EC or magnetic core/shell NPs (2%, w/v) was incubated for 24 h (at 25.0 ± 0.5 °C and under mechanical stirring: 50 rpm) with increasing amounts of diclofenac sodium (up to 0.01 M). The second method (entrapment procedure) followed for drug absorption into the pure polymer NPs and into the magnetic nanocomposites was similar to that above described for the preparation of these NPs, except that the aqueous phase contained also appropriate amounts of diclofenac sodium. The influence of the concentration of polymer and stabilizing agents on drug entrapment was also investigated. To that aim, the polymer added to the organic phase was varied between 0.2 and 1 g; also, the aqueous phase could contain between 0 and 2% (w/v) surfactant concentration (sodium dodecyl sulphate/polyethylene glycol 4000 always in the ratio 3/1).

2.2.2. Characterization methods

Mean particle diameters were determined in triplicate at 25.0 ± 0.5 °C by Quasi-Elastic Light Scattering (QELS) using a Nanosizer (Coulter® N4MD, Coulter Electronics Inc., USA). The scattering angle was set at 90° and the measurement was made after suitable dilution of the aqueous NP suspensions. Using the technique of photon correlation spectroscopy (PCS), the instrument evaluates the autocorrelation function of the scattered light intensity; in its standard mode of operation, the software provides an average diameter and a polydispersity index based on the second-order cumulant procedure. The stability of the formulations was evaluated by measuring the size of the particles after 2 weeks of storage at 4.0 ± 0.5 °C in water. To confirm the size measurements and to inspect the coating efficiency, the colloids were checked through analysis by dark-field high resolution transmission electron microscopy (HRTEM) pictures obtained using a STEM PHILIPS CM20 (The Netherlands) microscope set at 80 kV accelerating voltage. Prior to observation, dilute suspensions (≈0.1%, w/v) were sonicated for 5 min, and drops were placed on copper grids with formvar film. The grids were then dried at 35.0 ± 0.5 °C in a convection oven.

The magnetic properties of Fe and magnetic core/shell NPs were determined using a Manics DSM-8 vibrating magnetometer at 25.0 ± 0.5 °C. The magnetic field-responsive behaviour of Fe/EC NPs under the influence of an external magnetic field was also investigated by microscope visualization of a 0.5% (w/v) aqueous suspension under exposure to a 0.2 T permanent magnet, using a Nikon SMZ800 (Japan) stereoscopic zoom microscope.

2.2.3. Determination of diclofenac sodium loaded to iron, ethylcellulose and iron/ethylcellulose (core/shell) nanoparticles

UV absorption measurements, at the maximum absorbance wavelength (276 nm) in a PerkinElmer Lambda 11 UV–vis spectrophotometer (PerkinElmer, USA), were carried out to determine the drug concentration in all the systems investigated, using quartz cells of 1 cm path length. Good linearity was observed at this wavelength and the method has been validated and verified for accuracy, precision and linearity in all conditions tested.

The determination of the amount of diclofenac sodium loaded to the colloids was performed in triplicate by means of spectrophotometric determinations of the drug remaining in the supernatant (after NP centrifugation: 30 min at 20,000 rpm), which was deduced from the total amount of active agent in the NP suspension. For the method to be accurate, we considered the contribution to the absorbance of sources other than variations in drug concentration (mainly surfactant agents and electrolytes), by subtracting the absorbance of the supernatant produced in the same conditions but without diclofenac sodium. Drug incorporation to NPs was expressed in terms of diclofenac sodium entrapment efficiency (%) [encapsulated drug (mg)/total drug in the colloidal suspension (mg) × 100] and diclofenac sodium loading (%) [encapsulated drug (mg)/carrier (mg) × 100] (Brigger et al., 2004).

A qualitative follow-up of the adsorption process was done by electrophoretic mobility (u_e) determinations of the NPs in dilute suspensions (≈0.1%, w/v) with different drug concentrations, using a Malvern Zetasizer 2000 (England) electrophoresis device. Measurements were performed at 25.0 ± 0.5 °C, after 24 h of contact at this temperature under mechanical stirring (50 rpm). The experimental uncertainty of the measurements was <5%. In order to evaluate the effect of ionic strength variations, we performed the experiments both with and without 1 mM NaCl in solution.

2.2.4. In vitro release studies of diclofenac sodium from iron, ethylcellulose and iron/ethylcellulose (core/shell) nanoparticles

The study of drug release from NPs loaded with diclofenac sodium after a single adsorption process or after drug incorporation into the polymeric matrix was done with the NPs prepared following the best diclofenac sodium loading conditions: a 10^{-2} M drug concentration in the adsorption/absorption process.

Diclofenac release from NPs was performed *in vitro* following the dialysis bag method, and using PBS (pH = 7.4 ± 0.1) as the release medium. The bags were soaked in water at 25.0 ± 0.5 °C for 12 h before use. The dialysis bag (cut-off of 2000 Da, Spectrum® Spectra/Por® 6 dialysis membrane tubing, USA) retained the NPs, but allowed the free drug to diffuse into the dissolution medium. 2 mL of NP suspension (containing 3 mg/mL of active agent) were placed into the dialysis bag with the two ends fixed by clamps. The bags were placed in a glass beaker containing 100 mL of the dissolution medium and stirred at 200 rpm. The temperature was maintained at 37.0 ± 0.5 °C during the drug release experiments, which were performed in triplicate. At prefixed time intervals (0.25, 0.5, 0.75, 1, 1.5, 2, 3, 6, 9, 24, 48, 72 and 96 h), 5 mL of the medium were withdrawn and analyzed for the drug content using UV–vis spectrophotometry at 276 nm. An equal volume of PBS, maintained at the same temperature, was added after sample withdrawal to ensure the sink conditions. The same analytical procedure used for the estimation of the drug loading was followed in this study.

3. Results and discussion

3.1. Particle shape and size

From the dynamic light scattering measurements performed on the Fe nuclei, it was found that their average diameter (±standard deviation) was 350 ± 80 nm. The polydispersity index resulting from the cumulant analysis of the PCS data was 0.158, indicating a reasonably narrow size distribution of the nuclei. For the sake of comparison, let us mention that pure EC particles were 240 ± 25 nm in diameter, with a polydispersity index of 0.102.

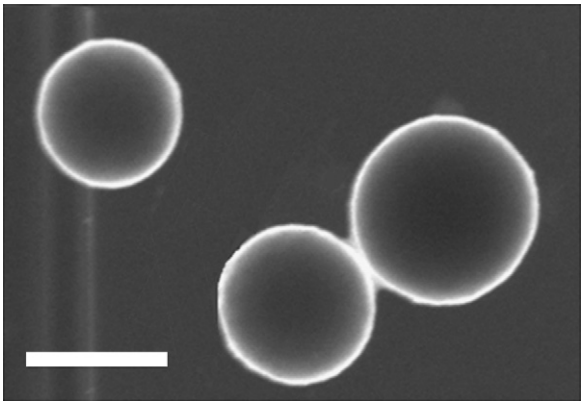

Fig. 1. Dark-field high resolution transmission electron microphotograph of iron/ethylcellulose nanocomposites. Bar length: 400 nm.

The emulsion solvent evaporation method used for the synthesis of Fe/EC colloids allowed the formation of well-stabilized spherical particles with an average diameter of 430 ± 40 nm and a narrow size distribution (polydispersity index: 0.067). Dark-field HRTEM microphotographs of these magnetic core/shell NPs (Fig. 1) allowed to observe that the Fe nuclei were covered by a well-defined polymeric shell of ≈35 nm thick. No presence of aggregates or bulky sediments was observed. The size of this magnetic colloid and the quality of the suspensions did not vary significantly when loaded with different amounts of diclofenac sodium. In addition, no drug precipitation or NP aggregation was observed, and no appreciable change in the size of NPs was detected after 2 weeks of storage at 4.0 ± 0.5 °C in water.

3.2. Magnetic properties

The magnetic responsiveness of Fe/EC nanocomposites was similar to that of the Fe nuclei, except that the polymeric matrix reduces the magnetization of the sample (Arias et al., 2007). From the linear portions (low field) of the first magnetization curve we determined the initial susceptibility: χ_i = 18.4 ± 0.5 for Fe, and 7.3 ± 0.4 for Fe/EC. It was also significant the reduction of saturation magnetization by the polymeric layer: 1755 ± 65 kA/m for Fe, and 745 ± 60 kA/m for Fe/EC. Microscopic observations of an initially homogeneous distribution of magnetic nanocomposites in a small drop of aqueous suspension and of the structures induced by a permanent magnet placed close to one of its faces are displayed in Fig. 2. Particle chaining in the field direction is an indication of strong magnetic dipolar interactions between the NPs. Such interactions overcome the double layer repulsions and could provide a useful tool in driving the diclofenac sodium-loaded NPs to their place of action, keeping them in the desired location.

3.3. Diclofenac sodium surface adsorption onto iron, ethylcellulose and iron/ethylcellulose (core/shell) nanoparticles

Fig. 3 shows the amount of this NSAID adsorbed at the surface of Fe, EC and magnetic composites as a function of the equilibrium drug concentration. A positive effect of diclofenac sodium concentration in the incubation medium on the adsorption efficiency (%) onto the NPs was observed. As can be seen for the three different

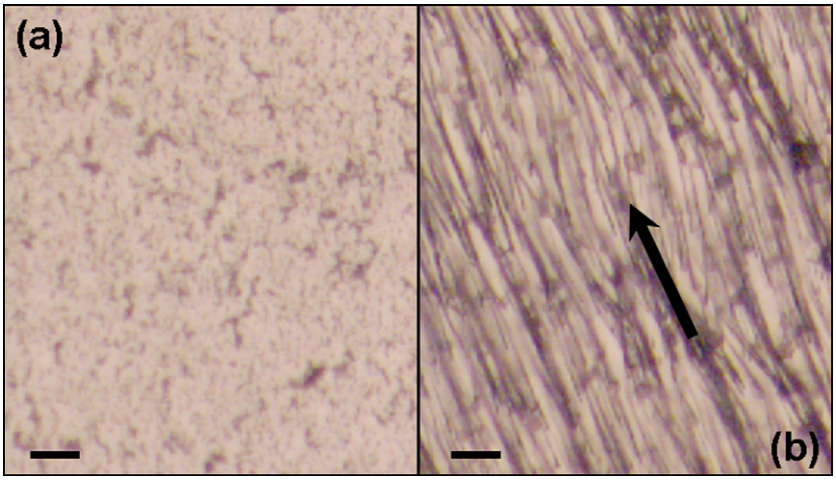

Fig. 2. Optical microscope photographs (magnification 63×) of a magnetic nanocomposite aqueous suspension without (a) and under the influence (b) of an external magnetic field (B = 0.2 T) in the direction of the arrow. Bar length: 10 μm.

types of materials, the adsorption graph is very similar in shape (an increase in drug concentration leads to larger adsorbed amounts, although a sort of saturation is suggested at high equilibrium concentrations), differing only in the incorporated absolute amounts of drug. The entrapment efficiency (%) of NSAID to Fe, EC and magnetic composites increased with the amount of drug in solution up to ≈8%, 11% and 12%, respectively for the range of concentrations investigated. However, NSAID loading (%) remained very low when using the surface adsorption procedure (maximum drug loading ≈1.3%, 1.7% and 1.9%, respectively). It is very interesting the fact that the amount of drug adsorbed by both the pure polymer and the core/shell particles is very similar, suggesting that the surface properties of the core/shell NPs are almost identical to those of pure polymer. Finally, the loading capacities of both EC and Fe/EC are slightly larger than those of the Fe cores, probably due to the highly porous nature of the EC surface (Arias et al., 2007).

The electrophoretic mobility (u_e) determinations qualitatively confirmed the low drug loading achieved (Fig. 4). Note first of all that, in the absence of active agent added, all the particles are negatively charged at the pH of the experiments (pH ≈ 6). In the case of Fe particles, this is the manifestation of the fact that the isoelectric point (the pH of zero zeta potential, that is, of zero u_e) of Fe has been experimentally determined to be in the vicinity of pH 5 (Arias et al., 2007), as a consequence of the existence of a thin iron oxide layer coating the metal in aqueous media. The negative surface charge of the polymer and composite particles comes from the presence of strong groups corresponding to dissociated molecules

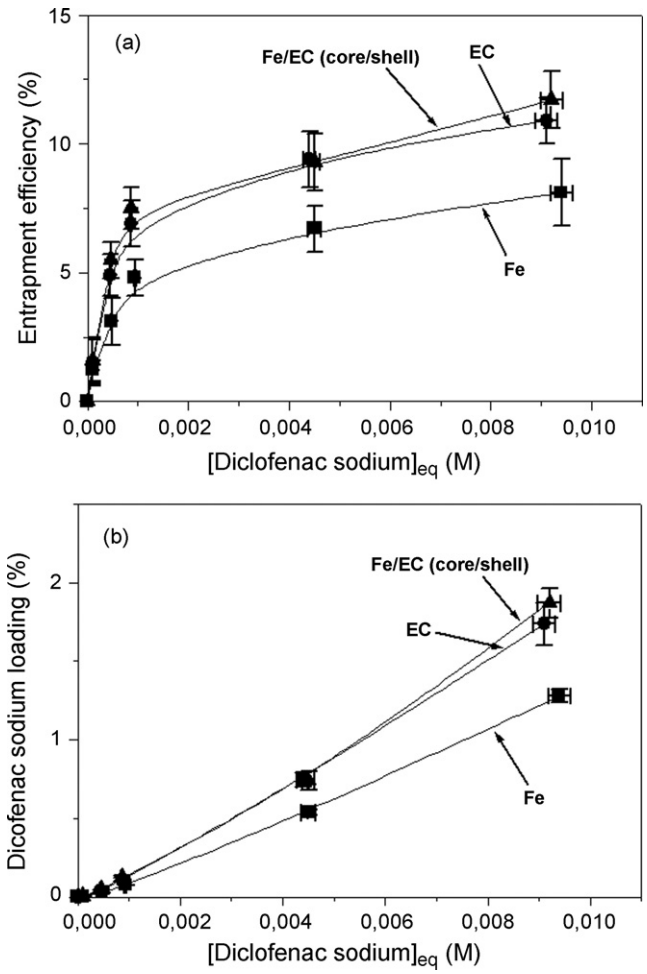

Fig. 3. Diclofenac sodium entrapment efficiency (%) (a) and diclofenac sodium loading (%) (b) on the surface of iron (■), ethylcellulose (●) and iron/ethylcellulose (core/shell) (▲) nanoparticles as a function of the equilibrium drug concentration. The lines are guides to the eye.

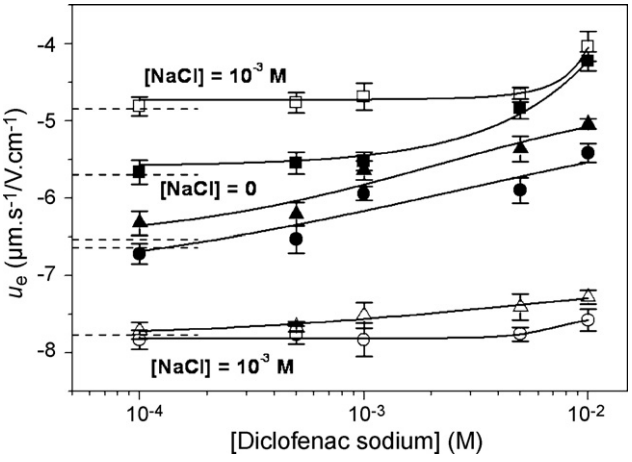

Fig. 4. Electrophoretic mobility (u_e) of iron (■, □), ethylcellulose (●, ○) and iron/ethylcellulose (core/shell) (▲, △) nanoparticles as a function of diclofenac sodium concentration, in the presence (open symbols) or in the absence (full symbols) of 10^{-3} M NaCl. The dashed lines indicate the mean u_e value of the nanoparticles in the absence of the drug for 1 mM NaCl or in water. The lines are guides to the eye.

of the sodium dodecyl sulphate surfactant used in the synthesis. These remain adsorbed on the particle surface even after the thorough cleaning procedure carried out. In addition, weak carboxylic acid groups of the polymer have also been reported to contribute to the charge generation and to the pH dependence of the surface charge of EC (Arias et al., 2007).

On the other hand, diclofenac sodium must be negatively charged in the aqueous medium (pH ≈ 6), as it will be in its ionized form ($pK_a \approx 4$) showing a net negative charge due to the lost of Na^+ (Maitani et al., 1991; Palomo et al., 1999). Thus, an unfavourable electrostatic interaction will exist with the negatively charged polymeric surfaces, thus leading to low drug adsorption. Nevertheless, this molecule provokes a tendency of u_e towards progressively less negative values as its concentration is increased. The main effect responsible for this trend is probably double layer compression, a consequence of the contribution of the drug to ionic strength changes. Finally, a clear influence of the addition of NaCl on the u_e values can also be observed. In the case of Fe, the presence of NaCl clearly yielded a u_e reduction because of the classical double-layer compression mechanism: the decrease of the electric potential with distance to the surface is faster the larger the ionic concentration in solution. As a consequence, the potential at the plane of shear (that is, the zeta potential, always assumed to correspond to the potential at a fixed distance to the surface) will be reduced. Such reduction of zeta potential explains the decrease in u_e (Hunter, 2001). However, EC and Fe/EC composites display a quite different trend: $|u_e|$ increased due to the manifestation of stagnant layer conductivity (Arias et al., 2007). As it was expected, the double layer compression (reduction of u_e) induced when the drug concentration increased was more significant when NaCl was absent from the medium.

3.4. Diclofenac absorption into ethylcellulose and iron/ethylcellulose (core/shell) nanoparticles

3.4.1. Effect of surfactant and polymer concentration

Results not shown for brevity demonstrated that the addition of the different concentrations of the surfactants (polyethylene glycol 4000 and sodium dodecyl sulphate) to the aqueous phase yielded homogeneous distributions of EC and magnetic (core/shell) NPs with reduced size and great uniformity, without influencing the drug incorporation into the NPs. Similar results were reported by Arias et al. (2008) in the case of poly(alkylcyanoacrylate) NPs loaded with 5-fluorouracil. Nevertheless, the presence of these surfactants is required to achieve significant drug loading, as the important role played by them in the drug vehiculization has also being documented: they are supposed to induce the opening of the polymer chains, thus creating a space within the polymeric network where the drug can be incorporated (Llovet et al., 1995; Soppimath et al., 2001).

Concerning the influence of the polymer concentration, no significant effect on drug loading was observed when the amount of polymer emulsified into the aqueous phase was enhanced. Results showed that only the absolute amounts of drug loaded increase when the EC concentration rises. On the contrary, the relative or specific drug loading was essentially independent of the amount of polymer added, staying constant at ≈6.6% for EC and ≈4.9% for Fe/EC composites. Hence, such an increase in the polymer emulsified simply leads to an increment in the formation of diclofenac sodium-loaded NPs (Llovet et al., 1995; Stolnik et al., 1995; McCarron et al., 2000).

3.4.2. Influence of the diclofenac sodium concentration

Under the acidic absorption conditions fixed (10^{-3} M HNO_3, pH ≈ 3), diclofenac sodium must be uncharged in the aqueous medium, as it will be in its non-ionized form ($pK_a \approx 4$) (Maitani et al., 1991; Palomo et al., 1999). Hence, the inclusion of diclofenac sodium species into the EC matrix will be not hindered by any electrostatic repulsion. Furthermore, the formulation conditions tried to minimize the escape of this NSAID: just after the beginning of the emulsion solvent evaporation process, it was observed that the polymer precipitated almost immediately in the aqueous phase thus leading to great mechanical trapping of the drug within the polymeric matrix.

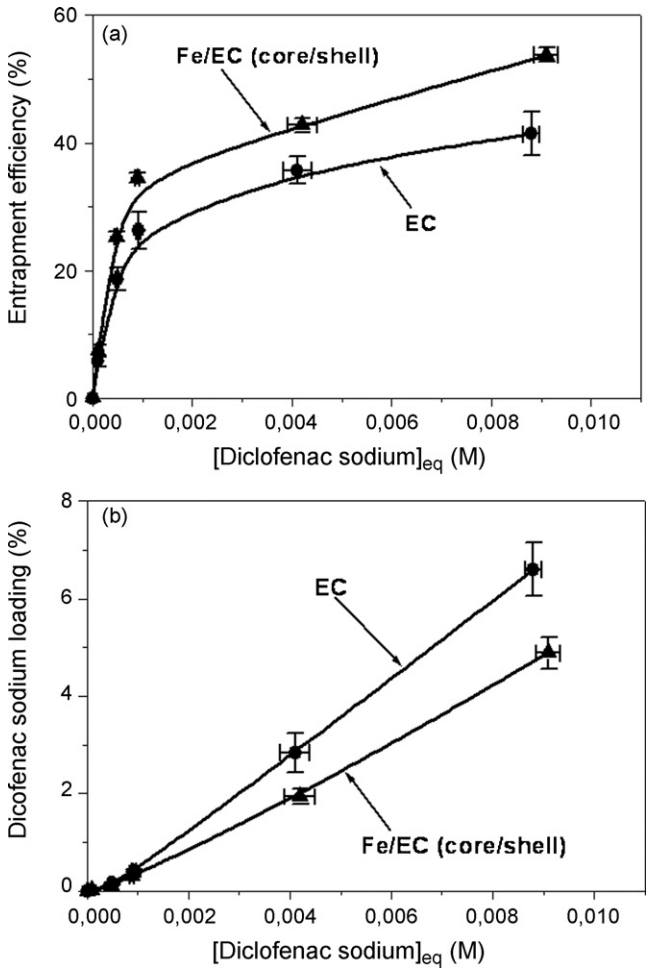

Fig. 5. Diclofenac sodium entrapment efficiency (%) (a) and diclofenac sodium loading (%) (b) into ethylcellulose (●) and iron/ethylcellulose (core/shell) (▲) nanoparticles as a function of the equilibrium drug concentration. The lines are guides to the eye.

Like the adsorption procedure, a positive effect of the drug concentration in the incubation medium on the absorption efficiency (%) into the NPs was observed. Fig. 5 shows the amount of diclofenac sodium absorbed by EC and Fe/EC composites as a function of the equilibrium drug concentration. In comparison to the surface adsorption procedure (Fig. 3), both the entrapment efficiency (%) and the drug loading (%) were significantly enhanced whatever the initial drug concentration fixed. As an example, in the case of EC these parameters rise from ≈11% and 1.7% (after drug adsorption onto the surface) to ≈41% and 6.6% (when the drug was absorbed into the polymer), respectively when the initial diclofenac sodium concentration was 10^{-2} M. With respect to the magnetic core/shell NPs, the entrapment efficiency (%) and the drug loading (%) rise from ≈12% and 1.9% (when the drug was surface adsorbed) to ≈54% and ≈4.9% (after drug absorption into the polymer shell), respectively when the initial diclofenac sodium concentration was 10^{-2} M.

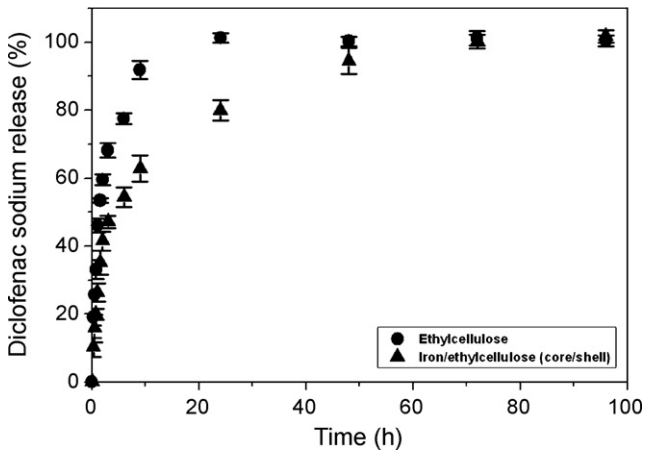

Fig. 6. Release of entrapped diclofenac sodium from ethylcellulose (●) and iron/ethylcellulose (core/shell) (▲) nanoparticles as a function of the incubation time in PBS (pH = 7.4 ± 0.1).

Compared to EC NPs, a higher diclofenac sodium entrapment efficiency was achieved into the magnetic nanocomposites may be due to additional NSAID trapping at the Fe/EC interface. This is favoured by the forces that contribute to the mechanical coating of Fe: attractive electrostatic interactions between the positively charged Fe cores and the negatively charged polymer at the acidic conditions in which the synthesis is performed (Arias et al., 2007). Furthermore, the vicinity of this positively charged Fe surface must be enriched in negative drug species due to attractive electrostatic interactions. With respect to drug loading (%), higher values were obtained in pure EC, a consequence of the lower amount of carrier used in this experiment (EC: 200 mg; magnetic core/shell: 350 mg).

3.5. Release of diclofenac sodium from iron, ethylcellulose and iron/ethylcellulose (core/shell) nanoparticles

The release of the drug adsorbed onto all the NPs investigated was almost complete after 1 h, due to a single and rapid desorption process. However, some differences were observed between Fe and polymer surfaces: drug release from EC and Fe/EC composites was slightly faster. This can be explained if we consider that a higher drug loading onto EC and magnetic composites could enhance the cumulative release (Brasseur et al., 1991). Regarding the release of drug absorbed into EC and Fe/EC NPs, it was a biphasic process with an early rapid release which took place within 1 h (up to ≈46% and 26%, respectively), the remaining diclofenac sodium being slowly liberated during the next 24 and 48 h, respectively (Fig. 6). *In vitro* release results of diclofenac sodium from magnetic carriers were also reported by Saravanan et al. (2004). These authors used magnetite as core particles and glutaraldehyde-crosslinked gelatin as drug-carrier coating; crosslinking apparently produced a more compact gelatin network, leading to slower release rates, extending to ≈18 days.

The rapid first phase release observed in Fig. 6 likely corresponded to the leakage of the surface-associated and/or poorly entrapped NSAID. Drug release during the slower release phase may result, on the contrary, from NSAID diffusion through the polymeric matrix. Such a biphasic profile, suggests that the major fraction of drug was entrapped into the polymeric network rather than adsorbed onto the NP surface. The slower release from the magnetic nanocomposites could be due to a more complex diffusion of the drug entrapped at the Fe/EC interface through the polymeric shell.

4. Conclusions

In this study, the optimal preparation conditions required to obtain diclofenac sodium-loaded Fe/EC (core/shell) nanoparticles suitable for parenteral administration have been identified. The incorporation of this NSAID into the magnetic nanocomposites during the emulsion solvent evaporation process has resulted in a higher drug loading and yielded a slower drug release profile, compared to single surface adsorption. This nanoplatform has a very suitable response to weak magnetic fields, suggesting that they are potential carriers for efficient delivery of diclofenac sodium to inflammation sites.

Acknowledgements

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