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Novel Chitosan Coated Magnetic Nanocarriers for the Targeted Diclofenac Delivery

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New magnetic devices consisting of magnetite functionalized with oleic acid and chitosan have been synthesized and employed to the loading of Diclofenac as potential tool for treatment of targeted inflammatory diseases. Magnetic loaded and un-loaded nanoparticles have been thoroughly characterized by infrared spectroscopy, transmission electron microscopy, determination of hydrodynamic diameter by Dynamic light scattering and zeta potential measurements at different pH conditions. A study of the release of Diclofenac has been performed *in vitro* and available mathematical models have been used to determine the release kinetic. Both properties and release data reveal that this nanomagnetic platform would be suitable for *in vivo* assays.

Keywords: Magnetic Nanoparticles, Oleic Acid, Chitosan, Diclofenac, Release Kinetics.

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1. INTRODUCTION

Magnetic nanoparticles (MNPs) are being actively investigated for many purposes. Especially in biomedicine field, because their application is much promising as targeted drug delivery systems, between other applications. The aim of the development of these systems is transporting a specific drug directly to the site of disease avoiding adverse effects over different parts of the body.[1] Several types of iron oxides have been employed in the field of MNPs, being magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) the preferred ones because of their biocompatibility. However iron oxide MNPs have a large surface-volume ratio with high surface energies as well as elevated chemical activity. They tend to aggregate and to be easily oxidized. By this way, surface coating and stabilization with polymers, small molecules or surfactants provides an effective strategy not only to keep stability but also to improve their reactivity with several substrates.[2]

Chitosan (CS), poly(1 → 4)-2-amino-2-deoxy-*d*-glucan, is a biopolymer that has been widely used in medicine field research because of its biological and chemical characteristics. In general, the size of NPs containing chitosan tends to be on the micrometer and cannot be applied in biomedicine.[3] Here the synthesis, characterization and application of novel magnetic carriers to the controlled release of a non steroidal anti-inflammatory drug (NSAID) are presented. To this purpose, MNPs were prepared using magnetite coated with oleic acid (OA) and CS, employing a simple and low cost method, previously studied.[4]

Diclofenac (Dic), a NSAID widely used for the treatment of inflammatory diseases was selected as model drug. The limitations associated to Dic. treatment lie in its short biological half-life, pre-systemic metabolism and several side effects.[5] The association of Diclofenac to MNPs is intending to construct a potential therapeutic formulation with an appropriate nanoparticle size to treat targeted inflammatory diseases, minimizing the side effects associated to the drug. The information regarding to the use of this NSAID with MNPs is limited in open literature and to the best of the author's knowledge, there are not published works reporting the loading of Diclofenac in this kind of MNPs.[6,7]

2. METHODS

2.1. Preparation and Characterization of MNPs

MNPs containing magnetite and oleic acid (Fe/OA) were prepared following the co-precipitation method:[8] 20 mL of solutions of $FeSO_4 \cdot 7H_2O$ and $FeCl_3 \cdot 6H_2O$ (Fe^{2+}/Fe^{3+}) molar ratio equal to 0.5 were used. Then 0.3 g of oleic acid was added in the reactor under nitrogen atmosphere at 70 °C; and 5 mL of NaOH 5 M were added dropwise.

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The resulting dispersion was transferred to a cold water bath leading decantation and finally dried at 45 °C.

Incorporaton of chitosan (CS) was conducted by nanoprecipitation method using experimental conditions previously studied:[4] 300 mg of the Fe/OA MNPs were dispersed in 75 mL acetone. After 15 min of sonication, 15 mL of a solution of chitosan (10.0 mg ml^{-1}) in acetic acid (50%) was added. The resulting precipitate was magnetically separated with a high-power Nd magnet, washed with water and dried at 45 °C for 24 h. This formulation is hereafter mentioned as Fe/OA/CS.

Dic loading onto the Fe/AO/CS nanoparticles was achieved by physical adsorption leading Fe/AO/CS-Dic formulation. It is worth noting that adsorption conditions (in terms of pH, concentration of Dic., etc.) have been fixed based on literature reports, aiming to solely evaluate the efficacy of physical adsorption method. Further assays are current in development using other incorporation of Dic. routes under similar conditions. This information will be part of a future extended work.

A suspension containing 50 mg of the nanoparticles in 4 mL of phosphate buffer (PBS) pH = 7.4 was added with Diclofenac achieving a final drug concentration of 5 mg mL^{-1}. The dispersion was incubated at room temperature under stirring for 24 h. Samples were withdrawn at different intervals of time to determine drug incorporation. UV absorption measurements were carried out at 276 nm using a UV/visible spectrophotometer Shimadzu 160 Japan. A calibrating curve relating absorbance (A) and Diclofenac concentration was constructed. The contribution to the absorbance of sources other than variations in drug concentration (mainly free stabilizers and electrolytes) was considered. To do this, MNPs were incubated under the same conditions but without Dic.

Drug incorporation was expressed as Diclofenac Encapsulation Efficiency (DEE%): [encapsulated drug (mg)/total drug in the suspension (mg) × 100] and Diclofenac Loading (DL%): [encapsulated drug (mg)/carrier (mg) × 100].

Particle hydrodynamic diameters (D_h) were determined by Dynamic Light Scattering (DLS) at 25 °C using a Malvern Zetasizer. Three different media were assayed for dispersion: distilled water, acetone and *N*,*N*′-dimethylformamide. Zeta potential (ζ) measurements were performed in a Malvern Zetasizer under different pH conditions. The composition of the nanoparticles was analyzed by FTIR-DRIFTS spectroscopy with a Thermo Scientific Nicolet 6700 spectrometer in the range 4000–400 cm^{-1}. Particle morphology was determined by transmission electron microscopy (TEM, JEOL100 CXII, JEOL, TOKIO, Japan, 1983 from CCT, Bahía Blanca, Argentina).

2.2. *In Vitro* Release Study of Diclofenac

The Dic release studies from MNPs were performed *in vitro* using PBS (pH = 7.4) as release medium. A dispersion of 5 mL containing 20 mg Fe/AO/CS-Dic was incubated at 37 °C under magnetic stirring. Samples of the supernatant were withdrawn at prefixed times (1, 2, 3, 5, 6 and 24 h) and analyzed for the drug content by UV/visible. The same treatment was applied to a sample of unloaded MNPs to discard the contribution to the absorbance of other sources.

2.3. Release Data Modeling

Data obtained from *in vitro* release assays were fitted to the following kinetics equations to predict the release kinetic: Zero order;[9] First order kinetics;[10] Higuchi model;[11] Hixson-Crowell model[12] and Korsmeyer-Peppas model.[13, 14] To establish the goodness of fit, r^2 coefficients determined to each model were compared. Models with best fit are those having highest (closer to 1) correlation coefficients.

3. RESULTS AND DISCUSSION

3.1. Characterization of MNPs and Dic Loaded MNPs

MNPs produced within this work yields 60%, with respect to the initial amounts of reactants. This implies that the synthetic pathways are highlyn effective. FTIR-DRIFTS (Fig. 1) analysis shows a band located near 570 cm^{-1} corresponding to the Fe—O stretching vibration of magnetite in the spectrum of both raw and Dic loaded MNPs. Bands located around 1650 cm^{-1} in spectra of both samples, are assignable to asymmetric stretching vibration of the COO group of OA and stretching vibrations of CO and NH groups of CS. Comparing spectra on Figures 1(B) and (C), bands related to Diclofenac are detected in Fe/OA/CS-Dic. spectrum. This fact ensures the Dic inclusion on MNPs.

From DLS measurements performed using dispersions of MNPs in various solvents (Table I) it can be seen that Dic loaded NPs present, in general, smaller D_h than those corresponding to non-loaded particles. The use of solvents of different polarity as dispersant gives an idea about the stability of the formulations in different media regarding to the possibility to form aggregates. From data on the Table I it is worth noting a different trend in the sizes of loaded and unloaded MNPs in the explored dispersants. According to the D_h, raw MNPs are better dispersed in aqueous media while Dic loaded MNPs exhibits smaller D_h in organic ones. This fact could be attributed to the presence of hydrophobic Dic moieties on the NPs surface. It is also important to highlight that the recorded sizes of MNPs-Dic are fully compatible to *in vivo* assays regarding to the biological process of elimination and capture of NPs in the body.[5]

As the polydispersity index in all cases ranged between 0.01 up to 0.5, it can be concluded that both MNPs formulations are rather monodispersed.[15] This aspect results crucial to select the route of administration of the drug during *in vivo* assays. In general, particles with sizes lower

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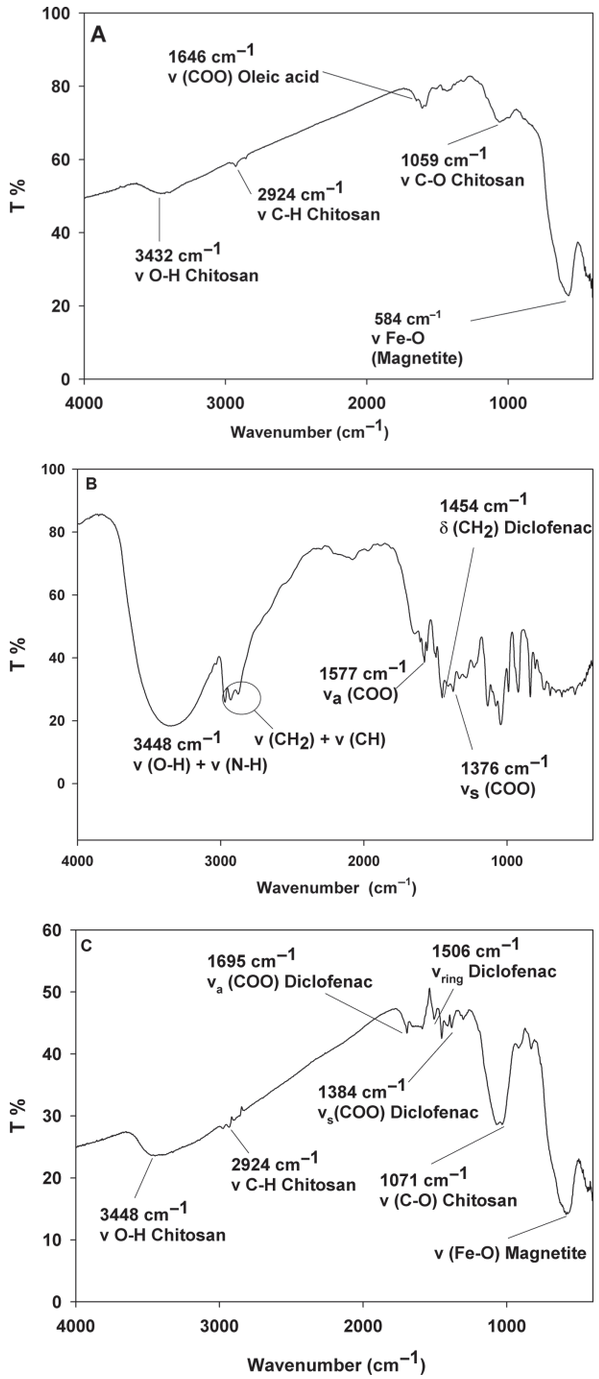


Fig. 1. FTIR-DRIFTS spectra of Fe/OA/CS(A), Diclofenac (B) and Fe/OA/CS-Dic(C) showing major bands for each formulation.

than 5 μm can be used for intravenous route.[5] By this way, these MNPs loaded with Dic could be suitable for intravenous injection as potential treatment of localized inflammatory diseases. Comparable results in terms of the carriers sizes were found by other authors exploring different magnetic devices. For instance, Saravanan et al. prepared magnetic devices containing the same therapeutic agent. Being in the micro-scale they demonstrated that those NPs were even useful and effective for *in vivo* applications.[5] Arias et al. obtained MNPs for Dic release with size around 350 nm and reached satisfactory results in *in vivo* analysis.[7]

Figure 2 shows TEM micrographies of Fe/OA/CS and Fe/OA/CS-Dic, where spherical and homogeneous in shape particles may be observed. Sizes estimated by this technique are about 10–15 nm for both Fe/OA/CS and Fe/OA/CS-Dic. It is worth to mention that size distribution obtained from DLS measurements corresponds to a higher average than the one obtained from TEM due to the intrinsic sensitivity of the technique to small aggregates present in the dispersion.

Stability studies on MNPs loaded Dic were performed by measuring hydrodynamic diameter of the samples after one month incubated in dispersion at room temperature. The results, listed in Table I, indicate that not significant differences in the D_h are detected after this period of time. Thus, the prepared systems may be considered stable enough regarding to their size, for *in vivo* applications.

Measurements of ζ as function of pH are included in Table I and reveal that a similar trend exists analyzing both loaded and unloaded NPs. This behaviour could be ascribed to two reasons: (i) Dic is adsorbed on NPs surface in a way in which its charged functional groups are not surface exposed. (ii) The amount of adsorbed Dic was not enough to coat all the exposed surface of NPs; hence surface charge of raw particles are being detected during ζ assays.

Furthermore, comparing ζ values of MNPs and Dic in the conditions of the adsorption (pH = 7.4; PBS), it is noted that both exhibit similar surface charge (see Table I). Hence ionic interactions were not feasible to occur. Interactions between Dic and MNPs could be of hydrophobic nature. Interactions, other than electrostatic between this drug and the components of MNPs (i.e., Fe or CS), have been reported in open literature.[16]

Based on the characterization results, a structure of the MNPs Dic loaded could be proposed and it is illustrated in Scheme 1.

Magnetic properties of NPs loaded Dic were qualitatively verified by exposing a dispersion of such particles to a high potency Nd magnet (See Graphical Abstract). Exhaustive magnetic analysis of raw MNPs has been included in a previous work[4] where it was demonstrated their superparamagnetic character and suitable magnetization saturation values for the intended applications.

3.2. Diclofenac Loading

The maximum quantity of Dic adsorbed was detected at the fifth hour of the adsorption assay. DEE was 54% and DL was 29%. These results are satisfactory and even better when comparing with other MNPs loaded with the same drug using roughly similar incorporation method. Arias et al. have developed iron/ethylcellulose MNPs loaded

Table I. Hydrodynamic diameters (D_h) of NPs/polydispersity index in different media and zeta potential determinations (ζ) as function of pH.

D_h/Polydispersity index					
Fe/OA/CS			Fe/OA/CS-Dic		
Water	Acetone	Dmf	Water	Acetone	Dmf
347 nm/0.321	516 nm/0.268	641 nm/0.370	368 nm/0.389	169 nm/0.349	324 nm/0.264
Average diameter/Polydispersity index after storage for 1 month at room temperature					
–	–	–	477 nm/0.266	236 nm/0.348	301 nm/0.333
Zeta potential (ζ) Diclofenac (pH 7.4): −17.6 mV					
Fe/OA/CS			Fe/OA/CS-Dic		
pH 2	pH 3	pH 7.4	pH 10	pH 2	pH 3	pH 7.4	pH 10
14.7 mV	10.5 mV	−18.4 mV	−22.9 mV	16 mV	11.5 mV	−23.3 mV	−30.9 mV

with Diclofenac by the physical adsorption method and found an encapsulation efficiency of 12% and a loading percentage of 1.9%.[7] Thereby, the MNPs here presented result very interesting in terms of the quantity of drug incorporated by means of a simple, low-cost and biosafe method.

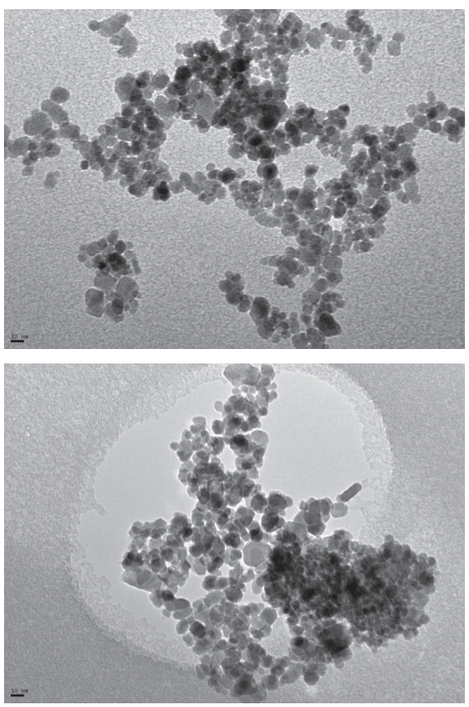
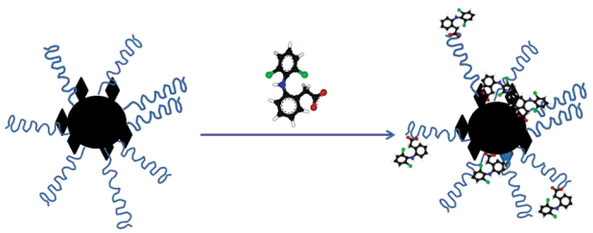


Fig. 2. TEM micrographies of Fe/OA/CS (up) and Fe/OA/CS-Dic (down). Bar length: 10 nm.



Scheme 1. Proposed structure for MNPs loaded with Diclofenac.

3.3. *In Vitro* Release and Kinetics Studies

The *in vitro* release study of Dic from MNPs was performed at 37 °C in PBS pH 7.4 to reproduce physiological conditions. Figure 3 shows the profile for Dic release.

The drug release was slow and burst effect was not detected. The maximum Dic released at 24 h was about 75% with respect to the amount of loaded Dic on MNPs. The kinetic is already suitable to obtain a long-term action of the drug along the time. These results become promising regarding to possible application of this formulation in the clinical treatment of targeted inflammatory diseases.

The data obtained was fitted to available mathematical models to determine the kinetic of drug release. The resulting correlation coefficients (r^2) were found to be 0.9852 for the zero order kinetics model; 0.9954 for the First order model; 0.9766 for the Higuchi mathematical model; 0.9564 and 0.9108 for Hixson-Crowell and Korsmeyer kinetics models, respectively. The model that best describes the release of Dic in this case, is the first order kinetics

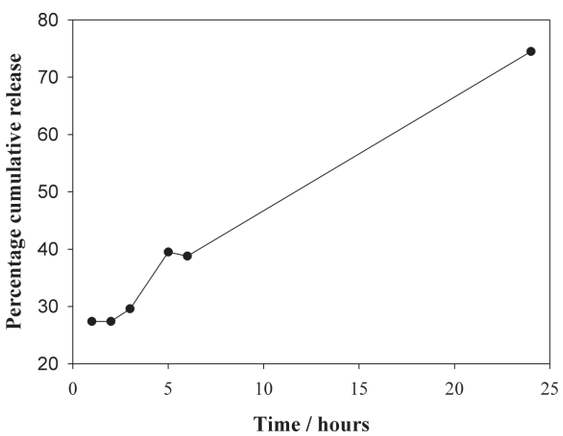


Fig. 3. *In vitro* release of Diclofenac from Fe/OA/CS-Dic MNPs in phosphate buffer (pH = 7.4) at 37 °C.

one in which the medicament release rate depends on its concentration.[7] The Korsmeyer-Peppas equation was applied to determine the mechanism of release of the AINE. First 60% of release data was considered to calculate the release exponent *n* which indicates the mechanism of release.[17] The n exponent found for Fe/OA/CS-Dic was 0.2987, being beyond the limits predicted from the Korsmeyer model which establishes a Fickian diffusion for n around 0.43; anomalous transport for n values between 0.43 and 0.85; and case II transport for values over 0.85. The result here found implies that this model is not useful to estimate the mechanism associated to Dic release from Fe/OA/CS-Dic. Other authors have also reported similar results for the release of Ibuprofen from a Hydroxypropylmethylcellulose matrix.[18] The mechanism for Dic release from magnetic NPs appears to be complex and further detailed studies are currently required to obtain more precise mechanistic information.

4. CONCLUDING REMARKS

In this work the application of a novel magnetic nanocarrier for the controlled release of Diclofenac has been presented. The efficiency of drug loading was very satisfactory employing the simple physical adsorption method. Characterization data suggests that the new formulation presents suitable properties in terms of size, stability and magnetic properties regarding to its potential to treat targeted inflammatory diseases. The results presented within this work are a first stage to the development of a new nanomagnetic platform suitable for target delivery of multiple drugs intended by different pathologies.

Complementary assays are currently in development to study others routes for Dic incorporation, extending the range of drug concentrations and exploring different conditions of release. Furthermore additional studies are intended aiming to apply these nanosystems in culture cells and *in vivo* animal models to evaluate the specific application of these devices as anti-inflammatory therapeutic agent.

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