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IJCPA, 2014; 1(4): 154-164

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

ISSN: 2348-0726

Research Article

Study of the Experimental Conditions and Mechanisms for Diclofenac Loading in Functionalized Magnetic Nanoparticles

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Received: 28 June 2014 / Revised: 18 July 2014 / Accepted: 29 August 2014/ Online publication: 30 August 2014

ABSTRACT

Magnetic nanoparticles (MNPs) composed of magnetite coated with oleic acid and functionalized with chitosan have been prepared to the targeted release of the non steroidal anti-inflammatory drug (NSAID) Diclofenac. Two procedures to incorporate the drug were explored: simple adsorption and covalent linkage. The impact of experimental variables such as the nature of the NSAID (commercial versus analytical sodium Diclofenac), its concentration and amount of coupling agent were evaluated in terms of the loading efficiency and the properties of interest of MNPs (size, surface functionality, stability). Loaded and unloaded MNPs were characterized by FTIR-DRIFTS spectroscopy, dynamic light scattering, zeta potential and transmission electron microscopy. UV-visible quantification of Diclofenac was achieved reaching values of loading efficiency between 11 and 27% depending on the experimental conditions. Probable mechanisms for drug-MNPs interactions have been proposed on the base of characterization data.

A nanocarrier of size lower than 150 nm, with satisfactory loading skill and high dispersion capability on water was obtained. The release behavior demonstrated to be independent on the presence of an external magnetic field. The properties found in these nanosystems result relevant in view of their effective *in vivo* implementation.

Keywords: Magnetic nanoparticles; Chitosan; Diclofenac; Drug target; *In vitro* release.

1. INTRODUCTION

Diclofenac is one of the non steroidal anti-inflammatory drug (NSAID) commonly chosen for the treatment of chronic inflammatory diseases because of its favorable pharmacodynamic characteristics[1,2]. Anyway, the chronic treatment with this NSAID leads to some side effects, being gastrointestinal and kidney damage the most frequent ones. In the last years several drug delivery systems have been developed aiming to diminishing these drawbacks, being most of them intended for oral intake or transdermal delivery[3,4]. Even the development of these systems has contributed to diminish the side effects of this NSAID, neither of them was designed as intended for the targeted delivery of this therapeutic agent to the specific injured tissue.

Magnetic systems of varied composition appear as an attractive strategy in order to target delivery of therapeutic agents to a specific site of the body by the aid of a magnetic field, avoiding or at least minimizing collateral effects associated to most drugs[8, 9, 10].

The implementation of magnetic nanocarreirs to target and release Diclofenac has not been explored enough according to the available literature[11.] For instance, Saravanan et al. obtained magnetic microspheres composed by gelatin to *in vivo* targeted Diclofenac delivery under the influence of a magnet. These authors found that only a 5% of the drug was concentrated in the target site. They concluded that further studies regarding to the carriers formulations are necessary to achieve higher drug

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amounts in the blank organ[8]. Arias et al. have prepared iron/ethylcellulose (core/shell) nanoparticles loaded with Diclofenac sodium for arthritis treatment. In their research they obtained drug loading levels of about 11% by surface adsorption and a prolonged drug release profile[12].

The design of a nanocarrier composed by a magnetic core and functionalized with chitosan appears as an interesting combination for drug delivery taking into account the compatible biological and chemical properties of the polymer with the magnetic capability of the iron oxide.

The aim of this manuscript lies in obtaining a suitable magnetic nanoplatform composed of magnetite coated with chitosan to the target of Diclofenac. The presence of the polymer is required, firstly to stabilize the magnetite NPs that have a strong tend to the aggregation after formed, and secondly to provide biocompatibility and reactive functional groups to the nanocarriers. Articles in open literature devoted to the preparation of magnetic systems containing chitosan are numerous[13, 14] and many of them are employed to the delivery of varied therapeutic agents[15, 16, 17]. However the design and evaluation of a nanocarrier based on a combination of magnetite/oleic acid/chitosan and Diclofenac have not been earlier reported as the best of these author´s knowledge.

In a previous work the simple adsorption of the drug was achieved in this kind of carrier[15]. The present contribution deals with the optimization of the mentioned preliminary formulation exploring new insights related to: i- the stability and sizes of the MNPs; ii- the covalent Diclofenac linkage and, iii- the release behavior and mechanisms for the drug-carriers interactions. This last aspect is missing in the actual literature regarding to the topic. All these issues are of relevant importance as the initial stage to obtain efficient nanodevices for *in vivo* studies.

2. MATERIALS AND METHODS

2.1 Synthesis of magnetic nanocarriers using different glutaraldehyde concentrations.

MNPs from magnetite and oleic acid (MAG/OA) were prepared by co-precipitation under previously studied conditions[15]. Briefly, 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 mixed. Then 0.3 g of oleic acid were added in the reactor under nitrogen atmosphere at 70°C; and 5 mL of NaOH 5M were added drop wise. The incorporation of the polymer was achieved by nanoprecipitation: 0.3 g of MAG/OA MNPs were dispersed in 75 mL acetone. After 15 minutes of sonication, 15 mL of a solution of chitosan (CS) (10.0 mg / ml) in acetic acid (50% v/v) were added. The resulting solid was magnetically separated with a high-power Nd magnet, washed with water three times and dried at 45°C for 24 hours. Crosslinking with glutaraldehyde (GA) was developed to fix CS to the magnetic core. To do this, 50 mg MAG/OA/CS were dispersed in water and then added with variable concentrations of the crosslinker (i.e: 1.5, 3 and 5 mL of an aqueous solution of glutaraldehyde (25% m/v) to obtain final GA concentrations of about 7.5 **(1)**; 15 **(3)** and 25% **(4)** respectively. After 2 hours of reaction at 45°C under magnetic stirring, samples were washed with water for three times and dried at 45°C in an oven. In order to evaluate the effect of ultrasound treatment in the efficiency of crosslinking procedure, the formulation containing 1.5% of GA was sonicated for 2 hours instead of magnetic stirring **(2)**.

2.2 Incorporation of Diclofenac

2.2.1 Simple adsorption method: aqueous dispersions containing 50 mg of nanocarriers and the corresponding amount of NSAID (25 or 50 mg) were magnetically stirred for 24 hours at room temperature. Samples were withdrawn at different intervals of time to determine drug incorporation.

2.2.2 Covalent linkage: Diclofenac was practiced by adding different quantities (50 or 100 µL) of GA to an aqueous dispersion containing 50 mg of the MNPs and the proper amount of the drug. Dispersions were magnetically stirred for 24 hours at room temperature. In order to determine drug incorporation, samples were withdrawn at different intervals of time.

In both methodologies, the influence of the nature of the drug was analyzed by using both a commercial available Diclofenac formulation and pure Diclofenac sodium.

After the loading of the drug, the purification of MNPs was performed by washing with distilled water to remove non interacting NSAID and dried at 45°C. The resulting powder as it

or redispersed in water was employed for whole physico-chemical characterization.

2.3 Diclofenac quantification

UV spectroscopic measurements were carried out to determine drug incorporation into the MNPs at 276 nm using a 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 by incubating MNPs under the same conditions but without the drug. Drug incorporation was expressed as Diclofenac Loading Efficiency (DLE %): [loaded drug (mg)/total drug in the suspension (mg) ×100].

2.4 Characterization

Particle hydrodynamic diameters (Dh) were determined by Dynamic Light Scattering (DLS) at 25°C. Dispersions were prepared using concentrations of about 0.1 mg/mL in both distilled water and acetone. Zeta potential (ζ) measurements were carried out in aqueous dispersions of concentration 0.1 mg/mL. Both determinations were performed in a Malvern Zetasizer for three independent measurements. Errors between samples were statistically analyzed and considered not significant.

The presence of different components of the nanoparticles as well as drug incorporation was verified by Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS-FTIR) using a Thermo Scientific Nicolet 6700 spectrometer, recording spectra in the range 4000–400 cm^{-1}. A few milligrams of the samples were mixed manually in a mortar with KBr powder to be placed in the sample unit of spectrometer.

Transmission electron microscopy (TEM, JEOL100 CXII, JEOL, TOKIO, Japan, 1983 from CCT, Bahía Blanca, Argentina) was employed to analyze particle morphology.

Stability studies were assayed by determination of Dh after two months of storage of MNPs samples in the different solvent dispersions and at room temperature.

High-resolution inductively coupled plasma atomic emission spectroscopy (ICP-AES, Shimadzu 9000) was used to evaluate the composition of the nanoparticles in terms of total iron content. For these measurements, 10 mg of MNPs were dissolved in 10 mL HCl 36% w/v.

The magnetic measurements were carried out in a vibrating sample magnetometer (VSM), and were reported in previous work[17].

2.5 *In vitro* release assays.

Diclofenac release has been studied *in vitro* using the best formulation in terms of the DLE and physicochemical properties. The influence of an external magnetic field during the assay was explored. By this way, 50 mg of the drug loaded nanocarrier were dispersed in 5 mL PBS (pH = 7.4) and incubated at 37°C under magnetic stirring for 24 hours. Samples of the supernatants were withdrawn at prefixed times (1, 2, 3, 5, 12 and 24 hours) and analyzed for the drug content by UV/visible spectroscopy. The same treatment was applied to the corresponding unloaded nanoparticles under both experimental conditions (presence and absence of the magnetic field) in order to be employed as blank of reaction to discard the contribution to the absorbance of other sources.

2.6 Release data modeling

Data obtained from in vitro release assays were fitted to the following mathematical models to predict the in vitro release kinetic:

(a) The zero-order rate describes those systems in which drug release does not depend on its concentration[18]:

$C = K_0 t$..... (1)

K_0 is the zero-order rate constant expressed in units of concentration/time and t is the time.

(b) The first-order kinetic model, where the drug release rate depends on its concentration [19]:

$\log c = \log c0 - kt / 2.303$..... (2)

being C_0, the initial concentration of drug and k, the first-order constant.

(c) The Higuchi model, in which the release of drugs from an insoluble matrix, is described as the square root of time dependent process[20]:

$Q = Kt^{1/2}$..... (3)

K is a constant reflecting the design variables of the system.

(d) The Hixson–Crowell model describes the release from systems in which there is a change in the surface area and in the diameter of particles or carrier[18]:

$Q_0^{1/3} - Q_t^{1/3} = K_{HC}t$..... (4)

Here, Q_t is the amount of drug released in time *t*, Q_0 is the initial amount of the drug and K_{HC} is the rate constant of the Hixson–Crowell rate equation.

(d) Finally, the Korsmeyer model employs a semi-empirical equation to describe the drug release from swelling controlled systems[21, 22]:

$M_t / M_\infty = Kt^n$ .....(5)

M_t/M_∞ is the fraction of drug released at time *t*, *K* is the rate constant and *n* is the release exponent. The *n* value is used to characterize different release mechanisms.

The goodness of fit was established by determining r^2 coefficients to each model. Highest (closer to 1) correlation coefficients are the models with best fit.

3. RESULTS AND DISCUSSION

3.1 Characterization of unloaded nanocarriers

GA was incorporated to MNPs formulation to crosslink the CS moieties, avoiding its disgregation and ensuring the stability of the nanosystems during application.

The influence of the GA was evaluated in terms of the evolution of the nanocarriers sizes. Figure 1 shows the tendency in Dh as a function of the GA nominal concentration using different solvents as dispersant media. It is not observable a straight trend between size and the solvent employed for the dispersion. It is worth noting that using the lowest concentration of GA (7.50 % m/v), the Dh diminishes with respect to the non-crosslinked nanocarrier. When ultrasonic treatment was implemented -at this GA concentration- a noticeable reduction in Dh was reached in the aqueous dispersion (see formulation 2 in Figure 1). Increasing GA concentration, higher Dh values are recorded, which is reasonable knowing the GA trend to form oligomers or even low molecular weigth polymers by interpenetration of its chains[23]. The obtained PDIs values resulted lower than 0.5 using all the tested solvents with all samples. This fact assures an almost monodispersed system.

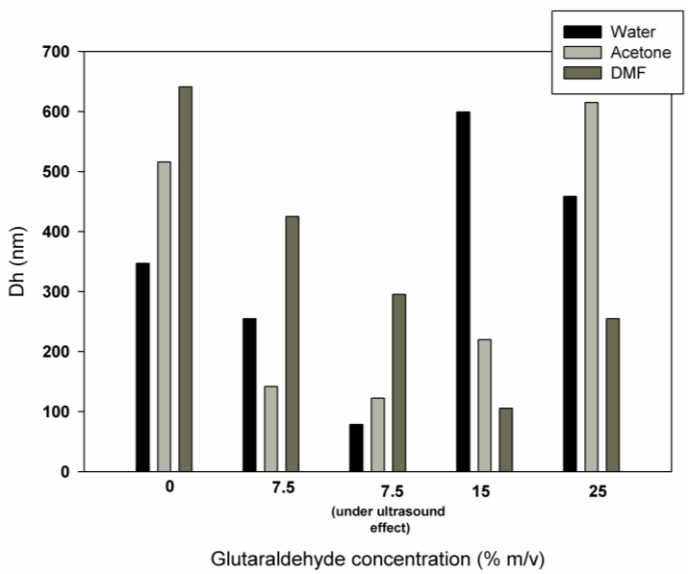


Figure 1: Dh of unloaded magnetic carriers in different dispersion media as a function of glutaraldehyde concentration.

Composition of all nanocarriers was analyzed by FTIR DRIFTS (Figure 2). MAG spectrum shows a strong band at 588 cm^{-1}, assignable to Fe-O stretching vibration. On the other hand, CS spectrum reveals the presence of bands at 3345 cm^{-1} (v O-H), 2920 and 2854 cm^{-1} (v C-H); 1560 cm^{-1} (N-H bending vibrations) and 1023 cm^{-1} (v CO). Spectra corresponding to MNPs composed by magnetite recovered with OA and functionalized with CS, reveals a band around 1646 cm^{-1} assignable to asymmetric stretching of COO group of the OA; bands present near 1510 and 1428 cm^{-1} could be attributable to COO^- coordinated to Fe.

When analyzing spectra corresponding to MNPs crosslinked with GA, the band associated to Fe-O stretching vibration as well as the bands assignable to CS above described, are detected. Signals corresponding to OA are masked by new bands from GA.

The crosslinking reaction with GA can be evidenced by the appearance of a band near 1650 cm^{-1}. This band is characteristic and indicative of the interaction between free amine groups from CS with aldehydic groups from GA to form stable imine

bonds leading to the formation of a Schiff base (v N=C). As described in literature, crosslinking reaction can involve two CS units belonging or not to the same polymeric chain[24].

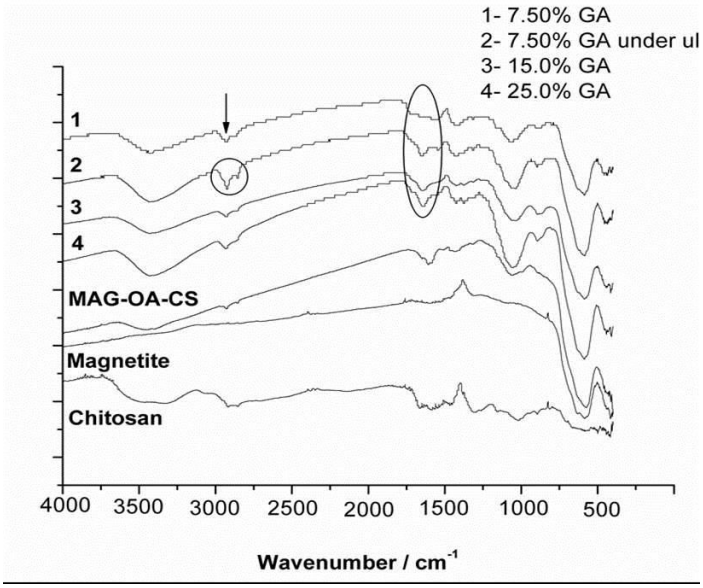


Figure 2: FTIR-DRIFTS spectra corresponding to: magnetite, chitosan, MNPs and MNPs crosslinked with different glutaraldehyde concentrations (% m/v).

Comparing FTIR spectra of formulations containing GA to the corresponding to raw MNPs, it is notable an increasing in the intensity of the band located near 2924 cm^{-1}, attributed to C-H and CH_2 stretching vibration of CS (See arrow in Figure 2). This fact is associated to the contribution of GA during crosslinking process of polymeric chains. It is worth noting that such band is clearly more intense in formulation **2** (See circled signals in Figure 2) comparing with the rest of nanocarriers and, especially to nanocarrier **1**, where the same concentration of crosslinking agent was used (7.50%).

FTIR DRIFTS analyses assure that prepared nanocarriers present all the components - i.e. CS, MAG/OA as well as GA. The application of ultrasound treatment favored the interactions GA-CS, providing extra stability to the formulation **2**.

Morphological analysis by TEM demonstrated that MNPs are spherical in shape with sizes between 15-30 nm. Figure 3 shows a TEM micrography corresponding to nanocarrier **2**, as example. In this case estimated size is between 5 and 10 nm and mainly corresponds to the magnetic core of the nanocarriers.

In general, the values of nanoparticles sizes, determined by DLS (see Figure 1), remain higher than the one estimated by TEM due to an intrinsic sensitivity of DLS to determine small aggregates in dispersion which present lower diffusion electrophoretic coefficient [15,17].

The ICP results revealed that the iron (from magnetite) content, expressed as mg Fe/mg MNP, in all the formulations was almost constant, of roughly 0.40 in average.

Regarding to the magnetic properties, an exhaustive analysis was developed in earlier works[17,25], then the same magnetic behaviour is expected and was qualitatively verified.

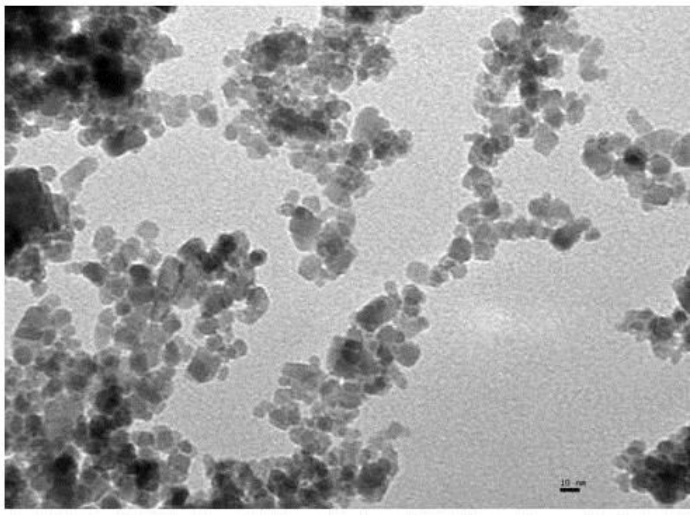


Figure 3: TEM micrography corresponding to unloaded magnetic nanocarrier 2

3.2 Z potential analysis: Proposed interacting mechanisms between carrier's components.

The evolution of Z potential as a function of the added GA concentration is depicted in Figure 4.

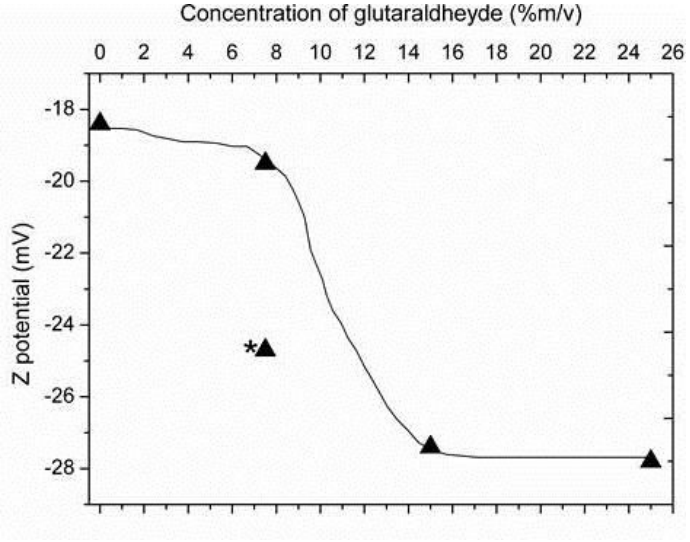


Figure 4: Evolution of the Z potencial as a function of the nominal concentration of GA.*corresponds to the formulation 2 containing 7.5% of GA under ultrasound treatment.

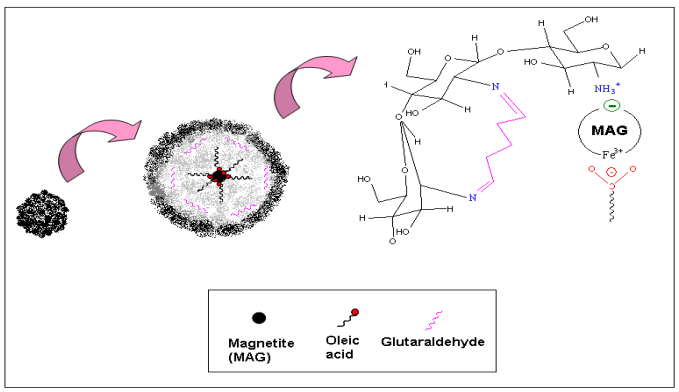
From the Figure it is clear that the surface charge diminishes as the concentration of GA increases.

This behaviour could be ascribed to interactions between GA and positive charged amino groups from CS available in the surface. The partial neutralization of these positive charged moieties by crosslinking would increment the negative surface of MNPs. Crosslinker interacting functional groups could be associated with monomeric GA as well as its oligomers or even polymers of varied molecular weights [23].

When increasing GA concentration up to 15%, not significant differences in ζ values are observed, indicating a possible saturation of the amine available groups from polymeric phase.

On the other hand it is noticeable the influence of the ultrasound treatment on the ζ of formulations containing 7.5% of GA. It is worth noting that the sample prepared by ultrasound exhibited a ζ lower than the similar prepared without ultrasound influence. This fact could be ascribed to the reduction of the aggregation during MNPs crosslinking leading to more available amine functional surface groups able to interact with GA moieties.

According to ξ and data regarding to the other characterization techniques a possible MNPs structure is proposed and included in Scheme 1.



Scheme 1: Proposed MNPs structure.

From the proposed structure and crosslinking mechanism it is deduced that interactions between CS and GA lead to the formation of stable imine bonds that would be the responsible in incrementing the stability of the nanocarriers by preventing polymer disggregation.

3.3 Experimental parameters influencing Diclofenac incorporation

The drug loading was assayed using formulation **2** .This sample was selected between the others regarding to its size and dispersability properties.

Two procedures to incorporate Diclofenac were performed such as simple adsorption and covalent linkage by a coupling agent (GA). Further experimental conditions were evaluated using both incorporation routes, i.e the nature of the drug (using a commercial formulation (DA) and pure sodic Diclofenac (SD)) and its concentration as well as the GA concentration to promote covalent linkages between the drug and the carrier. Characterization results in terms of Dh, ξ and DLE recovered from this screening are shown in Table 1.

Table 1: Diclofenac loading efficiency (DLE%), Dh and zeta potencial of all formulations obtained from varying different experimental conditions during drug encapsulation

MNPs formulation	Diclofenac form, quantity (mg/5mL)	GA initial concentration[1]	Dh (nm) / PDI		ζ mV	DLE %
			Water	Acetone		
Simple adsorption						
A	DA, 25	---	58.7/0.333	141.8/0.260	-15.1	0
B	DA, 50	---	255.0/0.263	122.4/0.472	1.79	11
E	SD, 25	---	825.0/ 0.080	458.7/0.282	-22.8	33
F	SD, 50	---	935/0,260	615/0.377	-24.1	3,8
Covalent linkage						
C	DA, 25	50 uL	396.1/0.370	220/0.307	-28.0	20
D	DA, 50	50 uL	615.0/0.210	955.0/0.297	-17.6	8
G	SD, 25	50 uL	149.3/ 0.268	235.9/0.348	-8.11	27
H	SD , 25	100 uL	825/ 0,394	955.4/0.253	-3.30	23

[1]Glutaraldehyde added as covalent linkage agent (µL/5mL)

3.3.1. Incorporation route

a. Simple Adsorption

The physical adsorption was unsuccessful- or at least was not detected by UV/visible methods- employing commercial Diclofenac, using the lower initial concentration of drug. When such concentration was duplicated, the DLE increased to roughly 11%.

ζ was almost -15.1 mV in the case of formulation A; which is similar to the one obtained for the unloaded nanocarriers. When duplicating the commercial drug amount, a significant

change in ζ value was registered. Surface charge turned into positive and could be considered as an evidence of drug incorporation, meaning that different functional groups remained exposed on surfaces of loaded MNPs.

When pure SD was employed, the DLE increased significantly in comparison to the one registered using the commercial drug. This could be due to possible interferences referring to drug-NPs surface interactions caused by excipients present in the commercial formulation. When concentration was incremented, DLE considerably diminished probably due to the saturation of available reactive sites on MNPs surface to interact with the SD.

In a previous work a DLE greatly higher, of about 54%, was reached using almost similar experimental conditions[15]. Differences are given by the chemical structure of the nanocarriers. In our earlier work the carriers were not crosslinked by GA. Therefore the NH_2/NH_3 and OH^- groups of CS remained accessible to interact with the drug. Whereas, in present case, the crosslinking is restricting polymeric-drug interactions. Similar results have been found by other researchers using chitosan-GA crosslinked beads for controlled release of Diclofenac[24].

The variation on the sizes of the loaded MNPs obtained by simple adsorption is strongly dependent on the nature of the drug and its concentration. It is observed that the increment of drug leads to an enlargement of the MNPs. In such cases the nanosystems are better dispersed in organic media. These evidences correlate well with the data on surface charge and are related to the surface exposed functional groups when the drug is effectively incorporated.

Using SD higher sized particles are obtained which could be ascribed to and increment of the aggregation caused by the presence of the drug moieties.

b. Covalent linkage with glutaraldheyde

The further addition of GA was performed to reinforce the drug-MNPs bonds. In general an enhancement of the loaded capability was observed with respect to the simple adsorption procedure under similar experimental conditions.

Exploring this loading procedure it is found that the loading levels are not improved by duplicating the drug concentration using either SD or DA drugs. Similar results have been reported by Boonsongrit et al.[16]. They have obtained nano and microparticles of CS for the controlled release of different drugs, including SD. They found an inverse ratio between the SD concentration and the loading level. They do not offer any justification for this observation. We propose that a saturation of the GA reactive sites occurred hence higher amounts of drug remain constant the DLE% or even reduced it by reaction with other moieties than the drug (DA).

From the zeta potential data it appears that the surface charge is different from the corresponding to the loaded carrier using simple adsorption procedure. Inducing covalent linkage, negatively charged MNPs were obtained. By the first these data could be considered as a proof that the commercial Diclofenac was effectively incorporated to the MNPs. And on the other side, comparing the value and fundamentally the sign of the zeta potential with the one registered for formulation **B**, it could be corroborated the different mechanism that take place for the DS incorporation when the coupling agent was employed.

Regarding to the average Dh, an increment is observed with respect to formulations prepared from simple adsorption. In general, this trend is a consequence of the GA used as coupling agent. As it was earlier mentioned, GA may form oligomers or polymers by interpenetration of its chains[23].

This fact would be the responsible of the increase in MNPs size, by forming a denser three-dimensional network structure.

Examining the effect of the nature of the drug, formulations **C** and **G** (where the drug was incorporated on its pure form, SD) are compared. It is observed that although the DLE values are comparable, the Dh of **G** is considerably lower and even more when measuring in water. The zeta potential is also different since appear to be less negative in **G** regarding to **C**. These findings could be attributed to the different mechanism to linkage the SD to the MNPs surface.

According to data on Table 1 the amount of GA added as coupling agent has not an influence on the % DLE, however the Dh of the MNPs greatly increase as GA concentration is higher.

Figure 5 shows FTIR-DRIFTS, highlighting significant signals corresponding to this formulation in comparison to the one obtained without GA as covalent linker (**E**). The presence of MAG and crosslinked CS is confirmed by the presence of bands

corresponding to stretching vibration Fe-O of MAG and stretching vibration of N=C from the imine bond between GA and CS (See Figure 2). It is also observable the incorporation of DS (See Figure 5 for band assignments and Figure 2 to compare with the unloaded nanocarrier **2**).

The analysis included above clearly reveals that formulation **G** is the most suitable, in terms of DLE and size, for *in vivo* further assays.

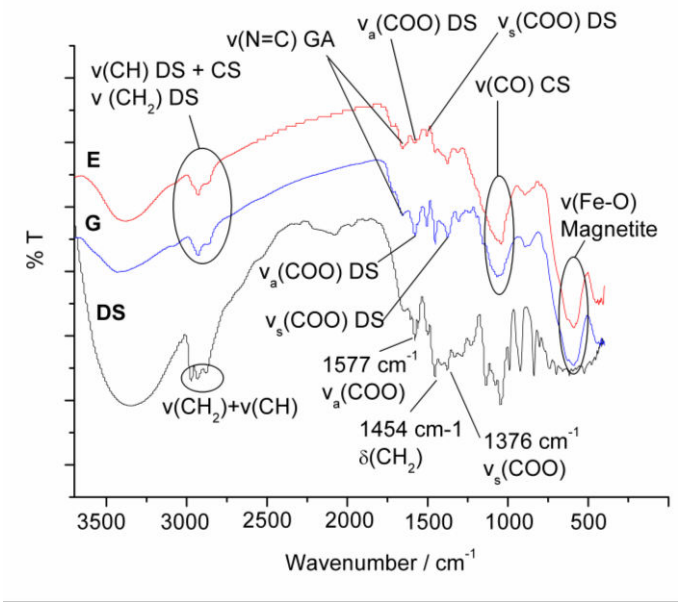
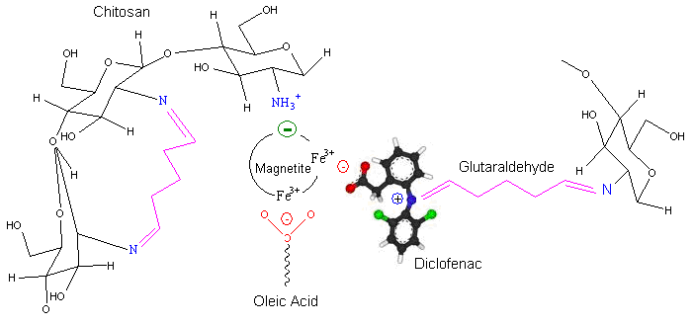


Figure 5: FTIR DRIFTS spectra of Diclofenac sodium (DS), loaded nanocarrier with DS (E) and DS loaded nanocarrier with GA as covalent linker (G).



Scheme 2: Proposed interactions of Diclofenac with nanocarrier´s components

3.3.2 Possible mechanisms for Diclofenac –MNPs interactions

It is expected that GA anchors Diclofenac by its NH group and leaving exposed the COO- group. This is feasible, taking into account that GA trends to crosslink with amino groups by forming imine bonds or by ionic interactions[27]. It is important to highlight that the loading procedure was performed at pH 6; and pKa of Diclofenac is about 4.15. Hence, deprotonation of the acid form occurred remained exposed the negative carboxylate group. Therefore two possible interactions could take place: by GA-Diclofenac as was described above but also between the drug and the regions on the MNPs not covered by GA. That means with the amine groups of CS by electrostatic interactions. It is well known that the cosslinking process is not fully effective, then it is feasible that part of the polymeric phase still remains exposed[28].

3.4 Release data

From previously performed analysis, formulation G has emerged as the most adequate in terms, not only of its loading capability but also from its stability and size properties. Hence it was selected for *in vitro* release tests.

The assays were performed in presence and absence of an external magnetic field and the obtained profiles are shown in Figure 6. It is important to know the influence of the magnetic field in the drug release, taking into account that the potential applications of these nanosystems relies in their targeted guiding to the desired organ or tissue by a magnet. On the other hand, the study was developed under physiological pH in order to approximate the *in vivo* conditions where the formulation is thought to be employed for the treatment of localized inflammatory diseases.

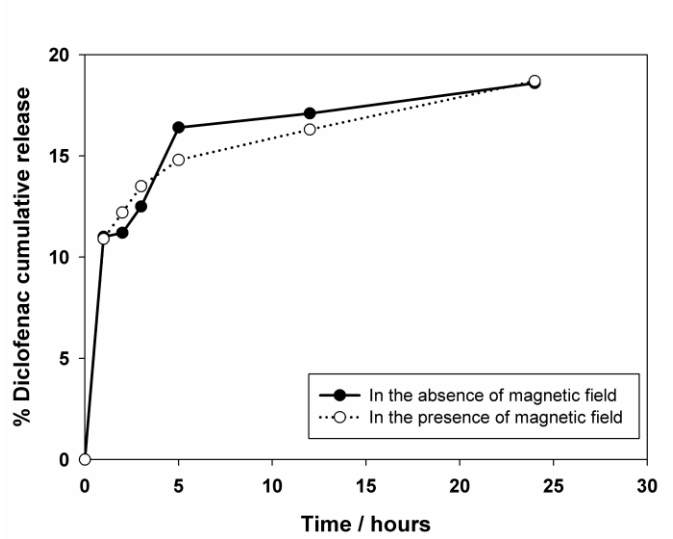


Figure 6: Cumulative percentage release of Diclofenac from G under the influence of a magnetic field.

From the Figure it is appreciated that differences in the percentage of Diclofenac cumulative release during the entire assay is not greatly affected by the presence of a magnetic field. After 24 hours, the drug release reached about 18% in

both conditions. Incomplete release of drugs from different carriers has been earlier reported. Ofokansi *et al.* found similar percentages for the release of Ibuprofen from microparticles composed by GA crosslinked chitosan[29]. V. Goncalves et al. have synthesized CS crosslinked with GA microspheres by a simple coacervation method for the controlled release of Diclofenac. Release study revealed that after 12 hours the drug released achieved 20%[30]. Similar results were found by Kulkarni et al. in their study based on the synthesis of CS microspheres in a w/o emulsion followed by crosslinking with different agents such as viz, GA and sulfuric acid[24]. Diclofenac loading between 23 and 30% were achieved with GA, finding the slowest release profile for this crosslinker agent among the others.

Among these data it is possible verify the strong covalent linkage promoting by the addition of GA, demonstrating that the interactions drug-carriers resulted relevant regarding to the pharmacokinetic of the formulation. For instance a previous work the release profile of Diclofenac physically adsorbed to MNPs composed by magnetite functionalized with OA acid and CS was explored and drug release reached 75% of the total NSAID loaded, after 24 hours of incubation in PBS buffer[15].

This behavior for Diclofenac release is interesting in terms of stability of the formulation: the delayed *in vitro* release would allow a more sustainable availability of the drug in time in the desired organ when extrapolating these results to *in vivo* conditions. By this way, the formulation would involve lower doses to achieve the physiological activity of the drug. Both directionality imparted by magnetism and sustained release in time could result in interesting synergic characteristics for potential *in vivo* applications.

The analysis of the results obtained by the application of different fitting kinetic models for drug release is listed in Table 2. These data reveal that the Korsmeyer model better fits Diclofenac release from MNPs in both explored conditions (absence and presence of a magnetic field). This model explains the release from swelling controlled systems, which means that liberation of the drug incorporated onto a swellable hydrophilic polymer occurs by concomitant sorption of water and desorption of the therapeutic agent[31] .

On the other hand, the use of GA changes the kinetic release of the drug in comparison to the non-containing GA nanocarrier. Since in this last case, the kinetic release was better fitted by the first order kinetic model as was reported elsewhere[15].

4. CONCLUSION

In this work different experimental conditions were assayed in order to improve the stability and loading ability of a magnetic nanocarrier composed by magnetite, oleic acid and chitosan for Diclofenac target and delivery. The screening of different glutaraldehyde concentrations as crosslinking agent permitted the selection of an optimum and stable formulation with a suitable size for *in vivo* applications. It was found that ultrasound treatment during crosslinking process greatly stabilize and improve the size of the carriers.

Exploring different conditions regarding to the incorporation of Diclofenac, a nanocarrier of size lower than 150 nm, with satisfactory loading skill (almost 30%) and with high dispersion capability on water was obtained. The characterization data allowed proposed potential mechanisms for both stabilization of MNPs with GA and interactions with the therapeutic agent.

Diclofenac release kinetic studies demonstrated that the magnetic field has not influence of the way in that the drug was released. This result is very interesting taking into account that the magnetic guidance of the particles has not an effect on the pharmacokinetic of the drug.

ACKNOWLEDGMENTS

The authors acknowledge the financial support from CONICET and the PGI 24/ZQ09 from UNS, (Argentina).

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