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Bionanocomposites containing magnetic graphite as potential systems for drug delivery

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ABSTRACT

New magnetic bio-hybrid matrices for potential application in drug delivery are developed from the assembly of the biopolymer alginate and magnetic graphite nanoparticles. Ibuprofen (IBU) intercalated in a Mg–Al layered double hydroxide (LDH) was chosen as a model drug delivery system (DDS) to be incorporated as third component of the magnetic bionanocomposite DDS. For comparative purposes DDS based on the incorporation of pure IBU in the magnetic bio-hybrid matrices were also studied. All the resulting magnetic bionanocomposites were processed as beads and films and characterized by different techniques with the aim to elucidate the role of the magnetic graphite on the systems, as well as that of the inorganic brucite-like layers in the drug-loaded LDH. In this way, the influence of both inorganic components on the mechanical properties, the water uptake ability, and the kinetics of the drug release from these magnetic systems were determined. In addition, the possibility of modulating the levels of IBU release by stimulating the bionanocomposites with an external magnetic field was also evaluated in *in vitro* assays.

1. Introduction

Nowadays, numerous studies are focused on the development of bio-hybrid systems, including those denoted as bionanocomposites that are based on biopolymers and nanoparticulated inorganic solids, with the aim of exploiting their properties for an extensive range of applications (Darder et al., 2007; Ruiz-Hitzky et al., 2008a,b, 2010a,b,c; Ruiz-Hitzky and Fernandes, 2013a,b). These type of materials are especially interesting in the biomedical field thanks to their safety and biocompatibility, being applied from wound dressing and tissue engineering to drug delivery (Fernandes et al., 2013; Lvov and Abdullayev, 2013; Park et al., 2013; Ruiz-Hitzky et al., 2008a, 2010b, 2013). The advance in the drug delivery systems (DDS) based on bionanocomposite materials provides materials with efficient chemical or physical barriers to control the speed of the drug release and the maintenance of the desired dose, combined with a simple, cheap, versatile and biocompatible synthesis process (Prabaharan and Jayakumar, 2011). The biocompatible character of biopolymers such as polysaccharides or proteins has been widely profited for application in the controlled release of drugs (Alvarez-Lorenzo et al., 2013; Coviello et al., 2007; Liu et al., 2005; Luo et al., 2011; Pongjanyakul and Puttipipatkhachorn, 2007; Young et al., 2005).

Among the polysaccharide group, alginate is commonly used in the development of DDS. It is a linear polysaccharide comprised of α-L-guluronic acid and β-D-mannuronic acid, which is extracted from brown seaweeds. It can be easily processed as beads, films or foams for many different applications. Alginate presents an advantageous property, as it can form a gel by cross-linking reactions with divalent cations, such as Ca^{2+} and Zn^{2+}, decreasing its solubility and often improving other properties such as mechanical resistance (Pongjanyakul and Puttipipatkhachorn, 2007). These types of reactions are very useful in the preparation of beads as well as in the stabilization of films or foams for the more diverse applications. Alginate-based bionanocomposites applied as DDS usually incorporate a hybrid

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material prepared by previous intercalation of the drug in a layered substrate such as clays (Fan et al., 2013; Iliescu et al., 2014) and double hydroxide (LDH), with the aim of increasing the stability of the system in order to procure an increased control over the release of the entrapped drug (Alcântara et al., 2010; Zhang et al., 2010). Layered double hydroxides (LDHs) consisting of brucite-like layers composed by hydroxides of divalent and trivalent metal cations, are commonly used as the inorganic counterpart of bio-hybrid systems for DDS purposes, due to their large anion exchange capacity and biocompatibility (Ruiz-Hitzky et al., 2010c). Besides a wide variety of drugs (Ambrogi et al., 2003; Costantino et al., 2009, 2012; Choy et al., 2004; Gordijo et al., 2005; Oh et al., 2012; Ribeiro et al., 2014; Tyner et al., 2004), several types of biomolecules have been intercalated into the brucite-like layers of LDHs, such as the anticoagulant heparin (Gu et al., 2008), phenylalanine and other amino acids (Aisawa et al., 2004) or even DNA (Choy et al., 1999).

Exclusive properties can be achieved by incorporating magnetic nanoparticles into the biopolymer matrices (Schexnailder and Schmidt, 2009). Actually, a large number of magnetic materials have been used for abundant technological and biomedical applications, including magnetic separation, MRI contrast agent, hyperthermia, thermal ablation and tissue engineering (Kim et al., 2012; Meenach et al., 2010; Reddy et al., 2011; Tartaj et al., 2003). For the DDS area, this is one of the most interesting purposes, especially for cancer therapy, where the levels of drug release can be tuned through stimulation by an external magnetic field (Chomoucka et al., 2010). In this context, LDH-drug containing materials provided with magnetic properties typically containing iron-oxide particles are currently explored in view to DDS applications (Ay et al., 2011; Pan et al., 2011; Wang et al., 2010). The incorporation of magnetic properties to LDH-based DDS can be used to procure a targeted controlled delivery of the drug as recently pointed out by Huang et al. (2013).

The major drawback of many of the abovementioned magnetic DDS is related to the use of toxic reagents (e.g., organic solvents, initiators or surfactants), frequently required in the synthesis process or in the preparation of the nanoparticles dispersion, as they are undesirable for biomedical applications. In this sense, novel magnetic graphite nanoparticles patented by Araújo-Moreira and Mombrú groups (Araújo-Moreira et al., 2005) were produced by a cheap and simple process based on the oxidation–reduction reaction of pristine graphite with controlled amounts of oxygen released from the decomposition of CuO at high temperature, allowing to obtain macroscopic amounts of magnetic pure graphite nanoparticles (Faccio et al., 2008; Mombrú et al., 2005; Pardo et al., 2006, 2012; Souza et al., 2012). These nanoparticles synthesized according to predetermined parameters (Araújo-Moreira et al., 2005) can be easily dispersed in bidistilled water only by sonication, in the absence of the usually required surfactants, organic solvents or initiators. Thus, this carbonaceous material may be potentially used in a broad range of applications, especially for biomedical purposes such as tissue engineering, hyperthermia and DDS.

In this work, we propose the development of magnetic bio-hybrid matrices based on the combination of alginate and magnetic graphite nanoparticles, in which the nonsteroidal anti-inflammatory drug ibuprofen (IBU) alone or intercalated in a Mg–Al layered double hydroxide (LDH) was chosen as the third component of the new type of magnetic bionanocomposite DDS. It is expected that the system involving the biopolymer and the two inorganic solids will combine three main advantages: (i) the protection afforded by the biocompatible alginate and its ability to be prepared as beads and films, (ii) the presence of magnetic graphite nanoparticles that improve the physical and mechanical properties of the biopolymer and afford hydrophobic and magnetic character, and (iii) the protective effect of the LDH layers entrapping the IBU molecules that slow down the release rate of the drug. For confirming these assumptions, *in vitro* tests were carried out in order to evaluate the behavior of these new magnetic bionanocomposite materials in the controlled delivery of the entrapped ibuprofen.

2. Experimental

2.1. Starting materials and reagents

Alginate (ALG) and sodium ibuprofen (IBU) were purchased from Sigma–Aldrich. Aqueous solutions were prepared from chemicals of analytical reagent grade: $AlCl_3{\cdot}6H_2O$ (>99%, Fluka), $MgCl_2{\cdot}6H_2O$ (Panreac), NaOH (98%, Fluka), NaCl (>99%, Sigma–Aldrich), Na_2CO_3 (>99%, Merck), $NaH_2PO_4{\cdot}H_2O$ (>99%, Sigma–Aldrich) and $CaCl_2$ $2H_2O$ (>99%, Sigma–Aldrich). Bidistilled water (resistivity of 18.2 MΩ cm) was obtained with a Maxima Ultrapure Water from Elga. Magnetic graphite (MG) was synthesized as described elsewhere (Araújo-Moreira et al., 2005).

2.2. Synthesis of the layered double hydroxide/ibuprofen intercalation compound

The intercalation compound of IBU in a $[Mg_{0.67}Al_{0.33}(OH)_6]Cl_{0.33}{\cdot}nH_2O$ (Mg_2Al-chloride) LDH was synthesized by co-precipitation method at constant pH, following the procedure described by Constantino and Pinnavaia (1995). A mixture of $MgCl_2{\cdot}6H_2O$ (18 mmol) and $AlCl_3.6H_2O$ (9 mmol) was dissolved in 400 mL of decarbonated bidistilled water. This aqueous solution and 1 M NaOH solution were added dropwise to 100 mL of aqueous solution containing 0.5 g IBU using a DOSINO pH module 800 (Metrohm). This system permitted a controlled addition of solutions in order to maintain a constant pH around 9.0 during the synthesis. The resulting suspension was kept under nitrogen flow to remove CO_2 and under magnetic stirring for 48 h at 60 °C. The solid product was isolated by centrifugation, washed several times with bidistilled water, and dried overnight at 60 °C. The resulting hybrid material was denoted as LDH–IBU.

2.3. Preparation of alginate–magnetic graphite bionanocomposite beads

Beads of the alginate–magnetic graphite bionanocomposites were prepared according to the following procedure: an aqueous solution of 2% (w/v) alginate was magnetically stirred with 1% (w/v) of pure IBU or the necessary amount of LDH–IBU intercalation compound containing 1% (w/v) IBU until homogenization. Then, a stable dispersion of 0.004% (w/v) magnetic graphite in bidistilled water was added to the alginate solution and homogenized by sonication. This mixture was introduced in a burette and then slowly poured as small droplets into a solution of 15% $CaCl_2$ for 3 h. The resulting beads were filtered and washed with abundant doubly distilled water to remove non-entrapped IBU and residual Ca^{2+} ions, frozen in liquid nitrogen and lyophilized in a freeze-dryer (Cryodos, Telstar) for later use. The beads containing pure IBU were denoted as ALG/IBU/MG, and those containing LDH–IBU as ALG/LDH–IBU/MG. For comparison, other batches without magnetic graphite nanoparticles were prepared following the same procedure except the addition of the magnetic graphite suspension. The resulting beads were denoted as ALG/IBU when incorporating IBU alone and as ALG/LDH–IBU when the hybrid was added. The IBU and MG content in the resulting batches of alginate-based beads are summarized in Table 1.

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