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Doxorubicin-loaded magnetic nanocapsules based on N-palmitoyl chitosan and magnetite: Synthesis and characterization

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HIGHLIGHTS

- A new type of magnetic nanocapsules has been prepared by a double emulsion method.
- Magnetic nanocapsules physico-chemical characteristics have been evaluated.
- Magnetic nanocapsules showed good ability to encapsulate a chemotherapeutic agent.
- Doxorubicin loaded-magnetic nanocapsules exhibited suitable magnetic properties.

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ABSTRACT

Doxorubicin-loaded magnetic nanocapsules, based on N-palmitoyl chitosan and magnetite, have been prepared using a double emulsion method. The morphology and size of the nanocapsules have been investigated by transmission and scanning electron microscopy. Magnetic nanocapsules were nearly spherical in shape, with a mean diameter in the nanometer range, and positive zeta potential. Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD) and Energy Dispersive X-ray analysis (EDX) confirmed the structure of composite magnetic nanocapsules. Magnetic nanocapsules exhibited suitable magnetic saturation, superparamagnetic behavior and good ability to incorporate a chemotherapeutic agent, properties which can be exploited in many different areas of biomedicine.

1. Introduction

The development of magnetic drug delivery systems for biomedical applications involves two types of formulations: nanoparticles and nanocapsules. In the last years, magnetic nanocapsules based on biocompatible polymers are emerging as excellent nanocarriers, especially for the delivery of chemotherapeutic drugs [1–3]. The advantages of nanocapsules systems as nanotherapeutic tools, include high encapsulation efficiency, maintenance of drug levels within a desired range by reducing their systemic distribution and the possibility to administrate lower but more accurately targeted doses of the cytotoxic compounds [4,5]. Furthermore, magnetic nanosystems exhibit remarkable characteristics given by their unique physical properties combined with the ability to be guided by an external magnetic field and to function at the cellular and molecular level of biological interactions [6]. Preliminary studies concerning the use of magnetic targeting in chemotherapy indicated that a total remission of animal tumors can be induced [7] without causing any known side effects [8].

These "intelligent" nanodevices include a magnetic inorganic material (used to direct the systems to the target and/or for molecular imaging), a biocompatible surface coating (that provides stabilization in physiological environment and multi-functionality) and a therapeutic agent (adsorbed/hosted within internal cavities of the particles) [9].

In most of biomedical applications, the magnetic core is represented by iron oxides (magnetite, or its oxidized form – maghemite) due to their biocompatibility, good stability and excellent magnetic properties (high magnetic saturation and

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superparamagnetic behavior) [10]. Moreover, the magnetic core is biodegradable and iron ions are reused by cells using normal biochemical pathways for Fe metabolism [11].

Biocompatible surface coatings with hydrophilic polymers such as poly (ethylene glycol), dextran or chitosan were carried out to improve the colloidal stability and to prolong circulation kinetics of magnetic nanoplatforms [12–14]. Chitosan, a heteropolymer composed of N-acetyl-2-amino-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose linked by (1–4)-β-glycosidic bonds offers the combined benefits of the required properties, biocompatibility, biodegradability and low toxicity [15–17] with functionalization ability given by the presence of amino and hydroxyl functional groups. Several representative works revealed the potential applications of chitosan-magnetic nanosystems in the drug delivery field [18,19]. However, these nanoplatforms had either an unstable state or limited drug loading capacity.

Current evidences suggest that incorporation of hydrophobic chains into chitosan structure, could improve drug delivery efficiency, mainly explained by the key role of hydrophobic interactions in the binding of amphiphilic compounds to biological and artificial lipid membranes, followed by enhanced endocytosis [20,21]. In this regard, several chemical modifications of chitosan with hydrophobic compounds have been performed in order to obtain amphiphilic chitosan derivatives [22,23]. The grafting of hydrophobic groups on chitosan confers new physicochemical properties, including the ability to self-associate in different mediums and to form various types of drug delivery platforms [24,25], with promising results as chemotherapeutic systems [26,27].

To our best knowledge, there are no studies concerning the development of magnetic nanocapsules based on N-hydroph obically modified chitosan with long alkyl chain (e.g. palmitoyl). The aim of this study was to present the synthesis, characterization and preliminary drug delivery evaluation of new composite magnetic nanocapsules which combines special abilities of N-palmitoyl chitosan with the versatility of magnetic material in order to fully exploit their potential as drug delivery system for breast cancer.

2. Materials and methods

2.1. Materials

Chitosan (Cs) with viscosity-averaged molecular weight of 50 kDa–190 kDa and a degree of deacetylation of 75–85%, palmitoyl chloride (PC), doxorubicin hydrochloride (DOX), sodium tripolyphosphate (TPP) 85%, polyoxyethylene sorbitan monooleate (Tween 80) and solvents were purchased from Sigma–Aldrich and used as received. MCF-7 human Caucasian breast adenocarcinoma cell line was purchased from ECACC (European Collection of Cell Cultures). Dulbecco's Modified Eagle Medium (DMEM – low glucose, Sigma–Aldrich), FBS (fetal bovine serum, sterile-filtered, suitable for cell culture, Sigma–Aldrich), P/S/N (penicillin/streptomycin/neomycin solution), PBS (phosphate buffered saline solution, sterilized, suitable for cell culture) and MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) were used as received. Lysozyme (from chicken egg) was obtained from Fluka.

2.2. Methods

2.2.1. Synthesis of N-palmitoyl chitosan

N-palmitoyl chitosan was prepared according to a previous report [28], with some modifications. A mixture of Cs (1 g, 5 mmols) and aqueous acetic acid (100 mL, 1% v/v) was stirred for 24 h to ensure total solubility; the pH was adjusted to 6.5 by slow addition of 1 M NaOH and then different volumes of PC (molar ratios Cs/PC: 1/0.5, 1/1, 1/2) were added dropwise and reacted for 24 h, with slowly agitation at room temperature. The acylated chitosan derivatives, further referred as PCs0.5, PCs1 and respectively PCs2 according to the molar ratio used, were precipitated by adding a methanol–ammonia mixture (50 mL, 7:3 v/v); washed with deionized water, an excess of hot methanol and diethyl ether to eliminate free fatty acids and dried in oven for 24 h at 40 ± 2 °C.

2.2.2. Evaluation of the degree of substitution by trinitrobenzene sulfonic acid (TNBS) assay

The degree of substitution in acylated chitosan derivatives was determined through quantification of remaining free amino groups by TNBS assay, according to a method previously described, with some modifications [29]. Briefly, 10 mg of each chitosan derivative was swelled in 2.5 mL of demineralized water (for 24 h) and then incubated with 2.5 mL of 4% NaHCO3 and 2.5 mL of 0.1% TNBS reagent at 37 °C, with slowly agitation. After 2 h, 2.5 mL of 2 M HCl was added and the absorbance was measured at 344 nm using a UV–visible spectrophotometer (UV-1700 PharmaSpec, Shimadzu). The amount of remaining free amino groups was calculated using a standard curve obtained by determining the free amine content of several solutions containing increasing amounts of native chitosan.

2.2.3. Synthesis of magnetic nanocapsules with dual entrapment (chemotherapeutic agent and hydrophobic magnetite)

Hydrophobic magnetite nanoparticles (sodium oleate-coated magnetite) were obtained in our laboratory. In a typical synthesis, the magnetic material was produced by co-precipitation from an aqueous solution containing Fe^{3+}/Fe^{2+} ions, with a molar ratio of 2:1, upon addition of aqueous sodium hydroxide solution. The black particles were collected by magnetic sedimentation and washed repeatedly with water until neutral pH. The obtained magnetic material had an average diameter of 13 nm and a saturation magnetization of 66.0 emu/g. In order to add a hydrophobic shell to the nanoparticles, the magnetite was subsequently functionalized using sodium oleate in aqueous suspension, and further used in this study as a magnetic material in the synthesis of nanocapsules. Doxorubicin-loaded magnetic nanocapsules have been obtained by a two-step, double emulsion method. First, hydrophilic phase I (4 mg DOX in 7.5 mL Tween 80 solution (4%)) and hydrophobic phase II (15 mL chloroform containing 35 mg magnetite) were mixed and emulsified with a Dremel Multipro ultraturax, (5 min, 5000 rpm) to form a W/O emulsion. Hydrophilic phase III (15 mL of mixed Tween 80 solution (4%) and 0.5% N-palmitoyl chitosan) was then added and emulsified (3 × 5 min, 14,000 rpm) to form a W/O/W emulsion. After being cross-linked with 9 mL TPP (0.5%), the blend was vigorously stirred for 4 h to remove the organic solvent. Magnetic nanocapsules (further referred as MN-DOX) were collected by centrifugation at 9000 rpm, purified with deionized water and freeze dried.

2.2.4. Characterization techniques of acylated chitosan derivatives and magnetic nanocapsules

Proton nuclear magnetic resonance (^{1}H NMR) spectra of native and acylated chitosan solutions (1% in D_2O with HCl, pH = 3) were recorded on a Brüker ADVANCE DRX 400 spectrometer operating at 400.1 MHz and equipped with a multinuclear broadband QNP 5 mm probe, at 330 K. A FT-IR spectrophotometer, model Vertex 70 (Brüker) was used to record the FT-IR spectra of all samples. FT-IR spectra were recorded on 500–4000 cm^{-1} domain, with a resolution of 4 cm^{-1}. Molecular weight averages (the number-average molecular weight, denoted by Mn, and weight-average molecular weight, denoted by Mw) and molecular weight distributions of polymeric samples were measured by a gel