Carbohydrate Polymers 151 (2016) 546–556

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Chondroitin sulfate-capped super-paramagnetic iron oxide nanoparticles as potential carriers of doxorubicin hydrochloride

Neha Mallick[a,*], Mohammed Anwar[a], Mohammed Asfer[b], Syed Hassan Mehdi[d], Mohammed Moshahid Alam Rizvi[d], Amulya Kumar Panda[c], Sushama Talegaonkar[a], Farhan Jalees Ahmad[a,*]

[a] Nanoformulation Research Lab., Faculty of Pharmacy, Hamdard University, New Delhi, India
[b] Department of Mechanical Engineering, IIT Kanpur, Uttar Pradesh, India
[c] Product Development Cell-II, National Institute of Immunology, Delhi, India
[d] Department of Biosciences, Jamia Millia Islamia, New Delhi, India

ARTICLE INFO

Article history:
Received 24 February 2016
Received in revised form 27 May 2016
Accepted 28 May 2016
Available online 29 May 2016

Chemical compounds studied in this article:
Chondroitin sulfate A (PubChem CID: 53477710)
Doxorubicin hydrochloride (PubChem CID: 443939)

Keywords:
Chondroitin sulfate
Glycosaminoglycan
Super-paramagnetic iron oxide nanoparticle
Doxorubicin
Drug loading
Box-Behnken design

ABSTRACT

Chondroitin-4-sulfate (CS), a glycosaminoglycan, was used to prepare CS-capped super-paramagnetic iron oxide nanoparticles, which were further employed for loading a water-soluble chemotherapeutic agent (doxorubicin hydrochloride, DOX). CS-capped SPIONs have potential biomedical application in cancer targeting. The optimized formulation had a hydrodynamic size of 91.2 ± 0.8 nm (PDI; 0.228 ± 0.004) and zeta potential of −49.1 ± 1.66 mV. DOX was loaded onto the formulation up to 2% (w/w) by physical interaction with CS. TEM showed nano-sized particles having a core-shell structure. XRD confirmed crystal phase of iron oxide. FT-IR conceived the interaction of iron oxide with CS as bidentate chelation and also confirmed DOX loading. Vibration sample magnetometry confirmed super-paramagnetic nature of nanoparticles, with saturation magnetization of 0.238 emu g^{-1}. *In vitro* release profile at pH 7.4 showed that 96.67% of DOX was released within 24 h (first order kinetics). MTT assay in MCF7 cells showed significantly higher ($p < 0.0001$) cytotoxicity for DOX in SPIONs than DOX solution (IC_{50} values 6.294 ± 0.4169 and 11.316 ± 0.1102 μg mL^{-1}, respectively).

1. Introduction

Polysaccharide-decorated nanoparticles have been attracting considerable attention by researchers involved in the field of surface-modified nanoparticles, as they provide a hydrophilic surface coating and prevent protein adsorption (Lemarchand, Gref, & Couvreur, 2004). Moreover, they additionally achieve active targeting by binding to specific receptors on certain cells or tissues, and exhibit well-documented biocompatibility and biodegradability (Lemarchand et al., 2004). A class of unbranched polysaccharides is formed by glycosaminoglycans, which comprise of repeating units of uronic acids and amino sugars (Yip, Smollich, & Götte, 2006). Chondroitin sulfates (CS) are the most abundant glycosaminoglycans of the body (Campo, Avenoso, Campo, Ferlazzo, & Calatroni, 2006), which comprise of a repeating disaccharide unit of glucuronic acid (GlcA) and *N*-acetyl-galactosamine (GalNAc), often modified by sulfate groups (Lauder, 2009). As they possess anti-angiogenic properties (via inhibition of transendothelial monocyte migration) (Yip et al., 2006), and have surface ligands on metastatic tumor cell lines (Monzavi-Karbassi et al., 2007), CS can be explored to formulate surface-modified NPs for tumor targeting.

Super-paramagnetic iron oxide nanoparticles (SPIONs) are sub-micron particles consisting of a ~15 nm iron oxide core (magnetite, Fe_3O_4 or maghemite, γ-Fe_2O_3) coated with a suitable organic or inorganic polymeric or non-polymeric capping

* Corresponding authors.
E-mail addresses: nehamallick.sch@jamiahamdard.ac.in, nehamallick@gmail.com (N. Mallick), md.anwar2008@gmail.com (M. Anwar), mdasfer@iitk.ac.in (M. Asfer), saeedimam@gmail.com (S.H. Mehdi), mrizvi@jmi.ac.in (M.M.A. Rizvi), amulya@nii.res.in (A.K. Panda), stalegaonkar@jamiahamdard.ac.in (S. Talegaonkar), fjahmad@jamiahamdard.ac.in, farhanja_2000@yahoo.com (F.J. Ahmad).

http://dx.doi.org/10.1016/j.carbpol.2016.05.102
0144-8617/

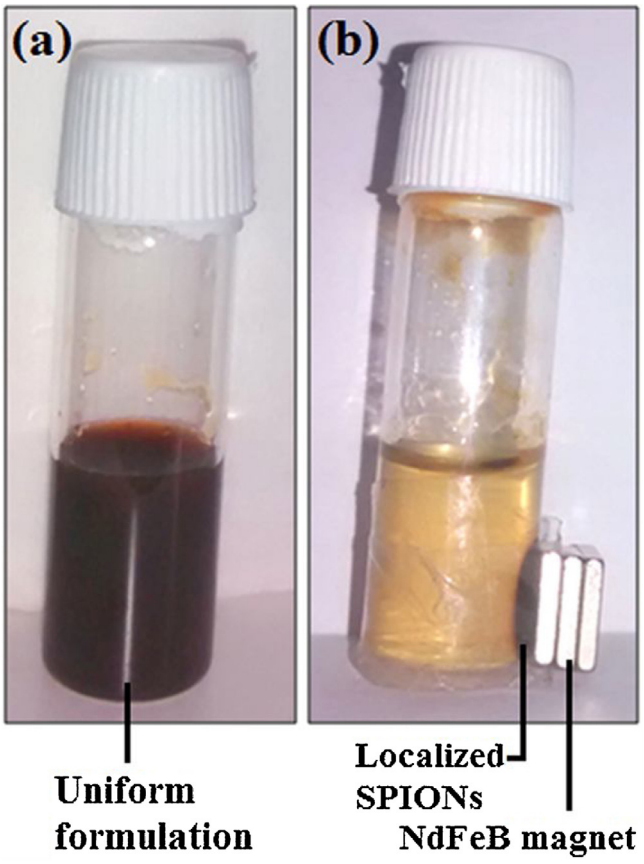

Fig. 1. (a) As prepared formulation of CS(0.5%)SPIONs; (b) CS(0.5%) SPIONs attracted to an external magnet.

agent to impart colloidal stability and surface functionalization (Gupta & Gupta, 2005b). They possess super-paramagnetic behavior and abundant potential for various biomedical applications, such as magnetically-assisted cancer targeting (Gupta & Gupta, 2005b). Numerous capping agents from carbohydrate and non-carbohydrate sources have been employed for stabilizing SPIONs during synthesis. Among polysaccharides and their derivatives, dextran (Perez & Nath, 2013), alginate (Ma, Qi, Maitani, & Nagai, 2007), carboxydextran (Laurent et al., 2008; Lunov et al., 2010), pegylated starch (Corot, Robert, Idée, & Port, 2006; Laurent et al., 2008), starch (Tancredi, Botasini, Moscoso-Londoño, Méndez, & Socolovsky, 2015) and carboxymethyl cellulose sodium (Mallick et al., 2015; Si et al., 2004) have been employed. CS has been previously used as a capping agent to prepare metallic nanoparticles including selenium nanoparticles (Han et al., 2012), magnetite nanoparticles (Kresse, Lawaczeck, & Pfefferer, 1995; Tóth, Illés, Szekeres, & Tombácz, 2015), gold nanoparticles (Cho et al., 2014), and silver nanoparticles (Cheng, Hung, Chen, Liu, & Young, 2014), and smart hollow microspheres inhabiting magnetite nanoparticles (Guilherme et al., 2010), for its non-toxic (Lauder, 2009), biocompatible and biodegradable nature.

Doxorubicin (DOX) is an anthracycline class chemotherapeutic, used for the treatment of various types of solid tumors, as well as hematological malignancies (Cortés-Funes & Coronado, 2007). It possesses numerous adverse effects, the main being dose-dependent cardiomyopathy (Swain, Whaley, & Ewer, 2003; Thorn et al., 2011), which is caused by production of reactive oxygen species in the myocardium (Chatterjee, Zhang, Honbo, & Karliner, 2010; Ichikawa et al., 2014). Since CS possesses antioxidant properties (Campo et al., 2006), it can be used in targeted drug delivery systems to counteract the side effect of DOX. Apart from that, CS, being an acidic mucopolysaccharide, can form ionic complexes with cationic drug molecules (Lee et al., 2007). This is evident from the formation of CS-chitosan polyelectrolyte complexes in the form of microspheres, which were used to entrap 5-fluorouracil (Huang, Sui, Wang, & Jiao, 2010). In the present work, CS, being anionic was used for loading DOX, which has cationic ammonium moiety.

In the present research work, we have aimed to prepare and characterize novel CS-capped SPIONs by a facile, one-pot green synthesis technique for loading of a chemotherapeutic agent, DOX, as a water-soluble model drug to investigate its application as a potential carrier for targeted drug delivery.

2. Materials and methods

2.1. Materials

Chondroitin-4-sulfate (CS, from bovine trachea, main component chondroitin sulfate A) was procured from Sigma Aldrich (Product no. 27042). Doxorubicin hydrochloride was a kind gift sample from Dabur Research Foundation, India. Sodium hydroxide flakes and HPLC-grade methanol were purchased from Thomas Baker (Mumbai, India). Ferrous sulfate heptahydrate and disodium hydrogen phosphate (anhydrous) were procured from S.D. Fine Chem. Ltd. (Mumbai, India). Potassium dihydrogen phosphate and sodium chloride were purchased from Qualigens Fine Chemicals (Mumbai, India). HPLC-grade acetonitrile was procured from Fischer Scientific (Thermo Electron LLS India Pvt. Ltd., Mumbai, India), and HPLC water from LOBA Chemie (Mumbai, India). Nylon syringe filters (0.22 μm, diameter 25 mm) were procured from Axiva Sichem Biotech (Delhi, India) and Dispovan 1-mL tuberculin syringes from Hindustan Syringes and Medical Devices Ltd. (Faridabad, India). Fetal Bovine Serum (FBS) was procured from Gibco (Thermo Fischer Scientific Inc., USA), and DMEM (Dulbecco's Modified Eagle's Medium) and 1% antibiotic solution containing penicillin, streptomycin and amphotericin B from HiMedia Laboratories (Mumbai, India). In-house produced deionized water was used for all the formulation experiments. MilliQ water was produced in-house by Millipore Water Purification System (Merck Millipore Corporation, USA).

2.2. Methods

2.2.1. Synthesis of chondroitin sulfate-capped SPIONs

SPIONs were prepared by wet chemical route, viz. controlled oxidation of ferrous (Fe^{2+}) ions by a modified form of a previously proposed method (Mallick et al., 2015; Si et al., 2004). An aqueous solution of ferrous sulphate heptahydrate (20 mL, 0.02 M) was added drop wise to a 20 mL capping agent aqueous solution (CS) of varying concentrations in deionized water. The mixture was stirred for 30 min at 400 rpm for the formation of iron-capping agent complex. The pH of the solution was gradually increased by adding sodium hydroxide (0.5 M NaOH) solution to obtain a pH ~10. The reaction mixture was subsequently aged for 2–3 h at room temperature at 1200 rpm for the precipitation of magnetite as CS-capped SPIONs and further passed through homogenizer (Silent Crusher S, Heidolph Instruments GmbH & Co.KG, Germany) at different rpm and time to get more uniformly small-sized SPIONs. Effect of concentration of capping agent and process variables (rpm and time) of homogenization on particle size (Z-average) and poly dispersity index (PDI) were investigated using quality by design (QbD) approach keeping the nature of capping agent and other reaction conditions (stirring rate, concentration of ferrous salt, sodium hydroxide (to maintain alkaline pH)) constant. The as-prepared formulation was centrifuged in Optimal LE-80K ultracentrifuge (Beckman Coulter Inc., USA) at 40,000 rpm at 4 °C