International Journal of Pharmaceutics 504 (2016) 110–116

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

Hydroxyl-modified magnetite nanoparticles as novel carrier for delivery of methotrexate

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ARTICLE INFO

Article history:
Received 21 February 2016
Received in revised form 13 March 2016
Accepted 14 March 2016
Available online 17 March 2016

Keywords:
Magnetite nanoparticles
Magnetic nanoparticle (MNP)
Methotrexate (MTX)
Anti-cancer
Drug delivery
MCF-7

ABSTRACT

In this work, novel hydroxyl-modified magnetite nanocarriers are introduced as efficient host for methotrexate conjugation. The modification was based on the Micheal type addition reaction between tris(hydroxymethyl) aminomethane and acrylamidopropyl functionalized, silica-coated magnetite nanoparticle. The chemical structure characterization was carried out by FT-IR and the organic content was determined by CHN analysis. The topography was studied by SEM, TEM, AFM. DLS was performed to show particles' mean diameter. Furthermore, the magnetite properties of modified particles were evaluated by VSM and the crystallinity was proved by XRD. To illustrate the efficiency of the modified particles, the anti-cancer drug methotrexate was conjugated to hydroxyl groups through estric bond formation. The controlled release activity of established nanoparticles was evaluated in simulated cellular fluid. Later, the anti-cancer behavior of drug conjugated nanoparticles was evaluated *in vitro* in MCF-7 cell line which showed enhanced toxicity after 48 h. Conclusively, the modified nanoparticles have remarked as powerful carrier to be applied as an anti-cancer agent.

1. Introduction

Developments in nanotechnology have revolutionized all aspects of scientific researches. Modern synthetic routes in preparation of metallic nanoparticles offered host of benefits in utilizing highly engineered and tailor-made nanocarriers (Fedlheim and Foss, 2001). Magnetite nanoparticles (MNPs) are among the most highly applied particles (Lu et al., 2007; Spaldin, 2010). The wide range of MNPs utilization are consisted of various fields such as catalysis (Gawande et al., 2013; Thangaraj et al., 2015), separation (Horak et al., 2007), magnetic resonance imaging [MRI] and drug delivery (McBain et al., 2008). MNPs have great potential to be nominated for drug delivery purposes due to their responsiveness to magnetic field, adjustable and uniform sizes between 1 and 100 nm, functionalization ability and the potential to carry a high dose of drug (Namdeo et al., 2008).

Recent approaches in nanomedicine have demonstrated the applicability of multifunctional MNPs for theranostic applications which include simultaneous diagnosis and therapeutic capability (Ahmed et al., 2012). Among highlighted concepts of MNPs, their negative contrast in MRI offer host of benefits in accurate diagnosis of malignant tumors (Sattarahmady et al., 2015). Besides, they offer unique possibilities for biomedical applications, especially through controlled release of drug. The concept of magnetic drug delivery was firstly developed for the delivery of cytotoxic drugs (Zimmermann and Pilwat, 1976). Subsequently, the creation of different engineering techniques for the decoration of MNPs with varying alignments have been established (Lu et al., 2007). The applications of nanoparticles with positive yields in drug delivery are highly dependent on their size and stability. Most synthetic methods involve co-precipitation and result in the formation of nanoparticles structures ranging of 10–20 nm, with the inevitable problem of agglomeration over longer periods of time. Moreover, naked MNPs are susceptible to oxidation and can lose their magnetic properties and dispersibility. For such multifunctional particles, developing protection strategies to chemically stabilize MNPs against degradation seems vital. With this goal in mind, encapsulating methods involving an overlay with organic precursor, including surfactant or polymer (Jeong et al., 2007), and inorganic layers such as silica shell (Yi et al., 2005) have been developed. MNPs coated with silica, composed with the inclusion of surface-reactive groups like functional alkoxysilanes, offer the chemical and thermal stabilization of MNPs while enabling multifunctional layer design for various purposes (Deng et al., 2005; Liu et al., 2004).

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http://dx.doi.org/10.1016/j.ijpharm.2016.03.022
0378-5173/

Introducing hydrophilic groups on the surface of MNPs could improve their affinity toward water soluble drugs (Yallapu et al., 2010). Among hydrophilic agents, tris(hydroxymethyl) aminomethane (TRIS) as available, inexpensive and biocompatible (Chen et al., 2014) precursor have been applied in MNP modification and utilized for lysozyme rapid purification (Zhang et al., 2011). Recently, Farjadian et al. (2015b) introduced novel method in silica modification with alkanol amine type precursors including TRIS which could be applied for MNP modification.

Among anti-cancer drug, methotrexate (MTX) has been known as a well-known therapeutics agent in treating autoimmune diseases, solid tumors and hematologic malignancies. However, the deviated pharmacokinetics and safety margin have bounded the therapeutic outcomes (Khan et al., 2012). For development in delivery systems of MTX several nanocarriers including carbon nanotubes, polymeric nanoparticles (*i.e.* micells, hydrogels), dendrimers, metallic nanoparticles (*i.e.* magnetic nanoparticles, gold nanoparticles and quantum dots) were applied (Khan et al., 2012). Herein, some recent approaches in this field are being reviewed. Folate conjugated carbon nanotubes for targeting MTX to inflammatory arthritic region have developed and evaluated *in vitro* and *in vivo* (Kayat et al., 2015). The mechanism of MTX release from chitosan hydrogel were studied by transmission electron microscopy which addresses release kinetics from hydrogels (Ashrafi and Azadi, 2016). In regard to treatment of psoriasis, MTX loaded gold nanoparticles were evaluated *in vitro* and *in vivo* (Bessar et al., 2016). KB cells were utilized in the evaluation of internalization and cytotoxicity of cysteine capped quantum dots (Johari-Ahar et al., 2016). The super paramagnetic iron-oxide nanoparticles encapsulated with chitosan and loaded with MTX assessed *in vitro* and proved to be good drug delivery systems (Mohammadi-Samani et al., 2013). Other systems consisting silica coated MNP coated with the amino group had constituted a self-assembled monolayer of conjugated MTX and evaluated in MCF-7 cell line which demonstrated effective anti-cancer behavior (Kohler et al., 2005). Furthermore, other systems including magneto-liposomes (Zhu et al., 2009), hyperbranched polyglycerol grafted MNP conjugated with MTX (Li et al., 2013) have been developed for the controlled release and targeted delivery system.

Through the advantages which stemmed from developing delivery systems of MTX on MNPs (Kohler et al., 2005; Li et al., 2013; Mohammadi-Samani et al., 2013; Zhu et al., 2009) and the advantages offered by TRIS on MNP (Zhang et al., 2011) and by following recent report on silica modification (Farjadian et al., 2015b) and mesoporous silica application as drug adsorbent (Farjadian et al., 2015a) in this study we reports novel synthesis of TRIS-modified MNP as novel nanocarrier for MTX conjugation based on acrylamidopropyl silica-coated MNP. The controlled release behavior of introduced particles evaluated *in vitro* while the anti-cancer behavior assessed in MCF-7 breast cancer cell line.

2. Experimental

2.1. General remarks

Sigma-Aldrich, Merck and Daejung Chemical Companies were the companies that chemicals were purchased from. Commercial MNP named nano-Fe_3O_4 (98% purity, coated with 1% PVP, containing impurities of Ca (0.18%), Cr (0.65%), K (0.12%), Mn (0.75%), SiO_2 (0.19%)) with sizes 20–30 nm were prepared from US Research Nanomaterials, Inc. After comparison with data from available literature, products were analyzed based on FT-IR spectra by utilizing a Bruker VERTEX70 spectrophotometer. Data from XRD was collected from D8, Advance, Bruker, AXS with X-ray diffractometer (CuKα radiation with λ = 1.540 A°, 2θ scan range = 10–90°, 25 °C). SEM (Scanning electron microscopy) with high voltage up to maximum 400 (Kv) was carried out with a KYKY-EM3200 instrument, after the samples had been sputter-coated with gold. Analysis via vibrating sample magnetometer (VSM) with instrument specification; VSM/AGFM (Meghnatis Danesh Pajouh; Iran) were fulfilled at room temperature. A Philips (model CM10) instrument was utilized to carry out transmission electron microscopy/(TEM) analysis. Atomic force microscopy/(AFM) analysis was performed with JPK nanowizard II instrument. Images were recorded with intermittent contact mode by using I-type cantilevers (length of about 230 μm) and 380 kHz resonance frequency. Dynamic light scattering (DLS) was carried out with Microtrac instrument. Methotrexate loading and release testing was carried out with UV–vis spectrophotometer (CECIL CE7250).

2.2. Synthesis of hydroxyl-modified magnetite nanoparticle (MNP-TRIS)

Commercial MNP were surface coated with silica through the reported procedure (Abu-Reziq et al., 2006). MNP US nanomaterial was used as the source of Fe_3O_4. The MNP (1 g) were premixed by ultrasonication in 50 ml of dried toluene. After that, (3-aminopropyl) trimethoxysilane (APTES, 4.0 ml) was added up and the containing composition stirred for four days at 80 °C. Collection of the silica coated nanoparticles was carried out using a permanent magnet. Particles were washed with ethanol and acetone (3×) and then were dried at 50 °C under vacuum for one full day. Next steps of modifications were accomplished according to recently reported method (Farjadian et al., 2015b). MNP modified aminopropylsilica (1 g) were ultrasonicated in dry THF (20 ml) for 30 min and then admixed with acryloylchloride in drop-wise adding manner (0.1 g, 1.1 mmole) at 0 °C while medium contains trimethylamine (0.15 g, 1.4 mmol). Afterwards, the resulting mix was stirred for 4 h at room temperature. The product isolation was performed by external magnet and washed with ethanol (50 ml × 3) and water (2 × 100 ml) and finally dried at 60C for 3 h. Third step of modifications was performed through Micheal type addition with TRIS. MNP modified acrylamidopropylsilica (1 g) was admixed with TRIS (0.13 g, 1.1 mmol) in 40 ml of ethanol for one day at 50 °C. The product separation, washing and drying steps was followed as previous.

2.3. Synthesis of the MTX-conjugated hydroxyl modified magnetite nanoparticle (MNP-TRIS-MTX)

MTX conjugation to the MNP-TRIS was accomplished through an esterification reaction among the hydroxyl groups of MNP and the carboxylic acid group of MTX. First, (20 mg, 0.04 mmol) of MTX was dissolved in DMSO (5 ml) and then N, N′-dicyclohexylcarbodiimide (DCC) (9.1 mg, 0.04 mmol) was added as carboxyl group activator in the presence of 4-dimethylaminopyridine (DMAP) (0.27 mg, 0.002 mmol). The mixture was stirred for 1 h and then 0.2 g of tris modified MNP was added up and the mix was shaken at 40 °C for a day. The resulting product was centrifuged and then washed with DMSO, deionized (DI) water and acetone, and dried at vacuum at room temperature.

2.4. Determination the amount of MTX on MNP-TRIS-MTX

To evaluate the amount of MTX conjugated to MNP-TRIS, samples containing 100 ppm of MNP-TRIS-MTX in hydrochloride aqueous acidic medium (pH 1.2, 2 ml) were prepared in micro-tubes. Then, the samples were shaked for 48 h in thermo-mixer at 40 °C. The amount of MTX released into the medium was determined through the use of a UV–vis spectrophotometer at 306 nm.