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Bovine Serum Albumin (BSA) coated iron oxide magnetic nanoparticles as biocompatible carriers for curcumin-anticancer drug

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ABSTRACT

The bovine serum albumin-coated magnetic nanoparticles (F@BSA NPs) were prepared as curcumin (CUR) carriers through desolvation and chemical co-precipitation process. The characteristics of CUR loaded F@BSA NPs (F@BSA@CUR NPs) were determined by X-ray diffraction (XRD), thermogravimetric analysis (TGA), fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), and vibrating-sampling magnetometry (VSM) techniques. It was found that the synthesized F@BSA@CUR NPs were spherical in shape with an average size of 56 ± 11.43 nm (mean ± SD (n = 33)), ζ-potential of −10.1 mV, and good magnetic responsivity. Meanwhile, the drug content of the nanoparticles was 6.88%. These F@BSA@CUR NPs also demonstrated sustained release of CUR at 37 °C in different buffer solutions. Cellular toxicity of F@BSA@CUR NPs was studied on HFF2 cell line. Also, the cytotoxicity of F@BSA@CUR NPs towards MCF-7 breast cancer cells was investigated. The results revealed that F@BSA@CUR NPs have significant cytotoxicity activity on MCF-7 cell line.

1. Introduction

Over 100 new natural products for their anticancer activities are in clinical progress, mostly [1–3]. Among the natural phytochemicals, curcumin (CUR) (diferuloyl methane), a low-molecular-weight, bright yellow natural polyphenolic compound which can be isolated from the rhizome of turmeric (*Curcuma longa*) has been used in inflammatory disorders and cancer treatment for a lot of years [4,5]. CUR can decrease tumor growth through various mechanisms, counting antitumor angiogenesis, proliferation suppression, apoptosis induction and prevention of metastasis [6,7]. However, CUR clinical applications are restricted because of its short biological half-life, poor solubility which resulting in poor absorption, and low bioavailability through the oral route [8]. Furthermore, free drugs may have nonspecific diffuse but a nanocarrier can escape into the tumor tissues through the leaky vessels by the EPR effect [9,10].

During the past decade, scientists have shown an increased interest in multifunctional nanomaterials which can reach to therapeutic goals for CUR and solve it's limitations [11,12].

Iron oxide magnetic nanoparticles have concerned a significant attention in drug delivery systems for cancer research [13–15], because of their multifunctional properties such as small size, magnetic susceptibility, biocompatibility, low toxicity, stability, availability for surface modification and further easily controlled by an external magnetic field application [16–18]. For successful use of magnetic nanoparticles, it is required to surface coating and specific modification [19]. Coating and modification of the surface of magnetic nanoparticles with various biocompatible and biodegradable materials has been widely studied [20]. Indicating a proper coating materials are important to obtain nanoparticles with suitable stability since these stabilizers are responsible for generating inter-particles repulsive force to prevent aggregations [21].

Also, albumin-based nanoparticles have received many attentions because of their biological origin, biodegradability, non-toxicity, non-immunogenicity, water solubility and easy availability [22,23]. Serum albumin as the main protein in the circulation system is a heart-shaped globular protein which is composed of∼585 amino acids and has the molecular weight of 66 kDa [24]. Human and bovine serum albumins (HAS and BSA) are the

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main available albumins widely used in the biomedical and pharmaceutical applications [25,26]. Abraxane is an antineoplastic medication albumin-based NP approved by FDA in 2005 used in the treatment breast cancer [27]. Among the diverse method for preparation of Albumin based nanoparticles desolvation or coacervation technique is known as a primary, main and simple method [28]. In this method, a desolvation agent such as acetone or ethanol can add to the aqueous solution of albumin under a constant stirring condition using a magnet. The flow rate and the volume of the added desolvating agent are an important parameter to obtain appropriate size of albumin NPs for biomedical applications. After finishing the adding of desolvation agent, a cross-linkers such as glutaraldehyde or EDC must be added to stabilize the nanoparticles by crosslinking of amino acids residues in the protein [28,29].

In view of our current studies on design and preparation of drug carriers [30–33], previously, our research group reported a simple improved method for the preparation of magnetic albumin based nano-carrier. Also, biocompatibility of the nanoparticles were evaluated [24]. In the present investigation, with optimal conditions in hand, the previous work was developed by evaluating of these nanoparticles in drug delivery applications. According to our information until now, this is the first report of CUR delivery with magnetic albumin based nano-carrier.

In this study, we prepared one kind of nano-scale carrier for CUR, using Fe_3O_4 nanoparticles and CUR as core and BSA as a biological macromolecular shell to form drug-loaded magnetic nanoparticles. The morphology, structure and characteristic of the F@BSA@CUR NPs were studied by XRD, TGA, FTIR, TEM, and VSM techniques. The application of F@BSA@CUR NPs as carriers of CUR was evaluated by measuring its drug content, encapsulation rate and *in vitro* anticancer activity on MCF-7 cell line.

2. Materials and methods

2.1. Materials

CUR, BSA, 1-ethyl-3-[3-(dimethylamino) propyl] carbodiimide (EDC) and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich Co (St. Louis, USA). $FeCl_3{\cdot}6H_2O$ and $FeCl_2{\cdot}4H_2O$ were purchased from Merck (Darmstadt, Germany). Ammonium hydroxide (25 wt% NH_3 in water) and acetone were purchased from Mojallali Company, Iran. All other chemicals and solvents were from general lab or HPLC grades, as needed, and were purchased from Emertat Chimi Company (Tehran, Iran).

2.2. Synthesis of bare Fe_3O_4

An aqueous solution (150 mL in deionized water) of $FeCl_3{\cdot}6H_2O$ (1.1 g) and $FeCl_2{\cdot}4H_2O$ (0.4 g), in the molar ratio 2Fe (III):1Fe (II) prepared and kept at a constant 60 ∘C for 15 min (min) under vigorous stirring. Then under vigorous stirring and N_2 gas a solution of ammonium hydroxide (20 mL NH_4OH (25%)) was added till the pH was reached to ~11 at which a black suspension was formed. This suspension was then stirred at 50 °C for 2 h, under vigorous stirring and N_2 gas. Fe_3O_4 magnetic nanoparticles, were separated from the aqueous solution by external magnet, washed with deionized water several times then dried in a vacuum oven overnight.

2.3. Synthesis of BSA coated magnetic nanoparticles (F@BSA NPs)

To an aqueous solution (32 mL in deionized water) of a mixture of $FeCl_3{\cdot}6H_2O$ (0.55 g) and $FeCl_2{\cdot}4H_2O$ (0.2 g), BSA (2 g) was added and kept at room temperature for 15 min under vigorous stirring. Then, 64 mL acetone was added to above solution drop wise at a rate of 2 mL/min under constant stirring at room temperature. For the stabilization of the unstable particles, 40 mg EDC was added for cross linking. The stirring condition was continued for 2 h to ensure the cross linking of all amino acid residues. Then the temperature was increased to 50 °C. Next, under vigorous stirring and N_2 gas a solution of ammonium hydroxide (15 mL NH_4OH (25%)) was added till the pH was raised to ~11 at which a black suspension was formed. This suspension was then stirred at room temperature for 3 h. The resulting BSA coated magnetic nanoparticles (F@BSA NPs) were purified by three cycles of centrifugation at 18,000 rpm for 15 min and washed with deionized water several times and dried in a vacuum oven overnight.

2.4. Synthesis of CUR loaded F@BSA NPs (F@BSA@CUR NPs)

To an aqueous solution (32 mL in deionized water) of a mixture of 0.55 g $FeCl_3{\cdot}6H_2O$, 0.2 g $FeCl_2{\cdot}4H_2O$ and 2 g BSA was added and kept at room temperature for 15 min under vigorous stirring. Then, CUR solution (750 mg CUR in 64 mL acetone) was added to above solution drop wise at a rate of 2 mL/min under constant stirring at room temperature in dark conditions. For the stabilization of the unstable particles, 40 mg EDC was added for cross linking. The stirring condition was continued for 2 h to ensure the cross linking of all amino acid residues. Then the temperature was increased to 50 °C. Next, under vigorous stirring and N_2 gas a solution of ammonium hydroxide (15 mL NH_4OH (25%)) was added till the pH was raised to ~11 at which a black suspension was formed. The suspension was stirred at room temperature for 3 h. The resulting CUR loaded F@BSA NPs (F@BSA@CUR NPs) were purified by three cycles of centrifugation at 18,000 rpm for 15 min and washed with deionized water several times and then dried in a vacuum oven overnight (Fig. 1).

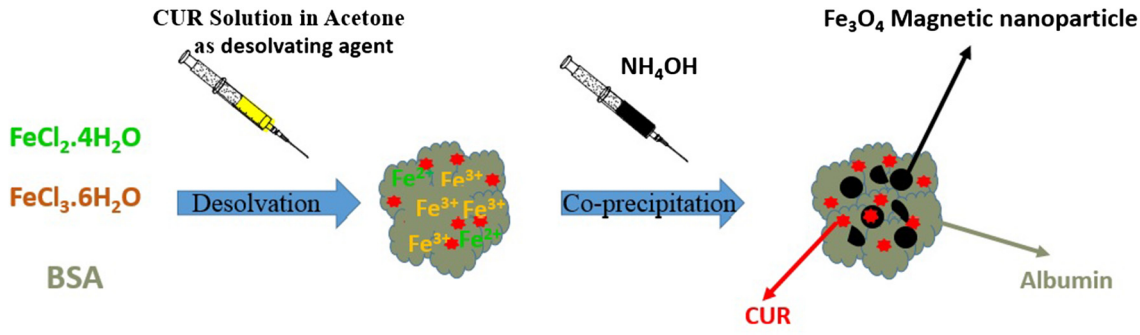

Fig. 1. Schematic illustration of the synthesis of F@BSA@CUR NPs.