Multifunctional gold coated iron oxide core-shell nanoparticles stabilized using thiolated sodium alginate for biomedical applications

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ARTICLE INFO

ABSTRACT

In this paper we report synthesis of aqueous based gold coated iron oxide nanoparticles to integrate the localized surface plasma resonance (SPR) properties of gold and magnetic properties of iron oxide in a single system. Iron oxide-gold core shell nanoparticles were stabilized by attachment of thiolated sodium alginate to the surface of nanoparticles. Transmission electron microscope (TEM) micrograph presents an average elementary particle size of 8.1 ± 2.1 nm. High resolution TEM (HR-TEM) and X-ray photon spectroscopy further confirms the presence of gold shell around iron oxide core. Gold coating is responsible for reducing saturation magnetization (M_s) value from ~41 emu/g to ~24 emu/g – in thiolated sodium alginate stabilized gold coated iron oxide core-shell nanoparticles. The drug (curcumin) loading efficiency for the prepared nanocomposites was estimated to be around 7.2 wt% (72 μg drug/mg nanoparticles) with encapsulation efficiency of 72.8%. Gold-coated iron oxide core-shell nanoparticles could be of immense importance in the field of targeted drug delivery along with capability to be used as contrast agent for MRI & CT.

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1. Introduction

Iron oxide nanoparticles have gained considerable interest nowadays of many researchers owing to their unique properties of superparamagnetism, high magnetic susceptibility, low Curie temperature, no coercivity value [1,2]. Two most important forms of iron oxide nanoparticles studied are magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) [3]. Today a wide range of applications are reported for iron oxide nanoparticles like drug delivery, magnetic cell sorting, magnetic fluid hyperthermia etc. [4–6]. Iron oxide nanoparticles are also known to play a vital role in magnetic resonance imaging (MRI) as they are a well-known contrast agents for MRI [7–9]. Many methods are reported for synthesis of iron oxide nanoparticles of which coprecipitation method is widely employed due to its simplicity and ease in controlling particle size [10,11]. Iron oxide nanoparticles of very small size also exhibit the property of superparamagnetism [12]. Iron oxide nanoparticles are very prone to aggregation. Thus, in order to protect these nanoparticles from aggregation, particles were surface modified with noble metals like gold, silver etc. and ligands/polymers [13]. Of lately increasing attention has been given in combining magnetic properties of iron oxide with optical properties of gold [14]. Properties like chemical stability, low cytotoxicity, biocompatibility along with well-known gold-thiol chemistry makes gold as a material of choice [15,16]. Iron oxide

(Fe_3O_4)/gold core–shell nanocomposites have been of much significance these days owing to their application in the field of targeted drug delivery, contrast agents for MRI, enhanced contrast in computed tomography (CT) etc. [17,18]. One of the drawbacks with this kind of core shell nanoparticles for in vivo applications is their elimination from the body by reticuloendothelial systems (RES) [19,20]. A common strategy to overcome these limitations is by stabilizing these nanocomposites with the help of polymers like alginates, glycols, vinyl alcohols etc. Alginates are natural block copolymers derived from brown algae and comprise of (1–4)-linked β-ᴅ-mannuronic acid (M-units) and α-ʟ guluronic acid (G-units) residues. Alginate polymers has carboxylic and hydroxyl groups on their surface which allows the functionalization via chemical or physical attachments of different moieties [21,22]. Biocompatibility, easy storage, non-immunogenic etc. are other benefits of alginate polymer which makes this polymer a potent choice [23]. Many well-known anti-cancer drugs like curcumin have limited usage in cancer treatment owing to minimal bioavailability and poor pharmacokinetics respectively [24]. These problems of low aqueous solubility of drugs could also be addressed by encapsulating drug using alginate based polymers stabilized nanoparticles. The present study reports the synthesis of gold coated iron oxide core-shell nanostructures further stabilized using thiol functionalized sodium alginate polymers. Thiols are known to have affinity towards gold as they tend to form strong bond with gold. X-ray diffractometer (XRD), UV–visible, energy dispersive X-ray spectroscopy (EDX) results confirmed coating of gold shell on to the surface of iron oxide nanoparticles. In addition High Resolution

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Transmission Electron Microscopy (HR-TEM), Fourier Transform Infrared Spectroscopy (FTIR), thermo gravimetric analyses (TGA), and X-ray photoelectron spectroscopy (XPS) were used to characterize the synthesized nanostructure. Vibrating Sample Magnetometer (VSM) was used to see the effect of gold shell coating on magnetic properties of core-shell iron oxide-gold nanoparticles. Curcumin was selected as potent anti-cancerous drug for encapsulation in above system and study *its* release behavior.

2. Materials and methods

Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, 99%), sodium alginate (($NaC_6H_7O_6$)$_n$), curcumin ($C_{21}H_{20}O_6$ 98%) and 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Himedia chemicals, India. Iron (II) sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$, 98%), conc. hydrochloric acid (HCl, 37%), sodium hydroxide (NaOH), citric acid ($C_6H_8O_7$, 99.5%) and L-cysteine hydrochloride ($C_3H_7NO_2S \cdot HCl \cdot H_2O$, 99%) and sodium chloride (NaCl, 99.5%) were purchased from Merck Chemicals and reagents, India. Hydrogen tetrachloroaurate ($HAuCl_4 \cdot 3H_2O$, 49–50% Au) was purchased from Thomas Baker, India. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC. HCl, 99%) and trisodium citrate dihydrate ($C_6H_5NO_3O_7 \cdot 2H_2O$, 99%) was purchased from Spectrochem, India. Experiments were carried out in double distilled water (purged with N_2 gas if required). All chemicals were of analytical grade and used as received.

2.1. Synthesis of citrate coated Fe_3O_4 nanocomposites (INPs)

Citrate capped Fe_3O_4 nanoparticles were prepared by co-precipitation method by procedure previously described by Cui et al. with slight modifications [25]. Briefly, aqueous solution of 0.1 M $FeSO_4 \cdot 7H_2O$ and 0.2 M $FeCl_3 \cdot 6H_2O$ was prepared in 100 ml of 0.1 M HCl. To this, 70 ml of 2 M NaOH was added drop wise under constant stirring condition for 60 min under inert atmosphere. Black colored suspension thus obtained was separated using high power neodymium alloy magnet (2500 G) and washed 3–4 times using nitrogen purged double distilled water. A part of iron oxide nanoparticles suspension was dried in vacuum oven and kept at 4 °C for further use. Iron oxide nanoparticles suspension thus obtained were stabilized using citric acid according to protocol used by Cheraghipour et al. [26]. Briefly stock solution of 0.1 g/ml of citric acid was prepared and used to stabilize suspension of Fe_3O_4 nanoparticles (70 mg of Fe_3O_4 nanoparticles dispersion in 50 ml distilled water) at a pH of 6.8. This mixture was under constant stirring condition and heated to around 80 °C for 2 h. The solution was kept as such to cool it to room temperature to obtain citric acid stabilized Fe_3O_4 nanoparticles. Part of thus obtained suspension was dried in vacuum oven for carrying out further studies.

2.2. Synthesis of gold coated Fe_3O_4 nanocomposites (GNPs)

Fe_3O_4/Au core-shell nanocomposites were prepared according to a modified protocol given by Banerjee et al. [27]. Briefly, 50 mg citrate coated Fe_3O_4 nanoparticle suspension was taken and diluted using 10 ml double distilled water and was sonicated for 20 min using water bath sonicator. This suspension was heated to 80–85 °C in water bath under constant stirring condition. To this 740 μl of freshly prepared 0.1 M gold chloride solution was added. Finally 3.5 ml of 0.1 M trisodium citrate dehydrate solution was added to this mixture. The solution was stirred for 60 min at 80–85 °C till a reddish tinge appeared in the suspension. Thus formed gold coated iron oxide nanoparticles were thoroughly washed 3–4 times using nitrogen purged double distilled water using neodymium alloy magnet (2500 G) to obtain gold coated iron oxide nanoparticle suspension (GNPs). Part of this suspension was dried in vacuum oven and kept at 4 °C for further use.

2.3. Synthesis of thiolated sodium alginate coated GNP (TGNPs)

Sodium alginate (SA) polymer was modified by covalent attachment of carboxylic group of alginate with amine group of cysteine according to the protocol given by Bernkop-Schnurch et al. [28] with slight modifications. Briefly, 2 g SA was dissolved in 200 ml of double distilled water and kept for overnight stirring in order to completely dissolve the polymer in water. To activate the carboxylic acid groups of SA, EDAC was added to a final concentration of 50 mM. The solution was stirred for 1 h at room temperature. L-Cysteine monohydrate hydrochloride was then added to the reaction mixture at ratio of polymer:cysteine of 2:1 (w/w). pH of the reaction was adjusted to 4 using 1 M HCl and was incubated for 2 h with constant stirring. After 2 h the pH of the solution was raised to 6 and it was stirred for additional 1 h. The polymer was then precipitated and washed 2–3 times using acetone to obtain thiolated SA (Fig. 1). Thus obtained thiolated SA was lyophilized and stored at 4 °C till further use. This thiolated SA (200 mg) was then mixed with 50 mg nanoparticles suspension of GNP in a ratio of 4:1 (w/w) and was stirred over night. Thus formed sample was washed 3–4 times using nitrogen purged DI water using magnetic separation to obtain TGNPs. TGNPs was lyophilized and stored at 4 °C for further use.

2.4. Determination of thiol content of thiolated SA polymer

Thiol content of thiolated SA polymer was determined by calorimetric reaction using Ellman's reagent (DTNB) [29]. 500 μl of Ellman's reagent [0.03% (w/v) DTNB in 0.5 M phosphate buffer pH 8.0] was added to 500 μl thiolated SA polymer solution in 0.5 M PBS (1 mg/ml). Absorbance was recorded at 412 nm using UV–Vis spectroscopy (Shimadzu model 2401 PC). Quantification of thiol group was done by comparing it with a standard curve of L-cysteine hydrochloride (0.001–0.01 mg/ml) in 0.5 M PBS (pH 8.0).

2.5. Drug loading in TGNPs

To load curcumin drug in TGNP, 3 ml aqueous dispersion of TGNPs (containing 10 mg thiolated SA modified GNP nanoparticles), 1 mg of curcumin drug dissolved in 300 μl of ethanol was added drop wise maintaining drug:nanoparticle ratio of 1:10 (w/w). The resultant solution was kept under magnetic stirring (approximately 400 rpm) overnight. Curcumin loaded TGNPs were separated using high power magnet (neodymium alloy magnet (2500 G)) and washed two times using distilled water.

2.6. Determination of encapsulation efficiency of TGNPs

In order to determine the encapsulation efficiency of TGNPs, curcumin loaded TGNPs were magnetically separated and the washings were pooled at the time of drug loading and dried. The concentration of curcumin (in ethanolic solution) was determined at 450 nm using a UV–Vis spectrometer and was compared with standard curve of curcumin drug (prepared in ethanol solution) in order to estimate the amount of drug encapsulated along with % encapsulation which could be determined using the following formulas:

$$Encapsulation\ efficiency\ (EE\%)$$
$$= \frac{Amount\ of\ drug\ loaded\ in\ nanoparticles}{total\ quantity\ of\ drug\ added\ initially} \times 100\%$$

$$Loading\ efficiency\ (LE) = \frac{Amount\ of\ drug\ added - Free\ drug}{Total\ amount\ of\ particles} \times 100\%$$