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Xanthan gum stabilized gold nanoparticles: Characterization, biocompatibility, stability and cytotoxicity

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ABSTRACT

Xanthan gum (XG) has been widely used in food, pharmaceutical and cosmetic industries. In the present study, we explored the potential of XG in the synthesis of gold nanoparticle. XG was used as both reducing and stabilizing agent. The effect of various formulation and process variables such as temperature, reaction time, gum concentration, gum volume and gold concentration, in GNP preparation was determined. The XG stabilized, rubey-red XGNP were obtained with 5 ml of XG aqueous solution (1.5 mg/ml). The optimum temperature was 80 °C whereas the reaction time was 3 h. The optimized nanoparticles were also investigated as drug delivery carrier for doxorubicin hydrochloride. DOX loaded gold nanoparticles (DXGP) were characterized by dynamic light scattering, TEM, FTIR, and DSC analysis. The synthesized nanoparticle showed mean particle size of 15–20 nm and zeta potential −29.1 mV. The colloidal stability of DXGP was studied under different conditions of pH, electrolytes and serum. Nanoparticles were found to be stable at pH range between pH 5–9 and NaCl concentration up to 0.5 M. In serum, nanoparticles showed significant stability up to 24 h. During toxicity studies, nanoparticles were found biocompatible and non-toxic. Compared with free DOX, DXGP displayed 3 times more cytotoxicity in A549 cells. In conclusion, this study provided an insight to synthesize GNP without using harsh chemicals.

1. Introduction

Xanthan gum (XG) is an anionic, high molecular weight, exopolysaccharide produced by aerobic fermentation of sugars by *Xanthomonas campestris*. Its basic chain consists of a β-(1 → 4) linked glucose backbone with the substitution of a charged trisaccharide side-chains of [β-(1 → 3)-mannose-α-(1 → 2)-glucuronic acid-β-(1 → 4)-mannose] on alternate glucose residues (Jian, Zhu, Zhang, Sun, & Jiang, 2012). It is non-toxic, hydrophilic and biodegradable bio-polymer. XG is being used in food, cosmetic and pharmaceutical industries. The industrial applications of XG are based upon its exceptional rheological properties. XG is soluble in both cold and hot water, hydrates quickly and produces high viscosity at low concentration (Sereno, Hill, & Mitchell, 2007; Sharma, Naresh, Dhuldhoya, Merchant, & Merchant, 2006). In pharmaceutical industries, XG has been reported for formulation of both solid and liquid dosage forms. In solid dosage formulation, it is used as controlled release agent (Jian et al., 2012; Phaechamud & Ritthidej, 2007; Santos, Veiga, Pina, & Sousa, 2005; Sinha, Mittal, Bhutani, & Kumaria, 2004) whereas in liquid formulations, it is used as thickening agent, suspending agent and emulsion stabilizer (Desplanques, Renou, Grisel, & Malhiac, 2012).

Recently, XG has also been used in the preparation and stabilization of inorganic iron and palladium nanoparticles (Comba, Dalmazzo, Santagata, & Sethi, 2011; Fan et al., 2013; Vecchia, Luna, & Sethi, 2009; Xue & Sethi, 2012). Nanoparticles are solid structures with a size below 200 nm and have found their applications in sensing, imaging and in drug and gene delivery. Gold nanoparticles (GNP) are one of the most commonly explored and used nanoparticles in drug delivery because of controlled size, improved efficacy and targeted delivery (Almeida, Figueroa, & Drezek; 2013; Pissuwan, Niidome, & Cortie, 2011). As drug delivery carrier, GNP has been used for the delivery of both hydrophilic and hydrophobic drugs (Aryal, Grailer, Pilla, Steeber, & Gong, 2009; Chen et al., 2007; Gibson, Khanal, & Zubarev, 2007; Oliveira et al., 2013; Prabaharan, Grailer, Pilla, Steeber, & Gong, 2009). But, the conventional

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synthesis of GNP involves the use of chemicals like sodium borohydrate, tri-sodium citrate etc. as reducing agent (Burygin et al., 2009; Duncan, Kim, & Rotello, 2010; Jung et al., 2013; Khan, Vishakantea, & Siddaramaiah, 2013; Saha et al., 2007). These GNP have been found to be very unstable and form aggregates with the slight change in pH and electrolyte concentration (Mirza & Shamshad, 2011; Rouhana, Jaber, & Schlenoff, 2007). The GNP prepared with gellan gum showed the stability between pH 4–8 and addition of NaCl up to 0.1 M (Dhar, Reddy, Shiras, Pokharkar, & Prasad, 2008). Natural gums stabilize the inorganic nanoparticles by two mechanisms: first, by adsorbing to the surface of nanoparticles which creates steric repulsion among the particles. In second mechanism, they increase the viscosity of nanoparticle suspension, and therefore slow down the aggregation processes (Comba & Sethi, 2009; Tiraferri, Chen, Sethi, & Elimelech, 2008; Xue & Sethi, 2012). The aim of this investigation was to synthesize the environment friendly gold nanoparticles using XG as reducing agent. The nanoparticle preparation was optimized for gum concentration and volume, gold concentration, temperature and reaction time. Gum stabilized GNP were studied for its utility as drug delivery carrier using Doxorubicin hydrochloride as model drug. Doxorubicin hydrochloride is hydrophilic in nature and has been clinically used for the treatment of various cancers, haematological malignancies, soft tissue sarcomas and solid tumours (Carvalho et al., 2009; Laginha, Verwoert, Charrois, & Allen, 2005). Two measure limitations of DOX are non-specific cytotoxicity and multi drug resistance. Multi drug resistance to DOX is due to drug efflux by P-glycoproteins. Doxorubicin is substrate for P-glycoproteins which efflux DOX and decrease intracellular drug level. DOX loaded GNP have shown to overcome both the problems of non-specific toxicity and drug resistance (Gu, Cheng, Man, Wong, & Cheng, 2012). The prepared nanoparticles were also investigated for biocompatibility, stability and cytotoxicity study in lung cancer cells.

2. Materials and methods

2.1. Materials

Xanthan gum and Tetrachloroauric acid ($HAuCl_4$) were purchased from Sigma–Aldrich (St Louis, MO, USA). Doxorubicin hydrochloride was received as gift sample from TherDose pharma Pvt. Ltd. (Hyderabad, India). The chemical used for buffer preparations were of analytical grade and were purchased from sd Fine-Chem Ltd. (Hyderabad, India). MTT (3-(4,5-dimethylthazol-2-yl)-2,5-diphenyl tetrazolium bromide, Dulbecco's modified eagle medium (DMEM), trypsin–EDTA, foetal bovine serum (FBS) and antibiotic solution (10,000 U/ml penicillin, 10 mg/ml streptomycin) were purchased from Sigma–Aldrich (St Louis, MO, USA). Cell culture plastic wares were obtained from Tarsons Products Pvt. Ltd. (Kolkata, India). All the formulations were prepared in MilliQ water.

2.2. Preparation of gum solution

The stock solution of gum was prepared by dissolving 500 mg of the gum in 100 ml water and was stirred overnight at room temperature. The solution was centrifuged to remove the insoluble materials and supernatant was lyophilized. The lyophilized dry powder was dissolved in water to get desired concentration of xanthan gum.

2.3. Synthesis of gold nanoparticles

Gold nanoparticles were prepared by reducing the aqueous solution of $HAuCl_4$ by heating at 80 °C in the presence of XG solution (1.5 mg/ml). The change in colour was obtained from colourless to purple to rubey-red after 2 h. The colloidal solution was cooled at room temperature and stored in amber colour vials at 4 °C. The nanoparticle formulation showed the absorption maxima at 525 nm.

2.4. Optimization of formulation and process variables

The effect of formulation and process variables such as gum concentration, gold concentration, gold to gum volume ratio, reaction time and temperature were studied by changing one parameter at a time and keeping other constant.

2.5. Characterizations of xanthan gum stabilized gold nanoparticles (XGNP)

2.5.1. Hydrodynamic diameter and polydispersity

The hydrodynamic diameter and polydispersity index of GNP were determined by dynamic light scattering using Zetasizer Nano-ZS (Malvern instrument Ltd., Malvern, UK). Before measurement, the samples were diluted appropriately to get particle count rate between 100 and 300 kcps and instrument was set up at 25 °C with a back scattering angle of 173°.

2.5.2. Transmission electron microscopy (TEM)

A drop of sample was placed on carbon coated copper grid, air dried at room temperature and stained with 2% uranyl acetate. The nanoparticle size measurement was done using transmission electron microscope (Hitachi, H-7500) and average of 10 nanoparticle size was considered as the size of the sample.

2.5.3. Surface charge

The electrokinetic properties of the GNP were determined by measuring the zeta potential using Zetasizer Nano-ZS. The samples were diluted 10 times with MilliQ water before the measurement.

2.5.4. Fourier transform infrared analysis

An amount of 2 mg of sample was mixed with 100 mg of potassium bromide and compressed to form a pellet. The pellet was placed in pellet holder and scanned for the measurement of % transmittance in the wave number range of 4000–450 cm^{-1} using FTIR spectrophotometer (Perkin Elmer, USA). The spectra requisition was carried out using the software Spectrum One (Perkin Elmer, USA).

2.6. Doxorubicin loading to nanoparticles

Blank gold nanoparticles were dispersed in phosphate buffer saline and were incubated with 1 mg of DOX solution (1 mg/ml) at room temperature overnight. The nanoparticles dispersion was centrifuged at 15,000 rpm for 30 min. DOX loading was determined by measuring the free drug content in supernatant. Percent drug loading was calculated as follows:

$$\%\ \text{DOX loading} = \frac{1 - D_S}{D_T} \times 100$$

whereas, D_S is the amount of DOX present in supernatant, D_T is the total amount of DOX loaded initially.

2.7. In vitro drug release studies

The in vitro drug release studies were performed using dialysis method. In dialysis tubing, the nanoparticles equivalent to 1 mg of DOX were dispersed in 1 ml of distilled water and placed in 100 ml of release medium (phosphate buffer saline pH 7.4 and sodium acetate buffer pH 4.5) at 37 °C in dark. Three millilitres of sample was withdrawn at different time intervals up to 12 h and was replaced with same volume of fresh medium. The samples were