



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.

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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 (Serenio, 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, & Dreze, 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; Prabakaran, Grailer, Pilla, Steeber, & Gong, 2009). But, the conventional

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synthesis of GNP involves the use of chemicals like sodium borohydride, 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 $4000\text{--}450\text{ cm}^{-1}$ 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

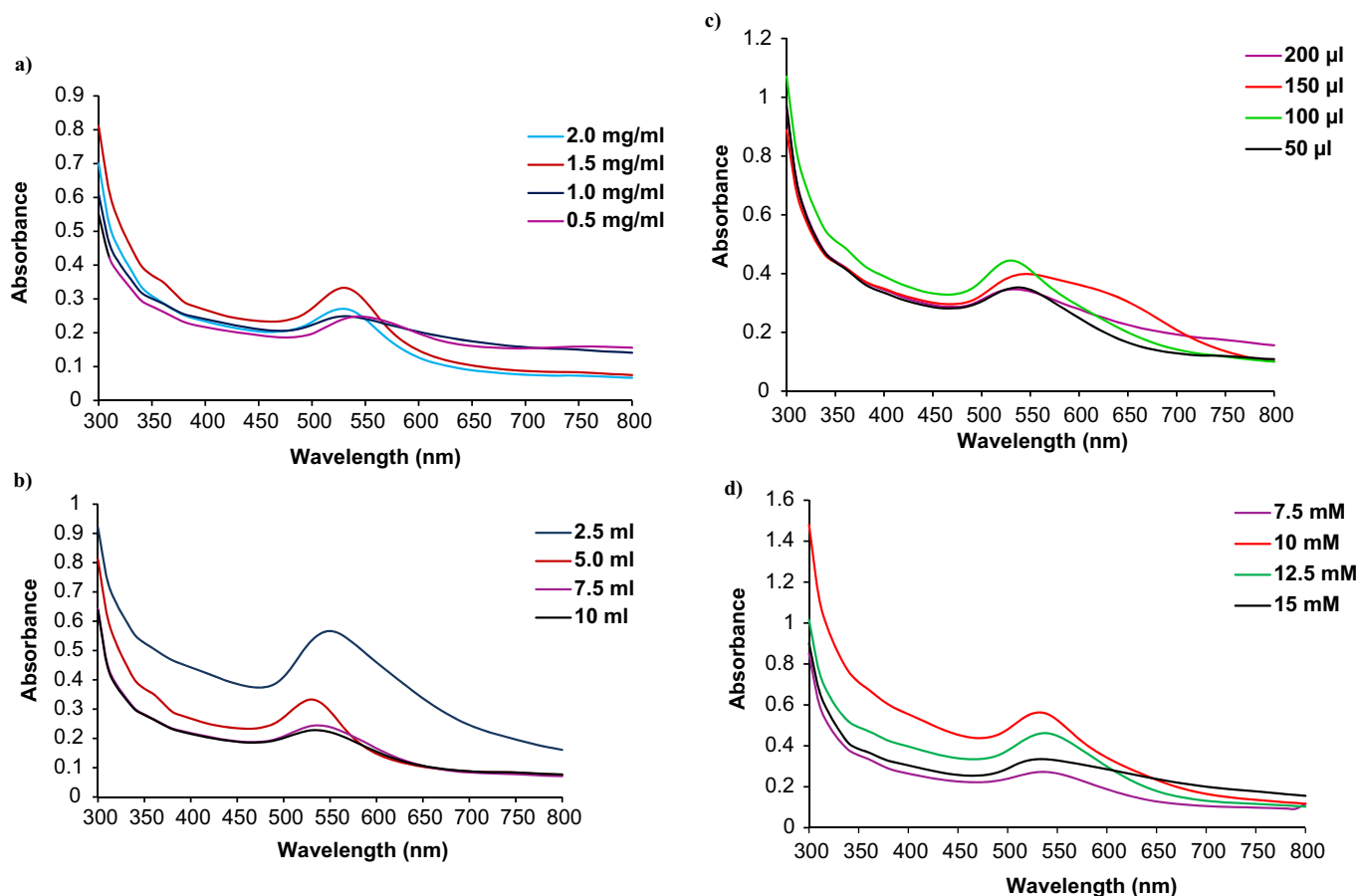


Fig. 1. Optimization of different formulation variables for the synthesis of Xanthan gum stabilized gold nanoparticles. Effect of (a) Xanthan gum concentration (b) gum volume (c) gold volume and (d) gold concentration.

analyzed for drug content using UV/vis spectrophotometer (Perkin Elmer, Lambda 25, USA) at 480 nm.

2.8. Hemolytic toxicity studies

The biocompatibility of prepared gold nanoparticles was determined by estimating the % hemolysis, caused by nanoparticles. The assay procedure reported by Kumar, Paul, & Sharma, 2011, was used with slight modification (Kumar et al., 2011). Blank gold nanoparticles were dispersed in normal physiological saline (0.9%, w/v NaCl). Varying concentrations (50–200 µg/ml) of the dispersion were added into red blood cells suspension (2%, v/v), mixed well and incubated at 37 °C. After 1 h, the samples were centrifuged at 3000 rpm for 5 min and absorbance of supernatant was measured at 540 nm spectrophotometrically. The supernatant absorbance of RBC suspension treated with distilled water and normal saline were taken as standard and control, respectively. The % hemolysis was calculated as: % hemolysis = $(A_{\text{sample}} - A_{\text{control}}) / (A_{\text{standard}} - A_{\text{control}}) \times 100$.

2.9. Cytotoxicity studies

Cellular toxicity of pure DOX and DXNP was evaluated by MTT assay in A549 human lung cancer cells. The assay is based on the mitochondrial reduction of yellow MTT, a tetrazolium dye, to a highly coloured blue formazan product. About 10,000 cells per well were cultured in 96 wells plate and allowed to attach overnight. Next day, cells were incubated with different concentration (0.1–10 µg/ml) of pure doxorubicin, blank gold nanoparticles

(positive control) and doxorubicin loaded gold nanoparticles. Cells were incubated in CO₂ incubator at 37 °C for 48 h. The solution of each well was replaced with fresh serum free media and 10 µl of MTT reagent (5 mg/ml) was added. After 4 h, the media was removed, 200 µl DMSO was added and incubated for 20 min. The absorbance was measured at 570 nm using Spectra Max plus 384 UV–vis plate reader. The cell viability was determined as a percent based on the absorbance measured relative to the absorbance of untreated or control cells. The half maximum inhibitory concentration (IC₅₀) values were determined by non-regression analysis using GraphPad Prism v. 5.03 (GraphPad Software Inc. CA).

2.10. Stability studies

2.10.1. Storage stability

Doxorubicin loaded nanoparticles were stored at 4 °C and were studied for particle size, zeta potential and drug content up to 1 month. Particle size and zeta potential were determined by particle size analyzer and DOX content was determined using UV/vis spectrophotometer.

2.10.2. Effect of pH and ionic concentration of medium

For the determination of effect of pH on the stability, DXGP were incubated in different pH medium (pH 3–11). Nanoparticles incubated in phosphate buffer saline pH 7.4 were taken as control. Absorbance was measured after 24 h and compared with absorbance of control.

The effect of ionic strength on the stability of DXNP was determined by incubating the nanoparticles in deionised water

containing varying concentrations (0.1–2 M) of sodium chloride (NaCl).

2.10.3. Serum stability of DXGP

Serum stability of DXGP was evaluated at two different serum concentration levels (10%, v/v, and 100% serum). Nanoparticles were dispersed in serum and particles size, zeta potential and absorbance were measured at different time intervals up to 72 h.

2.11. Statistical analysis

All the experiments were performed in triplicates. Data are expressed as Mean $\pm$ SD (standard deviation) ($n=3$). The values of in vitro drug release, cytotoxicity and stability studies were statistically analyzed using student's *t*-test. Statistical significance level was set at $p < 0.05$.

3. Result and discussion

3.1. Preparation and optimization of gold nanoparticles

Gold nanoparticles were prepared by chemical reduction method using xanthan gum as reducing agent. The role of gum concentration was studied by using gum solution in the range of 0.5–2 mg/ml containing 10 mM of HAuCl₄ concentration (Fig. 1a). The absorbance in the UV spectrum which describes the reduction of GNP, was found to be directly related to gum concentration. The intensity of the absorption band gradually increased and the maximum intensity of absorption was attained at 1.5 mg/ml which indicated the maximum gold nanoparticle concentration.

The effect of gold to gum volume ratio was evaluated by varying the gum volume in the range of 2.5–10 ml (Fig. 1b). At lower gum volume (2.5 ml) a peak shift was observed and the absorption band shifted to 550 nm which suggested that lower gum volume was not sufficient to reduce all Au ions. When the gum volume was changed from 5 to 7.5 ml followed by 10 ml, there was no significant shift in the characteristic absorption band but the absorbance was decreased. The maximum intensity of absorption was attained at 5 ml and was considered optimum gum volume for the synthesis of XGNP. The effect of gold concentration (Fig. 1c) and volume (Fig. 1d) was also studied and was found as per earlier reports. A volume of 100 μ l of 10 mM gold solution showed better absorbance at 525 nm.

Reaction temperature and time are two important process parameters in the synthesis of gold nanoparticles. In this study, the temperature was changed in the range of 60–90 °C with 10 mM HAuCl₄ concentration and 1.5 mg/ml gum solution (Fig. 2a). The maximum intensity of the characteristic absorption band was attained at 80 °C which indicates the maximum gold nanoparticle concentration. For the optimization of reaction time, nanoparticle synthesis was studied up to 4 h. The absorbance was found to be increased up to 2 h and after that there was no change in the absorbance (Fig. 2b).

3.2. Characterization of Xanthan gum stabilized gold nanoparticles

The hydrodynamic diameter and polydispersity index of optimized XGNP were determined using dynamic light scattering method. The observed mean hydrodynamic diameter was 41.2 ± 3.78 nm. The prepared nanoparticles showed high polydispersity (0.35–0.6) which can be attributed to presence of wide range of particles. The high negative surface potential (-47.2 ± 2.59) indicated the high stability of nanoparticles and presence of gum molecules on nanoparticle surface. The presence of acetyl group on O-6 position and pyruvic acid linked with mannose sugar are responsible for the negative charge. After DOX loading,

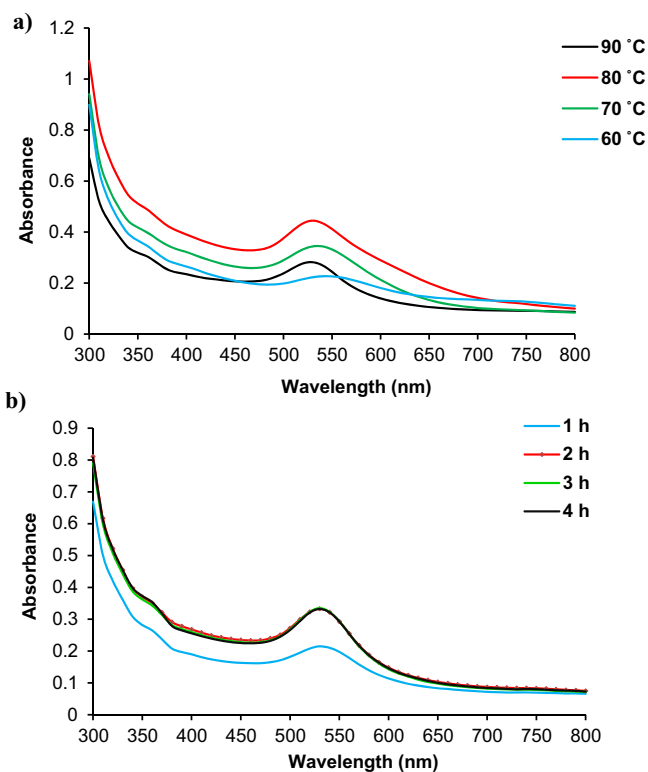


Fig. 2. Effect of temperature (a) and reaction time (b) in the synthesis of XG stabilized gold nanoparticles.

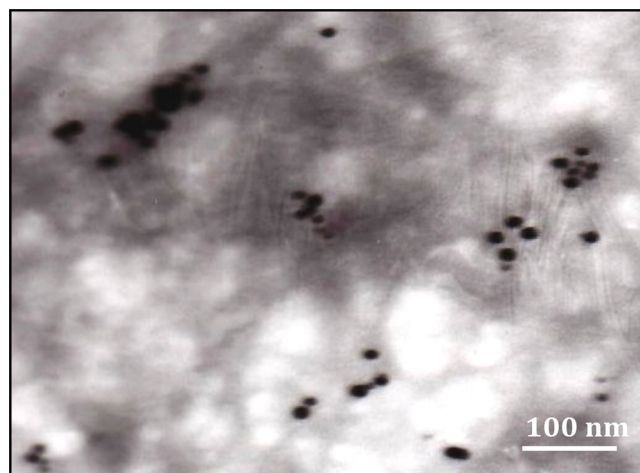


Fig. 3. Transmission electron microscopy (TEM) image of DOX loaded Xanthan gum stabilized gold nanoparticles (DXGP).

the surface charge was decreased to -29.1 ± 2.78 mV indicating the interaction between positively charged amine group of DOX and negative acidic group of gum. The nanoparticle surface morphology and actual particle size were determined by transmission electron microscopy. Fig. 3 showed that nanoparticles were spherical in shape and 15–20 nm in size.

The chemical interactions between DOX and nanoparticles were studied by FTIR analysis (Fig. 4). In XG spectra, the vibration peak of hydrogen bonded –OH and C–H stretching were observed at 3416 and 2902 cm^{-1} , respectively. The vibration peak of the acetal was found at 1051.8 cm^{-1} whereas C=O stretching peak was found at 1728.4 cm^{-1} . The FTIR spectra of blank nanoparticles exhibited all the characteristic peaks of XG at 3398, 1728 and 1042 cm^{-1} . The peak at 1254.2 cm^{-1} presented the C–O–C group. The DXGP

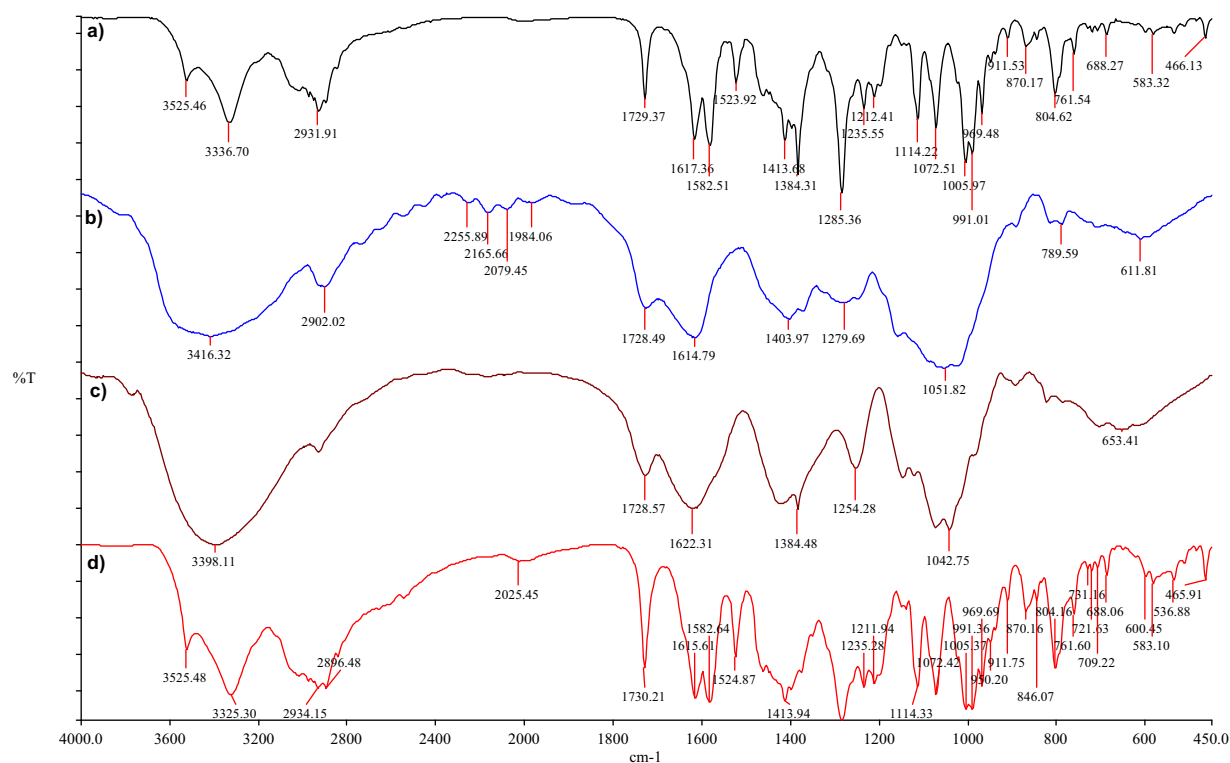


Fig. 4. FTIR Spectra of (a) pure Dox (b) Native XG (c) Blank GNP and (d) Dox loaded GNP.

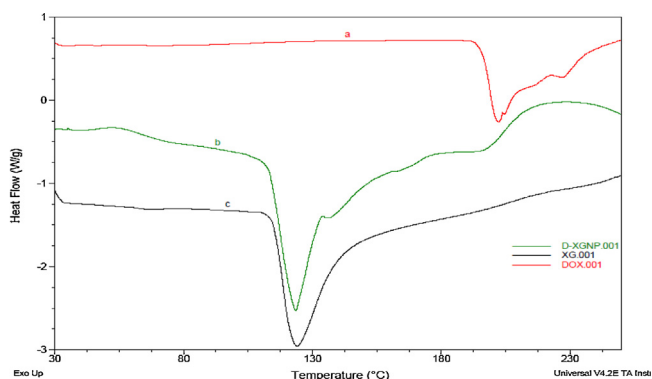


Fig. 5. Differential scanning thermogram of (a) pure DOX (b) Dox loaded xanthan gum stabilized nanoparticles and (c) native xanthan gum.

showed principal IR peaks of both XG and DOX showing the non-covalent binding of DOX to nanoparticles.

DSC is a useful technique to explain the thermal stability and phase transitions. Fig. 5 illustrates the DSC thermograms of pure DOX, native XG and DXGP. DSC scan of XG exhibited endothermic transition at 125 °C. The endothermic peak of DOX appeared at its melting point 195 °C. This peak was disappeared in DXGP spectra indicating that DOX was not precipitated on nanoparticle surface but was present as molecular or amorphous dispersion.

3.3. DOX loading

The observed doxorubicin loading was found to be $71.4 \pm 2.53\%$. The high drug loading can be attributed to presence of XG on the nanoparticle surface. Xanthan gum contains negatively charged moieties such as pyruvic acid which interacts with positively charged amine group of doxorubicin.

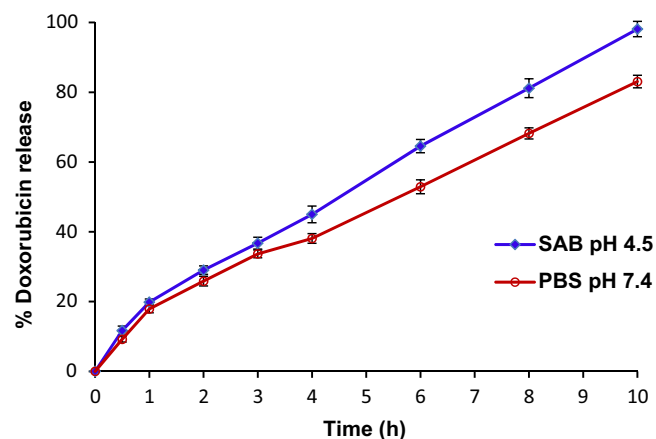


Fig. 6. In vitro drug release from DXNP in sodium acetate buffer pH 4.5 and phosphate buffer saline pH 7.4.

3.4. In vitro drug release studies

In vitro drug release from the nanoparticles was studied in PBS and SAB buffer (Fig. 6). The nanoparticles showed complete drug release within 10 h. Up to 4 h, there was no significant difference ($p > 0.05$) in doxorubicin release in both the medium but at the end DOX release was higher in SAB (98.1%) as compared to PBS (83.6%). The faster release of DOX in SAB can be attributed to protonation of amine groups of DOX molecules which increases hydrophilicity and solubility at lower pH. The protonation of amine group also leads to decreased interaction between DOX and GNP (Minati et al., 2012). The PBS pH 7.4 is a simulated condition for blood plasma whereas SAB pH 4.5 is for the internal environment of cancer cell. Thus the slower release of DOX in PBS and faster release in SAB would help to release the maximum amount of drug in tumour and hence to enhance the therapeutic efficacy of the formulation.

3.5. Hemolytic toxicity

Nanoparticles are reported to cause oxidative-stress induced hemolysis (Teodora, 2013). Therefore, the suitability for intravenous administration of nanoparticles is generally determined by hemolytic study. In this study, dose-dependent hemolytic toxicity of xanthan gum stabilized gold nanoparticle was carried out. The prepared nanoparticles did not show hemolysis up to 200 $\mu\text{g/ml}$.

3.6. Cytotoxicity studies

Anti-proliferative activity of doxorubicin loaded xanthan gum stabilized gold nanoparticles (DXGP) and free DOX was studied in A549 human lung cancer cells. The cell viability was determined by MTT assay after 48 h of incubation with different concentrations (Fig. 7). Blank xanthan stabilized gold nanoparticles (BXNP) were taken as positive control and did not decrease the cell viability at any test concentration. It suggested that the prepared nanoparticles were non-toxic and biocompatible. Drug loaded nanoparticles showed concentration-dependent cytotoxicity. The cell viability was decreased with the increase in DOX concentration. The IC_{50} value for pure DOX and DXGP was 2.46 and 0.79 $\mu\text{g/ml}$, respectively. The lower IC_{50} value of DXGP indicated the more cytotoxicity which can be attributed to enhanced cellular uptake and hence increased intracellular drug concentration. However, the exact mechanism and role of carbohydrate moieties present on nanoparticle in cellular uptake require detailed investigation. One possible reason may be due to the presence of mannose sugars in the composition of xanthan gum which was used for the stabilization of GNP. It is documented that mannose receptors are up regulated in human lung cancer cells A549 (Basuki et al., 2014; El-Boubbou et al., 2010). Basuki et al. reported the improvement in cell uptake of mannose functionalized iron nanoparticles in human lung cancer cells A549.

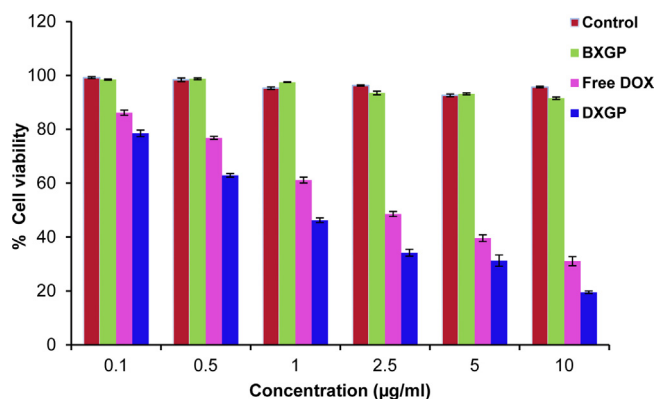


Fig. 7. Percent cell viability of A549 human lung cancer cells after 48 h of treatment with free DOX, BXNP and DXNP (mean $\pm$ SD, $n = 3$).

Hence, the presence of mannose in xanthan gum might have contributed to increase in uptake of drug through receptor-mediated endocytosis pathway.

3.7. Stability studies

The colloidal stability of DXGP was studied for both physical and chemical stabilities. The change in particle size and zeta potential are shown in Fig. 8. No significant ($p > 0.05$) changes in particle size and zeta potential were observed up to 15 days. But, chemical degradation of DXNP was faster than physical instability. The DOX content was less than 90% within 24 h. However, the DOX degradation was slower with DXGP in comparison to pure DOX. The observed drug degradation rate constant (K) was 8.28×10^{-3} and $5.07 \times 10^{-3} \text{ day}^{-1}$ for pure DOX and DXGP (Fig. 8c). Therefore,

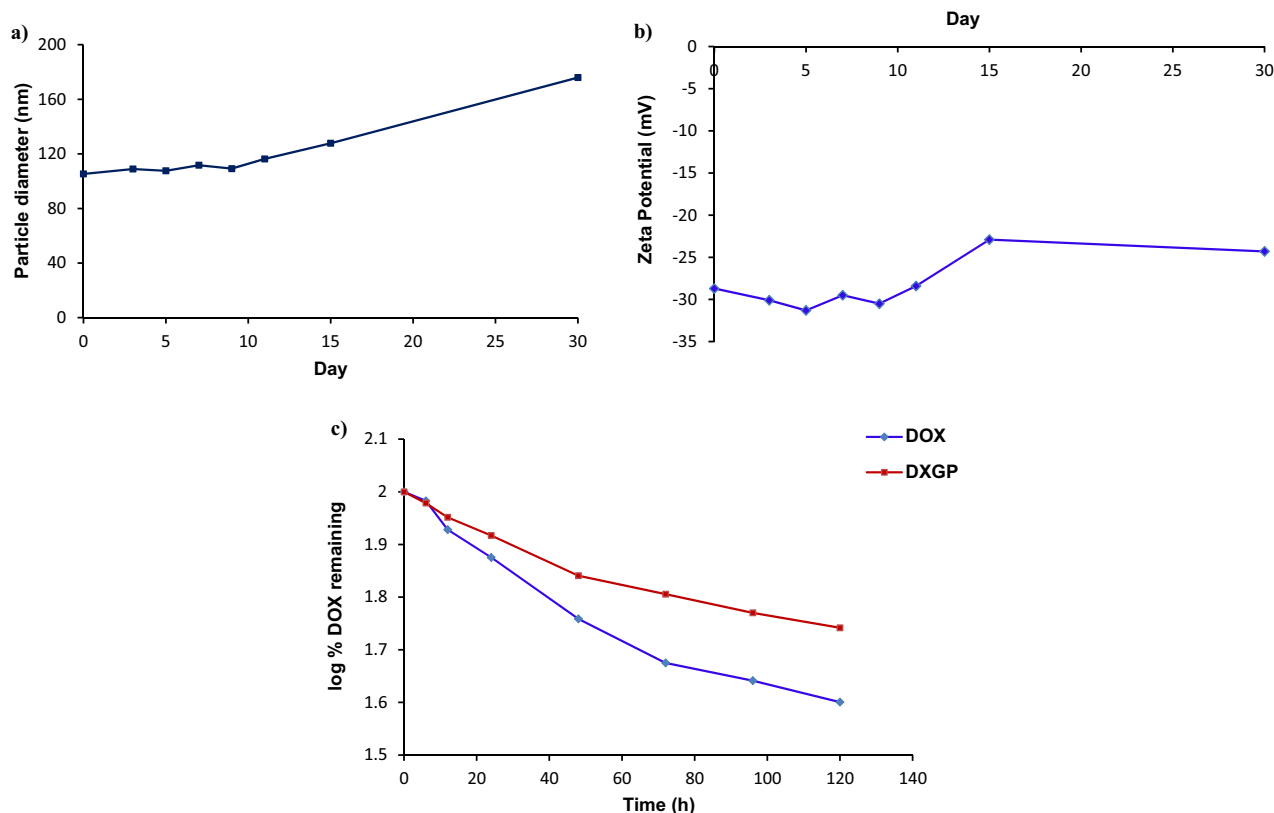


Fig. 8. Storage stability of DXGP: (a) particles diameter (b) zeta potential and (c) DOX content.

decrease in drug content was due to light sensitivity of DOX itself not because of formulation.

DXGP showed a wide range of pH stability (pH 5–9). The observed absorbance of DXGP at 480 nm was 1.109, 0.899, 0.908, 0.927 and 0.776 for pH 3, 5, 7, 9 and pH 11 respectively (Fig. 9a). The absorbance of control sample was 0.931. The results suggested that XG or carbohydrate capping of nanoparticles helped to increase the colloidal stability of gold nanoparticles. The decrease in absorbance at pH 11 may be due to gel formation properties of XG at higher pH levels (pH > 10) (Sharma et al., 2006).

In other study, DXGP were treated with different NaCl concentration to determine the stability of nanoparticles in varying ionic concentrations. Fig. 9b showed the absorbance pattern of DXGP after 24 h of incubation with different NaCl concentrations. The control group was incubated in deionised water without NaCl. The DXGP were found to be stable up to 0.5 M NaCl concentration.

The stability of DXGP was further evaluated at two serum conditions—diluted (10%, v/v serum) and undiluted or 100% serum. Fig. 10a showed the particle diameter of DXGP exposed to serum, after different time period. In both the conditions, particle size was increased continuously up to 72 h. But, the increase in particle diameter was more in undiluted serum than diluted serum. After 2 h, particle size was increased by 7.1 nm in 10% serum while it increased by 31.5 nm in 100% serum. From the Fig. 10a it is clear that DXGP were stable up to 24 h in both the conditions but after that size of nanoparticle was increased tremendously. The increase in particle size can be explained by the adsorption of serum proteins or other components on nanoparticle surface. (Yang, Zhang, Wang, White, & Jiang, 2009; Zhang & Zhang, 2010). The nanoparticle dispersion in distilled water had a high negative zeta potential of -30.2 mV and thus the electrostatic interactions between

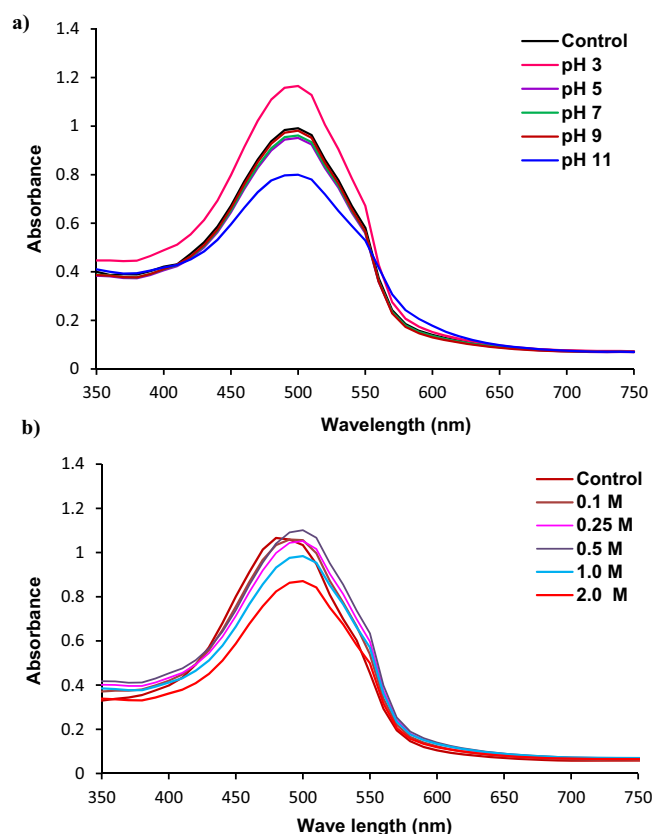


Fig. 9. Effect of pH and ionic strength on the stability of DXGP.

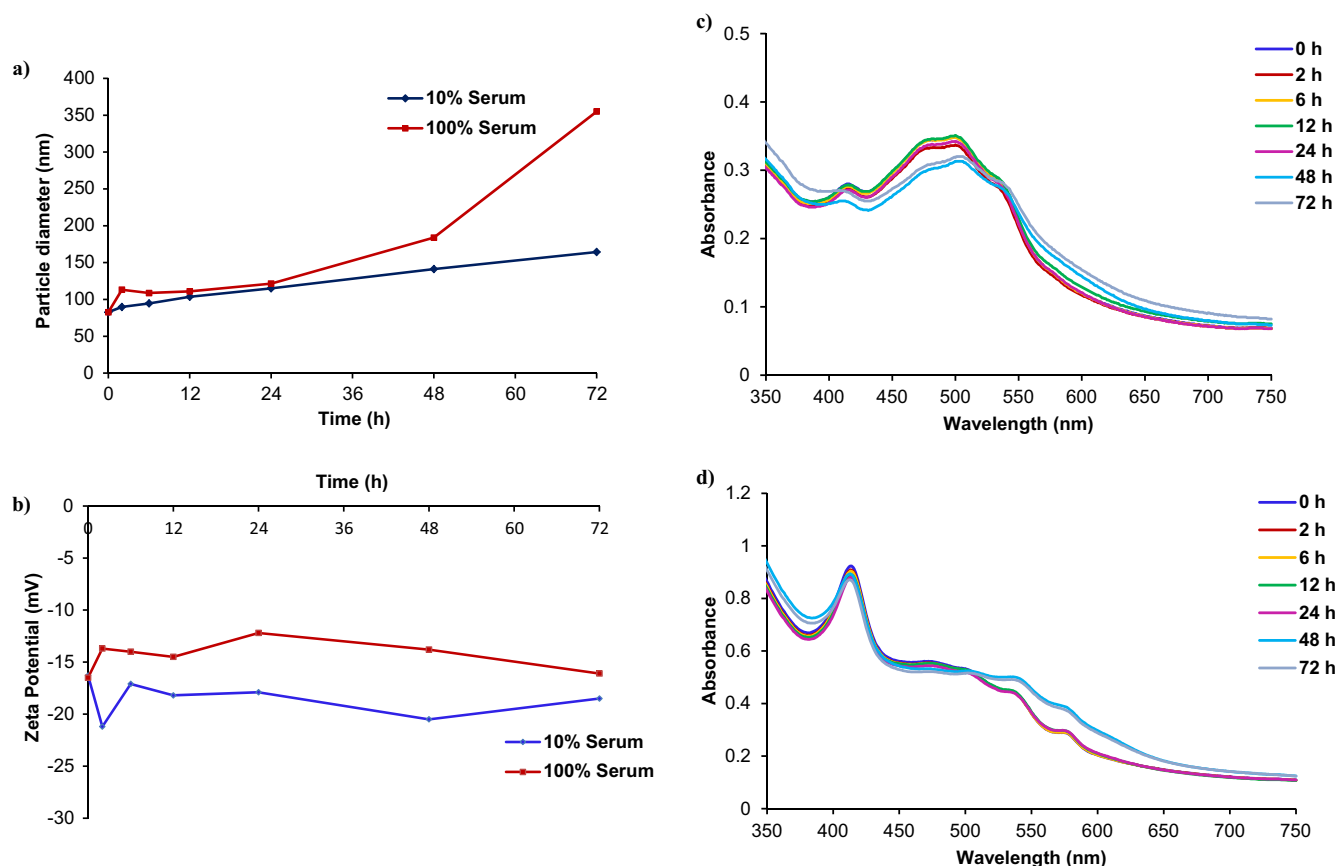


Fig. 10. Serum stability of DXGP (a) particle size (b) zeta potential (c) absorption spectra in diluted (10%) serum and (d) absorption spectra in undiluted (100%) serum.

electronegative nanoparticles and positive serum components can also not be ruled out. This possibility was well supported by the zeta potential values observed after addition in to serum (Fig. 10b). Zeta potential of nanoparticle was increased (less negative values) dramatically due to charge neutralization. After 24 h, surface charge of DXGP was -18.2 and -14.5 mV in 10% and 100% serum, respectively. Higher zeta potential in 100% serum indicated the more interactions between nanoparticles and serum proteins leading to higher particle size.

The change in absorption pattern of DXGP with time was also observed over the range of 350–750 nm. Interestingly, the absorption pattern in diluted serum (Fig. 10c) was similar to that of spectra observed throughout the study but new absorption spectrum was observed in 100% serum (Fig. 10d). It may be due to, as earlier mentioned, adsorbed or electro-statically interacted serum components. However, no change in absorption spectra was observed up to 24 h in both serum conditions. Overall, DXGP were stable physically and chemically up to 24 h even under the extreme conditions of serum.

4. Conclusions

The applications of gold nanoparticles as multifunctional vehicle (drug delivery carrier, imaging) are increasing because of ease of synthesis, biocompatibility and opportunity for surface conjugation. In this investigation, gold nanoparticles were synthesized using natural and biocompatible Xanthan gum as reducing and capping agent. Xanthan gum at very low concentration of 1.5 mg/ml converted the ionic gold to neutral gold particles with a particle size of 15–20 nm. Nanoparticles were found to be non-toxic and biocompatible in hemolysis study. Doxorubicin was loaded to the nanoparticles through non-covalent interaction. The prepared carbohydrate-rich gold nanoparticles showed high drug loading, good colloidal stability and enhanced cytotoxicity in A549 lung cancer cells. High physical stability of gum stabilized nanoparticles can be attributed to steric repulsion among the particles provided by same charge of gum moieties present on the nanoparticle surface. Receptor-mediated endocytosis of nanoparticles could be a reason for better anticancer activity of DXGP than free drug. Presence of mannose in xanthan gum might have contributed this effect. Hence, natural gum based approach could be a better method for the synthesis of gold nanoparticles.

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