Gold/Chitosan/Pluronic Composite Nanoparticles for Drug Delivery

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ABSTRACT: Composite nanoparticles were prepared and characterized as a sustained delivery system for paclitaxel, an anticancer drug. Gold nanoparticles were used as building blocks for constructing the composite nanoparticles. An ionic interaction between the anionic gold nanoparticles and cationic chitosan was induced to form the composite nanoparticles. Particle size analysis, field emission scanning electron microscopy, transmittance electron microscopy, and ultraviolet–visible were used to observe the formation of the composite nanoparticles. For the application of the composite nanoparticles as a drug carrier, paclitaxel was loaded into the composite nanoparticles, and the drug-release pattern was observed. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 108: 3239–3244, 2008

Key words: biomaterials; colloids; drug delivery systems; metal-polymer complexes; nanotechnology

INTRODUCTION

The incorporation of metal nanoparticles into polymer matrices is a field of particular interest for material engineering and the study of nanoparticle–matrix interactions.[1,2] There have been a number of attempts to achieve metal nanoparticle/polymer composites using the *in situ* preparation of the nanoparticles in the matrix or the polymerization of the matrix around the metal nanoparticles.[3,4] However, these approaches are affected by various experimental conditions such as the reduction of metal salts in the matrix and the polymerization of the matrix in an uncontrolled manner. A desirable approach would involve the blending of premade metal nanoparticles into polymers to form metal/polymer composites.[5]

Gold nanoparticles are building blocks for applications in a variety of biomedical areas and electronics.[6–8] In the past few years, many studies have investigated the control of the size of gold nanoparticles, their compositions, and their self-organization into a well-defined organization. Two-dimensional and three-dimensional lattices have been prepared by the self-assembly of monodispersed gold nanoparticles.

Chitosan is a polysaccharide composed of glucosamine and *N*-acetylglucosamine. Because of its biocompatibility, chitosan has found emerging applications in biomedicine.[9,10] In a slightly acidic solution, the amine group of chitosan is protonated and positively charged. This gives chitosan the ability to form complexes with oppositely charged materials. Chitosan has been used as a cationic polymer in layer-by-layer assembly with certain anionic substances such as gold nanoparticles for structural organization.[11]

In this study, composite nanoparticles were prepared and characterized as a delivery system for an anticancer drug. A number of studies have been performed to design chitosan-based drug delivery systems.[12–14] For this purpose, chemical modification was required to form the nanoparticles in the aqueous media by self-assembly, and hydrophobically modified chitosans were prepared. However, in this study, the gold nanoparticles were used as seed material for the formation of the composite nanoparticles, and the ionic interaction between the gold nanoparticles and chitosan was employed without chemical modification. During the formation of the composite nanoparticles, paclitaxel was loaded into the nanoparticles, which were characterized as a delivery system for the anticancer drug.

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EXPERIMENTAL

Materials

$HAuCl_4$, trisodium citrate dihydrate, low-molecular-weight chitosan (molecular weight = 4200), Tween 80, and paclitaxel were purchased from Sigma-Aldrich Inc. (St. Louis, MO). Pluronic F-127 [a poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) triblock copolymer] was obtained as a gift from BASF Corp. (Seoul, Korea) and used as received. F-127 can be represented by the formula $(EO)_{100}(PO)_{65}(EO)_{100}$, where EO is ethylene oxide and PO is propylene oxide, on the basis of its nominal molecular weight of 12,600 and its poly(ethylene oxide) concentration of 75%. The dialysis membrane [poly(vinylidene fluoride); molecular weight cutoff = 500,000] was a product of Spectrum (Houston, TX).

Preparation of the paclitaxel-loaded composite nanoparticles

Gold nanoparticles were prepared by citrate reduction of $HAuCl_4$ on the basis of a method described elsewhere.[15] Gold nanoparticles prepared in this study showed a uniform size distribution with a diameter of 16.1 nm. For drug loading, 150 mg of a drug suspension composed of a 1/2 (w/w) paclitaxel/Tween 80 mixture was prepared, in which an aqueous solution containing gold nanoparticles (50 μg/mL) was subsequently dispersed. A 2 wt % chitosan solution was prepared with acetic acid (pH 3.5). The ionic interaction between the gold nanoparticles and chitosan was induced to form paclitaxel-loaded gold/chitosan nanoparticles through the mixing of 150 mg of the drug suspension containing gold nanoparticles (the volume of the solution was 5.5 mL) and 1.5 mL of a chitosan aqueous solution. The solution mixture was dialyzed in distilled, deionized water for 12 h to solidify the chitosan matrix. To obtain the nanoparticles in the powdery state, gold/chitosan nanoparticles dispersed in the aqueous medium (the volume of the solution was ca. 8 mL) were freeze-dried with 4 mL of a 30 wt % F-127 aqueous solution. Figure 1 presents the preparation method schematically.

Particle size distribution and ζ-potential measurements

Solutions of 30 mg of freeze-dried nanoparticles in 30 mL of phosphate-buffered solution (PBS; pH 7.4) were prepared for the measurement of the particle size distribution and ζ potential. The intensity autocorrelation was measured at a scattering angle of 90° with electrophoretic light scattering (ELS 8000, Otsuka Electronics, Osaka, Japan) at 25 ± 0.1°C. When the difference between the measured and calculated baselines was less than 0.1%, the correlation function was accepted. A nonlinear, regularized, inverse Laplacian transformation technique was used to obtain the distribution of the decay constant. The mean diameter was evaluated by the Stokes–Einstein equation. Experiments were repeated three times.

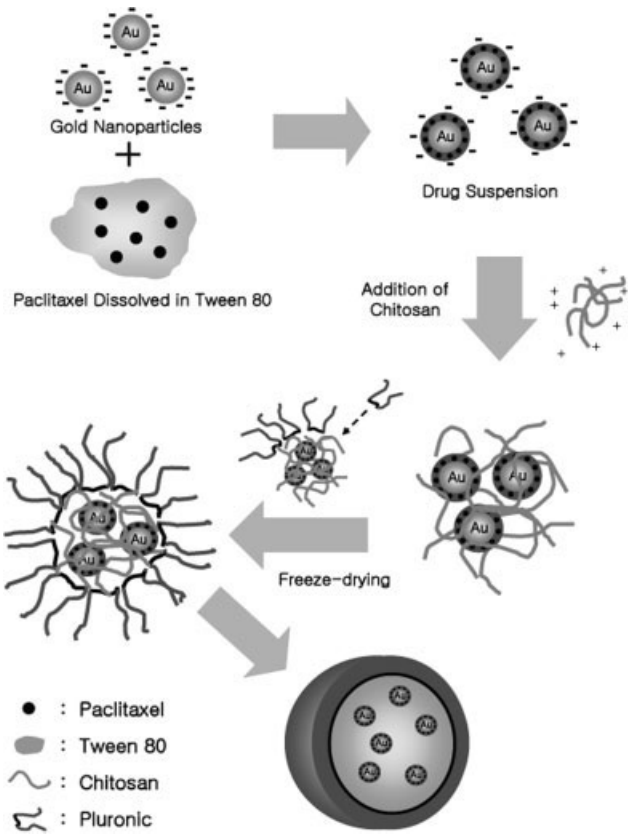

Figure 1 Schematic description of the formation of composite nanoparticles.

Field emission scanning electron microscopy (FESEM) measurements

The general view of composite nanoparticles was examined with FESEM (model JMS6700F, JEOL, Tokyo, Japan). For this purpose, freeze-dried nanoparticles were gold-coated *in vacuo* and examined at a tilt angle of 45°.

Transmittance electron microscopy (TEM) measurements

The freeze-dried nanoparticles were dispersed in distilled, deionized water to obtain a solution of 0.1 wt %. To prepare a sample for TEM, each solution was dropped onto a carbon-coated grid and then dried at 25°C in a vacuum oven for 24 h. Samples were examined by TEM (model 7600, Hitachi, Tokyo, Japan) at 100 kV.

In vitro release of paclitaxel from the gold/chitosan nanoparticles

To determine a loading amount, 10 mg of freeze-dried nanoparticles was dissolved in 20 mL of methanol for 2 days. Then, 1 mL of the solution was withdrawn and immediately filtered through a 0.45-μm membrane filter. Subsequently, this was added to 3 mL of a mixture of acetonitrile and water (50/50 v/v). Paclitaxel was determined by reverse-phase high-performance liquid chromatography (P2000, Spectra System, San Jose, CA) with a symmetry C_{18} column and an acetonitrile–water (50/50 v/v %) mobile phase over 20 min at a flow rate of 1 mL/min. The elute was monitored by ultraviolet absorption at 228 nm. The drug-loading amount is defined as the ratio of the amount of drug in the nanoparticles to the total weight of the nanoparticles, and the encapsulation efficiency is defined as the ratio of the amount of drug in the nanoparticles to the total amount of drug used in the preparation of the nanoparticles.

For measuring the release pattern of paclitaxel from the nanoparticles, 5 mg of freeze-dried nanoparticles was introduced into 20 mL of PBS (pH 7.4) containing 0.1% (w/v) Tween 80 in screw-capped tubes, which were placed in a shaking water bath maintained at 37°C and shaken horizontally at 130 cm^{-1}. At given time intervals, the tubes were centrifuged at 10,000 rpm for 10 min, 1 mL of the supernatant was withdrawn from the release medium (PBS) for analysis, and 1 mL of methylene chloride was added to the aliquot for extraction; this was followed by the addition of 3 mL of a mixture of acetonitrile and water (50/50 v/v). Evaporation of methylene chloride until a clear solution was obtained was carried out under a stream of nitrogen. High-performance liquid chromatography analysis was then conducted as previously described. The precipitated nanoparticles were resuspended in 20 mL of fresh PBS and placed back in the shaker bath.

Ultraviolet–visible measurements

For measuring the optical property of the gold nanoparticles, a 1 wt % concentration of nanoparticles in the aqueous media and bare gold nanoparticles in the aqueous media were examined with an ultraviolet–visible spectrophotometer (UV-2401PC, Shimadzu, Kyoto, Japan).

RESULTS AND DISCUSSION

Gold nanoparticles are usually stabilized by oppositely charged polyelectrolytes.[11] On the basis of these interactions between gold and polyelectrolytes, gold nanoparticles have been used as building blocks to construct composite nanoparticles for drug delivery.

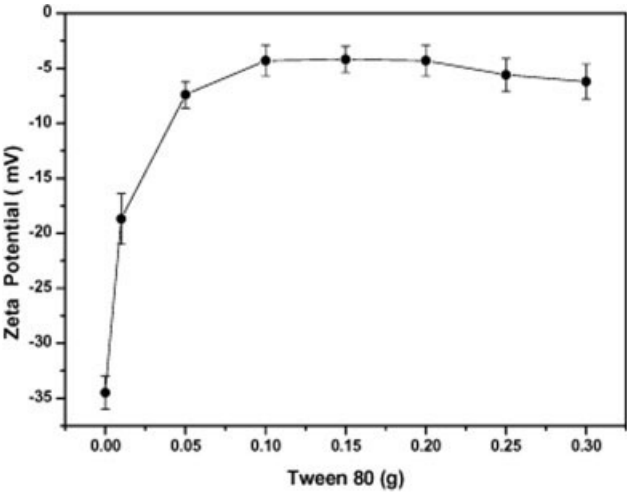

Figure 2 Change in the ζ potential as a function of the added amount of Tween 80 (the number of experiments was three).

For efficient drug loading, Tween 80 containing paclitaxel was mixed with gold nanoparticles in the aqueous media. Figure 2 shows the change in the ζ potential of gold nanoparticles as a function of the added amount of Tween 80. With the addition of Tween 80, the ζ potential was changed from −34.5 to −4.3 eV, and this indicated the diminution of the anionic character of the gold nanoparticles caused by the adsorption of Tween 80 onto the surface of the gold nanoparticles. The ζ potential did not show a significant change with more than 0.10 g of Tween 80. From the viewpoint of the loading amount of paclitaxel in the nanoparticles, the increased amount of Tween 80 was required. However, the leakage of Tween 80 was observed after the formation of composite nanoparticles with more than 0.15 g of Tween 80.

The ionic interaction between the gold nanoparticles and chitosan was induced to form the gold/chitosan nanoparticles. Bare gold nanoparticles, which showed a ζ potential of −34.5 eV, formed the ion complex with cationic chitosan with precipitation (agglomeration), and a similar phenomenon was observed with gold nanoparticles mixed with 0.01 g of Tween 80. However, no precipitation was observed in the mixture of gold nanoparticles mixed with 0.05 g of Tween 80, and the size distribution shows that the diameter of the formed nanoparticles was approximately 120 nm. This indicates that the control of the ionic interaction between the gold nanoparticles and chitosan plays an important role in forming composite nanoparticles and can be regulated by the added amount of Tween 80.

To determine the optimum ratio of chitosan to drug suspension (the drug suspension was 0.15 g of

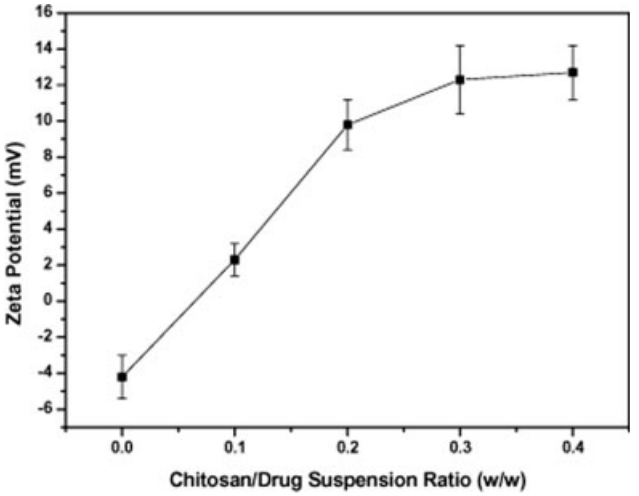

Figure 3 Change in the ζ potential as a function of the chitosan/drug suspension ratio (the number of experiments was three).

a mixture composed of 1/2 w/w paclitaxel/Tween 80 and 250 μg of gold nanoparticles), the ζ potential was measured as a function of the chitosan/drug suspension ratio (see Fig. 3). With the increase in the ratio, the ζ potential was increased, and this indicated that chitosan was dominant at the surface of the chitosan/drug suspension mixture. No significant change in the ζ potential was observed above the ratio of 0.2.

On the basis of these results, the gold/chitosan composite nanoparticles were prepared with 0.15 g of drug suspension and a chitosan/drug suspension ratio of 0.2.

To obtain the composite nanoparticles in the powdery state, gold/chitosan nanoparticles dispersed in the aqueous media were freeze-dried in the presence of F-127. Without F-127, gold/chitosan nanoparticles

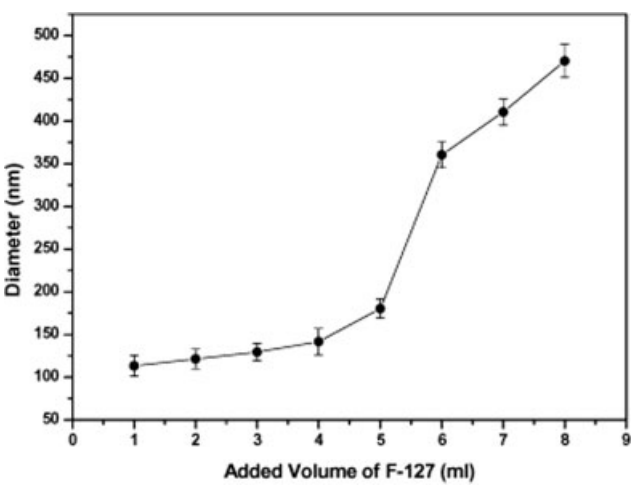

Figure 4 Diameter change in the composite nanoparticles as a function of the added volume of the 30 wt % F-127 aqueous solution (the number of experiments was three).

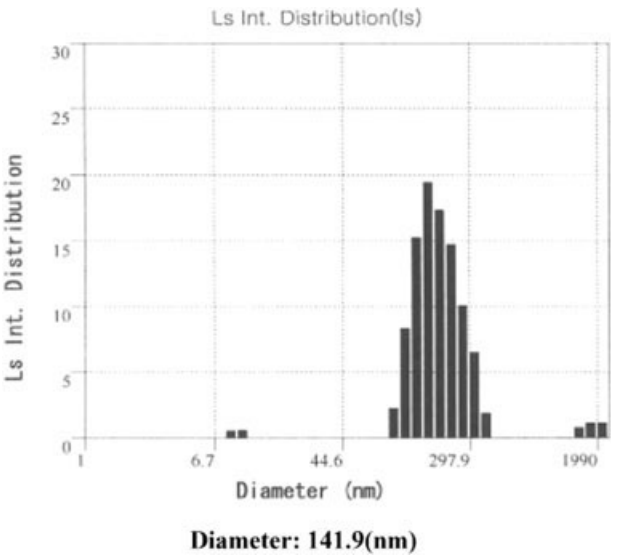

Figure 5 Size distribution of the composite nanoparticles with the optimized composition. Ls Int, light scattering intensity.

were aggregated during the freeze drying. As shown in Figure 4, the diameter of the composite nanoparticles was increased with the added volume of F-127 increasing. By consideration of the diameter of the composite nanoparticles, 4 mL of a 30 wt % F-127 aqueous solution was used. Figure 5 shows the size distribution of the freeze-dried composite nanoparticles resuspended in PBS (pH 7.4).

An FESEM picture was taken to observe the general view of the composite nanoparticles. Figure 6 shows the formation of nanoparticles with a spherical shape. With TEM measurements, the presence of

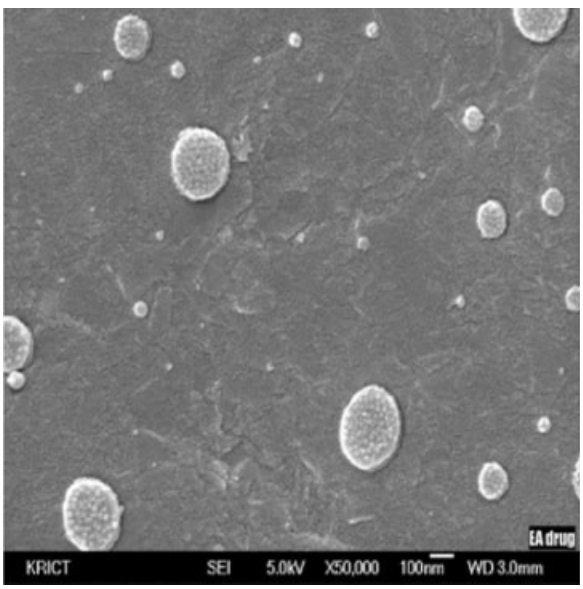

Figure 6 FESEM picture of the composite nanoparticles with the optimized composition.

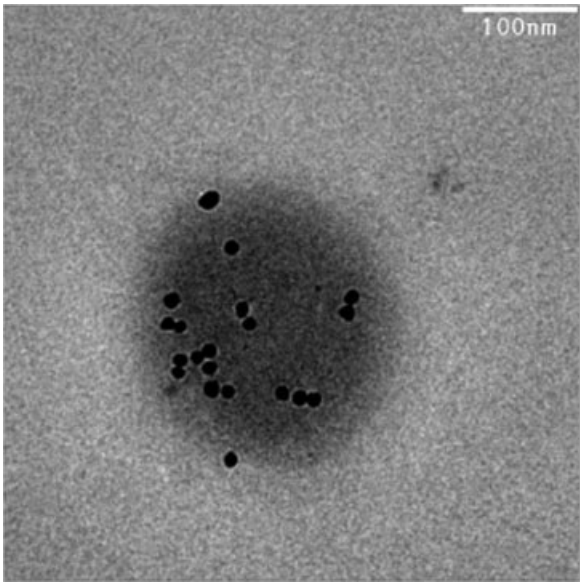

Figure 7 TEM picture of the composite nanoparticles with the optimized composition.

gold nanoparticles in the composite nanoparticles was verified, as shown in Figure 7. These results indicate that composite nanoparticles can be formed with the preparation method in this study.

For the application of composite nanoparticles as drug carriers, the release pattern of paclitaxel was measured, as shown in Figure 8. The loading amount of the nanoparticles was 3.52 ± 0.12 wt %. As described for the preparation method, the mixing of gold nanoparticles with Tween 80 containing paclitaxel was performed in aqueous media for the drug loading. If the ratio of paclitaxel to Tween 80 was more than 0.6, the precipitation of paclitaxel was observed during the mixing. This was due to the low solubility of paclitaxel in the aqueous media

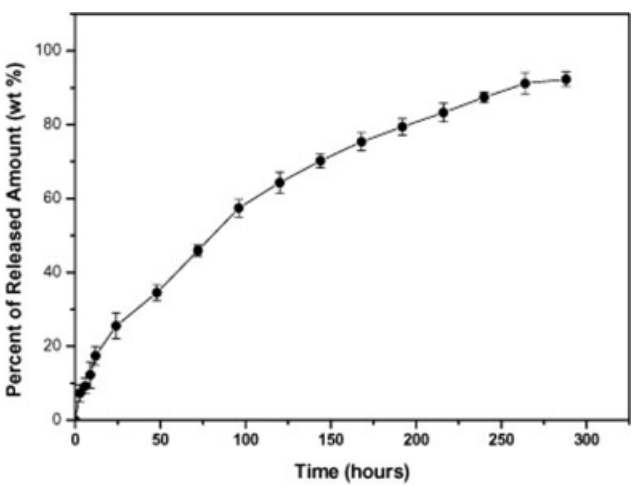

Figure 8 Release pattern of paclitaxel from the composite nanoparticles (the number of experiments was three). Ls Int, light scattering intensity.

(1 μg/mL).[16] With a minimal burst effect in the early stage of the release experiment, a sustained-release pattern was observed, about 90% of the initial loading amount being released during a 12-day period.

To observe the stability of the nanoparticles in the aqueous media, the size distribution was observed with composite nanoparticles after 2 weeks of equilibrium in PBS. If the particles were unstable in the aqueous medium, they tended to aggregate to form the agglomerate. This led to an unexpected change in the release of the loaded drug from the particles. As shown in Figure 9, the composite nanoparticles did not show aggregation, and this indicated that the composite nanoparticles were stable in the aqueous medium.

Because of the presence of gold nanoparticles in the matrix, the composite nanoparticles in this study are expected to improve radiotherapy. Hainfeld et al.[17] reported the effect of gold during radiotherapy. They measured the change in the tumor volume after intravenous gold injection followed by irradiation. In a comparison with controls (gold injection only or irradiation only), a significant reduction of the tumor volume was observed. As shown in Figure 10, the optical property of gold in the composite nanoparticles was not changed, and this indicated that the gold nanoparticles were stable in the matrix, and the gold nanoparticles in the composite nanoparticles were expected to behave like bare gold upon irradiation. Because of the presence of paclitaxel, a potent anticancer drug, an enhanced effect of radiotherapy is expected. The gold/chitosan/Pluronic composite nanoparticles can be delivered locally to the tumor site with angioplasty. After they are

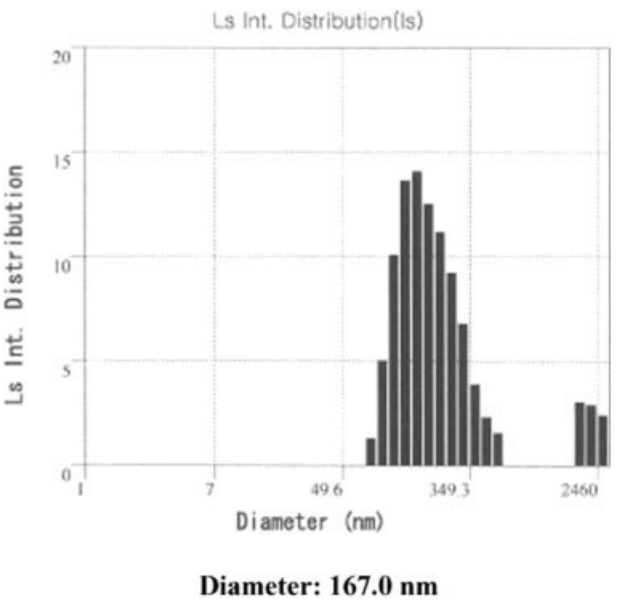

Figure 9 Size distribution of the composite nanoparticles after a 2-week equilibrium in PBS.

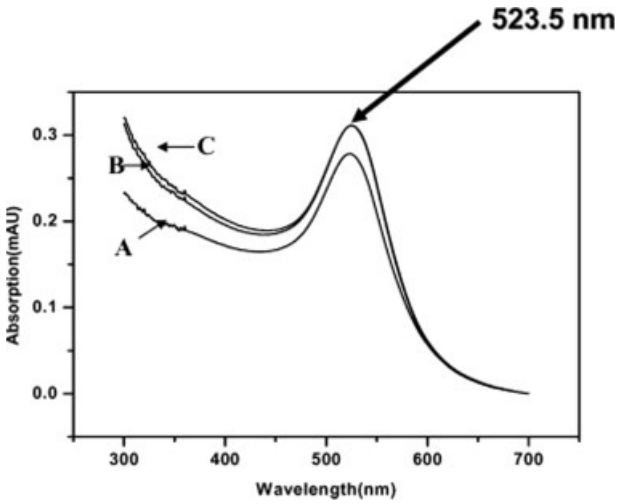

Figure 10 Changes in the optical properties of the gold nanoparticles in different environments: (A) gold nanoparticles in the aqueous media, (B) gold/chitosan nanoparticles, and (C) composite nanoparticles.

delivered to the tumor site, the target site will be irradiated, and paclitaxel will be released from the nanoparticles. The therapeutic level of paclitaxel is expected to be maintained at the tumor site because of the sustained release of paclitaxel from the nanoparticles. The feasibility of this approach will be reported later.

CONCLUSIONS

Gold/chitosan/Pluronic composite nanoparticles were prepared by a facile method. With the manipulation of the composition of the nanoparticles, composite nanoparticles were prepared in the powdery state, and efficient loading of paclitaxel into the composite nanoparticles was achieved. FESEM measurement showed the formation of nanoparticles with a spherical shape, and the presence of gold nanoparticles in the composite nanoparticles was verified by TEM measurements. On the basis of the stability in the aqueous media and the release pattern of paclitaxel, the composite nanoparticles in this study can be used as carriers for hydrophobic drugs.

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