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Preparation, characterization and *in vitro* release study of gallic acid loaded silica nanoparticles for controlled release

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Gallic acid (GA) is an interesting pharmaceutical component of plants. However, the short lifetime and the autoxidation of GA in aqueous solution significantly reduces its bioavailability and the residence time in the body system. In this study, GA was chemically bound to silica nanoparticles to control the release of GA based on the hydrolysis of the chemical bonds, and a silica nanoparticle drug delivery system was established. Gallic acid loaded silica nanoparticles (GA-SiO_2) were synthesized by a modified Stober method. The Fourier Transform Infrared Spectroscopy (FTIR) and X-Ray Diffraction (XRD) analysis proved that GA did conjugate to silica nanoparticles. The particle size of the GA-SiO_2 nanoparticles observed by Scanning Electron Microscope (SEM) was about 30 nm and the drug loading efficiency determined by Thermo Gravimetric Analysis/Differential Scanning Calorimetry (TGA/DSC) was 89.39%. The *in vitro* release study demonstrated that GA could be gradually released from the GA-SiO_2. In addition, the antioxidant capability increased continuously during the immersion time, so the GA could serve as an excellent antioxidant to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals in a long release period. Therefore, this study provided a novel drug delivery system for GA with controlled release capability and prolonged antioxidant activity.

1. Introduction

Gallic acid (3,4,5-trihydroxybenzoic acid, GA) has been well known as a strong natural antioxidant which is able to scavenge the Reactive Oxygen Species (ROS), e.g. superoxide anions, hydrogen peroxide, hydroxyl radicals and hypochlorous acid (Kim 2007). Furthermore, GA and its derivatives have been extensively evaluated for their antitumor activity against a variety of cell lines (Locatelli et al. 2012). However, the short lifetime and the autoxidation of GA in aqueous solution significantly reduces its bioavailability and the residence time in the body system. It has been demonstrated that GA administered orally exist in blood for 6 h at most, and more than half is metabolized to 4-O-methyl gallic acid, followed by excretion into urine (Zong et al. 1999). The radicals of GA, which were considered stable and long living, had a lifetime of 20 mins in aqueous solution (Giannakopoulos et al. 2006). Semi-quinone and quinone were generated by the autoxidation of oxygenated GA solution within minutes (Gil-Longo and González-Vázquez 2010).

A controlled-release system increases the overall efficacy (i.e., therapeutic efficiency) of a drug by maintaining the drug concentration in the body within the optimum therapeutic range and under the toxicity threshold (Barbe et al. 2004). In light of this, a wide range of drug delivery systems for GA have been designed, such as electrospun nano-fibers mats (Chuysinuan et al. 2009; Shao et al. 2011; Kim et al. 2012), microspheres or microcapsules (Pik-Ling Lam et al. 2012; Robert et al. 2012), hydrogels (Ciolacu et al. 2012). However, low blood stability has appeared to be a major problem for all the organic delivery systems. Because the adsorbed protein markers on the surface of hydrophobic carries delivered a signal to the immune system to evacuate these foreign objects from the body (Barbe et al. 2004). The modification of organic materials might solve this problem but makes the production process complicated. Thus inorganic materials are becoming increasingly popular attributed to a series of advantages, including their availability, high hydrophilicity and biocompatibility, promising capability of targeted delivery and controlled release of carried drugs (Kong et al. 2010). Recently, several reports have demonstrated that the phenolic radicals of GA could be stabilized for a long time and controlled release of GA could be achieved by embedding them in the interlayer space of Mg/Al layered double hydroxide (LDH) (Kong et al. 2010) and Zn–Al-LDH clay (Yeganeh Ghotbi and bin Hussein 2010).

Silica nanoparticles have been popular as inorganic drug delivery carriers due to their high surface absorbability, high surface activities and low requirements of experimental conditions of sol-gel technology etc. In the past decade, silica particles have been widely applied as delivery systems for drugs (Wang 2009), and microspheres and mesoporous silica were focused by most researchers. However, GA tends to diffuse into the solution during the microspheres preparation process, resulting in low encapsulation efficiency and drug uptake values, because GA is a small molecule with high water-solubility. Furthermore, the same reason led to the low encapsulation efficiency and drug uptake of GA for mesoporous silica.

Silica nanoparticles are usually generated by sol-gel technology, the process can be described as a two-step inorganic polycondensation as shown in Fig. 1(a) and (b). The carboxyl group of GA is attractive to react with the –Si-OH generated in the poly-

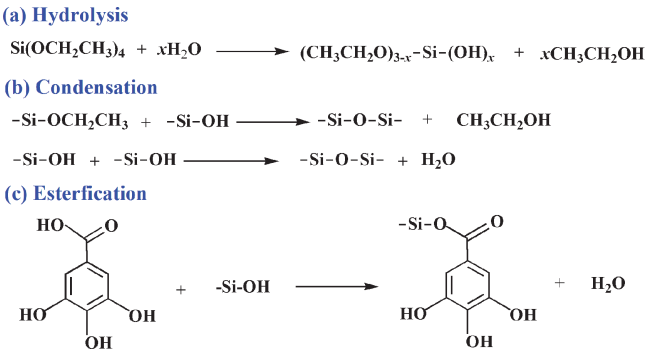

Fig. 1: Synthesis mechanisms of SiO_2 and GA-SiO_2 nanoparticles

condensation process. The generated gallic acid loaded silica nanoparticles (GA-SiO_2) administrated orally or delivered onto the stents or scaffolds could hydrolyze to GA and silica nanoparticles *in vivo*. The GA is cytotoxic against certain cancer cells *in vivo*, without harming the normal cells (de Mejia et al. 2006). It has been reported silica *in vivo* caused no abnormal inflammation or adverse reaction (Radin et al. 2005) and diffused through the local tissue surrounding the implant, enter the blood stream or lymph, and was excreted through the kidneys in the urine (Lai et al. 2002).

The aim of the present work was to investigate the use of silica nanoparticles as drug-delivery system for GA as anti-cancer agent whether orally administered or loaded onto ureteral stents and tissue engineering scaffolds, etc. The work was based on grafting GA onto the SiO_2 nanoparticles by the esterification of GA and -Si-OH as shown in Fig. 1(c). The synthesis of GA-SiO_2 was done by a modified Stober method (Arriagada and Osseo-Asare 1999). The chemical structures of samples were characterized by Transform Infrared Spectroscopy (FTIR) and X-Ray Diffraction (XRD). The drug loading efficiency and the particle size of the nanoparticles were determined and observed by Thermo Gravimetric Analysis/Differential Scanning Calorimetry (TGA/DSC) and Scanning Electron Microscopy (SEM). Furthermore, the release behavior of GA was investigated, and the antioxidant activity of GA was evaluated based on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity.

2. Investigations, results and discussion

2.1. FTIR and XRD analysis

The infrared absorption spectra of SiO_2, GA and GA-SiO_2 are shown in Fig. 2(A). The spectrum of GA showed bands around 3373 cm^{-1} due to the phenolic O-H stretching vibrations. The significant peaks at 1708 cm^{-1} and 1247 cm^{-1} were corresponding to the ν(C = O) and ν(C-O) of carboxyl groups, respectively. The bands at 1612 cm^{-1}, 1541 cm^{-1}, 1446 cm^{-1} and 1338 cm^{-1} were attributed to the aromatic C = C vibrations. The spectrum of SiO_2 showed a broad band at 3438 cm^{-1} due to O-H stretching of -Si-OH. The bands at 1118 cm^{-1} and 868 cm^{-1} were attributed to Si-O stretching of Si-O-Si and Si-OH respectively.

The spectrum of GA-SiO_2 showed the characteristic peaks of both GA and SiO_2. The peaks around 3386 cm^{-1} were assigned to the hydroxyl groups of GA and silanol groups. The significant peaks at 1708 cm^{-1} and 1247 cm^{-1} of GA slightly shifted to 1710 cm^{-1} and 1220 cm^{-1} due to the ν(C = O) and ν(C-O) of ester groups. The peaks at 1614 cm^{-1}, 1533 cm^{-1}, 1450 cm^{-1} and 1384 cm^{-1} were attributed to the aromatic C = C vibrations. A new but weak peak could be observed at 1670 cm^{-1} due to the hydrogen bonding of silanol groups to the hydroxyl groups of GA (Zhang et al. 2010). The new peak at 953 cm^{-1} was due to Si-O stretching of Si-O-CO (Silvestri et al. 2012). Based on the FTIR results, it was proved that GA-SiO_2 contained the chemical ingredients and functional groups of both GA and SiO_2.

Fig. 2(B) shows the XRD patterns of SiO_2, gallic acid and GA-SiO_2. The XRD pattern of SiO_2 nanoparticles was amorphous, and no significant sharp diffraction peaks were observed. Pure GA was highly crystalline in nature as indicated by the numerous peaks. It was observed that crystalline GA was detected in GA-SiO_2. The peaks at 16.3°, 25.4° and 27.8° of the crystalline GA were slightly shifted to 16.4°, 25.2° and 27.6° in the XRD patterns of GA-SiO_2, for the reason that the reaction between GA and silica nanoparticles induced a little change with the crystal structure of GA. The overall results of FTIR spectra and XRD patterns confirmed the esterification of GA and silica nanoparticles.

2.2. TGA/DSC study

The experimental drug uptake value and drug loading efficiency of GA in GA-SiO_2 were tested by the DSC/TGA study. As shown in Fig. 3, the DSC curve of GA-SiO_2 exhibited an endothermic peak at 241.6 °C and an exothermic peak at 441.6 °C, which corresponded to the evaporation of water of crystallization and the decomposition of gallic acid respectively. The TGA curve of GA-SiO_2 exhibited a total weight loss of 28.98%. Weight loss of 4.20% with temperature raising to 241.6 °C corresponded to the evaporation of water absorbed by SiO_2 nanoparticles. With the temperature raising to 800 °C, another weight loss of 24.78% before constant weight was achieved due to decomposition of GA. Therefore, the experimental drug uptake value was 24.78% which was close to theoretical drug uptake value (27.72%). The calculated drug loading efficiency was 89.39%.

2.3. SEM

The representative micrographs of SiO_2 and GA-SiO_2 nanoparticles observed by SEM are shown in Fig. 4(A) and (B) respectively. SiO_2 nanoparticles performed nearly monodispersed spherical shape. The average size of SiO_2 nanoparticles was about 30 nm. On the other hand, the synthetic GA-SiO_2 nanoparticles also performed nearly monodispersed spherical shape and an average size of about 30 nm as shown in Fig. 4(B). As known, GA is a kind of organic acid and could act as a catalyzer for the hydrolysis of TEOS (Barbe et al. 2004). However, no obvious differences between SiO_2 nanoparticles and GA-SiO_2 nanoparticles in the morphology and particle size were observed because the carboxyl group of GA was consumed in the esterification process of GA-SiO_2.

2.4. In vitro release of GA

In the release model of GA-SiO_2, the percentage of released drug was monitored for 120 h in two release media. The results are presented in Fig. 5. Fig. 5(A) presents the releasing performance of GA in HCl which simulated stomach conditions in the human body. It was found that about 30% of GA was released in the first hour in the HCl medium. Then, GA was continuously released at a slow speed in the following time in the HCl medium. Approximately 85% of GA was released after 120 h in HCl. Fig. 5(B) showed the release behavior of GA in the pH 5.33 buffer solution. It was also found that about 30% of GA was released in the first hour, and then continuously released in the following time. The fast release of GA in the first hour in the two studied media was due to the release of absorbed GA on the surface of SiO_2 nanoparticles, while the sustained release

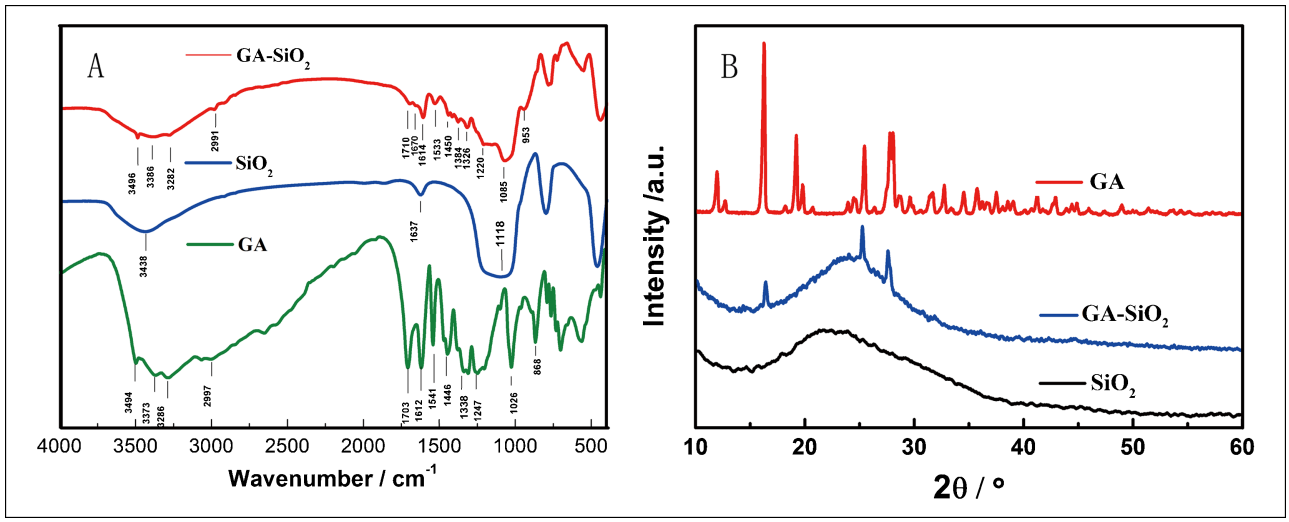

Fig. 2: FTIR spectra (A) and XRD patterns (B) of GA, SiO_2, and GA-SiO_2

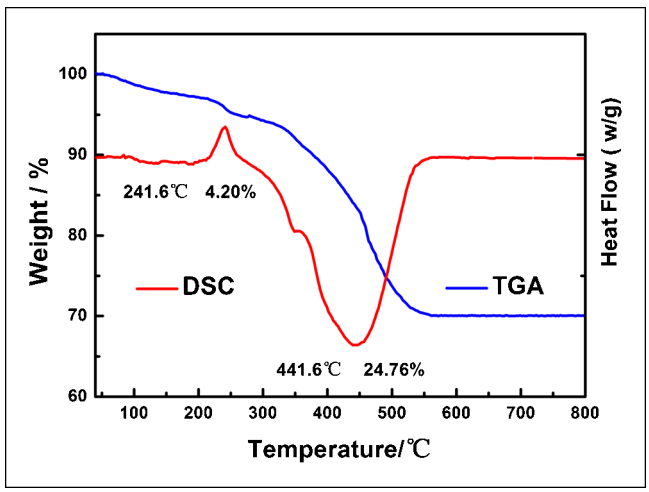

Fig. 3: TGA/DSC curves of GA-SiO_2 nanoparticles

of GA in the following time was due to the hydrolysis of ester groups of GA-SiO_2.

The results showed that GA was released at a slower speed in the medium of pH 5.33 buffer solution than in the HCl medium (pH 1.2). The total amount of GA released in pH 5.33 buffer solution was approximately 70%, which was less than that in the HCl medium. GA was more readily released from GA-SiO_2 when subjected to the HCl medium (pH 1.2) which simulated stomach conditions than in artificial urine. That was because the H^+ ions under acidic conditions promoted the hydrolysis of the ester groups of GA-SiO_2, leading to a faster release of GA in the HCl medium. The sustained release could improve the lifetime of GA in the body system. The drug efficacy could be significantly improved, and the trouble of multiple dosing could be reduced.

Gelatin-based nanoparticles have been proven to be relatively safe and effective nonviral gene delivery vehicles with a prolonged *in vivo* circulation time and high accumulation at the tumor site (Kaul and Amiji 2005). This indicated that the *in vivo* circulation time of the GA would be prolonged, so that the GA would be released and act as anti-cancer agent before metabolism and excretion.

2.5. *Antioxidant activity of GA*

The antioxidant activity of GA released from GA-SiO_2 is shown in Fig. 6. The antioxidant activity of GA increased with enhancing the immersion time whether in the HCl medium or the pH 5.33 buffer solution. In the same immersion time, the antioxidant activity of GA released into the HCl medium was a little greater than that into pH 5.33 buffer solution. Apparently, the antioxidant activity of the released GA was in excellent agreement with the release behavior of GA in the release media, since the antioxidant activity of GA was determined by the concentration. The results indicated that the antioxidant activity of GA gradually increased during the immersion time. GA could serve as an excellent antioxidant for a long release period, and the problem of autoxidation of GA in aqueous solution was significantly reduced.

3. Experimental

3.1. *Materials*

Gallic acid (GA) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Tetraethyl orthosilicate (TEOS) was purchased from Kelong Chemical Reagent Co., Ltd (Chengdu, China). 1,1-Diphenyl-2-picrylhydrazyl free radical (DPPH) was obtained from Tokyo Chemical Industry (TCI, Shanghai, China). All other chemicals were of analytical reagent grade and were used as received without further purification. Deionized water was prepared by ion exchange.

3.2. *The preparation of SiO_2 and GA-SiO_2 nanoparticles*

SiO_2 nanoparticles were prepared by the Stober method. In brief, H_2O, HCl and TEOS were mixed in a molar ratio of 4: 0.1: 1 and stirred by magnetic stirrers at 100 rpm for 4 h at room temperature, and then kept in room temperature for 12 h. The resulting product was freeze dried for 12 h. GA-SiO_2 nanoparticles were prepared by a modified Stober method. H_2O, HCl, TEOS and GA were mixed in a molar ratio of 8: 0.2: 2: 0.03 and stirred by magnetic stirrers at 100 rpm for 4 h at room temperature, and then kept at room temperature for 12 h. The resulting product was flushed by ethanol for three times and freeze dried for 12 h. The theoretically calculated drug uptake value was 27.72%. The drug loading efficiency was calculated as follows:

$$\text{Drug Loading Efficiency} = \text{Actual Drug Uptake}/\text{Theoretical Drug Uptake} \quad (1)$$

3.3. *Characterization of GA-SiO_2*

The FT-IR measurements were measured by a FT-IR system (VERTEX 70, Bruker) using a 32 mm round cell window with KBr as background spectrum. The data were obtained within the ranges of 4000 cm^{-1} to 400 cm^{-1} with a resolution of 1 cm^{-1}. The XRD measurements were made with a X-ray diffractometer (X-Pert PRO, PANalytical B.V.) equipped with a CuKα target. The X-rays were generated at 4 mA and 40 kV. Data were obtained from 10° to 60° at a step size of 0.02° and a scanning speed of 10°/min radiation. TGA/DSC analysis of the prepared samples was carried out with

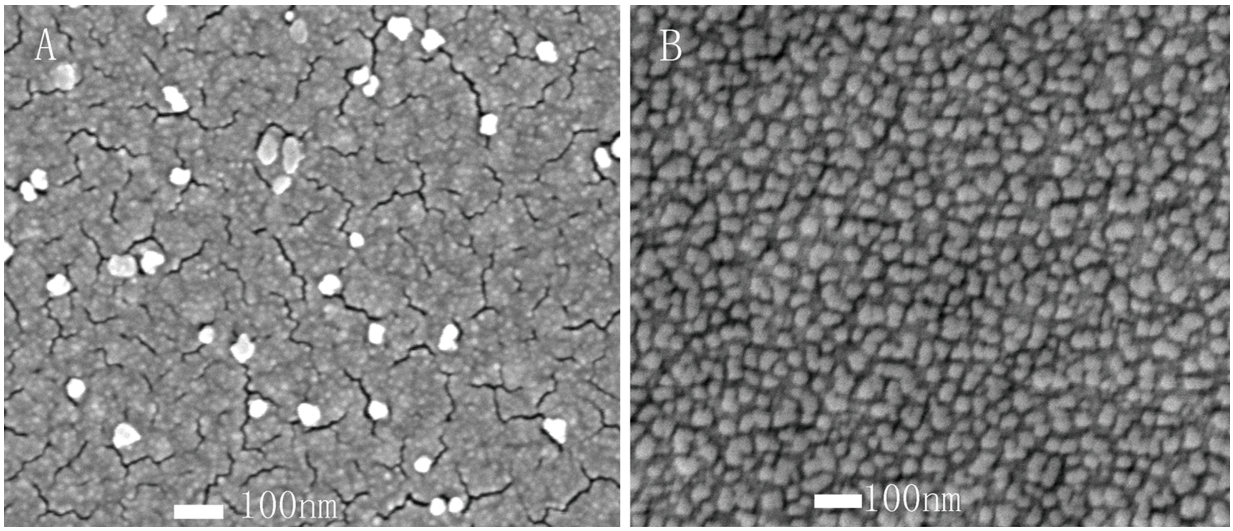

Fig. 4: Representative SEM micrographs of SiO_2 and GA-SiO_2 nanoparticles

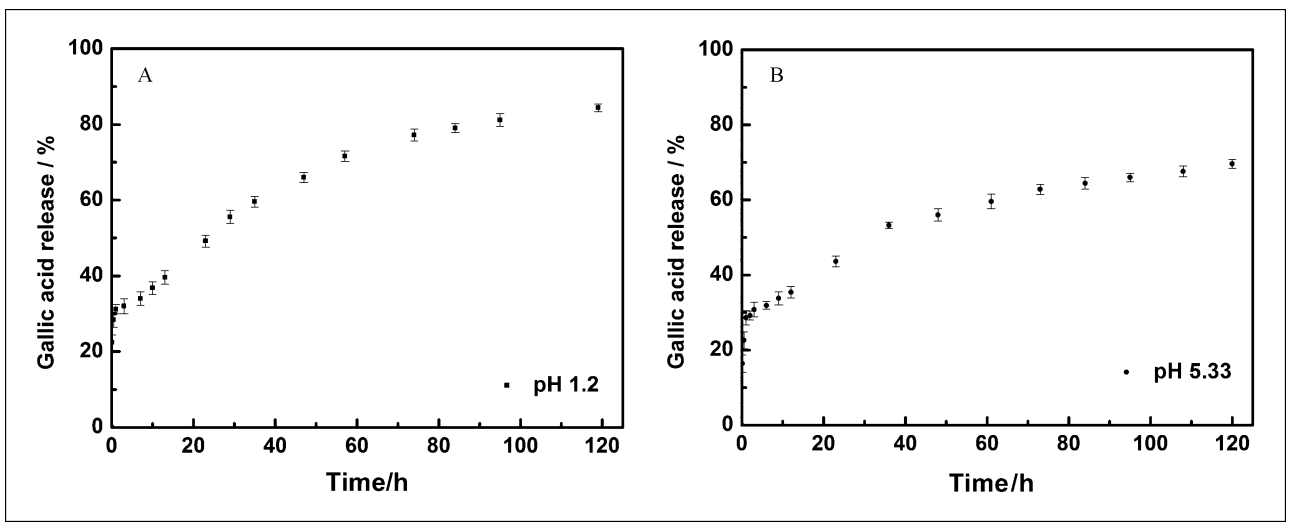

Fig. 5: Cumulative release of GA from GA-SiO_2 into the HCl medium (A) and the pH 5.33 buffer solution (B)

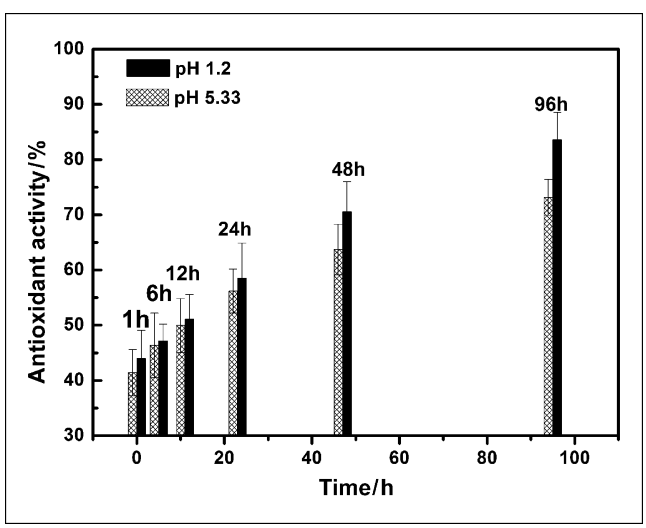

Fig. 6: The antioxidant activity of the released GA

a TG-DSC instrument (STA 449 F3 Jupiter, NETZSCH) using a heating rate of 10°/min under N_2/O_2 atmosphere. The morphologies and particle size of the prepared samples were characterized using SEM (Sirion 200, Holland FEI). The samples were gold-plated prior to imaging.

3.4. In vitro controlled release of GA

The *in vitro* release characteristics of GA from the GA-SiO_2 nanoparticles were investigated by the total immersion method in release medium. HCl solution (pH 1.2) and phosphoric acid-acetic acid-boric acid buffer solution (pH 5.33) were chosen to simulate gastric juice and urine, respectively. To prepare phosphoric acid-acetic acid-boric acid buffer solution, 4.95 g of boric acid were dissolved in 200 ml of distilled water, and then 9.22 g of phosphoric acid and 4.8 g of acetic acid were added to the solution. Then distilled water was added to the solution to fill the volume (2,000 ml). Finally, 37.5 ml of NaOH solution (0.2 M) was mixed with 100 ml of phosphoric acid-acetic acid-boric acid solution, the pH of the resulting solution was 5.33. A given amount of GA-SiO_2 nanoparticles were suspended in 100 ml of release medium and incubated at 37 °C. Aliquots were taken at desired time intervals. The suspensions were filtered using filter papers and the residues were returned to the suspension medium. The clear filtrates of GA-SiO_2 nanoparticle samples were analyzed by UV spectrophotometry (UV 2550, Shimadzu) at λ=259 nm for the determination of drug content. The measurements were carried out in triplicate.

3.5. Antioxidant activity of GA

The antioxidant activity of GA that had been released from GA-SiO_2 nanoparticles into the media was tested by a DPPH radical scavenging assay (Pasanphan and Chirachanchai 2008). In brief, a given amount of GA-SiO_2 nanoparticles were suspended in 100 ml of medium and incubated at 37 °C, and 1 ml of release medium was taken at a desired time intervals. The drawn samples were filtered using filter papers and the residues were returned to the suspension medium. The clear filtrates were mixed with 2 ml of DPPH ethanol solution (200 μM) and incubated for 30 min at room temperature in darkness. The absorbance value of the final solution was recorded at λ_{max} = 517 nm using UV spectrophotometer (UV 2550, Shimadzu). The relative percent of DPPH scavenging capacity was calculated according to the following equation:

$$\text{Scavenging capacity } \% = \frac{\lambda_0 - \lambda_t}{\lambda_0} \times 100 \quad (2)$$

where λ_0 and λ_t represent the absorbance value of DPPH solution with and without GA respectively. The tests were carried out in triplicate.

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