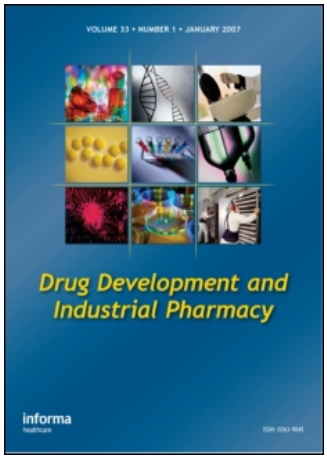

Drug Development and Industrial Pharmacy
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597245

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Online Publication Date: 01 January 2008
To cite this Article: Zhu, Xian, Zhang, Can, Wu, Xiaoli, Tang, Xiaoyan and Ping, Qineng (2008) 'Preparation, Physical Properties, and Stability of Gambogic Acid-Loaded Micelles Based on Chitosan Derivatives', Drug Development and Industrial Pharmacy, 34:1, 2 - 9
To link to this article: DOI: 10.1080/03639040701378001
URL: http://dx.doi.org/10.1080/03639040701378001

Drug Development and Industrial Pharmacy, 34:2–9, 2008

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040701378001

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Preparation, Physical Properties, and Stability of Gambogic Acid-Loaded Micelles Based on Chitosan Derivatives

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The objective of this study is to find out an optimized formulation of water insoluble anticancer drug gambogic acid (GA)-loaded chitosan derivatives micelles. After preliminary test, four factors (the amount of *N*-octyl-O-sulfate chitosan (AOSC), the amount of GA, volume of ethanol, and dialysis temperature) and three levels for each factor that might affect the formation of micelles were selected and arranged in L_9 (3^4) orthogonal experimental design to optimize the formulation of GA-loaded micelles. To compare each of the micellar formulations quantitatively, an overall desirability function was defined and calculated based on three assessment indices (drug content, loading efficiency, and entrapping efficiency of micelles). The optimized formulation was 8 mg of GA dissolved in 0.3 mL of ethanol, 12 mg of AOSC dissolved in 2 mL of H_2O, respectively. The drug solution and blank micellar solution were mixed, followed by dialysis against water at 25°C. The mean size of micelle was 100 nm approximately. Lyophilized samples could keep stable for at least 2 months when it was stored at 4°C. These data suggest that the amphiphilic chitosan derivative may improve the water solubility of GA and thus be used as its nano-carrier.

Keywords gambogic acid; chitosan micelles; orthogonal experimental design; preparation

INTRODUCTION

Gamboge, the resin from various Garcinia species, widely used as a coloring material due to its unique orange-yellow color (Asano, 1996; Lin 1993), is found to have a promising clinical anti-tumor activity. It has been developed as injections for clinical usage recently (Han, 2005). The studies from laboratories show that gambogic acid (GA, Figure 1), the major active component of gamboge, has potent cytotoxicity and inhibits proliferation of human lung carcinoma SPC-A1 cells in vivo and in vitro (Wu, 2004). However, the poor solubility of GA in water (< 1 μg/mL) requires the existence of solubilizing agents in the formulations of gambogic acid. In some patents of gambogic acid injection, L-arginine is added to form the complex (Dai, 2003; Jin, 2002; You, 2003), or polyoxylated castor oil (Cremophor EL) was used as solubilization agent. Although the largely improved solubility of GA was obtained, large amount of L-arginine has to be added to keep the solubility equilibrium, on the other hand, series of side-effects associated with Cremophor EL such as hypersensitivity reaction, nephrotoxicity, neurotoxicity, and cardiotoxicity will probably to occur (Feng, 2002; Lundberg, 2003; Mu, 2003). More researches of effective and safety materials are spurred for the solubilization of GA.

They offer attractive characteristics such as a generally small size (< 100 nm), a propensity to evade scavenging by the mononuclear phagocyte system (MPS) (Kwon, 1996). During the past decade, polymeric drug carriers including polymer-drug conjugates and polymeric micelles have proven to be useful in drug delivery, and several a simple preparation (Kataoka, 2001), controlled drug release (Nishiyama, 2005b; Nishiyama & Kataoka, 2006), as well as higher thermodynamic and kinetic stability (Aliabadi, 2006). These make them attractive carriers for those insoluble anticancer agents. Formulations have been studied in clinical trials (Duncan, 2003). Especially, polymeric micelles are currently recognized as one of the most promising modalities of drug carriers in cancer therapy (Allen, 1999; Kataoka, 1993, 2001; Lavasanifar, 2002; Nishiyama, 2005b; Nishiyama & Kataoka, 2006; Torchilin, 2002). Formed through the self-assembly of amphiphilic block copolymers, polymeric micelles possess a core-shell structure, in which the hydrophobic core acts as a microreservoir for the encapsulation of hydrophobic drugs like doxorubicin, cisplatin, and paclitaxol (Torchilin, 2001), and the outer surface modifies its physicochemical and biological properties.

A number of natural or synthetic polymers can be employed as the materials of polymeric micelles. Among them, chitosan, a polysaccharide copolymer consisting of glucosamine and *N*-acetylglucosamine (Fini, 2003), is an outstanding one. It is endowed with biochemical activity, excellent biocompatibility, and complete biodegradability, in combination with low toxicity (Muzzarelli, 2005), and therefore has wide applications in pharmaceutical fields. Being insoluble at alkaline or neutral

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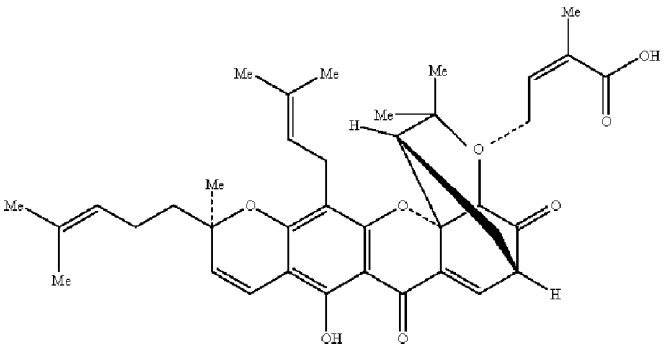

FIGURE 1. The chemical structure of gambogic acid.

pH, chitosan was subject to chemical modification on its amino groups and hydroxy groups, and *N*-octyl-*O*-sulfate chitosan (AOSC, Figure 2) was successfully developed in our laboratory (Zhang, 2004). It easily dissolves in water and core-shell micelles form self-assembly. We reported that the AOSC micellar solution had a high solubilization capacity for paclitaxel, raising its solubility by 1000 times (Zhang, 2003, 2004), and our studies showed the low toxicity, low sensitivity, and comparative anticancer activity with the commercial paclitaxel injection (to be published data).

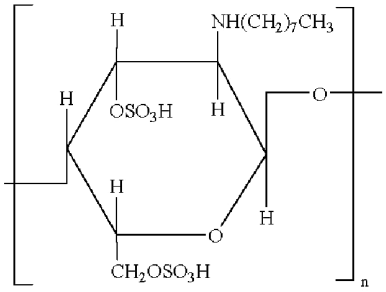

FIGURE 2. The chemical structure of *N*-octyl-*O*-sulfate chitosan (AOSC).

The occurrence of AOSC makes it possible to prepare an effective and safe GA injection. Present research investigated the potential of AOSC entrapping GA in the polymeric micelles and enhancing its solubility in aqueous media. The method of drug loading in the micellar system based on dialysis was studied. An orthogonal experimental design was utilized after preliminary factors screening to get an optimized formulation of GA-loaded AOSC micelles. The physical characteristics and the storage stability of GA loaded micelles after freeze-drying were determined by using photon correlation spectroscopy (PCS) and HPLC assay. This study may lead to a novel delivery systems for GA, and improve the efficacy and safety of its utilization in chemotherapy.

MATERIALS AND METHODS

Materials

Chitosan was provided by the Nantong Suanglin Biochemical Co. Ltd (China) with deacetylation degrees of 97% and viscosity average molecular weight of 65,000 D. GA was isolated from the resin by our group (purity 96.7%). Dialysis tubing cellulose membrane (Molecule Weight Cut-Off MWCO 10,000) was purchased from Sigma-Aldrich Co. (St. Louis, MO); Mannitol was obtained from Shantou chemical factory (Guangdong province, China). Methanol was of spectral grade and purchased from Hanbang Sci & Tech Co. Ltd (Jiangsu, China). All other reagents were analytical grade and used without further purification.

Methods

Synthesis of AOSC

AOSC was prepared following a procedure reported by our group (Zhang, 2003). Briefly, Chitosan (1.0 g) was first suspended in 50 mL methanol with stirring, and then octaldehyde (1.02 g) was added. After reaction of 24 h, KBH_4 (0.5 g) dissolved in 5 mL water was slowly added. After a further 24 h

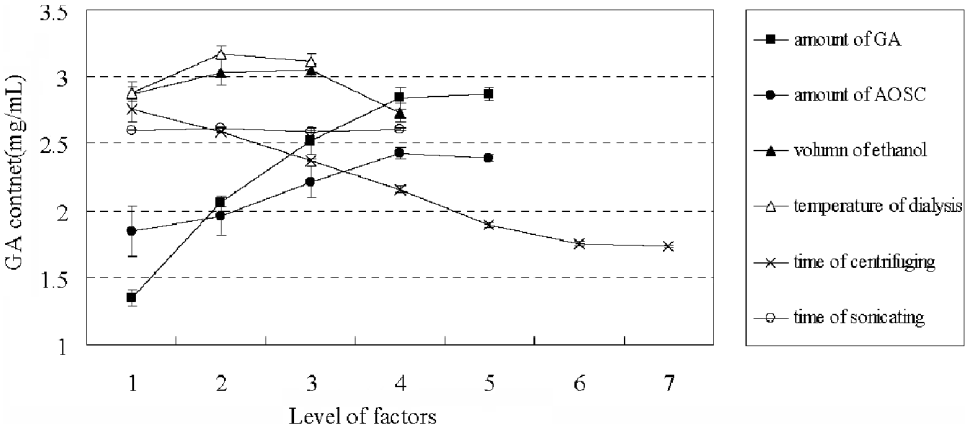

FIGURE 3. GA content of each level in factor screening.

continuous stirring, the reaction solution was neutralized with HCl and the product was precipitated with methanol. The precipitate was filtered and repeatedly washed with methanol and water. This *N*-octyl chitosan was dried under vacuum at 60°C overnight. *N*-octyl chitosan (1.05 g) suspended in *N*, *N*-dimethylformamide (DMF) (40 mL) stirred overnight. Chlorosulfonic acid (20 mL) was added dropwise into DMF (40 mL) at 0°C. After completely dripped, the solution was kept in agitation for 1 h, and then the suspension of *N*-octyl-chitosan and DMF was added to the above solution. The mixture was reacted at 10°C under N_2 atmosphere for 24 h. The reaction solution was neutralized with NaOH until pH 7, and the filtered solution was dialyzed (MWCO 10,000) against distilled water, and then lyophilized and the AOSC powder was obtained.

The chemical structure and substitution degree of the derivative were determined by FT-IR (Nicolet 2000), NMR spectroscopy (Bruker AVACEAV-500). According to the elemental analysis (Element Vario EL III analyzer) data, the substitution degree of octyl and sulfonic group of AOSC were 0.38 and 2.56, respectively.

Preparation of GA Loading Micelles of AOSC

GA loading micelles of AOSC were prepared by a dialysis method as described previously with modification (Zhang, 2003). Briefly, the predetermined amount of GA and AOSC were dissolved in ethanol and deionized water, respectively, according to the preliminary test or orthogonal experimental design. The two kinds of solution were mixed and sonicated (H66025T ultrasonic instrument, China), and then dialyzed in water. Before being measured, the dialyzed solution was centrifuged at 3000 rpm (TGL-16 Centrifuge, China) and filtrated through 0.22 μm pore-sized millipore films. Three samples were prepared at each level of every factor. The protective additive was added into the filtrate and prefreezed at −18°C in refrigerator for 4 h. The freeze dryer (LGJ-10 Freeze Dryer, China) had been running for 2 h before the sample was put into it and lyophilized first at −50°C and 0.01 mbar for 48 h. Lyophilized samples were stored at 4°C for further use.

GA-free polymeric micelles were produced in a similar manner without adding the drug.

HPLC Analysis

The HPLC method was used following the study (Hao, 2005). An acidified mobile phase mobile phase was composed by methanol and deionized water (94:6, v/v). The pH of solution was adjusted to 3.5 by phosphoric acid. The mixture is used at a flow rate of 1 mL/min. The wavelength of detector was 360 nm. Column (Lichrospher C_{18} China) temperature was maintained at 30°C. Injected volume of sample was 20 μL.

A series concentration of standard GA solution, from 10.4 μg/mL to 62.4 μg/mL was injected into HPLC with UV detector (Agilent 1050). Before being injected into HPLC, the micelles solution was diluted fifty times. GA content of each sample was calculated by the calibrate equation.

The standard GA solution with the concentration of 10.4, 31.2, and 62.4 μg/mL, was measured by HPLC and calculated by the calibrate equation to determine the recoveries and precision.

Preliminary Selection of Factors for Orthogonal Design

Many factors (the amount of added drug, the amount of AOSC, the volume of ethanol, dialysis temperature, and time of sonicating and centrifuging, as showing in Table 1) would affect the loading capacity of GA in the micellar system. To simplify the orthogonal experimental design, three assessment indices (the content of GA in the system, loading efficiency, and entrapping efficiency) were selected to evaluate the effect of different factors. For example, when the content of GA as the assessment index was studied at different level of added drug, other factors and their levels were kept invariable. If the GA content was not changed by one of the factors, the factor was regarded to have no effect on this index and kicked out of orthogonal design. The same conclusion could be reached when the drug loading and entrapping efficiency were selected as the assessment indices (data not shown).

GA content of each micellar sample was calculated by the standard equation using HPLC method. The other two indices, loading efficiency and entrapping efficiency, were calculated as follows

$$\text{loading efficiency (\%)} = \frac{\text{weight of entrapped drug (mg)}}{\text{weight of entrapped drug (mg)} + \text{weight of blank micelles (mg)}} \times 100$$

$$\text{entrapping efficiency (\%)} = \frac{\text{weight of entrapped drug (mg)}}{\text{weight of added drug (mg)}} \times 100$$

The average GA content of each level was showed in Figure 3. It changed obviously at different levels of GA, AOSC, ethanol, dialysis temperature, and centrifuging time.

TABLE 1
Factors and Levels in Factor Screening

	Level						
Factor	1	2	3	4	5	6	7
Amount of GA (mg)	4	6	8	10	12	–	–
Amount of AOSC (mg)	6	9	12	15	18	–	–
Volume of ethanol (mL)	0.1	0.2	0.3	0.4	–	–	–
Temperature of dialysis (°C)	0	25	35	–	–	–	–
Time of centrifuging (min)	0	10	20	30	40	50	60
Time of sonicating (min)	0	15	30	60	–	–	–

Orthogonal Experimental Design

After the single factor tests, it was found that the time of sonicating had no effect on the assessment indices of GA-loaded micelles. Furthermore, when the solution was centrifuged for more than 40 min, the indices changed slightly. In order to get better stability in following treatment and reduce the amount of experiments, all sample solution was dialyzed without sonicating and centrifuged at 3000 rpm for 40 min.

So the selected factors were the amount of GA, the amount of AOSC, the volume of ethanol, and dialysis temperature. The levels in which the assessment indices changed greatly were selected (Table 2).

An orthogonal experimental design was introduced to optimize the formation of GA-loaded micelles. Nine experiments were arranged according to L_9 (3^4) (Table 3).

To compare each of the micellar systems quantitatively, a desirability function (d_i) was defined and calculated as follows (Hassan, 1992):

$$d_i = \frac{Y_i - Y_{\min}}{Y_{\max} - Y_{\min}}$$

TABLE 2
Factors and Levels for Orthogonal Experimental Design

	Level		
Factor	1	2	3
A, amount of GA (mg)	8	10	12
B, amount of AOSC (mg)	9	12	15
C, volume of ethanol (mL)	0.1	0.2	0.3
D, temperature (°C)	0	25	35

TABLE 3
Experimental Arrangement According to L_9 (3^4) Orthogonal Experimental Table

	Factor			
Column No.	A	B	C	D
1	1	1	1	1
2	1	2	2	2
3	1	3	3	3
4	2	1	2	3
5	2	2	3	1
6	2	3	1	2
7	3	1	3	2
8	3	2	1	3
9	3	3	2	1

TABLE 4
Maximum ($Y_{\max}$) and Minimum ($Y_{\min}$) Values of the Three Assessment Indices Used for Normalization

Index	$Y_{\min}$	$Y_{\max}$
Drug Content (mg/mL)	1	3
Drug Loading Efficiency (%)	10	50
Drug Entrapping Efficiency (%)	15	100

The d_i value was calculated from the drug content, loading efficiency and entrapping efficiency. The maximum and minimum values ($Y_{\max}$ and $Y_{\min}$, respectively) for each assessment index were given for normalization in Table 4.

Physical Characteristics of GA-Loaded Micelles of AOSC

The size and ζ potential of the prepared GA-loaded micelles were measured by dynamic light scattering (DLS) using a Zetasizer $3000HS_A$ (Malvern Instruments) with 633 nm He-Ne lasers at 25°C. The micellar solutions were filtrated through micropore films (pore size 0.22 μm) before being measured. Both particle sizing and zeta potential measurements were triplicated for a single batch of micelles and the results were the average of the three measurements.

To study the configuration of GA-loaded micelles, the micellar solution was observed by transmission electron microscope JEM-200CX (TEM, JEOL, Japan) at 200 kV. Sample solution was placed on the copper grid covered with film and dried before observation.

Lyophilization of GA-Loaded Micellar System and Its Storage Stability

Different concentrations (1, 3, 5, 8, and 10%, w/w) of mannitol were added as protective additives, the optimized GA-loaded micellar solution from the orthogonal experiments was lyophilized at −50°C and 0.01 mbar after being prefreezed at −18°C in refrigerator for 4 h. Two days later, the temperature of freeze dryer was increased from −50 to 10°C in 1 h. Then the lyophilized powder was taken out and stored at 4°C.

Two months later, the powder was reconstituted by 5% glucose injection and filtrated through 0.22 μm pore-sized millipore films. The content of GA, the size and ζ potential of micelles were measured by HPLC and Zetasizer 3000HS as described above.

RESULTS AND DISCUSSION

Methodology of HPLC Assay

The standard equation about peak area (A) and concentration (C) of gambogic acid was as follows:

$$C = 0.0339 \times A + 2.1622 \ (r = 0.9999, \ n = 5)$$

TABLE 5
Precision for Determination of Gambogic Acid

	Intra-Day		Inter-Day	
Concentration (μg/mL)	Found (μg/mL)	RSD (%)	Found (μg/mL)	RSD (%)
10.4	10.7 ± 0.36	3.36	10.6 ± 0.56	5.28
31.2	30.1 ± 1.36	4.52	30.6 ± 1.11	3.63
62.4	61.7 ± 1.25	2.03	61.5 ± 1.45	3.36

The recoveries of standard equation at three concentrations were 95–103%. The intra-day and inter-day precision was showed in Table 5. Linear range and limitation of the standard equation was from 10.4 to 62.4 μg/mL and 10.4 μg/mL, respectively.

Orthogonal Design

The overall desirability function (DF), that is the geometric definition of the desirability function of three indices (the content of GA, loading efficiency, and entrapping efficiency), was calculated according to the following equation for each formulation.

$$\mathrm{DF} = \left[\prod_{i=1}^{3} d_i\right]^{1/3}$$

Based on the data obtained from each formulation (Table 6), the average value and range of DF under each factor were calculated (Table 7). According to DF, the most important factor, which affected three indices, was volume of ethanol (C). The affecting extent of other factors was gradually decreased in the order of dialysis temperature (D), the amount of AOSC (B), and the amount of GA (A), but there were no significant derivation differences among four factors ($F < F_{0.05}$).

TABLE 6
The Correlated Data of GA-Loaded Micelles Prepared on the Basis of Orthogonal Experimental Design

Column No.	Drug Content (mg/mL)	Loading Efficiency (%)	Entrapping Efficiency (%)	DF
1	1.621	27.44	42.54	0.618
2	2.364	30.23	65.83	0.838
3	2.175	25.01	61.75	0.749
4	1.652	28.77	36.35	0.600
5	2.298	30.40	52.84	0.773
6	2.203	23.46	45.81	0.666
7	2.174	35.46	42.02	0.740
8	1.988	25.65	34.79	0.605
9	2.392	26.10	43.48	0.697

TABLE 7
Straight Analysis of the Overall Desirability Function Values

Factor	A	B	C	D	DF
1	1	1	1	1	0.618
2	1	2	2	2	0.838
3	1	3	3	3	0.749
4	2	1	2	3	0.600
5	2	2	3	1	0.773
6	2	3	1	2	0.666
7	3	1	3	2	0.740
8	3	2	1	3	0.605
9	3	3	2	1	0.697
Average 1	0.745	0.663	0.640	0.706	–
Average 2	0.680	0.739	0.712	0.748	–
Average 3	0.681	0.704	0.754	0.651	–
Range	0.065	0.076	0.114	0.091	–
SS	0.006	0.011	0.024	0.014	–
F	1	1.833	4	2.333	–

$F_{0.05}(2,2) = 19$, $F_{0.01}(2,2) = 99$.

Based on the dialysis method, AOSC and the GA were dissolved in water and a water-miscible organic solvent (such as ethanol), respectively. AOSC forms the blank micelles in water automatically while dissolved. When the two solutions were mixed together, the blank micelles disassociated due to the change of AOSC solubility in the mixed solution. With the solution dialyzed against water, the organic solvent moved out of the dialysis tubing cellulose membrane and gradually was replaced by water, which triggered the self-association of AOSC and the entrapment of GA molecules in the assembled micelle structures. The semi-permeable membrane kept the micelles inside the dialysis bag, but allowed the removal of unloaded free drug as well as the organic solvent.

As is shown above, the volume of ethanol has the greatest influence on the assessment indices. The increased amount of ethanol leads to the significant decreases of the GA content, loading efficiency, and entrapping efficiency. The reason may be related to the amount of GA dissolved in ethanol. More ethanol is added, more GA is dissolved, and consequently more drug molecules remove from the semi-permeable membrane of dialysis tube. In addition, the existence of more ethanol inhibits the self-assembling of micelles and induces the breakage of the micelles, which results in the leakage of GA from the core of the AOSC micelles. In the formation process of the micellar system, a low-toxicity solvent, ethanol was used to dissolve the drug. The sonicating process was omitted and the process of dialysis could be undertaken at room temperature.

It is suggested that the temperature during dialysis process has a close relation with the penetrating rate of ethanol through the semi-permeable membrane, and therefore determination of the amount of ethanol left in the micellar solution, thus affects the overall desirability function indirectly. In this paper, it was found that the middle temperature, 25°C, was the best dialysis temperature.

The amount of AOSC and GA affected three indices at the same time. It could be seen in factor screening that the loading efficiency increased with the added GA increasing when the concentration of AOSC was 6 mg/mL. The content of GA was not found to change obviously when more than 10 mg drug was added. This indicated that the loading capacity of micelles (6 mg/mL) had been closed to its maximum. So the entrapping efficiency decreased. Similarly, loading capacity of micelles enhanced when the concentration of AOSC increased. According to the equation, loading efficiency decreased when the added drug invariable. However, in the orthogonal design, the amount of GA and AOSC was changing at the same time. So the changing tendency of loading and entrapped efficiency depended on the cooperated effect by them.

Finally, the optimum combination of factors and levels for the preparation of GA-loaded micelles of AOSC was established as $A_1B_2C_3D_2$. Therefore, the procedure was recommended to be as follows: 12 mg of AOSC and 8 mg of GA were dissolved in 2 mL of water and 0.3 mL of ethanol, respectively, and the mixed solution was subject to dialysis in 2 L of distilled water at 25°C. The water was replaced once after 8 h and the whole time of dialysis procedure was 12 h.

In preparation of GA-loaded micelles, many indices should be taken into account, such as GA content, loading efficiency, and entrapping efficiency, some of which will usually influence each other. In preliminary single-index test employed to evaluate the results of technical optimization and the indices studied separately, it is hard to find out an optimized formulation. In this paper, through orthogonal design and multi-index test, three indices (the content of GA, loading efficiency, and entrapping efficiency), which represented the quality of GA-loaded micellar system, were studied at the same time. An overall desirability function examining all the indices was used to assess the formation of micelles preparation.

To test the reproducibility of the optimal formulation, three samples were prepared following the procedure mentioned above. The average content of GA, loading efficiency, and entrapping efficiency were 2.23 ± 0.08 mg/mL, 29.8 ± 0.2%, and 63.8 ± 0.5% (mean ± *SD*, $n = 3$), respectively. No significant differences ($p < 0.01$) were observed in the three assessment indices of three samples, which indicated a good reproducibility of the micellar system by using the optimized process and formulation. Moreover, in the formation process of the micellar system, a low-toxicity solvent, ethanol was used to dissolve the drug instead of chloroform (Sezgin, 2006). The sonicating process was omitted and the process of dialysis could be undertaken at room temperature.

Compared with other GA preparations (GA content 1.25 mg/mL) (Dai, 2003), the micelles formed by AOSC provided higher drug content up to 2.23 mg/mL. As GA has high cytotoxicity, it is important to prevent its burst effect from micelles in blood. In our formulation, micelles solution had been centrifuged for 40 min, which made sure the complete removal of the free GA sticking to the surface of micelles, and the burst effect might therefore be avoided. All of these promise a bright future of GA-loaded micelles in pharmaceutical industry.

To get the same drug content (2 mg/mL, approximately), the solubilizing agents were added as the concentration of 11.1 mg/mL (L-arginine) in other gambogic injection (You, 2003), while the concentration of AOSC was about 5.22 mg/mL in our preparation. The reduced amount of solubilizing agents could prevent the potential side-effect in clinical use.

Physical Properties of the GA-Loaded Micelles

It is reported that nanoparticles with an average particle diameter range of 10–200 nm may reduce the reticuloendothelial system (RES) uptake in the body circulation system (Kataoka, 1993). It is believed that the mean size of micelles may affect both the passive targeting and delivery of the entrapped drug (Moghimi, 2001). Controlling particle size is one of most important approaches for developing drug carrier system. It was observed in the present study that the size and ζ potential of the GA-loaded micelles were 108.2 ± 0.8 nm and –34.1 ± 0.9 mv (mean ± *SD*, $n = 3$), respectively. The negative ζ potential revealed that the negative charged hydrophilic sulfonic group was located on the surface of micelles. The configuration of micelles was studied using TEM and it could be seen that the GA-loaded micelle of AOSC was a smooth spherical particle with a diameter of about 100 nm (Figure 4). This result of TEM was consistent with the data obtained by Zetasizer $3000HS_A$. The average size of GA-loaded micelles (100 nm approximately) makes it easy to pass the process of sterilizing

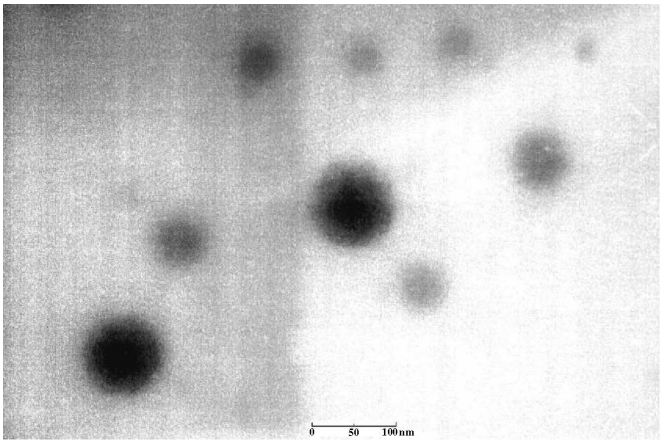

FIGURE 4. Gambogic acid loaded Micelle in water (×59,000) visualized by TEM.

and keep the integrity of micelles. This is helpful to the commercial production of an injection. The value of ζ potential has close relationship with the stability of particles. It also affected by the distribution and pharmacokinetics of particles in vivo (Su, 2004). If the charge on the particles is high, the particles repel one another and the colloid is stable. If the charge is near zero, the Brownian movement of the particles causes them to collide and to become attached to one another. It is desirable to maximize the particle charge in order to achieve greatest stability. A ζ potential of more than 61 mV usually indicates excellent stability, 41–60 mV good stability, 31–40 mV moderate stability, and 10–30 mV incipient instability (Plessis, 1996). The absolute potential value of GA-loaded micelles was above 30 mv, it means the micelles can be more stable in solution.

Lyophilized Micellar System and its Storage Stability

The lyophilized samples must have a good reconstitution (Zimmermann, 2000) and qualified appearance. For the unqualified appearance, the first two concentration of mannitol (1 and 3%) were not considered. According to the leakage rate of micelles, size and ζ potential of the lyophilized samples (Table 8), the concentration of mannitol of 5% was chosen. After 2 months' storage, no precipitation of GA was noted, and the GA concentration reduced from 1.95 ± 0.12 to 1.85 ± 0.01 mg/mL, with a leakage rate of 4.88 ± 0.33%. The size of micelles slightly increased from 108.2 ± 0.8 nm to 140.2 ± 0.9nm, which might be due to the aggregation of micelles. This indicated that the lyophilized drug-loaded micelles were stable during storage at 4°C for 2 months.

CONCLUSIONS

The chitosan derivative, AOSC may serve as potential material for encapsulation of hydrophobic anticancer drug GA by physical entrapment. Used an orthogonal experimental design, the optimum procedure of GA-loaded micelles were determined. The average content of GA was 2.23 ± 0.08 mg/mL. The size and ζ potential of micelles was 108.2 ± 0.8 nm and –34.1 ± 0.9 mv (mean ± *SD*, $n = 3$), respectively. Compared with paclitaxel-loaded AOSC micelles (250 nm) (Zhang, 2004), the gambogic acid-loaded AOSC micelles had a smaller size (110 nm). This indicated that the size of micellar system may have a close relationship with the kinds of drugs. The lyophilized powder could be stored at 4°C for 2 months without the obvious change of GA content. These data suggested that AOSC micelle may be useful as a carrier for GA. Additional research is required to further investigate the stability of GA loaded micelles in blood, in vitro release, in vivo pharmacokinetics and tissue distribution.

TABLE 8
Influence of Mannitol Content and Time of Preservation

Concentration of Mannitol (w/v)	Leakage Rate (%)	Size (nm)	ζ Potential (mV)
5%	3.70 ± 0.05	92.0 ± 3.3	–21.3 ± 0.3
8%	5.69 ± 1.08	86.8 ± 4.5	–22.9 ± 0.7
10%	5.44 ± 3.90	105.9 ± 5.4	–23.6 ± 1.1
5% (1 month later)	4.60 ± 1.30	140.6 ± 0.7	–25.7 ± 0.6
5% (2 months later)	4.88 ± 0.33	140.2 ± 0.9	–23.8 ± 0.6

ACKNOWLEDGMENTS

The authors would like to thank the Chinese Department of Science & Technology for International Science & Technology research cooperation (2005DFA30350) and Natural Science Foundation of Jiangsu, China (BK2006154).

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