Nanoencapsulation of *Centella asiatica* bioactive extract

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Introduction

Centella asiatica is a small herbaceous creeping plant found widespread in Thailand. It has been used for centuries as a medicinal herb in Ayurvedic medicine. It possesses anxiolytic activity (Bradwejn et al., 2000). It increases pentobarbitone-induced sleeping time and decreases immobility in the forced swim test (Sakina et al., 1990). It also elicits anti-anxiety effects in the elevated plus maze (Lucia et al., 1997). Its aqueous extract was reported to have cognitive-enhancing as well as antioxidant effects in rats (Kumar et al., 2002). Moreover, it was reported for the use in treatment of leprosy, wounds, cancer, fever, and syphilis. It is also used for the treatment of acnes and allergy (Van Wky et al., 1997). The most prominent group of biologically active compounds isolated from *C. asiatica* is the terpenes, e.g. asiaticoside, madecassic acid, madecassoside, and asiatic acid. Asiaticoside is the most abundant triterpene glycoside, which is effective in wound (Shukla et al., 1999). Several derivatives of asiaticoside (Inhee et al., 1999) and asiatic acid (Sang-sup et al., 2000) were found to show protective effect against beta amyloid-induced neurotoxicity associated with the dementia of Alzheimer's disease. The antitumor and cytotoxic properties of the crude extract and partially purified fractions of *C. asiatica* were reported by Babu et al. (1995). According to the authors, the partially purified extract was more effective on tumor cells than the crude extracts. Dermatologically, extract of *C. asiatica* has been used in scar management and in cosmetic formulation (Martelli et al., 2000). Even *C. asiatica* extract (CAE) possesses high potential biological activities; its clinical usage is limited to some extent due to its poor physical stability. CAE shows high hygroscopicity. The powder extract is promptly liquefied within a few minutes when exposed to normal environment. Therefore, the development of nanoparticles which the extract is entrapped inside could lead to significant advantage as the extract is protected from external moisture. The present study was focused on the development of chitosan-alginate nanoparticle of CAE. Chitosan and alginate were selected for production of nanoparticle base in this study because they are biodegradable, biocompatible, and low cost. We investigated certain basic properties of CAE in the preformulation study of the extract and the effect of various parameters in nanoparticle preparation process on the nanoparticles obtained.

Experimental

Materials

Standard asiaticoside, sodium alginate and chitosan (75% degree of deacetylation, MW 400,000) were of Sigma (Sigma-Aldrich, USA). *C. asiatica* extracts (CAE) was purchased from Changhai Top Genius Industrial Co.Ltd (CTG, China). Organic solvents were of analytical grade. Milli Q water was used as a solvent in aqueous system. Other ingredients were of the highest grade available.

Preparation of CAE nanoparticles

Nanaparticles of CAE were prepared by using ionic gelation principle. Calcium chloride solution was slowly drop-wised into the CAE containing sodium alginate solution with constant stirring speed of 700 rpm for 30 min. Solution of chitosan in 1% w/v acetic acid was then dropped into the previous mixture. The mixture was continued stirred at the same speed for 30 min. The obtained colloidal particle was centrifuged at 15,000 rpm for 30 min. The nanoparticles were collected and washed twice with Milli Q water then lyophilized. The powder was kept in desiccators at 4 degree C until used.

Characterization of CAE nanoparticles

The outer appearance and morphology of the obtained particles was observed by naked eyes and by using transmission electron microscopy (TEM) respectively. Particle size was measured. The mean size and size distribution of the CAE nanoparticles were measured by dynamic light scattering of PCS using Zetasizer 3000HS (Marvern Instrument, UK). The analysis was performed at a scattering angle of 173 at a temperature of $25^{o}C$ using samples diluted with Milli Q water. Each sample was repeatedly measured 3 times and the values reported are the mean diameter for three replicates.

Stability Study of CAE Nanoparticles

The physical stability of CAE entrapped in the nanopartcles was investigated in comparison with those non-entrapped. The samples were placed in closed containers and incubated separately at two different temperatures of 4°C and 30 °C for 6 weeks. The change of physical appearance, e.g. state of matter and color of all samples was observed.

Results and Discussion

The outer appearance of lyophilized CAE nanoparticles observed by naked eyes was pale yellow spongy mass whereas the color of nanoparticles without CAE was white as shown in Fig. 1. The morphology of nanoparticles obtained from TEM was shown in Fig. 2. It was noticed that the morphology of both nanoparticles with and without CAE was clearly observed as a spherical shape. The size of CAE entrapped nanoparticle was slightly bigger than the non-entrapped. This result indicated that the entrapped CAE played a major role on size expansion of the nanoparticles.

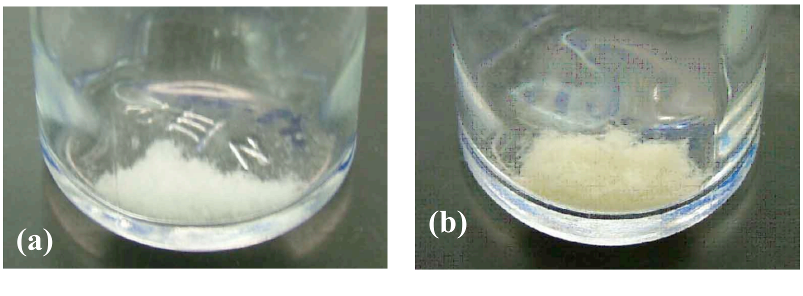

Fig. 1. The physical appearance of nanoparticles without CAE (a) and with CAE (b)

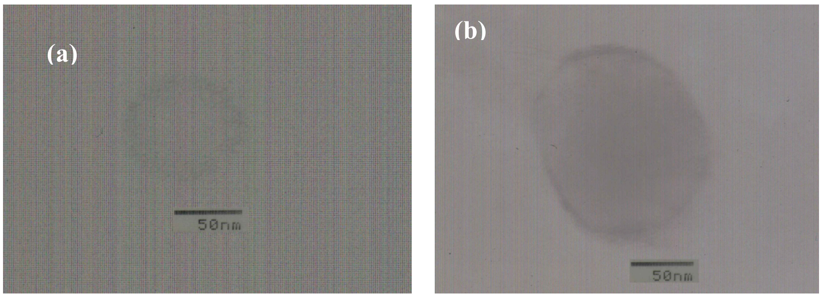

Fig. 2. Morphology by TEM of nanoparticles without CAE (a) and with CAE (b)

Results indicated that preparation parameters affected the size of CAE nanoparticles. The pH of the final mixture was one of the major effects. It was found that pH 4.72 was the most suitable among four studied pH as shown in Table 1. The alginate-CAE ratio also influenced the size of CAE nanoparticles obtained as shown in Table 2. It was found that the ratio of chitosan to alginate did strongly affect to the size of CAE particles as seen in Table 3. Hence, to obtain the optimum size of the nanoparticles, the pH of the reactant mixture, the ratio of polymer-extract and polymer-polymer should be adjusted.

Table 1 Effect of pH on CAE particle size

pH	**Size (nm)**
3.52	>5,000
3.65	>5,000
3.90	>5,000
4.72	331.40 ± 3.70

Table 2 Effect of alginate-extract ratio on CAE particle size

Weight Ratio (Alginate:Extract)	**Size (nm)**
1:1	457.20 ± 21.00
1:2	487.20 ± 19.50
1:3	490.50 ± 9.50
1:4	595.60 ± 19.60

Table 3 Effect of polymer ratio on CAE particle size

Weight Ratio (Chitosan:Alginate)	**Size (nm)**
1:1.2	> 5000
1:1.5	> 5000
1:2.0	> 5000
1:3.0	403.10 ± 5.00
1:6.0	331.40 ± 3.70

The outer appearance of CAE intact and CAE nanoparticles was comparatively shown in Fig. 3. It was noticed that after a few minutes exposure to ambient environment, the state of CAE intact was changed to liquid. CAE nanoparticles were still a powdery pale yellow solid state even stored for 6 weeks as shown in Fig. 4. The temperature showed no influence on the stability of CAE nanoparticles. From the physical point of view, nanoparticles can be considered as promising system for stabilizing CAE.

Fig. 4 The appearance of CAE entrapped in nanoparticles (A) and CAE intact (B)

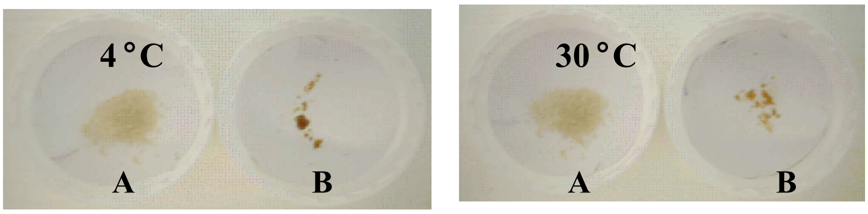

Fig. 5 The appearance of CAE entrapped in nanoparticles (A) and CAE intact (B) kept at 4 °C and 30 °C for 6 weeks

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