Encapsulation of Bioactive Compounds:
Preparation and Evaluation of Gallic Acid Nanoparticles

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1. Introduction

Gallic acid (GA) is the active compound found in various medicinal plants. It is known to have anti-inflammatory, antimutagenic, anticancer, and antioxidant activity (Soong *et al.*, 2006). It has been used as antioxidation additive in both food and pharmaceutical industry (Navita *et al.*, 2007). However, it was rapidly absorbed and eliminated with short mean half-life. The highest GA concentration observed in plasma is not high, limited the plasma level to exert its biological activities (Siranoush *et al.*, 2001). The design of dosage form for enhanced high plasma level could be done by using various kinds of polymer. The mucoadhesive property of the polymers could prolong drug residence time at the site of absorption (Dhawan *et al.*, 2004). Chitosan (CS) is a cationic polymer derived from the chitin of crustaceans. Its various applications have received considerable attention. CS has excellent biodegradable and biocompatible characteristics. Due to its unique polymeric cationic character, gel and filming properties, CS has been extensively examined in the pharmaceutical industry for its potential use in the development of drug delivery systems (Wen-Chuan *et al*, 2006). There are a variety of CS-based colloidal delivery vehicles for pharmaceutical applications which have already been published (Janes *et al*., 2001).

The aim of this work was to prepare and characterize nanoparticles of GA using CS and sodium carboxymethylcellulose (SCMC) as polymer matrix. The effect of polymer ratio on the nanoparticles was also investigated.

2. Materials and methods

2.1 Materials

CS, which molecular weight and degree of deacetylation was 83,000 and 92% respectively, was provided by Seafresh Chitosan (Lab) Co., Ltd. (Bangkok, Thailand). SCMC was supplied by Fluka (Finland). GA was from Sigma-Aldrich (German). Phosphoric acid, acetic acid, and methanol were from Merck (Germany). Hydrochloric acid 37% and sodium hydroxide were supplied by Labscan Analytical Science (Thailand).

2.2 Preparation of empty and GA loaded nanoparticles

The empty nanoparticles were prepared by adding drop-wise of 1%, w/w aqueous SCMC solution to certain amount of 0.1 % w/v CS solution in 1% acetic acid with continuous stirring rate of 700 rpm at room temperature for 1 h. In the case of loaded nanospheres, appropriate amount of GA was firstly dissolved in CS solution. All nanoparticles were purified by centrifugation at 15000 rpm for 40 min. Supernatant was discarded. The nanoparticles were freeze-dried before further use or analysis.

2.3 Characterization

The nanoparticles were characterized by following measurements. Particle size, size distribution and the zeta potential of CS nanoparticles were determined by using Zetasizer Nano-ZS (Malvern

Instruments, England). The analysis was performed at a scattering angle of 173 at 25^{o}C using samples diluted with de-ionized distilled water. The morphological measurement of the nanoparticles was performed by Transmission electron microscope (TEM) (JEOL JEM-2010, Japan)

2.4 Effect of CS concentration on nanoparticles

A 10-ml portion of 0.1, 0.15, 0.2, or 0.25% CS solution was diluted with de-ionized distilled water to 50 ml. The adjusted solutions were added drop-wise with 1ml of 1% aqueous SCMC solution with continuous stirring rate of 700 rpm at room temperature for 1 h. The size and zeta potential of the nanoparticles was determined.

2.5 Effect the mixing ratio of CS and SCMC on nanoparticles preparation

The nanoparticles were prepared in the same manner as mentioned above. The mixing ratio of CS and SCMC used in this experiment was 1:1, 2:1, 3:1, 4:1 and 5:1.

2.6 Determination entrapment efficiency

Each mixture of nanoparticles preparation was centrifuged at 15,000 rpm for 40 min at 4 $^{\circ}$C to separate the free GA in the supernatant from the GA incorporated in the nanoparticles. Concentration of GA in the supernatant was determined by HPLC-UV (Shimadzu, Japan). The mobile phase for GA was 20:80 methanol: 0.1% phosphoric acid at the flow rate of 1 ml/min. The UV detector was set at 280 nm. The entrapment efficiency (EE) was obtained from the difference of GA in the supernatant and the original given concentration.

3 Results and discussion

3.1 Morphological characterization of the chitosan (CS) nanoparticles

Figure 1 displayed the TEM microphotograph of nanoparticles with CS-SCMC ratio 1:1 of empty CS –SCMC nanoparticles and GA loaded nanoparticles. It could be seen that both types have a spherical shape with size around 200 nm.

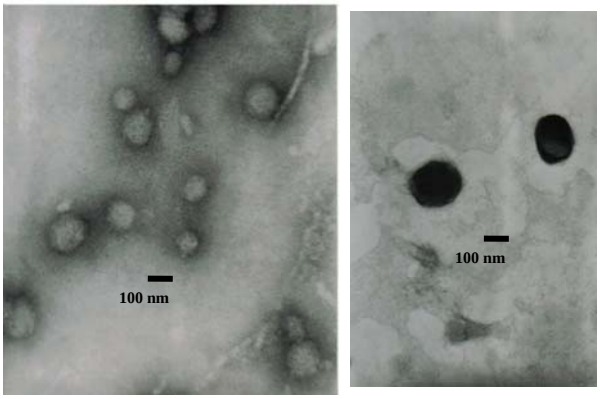
3.2 Effect of CS concentration on nanoparticles

The effects of CS concentration on the size and the size distribution of the nanoparticles were summarized in Table 1. Results revealed that at the lowest concentration of CS and fixed ratio of CS:SCMC as 1:1, the smallest size of nanoparticles with narrow range of size distribution was obtained.

3.3 Effect the mixing ratio of CS and SCMC on nanoparticles

The effect of mixing ratio of CS and SCMC on the size and the size distribution were shown in Table 2. In the study of this effect, the CS concentration was fixed at 0.1%, the ratio was varied 1:1 – 5:1 (CS : SCMC). It was found that the smallest size with narrow the size distribution range was obtained at the ratio of 1:1.

(A)



(B)

Figure 1 TEM of empty (A) and loaded (B) CS-SCMC nanoparticles

Table 1 Effect of CS concentration on nanoparticles

Concentration (%)	Particles size (nm)	Polydispersity index (PI)	Zeta potential (mV)
0.10	176.60 ± 52.06	0.23 ± 0.08	37.08 ± 0.93
0.15	306.27 ±131.85	0.35 ± 0.12	43.12 ± 12.29
0.20	270.93 ± 60.87	0.32 ± 0.01	40.80 ± 3.29
0.25	212.40 ± 67.17	0.30 ± 0.02	37.00 ± 3.02

Table 2 : Effect of ratio of mixing between CS and SCMC on the size and the size distribution of prepared nanoparticles

Ratio (w/w)	Particles size (nm)	Polydispersity index (PI)	Zeta potential (mV)
1:1	176.60 ± 52.06	0.23 ± 0.08	37.08 ± 0.93
2:1	491.60 ± 104.51	0.42 ± 0.01	45.88 ± 8.72
3:1	636.47 ± 65.53	0.49 ± 0.04	53.21 ± 1.37
4:1	1161.20 ± 281.76	0.62 ± 0.09	61.35 ± 2.23
5:1	1564.67 ± 261.14	0.78 ± 0.18	55.79 ± 1.61

3.4 Determination entrapment efficiency

The effect of various ratio of GA on the size, the size distribution and the entrapment efficiency (EE) were summarized in Table 3. The ratio 1:1:0.1 (CS:SCMC:GA) was the suitable systems to prepare nanoparticles with the highest % EE.

Table 3 Effect of GA on nanoparticles

CS:SCMC:GA	Particle size (nm)	Polydispersity index (PI)	% EE	Zeta potential (mV)
1:1:0.1	331.33 ± 6.50	0.294 ± 0.066	95.37	40.00 ± 1.85
1:1:0.2	221.50 ± 9.09	0.288 ± 0.043	92.70	40.30 ± 3.51
1:1:0.3	365.83 ± 15.94	0.298 ± 0.042	91.35	40.33 ±2.61
1:1:0.4	250.00 ±14.54	0.305 ± 0.047	89.98	39.70 ± 0.62
1:1:0.5	205.50 ± 5.57	0.197 ± 0.038	90.46	40.87 ± 3.98

4. Conclusion

It was concluded that GA nanoparticles could be prepared by simple self assembly complexation between the positive charge of CS and the negative charge of SCMC. The nanoparicles obtained were spherical with the size range around 200 - 300 nm. The entrapment efficiency of the prepared nanoparticles was found to be high enough. The results from this study encourage us for continuing further investigation of. release study and stability of GA existed in the nanoparticles and pharmaceutical application.

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6. Biblography

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