Nano – Size Encapsulation of Bioactive Compound of Green Tea

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1. Introduction

The demand for green tea (*Camellia sinensis* Theaceae) has recently increased due to human health concerns and preference. The main active component consisted in green tea is epigallocatechin gallate (EGCG). Various biological activities of EGCG have been reported. In some reports, EGCG is an even better antioxidant than α-tocopherol, butylated hydroxyanisole or butylated hydroxytoluene (Chun *et al*, 2001). Several *in vivo* experiments have shown chemopreventive effects of EGCG against cancer initiation, promotion and progression in animal models of oral, lung, duodenual, prostate, liver and colon cancers (Lam *et al.,* 2004). It has also been found to have anti-inflammatory properties (Clair *et al.*, 2002), antimutagenic, anti-angiogenic, antiproliferative, both *in vitro* and *in vivo*. In addition, EGCG is antibacterial, and it may inhibit human immunodeficiency virus, reduce platelet aggregation, and prevent the development of atherosclerosis (Xiaofeng *et al.*, 2006). Recently, investigators have found that EGCG has anti-HIV effects when bound to CD4 receptor (Samuel *et al.*, 2006).). However, EGCG has at least one limitation. It gives poor bioavailability. It is thought to be due to the poor stability of EGCG. It has been found that the stability of EGCG is pH dependent (Wai *et al.*, 2004).

A considerable number of polymeric microparticulate systems are under investigation to deliver substances to the intestine while protecting them from adverse conditions that affect their bioactivity (Borges *et al*, 2005). The micro/nanoparticulate drug delivery systems offer numerous advantages over the conventional dosage forms. These include improved efficacy, reduced toxicity, increase stability (Coppi *et al.*, 2002). Most of the methods of nanoparticles preparation involve the use of organic solvents (Barreiro-Iglesias, 2005) and a cross-linking agent, such as glutaldehyde. However, the chemical crosslinking agents have possibility of inducing undersirable effects (Sunil *et al.*, 2004). Self assembling nanoparticles can be formed spontaneously under mild conditions. This spontaneous formation is suitable to protect the drugs from inactivation by intense shear stress, heat, and pH (Sosaku *et al.*, 2005).

The aim of this work was to prepare and characterize nanoparticles of EGCG using chitosan (CS) and sodium carboxymethylcellulose (SCMC) as polymer matrix by means of self assembling nanoparticle formation. 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 15,000 and 90% respectively, was provided by Seafresh Chitosan (Lab) Co., Ltd. (Bangkok, Thailand). SCMC was supplied by Fluka (Finland). Green tea extract with 40% EGCG was obtained from Specialty Natural Product Co.,Ltd (Chonburi province, Thailand). EGCG standard was purchased from Sigma (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 extract 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 green tea extract 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 Determination of entrapment efficiency

Each mixture of nanoparticles preparation was centrifuged at 15,000 rpm for 40 min at 4 °C to separate the free EGCG in the supernatant from the EGCG incorporated in the nanoparticles. Concentration of EGCG in the supernatant was determined by HPLC-UV (Shimadzu, Japan). The mobile phase for EGCG was methanol: 0.1% phosphoric acid (20:80) 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 EGCG in the supernatant and the original given concentration.

3 Results and discussion

3.1 Morphological characterization of the nanoparticles

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

3.2 Effect of CS concentration

It was found that the CS concentration affected the size and size distribution of CS-SCMC nanoparticles as summarized in Table 1. Results also revealed that at lowest concentration of CS, the smallest size of nanoparticles with narrow range of size distribution was obtained.

3.3 Effect the mixing ratio of CS and SCMC

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 of 1:1 – 5:1

(CS : SCMC) was studied. It was found that the smallest size with narrow the size distribution range was obtained at the ratio of 1:1.

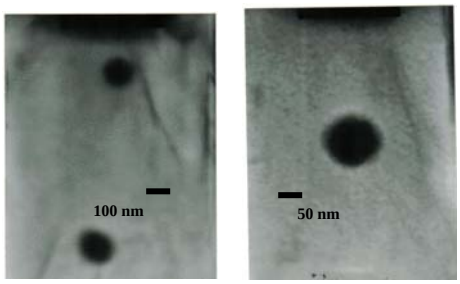

Figure 1 TEM of empty (A) and loaded (B) CS-SCMC nanoparticles

Table 1 Effect of CS concentration on nanoparticle preparation

Concentration (%)	Particles size (nm)	Polydispersity index (PI)	Zeta potential (mV)
0.10	264.00 ± 3.32	0.26 ± 0.02	37.43 ± 3.41
0.15	271.93 ± 4.02	0.24 ± 0.03	28.91 ± 5.14
0.20	193.73 ± 82.70	0.20 ± 0.08	35.11 ± 5.40
0.25	238.13 ± 20.90	0.27 ± 0.03	33.81 ± 3.89

Table 2 : The 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	264.00 ± 3.32	0.26 ± 0.02	37.43 ± 3.41
2:1	355.00 ± 112.33	0.31 ± 0.08	46.59 ± 4.12
3:1	697.00 ± 106.37	0.51 ± 0.04	52.71 ± 4.03
4:1	1204.00 ± 307.66	0.71 ± 0.15	57.93 ± 5.66
5:1	1109.27 ± 114.54	0.67 ± 0.04	52.53 ± 3.85

3.4 Determination entrapment efficiency

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

Table 3 Effect of EGCG extract on nanoparticles

CS:SCMC :EGCG	Particle size (nm)	Polydispersity index (PI)	%EE	Zeta potential (mV)
1:1:0.10	215.13 ± 15.13	0.29 ± 0.04	98.04 ± 0.60	38.27 ± 2.31
1:1:0.25	247.65 ± 60.10	0.30 ± 0.05	70.00 ±16.36	40.68 ± 2.24
1:1:0.50	241.40 ± 51.91	0.30 ± 0.05	11.46 ± 2.31	39.65 ± 5.83
1:1:1	257.60 ± 56.43	0.29 ± 0.05	07.75 ± 0.93	35.11 ± 2.96
1:1:2	234.46 ± 57.71	0.27 ± 0.05	ND	37.49 ± 4.18
1:1:5	270.13 ± 19.87	0.34 ± 0.06	ND	37.37 ± 3.49
1:1:10	286.46 ± 46.60	0.45 ± 0.09	ND	37.80 ± 2.63

4. Conclusion

It was concluded that EGCG 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 EGCG existed in the nanoparticles and pharmaceutical application.

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6. Biblography

Barreiro-Iglesias R., et al. (2005). *Preparation of chitosan beads by simultaneous cross-linking/insolubilization in basic pH rheological optimization and drug loading/release behaviour.* European Journal of Pharmaceutical Sciences 24, 77-84.

Borges O., et al. (2005). *Preparation of coated nanoparticles for a new mucosal vaccine delivery system.* Internantional Journal of Pharmaceutics 299, 155-166.

Chun H., et al. (2001) *Evaluation of antioxidant activity of epigallocatechin gallte in biphasic model systems in vitro.* Molecular and Cellular Biochemistry 218 (1-2), 147-155.

Clair A., et al. (2002). *Catechins from green tea (Camellia sinensis) inhibit bovine and human cartilage proteoglycan and type II collagen degradation in vitro*. Journal of Nutrition 132, 141-146.

Coppi G., et al. (2002). *Alginate microparticles for enzyme peroral administration*. International Journal of Pharmaceutics 242, 263-266.

Lam. W.H., et al. (2004). .*A potential prodrug for a green tea polyphenol proteasome inhibitor:evaluation of the peracetate ester of (-)-epigallocatechin gallate[(-)-EGCG]*. Bioorganic and Medicinal Chemistry 12, 5587-5593.

Samuel T.S., et al. (2006). *A method for fast determination of epigallocatechin galate (EGCG), epicatechin (EC), catechin (C) and caffeine (CAF) in green tea using HPLC.* Clênc.Tecnol. Aliment.Campinas 26(2), 394-400.

Sosaku I., et al. (2005). *Formation of biocompatible nanoparticles by self-assembly of enzymeatic hydrolysates of chitosan and carboxymethylcellulose*. Bioscience Biotechnology Biochemistry 69(9), 1637-1642.

Sunil A.A., et al. (2004). *Recent advances on chitosan-based micro- and nanoparticles in drug delivery.* Journal of Controlled Release 100, 5-28.

Xiaofeng Z., et al. (2006). *Electrochemical studies of (-)-epigallocatechin gallate and its interaction with DNA*. Analytical and Bioanalytical Chenistry 386 (6), 1913-1919.

Wai H.L., et al. (2004). *A potential prodrug for a green tea polyphenol proteasome inhibitor:evaluation of the percetate ester of (-)-epigallocatechin gallate[(-)-EGCG]*. Bioorganic and Medicinal Chemistry 12, 5587-5593.