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# Fabrication and optimization of EGCG-loaded nanoparticles by high pressure homogenization

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**ABSTRACT:** In this study, we investigated the effects of the number of high pressure homogenization cycles and alginate (AG)-to-chitosan (CS) ratio on the physicochemical properties (mean size, polydispersity index, surface charge, encapsulation efficiency, and free radical scavenging) of (-)-epigallocatechin-3-gallate (EGCG)-loaded nanoparticles. Nanoparticles prepared with alginate and chitosan concentrations of 0.01% and three cycles of high pressure homogenization exhibited a small size (293 nm) and a zeta potential of +37.49 mV, and were thus considered to be optimal for encapsulation. The highest encapsulation efficiency of 80.1% was achieved by using an EGCG concentration of 100 μg/g, which also resulted in the highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of 81.8% and 69.3% for pH 2.6 and pH 6.9, respectively. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43269.

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## INTRODUCTION

In addition to macronutrients, foods contain a range of bioactive compounds such as vitamins, antimicrobials, antioxidants, flavors, and colorants. These compounds exhibit diverse molecular properties and many of them are labile or have undesirable flavors, preventing their direct consumption in pure form.[1] Nutraceuticals, in particular, have been associated with health enhancement and disease prevention, thus, their incorporation to foods is of interest. Nevertheless, nutraceuticals can only be effective if their bioavailability is preserved, which is challenging because of their instability in the acidic pH of the stomach or the high temperatures used during food processing. For this reason, encapsulation of such compounds into delivery systems to ensure their protection against degradation and their effective release is necessary.[2–5]

Nanoencapsulation is a promising technology to capture and deliver bioactive compounds, many of which are notable for their instability and poor absorption in the body. Producing particles in the nanoscale range increases surface-to-volume ratio, which in turn increases reactivity and modifies their mechanical, electrical, and optical properties. Several encapsulation techniques have been developed to produce nanoparticles, including emulsification, coacervation, nanoprecipitation, and high pressure homogenization.[6] Nanoemulsions can be produced by forcing the mixture through a small orifice at high pressure (100∼2000 bar), a technique called high pressure homogenization. This technique has proven effective in stabilizing emulsions and inactivating microorganisms and enzymes.[7–10]

Among biopolymers used for encapsulation, alginate (AG) and chitosan (CS) have received considerable attention.[5] Alginate consists of linear chains of $\beta$-D-mannuronic acid and $\alpha$-L-guluronic acid, and is widely used in encapsulation because of its low toxicity, low cost, biodegradability, and ability to form gels.[11–13] Chitosan is the product of the deacetylation of chitin, an abundant polysaccharide found in the shells of crustaceans. Like alginate, chitosan is nontoxic, biocompatible, and biodegradable,[12–14] but has the advantage of being mucoadhesive, enhancing intestinal absorption of bioactive compounds.[15,16] Alginate beads coated with chitosan have been used for encapsulation because of their increased stability, and encapsulation efficiency compared with that of beads composed of alginate or chitosan alone.[12,13,17]

Bioactive compounds such as polyphenols and drugs have been encapsulated in nanoparticles made of alginate and/or chitosan.[18–21] Among polyphenols, tea catechins are known to be potent antioxidants and also show anticancer, neuroprotective, and cardioprotective effects. However, they are unstable and exhibit poor oral bioavailability, probably because of their degradation in the acidic environment of the stomach and alkaline environment of the intestine, as well as low intestinal

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absorption.[21–23] The major catechin in tea is (-)-epigallocatechin-3-gallate (EGCG), which besides being a powerful antioxidant, has antitumor and anticancer activities.[24] EGCG has been encapsulated in carriers such as chitosan-$\gamma$-PGA nanoparticles,[22] chitosan nanoparticles,[21] nanoliposomes,[23] and $\beta$-lactoglobulin nanoparticles.[25] Furthermore, high pressure homogenization has been applied to encapsulate EGCG in carrageenan-$\beta$-lactoglobulin nanoparticles.[26] Previous studies have reported that encapsulation of EGCG preserves its biological activity and enhances its absorption by the body.[21,25] Thus, encapsulation technology would allow incorporating EGCG to functional foods. To the best of our knowledge, this is the first attempt to encapsulate EGCG in alginate-chitosan nanoparticles (ACNs), using high pressure homogenization. In the current study, we investigated the effects of the number of high pressure homogenization cycles and alginate-to-chitosan ratio on the physicochemical properties (mean particle size, polydispersity index, surface charge, encapsulation efficiency, and free radical scavenging) of the EGCG-loaded alginate-chitosan nanoparticles generated by high pressure homogenization.

## MATERIALS AND METHODS

### Materials

Sodium alginate (AG, 90% purity) was obtained from Duksan Chemicals (Seoul, Korea). Chitosan (CS, molecular weight: 30 kDa, degree of deacetylation: 75%∼85%), (-)-epigallocatechin-3-gallate (EGCG, 95% purity), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich Co. (St. Louis, MO). All other chemicals were all of analytical grade.

### Preparation of AG-CS Nanoparticles

Alginate-chitosan nanoparticles (ACNs) were prepared by the electrostatic interactions between anionic AG and cationic CS. Briefly, the ACNs were prepared by the dropwise addition of a CS solution (0.01%∼0.5% w/v, 1% lactic acid) to an AG solution (0.01%∼0.5% w/v) while magnetic stirring for 120 min, followed by high speed homogenization (HG-15-A, DAIHAN Co., Seoul, Korea) at 10,000 rpm for 1 min. Subsequently, the AG-CS mixing solution was subjected to high pressure homogenization (EmulsiFlex®-C3, AVESTIN, Ottawa, Canada) at 10,000 psi and various cycles (0 to 5 cycles) to reduce the particle size to the nano scale range. The optimal concentration of AG and CS and the optimal process conditions for high pressure homogenization were determined.

### Preparation of EGCG-Loaded ACNs

EGCG-loaded ACNs were prepared using a similar process to that of the previous ACNs preparation. EGCG was firstly dissolved in an AG solution under constant magnetic stirring for 30 min (EGCG-AG). A CS solution was then added to the EGCG-AG solution, followed by stirring for 120 min and high speed homogenization at 10,000 rpm for 1 min. Subsequently, the mixing solution was subjected to high pressure homogenization at 10,000 psi and various cycles (0 to 5 cycles) to reduce the particle size of the EGCG-ACNs.

### Particle Size, Polydispersity Index, and Zeta Potential

The mean particle size, size distribution, and zeta potential of the ACNs and EGCG-ACNs were measured using a zeta potential and particle size analyzer (ELSZ-1000, Otsuka Electronics Co., Osaka, Japan) at a fixed detector angle of 90°. The particle size and size distribution were expressed as mean diameter (size, nm) and intensity, respectively. The sample was injected directly into the chamber of the ELSZ-1000 instrument, and the zeta potential of the particles was determined by measuring the direction and velocity of their droplet movement in a defined electric field.

### Encapsulation Efficiency of the EGCG-ACNs

The encapsulation efficiency of the EGCG-ACNs was determined by an isocratic high performance liquid chromatography (HPLC). The fabricated EGCG-ACNs were centrifuged at 4°C and 18,000 rpm for 30 min, and the supernatant was collected for quantitative analysis of non-encapsulated core material (free EGCG). The obtained supernatant was mixed with water in a ratio of 1:9 (v/v) and analyzed by isocratic HPLC. A Shimadzu D-20A HPLC (Kyoto, Japan) fitted with a UV absorbance detector (at 274 nm) and an ACE5 C18 column (4.6 × 250 mm, 5 $\mu$m; Advanced Chromatography Technologies, Aberdeen, UK) was used. The mobile phase was a mixture of acetonitrile : acetic acid : methanol : water (130:20:5:845, v/v/v/v), pumped at a flow rate of 1.0 mL/min. A sample volume of 20 µL was injected.

Encapsulation efficiency of the EGCG-ACNs was calculated using the following eq. (1):

$$\text{Encapsulation efficiency } (\%) = \frac{\text{Total EGCG} - \text{Free EGCG}}{\text{Total EGCG}} \times 100. \quad (1)$$

### Antioxidant Activity of the EGCG-ACNs by DPPH Assay

The antioxidant activity of the EGCG-ACNs was measured by the DPPH assay, performed at pH 2.6 and pH 6.9. Briefly, a stock solution of DPPH (0.15 m$M$) was prepared in 100% methanol, the sample was added to the DPPH solution and incubated for 30 min, and the absorbance was measured at 517 nm; 100% methanol was used as a control. The antioxidant activity was calculated using the following eq. (2):

$$\text{Antioxidant activity } (\%) = \frac{1 - \text{Sample OD}}{\text{Control OD}} \times 100. \quad (2)$$

### Statistical Analysis

All experiments were performed in triplicate, and all data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS version 20.0 (SPSS, Chicago, IL). Significant differences among treatments were determined by analysis of variance (ANOVA) and Duncan's multiple range test. The level of significance was set at $P < 0.05$.

## RESULTS AND DISCUSSION

### Effect of High Pressure Homogenization

In order to understand the effect of processing methods on the particle size and zeta potential of the ACNs, 0.01% alginate and 0.1% chitosan were used with various processing methods such as high speed homogenization and various cycles of high pressure homogenization (Table I). Figure 1 shows the particle size and zeta potential of the ACNs with various processing methods. The particle size of ACNs was about 880 nm after high

**Table I.** Processing Conditions of Alginate-Chitosan Nanoparticles (ACNs)

| Sample NO | AG (%) | CS (%) | HSH[a] | HPH1[b] | HPH2 | HPH3 | HPH4 | HPH5 |
|---|---|---|---|---|---|---|---|---|
| ACN-01 | 0.01 | 0.1 | O | X | X | X | X | X |
| ACN-02 | 0.01 | 0.1 | O | O | X | X | X | X |
| ACN-03 | 0.01 | 0.1 | O | O | O | X | X | X |
| ACN-04 | 0.01 | 0.1 | O | O | O | O | X | X |
| ACN-05 | 0.01 | 0.1 | O | O | O | O | O | X |
| ACN-06 | 0.01 | 0.1 | O | O | O | O | O | O |

O = applied; X = not applied.
[a] High speed homogenization at 10,000 rpm for 40 min.
[b] High pressure homogenization at 10,000 psi; HPH1 = one cycle, HPH2 = two cycles, HPH3 = three cycles, HPH4 = four cycles, HPH5 = five cycles.

speed homogenization and significantly ($P < 0.05$) decreased to 629 nm after one cycle of high pressure homogenization. The particle size of the ACNs was further decreased to about 400 nm with more high pressure homogenization cycles but it was not statistically significant ($P > 0.05$) over three cycles of high pressure homogenization. The high pressure homogenization could break down the most of ACNs but some of them may be passed without any damage. Two and three cycles of high pressure homogenization could make more homogeneous particle size of ACNs. However, high pressure homogenization could not make further size reduction of ACNs after most particles were broken down once.

The zeta potentials of all ACNs obtained here were ranged from +35.9 mV to +46.7 mV indicating that amine groups (–$NH^{3+}$) of chitosan were present on the surface.[14] It is known that high zeta potential above ±30 mV is required for good particle stability.[25] The zeta potential of ACNs was not significantly ($P > 0.05$) different after 1 to 4 cycles of high pressure homogenization but significantly ($P < 0.05$) decreased after five cycles of high pressure homogenization. The decrease of chitosan molecule chain caused by the severe high pressure may probably decrease the zeta potential after five cycles of high pressure homogenization.[27] High pressure homogenization not only has the advantage of producing small particles, but it can also make nanoparticles more stable and uniform and can help control their viscosity.[28] In addition, high pressure homogenization is very simple, efficient, and environmentally friendly as it does not require organic solvents. This technique can also be used to improve the water solubility and bioavailability of poorly soluble compounds.[29]

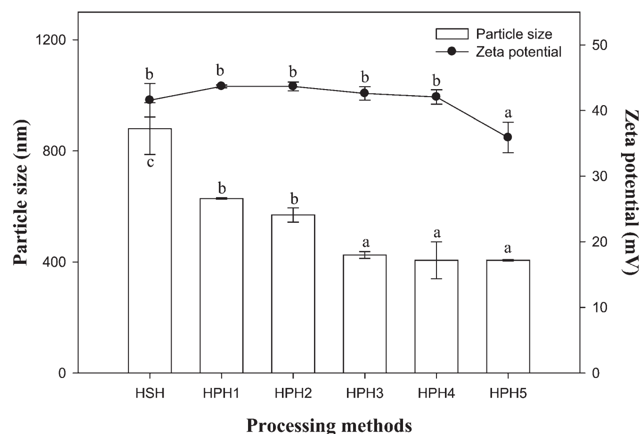


**Figure 1.** Effects of processing on particle size and zeta potential of the alginate-chitosan nanoparticles (ACNs). *Different letters indicate a significant difference at $P < 0.05$ by ANOVA test.

### Effect of AG and CS Ratios

Various AG and CS concentrations were evaluated (Table II), applying in all cases a 3-cycle high pressure homogenization under 10,000 psi, and their effects on the particle size and zeta potential are illustrated in Figure 2. The particle sizes of the ACNs were not significantly ($P > 0.05$) increased with CS contents from 0.01% to 0.05% as shown in ACN-11 to ACN-13 of Figure 2(a). However, their particle sizes were significantly ($P < 0.05$) increased when more than 0.5% CS was used (ACN-15). This outcome agrees with previous studies reporting that an increase in the amount of CS resulted in an increase in particle size.[29] Likewise, when the AG concentration was high (0.5%, ACN-19), the particle size was substantially larger (696 nm) than that obtained at lower AG concentrations, although not as large as that obtained with a high CS concentration. The zeta potential was slightly increased from +37.5 mV to +43.7 mV with CS concentrations of 0.01–0.5%. The highest zeta potential (+43.7 mV) corresponded to the particles with the highest CS concentration because of the cationic nature of CS.[12] By contrast, the zeta potential was significantly ($P < 0.05$) decreased from +37.5 mV to −29.7 mV with AG

**Table II.** Concentrations of Alginate and Chitosan in EGCG-ACNs Preparation

| Sample NO | AG (%) | CS (%) |
|---|---|---|
| ACN-11 | 0.01 | 0.01 |
| ACN-12 | 0.01 | 0.02 |
| ACN-13 | 0.01 | 0.05 |
| ACN-14 | 0.01 | 0.10 |
| ACN-15 | 0.01 | 0.50 |
| ACN-16 | 0.02 | 0.01 |
| ACN-17 | 0.05 | 0.01 |
| ACN-18 | 0.10 | 0.01 |
| ACN-19 | 0.50 | 0.01 |

Processed by three cycles of high pressure homogenization.

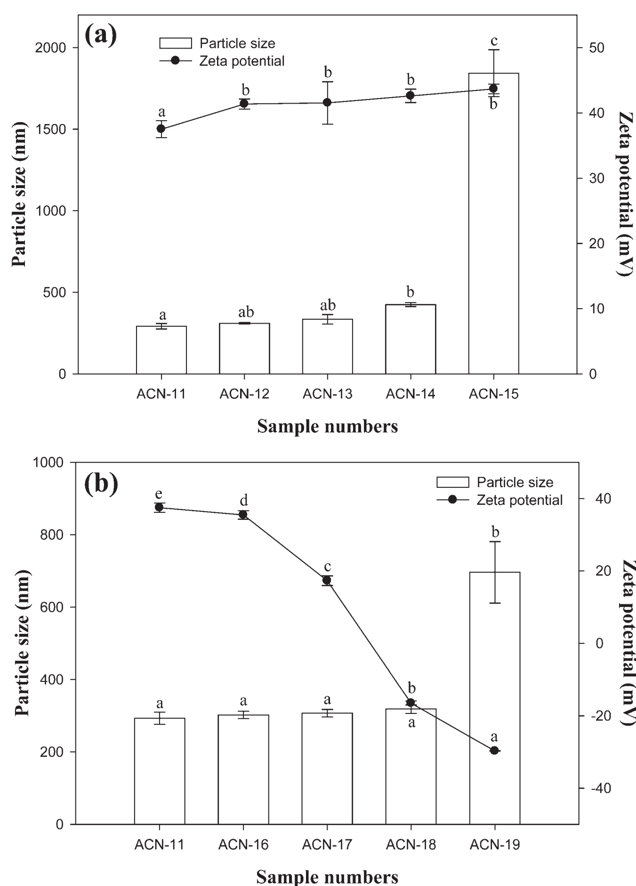


**Figure 2.** Effects of (a) chitosan concentration and (b) sodium alginate concentration on particle size and zeta potential of ACNs. *Different letters indicate a significant difference at $P < 0.05$ by ANOVA test.

concentrations of 0.01–0.5%. The highest AG concentration resulted in the lowest zeta potential (−29.7 mV). The surface of the ACNs would be negatively charged with hydroxyl groups (–OH) of AG when the AG content was higher than 0.05%. The sample with the lowest CS and AG concentrations (0.01%, ACN-11) exhibited the smallest particle size (293 nm) and a zeta potential of +37.49 mV. Thus, a ratio of 1 : 1 corresponding to AG and CS concentrations of 0.01% was used for further experiments. ACN sizes under 500 nm are desirable because such nanoparticles can translocate through the mucous barrier of the intestine and interact with the absorptive cells in the intestinal epithelium. Furthermore, a positive zeta potential is desirable because it increases the stability of the nanoparticles, preventing particle aggregation, and can also enhance mucoadhesion and release in the intestine.[21,30] Both AG and CS have been previously used for nanoencapsulation because of their advantages of being nontoxic, biocompatible, and biodegradable.[12–14] However, ACNs showed greater advantages of smaller, more stable particles and higher encapsulation efficiency than those obtained using AG or CS alone.[13,17]

### Effect of EGCG Loading on Particle Size and Zeta Potential

To prepare EGCG-loaded ACNs, a concentration of 0.01% AG and CS was used, and the nanoparticles were homogenized at high pressure at 10,000 psi for three cycles after high speed homogenization. EGCG concentrations (based on the AG content) of 1–50% were evaluated. Furthermore, three additional samples were prepared at higher CS concentrations of 0.01–0.1% as shown in Table III. The effect of the EGCG loading on the particle size and zeta potential of the nanoparticles is illustrated in Figure 3. Although the particle size tended to increase as the EGCG concentration increased, it did not change significantly ($P > 0.05$) for concentrations between 1% and 10% (ACN-20 to ACN-23). Nevertheless, at 50% EGCG concentration (ACN-24), the particle size significantly ($P < 0.05$)

**Table III.** Concentrations of EGCG and Chitosan in EGCG-ACNs Preparation

| Sample NO | AG (%) | CS (%) | EGCG (%)[a] |
|---|---|---|---|
| ACN-20 | 0.01 | 0.01 | 1 |
| ACN-21 | 0.01 | 0.01 | 5 |
| ACN-22 | 0.01 | 0.01 | 10 |
| ACN-23 | 0.01 | 0.01 | 20 |
| ACN-24 | 0.01 | 0.01 | 50 |
| ACN-25 | 0.01 | 0.02 | 10 |
| ACN-26 | 0.01 | 0.05 | 10 |
| ACN-27 | 0.01 | 0.10 | 10 |

[a] EGCG (%) = EGCG/sodium alginate × 100.

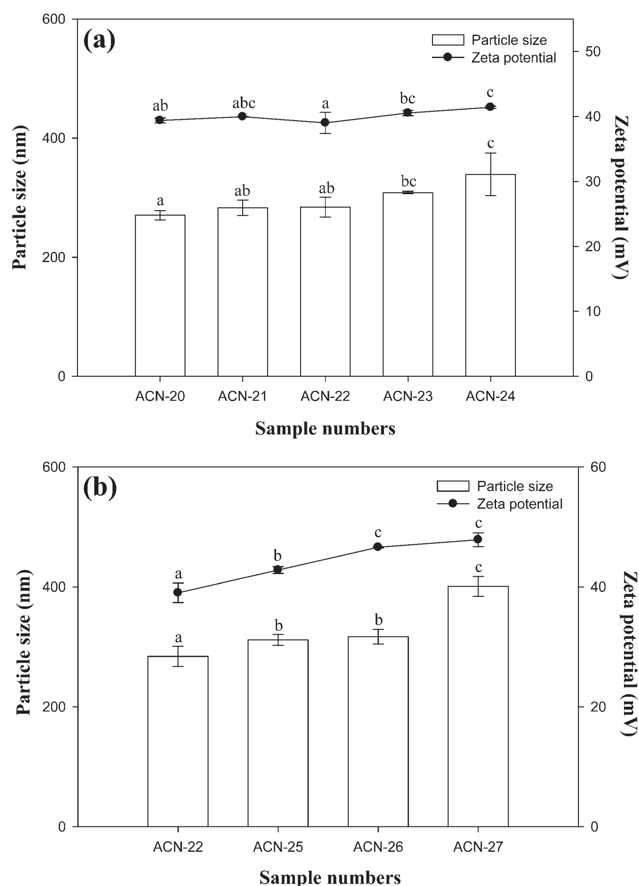


**Figure 3.** Effects of (a) EGCG concentration and (b) chitosan concentration particle size and zeta potential of EGCG-ACNs. *Different letters indicate a significant difference at $P < 0.05$ by ANOVA test.

increased. The zeta potential also exhibited a slight increase. For this EGCG concentration range, the largest particle size (339.1 nm) and the highest zeta potential (+41.4 mV) corresponded to nanoparticles fabricated using 0.01% AG and 0.01% CS. In addition, an increase in the CS concentration while using 10% EGCG resulted in an increase in the particle size and zeta potential. As expected, the particle size and zeta potential of ACNs with 10% EGCG load significantly ($P < 0.05$) increased from 283.9 nm and +39.0 mV to 400.8 nm and +47.9 mV, respectively, for chitosan concentrations of 0.01%~0.1%. The largest particle size was obtained when using a concentration of 0.01% AG and 0.1% CS (ACN-27). By contrast, low AG and CS concentrations (0.01%) when using a 10% EGCG load resulted in the smallest particle size of 283.90 nm (ACN-22). Therefore, 0.01% AG, 0.01% CS, and 10% EGCG was used in further experiments. Figure 4 illustrates the size distribution by intensity of the nanoparticles obtained using these conditions. Dynamic light scattering revealed that the nanoparticles had diameters in the range 100~500 nm, and more than 50% had diameters between 200~300 nm.

### Encapsulation Efficiency

The encapsulation efficiency was determined by evaluating various CS and EGCG concentrations, and the results are summarized in Figure 5. The encapsulation efficiency of the ACNs ranged from 70.4% to 83.8% for CS concentrations between 0.01% and 0.1%. The maximum encapsulation efficiency was obtained with the ACNs using 0.1% CS, whereas the lowest encapsulation efficiency was obtained using 0.02% CS. With 0.01% CS concentration, the encapsulation efficiency was 80.1%, which was not significantly ($P > 0.05$) different to the maximum value of 83.8% as shown in Figure 5(a). However, the particle size of the ACNs obtained using 0.1% CS was 400 nm, being almost 70% larger than those (284 nm) obtained using 0.01% CS. Figure 5(b) shows the effect of the EGCG concentration on the encapsulation efficiency. The concentration of EGCG was the most influential factor for the encapsulation efficiency. A low EGCG concentration (10 μg/mL) resulted in low encapsulation efficiency of 8.4%. However, when the EGCG concentration was increased to 50 μg/mL, the encapsulation

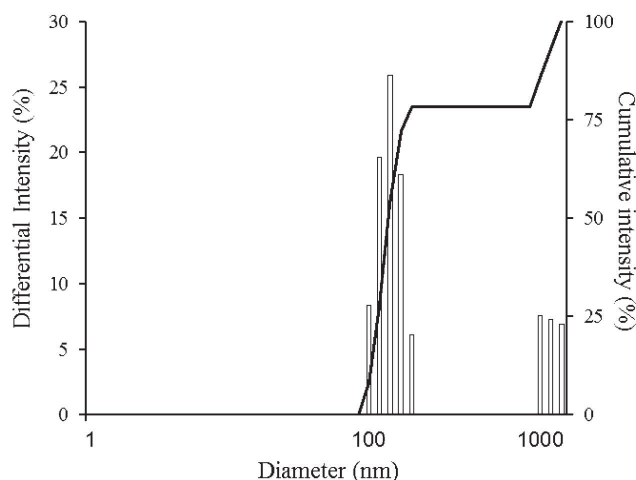


**Figure 4.** Size distribution of EGCG-ACNs in differential intensity (%) and cumulative intensity (%).

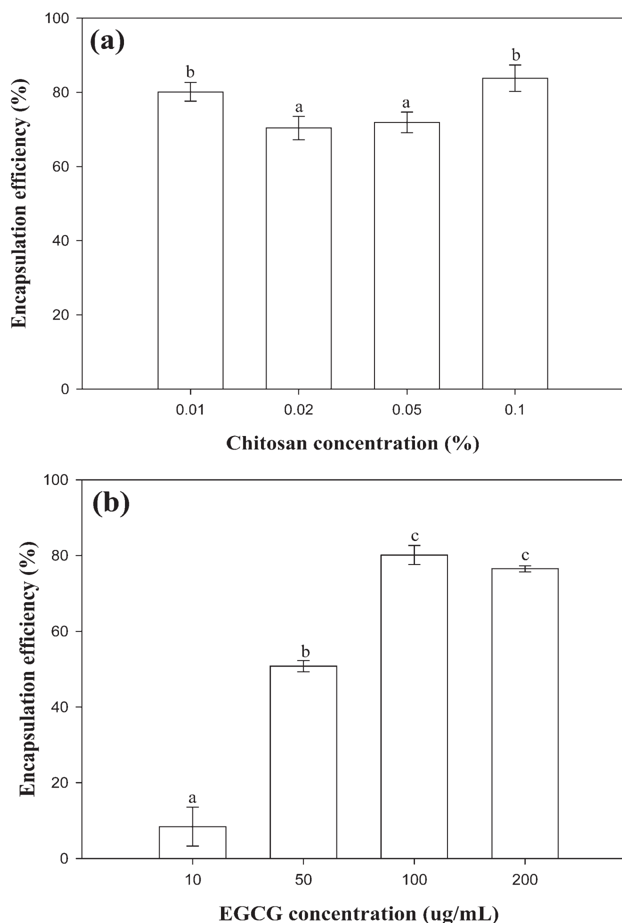


**Figure 5.** EGCG encapsulation efficiency according to (a) chitosan concentration and (b) EGCG concentration. *Different letters indicate a significant difference at $P < 0.05$ by ANOVA test.

efficiency exhibited a significant ($P < 0.05$) increase to 50.8%. The highest encapsulation efficiency of 80.1% was achieved by using an EGCG concentration of 100 μg/mL, but further increases in the EGCG concentration resulted in a slight decrease in the encapsulation efficiency. Therefore, the highest encapsulation efficiency was attained when using a CS concentration of 0.01% and an EGCG concentration of 100 μg/mL. The encapsulation efficiency of EGCG nanoparticles has been reported to be in the range of 20%~80%[23]; hence, we attained high encapsulation efficiency. The encapsulation efficiency obtained here was also higher than that reported for tea catechins encapsulated in chitosan-tripolyphosphate nanoparticles (24%~53%),[31] chitosan-$\gamma$-PGA nanoparticles (14%~23.5%),[22] and $\beta$-lactoglobulin nanoparticles (32%~73%).[25] Harris *et al.*[14] prepared antioxidant-loaded CS microspheres, obtaining an encapsulation efficiency above 85% after 3 months, demonstrating that CS is a suitable material for encapsulating antioxidants. In addition, using a combination of CS and AG for encapsulation has been reported to yield higher encapsulation efficiency than that obtained when using AG alone.[18]

### DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was evaluated at two pH values, one acidic (pH 2.6) and one close to neutral (pH 6.9) as

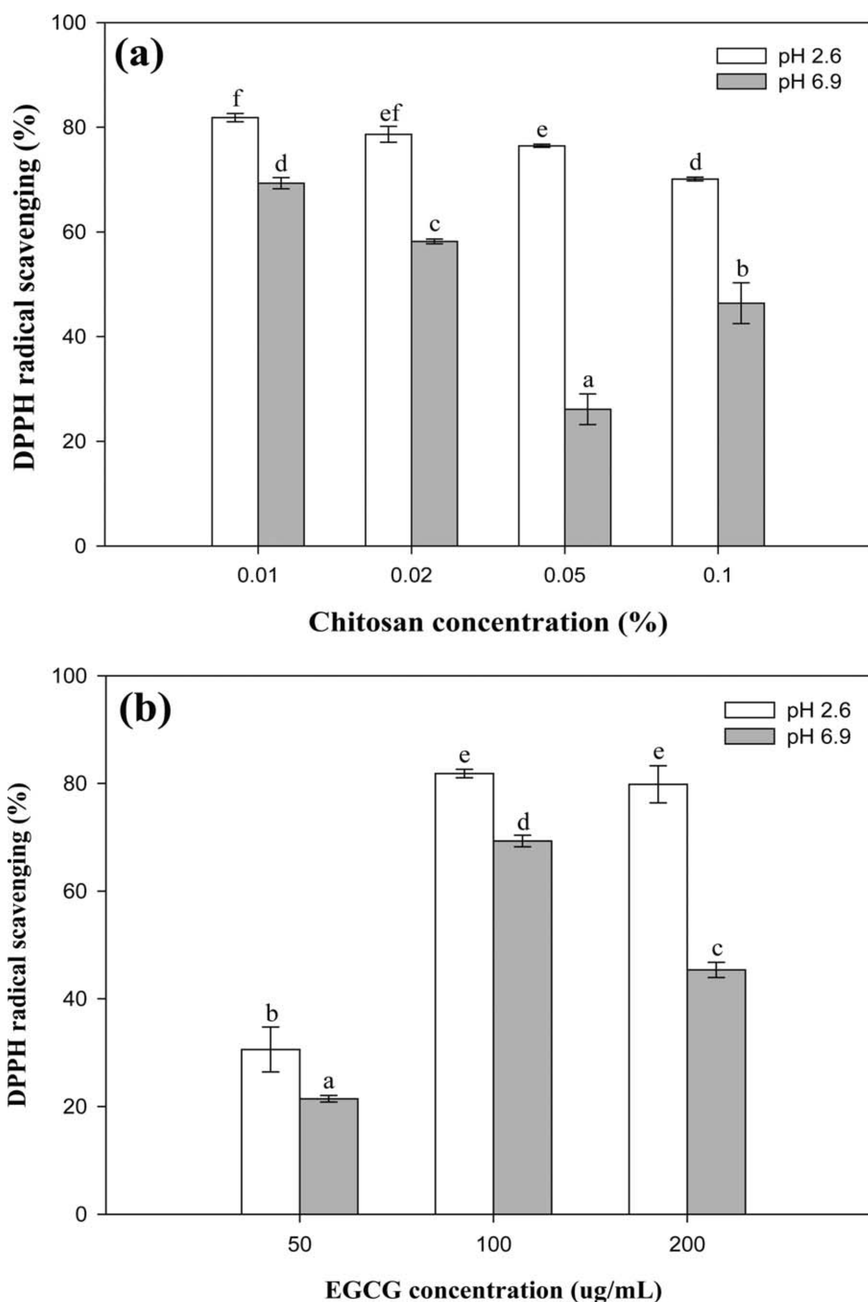


**Figure 6.** DPPH radical scavenging according to (a) chitosan concentration and (b) EGCG concentration. *Different letters indicate a significant difference at $P < 0.05$ by ANOVA test.

shown in Figure 6. The selected pH values are close to those in the stomach (pH 1.0∼4.0) and proximal ileum (pH 6.6∼7.0).[22] Various CS and EGCG concentrations were evaluated. In both environments, samples with the lowest CS concentration (0.01%) and 100 μg/g of EGCG exhibited the highest DPPH radical scavenging activities, with values of 81.8% and 69.3% for pH 2.6 and pH 6.9, respectively. Delivery systems containing chitosan have mucoadhesive and absorption enhancement properties;[14] thus, the high DPPH radical scavenging activities attained here suggest that the EGCG-loaded ACNs could be an effective vehicle for antioxidant delivery. Dube *et al.*[21] compared the intestinal absorption of tea catechins including EGCG in encapsulated and non-encapsulated forms, and reported that nanoencapsulation of catechins in CS substantially increased their absorption in the intestine. Similarly, Zou *et al.*[23] described the enhanced stability and release of nanoliposome-encapsulated EGCG in simulated intestinal fluid. These results indicate that encapsulation is an effective strategy to overcome the poor stability and bioavailability of tea catechins.

## CONCLUSIONS

ACNs for encapsulating EGCG were prepared and optimized in this study. Parameters that can affect the performance of the

nanoencapsulation, namely the number of high pressure homogenization cycles, AG-to-CS ratio, and EGCG loading were evaluated. The optimal conditions for preparing the nanoparticles were a 1:1 AG : CS ratio, using 0.01% of each, 100 μg/mL EGCG, and 3-cycle high pressure homogenization. Applying these conditions, encapsulation efficiency of 80.1% and DPPH radical scavenging activities of 81.8% and 69.3% for pH 2.6 and pH 6.9, respectively, were obtained. Therefore, the obtained nanoparticles successfully encapsulated EGCG and preserved its antioxidant activity. The methodology presented here could be applied to other tea catechins and antioxidants, and shows potential for increasing the stability of these compounds and their oral bioavailability.

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## REFERENCES

1. Relkin, P.; Shukat, R.; Moulin, G. *Food Res. Int.* **2014**, *63*, 9.
2. Relkin, P.; Yung, J. M.; Kalnin, D.; Ollivon, M. *Food Biophys.* **2008**, *3*, 163.
3. Chen, L.; Remondetto, G. E.; Subirade, M. *Trends Food Sci. Technol.* **2006**, *17*, 272.
4. Gibbs, B. F.; Kermasha, S.; Alli, I.; Mulligan, C. N. *Int. J. Food Sci. Nutr.* **1999**, *50*, 213.
5. Fang, Z.; Bhandari, B. *Trends Food Sci. Technol.* **2010**, *21*, 510.
6. Ezhilarasi, P. N.; Karthik, P.; Chhanwal, N.; Anandharamakrishnan, C. *Food Bioprocess Technol.* **2013**, *6*, 628.
7. Desrumaux, A.; Marcand, J. *Int. J. Food Sci. Nutr.* **2002**, *37*, 263.
8. Cruz, N.; Capellas, M.; Hernández, M.; Trujillo, A. J.; Guamis, B.; Ferragut, V. *Food Res. Int.* **2007**, *40*, 725.
9. Diels, A. M. J.; Wuytack, E. Y.; Michiels, C. W. *Int. J. Food Microbiol.* **2003**, *87*, 55.
10. Hayes, M. G.; Fox, P. F.; Kelly, A. L. *J. Dairy Res.* **2005**, *72*, 25.
11. Braccini, I.; Pérez, S. *Biomacromolecules* **2001**, *2*, 1089.
12. Gazori, T.; Khoshayand, M. R.; Azizi, E.; Yazdizade, P.; Nomani, A.; Haririan, I. *Carbohydr. Polym.* **2009**, *77*, 599.
13. Rafiee, A.; Alimohammadian, M. H.; Gazori, T.; Riazi-rad, F.; Fatemi, S. M. R.; Parizadeh, A.; Haririan, I.; Havaskary, M. *Asian Pac. J. Trop. Dis.* **2014**, *4*, 372.
14. Harris, R.; Lecumberri, E.; Mateos-Aparicio, I.; Mengíbar, M.; Heras, A. *Carbohydr. Polym.* **2011**, *84*, 803.
15. Lei, F.; Wang, X.; Liang, C.; Yuan, F.; Gao, Y. *J. Appl. Polym. Sci.* **2014**, *131*, 39732.
16. Thanou, M.; Verhoef, J. C.; Junginger, H. E. *Adv. Drug Delivery Rev.* **2001**, *52*, 117.
17. Yan, X. L.; Khor, E.; Lim, L. Y. *J. Biomed. Mater. Res.* **2001**, *58*, 358.
18. Belšçak-Cvitanović, A.; Stojanović, R.; Manojlović, V.; Komes, D.; Cindrić, I. J.; Nedović, V.; Bugarski, B. *Food Res. Int.* **2011**, *44*, 1094.
19. Mukhopadhyay, P.; Sarkar, K.; Soam, S.; Kundu, P. P. *J. Appl. Polym. Sci.* **2013**, *129*, 835.
20. Elzatahry, A. A.; Mohy Eldin, M. S.; Soliman, E. A.; Hassan, E. A. *J. Appl. Polym. Sci.* **2009**, *111*, 2452.
21. Dube, A.; Nicolazzo, J. A.; Larson, I. *Eur. J. Pharm. Sci.* **2010**, *41*, 219.
22. Tang, D. W.; Yu, S. H.; Ho, Y. C.; Huang, B. Q.; Tsai, G. J.; Hsieh, H. Y.; Sung, H. W.; Mi, F. L. *Food Hydrocolloid.* **2013**, *30*, 33.
23. Zou, L. Q.; Peng, S. F.; Liu, W.; Gan, L.; Liu, W. L.; Liang, R. H.; Liu, C. M.; Niu, J.; Cao, Y. L.; Liu, Z.; Chen, X. *Food Res. Int.* **2014**, *64*, 492.
24. Singh, B. N.; Shankar, S.; Srivastava, R. K. *Biochem. Pharmacol.* **2011**, *82*, 1807.
25. Li, B.; Du, W.; Jin, J.; Du, Q. *J. Agric. Food Chem.* **2012**, *60*, 3477.
26. Ru, Q.; Yu, H.; Huang, Q. *J. Agric. Food Chem.* **2010**, *19*, 10373.
27. Wang, Y.; Li, D.; Wang, L. J.; Xue, J. *Carbohydr. Polym.* **2011**, *83*, 489.
28. Villay, A.; Lakkis de Filippis, F.; Picton, L.; Le Cerf, D.; Vial, C.; Michaud, P. *Food Hydrocolloid.* **2012**, *27*, 278.
29. Donsi, F.; Wang, Y.; Li, J.; Huang, Q. *J. Agric. Food Chem.* **2010**, *58*, 2848.
30. Mukhopadhyay, P.; Chakraborty, S.; Bhattacharya, S.; Mishra, R.; Kundu, P. P. *Int. J. Biol. Macromol.* **2015**, *72*, 640.
31. Hu, B.; Pan, C.; Sun, Y.; Hou, Z.; Ye, H.; Hu, B.; Zeng, X. *J. Agric. Food Chem.* **2008**, *56*, 7451.