P-079 **Entrapment of *Tacca chantrieri* extract in chitosan- alginate nanoparticles**

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INTRODUCTION AND OBJECTIVES

The genus Tacca (Taccaceae) is mainly distributed in tropical region of Asia, Pacific Islands, and Australia. The rhizome of *Tacca chantrieri* has been used in Chinese medicines for treatment of various diseases including high blood pressure, burns, gastric ulcers, enteritis, and hepatitis (Yokosuka 2002; Tiamjan 2007). It was reported that the main active component existing in its rhizome is a wide array of saponin, a group known as non-volatile, surface-active substance widely distributed in plants. However, the saponin found is not stable and shows strong acidic irritation property (Tava 2003). The aim of this study was to entrap the active extract of *T. chantrieri* in chitosan-alginate nanoparticles in order to improve its stability and reduce the irritation from its acidic property. The effect of molecular weight and degree of deacetylation of chitosan as well as the extract concentration on nanoparticle characteristics was demonstrated in this paper.

MATERIALS AND METHODS

Extraction method The dried rhizome powder of *T. chantrieri* was extracted using the maceration method with 95%ethanol to yield *T. chantrieri* crude extract (TCE). The TCE was further purified by column chromatography. After the solvent of the elucidated solution was removed and the residue was lyophilized, the partial purified extract (PPE) was obtained.

Test for solubility and pH An excess amount of PPE was added with an exact amount of the test solvents and shaked until equilibrium then filtered through 0.45 μm membrane filter. The filtrate was analyzed by HPLC for saponin active compound. The 1.0%w/v of PPE in distilled water was tested for pH value.

Preparation of Chitosan–alginate nanoparticles Chitosan-alginate nanoparticles were prepared by ionotropic polyelectrolyte pre-gelation method (Sarmento 2007). A drop of 0.18 mM calcium chloride solution was added under gentle stirring into a beaker containing 0.06% alginate and an exact amount of PPE solution to provide an alginate pre-gel. Then, 0.10% chitosan solution was added dropwise into the pre-gel. The effect of molecular weight (MW), degree of deacetylation (DD) and concentration of PPE was investigated. The concentration of PPE was fixed at 1 mg/ml when the effect of MW and DD was investigated; the DD was fixed at 95% when the effect of MW and PPE concentration was examined. Chitosan at MW of 425000 and DD of 95% was used when the effect of PPE concentration was investigated.

Characterization of nanoparticles The determination of particle size diameter, polydispersity index (PDI) and zeta potential of the nanoparticles obtained was performed by using photon correlation spectrophotometer (PCS) (Zetasizer ZS, Malvern Instruments, UK). The supernatant after particle formation was analyzed by HPLC for encapsulation efficiency.

Statistical analysis The obtained data were evaluated by one way-ANOVA. $P < 0.05$ was considered as the significant level.

RESULTS AND DISCUSSION

Solubility and pH of PPE
The result of the solubility test as shown in Table 1 indicated that the PPE is high soluble in the solvent with high polarity index such as water, DMSO, and ethanol. Acording to this result, the PPE was considered as a polar compound. The pH value of 1.0%w/v PPE in distilled water was 3.48±0.05. This indicated that the PPE was an acidic compound.

Table 1. The solubility of PPE

Solvents	Part of solvent required to dissolve 1 part of PPE
Distilled water	1:10
Dimethylsulfoxide	1:10
Absolute ethanol	1:10
n-Buthanol	>1:10,000
Hexane	>1:10,000

Influence of MW and DD of Chitosan and PPE concentration on particle size It was found that the mean size of the particles was affected by molecular weight and degree of deacetylation of chitosan as well as the concentration of PPE as shown in Table 2, 3, and 4 respectively. The results demonstrated that the particle size tended to increase when increasing of molecular weight of chitosan and concentration of PPE but decreased with the increasing of %DD.

Table 2 Effect of chitosan MW on particle size

MW(kDa)	Particle size (nm)
22,000	230.67±2.42
425,000	256.63±0.89
760,000	277.80±2.20

Table 3 Effect of chitosan DD on particle size

DD (%)	Particle size, (nm)
76	230.37±4.31
87	314.03±3.31
94	230.67±2.42

Table 4 Effect of PPE concentration on particle size

PPE Conc. (mg/ml)	Mean of particle size(nm)±SD
0.00	212.07±06.71
1.00	256.63±00.89
2.00	321.90±11.27
4.00	325.63±09.11

Influence of MW and DD of chitosan and Concentration of PPE on PDI and zeta potential The PDI and zeta potential of the particles prepared from different MW and DD of chitosan and various concentration of PPE are shown in Table 5, 6 and 7, respectively. The results indicated that the MW of chitosan increased the PDI where the DD increased the zeta potential of the particles. Moreover, it was found that when the concentration of PPE increased, the PDI of the particles tended to increase where the zeta potential of the particles was decreased.

Table 5 Effect of chitosan MW on PDI and zeta potential

MW (kDa)	PDI	Zeta potential
22,000	0.25±0.00	-26.87±0.78
425,000	0.32±0.02	-25.67±1.76
760,000	0.36±0.01	-24.17±1.29

Table 6 Effect of chitosan DD on PDI and zeta potential

DD (%)	PDI	Zeta potential
76	0.33±0.01	-22.57±0.42
87	0.32±0.02	-35.10±0.73
94	0.36±0.01	-36.87±0.78

Table 7 Effect of PPE concentration on PDI and zeta potential

PPE (mg/ml)	PDI	Zeta potential
0.00	0.39±0.05	-28.23±0.84
1.00	0.32±0.05	-25.67±1.76
2.00	0.39±0.03	-25.50±0.33
4.00	0.48±0.06	-22.27±0.42

Influence of MW and DD of Chitosan and concentration of PPE on Encapsulation Efficiency As shown in Figure 1 and 2, the encapsulation efficiency was affected by MW and DD of chitosan and concentration of PPE. It was found that the encapsulation efficiency decreased when MW of chitosan and concentration of PPE increased. On the other hand, it was increased with the increase of chitosan DD.

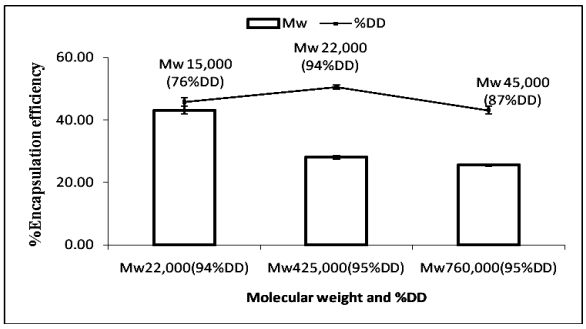

Figure 1 : Effect of MW and DD of chitosan on encapsulation efficiency

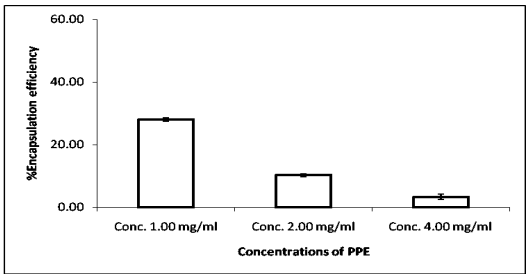

Figure 2 : Effect of PPE concentration on encapsulation efficiency

CONCLUSION

The results from this study could be concluded that the PPE of *T. chantrieri* could be dissolved easily in water, DMSO, and ethanol but not in nonpolar solvents. The aqueous solution of the extract gave acidic pH. Moreover, this paper demonstrated that that the size and size distribution, and the zeta potential of the particles obtained as well as the encapsulation efficiency were influenced by the MW and DD of chitosan and the concentration of PPE.

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