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# Optimisation of preparation conditions and properties of phytosterol liposome-encapsulating nattokinase

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Phytosterol liposomes were prepared using the thin film method and used to encapsulate nattokinase (NK). In order to obtain a high encapsulation efficiency within the liposome, an orthogonal experiment (L9 $(3)^4$) was applied to optimise the preparation conditions. The molar ratio of lecithin to phytosterols, NK activity and mass ratio of mannite to lecithin were the main factors that influenced the encapsulation efficiency of the liposomes. Based on the results of a single-factor test, these three factors were chosen for this study. We determined the optimum extraction conditions to be as follows: a molar ratio of lecithin to phytosterol of 2 : 1, NK activity of 2500 U $mL^{-1}$ and a mass ratio of mannite to lecithin of 3 : 1. Under these optimised conditions, an encapsulation efficiency of 65.25% was achieved, which agreed closely with the predicted result. Moreover, the zeta potential, size distribution and microstructure of the liposomes prepared were measured, and we found that the zeta potential was $-51 \pm 3$ mV and the mean diameter was 194.1 nm. From the results of the scanning electron microscopy, we observed that the phytosterol liposomes were round and regular in shape and showed no aggregation.

**Keywords:** liposome; nattokinase; phytosterol preparation; encapsulating properties

## 1. Introduction

Natto is a traditional high-protein food commonly consumed in Asia and is produced using the bacterium *Bacillus natto* to ferment a protein source over a short period of time. In the recent years, this hypothesis has been confirmed by several clinical trials using animals and humans. Nattokinase (NK) is a potent fibrinolytic enzyme produced by *B. natto*, and has been isolated from natto by Sumi et al. (1990), Sumi (1987) and Fujita, Nomura, and Hong (1993). NK can break down fibrin directly, while enhancing the body's production of both plasmin and other

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