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An investigation of propranolol-loaded chitosan nanoparticles for transmucosal delivery: Physical characterization

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Objectives: The objectives of this study were to develop the propranolol-loaded chitosan nanoparticles for transmucosal delivery and investigate the effect of molecular weight of chitosan and concentrations of propranolol on physical properties, i.e. pH, zeta potential and particle size of nanoparticles using the Design Expert®.

Methods: The investigated nanoparticles composed of tripolyphosphate (0.1 %w/v), various molecular weights of chitosan solution (0.2% w/v) (at 20, 35, 45 kDa), and various concentrations of propranolol at 10, 20 and 30 mg/mL were prepared and characterized for pH, zeta potential and particle size. The data obtained were analyzed using conventional comparative and response surface method.

Results: The results indicated that the response surface method showed the relationship between the formulation factors and the physical properties of nanoparticles was more markedly than the conventional comparative method. Using the response surface, the physical properties of other nanoparticles over the model formulations can be predicted. Whiles the conventional comparative method can be explained only the physical properties of model formulations. The formulation factors with a significant influence on pH, zeta potential and particle size were the molecular weight of chitosan (X_1) and concentrations of propranolol (X_2). The X_1 supported a positive influence on pH and particle size, whiles X_2 supported a positive influence on particle size. The linear correlation coefficient results for pH and zeta potential were relatively high (0.7–0.9) indicating the reliability of the response surface. Moreover, the vertical spread of the internally studentized residuals was not out of the line from bottom-to-top, indicating all points fall within the limits (at 95% confidence level)

Conclusion: The formulation factors i.e. the molecular weight of chitosan and the concentrations of propranolol significantly affected the physical properties, i.e. pH, zeta potential and particle size of the nanoparticles. Using the response surface method, the relationship between the formulation factors and the physical properties was clearly understood. In this study, we were successful in showing the feasibility of the development of transmucosal delivery of propranolol using nanoparticles. Further study is required to confirm the potential of nanoparticels by *in vitro and in vivo* drug penetration study.

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Introduction

Nanoparticulate delivery systems to deliver drugs into the body have received much attention. Several reports focus on the use of nanoparticles for enhancing transmucosal transport of drugs and biological substances.[1] However, it is well known that in most cases, nanoparticles are of little or no value as transmucosal carriers because the effect of gastrointestinal enzymetics. Therefore, many techniques for preparing nanoparticles have been developed to formulate the appropriate nanoparticles for transmucosal delivery including ionic cross-linking technique, one of a popular technique.[1)] The ionic cross-linking technique is concerned with the interaction between cationic and anionic molecules. Chitosan, a cationic natural polysaccharide, is the most generally used as cationic polymer in nanoparticles. Chitosan has received great attention in the many fields due to the many advantages of its properties such as biocompatible, biodegradable, low toxicity, good mucoadhesion and membrane permeable enhancing properties.[1, 2] In addition, the cationic molecules of chitosan can be interacted with anionic molecules especially tripolyphosphate (TPP) to form nanoparticles for drug delivery system.[2, 3] In the development of the proper nanoparticulate delivery systems, the optimal formulation factor (types and amount of compositions) and the physical properties (pH, charge, particle size, drug content) of nanoparticles should be considered. However, the complicated relationship between formulation factor and physical properties is not fully understood. Various formulation factors in the nanoparticles may affect one or several

physical properties, simultaneously. Recently, the computer program (Design Expert®) has been successfully applied to development of the optimal formulation, and to understand the complicated relationship between formulation factors and response variables of several pharmaceutical formulations, i.e. liposomes[4], microemulsions[5]. So, the aims of this study were to develop the propranolol-loaded chitosan nanoparticles for transmucosal delivery, and to investigate the effect of molecular weight of chitosan and concentrations of propranolol on physical properties, i.e. pH, zeta potential and particle size of the nanoparticles using the Design Expert®. The data obtained were analyzed using conventional comparative and response surface method.

Methods

***Preparation of propranolol-loaded chitosan nanoparticles*:** Model formulations of propranolol-loaded chitosan nanoparticles were prepared according to formulations obtained from a regular two-level factorial design. Propranolol-loaded chitosan nanoparticles were prepared by ionic interaction method. Chitosan solution (0.2% w/v) with various molecular weights (20, 35, 45 kDa) were prepared in 1% v/v acetic acid solution. TPP (0.1 %w/v) and propranolol (10 mg in 1 mL of water) were dissolved in purified water. The 10 mL of chitosan solution was constantly stirred by magnetic stirrer at room temperature. The propranolol solution was mixed with chitosan solution for 5 min and 5 mL of TPP solution was then added. The colloidal dispersion was further stirred for 5 min. The effect of molecular weight of chitosan and the concentrations of propranolol (10, 20, 30 mg) on formation of the nanoparticles were investigated and particle size, zeta potential and pH were further examined.

Investigation of propranolol-loaded chitosan nanoparticles: The pH of the nanoparticles was measured using pH meter (Mettler Toledo seveneasy, Switzerland). The zeta potential was measured by the Zeta Plus (Brookhaven Instruments Co., New York, NY, USA) and the particle size of the nanoparticles was detected by the dynamic light scattering technique (Horiba, LA-950, Kyoto, Japan). All measurements were carried out in triplicate.

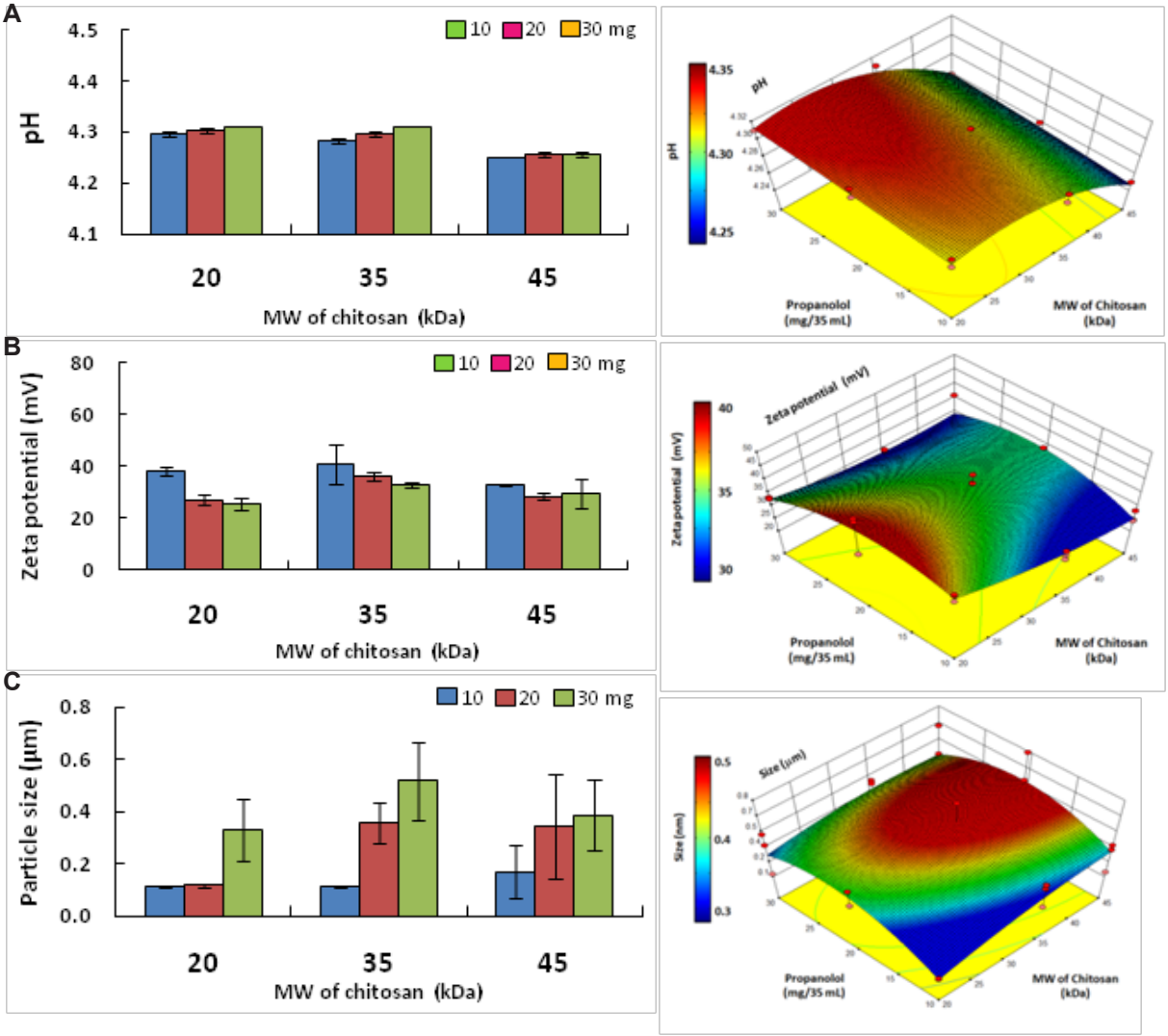

Figure 1 The influences of the formulation factor on (A) pH, (B) zeta potential and (C) particle size of propranolol-loaded chitosan nanoparticles analyzed using (left) the conventional comparative method and (right) the response surface method

Data analysis: The dataset obtained from the investigation was analyzed using a conventional comparative and response surface method. The response surfaces of all response variables were evaluated and sketched using the Design Expert® Software, Version 9 (Stat-Ease, Inc., MN,
U.S.A., No.54B0913). The best fitting mathematical curves (linear, cubic, special cubic and quadratic) were revealed based on the summary statistics for the model, which were the standard deviation (SD), the multiple correlation coefficient (R^2), the adjusted multiple correlation coefficient (adjusted R^2), the predicted multiple correlation coefficient (predicted R^2) and the Adequate precision. These statistics were all verified with the Design Expert® Software.

Results and Discussion

The influences of the formulation factors (i.e. molecular weight of chitosan and concentrations of propranolol HCl) on physical properties (i.e. pH, zeta potential and particle size), are shown in Figure 1. The response surface method showed the relationship between the formulation factors and the physical properties of nanoparticles were more markedly than the conventional comparative method (clustered column charts). Since, the response surface of response surface method can predict influences of the formulation factors on the physical properties of other nanoparticles over the model formulations. The reliability of the response surface was evaluated and shown in Figure 2.

The charts of pH indicated that the pH of the nanoparticles gradually increased with increasing concentration of propranolol. Whiles, the increase in the molecular weight of chitosan resulted in a decrease of pH of the nanoparticles systems. These results agreed well with the response surface of pH, which suggested that an increase in the concentration of propranolol led to an increase in the pH. The zeta potential of all nanoparticles showed a positive charge. Under the experimental condition, the pH of the system was approximately 4.3, the chitosan and the propranolol may exhibit the positive charge due to the pKa of chitosan and propranolol were around 6.2-6.5 and 9.4-9.5, respectively. However, the increase in concentration of propranolol, the zeta potential was decreased due to the competitiveness of unoccupied amine group between chitosan and propranolol on the negative surface charge of the particles. So, it was attributed to the difficulty in interaction with negative charge of TPP molecules. The zeta potential of 35 kDa-chitosan nanoparticles displayed the highest value when comparing with the other molecular weight of chitosan nanoparticles. The 35 kDa-chitosan molecules may probably adopt a spread conformation in solution more than the other molecular weights of chitosan, because the electrostatic repulsion force existing between amine groups along the molecular chain of 35 kDa-chitosan molecules affect the zeta potential higher than the other molecular weights of chitosan.[6] The relationship between the molecular weight of chitosan and the concentration of propranolol on zeta potential using the response surface method were more clearly comprehensible than the result obtained from the charts. The chart of particle size revealed that the particle size of the nanoparticles was significantly increased when increasing the concentration of propranolol at all molecular weight of chitosan. This result was corresponding with the response surface of particle size. The particle size of the colloid was in 0.115 - 0.520 µm size range. The increase in particle size correlated with the reduction in zeta potential such that the particle became closer and hence the larger size obtained.[7)] Therefore, the optimum concentrations of chitosan, TPP and propranolol contributed to the balance between the attractive force and repulsive force from the anionic and cationic molecules leading to the formation of the nanoparticles.

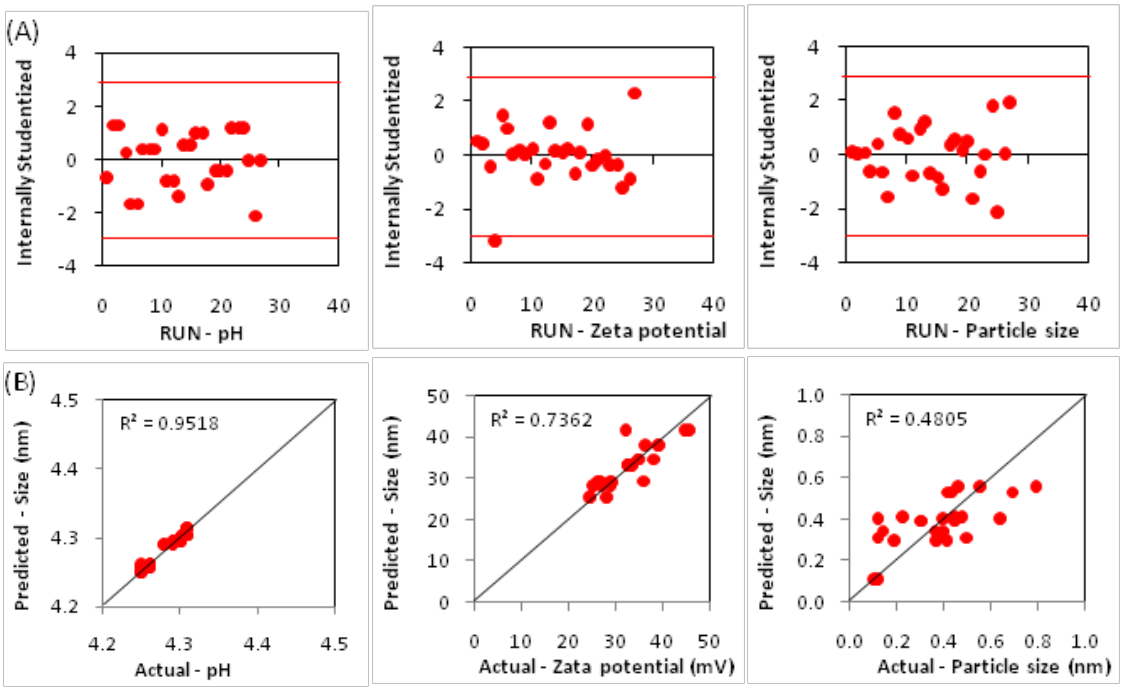

Figure 2 (A) the corresponding residual plot between run number and internally studentized residuals and (B) the linear correlation plot between the predicted and actual values for various physical properties

Moreover, the term of significant model, regression coefficient value, analysis of variance and adequate precision, for the physical properties are shown in Table 1.The quadratic model for all properties was significant (except particle size), and the model maximized the R^2 coefficients. Adequate precisions of 23.6493 (pH), 10.5908 (zeta potential), and 6.4022 (particle size) indicated an adequate signal/noise ratio (an adequate precision ratio greater than 4 is desirable). Non-significant lack of fit values represented a good model in this study. The results indicated that the formulation factors with a significant influence on pH were X_1 and X_2. The X_1 supported a positive influence (coefficient = 0.0078), as the increase in X_1 leads to increase in pH. While, the X_2 supported a negative influence (coefficient = -0.0243), as the increase in X_2 leads to decrease in pH. The formulation factors with a significant influence on zeta potential were X_1 and X_2. Both formulation factors (X_1 and X_2) supported a negative influence (coefficient = -3.9883 and -0.1283, respectively), as the increase in formulation factors leads to decrease in zeta potential. Furthermore, the X_1 and X_2 supported a positive influence on particle size (coefficient = 0.0797 and 0.0642, respectively), however the statistical analysis show not significantly different.

Table 1 Terms of the significant model, regression coefficient value, and analysis of variance (p value) for the response variables

Polynomial term	pH		Zeta potential		Particle size	
	coefficient	p value	coefficient	p value	coefficient	p value
Model	Quadratic	<0.0001*	Quadratic	<0.0001*	Quadratic	0.01193*
Intercept	4.2958	-	35.4059	-	0.5183	-
X_1: MW of chaitosan	0.0078	<0.0001*	-3.9883	<0.0001*	0.0797	0.0319
X_2: Propranolol conc.	-0.0243	<0.0001*	-0.1283	<0.0001*	0.0642	0.0794
X_1X_2	-0.0015	0.3635	2.2913	0.0235	-0.0380	0.3785
X_1^2	0.0004	0.8530	2.0817	0.1521	-0.0441	0.4914
X_2^2	-0.0178	<0.0001*	-0.6196	0.0001	-0.1827	0.0062
R^2	0.9518	-	0.7362	-	0.4805	-
Adjusted R^2	0.9403	-	0.6734	-	0.3568	-
Predicted R^2	0.9217	-	0.5736	-	0.1161	-
Adequate precision	23.6493	-	10.5908	-	6.4022	-
Lack of Fit	-	0.0165	-	0.7605	-	0.7850

Conclusion

The formulation factors i.e. the molecular weight of chitosan and the concentrations of propranolol significantly affected the physical properties, i.e. pH, zeta potential and particle size of the nanoparticles. Using the response surface method, the relationship between the formulation factors and the physical properties was clearly understood. In this study, we were successful in showing the feasibility of the development of transmucosal delivery of propranolol using nanoparticles. Further study is required to confirm the potential of nanoparticels by *in vitro and in vivo* drug penetration study.

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