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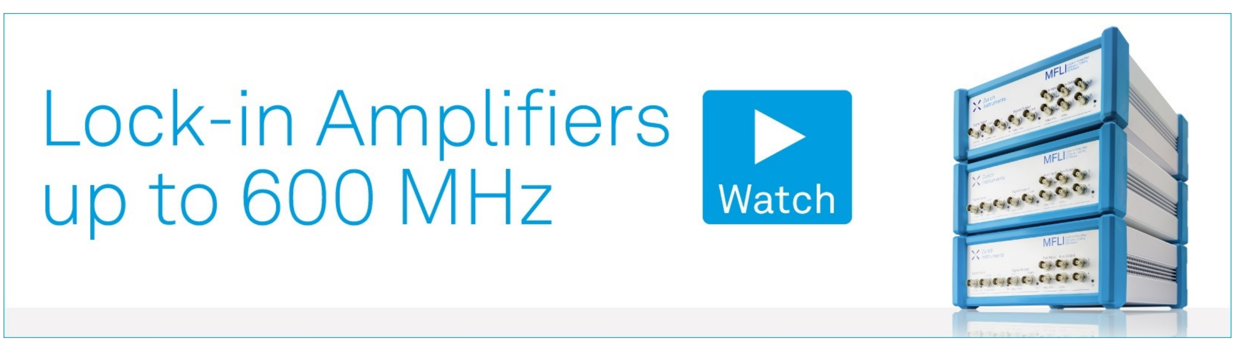

Determination of the Optimum Composition to Produce Minimum Particle Size of β-carotene Microencapsulated in Acid Hydrolyzed Starch-Chitosan/TPP (Tripolyphosphate) Matrices Using Taguchi Method

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Abstract. In this study, the combined factors of acid hydrolyzed starch-to-chitosan weight ratio (weight ratio SC), β-carotene addition level, and tripolyphosphate ion (TPP) addition level were investigated to produce minimum particles size of β-carotene microencapsulated in acid hydrolyzed starch-chitosan/TPP matrices using a precipitation method. For this purpose, the design of experiment Taguchi has been applied. The results showed that the optimum composition to produce the smallest particle size was obtained at the weight ratio SC 1:1, β-carotene addition level 10 mg and TPP addition level 600 mg. The three factors above had significant influences on the resulting particle size with rank from the largest to the smallest was weight ratio SC, TPP addition level, and β-carotene addition level respectively. The results of the confirmation test were in line with the calculation from the full factorial design of experiment. These confirmed that the Taguchi method could be a useful tool to determine the optimum composition in producing an optimum particle size of microencapsulation product using limited data.

INTRODUCTION

Drug delivery system remains to be an interesting topic to study up to present days. The success of drug therapy is not only determined by the efficacy of the drug, but also by the delivery of the drug to the target site in the human body. Particle size parameters are important in drug dissolution and drug absorption. Smaller particle size has a wider surface area that is easier for the drug to dissolve by increasing wetted area. Additionally, the smaller particle size also increases drug solubility and hence it increases, in turn, the absorption of the drug in target site [1].

In our previous study [2], we found that native starch-chitosan/TPP combination was a compatible matrix that could be used to encapsulate β-carotene, poorly soluble drug. This matrix consists of natural polymers and has the advantage of being able to produce encapsulated β-carotene particles in micron size through a precipitation process.

Taguchi experimental design or generally called robust design is the one used in engineering to optimize process parameters in an effective- and efficiently producing products [3]. Taguchi method is good to optimize experimental parameters as it reduces the number of experiments and useful to screen the influence rank of factors in determining the size of silica nanoparticles [4]. The method has been used in previous studies to investigate production condition in preparing the smallest particle size of bovine serum albumin (BSA) nanoparticles [5]. The combination of the determinant factors of the BSA particle size can be predicted even though the combination has not been applied in Taguchi orthogonal array. Taguchi prediction value can be very close to experimental confirmation value, with insignificant deviation value.

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In the present study, the Taguchi experimental design will be applied to determine the combination of composition factors in generating the smallest particle size of β-carotene encapsulated in the acid hydrolyzed starch-chitosan/TPP matrices, including weight ratio of acid hydrolyzed starch-to-chitosan (weight ratio SC), β-carotene addition level, and TPP addition level. The results of the determination of the optimum particle size will be confirmed using a dynamic light scattering (DLS) measurement. Proving that Taguchi design can be used in particle size optimization with limited data of the study, the results of the experiments will be compared to the value resulting from a full factorial experimental design.

EXPERIMENTAL

Materials

β-carotene, corn starch, chitosan and TPP from Sigma Aldrich, glacial acetic acid, HCl, and absolute ethanol from Merck, ethanol 96%, demineralized water.

Methods

The method used in this study was in accordance with the method in the previous study [2].

β-carotene microencapsulation in the acid hydrolyzed starch-chitosan/TPP matrix

The microencapsulation process of β-carotene in the acid hydrolyzed starch-chitosan/TPP matrix was carried out in a similar way to our previous study 2 which followed the steps in Kim and Huber's research 6. A blending matrix was prepared before the microencapsulation step. The matrix consisted of a mixture of acid hydrolyzed starch, chitosan, and TPP. The acid hydrolyzed starch was synthesized using 0.15 M HCl 7. Twenty mL of a certain concentration of TPP solution was added to 1% (w/v) acid hydrolyzed starch dispersion according to the weight ratio SC as described in Table 1. A certain amount of cationic solution (1% (w/v) chitosan) according to weight ratio SC was added to the TPP-acid hydrolyzed starch mixture under magnetic stirring.

Once the matrix mixture has been prepared, a 220 mL mixture of the matrix was heated in a water bath at 90 °C for 5 minutes under magnetic stirring. A hundred mL of a certain concentration of β-carotene dissolved in ethanol (Table 1) was added dropwise under continuous stirring. The additions of β-carotene dissolved in ethanol were divided into 2 steps, the first was in temperature 90 °C, and the second was in room temperature. The mixture was then centrifuged (7000×g, 10 min) to recover the encapsulation products. The β-carotene encapsulated in the acid hydrolyzed starch-chitosan/TPP matrices was dried using freeze-dryer for 13 hours, crushed, and sieved through a 200 mesh sieve.

TABLE 1. Composition of matrices

Sample code*	Weight ratio SC	β-carotene conc. (mg/200mL)	TPP conc. (%)	Volume of 1% acid hydrolyzed starch dispersion (mL)	Volume of 1% chitosan solution (mL)	Volume of TPP solution (mL)
HSC1:1B10T400	1:1	10	2	100	100	20
HSC1:1B20T600	1:1	20	3	100	100	20
HSC4:1B10T600	4:1	10	3	160	40	20
HSC4:1B20T400	4:1	20	2	160	40	20
HSC4:1B10T400	4:1	10	2	160	40	20
HSC1:1B10T600	1:1	10	3	100	100	20
HSC4:1B20T600	4:1	20	3	160	40	20
HSC1:1B20T400	1:1	20	2	100	100	20

*sample codes were designated as follows: the HSC letters abbreviate hydrolyzed starch-chitosan, the numbers following the HSC letters were the weight ratio SC, the B letter represented β-carotene, the number after the B letter was the addition level of β-carotene, the T letter was stand for TPP, and the number at the end of the sample code denoted he TPP addition level.

Analysis of Particle Size

The size of the micro particles was determined by Horiba SZ-100 (Horiba, Ltd., Japan). The product of microencapsulation was added demineralized water then homogenized for 1 minute and analyzed using DLS.

Statistical Analysis

Statistical analysis in this study was carried out using the Minitab for Windows 19 software with the following steps: (1) Select the L4 orthogonal array in design of experiment Taguchi, (2) Input data into Minitab for analysis of ANOVA general linear model, (3) Input data into Minitab for Taguchi analysis, (4) Analyze data, (5) Confirm the results of Taguchi design using the full factorial design, (6) Interpret the results, and (7) Draw conclusions.

RESULTS AND DISCUSSION

This study investigated the optimum formulation in producing the minimum particle size of β-carotene encapsulated in the acid hydrolyzed starch-chitosan/TPP. We selected three critical factors in two levels for each that affected particle size: the weight ratio SC, β-carotene addition level, and TPP addition level according to the preliminary experiment. The first step in analyzing data was made using Taguchi statistical experiment design by choosing an orthogonal array that fitted the existing resources. We used L4 orthogonal array in Taguchi. Table 2 contained the experiment results of the particle size measurement using DLS instrument in duplicate replications.

TABLE 2. Results of particle size measurements with the DLS instrument

Sample Code	Weight ratio SC	β-carotene addition level (mg)	TPP addition level (mg)	Particle size 1 (nm)	Particle size 2 (nm)	Average Particle size (nm)
HSC1:1B10T400	1:1	10	400	861.6	815.7	838.7
HSC1:1B20T600	1:1	20	600	823.4	848.0	835.7
HSC4:1B10T600	4:1	10	600	934.2	958.2	946.2
HSC4:1B20T400	4:1	20	400	1231.6	1433.5	1332.6

ANOVA analysis was made to find out significant differences among the independent factors affecting the particle size of the β-carotene encapsulated in the acid hydrolyzed starch-chitosan/TPP. Table 3 listed the results of the ANOVA analysis. The P-value of the ANOVA results of the factors of the weight ratio SC, the β-carotene addition level, and the TPP addition level was below 0.05. It meant that the factors of the weight ratio SC, the β-carotene addition level, and the TPP addition level in the study had significant effect on the particle size in the following respective order from the most significant to the least significant: the weight ratio SC, the TPP addition level, and the β-carotene addition level.

TABLE 3. ANOVA analysis results

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Weight ratio SC	1	182650	182650	33.17	0.005
BC addition level	1	73498	73498	13.35	0.022
TPP addition level	1	75777	75777	13.76	0.021
Error	4	22026	5506		
Total	7	353950			

The next step was determining the optimum compositions that generated a minimum particle size using the Taguchi method. Response table for the signal to noise ratios was shown in Table 4, meanwhile, Table 5 depicted the response table for means.

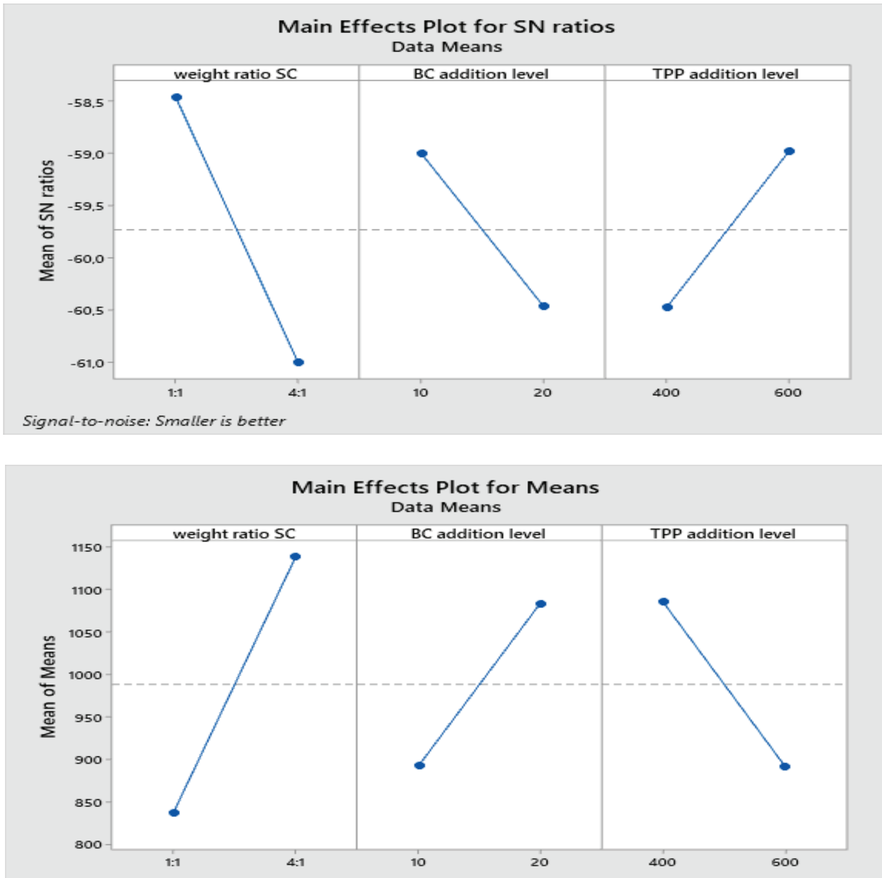

FIGURE 1. Main effect plot for SN ratios (a) and main effect plot for means (b)

TABLE 4. Response table for the signal to noise ratios (smaller is better) using the design of experiment Taguchi

Level	Weight ratio SC	β-carotene addition level	TPP addition level
1	-58.46	-59.00	-60.48
2	-61.01	-60.47	-58.98
Delta	2.55	1.47	1.50
Rank	1	3	2

TABLE 5. Response table for means using the design of experiment Taguchi

Level	Weight ratio SC	β-carotene addition level	TPP addition level
1	837.2	892.4	1085.6
2	1139.4	1084.1	891.0
Delta	302.2	191.7	194.6
Rank	1	3	2

Taguchi rank results were consistent with ANOVA. The respective order of the significance of the effect of the factors affecting the particle size from the most significant to the least significant was: the weight ratio SC, the TPP addition level, and the β-carotene addition level. The graph S/N and the mean values were indicative of the combination of the factors that gave the optimum particle size (in this case the smallest one), which were the weight ratio SC 1:1, the β-carotene addition level of 10 mg, and the TPP addition level of 600 mg.

The Prediction of the Optimum Particle Size

Optimum particle size = 988.3 + (837.2-988.3) + (892.5-988.3) + (891.0-988.3) = 644.0 nm
Confirmation value = 816.3 nm (replication 1) and 804.6 nm (replication 2)

The Confirmation of the Results of the Taguchi Method

The step was made to validate the optimum particle size by conducting experiments with the optimum combination of the factors above. The results of the experiments showed that the particle size at the weight ratio SC 1:1, in the β-carotene addition level of 10 mg, and the TPP addition level of 600 mg was 810.5 nm (the mean of confirmation value) with the deviation of the Taguchi prediction value of 25.85%. The experimental value had a very significant deviation. Evaluating the results, a set of particle size measurements was carried out using a full factorial design of 3 factors in 2 levels shown in Table 5.

TABLE 6. DLS data for full factorial design

Sample Code	Weight ratio SC	β-carotene addition level (mg)	TPP addition level (mg)	Particle size 1 (nm)	Particle size 2 (nm)	Average Particle size (nm)
HSC1:1B10T400	1:1	10	400	861.6	815.7	838.7
HSC1:1B20T600	1:1	20	600	823.4	848.0	835.7
HSC4:1B10T600	4:1	10	600	934.2	958.2	946.2
HSC4:1B20T400	4:1	20	400	1231.6	1433.5	1332.6
HSC4:1B10T400	4:1	10	400	1141.8	1131.8	1136.8
HSC1:1B10T600	1:1	10	600	816.3	804.6	810.5
HSC4:1B20T600	4:1	20	600	1127.4	1104.0	1115.7
HSC1:1B20T400	1:1	20	400	1046.2	1069.9	1058.1

Table 6 showed that particle size increased with increasing the weight ratio SC, increasing the β-carotene addition level and decreasing the TPP addition level therefore the HSC4:1B20T400 sample has the largest particle size among the samples in this current experiment. The excess starch fraction in the weight ratio SC caused an increase in the number of chains that were freer to conduct a hydrogen bonding with water, thereby increasing the amount of water that was retained in the matrix [8]. Similar results were found in other study [9]. The increase in the addition of non-polar substances (β-carotene) caused the bond between polymers to be less strong thus the polymers were easier to bind to water. Decreasing the degree of TPP crosslinking could increase the probability of a polymer that was not cross-linked to hold a hydrogen bond with water [10]. Increased probability of hydrogen bond with water could increase swelling capability thereby increasing particle size measured by DLS instrument [11].

TABLE 7. The output of response optimization (particle size) using the design of experiment full factorial

Parameters						
Response	**Goal**	**Lower**	**Target**	**Upper**	**Weight**	**Importance**
Particle size	Minimum		804.6	1433.5	1	1

Solution					
Solution	**Weight ratio SC**	**β-carotene addition level**	**TPP addition level**	**Particle size Fit**	**Composite Desirability**
1	1:1	10	600	810.45	0.990698

Multiple Response Prediction	
Variable	**Setting**
weight ratio SC	1:1
β-carotene addition level	10
TPP addition level	600

Response	Fit	SE Fit	95% CI	95% PI
Particle size	810.4	37.7	(723.6; 897.3)	(660.0; 960.9)

The results of the statistical calculations using the full factorial experiment design (Table 7) showed that the optimum particle size (the smallest one) was in the combination of the weight ratio SC 1:1, β-carotene addition level 10 and TPP addition level 600 mg. It showed that the results of the Taguchi calculation using the four experiment data in 2 replications (L4 orthogonal array) was consistent with those of the full factorial experiment design with eight samples in two replications. The results proved that Taguchi design can be applied in the determination of the optimum composition to produce the minimum particle size of the β-carotene encapsulated in the acid hydrolyzed starch-chitosan/TPP using a limited number of experiments. Another experiment also found that Taguchi method is an effective and efficient method in optimizing drug encapsulation [8, 9].

CONCLUSION

The Taguchi L4 orthogonal array has been used to determine the optimum composition of the β-carotene encapsulated in an acid hydrolyzed starch-chitosan/TPP matrix with independent factors were the weight ratio SC, β-carotene addition level, and TPP addition level. The results of ANOVA analysis showed that the three factors significantly influenced the particle size of the β-carotene encapsulated in the acid hydrolyzed starch-chitosan/TPP matrices. The results of the data processing using the design of experiment Taguchi showed that the smallest particle size resulted from the use of the weight ratio SC 1:1, β-carotene addition level 10 mg and the TPP addition level 600 mg. The results of the comparisons to other experimental designs, namely the full factorial experimental design, showed that the Taguchi method in this study can be used to determine the optimum composition of the weight ratio SC, β-carotene addition level, and TPP addition level that produces the smallest particle size using limited data.

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