CHAPTER IV

RESULTS AND DISCUSSION

1. Preparation of curcuminoids-PLGA nanoparticles using vitamin E TPGS as the stabilizer

The formulation number 1 to 15 of curcuminoids-PLGA nanoparticles were prepared as described in Chapter III. In these formulations, vitamin E TPGS was used as the stabilizer. Each formulation composed of PLGA at different ratio of PLA and PGA (50:50, 75:25 or 85:15), various amounts of curcuminoids (2 %, 6 %, or 10 %) and various concentrations of vitamin E TPGS (3 %, 5 %, or 7 %). The detailed formulations were shown previously in Table 3-2 of Chapter III.

1.1 Determination of Responses of curcuminoids-PLGA nanoparticles using vitamin E TPGS as the stabilizer

The fifteen formulations of curcuminoids-PLGA nanoparticles were subjected to investigation on five responses, which were % recovery, particle size, polydispersity index, and % curcuminoids content and entrapment efficiency. The results were summarized in Table 4-1.

According to Table 4-1, it was found that % recoveries of nanoparticles were slightly different among all formulations, ranging from 61.31 to 68.68 %. The particle sizes of nanoparticles of all formulations were almost similar with the mean of 300-330 nm. The polydispersity indexes were also slightly different from each other. The values were between 0.59 and 0.84, indicating the fairly broad distribution of particle size. The % curcuminoids content and entrapment efficiency of all formulations were quite low. The % curcuminoids contents varied from 0.02 to 0.14 % while the entrapment efficiency ranged from 0.59 % to 1.85 %.

Table 4-1. Responses of curcuminoids-PLGA nanoparticles using vitamin E TPGS as the stabilizer (formulation number 1 to 15)

Number	Responses				
	Recovery (%)	Mean Particle Size (nm)	Polydispersity Index	Curcuminoids Content (%)	Entrapment Efficiency (%)
1	65.26	307	0.59	0.0244	0.80
2	66.11	320	0.83	0.0972	1.07
3	64.82	320	0.77	0.0625	0.67
4	65.93	320	0.74	0.1101	0.73
5	68.68	330	0.84	0.0540	1.85
6	62.5	313	0.75	0.0454	1.41
7	66.42	320	0.81	0.0950	1.05
8	61.31	320	0.71	0.0952	0.97
9	62.08	300	0.69	0.0864	0.89
10	62.26	320	0.72	0.1209	0.75
11	63.54	310	0.73	0.0932	0.59
12	61.35	306	0.76	0.0430	1.32
13	67.54	320	0.77	0.0984	1.11
14	63.99	310	0.81	0.0618	0.66
15	68.02	303	0.84	0.1389	0.94

1.2 Effect of PLA-PGA ratio, curcuminoids amounts, and vitamin E TPGS concentration on the nanoparticles recovery

According to Table 4-1, the % recovery of nanoparticles were statistically analyzed, in order to correlate with the variables of formulation ingredients, which were PLA-PGA ratio of PLGA (A), curcuminoids amount (B) and vitamin E TPGS concentration (C). The fit summary of regression models to the data was shown in Table Appendix D-1. The sequential model sum of squares of all models gave small F-value and the large p-values, which were greater than 0.05. This implied that the models were not significantly fitted with the data. In addition, the model summary statistic results indicated that the adjusted-R^2 of all models were not close to 1, suggesting that the models were not appropriate. Moreover, both data transformation and model reduction were used in order to correlate the % recovery with the three variables but neither of them could improve the fit of all models to the data (the results of data transformation are not shown). The analysis of variance (ANOVA) output with model reduction was shown in Table Appendix D-2. The correlation equation between % recovery and the three variables was shown in Equation 4-1.

Final Equation: **Recovery (%) = + 64.6540** (Equation 4-1)

It was found that all model terms (A, B and C) were eliminated, suggesting that these terms were not significant in the regression model. The final equation as shown in Equation 4-1 composed of only a constant value, which was the intercept or the average value of % recovery of all 15 formulations. In other words, the equation implied that the difference of % recovery of the formulation number 1 to 15 were not statistically significant.

According to the results above, it can be interpreted that there was no effect of PLA-PGA ratio, curcuminoids amount, and vitamin E TPGS concentration, within the range used in this experiment, on the % recovery of curcuminoids-PLGA nanoparticles using vitamin E TPGS as the stabilizer. It is possible that the % recovery of nanopraticles is dependent on other parameters, e.g. the preparation method, separation procedure, etc., rather than these three formulation ingredients.

1.3 Effect of PLA-PGA ratio, curcuminoids amounts, and vitamin E TPGS concentration on the particle size

According to Table 4-1, the mean particle sizes of nanoparticles were statistically analyzed, in order to correlate with the variables of formulation ingredients, which were PLA-PGA ratio of PLGA (A), curcuminoids amount (B) and vitamin E TPGS concentration (C). The fit summary of regression models to the data was shown in Table Appendix D-3. The sequential model sum of squares of all models gave small F-value and the large p-values, which were greater than 0.05. This implied that the models were not significantly fitted with the data. In addition, the model summary statistic results indicated that the adjusted-R^2 of all models were not close to 1, suggesting that the models were not appropriate. Furthermore, both data transformation and model reduction were used in order to correlate the mean particle size with the three variables but neither of them could improve the fit of all models to the data (the results of data transformation are not shown). The analysis of variance (ANOVA) output with model reduction was shown in Table Appendix D-4. The correlation equation between the mean particle size and the three variables was shown in Equation 4-2.

Final Equation: **Mean particle size (nm) = + 314.7333** (Equation 4-2)

It was found that all model terms (A, B and C) were eliminated, suggesting that these terms were not significant in the regression model. The final equation as shown in Equation 4-2 composed of only a constant value, which was the intercept or the average value of the mean particle size of all 15 formulations. In other words, the equation implied that the difference of the mean particle size of the formulation number 1 to 15 were not statistically significant.

According to the results above, it can be interpreted that there was no effect of PLA-PGA ratio, curcuminoids amount, and vitamin E TPGS concentration, within the range used in this experiment, on the particle size of curcuminoids-PLGA nanoparticles using vitamin E TPGS as the stabilizer. An extension of experimental range might be useful for further study dealing with this response. On the other hand, the particle size of nanopraticles might be dependent on other parameters, e.g. the preparation method, etc., rather than these three formulation ingredients.

1.4 Effect of PLA-PGA ratio, curcuminoids amounts, and vitamin E TPGS concentration on the particle size distribution

According to Table 4-1, the particle size distribution of nanoparticles were statistically analyzed, in order to correlate with the variables of formulation ingredients, which were PLA-PGA ratio of PLGA (A), curcuminoids amount (B) and vitamin E TPGS concentration (C). The model fit results were summarized in Table Appendix D-5. The sequential model sum of squares of all models gave small F-value and the large p-values, which were greater than 0.05. This implied that the models were not significantly fitted with the data. In addition, the model summary statistic results provided adjusted-R^2 of all models were not close to 1, indicating that the models were not appropriate.

Consequently, both data transformation and model reduction were used in order to correlate the particle size distribution with the three variables but neither of them could improve the fit of all models to the data (the results of data transformation are not shown). The analysis of variance (ANOVA) output with model reduction was shown in Table Appendix D-6. The correlation equation between the particle size distribution and the three variables was shown in Equation 4-3.

Final Equation: **Particle size distribution = + 0.75733** (Equation 4-3)

It was found that all model terms (A, B and C) were eliminated, suggesting that these terms were not significant in the regression model. The final equation as shown in Equation 4-3 composed of only a constant value, which was the intercept or the average value of particle size distribution of the 15 formulations. In other words, the equation implied that the difference of the particle size distribution of the formulation number 1 to 15 were not statistically significant.

According to the results above, it can be concluded that there was no effect of PLA-PGA ratio, curcuminoids amount, and vitamin E TPGS concentration, within the range used in this experiment, on the particle size distribution of curcuminoids-PLGA nanoparticles using vitamin E TPGS as the stabilizer. An extension of experimental range might be needed for further study dealing with this response. On the contrary, the particle size distribution of nanopraticles might be dependent on other parameters, e.g. the preparation method, etc., rather than these three formulation ingredients.

1.5 Effect of PLA-PGA ratio, curcuminoids amounts, and vitamin E TPGS concentration on the curcuminoids content of nanoparticles

According to Table 4-1, the % curcuminoids content of nanoparticles were statistically analyzed, in order to correlate with the variables of formulation ingredients, which were the PLA-PGA ratio of PLGA (A), curcuminoids amount (B) and vitamin E TPGS concentration (C). The model fit results were summarized in Table Appendix D-7. The sequential model sum of squares exhibited tnat the linear model gave the relatively high F-value and the small p-value, which was less than 0.05 ($p < 0.0001$), while the 2FI and quadratic models gave small F-values and the large p-values, which were greater than 0.05. In addition, the lack of fit test and model summary statistic results expressed that the linear model provided p-value of 0.1369 (>0.05) and adjusted-R^2 of 0.8660 (close to 1). This implied that the linear model was significantly fitted with the data, thus the linear model was appropriate for these data. An attempt to correlate the % curcuminoids content with the transformed data did not give better fit of any model (the data are not shown).

The analysis of variance (ANOVA) output with model reduction was shown in Table Appendix D-8. The correlation equations between % curcuminoids content and the three variables, in terms of coded factors and actual factors, were shown in Equation 4-4 and 4-5, respectively.

Final Equation in Terms of Coded Factors:

$$\textbf{Content} = +0.082 + 0.037\ B - 0.013\ C \qquad \text{(Equation 4-4)}$$

Final Equation in Terms of Actual Factors:

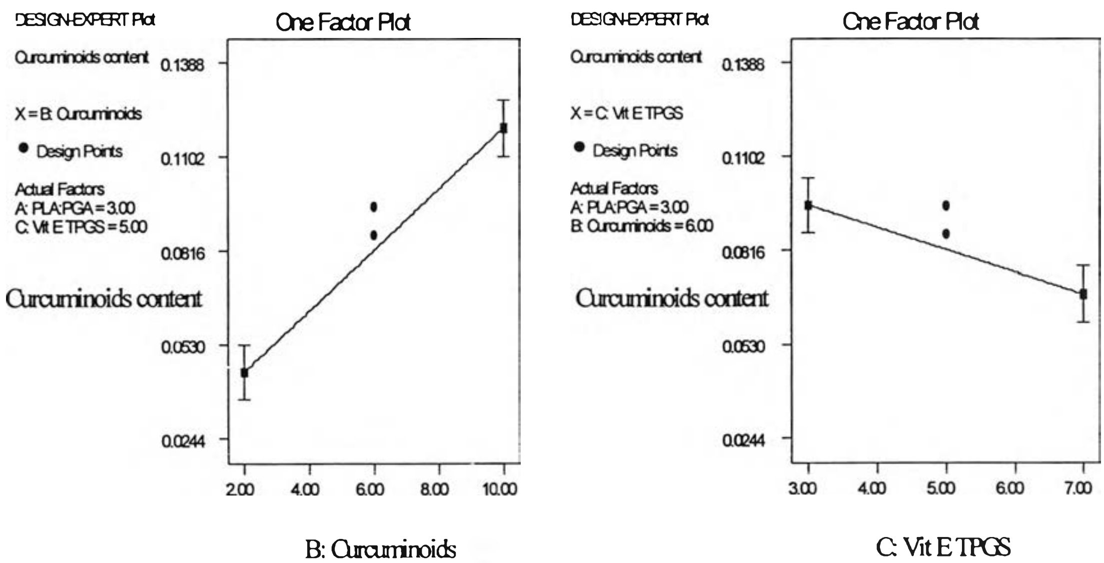
$$\textbf{Content} = +0.059828 + 9.25625 \times 10^{-3}(\text{Curcuminoids}) - 6.72500 \times 10^{-3}(\text{Vit E TPGS}) \qquad \text{(Equation 4-5)}$$

Where: Curcuminoids = %curcuminoids loaded in the formulation

Vit E TPGS = %vitamin E TPGS concentration

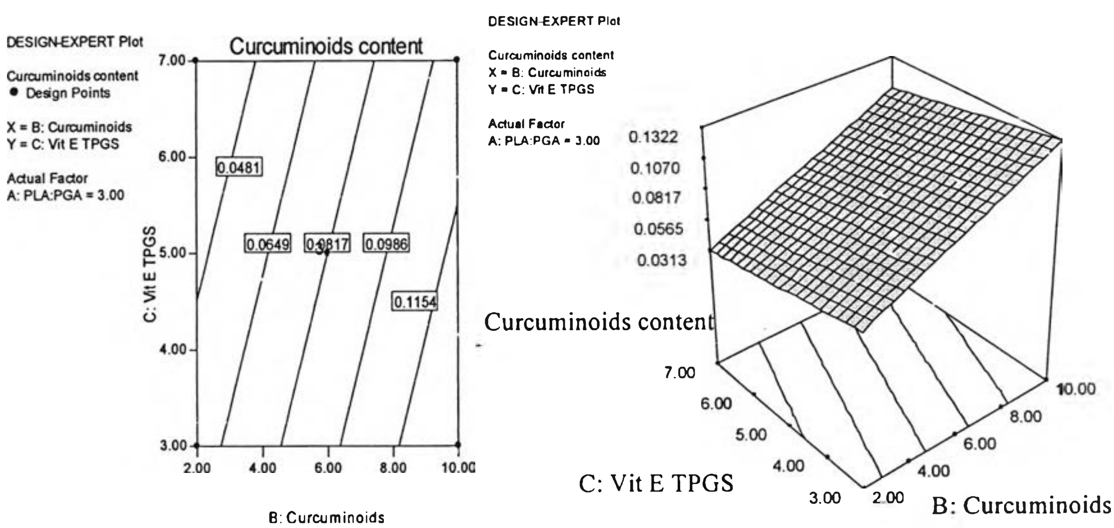
According to the equation, it was found that only the curcuminoids amount (B) and the vitamin E TPGS concentration (C) remained in the equation, suggesting that these terms were significant in the regression model. From the results above, it can be interpreted that the curcuminoids content in curcuminoids-PLGA nanoparticles was increased as an increase of % curcuminoids loaded in the formulations. It is possible that the amount of curcuminoids used in the formulation (2-10%) did not reach the maximum capacity to entrap the curcuminoids of nanoparticles. However, the curcuminoids content in PLGA nanoparticles was reduced as an increase of vitamin E TPGS concentration. This may be due to the emulsifying effect of vitamin E TPGS, causing an increase of solubility of curcuminoids in the aqueous phase during the preparation and, hence, lower the amount of curcuminods entrapped in the nanoparticles.

The % curcuminoids content found in the nanoparticles was plotted against %curcuminoids loaded in the formulation (Figure 4-1a) and against vitamin E TPGS concentration (Figure 4-1b). The Contour and 3-D plots of the %curcuminoids content versus % curcuminoids loading and vitamin E TPGS concentration were shown in Figure 4-2a and 4-2b, respectively.



4-1a 4-1b

Figure 4-1. Plot % curcuminoids content gainst % curcuminoids loaded in the formulation (4-1a) and against vitamin E TPGS concentration (4-1b).



4-2a 4-2b

Figure 4-2. Contour plot (4-2a) and 3-D plot (4-2b) of the %curcuminoids content versus % curcuminoids loading and vitamin E TPGS concentration

1.6 Effect of PLA:PGA ratio, % curcuminoids, and vitamin E TPGS concentration on the entrapment efficiency

According to Table 4-1, the entrapment efficiency of nanoparticles were statistically analyzed, in order to correlate with the variables of formulation ingredients, which were the PLA-PGA ratio of PLGA (A), curcuminoids amount (B) and vitamin E TPGS concentration (C). The model fit results were summarized in Table Appendix D-9. The sequential model sum of squares exhibited that linear model gave the relatively high F-value and the small p-value, which was less than 0.05 ($p = 0.0089$), while the 2FI and quadratic models gave small F-values and the large p-values, which were greater than 0.05. In addition, the lack of fit test and model summary statistic results expressed that the linear model provided p-value of 0.0929 (>0.05) and adjusted-R^2 of 0.5383, which was greater than the adjusted-R^2 of the 2FI and quadratic models. This implied that the linear model was more appropriate for these data than the 2FI and quadratic model. However, the adjusted-R^2 of the linear model was still fairly low. All forms of the data transformation could not provide better fit of any model (the data are not shown). Therefore, only the model reduction was used to improve the fit of the linear regression model.

The analysis of variance (ANOVA) output with model reduction was shown in Table Appendix D-10. The correlation equations between the entrapment efficiency and the three variables, in terms of coded factors and actual factors, were shown in Equation 4-6 and 4-7, respectively.

Final Equation in Terms of Coded Factors:

Entrapment Efficiency = +0.99 - 0.30 B (Equation 4-6)

Final Equation in Terms of Actual Factors:

Entrapment Efficiency = **+1.43182**

- 0.074172 (Curcuminoids) (Equation 4-7)

Where: Curcuminoids = % curcuminoids loaded in the formulation

Vit E TPGS = % vitamin E TPGS concentration

According to the equation, it was found that only the curcuminoids amount (B) remained in the equation, suggesting that this term was significant in the regression model. From the results above, it can be interpreted that the entrapment efficiency of the curcuminoids-PLGA nanoparticles decreased as an increase of % curcuminoids loaded in the formulations. It was in conflict with the case of curcuminoids content, which increased as the increase of curcuminoids amount loaded in the formulation. This is possible that only some of the entire increased amount of curcuminoids in the formulation could be entrapped. In other words, the rate of increase of curcuminoids content might be not proportional to the rate of increase of curcuminoids amount loaded in the formulation.

The plot of the entrapment efficiency of the nanoparticles against %curcuminoids loaded in the formulation is shown in Figure 4-3.

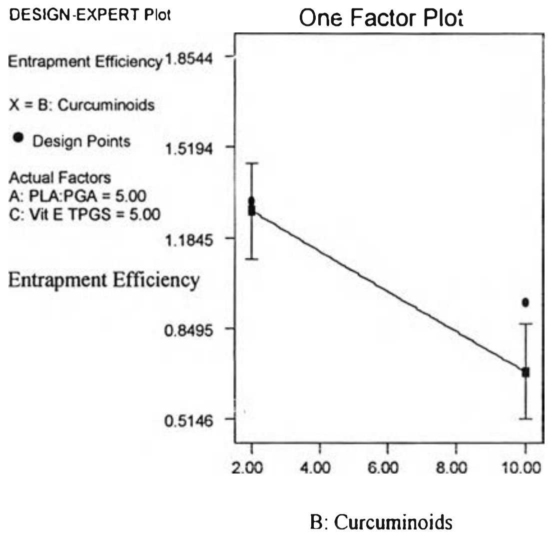


Figure 4-3. Plots of the entrapment efficiency of the nanoparticles against %curcuminoids loaded in the formulation

1.7 Optimization of curcuminoids-PLGA nanoparticles preparation usingvitamin E TPGS as the stabilizer

According to the statistical results of the effects of the PLA-PGA ratio of PLGA, curcuminoids amount loaded in the formulation and vitamin E TPGS concentration on five responses as described in 1.1 to 1.6, it was attempted to determine a suitable formulation that could provide the satisfactory responses. The method to obtain such optimized formulation was the use of simultaneous optimization technique, which correlated all effects with all responses simultaneously. The formulation optimization was performed using desirability function approach, in which the goal of both independent (factor) and dependent variables (response) were set. The upper and lower limits of all parameters were depending upon the upper and lower value of all criteria. The setting criteria were summarized under the "constraints" section in the Table 4-2. For example, the PLA-PGA ratio could be any ratio between 1 and 5 and, hence, the goal should be “is in range”. In case of % recovery, the values were between 60 and 70 but the higher % recovery was preferable and, hence, the goal should be “maximize”. In details, The PLA-PGA ratio, curcuminoids amount, and vitamin E TPGS concentration were set as “is in range” of 1 to 5, 2 to 10, and 3 to 7, respectively. The % recovery of nanoparticles was set to be “maximize” between 60 and 70 %. The particle size and the polydispersity index were set to be “minimize” within the range of 300 to 330, and 0.5 to 0.8, respectively. Curcuminoids content and entrapment efficiency were set to be “maximize” within the range of 0.02 to 0.2 %, and 0.5 to 2 %, respectively. The upper and lower values of all effects and responses were equally weighed. All effects and responses were considered to be equally important in the selection process for optimal formulations. Three optimal formulations of curcuminoids-PLGA nanoparticles were achieved according to the highest desirability as seen under the “solutions” in Table 4-2. The desirability values of all three optimal formulations were equal to 0.497. The first formulation composed of PLGA with PLA-PGA ratio of 50:50, curcuminoids amount of 10.00 % and vitaminE TPGS concentration of 3.00 %. The second formulation composed of PLGA with PLA-PGA ratio of 75:25, curcuminoids amount of 9.96 % and vitaminE TPGS concentration of 3.00 %. The last formulation composed of PLGA with PLA-PGA ratio 85:15, curcuminoids amount 9.94 % and vitaminE TPGS concentration 3.00 %. Although all three formulations gave the same desirability value, the formulation containing PLGA with the PLA-PGA ratio of 50:50 was chosen. This was due to the notice that the formulations using poloxamer 407 and polyvinyl alcohol (PVA) as stabilizer provided the optimal formulations using PLGA with PLA-PGA ratio of 50:50 (see further details in 2.7 and 3.7). In addition, the higher PGA monomer in the PLGA provided more hydrophilicity of the copolymer and, hence, the ratio of PLA-PGA of 50:50, compared to 75:25 and 85:15, was easily prepared following this method in which water miscible solvents i.e. methanol, was used.

Table 4-2. Design-Expert® output of the simultaneous optimization of curcuminoids-PLGA nanoparticles using vitamin E TPGS as the stabilizer

Constraints

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
PLA-PGA ratio	is in range	1	5	1	1	3
Curcuminoids amount	is in range	2	10	1	1	3
Vit E TPGS concentration	is in range	3	7	1	1	3
% Recovery	maximize	60	70	1	1	3
Particle Size	is in range	300	330	1	1	3
Polydispersity	minimize	0.5	0.8	1	1	3
Curcuminoids content	maximize	0.02	0.2	1	1	3
Entrapment efficiency	minimize	0.1	2.0	1	1	3

Solutions

No.	PLGA	Curcuminoids	Vit E TPGS	Recovery	Size	Polydispersity	Content	Entrapment	Desirability
1.	1	10.00	3.00	64.65	314.73	0.76	0.1322	1.179	0.497
2.	3	9.96	3.00	64.65	314.73	0.76	0.1319	1.176	0.497
3.	5	9.94	3.00	64.65	314.73	0.76	0.1317	1.174	0.497

2. Preparation of curcuminoids-PLGA nanoparticles using poloxamer 407 as the stabilizer

The formulation number 16 to 30 of curcuminoids-PLGA nanoparticles were prepared as described in Chapter III. In these formulations, poloxamer 407 was used as the stabilizer. Each formulation composed of PLGA at different ratio of PLA and PGA (50:50, 75:25 or 85:15), various amounts of curcuminoids (2 %, 6 %, or 10 %) and various concentrations of poloxamer 407 (9 %, 12 %, or 15 %). The detailed formulations were shown previously in Table 3-2 of Chapter III.

2.1 Determination of effect of PLA-PGA ratio, curcuminoids amount, and poloxamer 407 concentration on the nanoparticles

Fifteen formulations of PLGA nanoparticles containing curcuminoids were subjected to investigation on five different responses, which were %recovery, mean particle size, polydispersity index, %curcuminoids content, and %entrapment efficiency. The results were summarized in Table 4-3.

According to Table 4-3, it was found that %recoveries of nanoparticles after preparation were slightly different among all formulations, ranging from 46.43 to 57.23%. The particle sizes of nanoparticles obtained from each formulation were different from each other. The mean particle sizes were around 270 to 333 nm. The polydispersity indexes of nanoparticles were between 0.80 and 1.20, indicating the very broad distribution of particle size. The curcuminoids content and entrapment efficiency of all formulations were low, but different from each other. The curcuminoids contents varied from 0.5236 % to 2.9824 %. The entrapment efficiency ranged from 7.13 % to 25.33 %.

Table 4-3. Responses from of curcuminoids-PLGA nanoparticles using poloxamer 407 as stabilizer (Formulation Number 16 to 30)

Formulation Number	Responses				
	Recovery (%)	Mean Particle Size (nm.)	Polydispersity Index	Curcuminoids Content (%)	Entrapment Efficiency (%)
16	53.52	297	1.04	0.6421	17.18
17	52.67	333	1.20	2.2860	20.06
18	55.03	280	0.80	0.9896	9.08
19	54.1	317	1.13	1.6756	9.07
20	46.43	320	1.33	0.9025	20.95
21	56.14	280	0.71	0.5236	14.68
22	50.17	330	0.87	1.3912	11.64
23	57.23	320	0.97	1.3908	13.25
24	55.84	320	1.01	1.1052	10.29
25	48.65	323	1.03	2.9824	14.51
26	51.33	317	0.89	1.3881	7.12
27	55.15	307	1.13	0.7308	20.15
28	56.38	313	1.55	1.9241	18.08
29	51.89	270	1.08	0.9616	8.31
30	52.44	333	0.89	1.8614	9.76

1.8 Effect of PLA-PGA ratio, curcuminoids amounts, and poloxamer 407 concentration on the nanoparticles recovery

According to Table 4-3, the % recovery of nanoparticles were statistically analyzed, in order to correlate with the variables of formulation ingredients, which were the PLA-PGA ratio of PLGA (A), curcuminoids amount (B) and poloxamer 407 concentration (C). The fit summary of regression models to the data was shown in Table Appendix D-11. The sequential model sum of squares of all models gave small F-values and the large p-values, which were greater than 0.05. This implied that the models were not significantly fitted with the data. In addition, the model summary statistic results indicated that the adjusted-R^2 of all models were not close to 1, suggesting that the models were not appropriate. An attempt to correlate the % recovery with the transformed data failed to improve the fit of all models to the data (the data are not shown).

The analysis of variance (ANOVA) output with model reduction was shown in Table Appendix D-12. The correlation equation between the % recovery and the three variables was shown in Equation 4-8.

Final Equation: **Recovery (%) = + 53.1313** (Equation 4-8)

It was found that all model terms (A, B and C) were eliminated, suggesting that these terms were not significant in the regression model. The final equation as shown in Equation 4-8 composed of only a constant value, which was the intercept or the average value of % recovery of the 15 formulations. In other words, the equation implied that the difference of the % recovery of the formulation number 1 to 15 were not statistically significant.

According to the results above, it can be translated that there was no effect of PLA-PGA ratio, curcuminoids amount, and poloxamer 407 concentration, within the range used in this experiment, on the % recovery of curcuminoids-PLGA nanoparticles using poloxamer as the stabilizer. It is possible that the % recovery of nanopraticles might be dependent on other parameters, e.g. the preparation method, separation procedure, etc., rather than these three formulation ingredients.

2.3 Effect of PLA-PGA ratio, curcuminoids amounts, and poloxamer 407 concentration on the mean particle size

According to Table 4-3, the mean particle size of nanoparticles were statistically analyzed, in order to correlate with the variables of formulation ingredients, which were the PLA-PGA ratio of PLGA (A), curcuminoids amount (B) and poloxamer 407 concentration (C). The model fit results were summarized in Table Appendix D-13. The sequential model sum of squares exhibited that linear model gave the relatively high F-value and the small p-value, which was less than 0.05 ($p = 0.0122$), while the 2FI and quadratic models gave small F-values and the large p-values, which were greater than 0.05. In addition, the lack of fit test and model summary statistic results expressed that the linear model provided p-value of 0.1317 (>0.05). This implied that the linear model was significantly fitted with the data, thus the linear model was appropriate for these data. However, adjusted-R^2 of the linear model was fairly low (adjusted-R^2 = 0.5090). An attempt to correlate the mean particle size with the transformed data did not give better fit of any model (the data are not shown). Therefore, only the model reduction was used to improve the fit of the linear regression model.

The analysis of variance (ANOVA) output with model reduction was shown in Table Appendix D-14. The correlation equations between the mean particle size and the three variables, in terms of coded factors and actual factors, were shown in Equation 4-9 and 4-10, respectively.

Final Equation in Terms of Coded Factors:

Mean particle size = +310.67 + 10.75 B - 17.75 C (Equation 4-9)

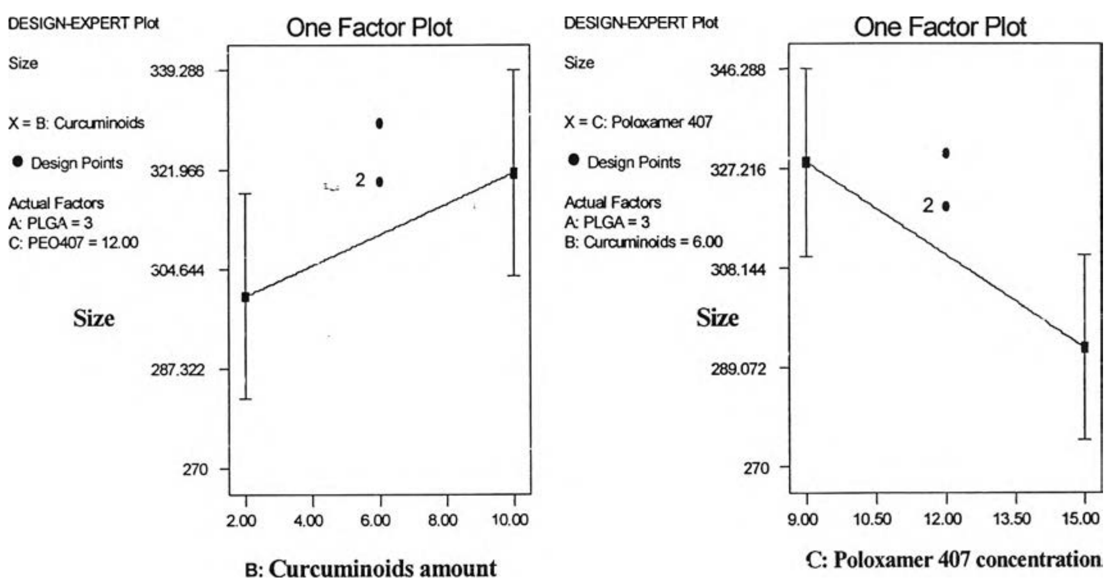
Final Equation in Terms of Actual Factors:

Mean particle size = +365.54167 + 2.68750 (Curcuminoids)
- 5.91667 (Poloxamer 407) (Equation 4-10)

Where: Curcuminoids = %curcuminoids loaded in the formulation
Poloxamer407 = % poloxamer 407 concentration

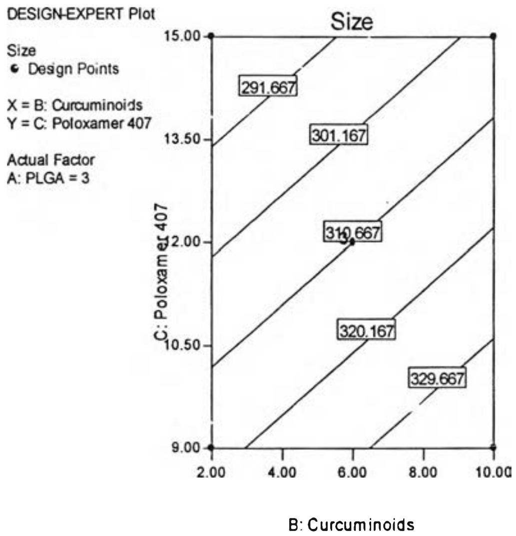
It was found that only the curcuminoids amount (B) and the concentration of poloxamer 407 (C) remained in the equation, suggesting that these terms were significant in the linear regression model. According to the final equations, it was indicated that the mean particle size of curcuminoids-PLGA nanoparticles was increased as an increase of % curcuminoids loaded in the formulations, but was reduced as an increase of . From the results, it is possible that when the amount of curcuminoids in the formulation increase, while the amount of PLGA was constant, the polymer would try to entrap more content by forming bigger particles. However, the mean particle size of nanoparticles was reduced as an increase of poloxamer 407 concentration. This is possible that the larger amount of poloxamer 407 could cover larger surface area of nanoparticles, causing an increase of emulsifying property of poloxamer 407 for suspending of the polymer in the aqueous during preparation process and, hence, the smaller particles could be formed.

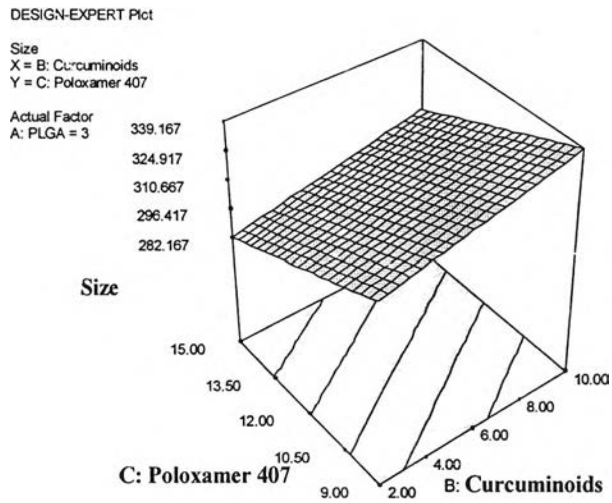
The mean particle size of the nanoparticles was plotted against % curcuminoids loaded in the formulation (Figure 4-4a) and against poloxamer 407 concentration (Figure 4-4b). The Contour and 3-D plots of the particle size versus % curcuminoids loading and poloxamer 407 concentration were shown in Figure 4-5a and b, respectively.



4-4a 4-4b

Figure 4-4. Plot of particle size against %curcuminoids loaded in the formulation (Figure 4-4a) and against poloxamer 407 concentration (Figure 4-4b).





4-5a 4-5b

Figure 4-5. Contour plot (4-5a) and 3-D plot (4-5b) of the particle size versus % curcuminoids loading and poloxamer 407 concentration

2.4 Effect of PLA-PGA ratio, curcuminoids amounts, and poloxamer 407 concentration on particle size distribution

According to Table 4-3, the particle size distribution of nanoparticles of nanoparticles were statistically analyzed, in order to correlate with the variables of formulation ingredients, which were the PLA-PGA ratio of PLGA (A), curcuminoids amount (B) and poloxamer 407 concentration (C). The model fit results were summarized in Table Appendix D-15. The sequential model sum of squares exhibited that linear model gave the relatively high F-value and the small p-value, which was less than 0.05 (p = 0.0183), while the 2FI and quadratic models gave small F-values and the large p-values, which were greater than 0.05. In addition, the lack of fit test and model summary statistic results expressed that the linear model provided p-value of 0.1656 (>0.05). This implied that the linear model was significantly fitted with the data, thus the linear model was appropriate for these data. However, adjusted-R^2 of the linear model was fairly low (adjusted-R^2 = 0.4703). An attempt to correlate the mean particle size with the transformed data did not give better fit of any model (the data are not shown). Therefore, only the model reduction was used to improve the fit of the linear regression model.

The analysis of variance (ANOVA) output with model reduction was shown in Table Appendix D-16. The correlation equations between the mean particle size and the three variables, in terms of coded factors and actual factors, were shown in Equation 4-11 and 4-12, respectively.

Final Equation in Terms of Coded Factors:

Polydispersity index = +1.04 - 0.20 C (Equation 4-11)

Final Equation in Terms of Actual Factors:

Polydispersity index = +1.85700
- 0.067917 (Poloxamer 407) (Equation 4-12)

Where: Poloxamer 407 = % poloxamer 407 concentration

It was found that only the factor concentration of poloxamer 407 (C) remained in the equation, suggesting that this term was significant in the model. According to the final equations, it was indicated that the particle size distribution of curcuminoids-PLGA nanoparticles was decreased as an increase of poloxamer 407 concentration. This is possible that the increase of poloxamer 407 concentration could provide more emulsifying property of poloxamer 407, which would provide more regular size of the nanoparticles suspended in the aqueous phase. Consequently, the particle size distribution was reduced. The particle size distribution of the nanoparticles was plotted against poloxamer 407 concentration as shown in Figure 4-6.

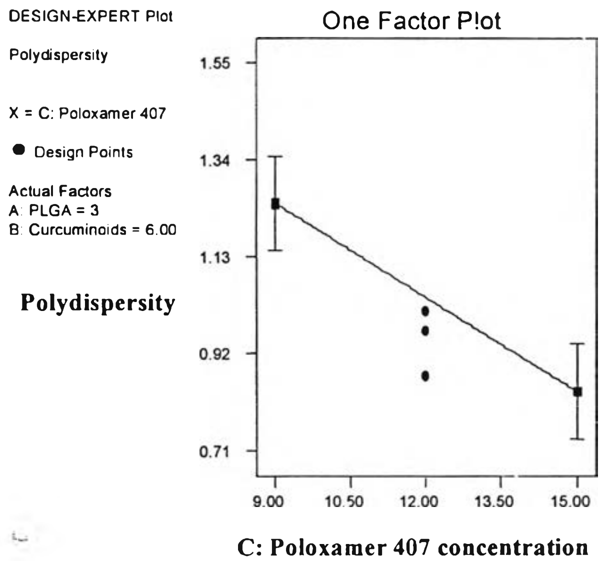


Figure 4-6. Plot of particle size distribution (polydispersity) against poloxamer 407 concentration

2.5 Effect of PLA-PGA ratio, curcuminoids amounts, and poloxamer 407 concentration on the curcuminoids content in nanoparticles

According to Table 4-3, the % curcuminoids content of nanoparticles were statistically analyzed, in order to correlate with the variables of formulation ingredients, which were the PLA-PGA ratio of PLGA (A), curcuminoids amount (B) and poloxamer 407 concentration (C). The model fit results were summarized in Table Appendix D-17. The sequential model sum of squares exhibited that the linear model gave the relatively high F-value and the small p-value, which was less than 0.05 ($p < 0.0001$), while the 2FI and quadratic models gave small F-values and the large p-values, which were greater than 0.05. In addition, the lack of fit test and model summary statistic results expressed that the linear model provided p-value of 0.2627 ($p > 0.05$) and adjusted-R^2 of 0.8329 (close to 1). This implied that the linear model was significantly fitted with the data, thus the linear model was appropriate for these data. An attempt to correlate the % curcuminoids content with the transformed data did not give better fit of any model (the data are not shown).

The analysis of variance (ANOVA) output with model reduction was shown in Table Appendix D-18. The correlation equations between % curcuminoids content and the three variables, in terms of coded factors and actual factors, were shown in Equation 4-13 and 4-14, respectively.

Final Equation in Terms of Coded Factors:

$$\textbf{Content} = +1.38 + 0.64\,B - 0.53\,C \qquad \text{(Equation 4-13)}$$

Final Equation in Terms of Actual Factors:

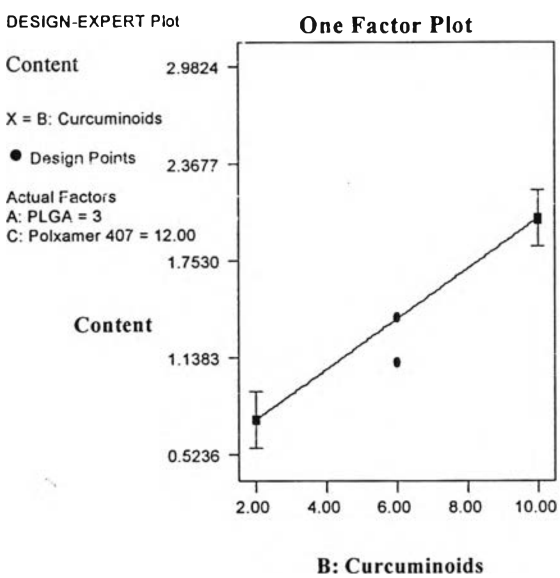
$$\textbf{Content} = +2.54186 + 0.15964\,(\text{Curcuminoids}) - 0.17634\,(\text{Poloxamer407}) \qquad \text{(Equation 4-14)}$$

Where: Curcuminoids = %curcuminoids loaded in the formulation

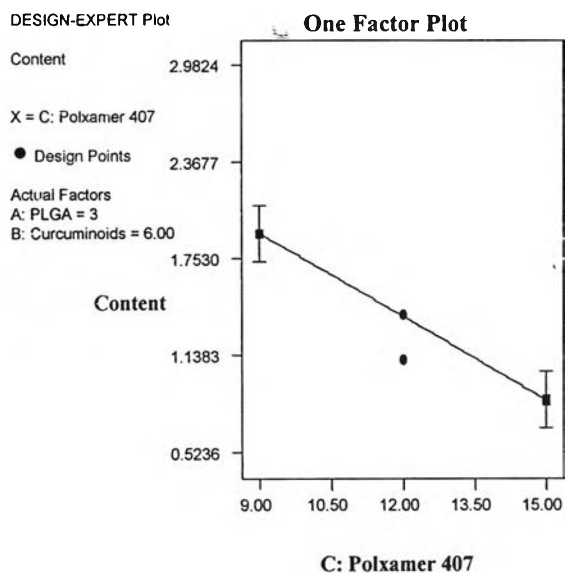
Poloxamer407 = %poloxamer 407 concentration

According to the final equations, it was found that curcuminoids amount (B) and poloxamer 407 concentration (C) remained in the equations, suggesting that these factors were significant in the linear regression model. It was indicated that curcuminoids content in PLGA nanoparticles increased as an increase of % curcuminoids loaded in the formulations. It is possible that the amount of curcuminoids used in the formulation (2-10%) did not reach the maximum capacity to entrap the curcuminoids of nanoparticles. However, the curcuminoids content in PLGA nanoparticles was reduced as an increase of poloxamer 407 concentration. This may due to the emulsifying effect of poloxamer 407, causing an increase of solubility of curcuminoids in the aqueous during the preparation and, hence, lower the amount of curcuminods trapped in the nanoparticles.

The % curcuminoids content in the nanoparticles was plotted against %curcuminoids loaded in the formulation (Figure 4-7a) and poloxamer 407 concentration (Figure 4-7b). The Contour and 3-D plots of the %curcuminoids content versus % curcuminoids loading and vitamin E TPGS concentration were shown in Figure 4-8a and 4-8b, respectively.

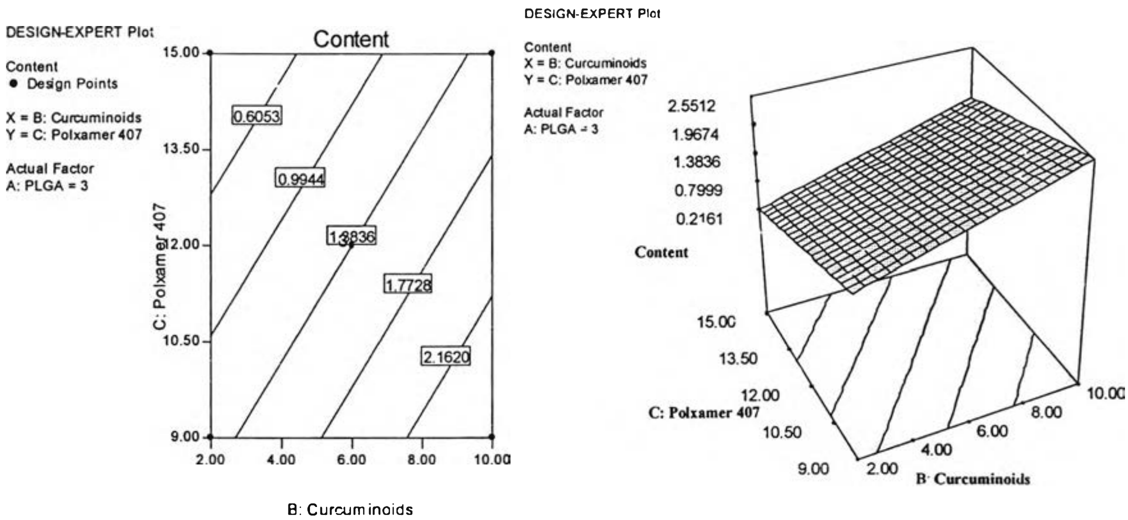


4-7a



4-7b

Figure 4-7. Plot of %curcuminoids content against %curcuminoids loaded in the formulation and poloxamer 407 concentration



4-8a 4-8b

Figure 4-8. Contour plot (4-8a) and 3-D plot (4-8b) of the %curcuminoids content versus % curcuminoids loading and poloxamer concentration

2.6 Effect of PLA-PGA ratio, curcuminoids amount, and poloxamer 407 concentration on the entrapment efficiency

According to Table 4-3, the entrapment efficiency of nanoparticles were statistically analyzed, in order to correlate with the variables of formulation ingredients, which were the PLA-PGA ratio of PLGA (A), curcuminoids amount (B) and poloxamer 407 concentration (C). The model fit results were summarized in Table Appendix D-19. The sequential model sum of squares exhibited that the linear model gave the relatively high F-value and the small p-value, which was less than 0.05 ($p < 0.0001$), while the 2FI and quadratic models gave small F-values and the large p-values, which were greater than 0.05. In addition, the lack of fit test and model summary statistic results expressed that the linear model provided p-value of 0.4675 ($p > 0.05$) and adjusted-R^2 of 0.8619 (close to 1). This implied that the linear model was significantly fitted with the data, thus the linear model was appropriate for these data. An attempt to correlate the entrapment efficiency of nanoparticles with the transformed data did not give better fit of any model (the data are not shown).

The analysis of variance (ANOVA) output with model reduction was shown in Table Appendix D-20. The correlation equations between the entrapment efficiency of nanoparticles and the three variables, in terms of coded factors and actual factors, were shown in Equation 4-15 and 4-16, respectively.

Final Equation in Terms of Coded Factors:

Entrapment Efficiency = +13.61 - 4.07 B - 4.30 C (Equation 4-15)

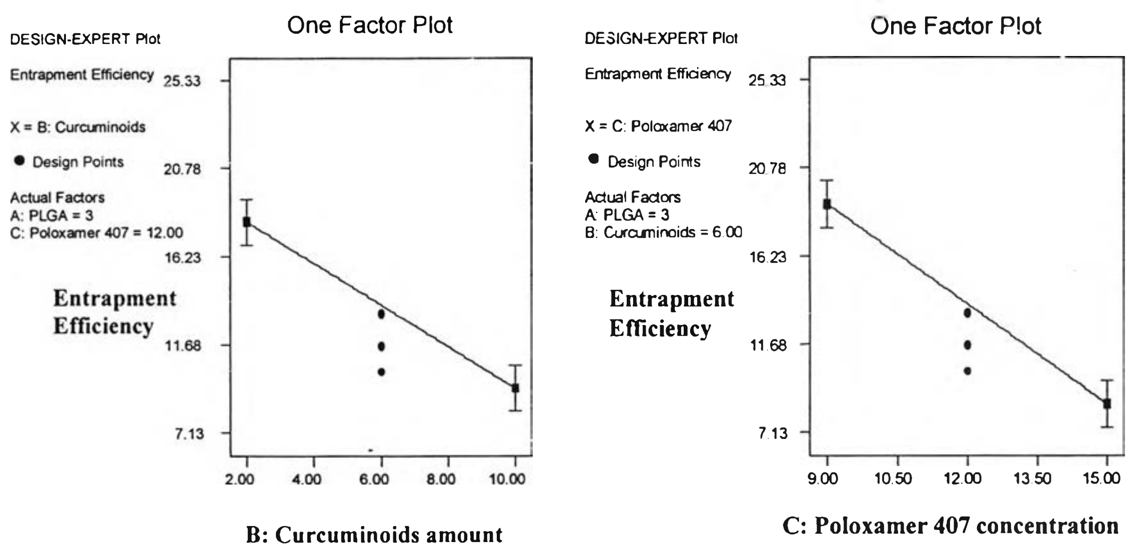
Final Equation in Terms of Actual Factors:

Entrapment Efficiency = +36.90571 - 1.01656 (Curcuminoids)
- 1.43292 (Polxamer 407) (Equation 4-16)

Where: Curcuminoids = %curcuminoids loaded in the formulation
Poloxamer407 = % poloxamer 407 concentration

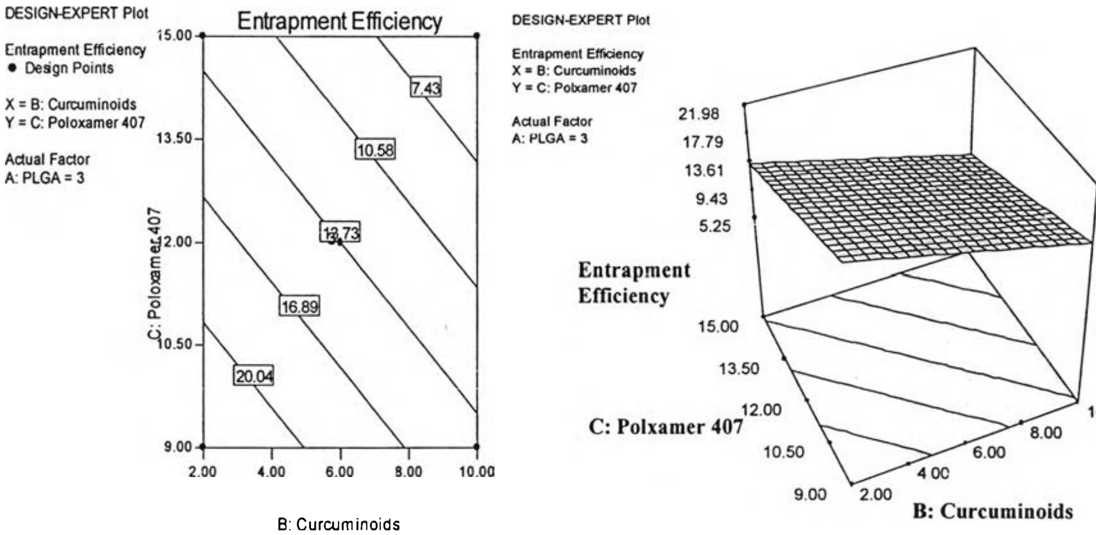
According to the final equations, it was found that curcuminoids amount (B) and poloxamer 407 concentration (C) remained in the equations, suggesting that these factors were significant in the linear regression model. It was indicated that the entrapment efficiency of curcuminoids-PLGA nanoparticles decreased as an increase of %curcuminoids loaded in the formulations. It was in conflict with the case of curcuminoids content, which increased as the increase of curcuminoids amount loaded in the formulation. This may be due to the limited capacity of nanoparticles thus only some of the entire increased amount of curcuminoids in the formulation could be entrapped. In other words, the rate of increase of curcuminoids content might be not proportional to the rate of increase of curcuminoids amount loaded in the formulation. In addition, the entrapment efficiency of curcuminoids-PLGA nanoparticles was reduced as an increase of poloxamer 407 concentration. This may due to the emulsifying effect of poloxamer 407, causing an increase of solubility of curcuminoids in the aqueous during the preparation collaborated with the limited capacity of nanoparticles, hence, lower the amount of curcuminods entrapped in the nanoparticles.

The entrapment efficiency of the nanoparticles was plotted against %curcuminoids amount in the formulation (Figure 4-9a) and against poloxamer 407 concentration (Figure 4-9b). The Contour and 3-D plots of the entrapment efficiency of the nanoparticles versus % curcuminoids loading and poloxamer 407 concentration were shown in Figure 4-10a and 4-10b, respectively.



4-9a 4-9b

Figure 4-9. Plot of the entrapment efficiency of nanoparticles against % curcuminoids amount (4-9a) and against poloxamer 407 concentration (4-9b).



4-10a 4-10b

Figure 4-10. Contour plot (4-10a) and 3-D plot (4-10b) of the particle size versus % curcuminoids loading and poloxamer 407 concentration

2.7 Optimization of curcuminoids-PLGA nanoparticles formulation using poloxamer 407 as the stabilizer

According to the statistical results of the effects of the PLA-PGA ratio of PLGA, curcuminoids amount loaded in the formulation and poloxamer 407 concentration on five responses as described in 2.1 to 2.6, it was attempted to determine a suitable formulation that could provide the satisfactory responses. The method to obtain such optimized formulation was the use of simultaneous optimization technique, which correlated all effects with all responses simultaneously. The formulation optimization was performed using desirability function approach, in which the goal of both independent (factor) and dependent variables (response) were set. The upper and lower limits of all parameters were depending upon the upper and lower value of all criteria. The setting criteria were summarized under the "constraints" section in the Table 4-4. For example, the PLA-PGA ratio could be any ratio between 1 and 5 and, hence, the goal should be "is in range". In case of % recovery, the values were between 45 and 60 but the higher % recovery was preferable and, hence, the goal should be "maximize". In details, The PLA-PGA ratio, curcuminoids amount, and poloxamer 407concentration were set as "is in range" of 1 to 5, 2 to 10, and 3 to 7, respectively. The % recovery of nanoparticles was set to be "maximize" between 45 and 60 %. The particle size and the polydispersity index were set to be "minimize" within the range of 270 to 330, and 0.7 to 1.0, respectively. Curcuminoids content and entrapment efficiency were set to be "maximize" within the range of 0.5 to 5.0 %, and 10.0 to 30.0 %, respectively. The upper and lower values of all effects and responses were equally weighed. All effects and responses were considered to be equally important in the selection process for optimal formulations.

Finally, three optimal formulations of curcuminoids-PLGA nanoparticles were achieved according to the highest desirability as seen under the "solutions" section in Table 4-4. The desirability values of all the three optimal formulations were equal to 0.338. The first formulation composed of PLGA with PLA-PGA ratio of 50:50, curcuminoids amount of 4.56 % and poloxamer 407 concentration of 15.00 %. The second formulation composed of PLGA with PLA-PGA ratio of 75:25, curcuminoids amount of 4.57 % and poloxamer 407 concentration of 15.00 %. The last formulation composed of PLGA with PLA-PGA ratio 85:15, curcuminoids amount 4.57 % and poloxamer 407 concentration 15.00 %. Although all three formulations gave the same desirability number, the formulation containing PLGA with the PLA-PGA ratio of 50:50 was chosen. This was due to the notice that the formulation using vitamin E TPGS and polyvinyl alcohol (PVA) as stabilizers provided the optimal formulations using PLGA with PLA-PGA ratio of 50:50 (see further details in 1.7 and 3.7). In addition, the higher

PGA monomer in the PLGA provided more hydrophilicity of the copolymer and, hence, from the PLGA copolymer with the PLA-PGA ratio of 50:50, compared to 75:25 and 85:15, the nanoparticles were easily prepared following this method in which water miscible solvents i.e. methanol, was used.

Table 4-4. Design-Expert® output of the simultaneous optimization of curcuminoids-PLGA nanoparticles using using poloxamer 407 as the stabilizer.

Constraints

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
PLA-PGA ratio	is in range	1	5	1	1	3
Curcuminoids content	is in range	2	10	1	1	3
Polxamer 407 concentration	is in range	9	15	1	1	3
% Recovery	maximize	45	60	1	1	3
Particle size	minimize	270	330	1	1	3
Polydispersity	minimize	0.7	1.0	1	1	3
% Curcuminoids content	maximize	0.5	5.0	1	1	3
Entrapment efficiency	maximize	10.0	30.0	1	1	3

Solutions

No.	PLGA	Curcuminoids	Poloxamer	Recovery	Size	Polydispersity	Content	Entrapment	Desirability
1	1	4.56	15.00	53.13	289.06	0.84	0.85	10.77	0.338
2	3	4.57	15.00	53.13	289.08	0.84	0.85	10.76	0.338
3	5	4.57	15.00	53.13	289.06	0.84	0.85	10.77	0.338

3. Preparation of curcuminoids-PLGA nanoparticles using polyvinyl alcohol as the stabilizer

The last set of formulations, formulation number 31 to 45, of PLGA nanoparticles containing curcuminoids were prepared as described in Chapter III. In these formulations, polyvinyl alcohol (PVA) was used as the stabilizer. Each formulation composed of PLGA at different ratio of PLA and PGA (50:50, 75:25 or 85:15), various amounts of curcuminoids (2 %, 6 %, or 8 %) and various concentrations of vitamin E TPGS (3 %, 5 %, or 7 %). The detailed formulations were previously shown in Table 3-3 of chapter III.

3.1 Determination of effect of PLA-PGA ratio, curcuminoids amount, and polyvinyl alcohol concentration on the nanoparticles

The fifteen formulations of PLGA nanoparticles containing curcuminoids were subjected to investigation on five different responses, which were %recovery, particle size, polydispersity index, %curcuminoids content, and %entrapment efficiency. The results were summarized in Table 4-5.

According to Table 4-5, it was found that %recoveries of nanoparticles after preparation were slightly different among all formulations, ranging from 47.54 to 55.04%. The particle sizes of nanoparticles obtained from each formulation were different from each other. The mean particle sizes were around 327 to 423 nm. The polydispersity indexes of nanoparticles from all formulation were between 0.85 and 0.99. The curcuminoids content and entrapment efficiency of all formulations were different. Curcuminoids contents varied from 1.05 % to 5.00 %. The entrapment efficiency ranged from 21.85 to 32.51%.

Table 4-5. Responses from of curcuminoids-PLGA nanoparticles using polyvinyl alcohol as stabilizer (Formulation Number 31 to 45)

Formulation Number	Responses				
	Recovery (%)	Particle Size (nm)	Polydispersity Index	Curcuminoids Content (%)	Entrapment Efficiency (%)
31	51.40	363	0.92	1.1291	29.01
32	53.63	327	0.87	2.8942	25.88
33	51.68	383	0.95	2.9025	24.98
34	49.92	353	0.88	4.5606	22.77
35	52.04	363	0.92	1.0826	28.18
36	47.54	397	0.85	1.0545	25.05
37	51.59	390	0.96	2.6369	22.67
38	54.51	387	0.93	2.4048	21.84
39	52.94	383	0.99	2.7115	23.91
40	54.33	380	0.97	4.1865	22.84
41	55.04	400	0.87	4.4771	24.64
42	54.76	400	0.97	1.1872	32.50
43	53.24	393	0.93	2.9669	26.34
44	52.38	423	0.94	3.3239	29.02
45	53.51	397	0.96	5.0000	26.79

3.2 Effect of PLA-PGA ratio, curcuminoids amounts, and polyvinyl alcohol concentration on the nanoparticles recovery

According to Table 4-5, the % recovery of nanoparticles were statistically analyzed, in order to correlate with the variables of formulation ingredients, which were PLA-PGA ratio of PLGA (A), curcuminoids amount (B) and PVA concentration (C). The fit summary of regression models to the data was shown in Table Appendix D-21. The sequential model sum of squares of all models gave small F-value and the large p-values, which were greater than 0.05. This implied that the models were not significantly fitted with the data. In addition, the model summary statistic results indicated that the adjusted-R^2 of all models were not close to 1, suggesting that the models were not appropriate. Moreover, both data transformation and model reduction were used in order to correlate the % recovery with the three variables but neither of them could improve the fit of all models to the data (the results of data transformation are not shown).

The analysis of variance (ANOVA) output with model reduction was shown in Table Appendix D-22. The correlation equation between % recovery and the three variables was shown in Equation 4-17.

Final Equation: **Recovery (%) = + 52.56733** (Equation 4-17)

From the final equation, it was found that all model terms (A, B and C) were eliminated, suggesting that these terms were not significant in the regression model. The final equation as shown in Equation 4-17 composed of only a constant value, which was the intercept or the average value of % recovery of the 15 formulations. In other words, the equation implied that the difference of the % recovery of the formulation number 1 to 15 were not statistically significant.

According to the results above, it can be concluded that there was no effect of PLA-PGA ratio, curcuminoids amount, and PVA concentration, within the range used in this experiment, on the %recovery yield of curcuminoids-PLGA nanoparticles using PVA as the stabilizer. It is possible that the % recovery of nanopraticles is dependent on other parameters, e.g. the preparation method, separation procedure, etc., rather than the formulation ingredients.

3.3 Effect of PLA-PGA ratio, curcuminoids amounts, and polyvinyl alcohol concentration on the particle size

According to Table 4-5, the mean particle size of nanoparticles were statistically analyzed, in order to correlate with the variables of formulation ingredients, which were the PLA-PGA ratio of PLGA (A), curcuminoids amount (B) and PVA concentration (C). The model fit results were summarized in Table Appendix D-23. The sequential model sum of squares exhibited that linear model gave the relatively high F-value and the small p-value, which was less than 0.05 ($p < 0.0001$), while the 2FI and quadratic models gave small F-values and the large p-values, which were greater than 0.05. In addition, the lack of fit test and model summary statistic results expressed that the linear model provided p-value of 0.1397 (>0.05) and the adjusted-R^2 of 0.8740 (close to 1). This implied that the linear model was significantly fitted with the data, thus the linear model was appropriate for these data. An attempt to correlate the mean particle size with the transformed data did not give better fit of any model (the data are not shown).

The analysis of variance (ANOVA) output with model reduction was shown in Table Appendix D-24. The correlation equations between the mean particle size and the three variables, in terms of coded factors and actual factors, were shown in Equation 4-18 and 4-19, respectively.

Final Equation in Terms of Coded Factors:

Mean particle size = +382.60 + 23.37A + 17.50C (Equation 4-18)

Final Equation in Terms of Actual Factors:

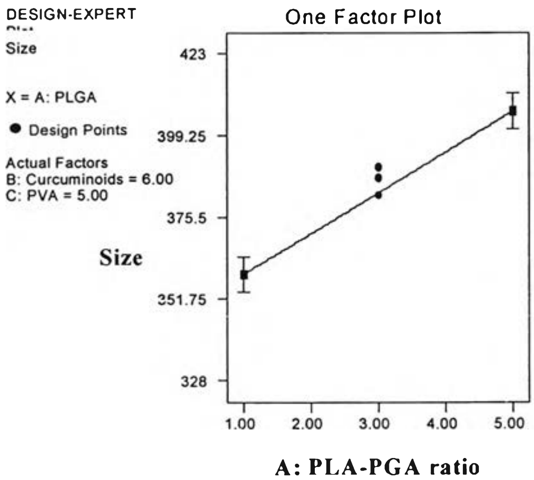
Mean particle size = +303.78750 + 11.68750 (PLA-PGA ratio) + 8.75000 (PVA) (Equation 4-19)

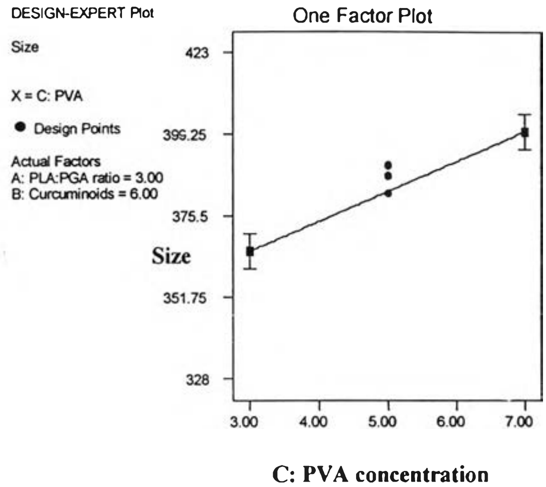
Where: PLA-PGA ratio = PLA-PGA ratio of PLGA copolymers

PVA = % PVA concentration

It was found that only PLA-PGA ratio (A) and concentration of PVA (C) remained in the equation, suggesting that these terms were significant in the model. According to the final equations, this indicated that the mean particle size of curcuminoids-PLGA nanoparticles was increased as an increase PLA-PGA ratio and PVA concentration. From the results, it is possible that the increase of PLA-PGA ratio causing an increase of hydrophobicity of PLGA copolymer, and hence it provided the difficulty suspending of PLGA copolymer in aqueous phase. In case of PVA concentration, it is possible that an increase of PVA concentration caused an increase of viscosity of the aqueous phase, thus it also provided the difficulty of suspending of PLGA copolymer in aqueous phase. Therefore the large size of nanoparticles could be obtained.

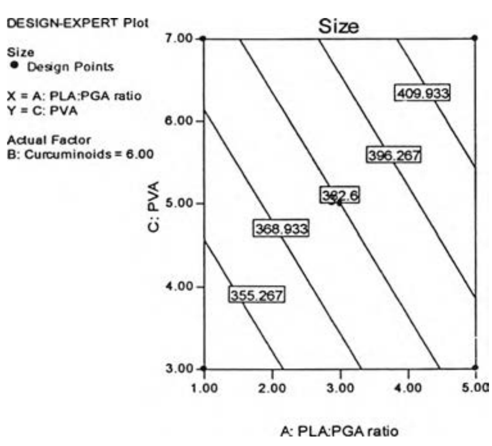
The particle size of the nanoparticles was plotted against PLA-PGA ratio (Figure 4-11a) and against PVA concentration (Figure 4-11b). The Contour and 3-D plots of the particle size versus PLA-PGA ratio and PVA concentration were shown in Figure 4-12a and 4-12b, respectively.

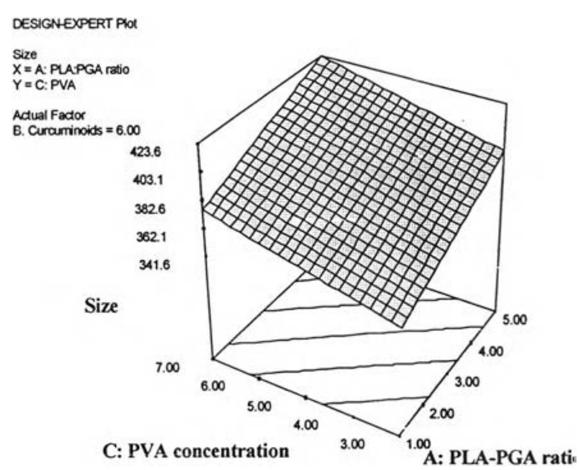




4-11a 4-11b

Figure 4-11. Plot of particle size against %curcuminoids loaded in the formulation (Figure 4-11a) and against PVA concentration (Figure 4-11b)





4-12a 4-12b

Figure 4-12. Contour plot (4-5a) and 3-D plot (4-5b) of the particle size versus % curcuminoids loading and poloxamer 407 concentration

3.4 Effect of PLA-PGA ratio, curcuminoids amounts, and polyvinyl alcohol concentration on the particle size distribution

According to Table 4-5, the % recovery of nanoparticles were statistically analyzed, in order to correlate with the variables of formulation ingredients, which were the PLA-PGA ratio of PLGA (A), curcuminoids amount (B) and poloxamer 407 concentration (C). The fit summary of regression models to the data was shown in Table Appendix D-25. The sequential model sum of squares of all models gave small F-values and the large p-values, which were greater than 0.05. This implied that the models were not significantly fitted with the data. In addition, the model summary statistic results indicated that the adjusted-R^2 of all models were not close to 1, suggesting that the models were not appropriate. An attempt to correlate the % recovery with the transformed data failed to improve the fit of all models to the data (the data are not shown).

The analysis of variance (ANOVA) output with model reduction was shown in Table Appendix D-26. The correlation equation between the % recovery and the three variables was shown in Equation 4-20.

Final Equation: **Polydispersity index = + 0.92733** (Equation 4-20)

It was found that all model terms (A, B and C) were eliminated, suggesting that these terms were not significant in the regression model. The final equation as shown in Equation 4-20 composed of only a constant value, which was the intercept or the average value of the polydispersity index of all 15 formulations. In other words, the equation implied that the difference the polydispersity index of the formulation number 1 to 15 were not statistically significant.

According to the results above, it can be concluded that there was no effect of PLA-PGA ratio, curcuminoids amount, and PVA concentration, within the range used in this experiment, on the particle size distribution of curcuminoids-PLGA nanoparticles using PVA as stabilizer. It is possible that the particle size distribution of nanopraticles is dependent on other parameters, e.g. the preparation method, etc.

3.5 Effect of PLA-PGA ratio, curcuminoids amounts, and polyvinyl alcohol concentration on the curcuminoids content in nanoparticles

According to Table 4-5, the % curcuminoids content of nanoparticles were statistically analyzed, in order to correlate with the variables of formulation ingredients, which were the PLA-PGA ratio of PLGA (A), curcuminoids amount (B) and PVA concentration (C). The model fit results were summarized in Table Appendix D-27. The sequential model sum of squares exhibited that the linear model gave the relatively high F-value and the small p-value, which was less than 0.05 ($p < 0.0001$), while the 2FI and quadratic models gave small F-values and the large p-values, which were greater than 0.05. In addition, the lack of fit test and model summary statistic results expressed that the linear model provided p-value of 0.2961 ($p > 0.05$) and adjusted-R^2 of 0.9647 (close to 1). This implied that the linear model was significantly fitted with the data, thus the linear model was appropriate for these data. An attempt to correlate the % curcuminoids content with the transformed data did not give better fit of any model (the data are not shown).

The analysis of variance (ANOVA) output with model reduction was shown in Table Appendix D-28. The correlation equations between % curcuminoids content and the three variables, in terms of coded factors and actual factors, were shown in Equation 4-21 and 4-22, respectively.

Final Equation in Terms of Coded Factors:

Content = +2.83 + 1.72B (Equation 4-21)

Final Equation in Terms of Actual Factors:

Content = +0.25250 + 0.43034 (Curcuminoids) (Equation 4-22)

Where: Curcuminoids = %curcuminoids loaded in the formulation

It was found that only the term of curcuminoids amount (B) remained in the finaql equation, suggesting that this factor was significant in the model. This can be interpreted that curcuminoids content in PLGA nanoparticles was increased as an increase of % curcuminoids loaded in the formulations. It is possible that the amount of curcuminoids used in the formulation (2-10%) did not reach the maximum capacity to entrap the curcuminoids of nanoparticles. The %curcuminoids content found in the nanoparticles was plotted against %curcuminoids loaded in the formulation. The plot is shown in Figure 4-13.

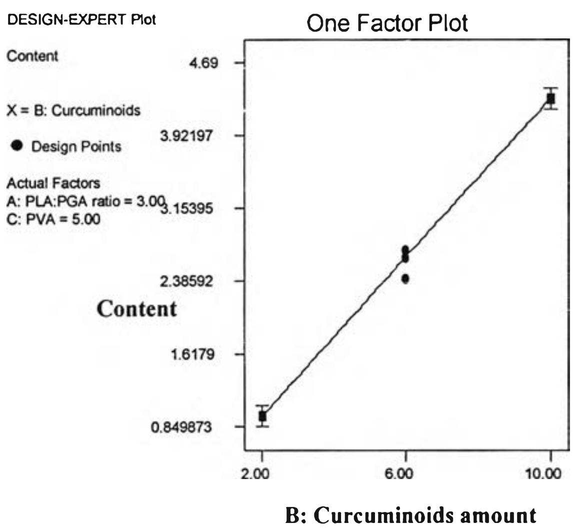


Figure 4-13. Plot of %curcuminoids content against %curcuminoids loaded in the formulation

3.6 Effect of PLA-PGA ratio, curcuminoids amount, and polyvinyl alcohol concentration on the entrapment efficiency

According to Table 4-5, the entrapment efficiency of nanoparticles were statistically analyzed, in order to correlate with the variables of formulation ingredients, which were the PLA-PGA ratio of PLGA (A), curcuminoids amount (B) and PVA concentration (C). The model fit results were summarized in Table Appendix D-29. The sequential model sum of squares exhibited that the quadratic model gave the relatively high F-value and the small p-value, which was less than 0.05 ($p = 0.0215$), while the 2FI and linear models gave small F-values and the large p-values, which were greater than 0.05. In addition, the lack of fit test and model summary statistic results expressed that the quadratic model provided p-value of 0.1425 ($p > 0.05$) and adjusted-R^2 of 0.7901. This implied that the quadratic model was significantly fitted with the data, thus the quadratic model was appropriate for these data. An attempt to correlate the % curcuminoids content with the transformed data did not give better fit of any model (the data are not shown).

The analysis of variance (ANOVA) output with model reduction was shown in Table Appendix D-28. The correlation equations between % curcuminoids content and the three variables, in terms of coded factors and actual factors, were shown in Equation 4-21 and 4-22, respectively.

It was found that only PLA-PGA ratio (A) and curcuminoids amount (B) remained in the equation, suggesting that these terms were significant in the model. According to Equation 4-23 and 4-24, it can be interpreted that the entrapment efficiency of curucminoids-PLGA nanoparticles in as an increase of PLA-PGA ratio of PLGA copolymer. In contrast, the entrapment efficiency of curucminoids-PLGA nanoparticles decrease when the % curcuminoids loaded in the formulations increase. It may possible that when the PLA-PGA ratio of PLGA copolymer increase, the particle size tend to increasse, thus, the entrapment efficiency also increase. In case of curcuminoids amount, it was in conflict with the case of curcuminoids content, which increased as the increase of curcuminoids amount loaded in the formulation. This may be due to the limited capacity of nanoparticles thus only some of the entire increased amount of curcuminoids in the formulation could be entrapped. In other words, the rate of increase of curcuminoids content might be not proportional to the rate of increase of curcuminoids amount loaded in the formulation.

Final Equation in Terms of Coded Factors:

$$\textbf{Entrapment Efficiency} = +23.16 + 1.49A - 2.23B + 3.13A^2 + 1.73B^2 \quad \text{(Equation 4-23)}$$

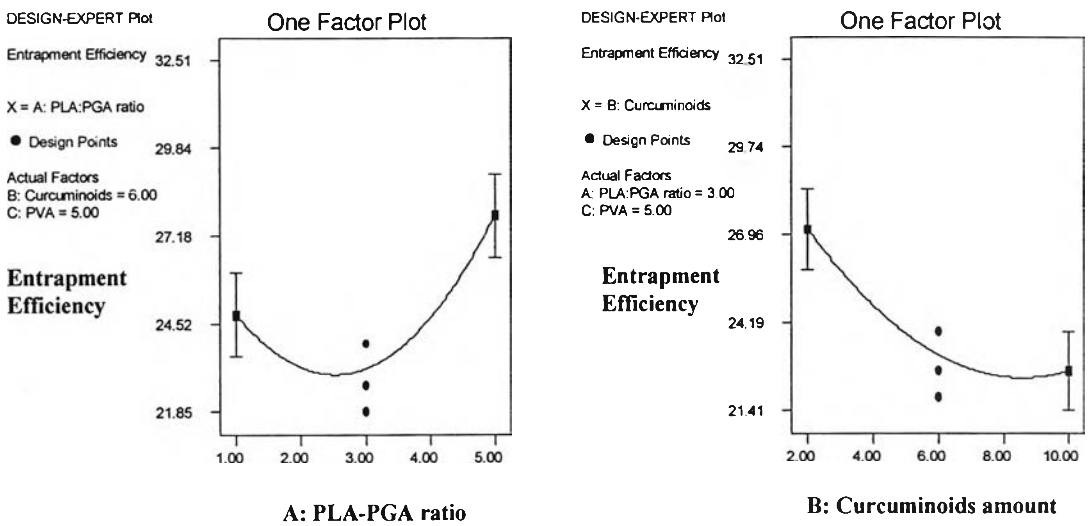
Final Equation in Terms of Actual Factors:

$$\textbf{Entrapment Efficiency} = +35.21010 - 3.94755\,(\text{PLA-PGA ratio}) - 1.85721\,(\text{Curcuminoids}) - 0.78240\,(\text{PLA-PGA ratio})^2 + 0.10826\,(\text{Curcuminoids})^2 \quad \text{(Equation 4-24)}$$

Where: PLA-PGA ratio = PLA-PGA ratio of PLGA copolymers

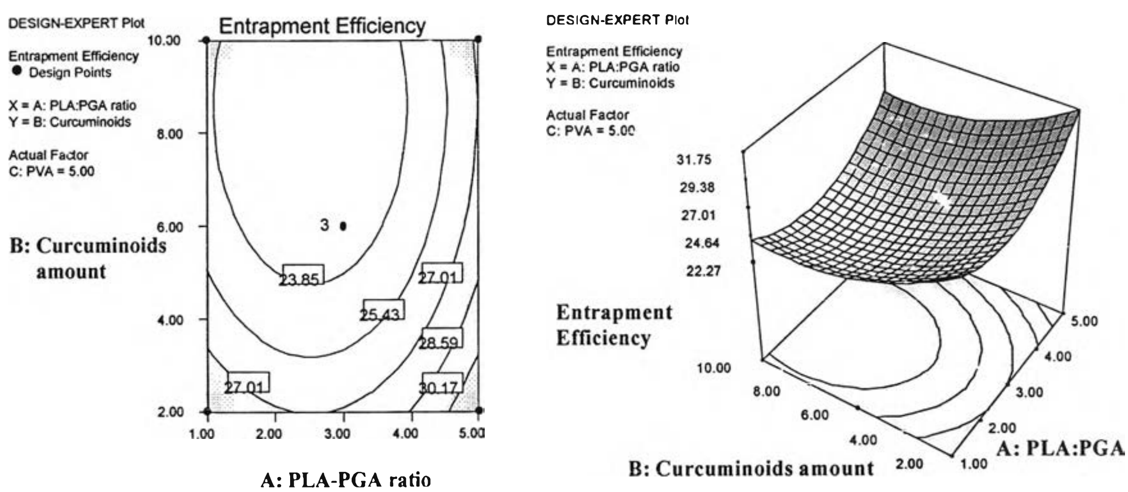
Curcuminoids = %curcuminoids loaded in the formulation

The entrapment efficiency of the nanoparticles was plotted against PLA-PGA ratio (Figure 4-15a) and against %curcuminoids loaded in the formulation (Figure 4-15b). The Contour and 3-D plots of the entrapment efficiency versus PLA-PGA ratio and % curcuminoids loading were shown in Figure 4-16a and 4-16b, respectively.



4-15a　　4-15b

Figure 4-15. Plot of entrapment efficiency against PLA-PGA ratio (Figure 4-15a) and against %curcuminoids loaded in the formulation (Figure 4-15b).



4-16a　　4-16b

Figure 4-16. Contour plot (4-16a) and 3-D plot (4-16b) of the entrapment efficiency versus PLA-PGA ratio and %curcuminoids loading

3.7 Optimization of curcuminoids-PLGA nanoparticles formulation using polyvinyl alcohol as stabilizer

According to the statistic results of the effects of the PLA-PGA ratio of PLGA, curcuminoids amount and PVA concentration on five responses as described in 3.2-3-6, it was attempted to determine a suitable formulation that could provide the satisfactory responses. The method to obtain such optimized formulation was the use of simultaneous optimization technique, which correlated all effects with all responses simultaneously. The formulation optimization was performed using desirability function approach, in which the goal of both independent (factor) and dependent variables (response) were set. The upper and lower limits of all parameters were dependent upon the upper and lower value of all criteria. The information of all parameters and criteria was summarized in Table 4-6. For example, the effect of PLA-PGA ratio could be any ratio between 1 and 5 and, hence, the term "is in range" was used. In case of %recovery, the response between 45 and 60 could be used but the higher %recovery was preferable and, hence, the term "maximize" was used. In details, The PLA-PGA ratio, curcuminoids amount, and PVA concentration were set as "is in range" of 1 to 5, 2 to 10, and 3 to 7, respectively. The % recovery of nanoparticles was set to be "maximize" between 45 and 60 %. The particle size and the polydispersity index were set to be "minimize" between the value of 300 and 400, and 0.8 and 1.0, respectively. Curcuminoids content and entrapment efficiency were set to be "maximize" between the value of 1.0 and 5.0 %, and 20 to 40 %, respectively. The upper and lower values of all effects and responses were equally weighted. All effects and responses were considered to be equally important in the selection process for optimal formulations. The above setting criteria were demonstrated under the "constraints" section in Table 4-6. Three optimal formulations of curcuminoids-PLGA nanoparticles were achieved according to the highest desirability as seen under the "solutions" section in Table 4-6. The desirability of the first formulation was higher than other formulations, thus it was chosen. The first formulation composed of PLGA with PLA-PGA ratio of 50:50, curcuminoids amount of 10.0 %, PVA concentration of 3.00 %. In addition, the higher PGA monomer in the PLGA provided more hydrophilicity of the copolymer and, hence, the ratio of PLA-PGA of 50:50, compared to 75:25 and 85:15, was easily used in the preparation using water miscible solvents i.e. methanol.

Table 4-6. Design-Expert® output of the constrained optimization of curcuminoids-PLGA nanoparticles using polyvinyl alcohol (PVA) as the stabilizer

Constraints

Name	**Goal**	**Lower Limit**	**Upper Limit**	**Lower Weight**	**Upper Weight**	**Importance**
PLA-PGA ratio	is in range	1	5	1	1	3
Curcuminoids amount	is in range	2	10	1	1	3
PVA concentration	is in range	3	7	1	1	3
% Recovery	maximize	45	60	1	1	3
Particle Size	minimize	300	400	1	1	3
Polydispersity	minimize	0.8	1	1	1	3
% Curcuminoids content	maximize	1	5	1	1	3
Entrapment efficiency	maximize	20	40	1	1	3

Solutions

No.	**PLA:PGA**	**Curcuminoids**	**PVA**	**Recovery**	**Size**	**Polydispersity**	**Content**	**Entrapment**	**Desirability**
1.	1.00	10.00	3.00	52.57	341.73	0.93	4.59	24.30	0.460
2.	4.96	10.00	3.00	52.57	388	0.93	4.82	27.13	0.376
3.	2.96	10.00	3.00	52.57	364.64	0.93	4.37	22.63	0.373

4. Characterization of the optimal formulations

The three curcuminoids-PLGA nanoparticles formulations (Formulation number 46, 47 and 48) obtained from the optimization processes were summarized in Table 4-7. These formulations were chosen according to the highest desirability that satisfied the required conditions as previously mentioned. The first formulation with the desiability of 0.497, consisted of PLGA with the PLA-PGA ratio of 50:50, 10% of curcuminiods and 3% of vitamin E TPGS (Formulation number 46). Formulation number 47 with the desirability of 0.338 consisted of PLGA with the PLA-PGA ratio of 50:50, 4.56% of curcuminiods and 15% of poloxamer. The last formulation (Formulation Number 48) with the desirability of 0.460 consisted of PLGA with the PLA-PGA ratio of 50:50, 10% of curcuminiods and 3% of PVA.

Table 4-7. The optimal formulation of curcuminoids-PLGA nanoparticles

Formulation Number	PLGA	Curcuminoids (%)	Stabilizer		
			Vitamin E TPGS	Poloxamer 407	PVA
46	50:50	10.00	3.0	-	-
47	50:50	4.56	-	15.0	-
48	50:50	10.00	-	-	3.0

The three formulations (formulation number 46-48) were prepared and evaluated on the %recovery, particle size, size distribution, %curcuminoids content and %entrapment efficiency. The responses of prepared curcuminoids-PLGA nanoparticles were summarized in Table 4-8.

Table 4-8. Observed resposes of the optimal formulation of curcuminoids-PLGA nanoparticles (Formulation Number 46-48)

Formulation Number	Characterization				
	Recovery (%)	Particle size (nm)	Polydispersity Index	Curcuminoids content (%)	Entrapment Efficiency (%)
46	53.89	313	0.81	0.10	0.55
47	56.24	287	0.86	0.98	12.29
48	50.04	333	0.92	2.39	11.95

For the curcuminoids-PLGA nanoparticles using vitamin E TPGS as stabilizer (formulation number 46), the observed values of particle size, polydispersity, %curcuminoids content, and entrapment efficiency (Table 4-8) were in the range of 95% predicted interval (Table 4-9), between 95% PI low and 95% PI high. However, the %recovery of 53.89% was found to be slightly lower than the value of 95% PI low (59.23%). This indicated the regression model could predict almost all responses effectively, except %recovery.

Table 4-9. Predicted values from the simultaneous optimization of all responses of curcuminoids-PLGA nanoparticles using vitamin E TPGS as stabilizer

Factor	Name	Level	Low Level	High Level	Std. Dev.
A	PLA-PGA ratio	3.00	1.00	5.00	0.050
B	Curcuminoids amount	6.00	2.00	10.00	0.050
C	Vit E TPGS	5.00	3.00	7.00	0.050

	Prediction	SE Mean	95% CI low	95% CI high	SE Pred	95% PI low	95% PI high
%Recovery	64.65	0.63	63.30	66.01	2.53	59.23	70.08
Particle Size	315	2.11	310.21	319.26	8.44	296.62	332.85
Polydispersity	0.76	0.017	0.72	0.79	0.069	0.61	0.90
%Curcuminoids content	0.08	3.209E-003	0.075	0.089	0.013	0.054	0.11
Entrapment	0.99	0.068	0.84	1.13	0.27	0.40	1.57

For the curcuminoids-PLGA nanoparticles using poloxamer as stabilizer (formulation number 47), the observed values of all responses (Table 4-8) were in the range of 95% predicted interval (Table 4-10) since all of them were between 95% PI low and 95% PI high. This indicated the regression model could predict all responses effectively.

Table 4-10. Predicted values from the simultaneous optimization of all responses of curcuminoids-PLGA nanoparticles using poloxamer 407 as stabilizer

Factor	NameLevelLow LevelHigh LevelStd. Dev.				
A	PLA-PGA ratio	3.00	1.00	5.00	0.50
B	Curcuminoids amount	6.00	2.00	10.00	0.050
C	Polxamer 407	12.00	9.00	15.00	0.050

	Prediction	SE Mean	95% CI low	95% CI high	SE Pred	95% PI low	95% PI high
%Recovery	53.1313	0.79	51.43	54.83	3.17	46.34	59.93
Particle size	310.667	3.46	303.12	318.21	13.86	280.47	340.86
Polydispersity	1.042	0.039	0.96	1.13	0.16	0.70	1.38
%Curcuminoids content	1.26	0.060	1.12	1.39	0.17	0.88	1.64
Entrapment Efficiency	13.61	0.44	12.66	14.56	1.74	9.81	17.41

For the curcuminoids-PLGA nanoparticles using PVA as stabilizer (formulation number 48), the observed values of %recovery, polydispersity and %curcuminoids content (Table 4-8) were in the range of 95% predicted interval (Table 4-11), between 95% PI low and 95% PI high. However, the particle size (333 nm) and the %recovery of 53.89% was found to be lower than the value of 95% PI low (63.30%). (11.95%) was found to be lower than the value of 95% PI low (364.73 nm and 19.68%, respectively). This indicated the regression model could possible use to predict some responses effectively with an exception of particle size and %entrapment efficiency.

Table 4-11. Predicted values from the simultaneous optimization of all responses of curcuminoids-PLGA nanoparticles using PVA as stabilizer

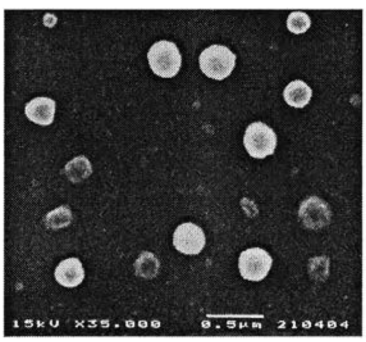
Factor	Name	Level	Low Level	High Level	Std. Dev.
A	PLA-PGA ratio	3.00	1.00	5.00	0.050
B	Curcuminoids amount	6.00	2.00	10.00	0.050
C	PVA	5.00	3.00	7.00	0.050

	Prediction	SE Mean	95% CI low	95% CI high	SE Pred	95% PI low	95% PI high
%Recovery	52.57	0.520	51.46	53.68	2.07	48.13	57.00
Particle size	382.6	2.050	378.13	387.07	8.20	364.73	400.47
Polydispersity	0.93	0.011	0.90	0.95	0.044	0.83	1.02
%Curcuminoids content	2.65	0.061	2.52	2.78	0.17	2.27	3.03
Entrapment cfficiency	23.16	0.680	21.65	24.67	1.56	19.68	26.65

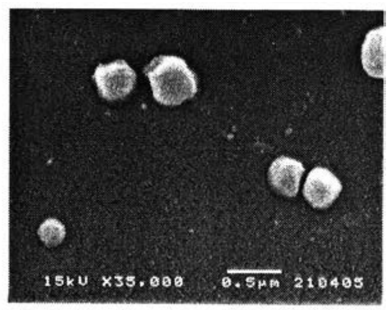
From the results of the comparison between predicted responses and actual responses obtained from the optimal formulation, it was suggested that the optimization method used here was suitable to obtain the formulation with specified requirements of the responses. It was noticed that slightly differences in the predicted values and actual values occurred. However, it is possible that only one experiment was performed for each formulation, causing the response results fail to fall within the 95% PI values. It was therefore recommended that a number of experiments should be done in order to improve the confident results of the actual responses.

5. Morphology of the nanoparticles from optimal formulations

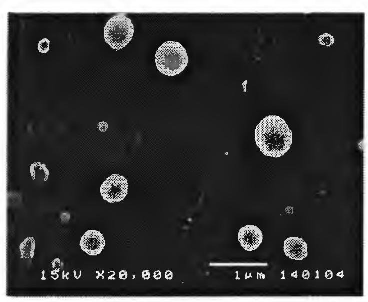
The scanning electron micrograph of all three curcuminoids-PLGA nanoparticles obtained from the optimal formulations number 46, 47, and 48 were shown below in Figure 4-17a, 4-17b, and 4-17c, respectively. From the photos, it can be noticed that all of nanoparticles were almost spherical in shape with nearly smooth surfaces, especially the nanoparticles prepared using PVA as stabilizer. Since curcuminoids precipitate or crystal was not observed, suggesting that the washing process was effective to remove un-entrapped curcuminoids.



4-17a



4-17b



4-17c

Figure 4-17. Scanning electron micrograph of curcuminoids-PLGA nanoparticles obtained from the optimal formulation number 46 (4-17a), number 47 (4-17b), and number 48 (4-17c)

6. *In Vitro* Release Study

The cumulative percentage of curcuminoids releases of the three formulations was exhibited in Table 4-12. The release patterns of curcuminoids from PLGA nanoparticles obtained from the three optimal formulations were shown in Figure 4-18. It was found that the results were similar in the release profile with slightly different amount of curcuminoids released. The patterns seemed to be biphasic with rapidly initial release in the first five hours. After that, curcuminoids were slower released and constant after 20 hours. The initial rapidly release might be due to the release of curcuminoids located near the surface or adsorbed on the surface of nanoparticles, thus they could be released immediately. The release profile also demonstrated that the burst effect was not observed in these formulations. This result may be partially explained by the smooth surface of nanoparticles observed under electron microscope.

Table 4-12. Cumulative percentage of curcuminoids released from the nanoparticles obtained from the optimal formulation (Number 46 to 48)

Time (hour)	**Cumulative percentage curcuminoids released**		
	Number 46	**Number 47**	**Number 48**
0.25	9.41	10.73	6.37
0.5	14.37	15.49	10.35
0.75	17.63	19.68	13.94
1	21.84	23.36	17.36
2	29.79	32.84	24.83
3	35.86	40.51	29.34
4	41.05	46.60	34.18
5	44.64	50.76	38.11
6	48.35	53.47	40.55
8	52.38	58.23	44.62
10	55.91	61.71	49.14
12	59.17	64.52	52.29
24	65.43	70.95	62.08
36	69.25	72.56	65.49
48	71.87	73.24	68.98

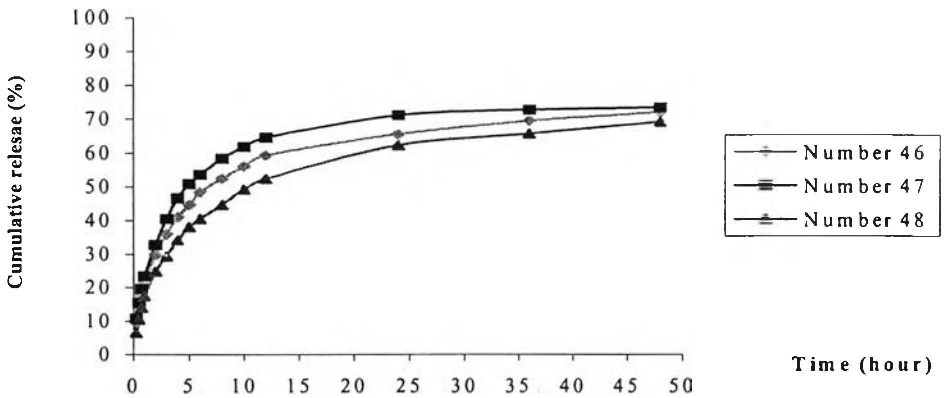


Figure 4-18. Release patterns of curcuminoids from PLGA nanoparticles obtained from the optimal formulation (Number 46 to 48)