# CHAPTER IV

# RESULTS & DISCUSSION

## 1. Preparation of Microcapsules by Interfacial Cross- linked Proteins with Terephtaloyl Chloride.

In this investigation IFP was used to prepare microcapsules from protein and TC because this process is a unique polymerization making reaction between the two immiscible phases to prepare microcapsules. And it was considerably use as an easy method for microencapsulation. In this study, the Schotten –Bauman reaction of diacid chloride with compound containing two or more active atoms such as –NH can be used for preparation. For preliminary studies, the reaction of –NH group of protein in an aqueous solution was reacted with diacid chloride group of terephthaloyl chloride in solvent mixture of chloroform and cyclohexane (1:4) at room temperature. Poly (terephthalamide) film was observed at the interface of the two solutions. The polymer formation can be enhanced by the presence of an alkali in the aqueous phase that served to neutralize hydrogen chloride generated during the polycondensation reaction. This technique is superior to other techniques because the polymerization reactions can be run in common equipment under atmospheric condition. A surfactant was used as an emulsifying agent and to enhance the transfer of diamine to organic phase. Bovine serum albumin, ovalbumin and galatin were three types of proteins that were investigated. In this experiment proteins were dissolved in carbonated buffer pH 9.8 in order to neutralize HCl generated during the reaction. Span 85® was the emulsifying agent use to enhance the transfer of diamine group to the organic phase. TC (cross-linking agent) was dissolved in chloroform and cyclohexane mixture. Stirring was continued for 30 min in order to ensure complete reaction. Microcapsules from BSA and ovalbumin were obtained easily after 5 min., yellowish precipitate happened when reaction was continued for 30 min.

2. Preparation of BSA-TC Microcapsules and BSA-TC Walled D-panthenol Microcapsules

The result from previous study showed that both BSA and ovalbumin could be used to prepare microcapsules, which were stable enough to use in topical preparations. However, BSA was more preferable than ovalbumin when dissolved in buffer pH 9.8. BSA gave clear solution and was easy to dissolve while ovalbumin was difficult and gave a lot of bubble in solution.

This experiment was studied more in parameters affected microcapsules formation such as BSA concentration, TC concentration and stirring rate. From previous result in table 3, BSA were easily obtained when using 20% BSA with 1.25-5% TC at ambient temperature. Reaction time was fixed at 30 minutes and the stirring rate was controlled at 800 rpm. The microcapsules appeared as transparent and sphere under optical microscope.

2.1 Effect of BSA Concentration

Concentration of BSA did not affect much on physical appearances of microcapsules while percent of TC was fixed at 2.5. It could be explained that at excess amount of TC, microcapsules were still formed at various concentration of BSA. The result was also consistent with the study by Ishizaka et al. (1981). BSA microcapsules were not different in shape, size and morphology at different concentration of BSA. Furthermore, they were completely encapsulated and stable in both forms.

2.2 Effect of Terephthaloyl Chloride Concentration

The physical appearances of BSA microcapsules when concentrations of TC were varied from 1.25, 2.5 and 5%w/v. The BSA-TC showed difference in shape, thickness of walls and stability. Increasing in TC concentration to 5% w/v resulted in thicker of walls. BSA-TC microcapsules with 2.5% and %5w/v TC exhibited similar characteristics. They were both transparent spheres with distinct membrane and were collapsed after drying. However, at lower concentration of 1.25 %w/v TC, the microcapsules appeared to be aggregated and easily broken. Also the wall of

microcapsules was very thin when compared with other preparations that were obtained from high TC concentration.

2.3 Effect of Stirring Rate

Size and size distributions of microcapsules obtained from varied stirring rates were different in mean diameters. Microcapsules of average particle sizes of 91.15-377.29 μm were obtained from lower stirring rate of 800 rpm while smaller microcapsules of 31.15-μm were obtained when the stirring rate was used at 11000 rpm. The result was consistent with a report study by Ishizaka et al. (1981). The stirring rate is the critical variable and affects the molecular weight of polymer. The higher the stirring rate the smaller the size and shape was obtained.

2.4 Effect of BSA Concentration and D-panthenol Concentration

BSA –TC walled D-panthenol microcapsules were obtained by interfacial cross-linked BSA with TC where D-panthenol was loaded from10.0 w/v to 20% w/v in BSA solution. According to the result from preparation of BSA-TC microcapsules, BSA was used at 13.3% and 20%w/v and TC at 5%w/v. BSA-TC Walled D-panthenol microcapsules obtained from formulation D1-D6 were different from blank BSA-TC microcapsules, size and shape were bigger and easily aggregated. They were irregular and robust. This result indicated that concentration of BSA and drug loading influenced the morphology of microcapsules, particle sizes and size distribution. In addition %yield and % entrapment were different as shown in topic 3 (characterization of BSA-TC walled microcapsules).

3. Characterization of Blank Microcapsules, BSA-TC and BSA-TC Walled D-Panthenol Microcapsules

3.1 Optical Microscopy

Microcapsules obtained from proteins cross-linked with TC were spherical like shape and some were irregular. Both BSA and ovalbumin microcapsules were collapsed after drying. In the case of gelatin microcapsules when prepared by this technique, it was found that gelatin microcapsules were big, irregular shapes and

aggregated together. The gelatin microcapsules could not obtain as BSA and ovalbumin microcapsules. When gelatin microcapsules dispersed in water, they were swelled and dissolved in water. Table 8 shows the appearances of microcapsules from formulations A1-A9. The photograph of BSA, ovalbumin and gelatin microcapsules are shown in figures 12-14, respectively.

Table 8 Appearance of microcapsules from different cross-linked proteins with TC
BSA= bovine serum albumin, OVAL= ovalbumin, GEL= gelatin

| Formulation | %w/v Proteins | %w/v TC | appearance |
|---|---|---|---|
| A1 | BSA 20 | 1.25 | sphere, thin wall |
| A2 | BSA 20 | 2.50 | sphere, stable |
| A3 | BSA 20 | 5.00 | sphere, stable |
| A4 | OVAL 20 | 1.25 | sphere, unstable |
| A5 | OVAL 20 | 2.50 | sphere, stable |
| A6 | OVLA 20 | 5.00 | sphere, stable |
| A7 | GEL 20 | 2.50 | big and irregular |
| A8 | GEL 6 | 2.50 | big and irregular |
| A9 | GEL 6 | 1.25 | big and irregular |

The morphology of BSA-TC microcapsules was examined by using an optical microscopy. Not only the spherical shape with smooth surface was found, the collapsed and irregular particles also found in the preparations.

Figure 15 shows photographs of BSA-TC microcapsules prepared by various concentration of BSA. Increasing concentration of BSA resulted in increasing diameter of microcapsules. All of them showed spherical shape but B1 and B2 showed some aggregation of microcapsules.

Figure 16 shows photographs of BSA-TC microcapsules prepared by different concentration of TC. The wall thickness and shape of microcapsules were different. Increasing in TC concentration resulted in increasing wall thickness.

Figure 17 shows photographs of BSA-TC microcapsules prepared by different stirring rate. Microcapsules were spherical. There was an evidence of robust when the stirring rate was increased to 11000 rpm.

Figure 18 shows the photographs of BSA-TC walled D-panthenol microcapsules prepared by using 13.3% w/v of BSA and 5%w/vTC with different drug loading (D1-D4). Some of D-panthenol microcapsules were irregular and robust when D-panthenol was increased to 13.3%, 16.6%and 20%w/v, respectively.

Figure 19 shows the photographs of BSA-TC walled D-panthenol microcapsules prepared by using 20%w/v BSA and 5%w/v TC (D5-D7). When the concentration of BSA was increased to 20%w/v, more D-panthenol could be loaded than that of 13.3% w/v BSA. The microcapsules show spherical in 13.3%w/v and 16.6%w/v D-panthenol loading, respectively. When D-panthenol was increased to 20%w/v the microcapsules was robust as shown in figure 19 formulation D7.

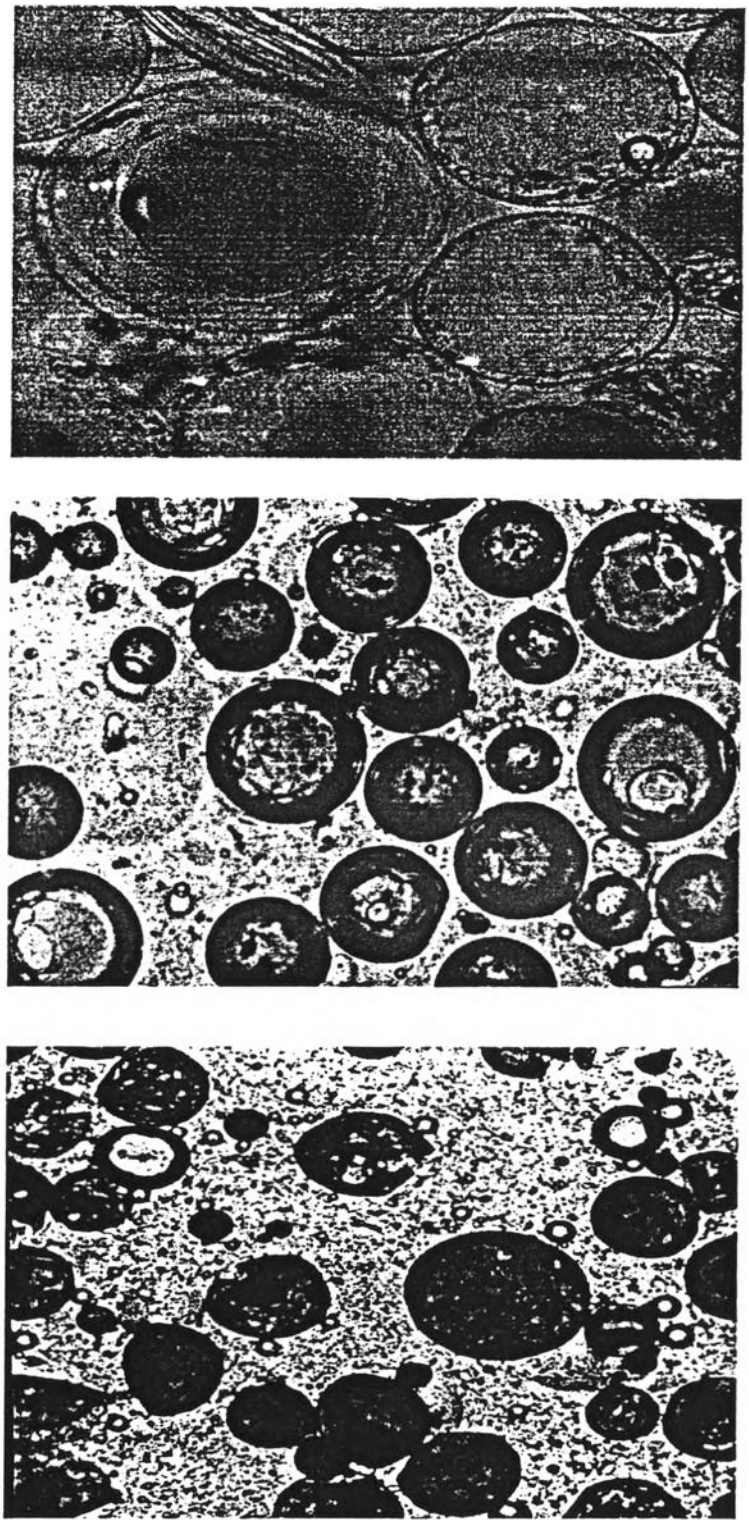


Figure 12 The photographs of microcapsules prepared by BSA and different concentrations of TC from optical microscope. Magnification 40x10

A1=1.25%w/vTC, A2=2.5%w/vTC, A3=5%w/vTC

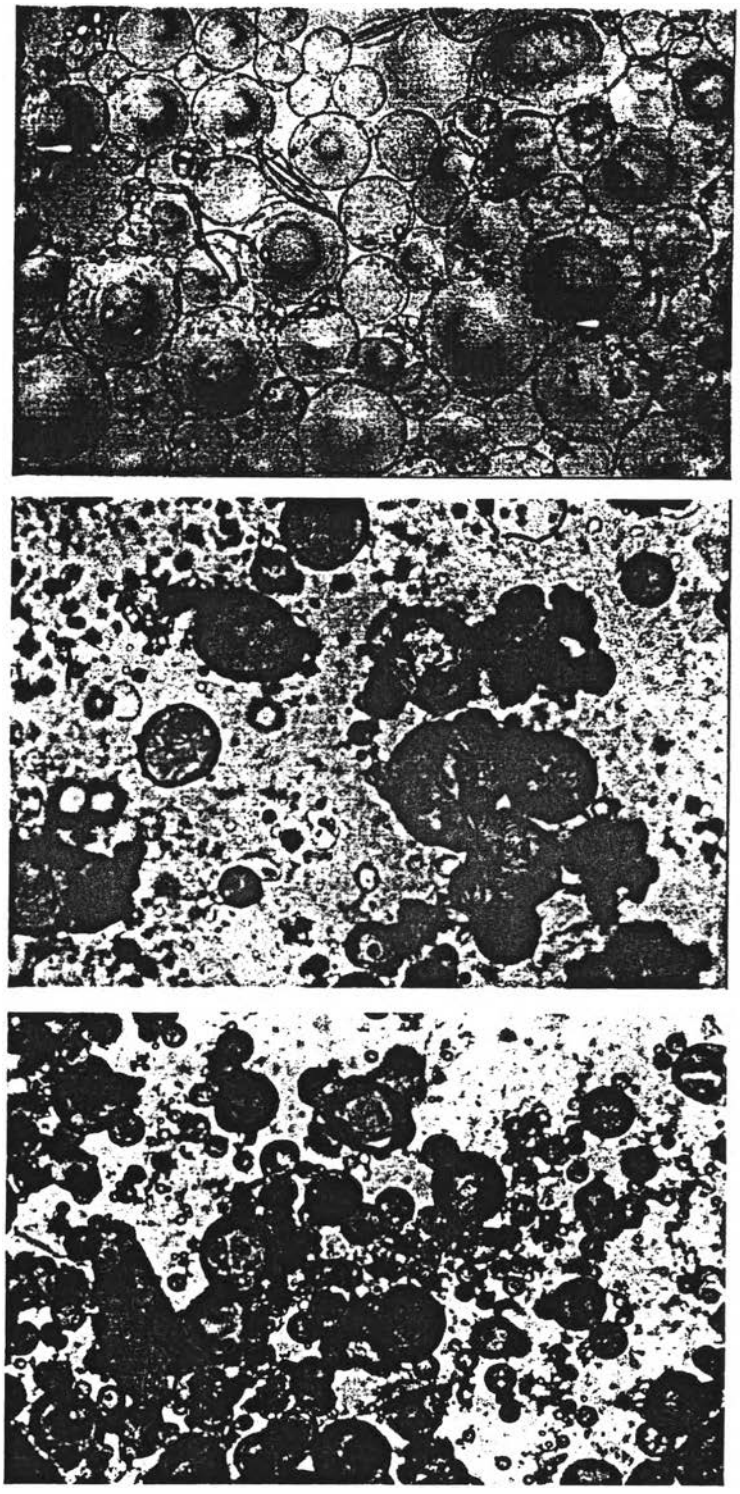


Figure13 The photographs of microcapsules prepared by ovalbumin and different concentrations of TC from optical microscope. Magnification 40x10

A4=1.25%w/vTC, A5=2.5%w/vTC, A6=5.0%w/vTC

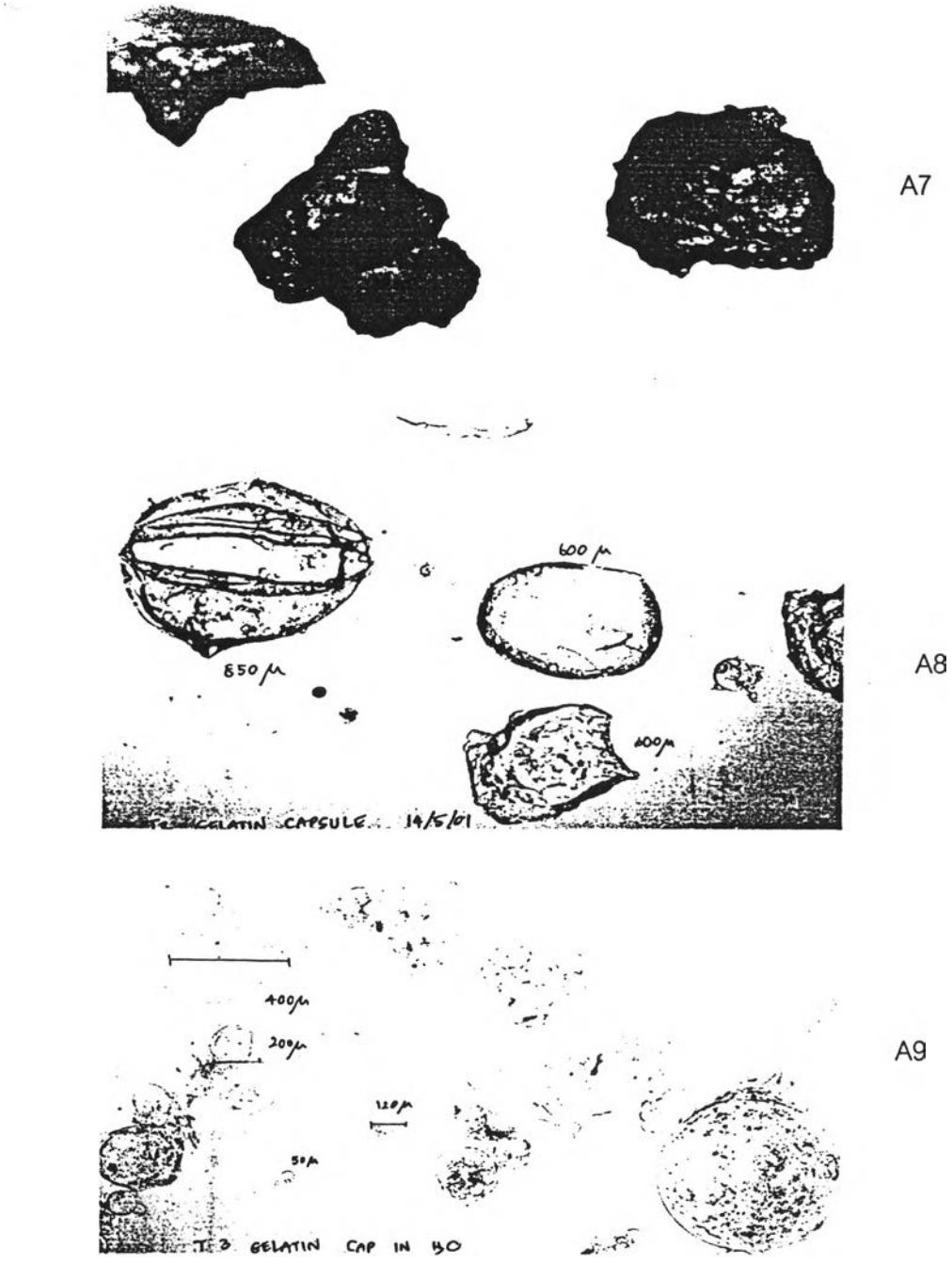


Figure 14 The photographs of microcapsules prepared by gelatin and different concentrations of TC from optical microscope. Magnification 40x10

A7=2.5% w/v TC, 20%w/v gelatin, A8=2.5% w/v TC, 6% w/v gelatin

A9=1.25%w/v TC, 6%w/v gelatin

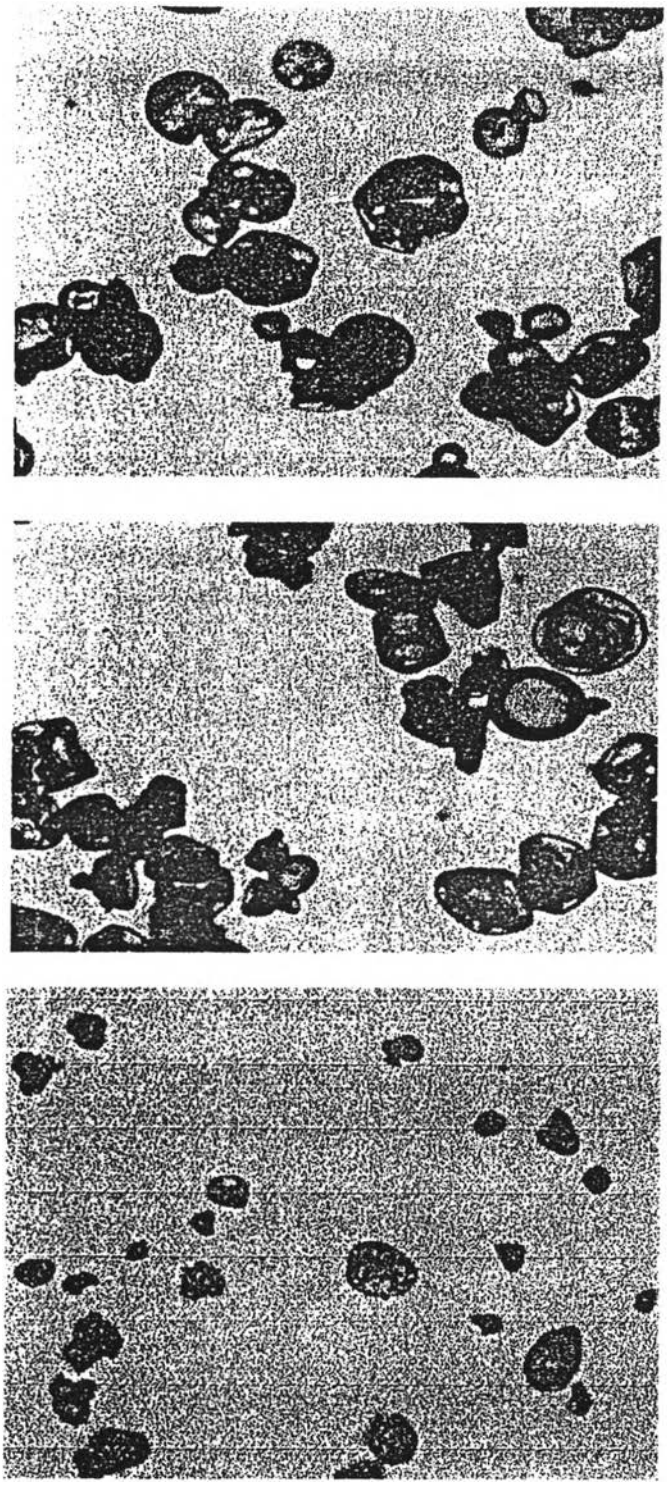


Figure 15 The photographs of BSA-TC microcapsules prepared from different concentrations of BSA (B1-B3). Magnification 40x10

B1=20%w/v BSA, B2=15%w/v BSA, B3=10%w/v BSA

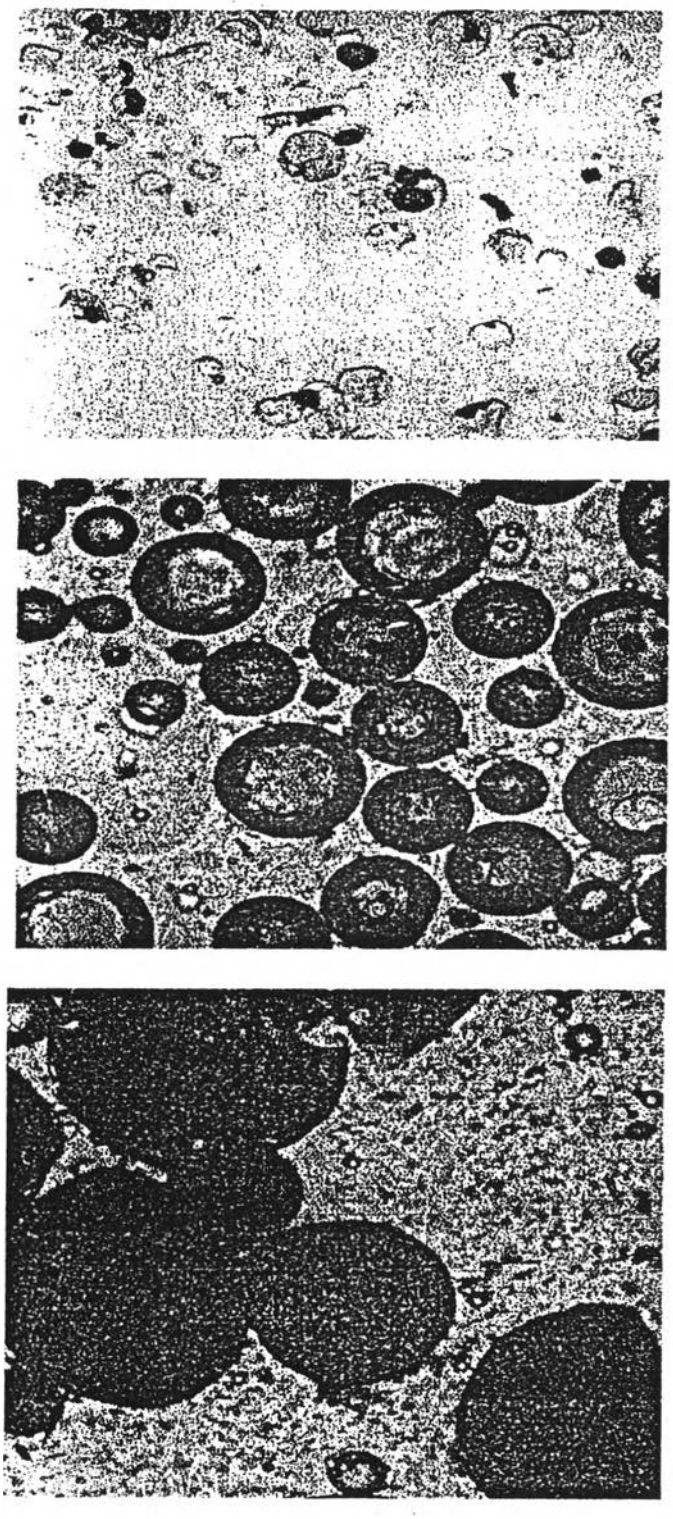


Figure 16 The photograph of BSA-TC microcapsules prepared from20%w/v BSA with different concentrations of TC. Magnification 40x10

B4=1.25%w/v TC, B1=2.5%w/v TC, B5=5%w/v TC

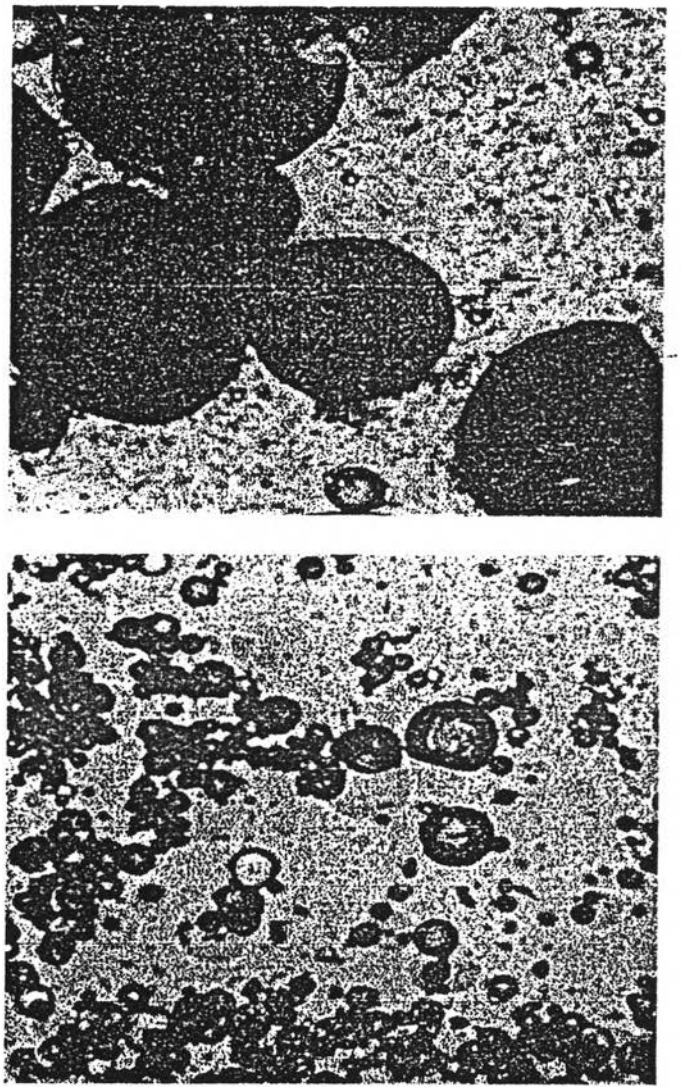


Figure 17 The photographs of BSA-TC microcapsules prepared from different stirring rates. Magnification 40x10

B5= 800 rpm, B6= 11,000 rpm

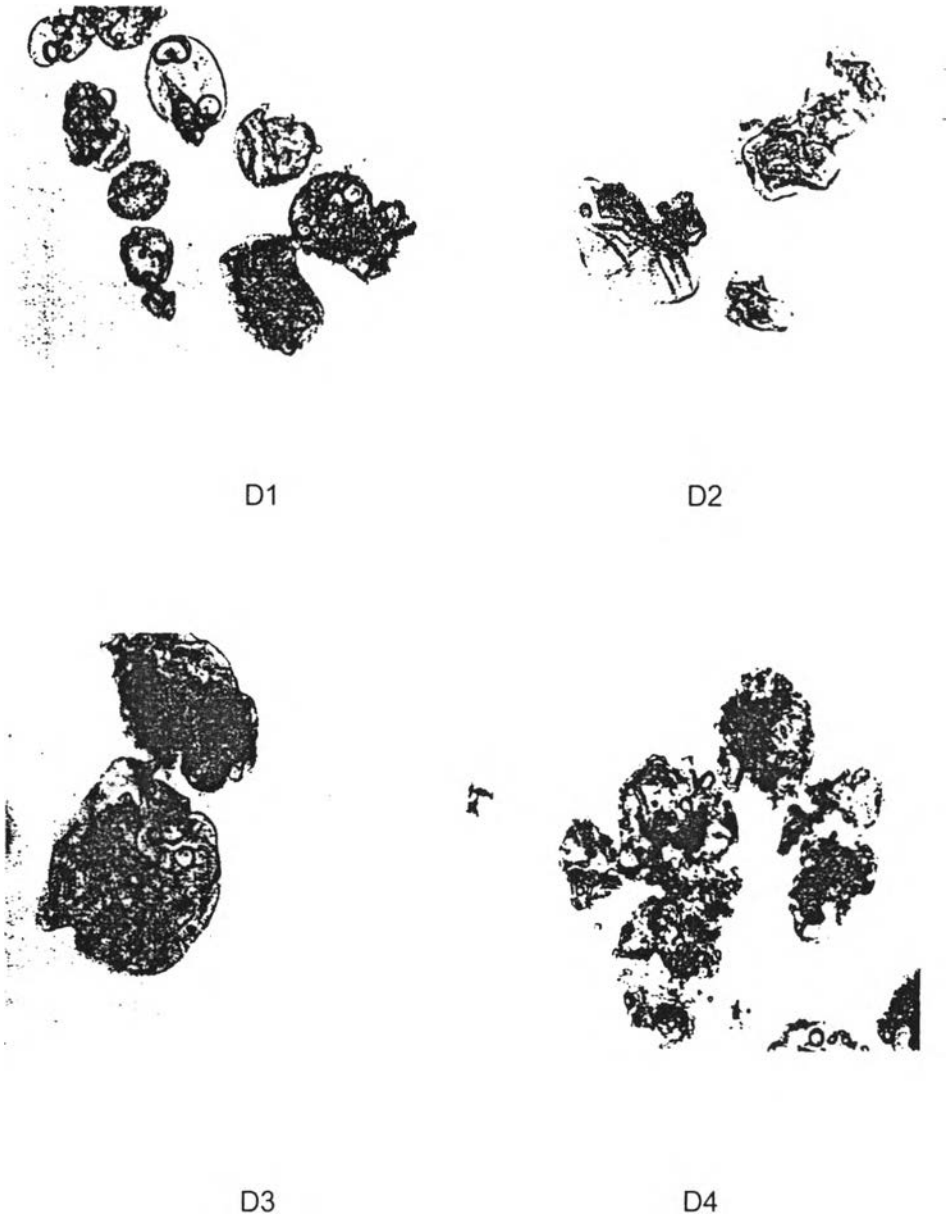


Figure 18 The photographs of BSA-TC walled D-panthenol microcapsules prepared by different concentrations of D- panthenol in carbonate buffer with 5%w/v TC and 13.3%w/v BSA (D1-D4). Magnification 40x10

D1= 10.0% w/v D-panthenol

D2= 13.3% w/v D-panthenol

D3= 16.6% w/v D-panthenol

D4= 20.0% w/v D-panthenol

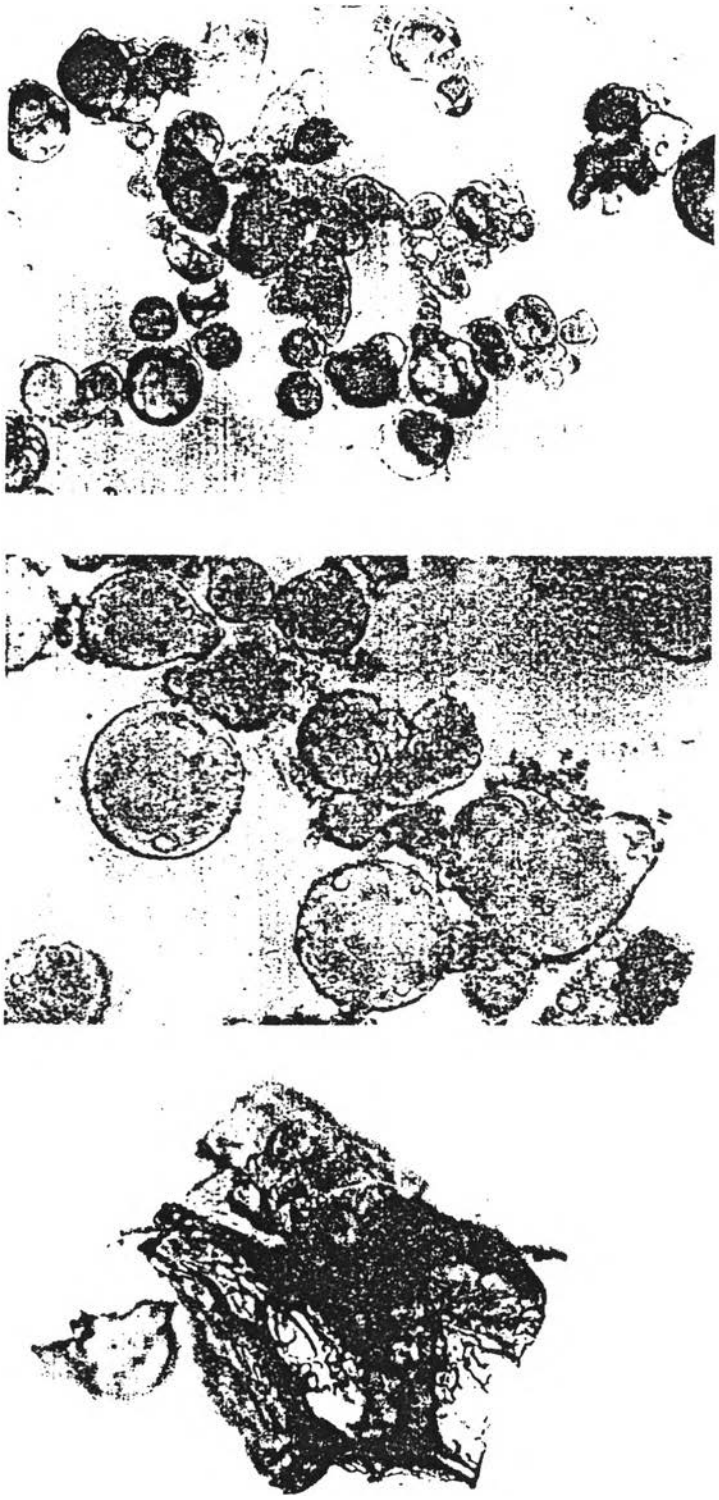


Figure19 The photographs of BSA-TC walled D-panthenol microcapsules prepared by using 20%w/vBSA with different concentrations of D-panthenol in carbonate buffer (D5-D7). Magnification 40x10

D5=13.3% w/v D-panthenol, D6=16.6% w/v D-panthenol, D7=20% w/v D-panthenol

3.2 Scanning Electron Microscope

BSA-TC Microcapsules

SEM of BSA-TC microcapsules prepared by using 20% BSA and 2.5% TC was shown in figure 20. BSA-TC microcapsules were spherical shape with smooth surface. Figure 21 shows surface morphology of BSA-TC microcapsules. There was an excess of BSA deposit outside the wall of microcapsules.

BSA-TC Walled D-panthenol Microcapsule

Figures 22-32 show morphology and surface characteristics of BSA-TC walled D-panthenol microcapsules. The shape of microcapsules showed the evidence of robust and collapsed in irregular shape with rough surface. Some of them were aggregated and some broken. When the percentage of BSA was fixed at 13.3%w/v and the percentage of D-panthenol were increased from 10,13.3,16.6 and 20%w/v respectively, the shapes of microcapsules were more rounded. D-panthenol was completely filled in microcapsules as shown in figures 22, 24, 26 and 28 respectively. The more increasing in D-panthenol loading, the less of deposit of BSA at the surface. The final one in figure 29 showed more porous surface.

When the concentration of BSA was increased to 20% w/v and D-panthenol loading was varied from 13.3, 16.6 and 20 %w/v respectively. Microcapsules were bigger and the surfaces were thick as shown in figures 30-33 respectively. The more concentration of D-panthenol at 20%w/v there was aggregation, the microcapsules was not formed.

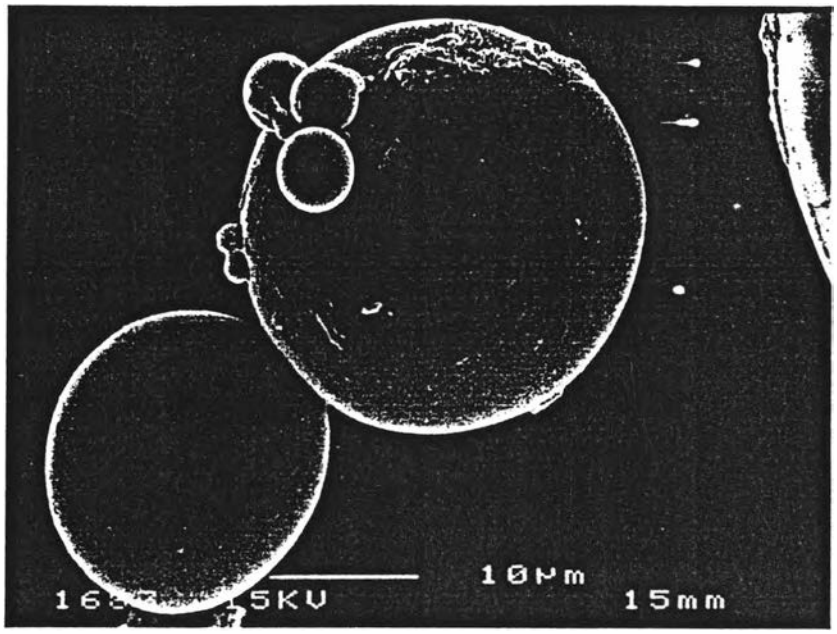


Figure 20 Scanning electron micrograph of BSA-TC microcapsules prepared by using 20%w/v BSA and 2.5%w/v TC formulation B1. Magnification x750

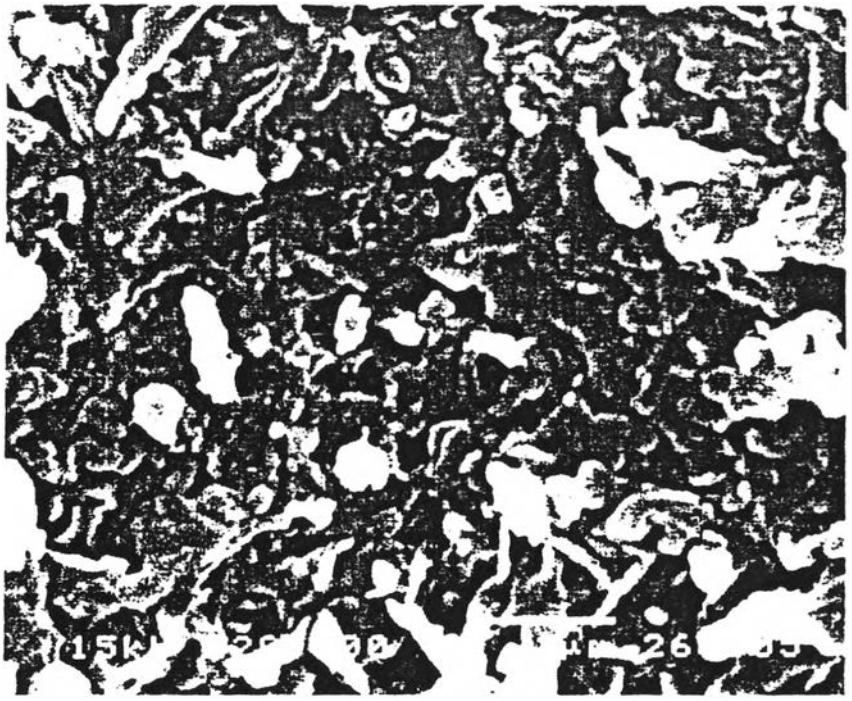

Figure 21 Surface morphology of BSA-TC microcapsules prepared by using 20%w/v BSA and 2.5%w/v TC formulation B1. Magnification x 20,000

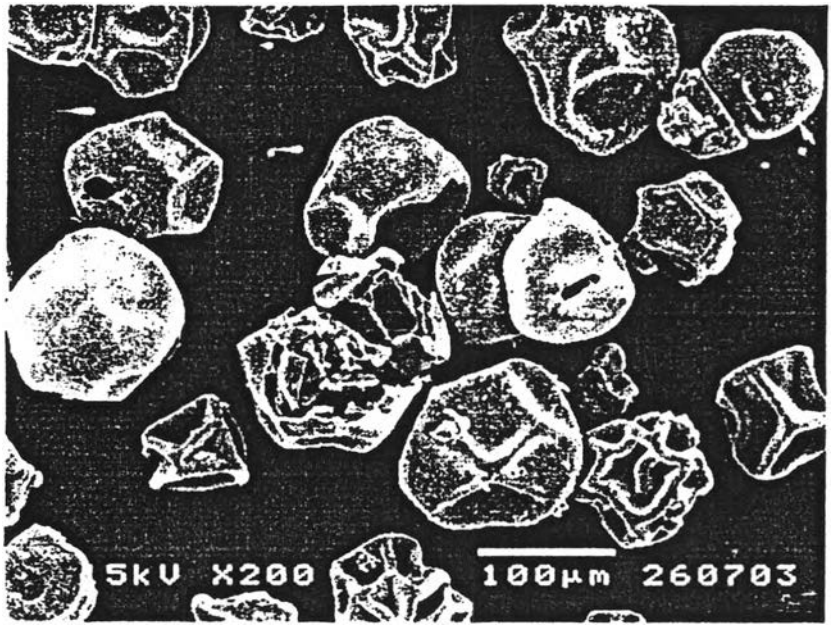


Figure 22 Scanning electron micrograph of BSA-TC walled D-panthenol microcapsules by using 13.3%w/v BSA and 10%w/v D-panthenol loading formulation D1. Magnification x 200


Figure 23 Surface morphology of BSA-TC walled D-panthenol microcapsules by using 13.3%w/v BSA and10%w/v D-panthenol loading formulation D1. Magnification x 20000

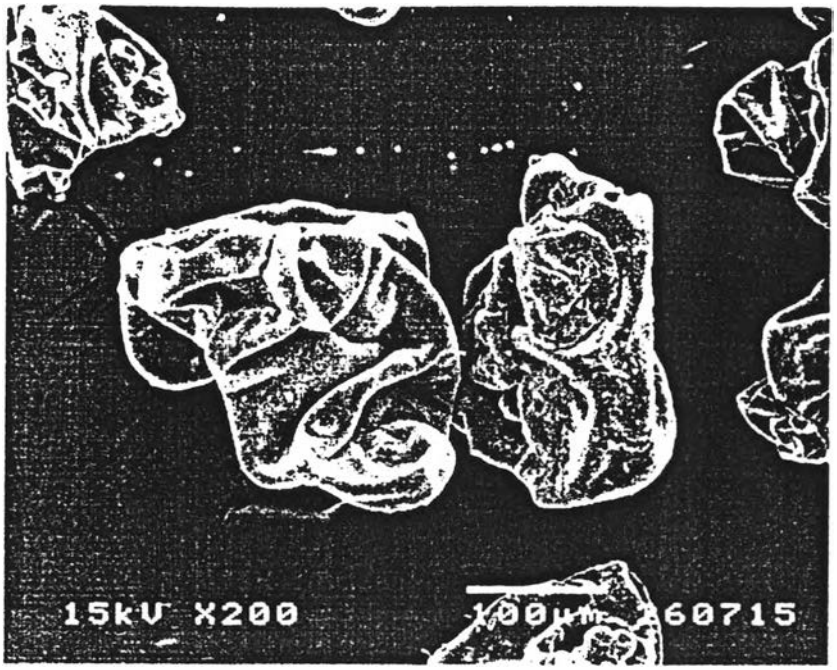


Figure 24 Scanning electron micrograph of BSA-TC walled D-panthenol microcapsules by using 13.3%w/v BSA and 13.3%w/v D-panthenol loading formulation D2.

Magnification x 200

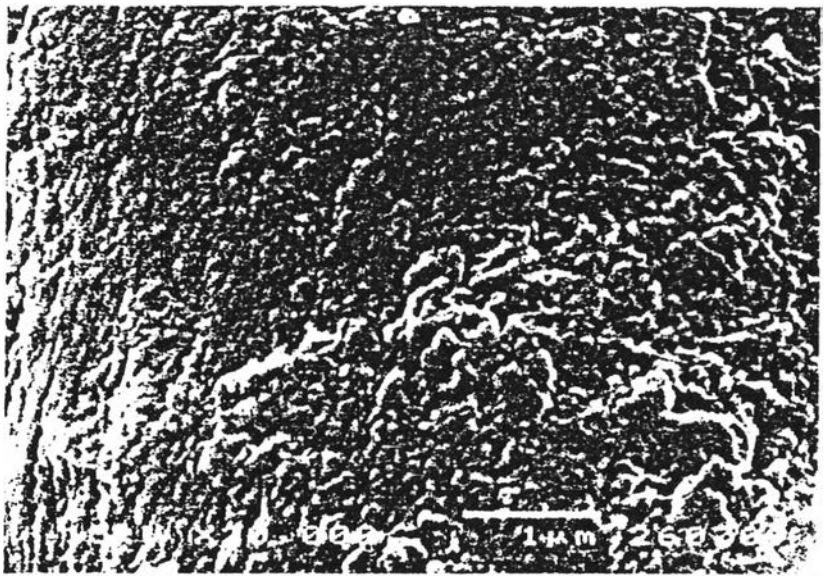

Figure 25 Surface morphology of BSA-TC walled D-panthenol microcapsules by using 13.3%w/v BSA and 13.3%w/v D-panthenol loading formulation D2.

Magnification x 20000

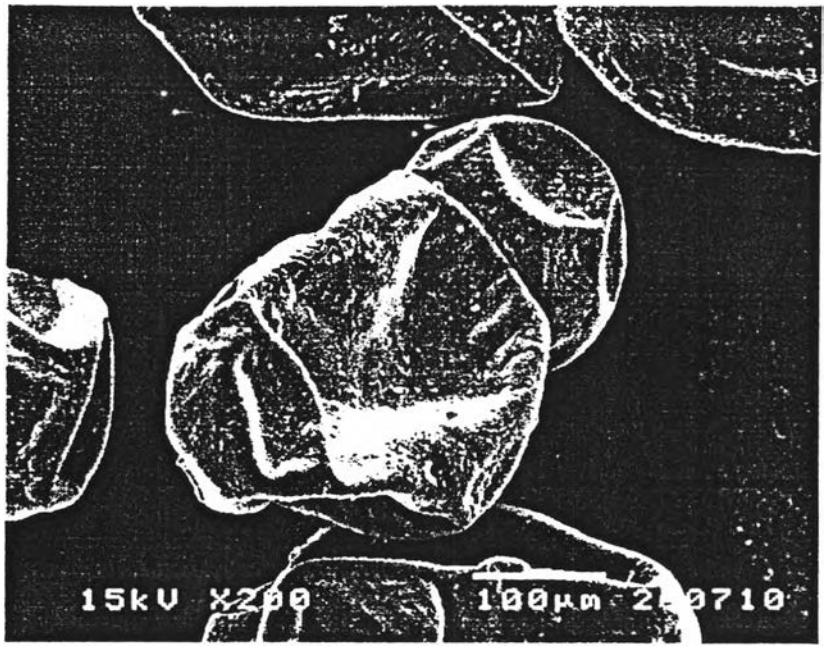


Figure 26 Scanning electron micrograph of BSA-TC walled D-panthenol microcapsules by using 13.3%w/v BSA and 16.6%w/v D-panthenol loading formulation D3.
Magnification x 200

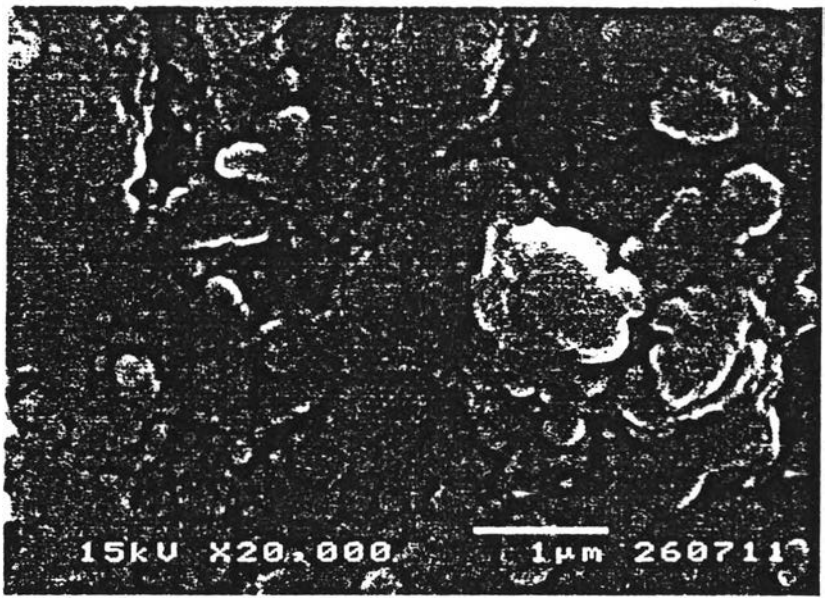


Figure 27 Surface morphology of BSA-TC walled D-panthenol microcapsules by using 13.3%w/v BSA and 16.6% w/v D-panthenol loading formuiation D3.
Magnification x 20000

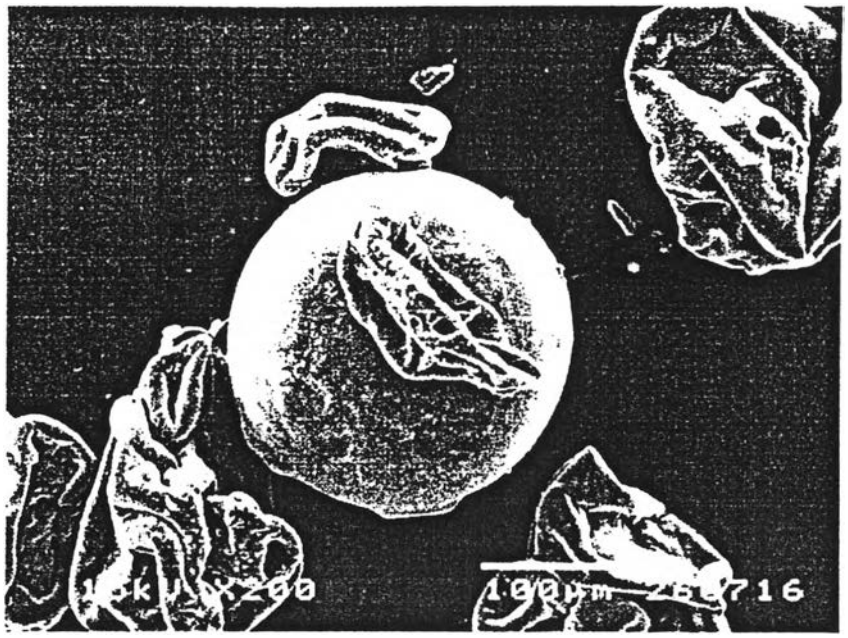

Figure 28 Scanning electron micrograph of BSA-TC walled D-panthenol microcapsules by using 13.3%w/v BSA and 20%w/v D-panthenol loading formulation D4. Magnification x 200

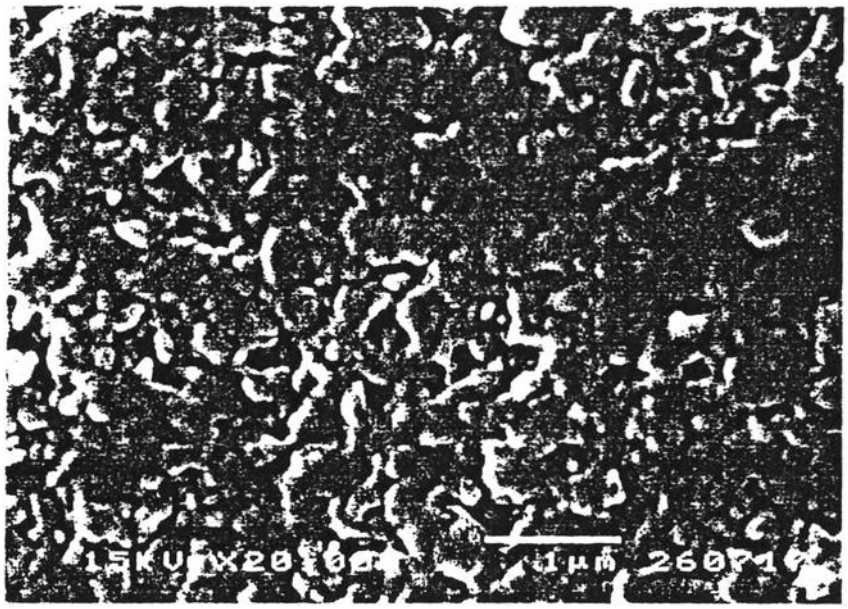

Figure29 Surface morphology of BSA-TC walled D-panthenol microcapsules by using 13.3%w/v BSA and 20% w/v D-panthenol loading formulation D4. Magnification x 20000

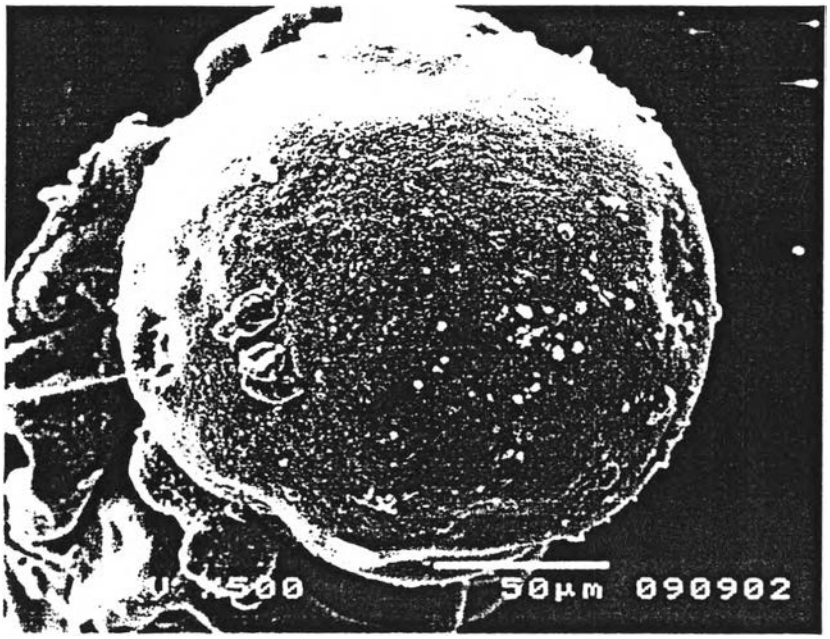


Figure 30 Scanning electron micrograph of BSA-TC walled D-panthenol microcapsules by using 20%w/v BSA and 13.3%w/v D-panthenol loading formulation D5. Magnification x 200

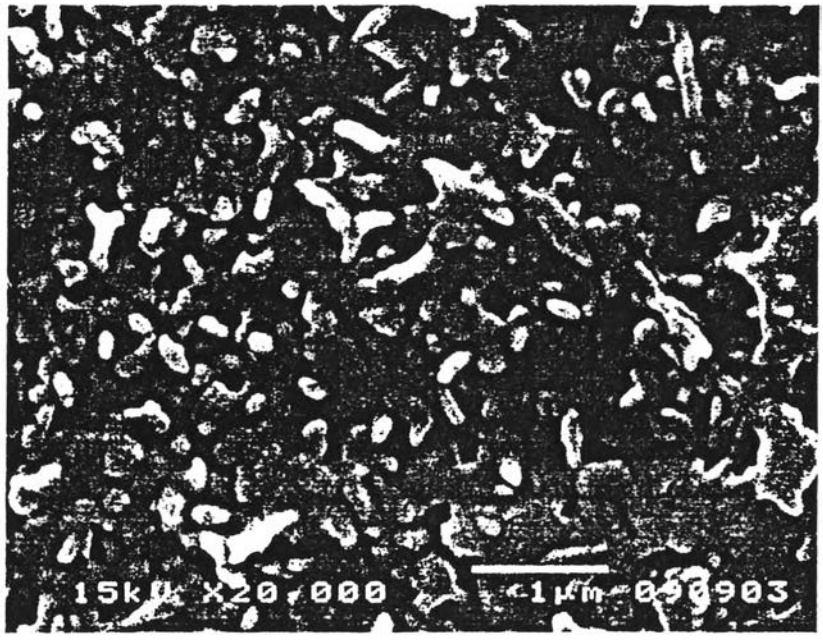


Figure 31 Surface morphology of BSA-TC walled D-panthenol microcapsules by using 20%w/v BSA and 13.3%w/v D-panthenol loading formulation D5. Magnification x 20000

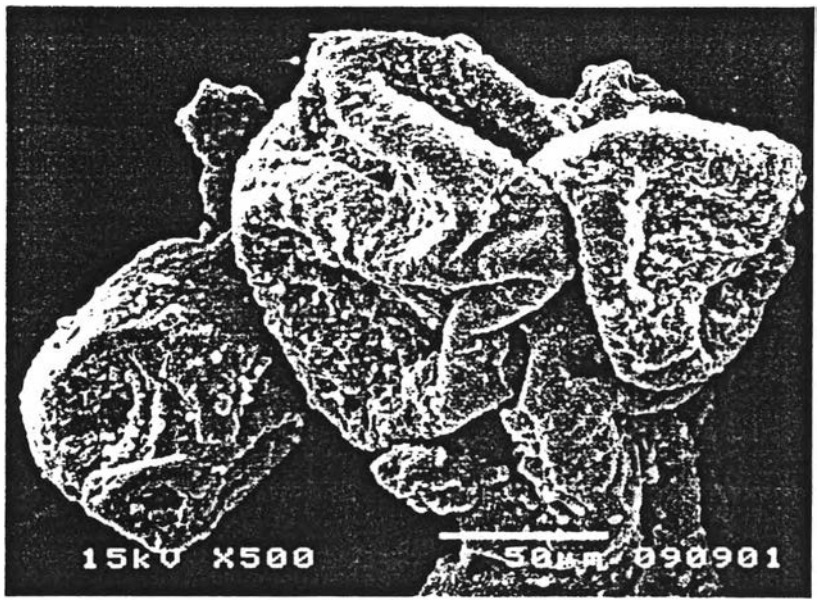


Figure 32 Scanning electron micrograph of BSA-TC walled D-panthenol microcapsules by using 20% w/v BSA and16.6% w/v D-panthenol loading formulation D6. Magnification x 200

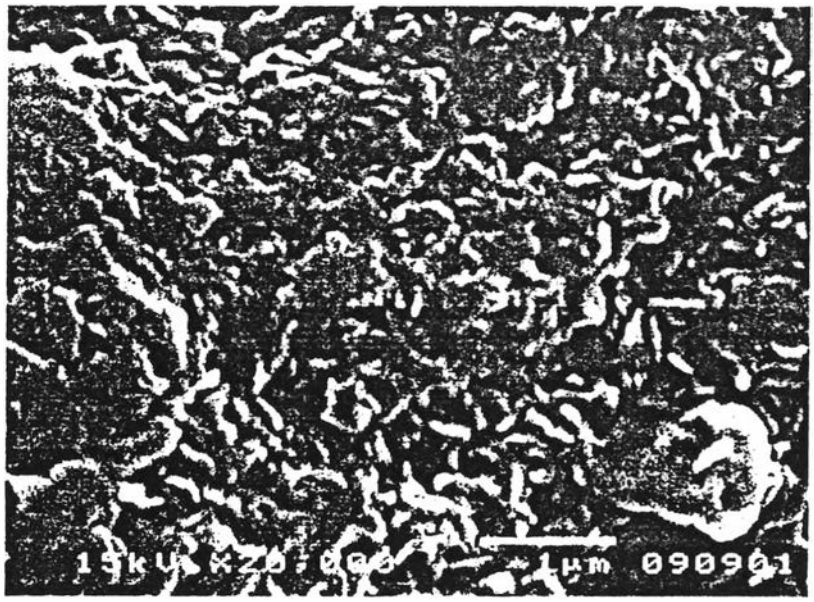


Figure 33 Surface morphology of BSA-TC walled D-panthenol microcapsules by using 20%w/v BSA and 16.6%w/v D-panthenol loading formulation D6. Magnification x 20000

3.3 Particle Size and Size Distribution

BSA-TC microcapsules

Table 9 shows the average mean diameter of BSA-TA microcapusules prepared by varying concentrations of BSA and the percentage of TC was fixed at 2.5%w/v. The mean diameter was between 107.27-128.94 μm. The mean sizes were significant by different when they were compared by using ANOVA.

Table 9 Mean diameter of BSA-TC microcapsules prepared by using 2.5% w/v TC and varied concentrations of BSA from 20%, 15% and 10% w/v.

| Formulation No | % w/v BSA | % w/v TC | Mean size(μm) | | | | |
|---|---|---|---|---|---|---|---|
| | | | N1 | N2 | N3 | Average | SD |
| B 1 | 20 | 2.5 | 122.58 | 123.41 | 128.24 | 124.74 | 3.06 |
| B 2 | 15 | 2.5 | 127.60 | 127.80 | 131.41 | 128.94 | 2.14 |
| B 3 | 10 | 2.5 | 105.82 | 107.30 | 108.7 | 107.27 | 1.44 |

Table 10 shows the average mean diameter of BSA-TC microcapsules prepared by using 20% w/v BSA and varied concentration of TC from 1.25, 2.5 and 5% w/v. An increase in concentration of TC resulted in decreased mean diameter from 377.29 μm to 91.15 μm. The increasing concentration of TC made the wall more hardening and stable, therefore the particle size was not changed. They showed as small particles. When the concentration of TC was reduced, the hardening of the wall was reduced resulted in collision of droplets, which fused together, so the mean size was bigger.

Table 10 Mean diameter of BSA-TC microcapsules prepared by using 20% w/v BSA and varied concentrations of TC from 1.25, 2.5 and 5% w/v.

| Formulation No | % w/v BSA | % w/v TC | Mean size(μm) | | | | |
|---|---|---|---|---|---|---|---|
| | | | N1 | N2 | N3 | Average | SD |
| B 4 | 20 | 1.25 | 377.27 | 375.64 | 378.95 | 377.29 | 165 |
| B 1 | 20 | 2.5 | 122.58 | 123.41 | 128.24 | 124.74 | 3.34 |
| B 5 | 20 | 5.0 | 90.85 | 90.82 | 91.79 | 91.15 | 0.55 |

Table 11 shows average mean diameter of BSA-TC microcapsules prepared by 20% w/v BSA and 5%W/V TC with different stirring rates. The average mean diameter was changed from 91.15 μm to 31.15 μm due to the high shear rate of stirrer resulted in reduction of energy at the surface of microcapsules, then the average mean size was reduce with significant difference. The result was agreed with the previous report (Wakamatsu, Koishi and Kondo, 1974).

Table 11 Mean diameter of BSA-TC microcapsules prepared by using 20%w/v BSA and 5%w/v TC with different stirring rates

| Formulation No | % w/v BSA | Stirring Rate (rpm) | Mean size (μm) | | | | |
|---|---|---|---|---|---|---|---|
| | | | N1 | N2 | N3 | Average | SD |
| B 5 | 20 | 800 | 90.85 | 90.82 | 91.79 | 91.15 | 0.55 |
| B 6 | 20 | 11000 | 30.26 | 30.93 | 32.26 | 31.15 | 1.02 |

BSA-TC walled D-panthenol Microcapsules

Table 12 shows the average mean diameter of BSA-TC walled D-panthenol microcapsules prepared from 13.3% w/v BSA and 5% w/v TC with varying concentrations of D-panthenol from 10, 13.3, 16.6 and 20.0% w/v respectively. The mean diameter was between 349.33-660.41μm. The increased concentration of D-panthenol resulted in increased mean diameter of particle sizes due to the partition coefficient of D-panthenol in aqueous was higher than in organic phase. Higher encapsulation of D-panthenol inside microcapsules resulted in larger mean diameter. The optimum concentration of D-panthenol was 16.6%w/v. It the concentration was higher than 16.6%w/v, the average mean diameter was reduced due to the limitation of concentration of BSA and TC.

Table 12 Mean diameter of BSA-TC walled D-panthenol microcapsules prepared from 13.3%w/v BSA with 5% w/v TC with varying concentrations of D-panthenol from 10-20% w/v

| Formulation No | % w/v BSA | % w/v D-panthenol | Mean size (μm) | | | | |
|---|---|---|---|---|---|---|---|
| | | | N1 | N2 | N3 | Average | SD |
| D 1 | 13.3 | 10.0 | 348.56 | 354.90 | 344.54 | 349.33 | 5.2 |
| D 2 | 13.3 | 13.3 | 375.49 | 385.90 | 378.79 | 380.06 | 5.3 |
| D 3 | 13.3 | 16.6 | 657.91 | 678.99 | 644.32 | 660.41 | 17 |
| D 4 | 13.3 | 20.0 | 537.04 | 552.33 | 540.44 | 543.27 | 8.0 |

Table 13 shows the average mean diameter of BSA-TC walled D-panthenol microcapsules prepared from 20%w/v BSA and 5%w/v TC with varying concentration of D-panthenol from 13.3%, 16.6% and 20% w/v respectively. The results were corresponded. The mean particle size was 466.76-572.5 μm. An increase in concentration of D-panthenol resulted in increased mean diameter of particle sizes due

to the partition coefficient of D-panthenol in the aqueous phase was higher than that in the organic phase resulted in higher encapsulation of D-panthenol inside microcapsules

Table 13 Mean diameter of BSA-TC walled D-panthenol microcapsules prepared from 20% w/v BSA 5%w/v TC with varying concentrations of D-panthenol from 13.3-20% w/v

| Formulation No | % w/v BSA | % w/v D-panthenol | Mean size (μm) | | | | |
|---|---|---|---|---|---|---|---|
| | | | N1 | N2 | N3 | Average | SD |
| D 5 | 20 | 13.3 | 460.44 | 455.10 | 484.74 | 466.76 | 15.8 |
| D 6 | 20 | 16.6 | 568.75 | 559.94 | 588.83 | 572.51 | 14.8 |
| D 7 | 20 | 20.0 | NA | NA | NA | NA | NA |

NA= not applicable

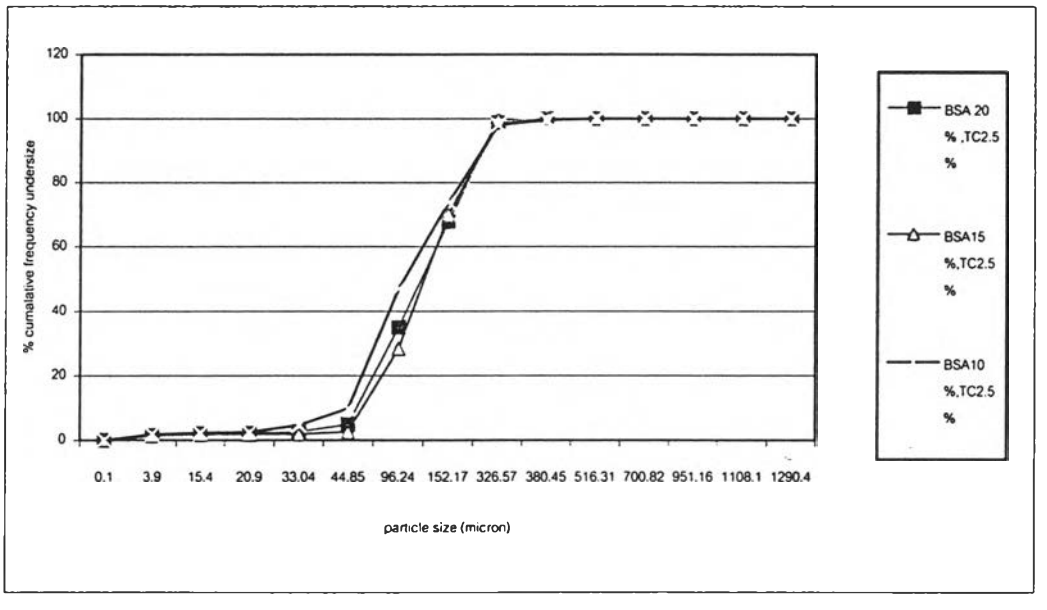


Figure 34 The percentage cumulative frequency undersize of BSA-TC microcapsules prepared from different concentrations of BSA (B1-B3).

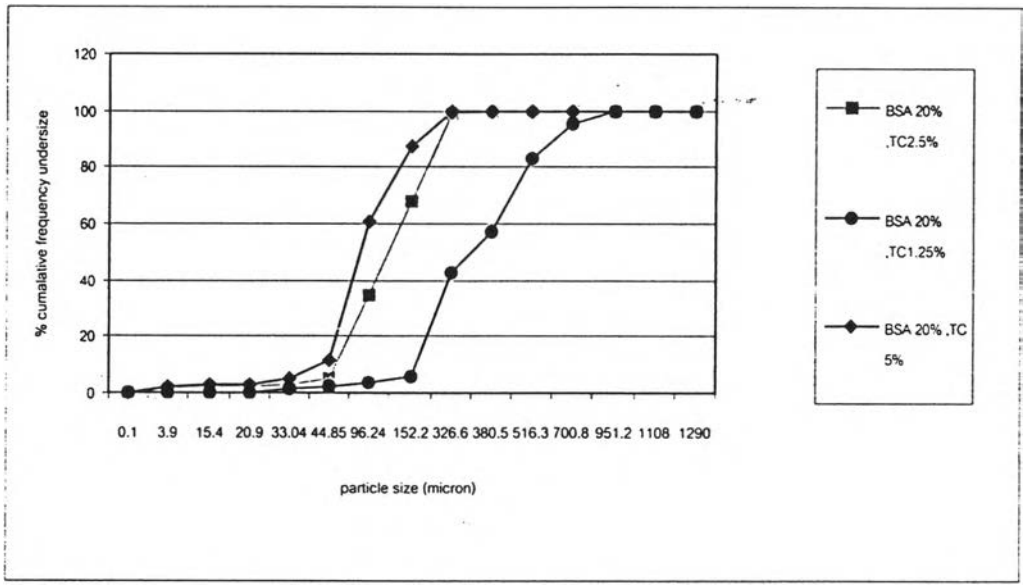


Figure 35 The percentage cumulative frequency undersize of BSA-TC microcapsules prepared from different concentrations of TC (B1,B4, B5).

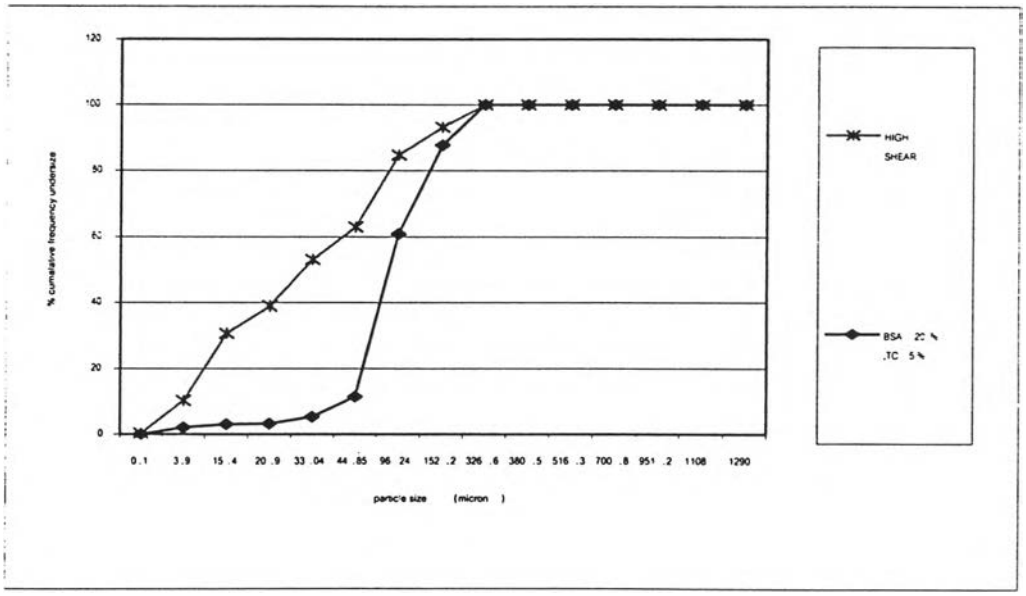


Figure 36 The percentage cumulative frequency undersize of BSA-TC microcapsules prepared from different stirring rates (B5, B6).

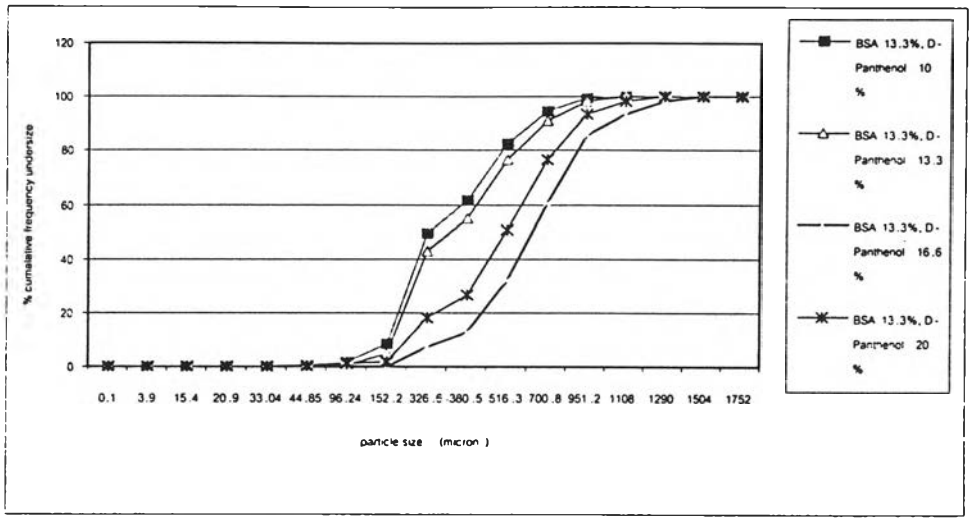


Figure 37 The percentage cumulative frequency undersize of BSA-TC walled D-panthenol microcapsules prepared by different concentrations of D-panthenol with 13.3%w/v BSA and 5%w/v TC (D1-D4).

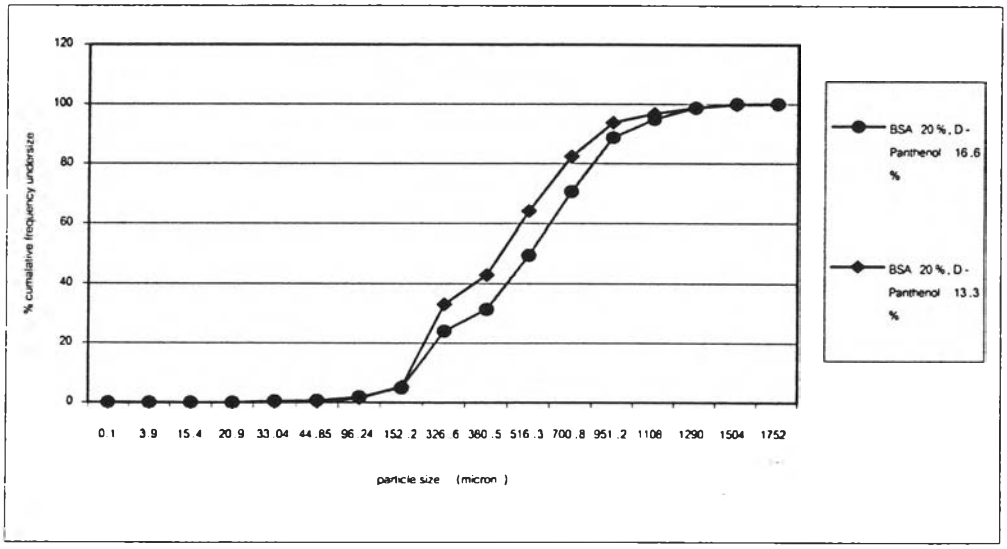


Figure 38 The percentage cumulative frequency undersize of BSA-TC walled D-panthenol microcapsules prepared by different concentrations of D-panthenol with 20%w/v BSA and 5%w/v TC (D5-D6).

3.4 Yield of Microcapsules

BSA-TC microcapsules were shrunk and powdery with free flowing properties after drying. The percent yield was between 60-70% as shown in table 14 and illustrated as histogram in figure 39 when percentage of TC was reduced to 1.25%w/v. While the percentage of BSA increased the ratio of BSA and TC was not suitable then the microcapsules were not formed. Table 15 shows percent yield of BSA-TC walled D-panthenol microcapsules, which was between 80-90%. Figure 40 shows histogram of percent yield of BSA-TC wall D-panthenol microcapsules. The difference of percent yield indicated that the formation of concentration of core material influenced the formation of microcapsules. In interfacial polymerization reaction, w/o emulsion was formed and surfactant played an important role for emulsification of polymer solution in the organic solvent phase and ingredient in the aqueous phase. For this reason, if the concentration of surfactant was not enough to emulsify the aqueous phase, the polymerization reaction would be incompleted. These BSA-TC walled D-panthenol microcapsules could not be formed in formulas D4, and D7 due to their high concentrations of D-panthenol. Formula D2 shows highest percent yield of 90.4%, this formula contains the same percentage of BSA and D-panthenol. If the active ingredient concentration was greater or less than the BSA concentration, then lower percent yield was obtained.

Table 14 The percent yield of BSA-TC microcapsules

| Formulation No | Variable | | Speed (rpm) | Wt of microcapsules (g) | % Yield |
|---|---|---|---|---|---|
| | %BSA | %TC | | | |
| B 1 | 20 | 2.5 | 800 | 1.0069 | 67.13 |
| B 2 | 15 | 2.5 | 800 | 0.8386 | 67.09 |
| B 3 | 10 | 2.5 | 800 | 0.6504 | 65.04 |
| B 4 | 20 | 1.25 | 800 | NA | NA |
| B 5 | 20 | 5.0 | 800 | 1.2150 | 60.75 |
| B 6 | 20 | 5.0 | 11000 | 1.2710 | 70.50 |

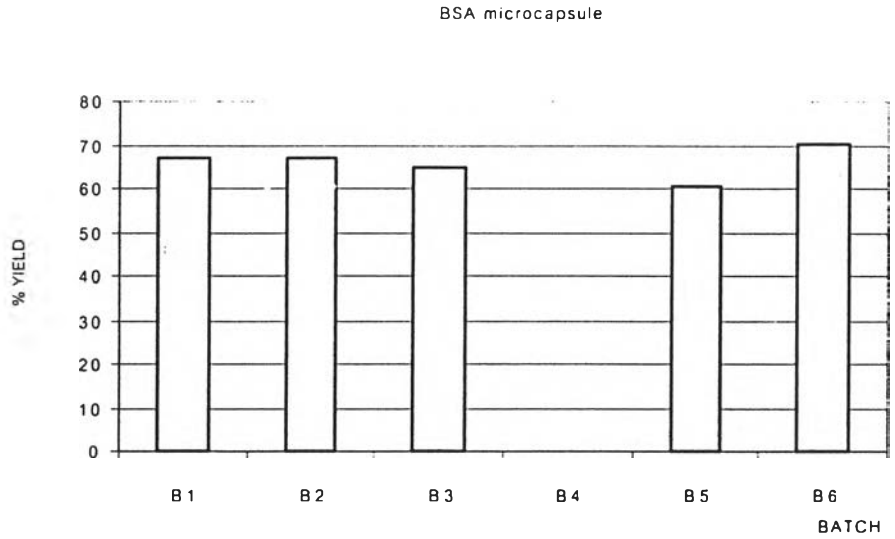


Figure 39 The percent yield of BSA –TC microcapsules.

Table 15 The percent yield of D-panthenol microcapsules prepared from 13.3%w/v BSA and 20%w/v BSA with 5% w/v TC

| Formulation No | Variable | | Wt.of microcapsules (g) | % Yield |
|---|---|---|---|---|
| | %BSA | %D-Panthenol | | |
| D 1 | 13.3 | 10.0 | 4.8412 | 87.47 |
| D 2 | 13.3 | 13.3 | 5.4240 | 90.40 |
| D 3 | 13.3 | 16.6 | 5.8010 | 89.24 |
| D 4 | 13.3 | 20.0 | NA | NA |
| D 5 | 20.0 | 13.3 | 6.414 | 80.17 |
| D 6 | 20.0 | 16.6 | 7.0647 | 83.12 |
| D 7 | 20.0 | 20.0 | NA | NA |

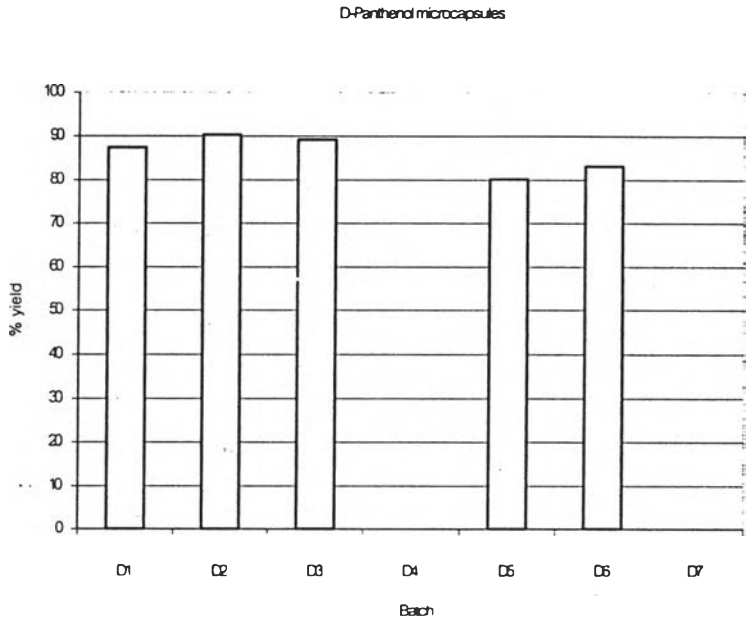


Figure 40 The percent yield of BSA-TC walled D-panthenol microcapsules.

4. Method of Quantitative Analysis of D-panthenol

4.1 UV Spectrophotometer

Both D-panthenol and pantothenyl ethyl ether show the maximum absorption wavelength at 210 nm. in figures 41-42. The UV detector of HPLC was set at 210 nm in order to obtain the accurately quantitative analysis of D-panthenol microcapsules.

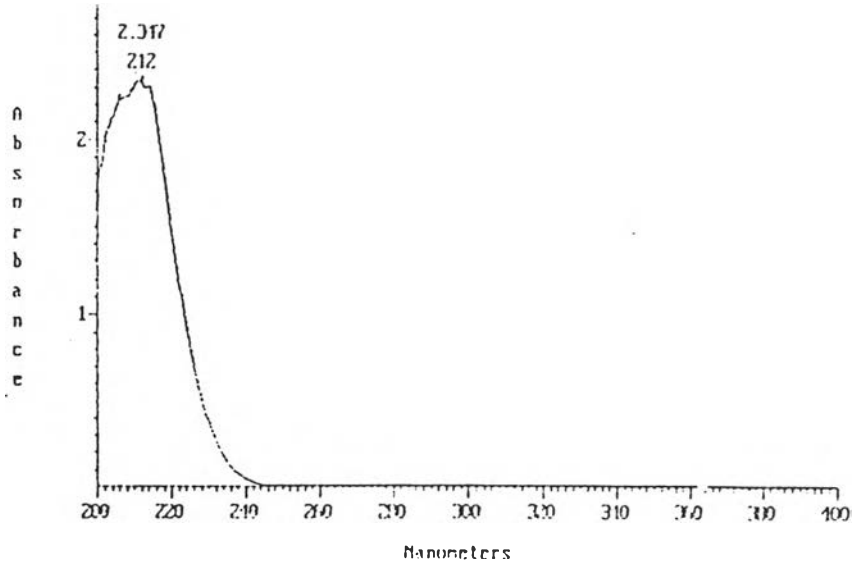


Figure 41 The UV spectrum of D-panthenol.

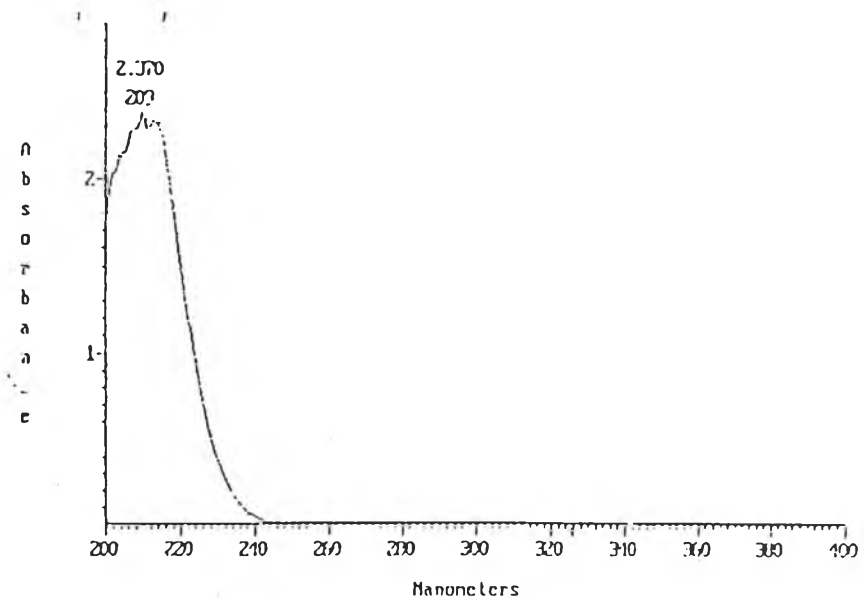


Figure 42 The UV spectrum of D-pantothenyl ethyl ether.

4.2 HPLC Assay for D-panthenol Analysis

4.2.1 Accuracy

Three sets of standard solutions of D-panthenol were prepared and injected. Each sample was analyzed by HPLC. The accuracy data was presented in table 16. The values of % recovery were between 98.60- 109.49%, which indicated that the HPLC condition could be used to determine D-panthenol content within the concentration range studied.

Table 16 Accuracy data of D-panthenol standard solution

| D-Panthenol concentration (mg/ml) | Peak area ratio | Analytical concentration (mg/ml) | % Recovery |
|---|---|---|---|
| 0.2505 | 0.7920 | 0.2457 | 98.09 |
| | 0.7960 | 0.2515 | 100.47 |
| | 0.8000 | 0.2573 | 102.73 |
| Mean | | | 100.43 |
| SD | | | 2.32 |
| %CV | | | 2.31 |
| 0.5010 | 0.9726 | 0.5078 | 101.36 |
| | 0.9489 | 0.4734 | 94.50 |
| | 0.9486 | 0.4730 | 94.50 |
| Mean | | | 98.60 |
| SD | | | 3.52 |
| %CV | | | 3.57 |
| 1.002 | 1.3513 | 1.0574 | 105.54 |
| | 1.3888 | 1.1119 | 110.97 |
| | 1.3957 | 1.1219 | 111.97 |
| Mean | | | 109.49 |
| SD | | | 3.46 |
| %CV | | | 3.16 |

Note Accuracy (CV%) $= \frac{SD \times 100}{X}$

Where SD = Standard deviation of sample of each concentration

X = Mean experimental condition

4.2.2 Linearity

Table 17 shows the peak area ratio of standard solution of D-panthenol. The standard calibration curve of D-panthenol is shown in figure 43. Linear regression analyses of the peak area ratio was performed in the concentration of 0.25 – 1.5 mg/ml of D-panthenol with resulted in a coefficient of determination of 0.999.

The regression equation of the line is $Y= 0.689X+0.6227$

Where Y is the peak area ratio of D-panthenol

X is the concentration of D-panthenol solution in mg/ml

Table 17 Peak area ratio of D-panthenol standard solution.

| STD. NO | Concentration (mg/ml) | Peak area ratio |
|---|---|---|
| 1 | 0.2505 | 0.7944 |
| 2 | 0.5010 | 0.9661 |
| 3 | 0.7525 | 1.1546 |
| 4 | 1.0020 | 1.2960 |
| 5 | 1.2525 | 1.4918 |
| 6 | 1.5030 | 1.6587 |

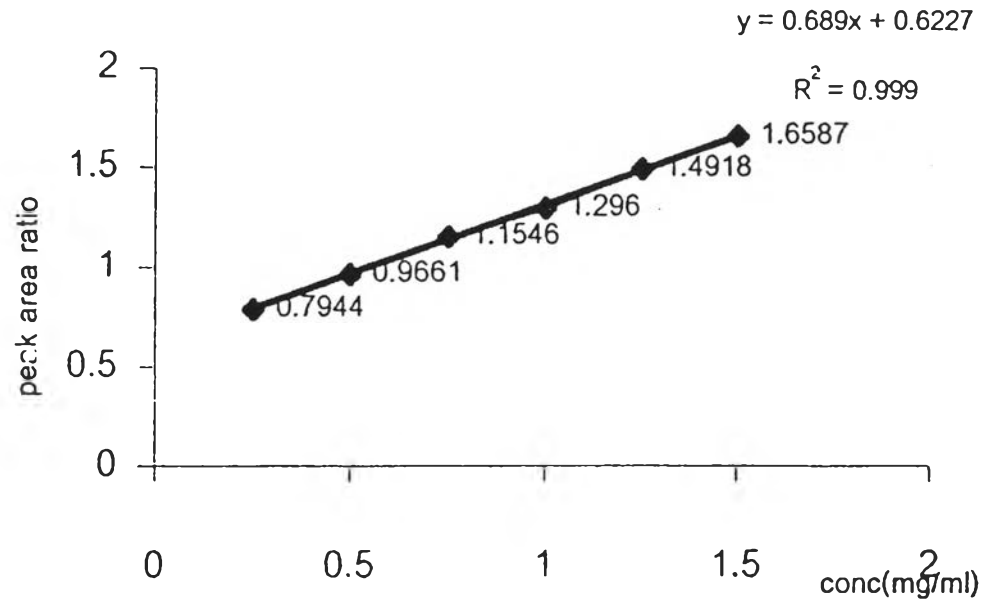


Figure 43 Standard calibration curve of D-panthenol.

4.2.3 Specificity

Figure 44 shows the chromatograms of D-panthenol and pantothenyl ethyl ether in standard solution, blank microscapsules with PEE and D-panthenol in blank microcapsules. The retention times for D-panthenol and pantothenyl ethyl ether in standard solution were approximately 2.5-2.6 and 5.1-5.2 minutes respectively. The chromatograms show no interference from the constituents in the preparation. Therefore, the conditions of HPLC for quantitative analysis of D-panthenol were appropriately applicable to the experiment without any interference.

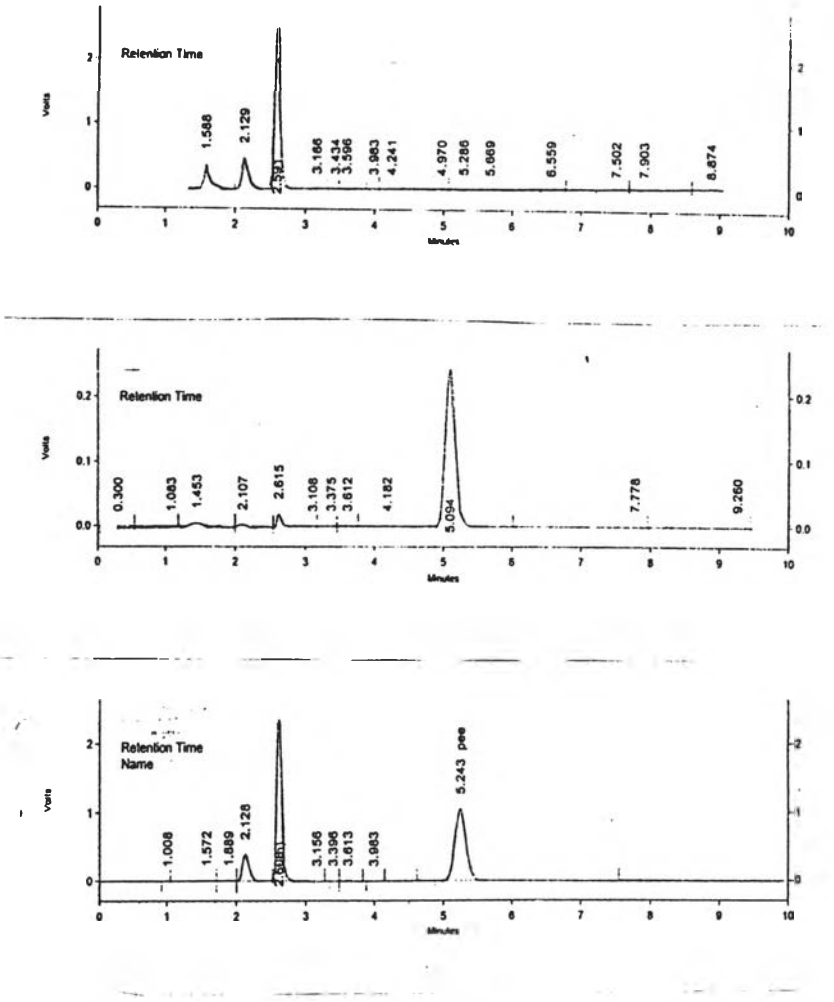


Figure 44 The chromatogram of D-panthenol (a), pantothenyl ethyl ether (b) and sample of D-panthenol microcapsules (c).

4.2.4 Precision

Tables 18-19 show the peak area ratio of D-panthenol and pantothenyl ethyl ether analyzed by HPLC both in the same day and different days. The concentration of standard solution was in the range of 0.25 mg/ml – 1.5 mg/ml for within run analysis, the percentage of coefficient of variation (%CV) was within the range of 0.5 - 2.2%. So this method could be used to determine the amount of D-panthenol.

Table 18 Within run precision data

| Concentration (mg/ml) | Peak area ratio | | | Mean | SD | %CV |
|---|---|---|---|---|---|---|
| | No.1 | No.2 | No.3 | | | |
| 0.2505 | 0.7920 | 0.7960 | 0.8000 | 0.7962 | 0.004 | 0.50 |
| 0.5010 | 0.9726 | 0.9489 | 0.9486 | 0.9567 | 0.0137 | 1.43 |
| 0.7515 | 1.1709 | 1.2088 | 1.2133 | 1.1976 | 0.023 | 1.92 |
| 1.0020 | 1.3513 | 1.3888 | 1.3957 | 1.3786 | 0.0238 | 1.73 |
| 1.2525 | 1.6064 | 1.6730 | 1.6664 | 1.9486 | 0.0.66 | 2.22 |
| 1.5030 | 1.7925 | 1.7858 | 1.7309 | 1.7697 | 0.0338 | 1.91 |

Table 19. Between run precision data

| Concentration (mg/ml) | Peak area ratio | | | Mean | SD | %CV |
|---|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | | | |
| 0.25 | 0.7972 | 0.7836 | 0.7944 | 0.8006 | 0.008 | 0.99 |
| 0.50 | 0.9638 | 0.9920 | 0.9661 | 0.9773 | 0.013 | 1.33 |
| 0.75 | 1.144 | 1.1715 | 1.1546 | 1.1567 | 0.014 | 1.21 |
| 1.00 | 1.3152 | 1.3510 | 1.2960 | 1.3207 | 0.028 | 2.12 |
| 1.25 | 1.5613 | 1.5994 | 1.4918 | 1.5508 | 0.0545 | 3.51 |
| 1.50 | 1.6573 | 1.6364 | 1.6220 | 1.6386 | 0.0178 | 1.08 |

The percentage of coefficient of variation (%CV) forms between run analysis was in the range of 0.9-3.51 %. Therefore, this analytical method could be used to analyze D-panthenol.

4.3 Analysis of D-panthenol in Microcapsules

Three batches of D-panthenol microcapsules D3, D5 and D6 were analyzed. 500 mg of microcapsules was crushed in mortar with two times 25.0 ml of water and filtered by filter paper to 100-ml volume flask. 5 ml of pantothenyl ethyl ether was pipetted to the solution and adjusted to volume with water. Forty microliters was analyzed and the result of percent entrapment was calculated by the equation that was shown in appendix IV. Table 20 shows peak area ratio of D-panthenol from HPLC analysis and amount of D –panthenol in products.

Table 20 The amount of entrapped D-panthenol in microcapsules prepared by varied BSA and D-panthenol in different ratio from formulation $D_3$, $D_5$ and $D_6$

| Formulation No. | Amount D-panthenol (g) | BSA (g) | Peak area ratio | Entrapped D-panthenol (mg/ml) | % Entrapment |
|---|---|---|---|---|---|
| D3 | 2.5 | 2 | 1.2992 | 0.9819 | 51.07 |
| | | 2 | 1.2452 | 0.9035 | 46.98 |
| | | 2 | 1.2900 | 0.9685 | 50.38 |
| Mean (X) | | | 1.2781 | 0.9512 | 49.45 |
| SD | | | | | 2.06 |
| D5 | 2.0 | 3 | 1.3878 | 1.1104 | 69.24 |
| | | 3 | 1.3675 | 1.0810 | 67.40 |
| | | 3 | 1.3640 | 1.0759 | 67.92 |
| Mean | | | 1.3731 | 1.0891 | 68.19 |
| SD | | | | | 0.95 |
| D6 | 2.5 | 3 | 1.2597 | 0.9245 | 44.47 |
| | | 3 | 1.2726 | 0.9432 | 45.60 |
| | | 3 | 1.2851 | 0.9614 | 46.22 |
| Mean | | | 1.2725 | 0.9431 | 45.34 |
| SD | | | | | 0.89 |

Table 21 The percent observed content, percent theoretical and percent entrapment of BSA- TC walled D-panthenol microcapsules

| Formulation No | %w/v D-panthenol conc. | % Observed content | | | | | % Theoretical content |
|---|---|---|---|---|---|---|---|
| | | N1 | N2 | N3 | Mean | SD | |
| D3 | 16.6 | 19.64 | 18.07 | 19.34 | 19.02 | 0.83 | 38.46 |
| D5 | 13.3 | 17.31 | 16.85 | 16.77 | 16.98 | 0.29 | 25.0 |
| D6 | 16.6 | 13.08 | 13.41 | 13.61 | 13.37 | 0.27 | 29.41 |

The D-panthenol entrapment by IFP was in the range of 40-70%. Since the D-panthenol was a water-soluble drug in aqueous phase and it should be emulsified by surfactant in the preparation. The mixture of BSA and D-panthenol were completely soluble in carbonate buffer pH9.8 when surfactant was fixed in this preparation, the emulsification of aqueous phase and organic phase depended on the amount of surfactant. Since increased the amount of surfactant increase in amount of diamine and drug to the organic phase where polymerization happened and microcapsules were formed. In addition the amount of Span 85® included in microcapsules was unknown, the effect of concentration of Span 85® influenced the microcapsules was not clear. For this reason, it could not indicate the relation between the concentration of BSA and D-panthenol for %entrapment. However, the highest percentage entrapment was 68.19 $\pm$0.95% when 20%w/v of BSA and 13.3%w/v of D-panthenol were used in this experiment. The higher amount of BSA with lower amount of D-panthenol resulted in higher percent entrapment.