CHAPTER IV

RESULTS AND DISCUSSION

1. UV Analysis

The amount of minocycline hydrochloride of the prepared microcapsules could be determined by UV spectrophotometric method. The validation of the UV spectrophotometric method used is presented as follows: (Gorog, 1995)

1.1 Specificity

The absorption spectrum of minocycline hydrochloride in isotonic borate buffer pH 7.5±0.1 showed 3 maximum peaks of absorbance at 244, 278 and 381 nm, respectively as shown in Figure 8. Figure 9 shows the spectrum of the solution extracted from minocycline hydrochloride microcapsules and blank microcapsules by the method used for analysis of drug content (page 35). The spectrum also shows the same maximum absorbance at 3 peaks of 244, 278 and 381 nm. Therefore the wavelength used to analyse drug content of minocycline hydrochloride microcapsules was 244 nm since it was the λ_{max} of minocycline hydrochloride in this medium and this wavelength was not interferred by other components of a combined formulation (Figure 9(b)).

Figure 10 shows the spectrum of the solution released from minocycline hydrochloride microcapsules and blank microcapsules. The solution released from minocycline hydrochloride microcapsules showed broad peaks at 244 and 278 nm. The wavelength used to analyse drug release of minocycline hydrochloride microcapsules was 381 nm since it was not interferred by other components of a combined formulation (Figure 10(b)).

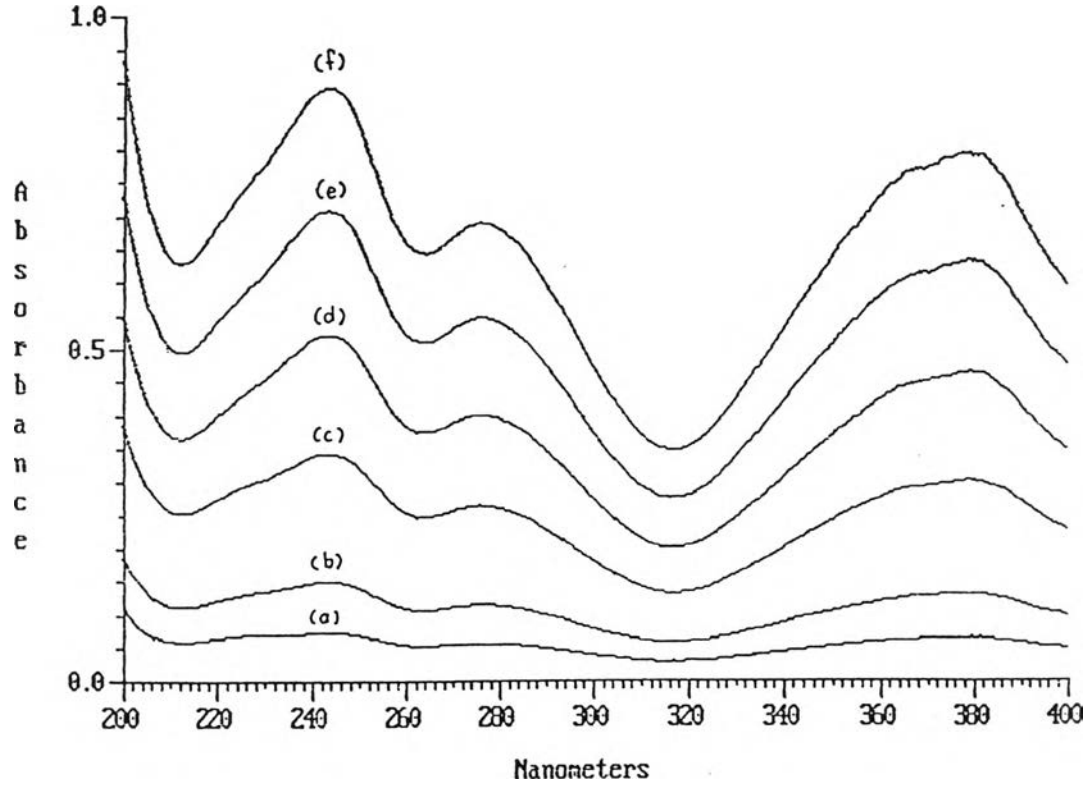


Figure 8 Spectrum of minocycline hydrochloride standard solutions at the concentration of 2 (a), 4 (b), 9 (c), 14 (d), 19 (e), and 24 (f) μg/mL, respectively.

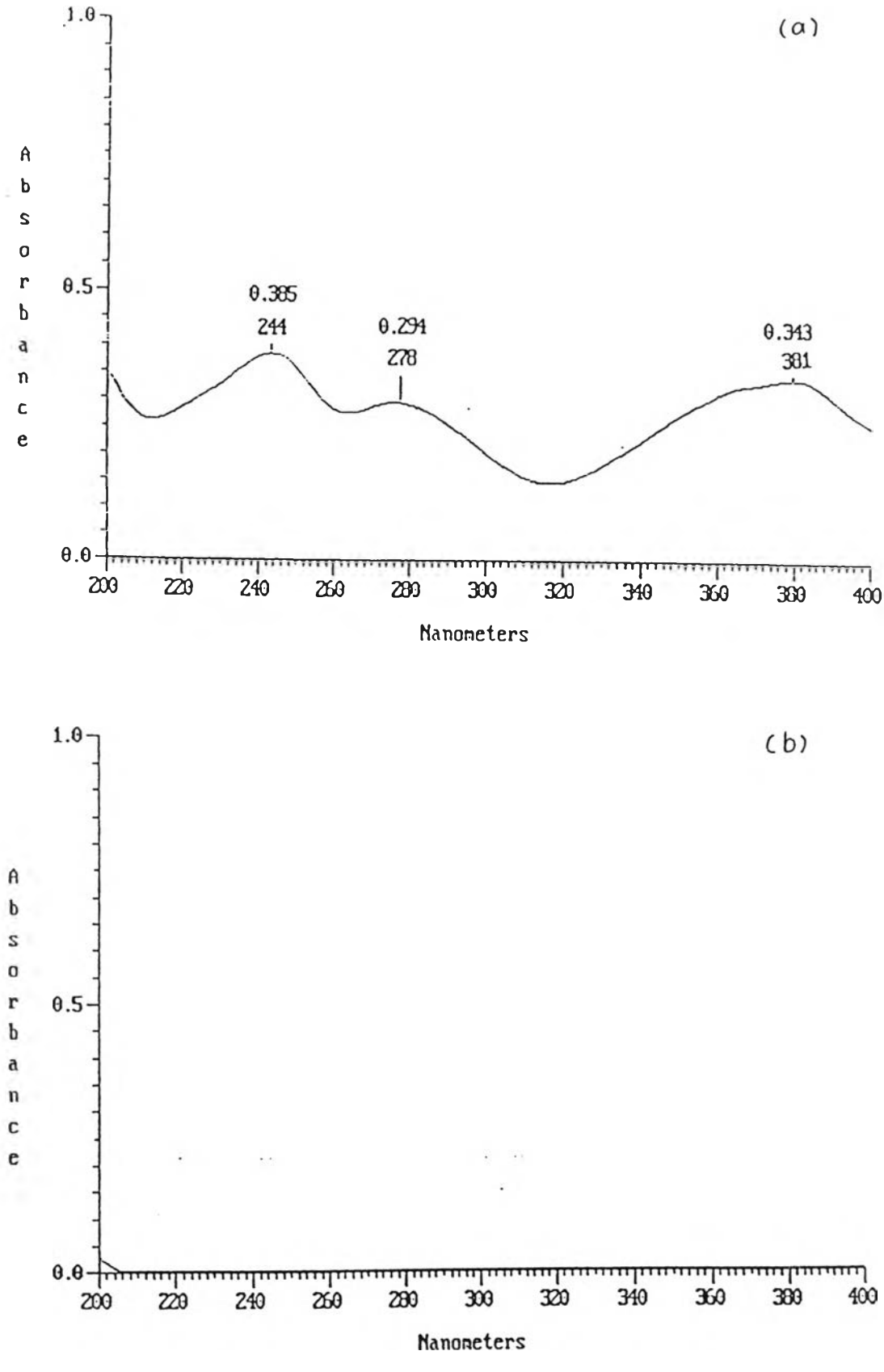


Figure 9 Spectrum of the solution extracted from minocycline hydrochloride microcapsules (a) and blank microcapsules (b).

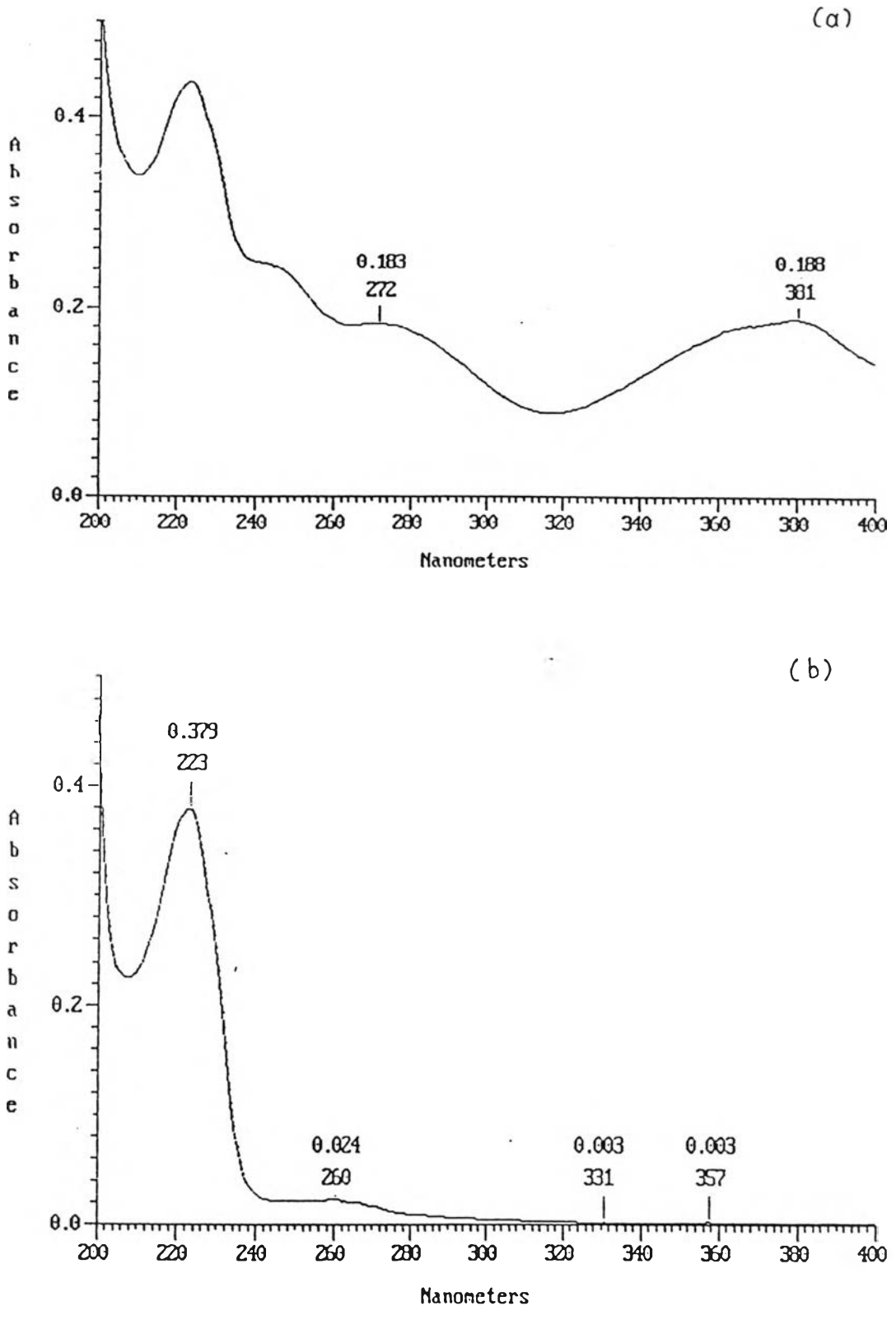


Figure 10 Spectrum of the solution released from minocycline hydrochloride microcapsules (a) and blank microcapsules (b).

1.2 Linearity

Figure 8 shows the spectrum of minocycline hydrochloride standard solutions at various concentrations. The standard curve data of minocycline hydrochloride assayed by UV spectrophotometer at 244 nm is shown in Table 7. A standard curve plotted between the absorbance and concentration in μg/mL of minocycline hydrochloride at 244 nm is shown in Figure 11.

The linear regression analysis was applied for fitting the data obtained. A straigth line was obtained with a coefficient of determination (R^2) of 0.9999. The regression equation of the line is

$$y = 0.0366\,x + 0.0050 \qquad (10)$$

where y is the absorbance of minocycline hydrochloride and x is the concentration of minocycline hydrochloride solution in μg/mL.

The standard curve data of minocycline hydrochloride assayed by UV spectrophotometer at 381 nm is shown in Table 8. A standard curve plotted between the absorbance and concentration in μg/mL of minocycline hydrochloride at 381 nm is shown in Figure 12.

The linear regression analysis was applied for fitting the data obtained. A straigth line was obtained with a coefficient of determination (R^2) of 0.9997. The regression equation of this line is

$$y = 0.0328x + 0.0060 \qquad (11)$$

where y is the absorbance of minocycline hydrochloride and x is the concentration of minocycline hydrochloride solution in μg/mL.

Table 7 Calibration curve data of minocycline hydrochloride assayed by UV spectrophotometer at 244 nm

Standard solution no.	Concentration (μg/mL)	Absorbance at 244 nm
1	2	0.077
2	4	0.152
3	9	0.335
4	14	0.517
5	19	0.705
6	24	0.881

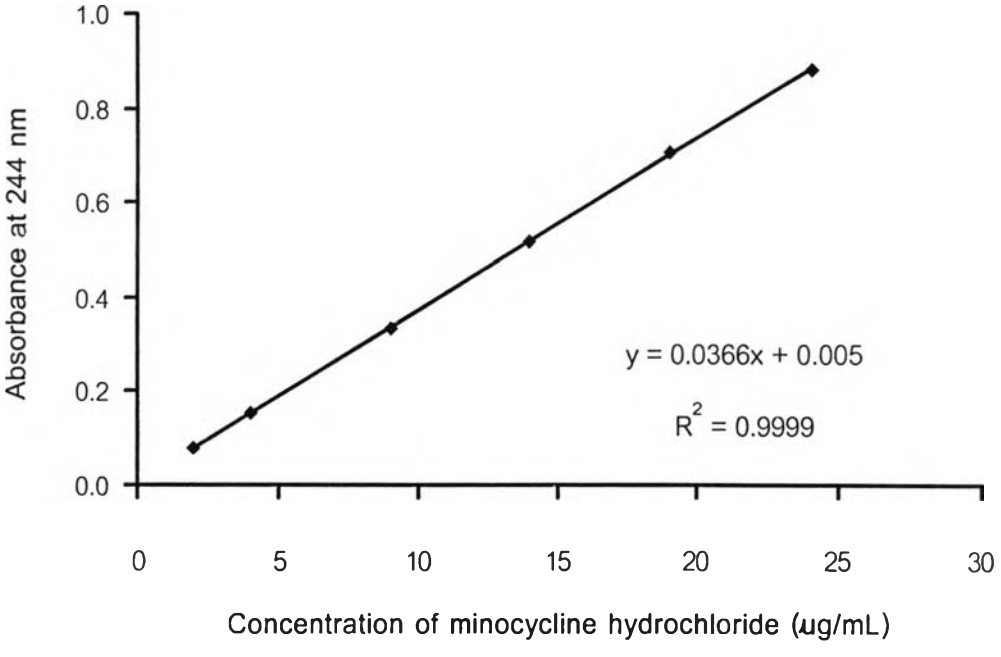


Figure 11 Calibration curve of minocycline hydrochloride assayed by UV spectrophotometer at 244 nm.

Table 8 Calibration curve data of minocycline hydrochloride assayed by UV spectrophotometer at 381 nm

Standard solution no.	Concentration (μg/mL)	Absorbance at 381 nm
1	2	0.070
2	4	0.135
3	9	0.306
4	14	0.469
5	19	0.622
6	24	0.795

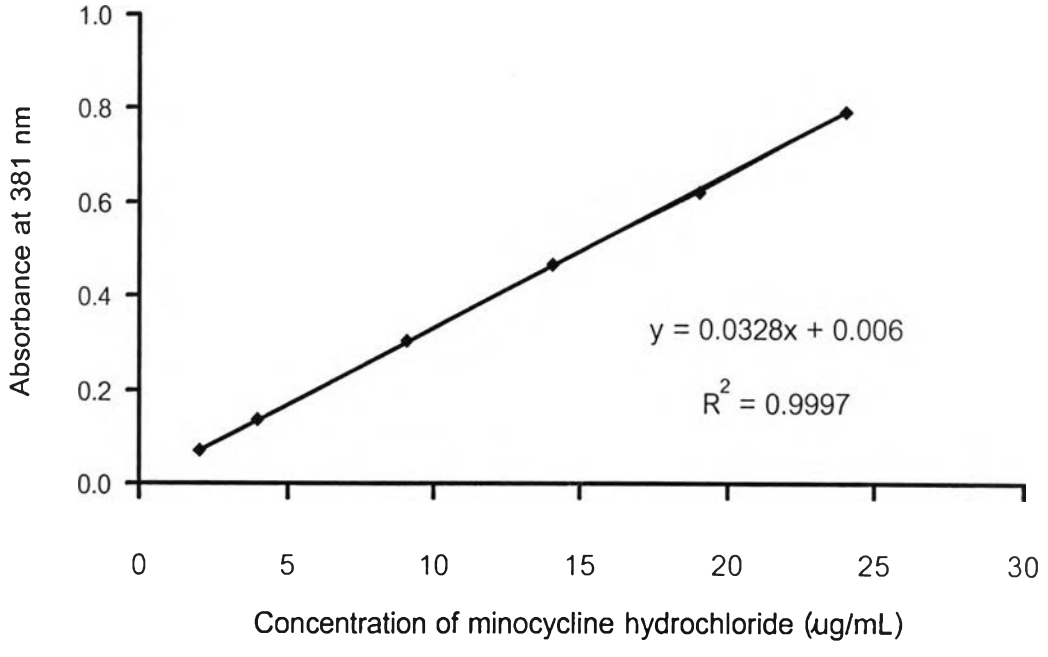


Figure 12 Calibration curve of minocycline hydrochloride assayed by UV spectrophotometer at 381 nm.

1.3 Accuracy

The accuracy of the analysis of minocycline hydrochloride by UV spectrophotometric method at 244 and 381 nm was performed by analyzing the percent analytical recoveries of standard solution (Tables 9 and 10). The obtained percentage recoveries were ranged from 97.00–100.59% and 97.50–101.67% for 244 and 381 nm, respectively (acceptance criteria of the percent recovery should not be less than 90% and not more than 110% (Gorog, 1995)) and the percent coefficients of variation (%CV) of percentage recoveries were low (1.17 and 1.03) which indicated that the UV spectrophotometric method could be used to determine the amount of minocycline hydrochloride within the concentration range studied.

1.4 Precision

The precision of the analysis of minocycline hydrochloride by UV spectrophotometric method at 244 and 381 nm was determined for both within-run and between-run as illustrated in Tables 11, 12, 13, and 14. The percent coefficients of variation (%CV) of all precision determinations were 0.07-1.41 (accepted criteria of the percent coefficients of variation are not more than 2% (Gorog, 1995)) which indicated that the UV spectrophotometric method could be used to determine the amount of minocycline hydrochloride over the period studied.

1.5 Limit of Quantitation

Limit of quantitation of the analysis of minocycline hydrochloride by UV spectrophotometric method at 244 and 381 nm was 2 μg/mL. The UV spectrophotometric method could be used to determine the amount of minocycline hydrochloride at 2 μg/mL with acceptable accuracy and precision (Gorog, 1995) as shown in Tables 9,10,11,12,13, and 14.

In conclusion specificity, linearity, accuracy, precision, and limit of quantitation in the assay of minocycline hydrochloride by the UV spectrophotometric method were established and they were in the acceptance criteria. Therefore, the UV spectrophotometric method could be used to determine the amount of minocycline hydrochloride in the concentration range and over the period of time studied.

Table 9 Accuracy data of minocycline hydrochloride assayed by UV spectrophotometric method at 244 nm

Minocycline hydrochloride conc. (μg/mL)	Estimated conc. (μg/mL)	% Recovery
2	1.99	99.50
2	1.94	97.00
2	1.99	99.50
12	11.99	99.92
12	11.89	99.08
12	11.75	97.92
22	21.99	99.95
22	22.13	100.59
22	22.08	100.33
	mean	99.31
	SD	1.16
	%CV	1.17

Table 10 Accuracy data of minocycline hydrochloride assayed by UV spectrophotometric method at 381 nm

Minocycline hydrochloride conc. (μg/mL)	Estimated conc. (μg/mL)	% Recovery
2	1.95	97.50
2	1.95	97.50
2	1.98	99.00
12	11.95	99.58
12	12.20	101.67
12	12.16	101.33
22	21.89	99.50
22	22.13	100.59
22	21.83	99.23
	mean	100.32
	SD	1.03
	%CV	1.03

Table 11 Data of within-run precision of minocycline hydrochloride assayed by UV spectrophotometric method at 244 nm

conc. (μg/mL)	Absorbance at 244 nm					
	Samp.1	Samp.2	Samp.3	Mean	SD	%CV
2	0.077	0.076	0.078	0.077	0.001	1.30
12	0.440	0.443	0.442	0.442	0.002	0.35
22	0.813	0.812	0.813	0.813	0.001	0.07

Table 12 Data of between-run precision of minocycline hydrochloride assayed by UV spectrophotometric method at 244 nm

conc. (μg/mL)	Absorbance at 244 nm					
	Day1	Day2	Day3	Mean	SD	%CV
2	0.077	0.076	0.078	0.077	0.001	1.30
12	0.440	0.441	0.438	0.440	0.002	0.35
22	0.813	0.816	0.813	0.814	0.002	0.21

Table 13 Data of within-run precision of minocycline hydrochloride assayed by UV spectrophotometric method at 381 nm

conc. (μg/mL)	Absorbance at 381 nm					
	Samp.1	Samp.2	Samp.3	Mean	SD	%CV
2	0.070	0.071	0.071	0.071	0.001	0.82
12	0.403	0.402	0.401	0.402	0.001	0.25
22	0.726	0.725	0.720	0.724	0.003	0.44

Table 14 Data of between-run precision of minocycline hydrochloride assayed by UV spectrophotometric method at 381 nm

conc. (μg/mL)	Absorbance at 381 nm					
	Day1	Day2	Day3	Mean	SD	%CV
2	0.070	0.072	0.071	0.071	0.001	1.41
12	0.403	0.405	0.402	0.403	0.002	0.38
22	0.726	0.729	0.725	0.727	0.002	0.29

2. Preparation of Minocycline Hydrochloride Microcapsules by Solvent Evaporation Technique

Water in oil in water solvent evaporation technique was used to prepare minocycline hydrochloride microcapsules because minocycline hydrochloride is soluble in water and the multiple emulsion solvent evaporation technique was one of the most efficient incorporation of water soluble drug (Watts, Devies, and Melia, 1990). In this method the polymer was dissolved in organic solvent (dichloromethane) while minocycline hydrochloride was dissolved in water. In order to make the emulsion stable during dispersing water phase in oil phase, sodium carboxymethylcellulose was used as stabiliser.

For further step, water in oil emulsion was reemulsified into aqueous solution containing polyvinyl alcohol as an emulsifier to produce a multiple water in oil in water emulsion. Dichloromethane acts as a barrier between the two aqueous compartments, preventing the diffusion of minocycline hydrochloride toward the external phase.

Stirring was continued for 3 hours in order to ensure complete evaporation of dichloromethane resulting in a thin polymer film coated around the minocycline hydrochloride droplets. Microcapsules with spherical-like shape were produced and they had free-flowing properties after drying.

2.1 Effect of Type of Polymer and Stabiliser Concentration

Physicochemical properties and concentration of emulsifier or stabiliser influenced morphology and encapsulation efficiency (Esposito et al., 1997; Schugens et al., 1994; Yeh et al., 1995). In preliminary study it was found that minocycline hydrochloride microcapsules prepared using sodium carboxymethylcellulose (SCMC) as stabiliser gave better result than gelatin and methylcellulose in terms of microparticle morphology and stability. Then SCMC was used as stabiliser in this study and the concentration of SCMC was also varied from 0-0.20% w/v in each polymer.

Physical appearance of all microcapsule formulations showed yellow color due to the color of minocycline hydrochloride and the microcapsules were free-flowing. Percent entrapment of minocycline hydrochloride microcapsules is shown in Table 15.

Split-plot design, randomized block design and John Tukey's Hornestly significant difference were used for data analysis of the percent entrapment. Split-plot design was used for comparing difference of each SCMC concentration in all polymers. It was found that the percent entrapment of microcapsules prepared with different concentrations of SCMC had significant difference ($p < 0.05$) as shown in Table 31 of Appendix E.

Randomized block design was used for comparing difference of each SCMC concentration in each polymer on percent entrapment of minocycline hydrochloride microcapsules. It was found that the percent entrapment of microcapsules prepared with different concentrations of SCMC in every polymer had significant difference ($p < 0.05$) as shown in Tables 32, 34, 36, and 38 of Appendix E.

After that John Tukey's Hornestly significant difference was used for multiple comparison of SCMC concentration which gave the most drug entrapment in each polymer. It was found that microcapsules prepared with 0% SCMC for every polymer had the highest percent entrapment ($p < 0.05$) as shown in Tables 33, 35, 37, and 39 of Appendix E. This result is consistent with a previous study by Yeh et al.(1995). The mechanism underlying this observation is not known.

Physicochemical properties of polymers such as molecular weight, and hydrophilic properties might influence the properties of microcapsules (Polard et al., 1996). In this study 4 types of biodegradable polymers namely poly (L-lactide) (L-PLA), poly (DL-lactide) (DL-PLA), poly (DL-lactide-co-glycolide) 75:25 (PLGA 75:25), and poly (DL-lactide-co-glycolide) 50:50 (PLGA 50:50) were investigated due to their biocompatibility and biodegradability (Jain et al., 1998). L-PLA, DL-PLA, and PLGA 75:25 have molecular weight within the same range (85,000-160,000) while PLGA 50:50 has the lowest molecular weight (59,000).

Table 15 Percent entrapment of minocycline hydrochloride microcapsules prepared by w/o/w solvent evaporation technique with different polymers and sodium carboxymethylcellulose concentrations (mean ± SD, n=3)

Coating polymer	SCMC concentration (%W/V)	%Entrapment (mean ± SD)
L-PLA MW 85,000-160,000	0%	4.99 ± 0.03
	0.05%	2.87 ± 0.00
	0.10%	1.45 ± 0.00
	0.20%	1.46 ± 0.03
DL-PLA MW 106,000	0%	4.17 ± 0.03
	0.05%	0.54 ± 0.00
	0.10%	0.91 ± 0.00
	0.20%	0.90 ± 0.00
PLGA(75:25) MW 90,000-126,000	0%	4.87 ± 0.03
	0.05%	2.28 ± 0.03
	0.10%	4.68 ± 0.00
	0.20%	1.37 ± 0.03
PLGA(50:50) MW 59,000	0%	2.99 ± 0.03
	0.05%	1.77 ± 0.03
	0.10%	0.85 ± 0.00
	0.20%	0.81 ± 0.03

Randomized block design and John Tukey's Hornestly significant difference were used for data analysis of percent entrapment in different polymers with 0% SCMC. Randomized block design was used for comparing difference of each polymer. It was found that the percent entrapment of microcapsules prepared with different polymers had significant difference ($p < 0.05$) as shown in Table 40 of Appendix E.

After that John Tukey's Hornestly significant difference was used for multiple comparison of polymer types. It was found that percent entrapments of minocycline

hydrochloride microcapsules prepared using L-PLA, DL-PLA and PLGA 75:25 were higher than microcapsules prepared using PLGA 50:50 ($p < 0.05$) as shown in Table 41 of Appendix E.

This can be explained that PLGA 50:50 contains more glycolic acid than L-PLA, DL-PLA, and PLGA 75:25. Thus it is more hydrophilic and can absorb more water (Jain et al., 1998). Consequently, minocycline hydrochloride dissolved in internal aqueous phase might penetrate through PLGA 50:50 better than L-PLA, DL-PLA and PLGA 75:25. Thus, percent entrapment of minocycline hydrochloride microcapsules prepared using PLGA 50:50 was the lowest.

Furthermore, it was found that all formulations gave low drug entrapment (Table 15). This can be explained that minocycline hydrochloride can be partially soluble in dichloromethane which was supposed to act as a barrier between the two aqueous components. Thus, minocycline hydrochloride might penetrate into the outer aqueous compartment, resulting in low entrapment of the drug.

2.2 Effect of Core to Wall Ratio

Core to wall ratio influenced the properties of microcapsules (Ruiz, Sakr and Sprockel, 1990; Vanichtanunkul, Vayumhasuwan and Nimmannit, 1998; Herrmann and Bodmeier, 1995).

From the investigation of stabilising agent concentration effect, the 0% SCMC of each coating polymer was chosen for futher studies. The microcapsules were prepared with various core to wall ratios such as 1:5, 1:1, and 5:1 by fixed amount of drug and varying amount of polymer.

The core to wall ratios affected to percent yield, percent content, core entrapment, morphology, particle size, and the release characteristic of microcapsules as shown in topic 3.

3. Physicochemical Characterization of Minocycline Hydrochloride Microcapsules

3.1 Yield of Microcapsules

Physical appearance of all microcapsule formulations showed yellow color due to the color of minocycline hydrochloride and the microcapsules were free-flowing. Table 16 shows percent yield of minocycline hydrochloride microcapsules.

Every polymer showed the same trend that when the core to wall ratio increased the percent yield decreased. The microcapsules prepared with 1:5 core to wall ratio in each coating polymer gave the highest percent yield while the microcapsules prepared with 5:1 core to wall ratio gave the lowest percent yield as shown in Table 16. Increasing the amount of polymer in the organic phase increased the viscosity of the primary w/o emulsion. This possibly stabilised the internal aqueous phase against coalescence, reduced mixing with the external aqueous phase, and hence reduced drug loss to the external phase. This result is consistent with a previous study by Herrmann and Bodmeier (1995).

The microcapsules prepared with 5:1 core to wall ratio gave so low percent yield that it was not investigated in the next topics.

Table 16 Percent yield of minocycline hydrochloride microcapsules prepared by the w/o/w solvent evaporation technique with different polymers and core to wall ratios

Coating polymer	Core to wall ratio	%Yield
L-PLA	1:5	83.22
	1:1	52.00
	5:1	23.81
DL-PLA	1:5	79.67
	1:1	49.88
	5:1	26.03
PLGA 75:25	1:5	72.97
	1:1	46.38
	5:1	17.43
PLGA 50:50	1:5	81.85
	1:1	46.91
	5:1	16.67

3.2 Percent Content and Percent Core Entrapment

The microcapsules prepared with 1:5 core to wall ratio in each coating polymer gave higher percent entrapment than the microcapsules prepared with 1:1 core to wall ratio (Table 17). There was a statistically significant difference between 1:5 and 1:1 core to wall ratio with $p < 0.05$ when evaluated by randomized block design (Table 42-45 of Appendix E).

Table 17 The percent content and percent entrapment of minocycline hydrochloride microcapsules prepared by the w/o/w solvent evaporation technique with different polymers and core to wall ratios (mean ± SD, n=3)

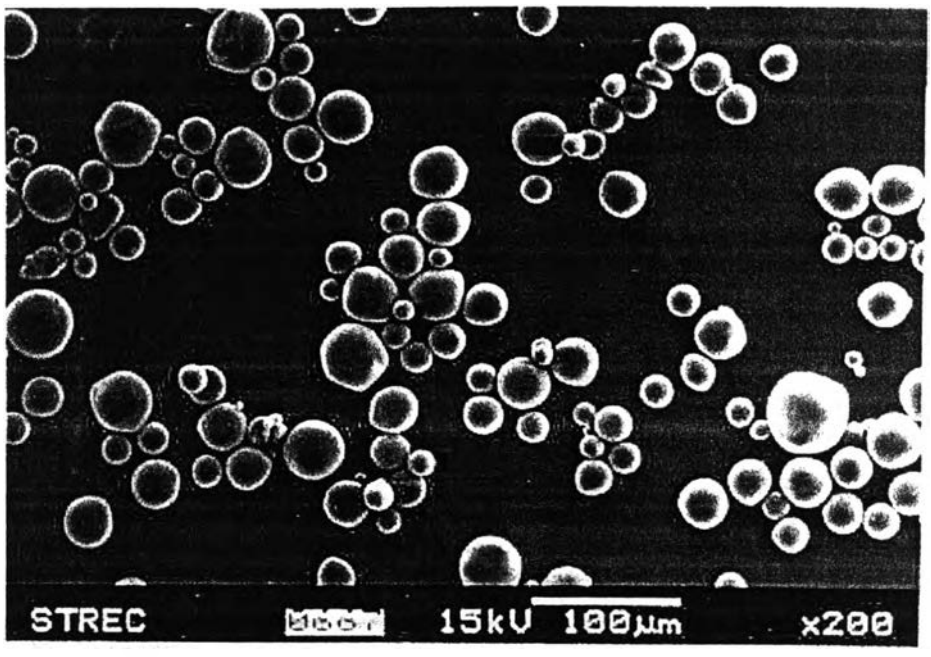
Coating polymer	Core to wall ratio	% Content (mean ± SD)	% Entrapment (mean ± SD)
L-PLA	1:5	0.84 ± 0.01	4.99 ± 0.03
	1:1	0.02 ± 0.01	0.05 ± 0.01
DL-PLA	1:5	0.71 ± 0.01	4.17 ± 0.03
	1:1	0.02 ± 0.00	0.04 ± 0.00
PLGA 75:25	1:5	0.82 ± 0.01	4.87 ± 0.03
	1:1	0.03 ± 0.00	0.06 ± 0.00
PLGA 50:50	1:5	0.49 ± 0.01	2.99 ± 0.03
	1:1	0.03 ± 0.00	0.06 ± 0.00

This result can be explained as described earlier (Topic 3.1). This result agrees with a previous research by Herrmann and Bodmeier (1995).

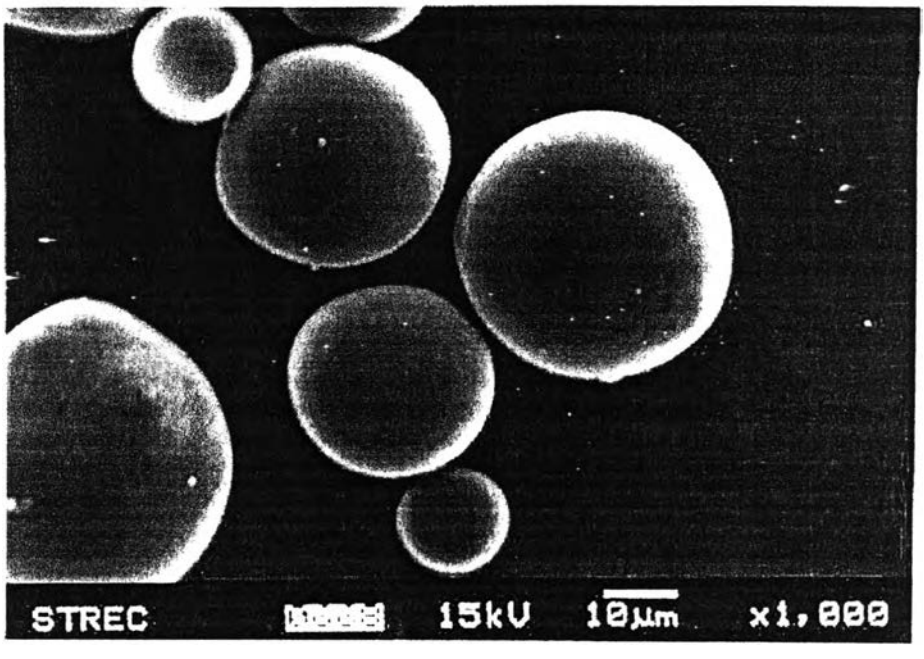
The microcapsules prepared with 1:1 core to wall ratio gave so low percent content and percent core entrapment that it was not investigated in the next topics.

3.3 Morphology

Morphology of the microcapsules prepared with 1 : 5 core to wall ratio in each coating polymer was shown in Figures 13, 14, 15, and 16. The microcapsules prepared from poly (L-lactide), poly (DL-lactide), poly (DL-lactide-co-glycolide) 75:25, and 50:50 had spherical-like shape with some aggregation.

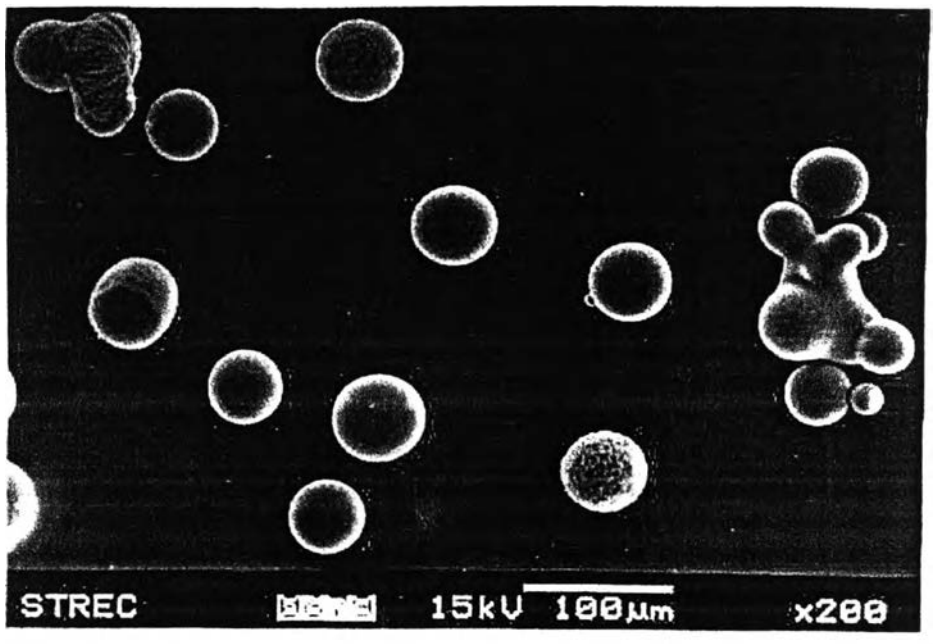


(a)

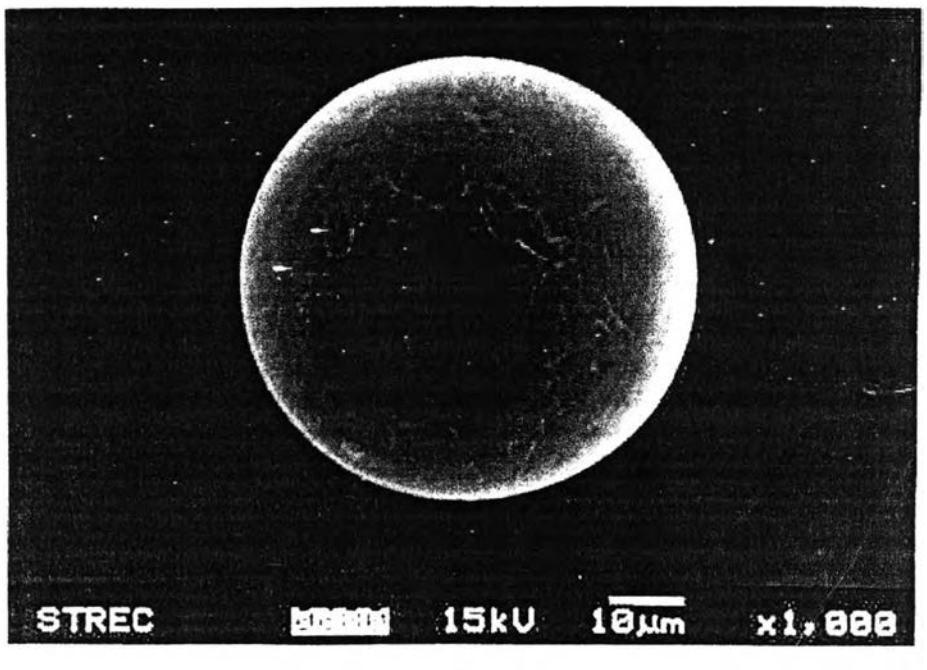


(b)

Figure 13 Scanning electron micrographs of minocycline hydrochloride microcapsules prepared by the w/o/w solvent evaporation technique using poly (L-lactide) with 1:5 core to wall ratio. Magnification 200 x (a) and 1000 x (b), respectively.

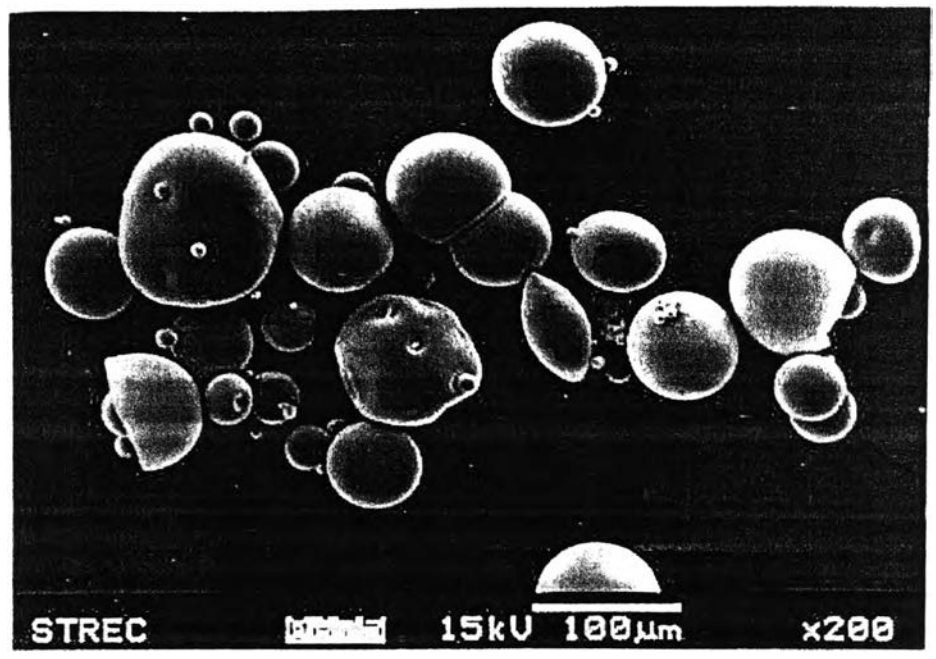


(a)

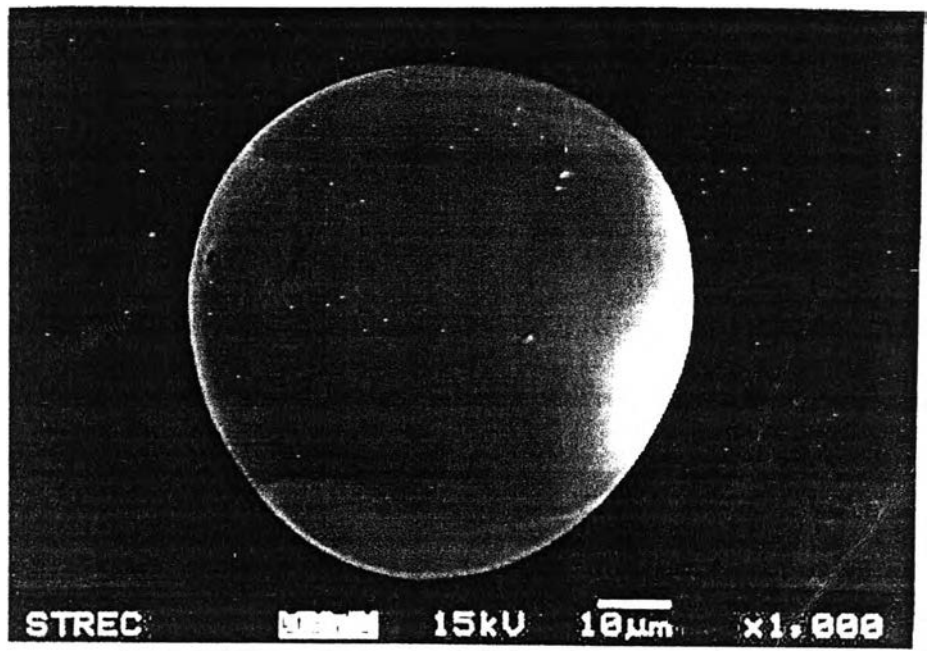


(b)

Figure 14 Scanning electron micrographs of minocycline hydrochloride microcapsules prepared by the w/o/w solvent evaporation technique using poly (DL- lactide) with 1:5 core to wall ratio. Magnification 200 x (a) and 1000 x (b), respectively.

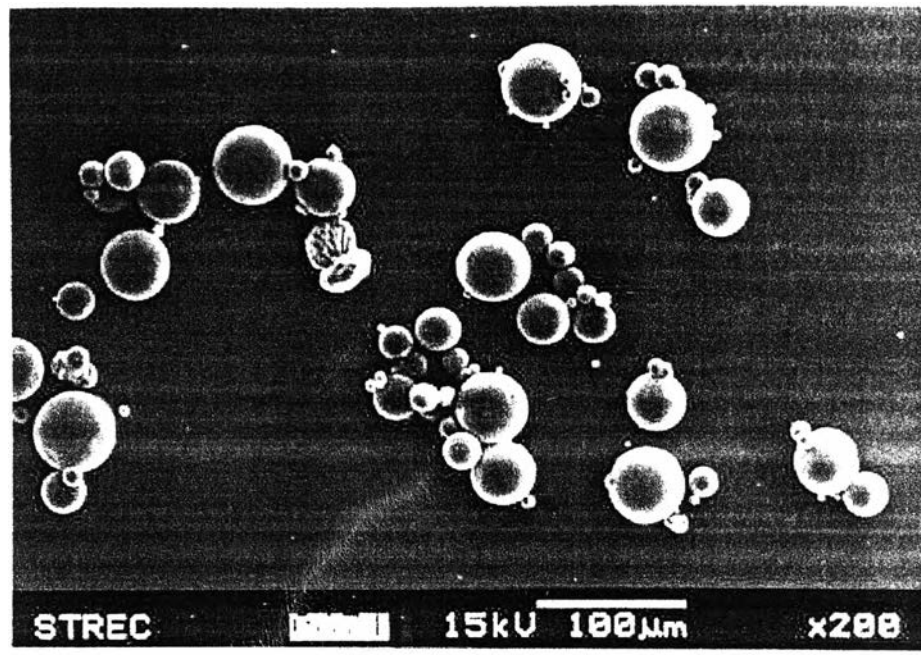


(a)

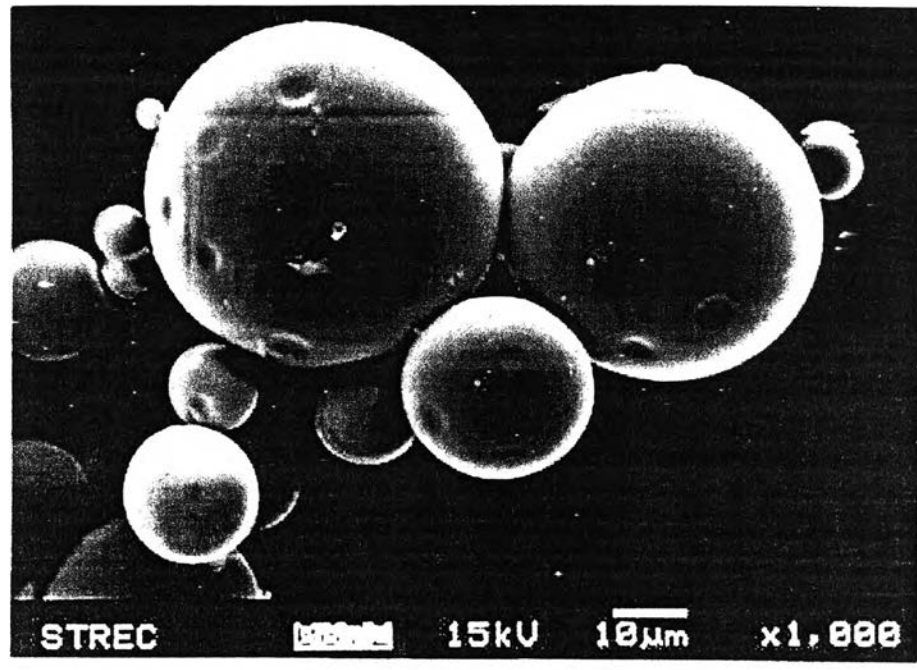


(b)

Figure 15 Scanning electron micrographs of minocycline hydrochloride microcapsules prepared by the w/o/w solvent evaporation technique using poly (DL-lactide-co-glycolide) 75:25 with 1:5 core to wall ratio. Magnification 200 x (a) and 1000 x (b), respectively.



(a)



(b)

Figure 16 Scanning electron micrographs of minocycline hydrochloride microcapsules prepared by the w/o/w solvent evaporation technique using poly (DL-lactide- co-glycolide) 50:50 with 1:5 core to wall ratio. Magnification 200 x (a) and 1000 x (b), respectively.

The surfaces of microcapsules prepared from poly (L-lactide) and poly (DL-lactide) were smooth while the surfaces of microcapsules prepared from poly (DL-lactide-co-glycolide) 75:25 and 50:50 showed porosity and polymer attachment. This can be explained that poly (DL-lactide-co-glycolide) 75:25 and 50:50 contain glycolic acid then they are more hydrophilic and more permeable to water (Jain et al., 1998). Consequently, water may penetrate between the two aqueous compartment, resulting in surfaces of microcapsules with porosity.

3.4 Particle Size and Size Distribution

Laser particle size analyser was used to measure the size and size distribution of the microcapsules prepared with 1:5 core to wall ratio in each coating polymer. Size distribution of all preparations was not normal distribution (Appendix C) then the mode size was used to investigate as shown in Table 18.

Table 18 Mode size of minocycline hydrochloride microcapsules prepared with 1:5 core to wall ratio and various coating polymers

Coating polymer	Mode size (μm)
L-PLA	88.91-103.58 (with right shoulder)
DL-PLA	56.23-65.51
PLGA 75:25	76.32-88.91 (with right shoulder)
PLGA 50:50	56.23-65.51 and 258.95-301.68 (bimodal distribution)

PLGA 50:50 had the lowest molecular weight then it might have rapid rate of polymer precipitation at the droplet surface (Polard et al., 1996). Therefore, some parts of microcapsules showed lower mode size (Table 18). Furthermore, PLGA 50:50 is more hydrophilic and absorb more water (Jain et al., 1998) thus each droplet of primary emulsion might agglomerate, resulting in microcapsules with higher mode size.

3.5 The Release of Minocycline Hydrochloride from Microcapsules

In preliminary study, it was found that phosphate buffer could not be used for study the release of minocycline hydrochloride from microcapsules because it interfered with analysis of drug released from minocycline hydrochloride microcapsules. Thus isotonic borate buffer pH 7.5 ± 0.1 was used as the medium of drug release (Esposito et al., 1997).

The effect of coating polymer on the *in vitro* drug release within 48 hours from the microcapsules is illustrated in Figure 17. All formulations showed two release phases when percent released was plotted against time; one initial fast release phase (burst effect) followed by second slow release phase. The burst effect could be attributed to drug diffusion from near surface and preformed pores within the microcapsules rather than the polymer degradation rate. The drug release in the second slow phase could be produced by diffusion from the interior together with the erosion of the polymer. The aqueous medium slowly penetrated through the internal structure of the particles causing progressive degradation of polymer chain (Martinez et al., 1997).

Poly (DL-lactide-co-glycolide) 50:50 (PLGA 50:50) gave the lowest release profile. This result disagrees with previous study by Esposito et al. (1997) and Lacasse et al. (1997) in that lower molecular weight polymer gave higher drug release. Martinez et al. (1997) reported that PLGA 50:50 showed higher release profile than PLGA 75:25 due to more rapid degradation of the copolymer. However this result is consistent with previous studies by Ramtoola, Corrigan and Bourke (1991) and Suzuki and Price (1985) that lower drug loading gave lower drug release and greater particle size gave lower drug release. This result could be explained that PLGA 50:50 had the lowest molecular weight but it had the lowest percent entrapment (2.99 ± 0.03%), the largest mode size (258.95 – 301.68 μm) and aggregated particles (Figure 18(d)) then the release profile of PLGA 50:50 was the lowest.

Poly (L-lactide) (L-PLA), poly (DL-lactide) (DL-PLA), and poly (DL-lactide-co-glycolide) 75:25 (PLGA 75:25) had molecular weight within the same range (85,000 – 160,000) but they had different percent entrapment. L-PLA had similar percent

entrapment to PLGA 75:25, and higher than DL-PLA. Thus, release profile of L-PLA and PLGA 75:25 was higher than DL-PLA. This result agrees with previous studies by Ramtoola, Corrigan and Bourke (1991) and Suzuki and Price (1985).

The release profile of PLGA 75:25 was higher than L-PLA. This result can be explained that PLGA 75:25 contains glycolic acid which was hydrolyzed faster than L-PLA (Shih, Waldron and Zenther, 1996). Furthermore, microcapsules prepared using PLGA 75:25 had porous surface (Figure 18(c)), thus drug could penetrated through pore to medium faster than other polymers.

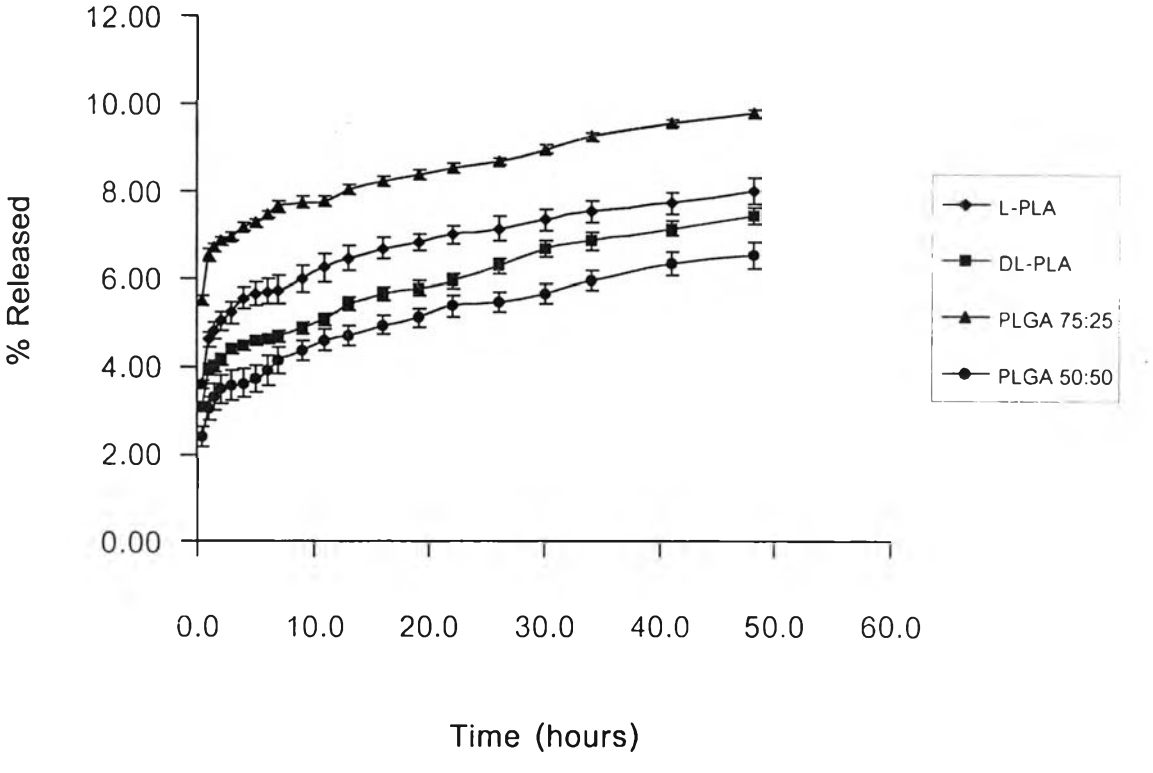


Figure 17 The release profiles of minocycline hydrochloride microcapsules prepared with poly (L-lactide), poly (DL-lactide), poly (DL-lactide-co-glycolide) 75:25, and poly (DL-lactide-co-glycolide) 50:50 in isotonic borate buffer pH 7.5 ± 0.1.

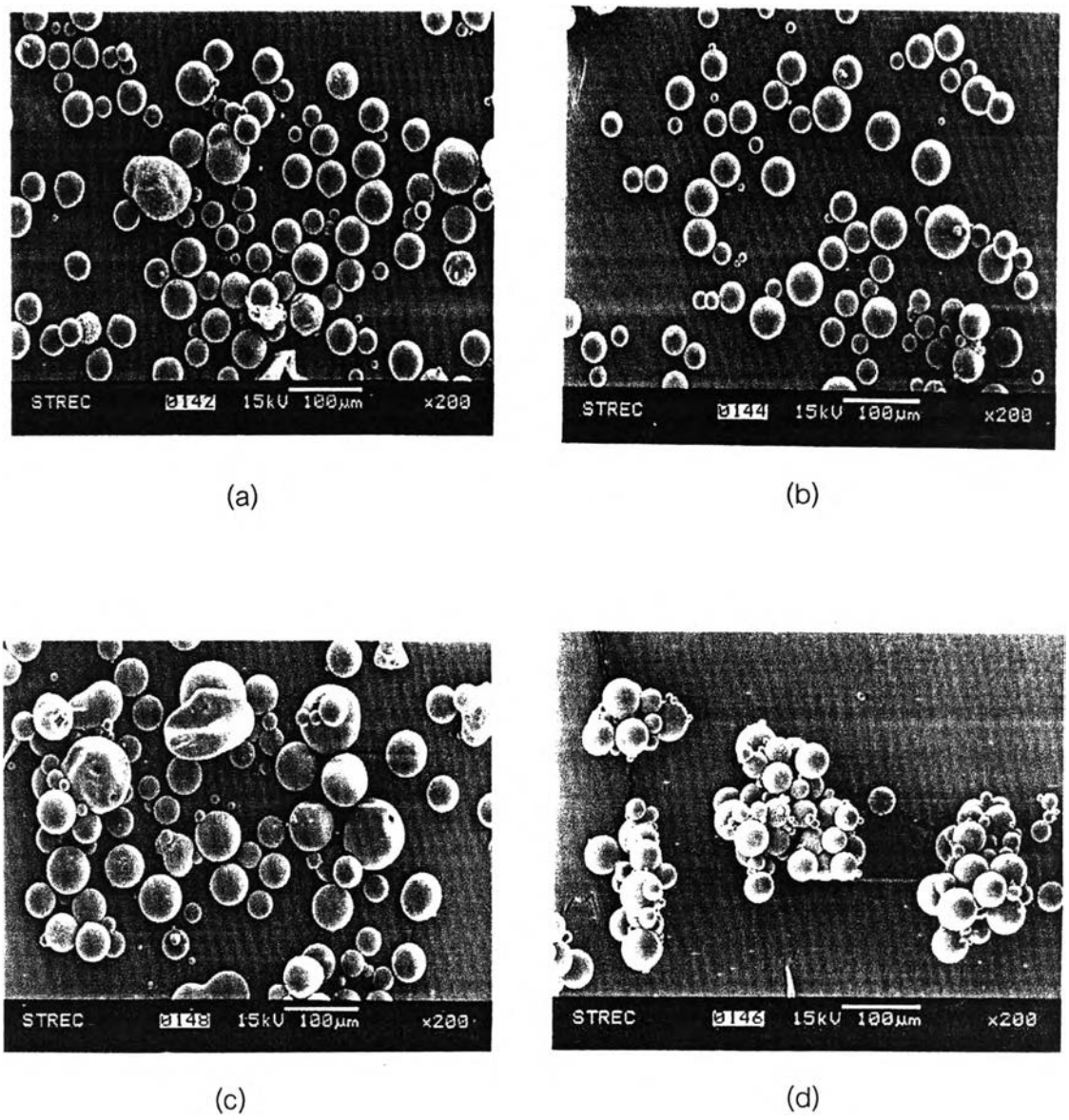


Figure 18 Scanning electron micrographs of minocycline hydrochloride microcapsules prepared with poly (L-lactide) (a), poly (DL-lactide) (b), poly (DL-lactide-co-glycolide) 75:25 (c), and poly (DL-lactide-co-glycolide) 50:50 (d) after 48 hours drug release.

This study was designed to develop sustained release microcapsules of minocycline hydrochloride. Thus, the release rate constant (k), the coefficient of determination (R^2), and intercept of graph were calculated from release profile at 11-48 hours. The appropriate release kinetic was selected from these values.

The zero-order, first-order, and Higuchi model had the coefficient of determination (R^2) within the same range but standard error of the release rate constant (k) and graph intercept of first-order had the narrowest range as shown in Table 19. The release kinetics of minocycline hydrochloride from microcapsules prepared from L-PLA, DL-PLA, PLGA 75:25, and PLGA 50:50 followed first-order. A fit with the first-order suggested controlled dissolution process.

Table19 The release rate constant, the coefficient of determination (R^2), and graph intercept of zero-order (k_0), first-order (k_1), and Higuchi model (k_h) of minocycline hydrochloride microcapsules prepared with 1:5 core to wall ratio and various coating polymers in pH 7.5 ± 0.1 (mean ± SE, n=6)

Coating	Zero-order			First-order			Higuchi Model		
polymer	k_0	R^2	Intercept	k_1	R^2	Intercept	k_h	R^2	Intercept
L-PLA	0.0451± 0.0010	0.9737± 0.0043	5.9264± 0.0562	0.0028± 0.0001	0.9622± 0.0051	0.0094± 0.0038	0.4650± 0.0104	0.9918± 0.0019	4.7863± 0.0788
DL-PLA	0.0628± 0.0031	0.9692± 0.0038	4.5990± 0.1290	0.0044± 0.0002	0.9526± 0.0053	0.6779± 0.0107	0.6466± 0.0313	0.9849± 0.0025	3.0157± 0.1901
PLGA 75:25	0.0530± 0.0009	0.9838± 0.0032	7.3299± 0.0575	0.0026± 0.00004	0.9787± 0.0034	0.8706± 0.0039	0.5441± 0.0094	0.9910± 0.0016	6.0042± 0.0564
PLGA 50:50	0.0531± 0.0018	0.9853± 0.0026	4.0948± 0.0859	0.0042± 0.0001	0.9762± 0.0047	0.6271± 0.0088	0.5439± 0.0182	0.9889± 0.0016	2.7716± 0.1025

The result of this study showed that minocycline hydrochloride microcapsules prepared using this technique had low core entrapment and drug release. However, the concentration of drug released was within therapeutic range (>1μg/mL) (Mashimo et al., 1981). For further study, this technique should be developed for increasing the percent entrapment of minocycline hydrochloride microcapsules.

3.6 Determination of Residual Dichloromethane Content in the Microcapsules

Amount of residual organic solvent after production is important because the toxicity of dichloromethane which is classified as class 2 solvent: suspect animal carcinogens or possible cause of other irreversible toxicity, such as neurotoxicity or teratogenicity. The United States Pharmacopeia that the limits for dichloromethane in preparation is 500 ppm.

Amounts of the residual dichloromethane prepared from poly (L-lactide), poly (DL-lactide) and poly(DL-lactide-co-glycolide) 50:50 were lower than the USP limits (Table 20). The residual dichloromethane prepared from poly(DL-lactide-co-glycolide) 75:25 was 4% higher than the USP limits. It could be explained that different duration of storage might influence the residual dichloromethane content.

Table 20 Residual dichloromethane in various coating polymers of minocycline hydrochloride microcapsules prepared with 1:5 core to wall ratio (n=1)

Coating polymer	Residual DCM (ppm)
L-PLA	107.3
DL-PLA	lower than limit of quantitation
PLGA 75:25	520.6
PLGA 50:50	474.3